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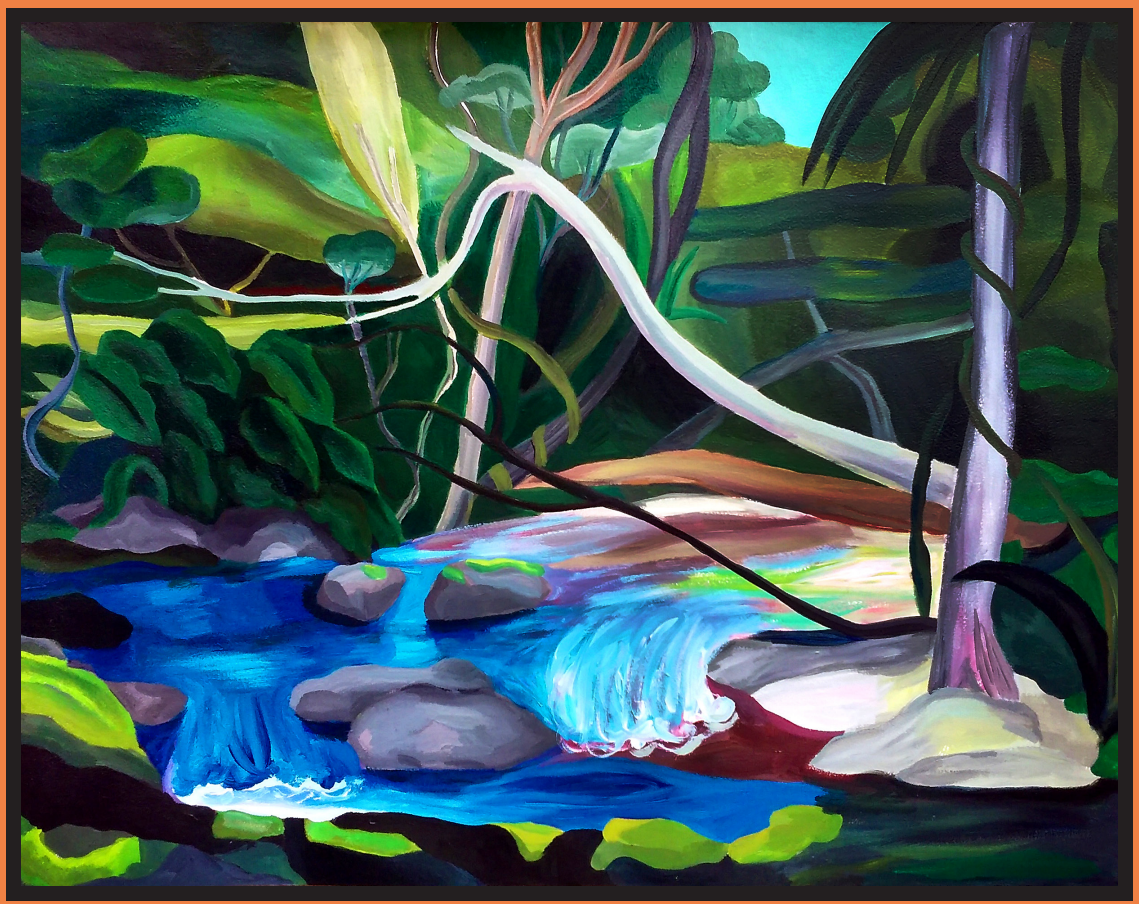

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La Tapa
Todo, 2016
Daniela Kantor

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REUNIÓN CONJUNTA SAIC SAB AAFE AACYTAL 2023

LXVIII REUNIÓN ANUAL DE LA SOCIEDAD ARGENTINA DE INVESTIGACIÓN CLÍNICA (SAIC)

XXV JORNADAS ANUALES DE LA SOCIEDAD ARGENTINA DE BIOLOGÍA (SAB)

LV REUNIÓN ANUAL DE LA ASOCIACIÓN ARGENTINA DE FARMACOLOGÍA EXPERIMENTAL (AAFE)

VIII REUNIÓN CIENTÍFICA REGIONAL DE LA ASOCIACIÓN ARGENTINA DE CIENCIA Y TECNOLOGÍA DE ANIMALES DE LABORATORIO (AACYTAL)

15-17 de noviembre de 2023
Hotel 13 de Julio – Mar del Plata

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Dra. Isabel Luthy
Dra. Silvina Pérez Martínez
Dr. Ventura Simonovich
Dr. Gabriel Pinto

JOINT MEETING SAIC SAB AAFE AACYTAL 2023

LXVIII ANNUAL MEETING OF
SOCIEDAD ARGENTINA DE INVESTIGACIÓN CLÍNICA
(SAIC)

XXV ANNUAL CONFERENCES OF SOCIEDAD
ARGENTINA DE BIOLOGÍA
(SAB)

LV ANNUAL MEETING OF ASOCIACIÓN ARGENTINA
DE FARMACOLOGÍA EXPERIMENTAL
(AAFE)

VIII REGIONAL SCIENTIFIC MEETING OF
ASOCIACIÓN ARGENTINA DE CIENCIA Y
TECNOLOGÍA DE ANIMALES DE LABORATORIO
(AACyTAL)

November 15-17, 2023
13 de Julio Hotel – Mar del Plata

RESPONSIBLE EDITORS
Dra. Isabel Luthy
Dra. Silvina Pérez Martínez
Dr. Ventura Simonovich
Dr. Gabriel Pinto

LA TAPA

Daniela Kantor. Todo, 2016

Técnica: Acrílico sobre cartón. Medidas 50 x 70 cm.

Diseñadora gráfica (FADU-UBA), docente, historietista e ilustradora. Habla idioma inglés, se desenvuelve con el francés, italiano y portugués. Es Jefa de Trabajos Prácticos en la materia Ilustración inicial (cátedra Daniel Roldan, FADU/UBA). En 2019 gano la Beca UBA Internacional en el marco del programa de intercambio docente con la Universidad Regiomontana (México) donde fue invitada a participar en la Feria del libro de los Universitarios de UNAM para presentar el libro de catedra “Palabra de ilustrador” del cual fue coordinadora. Dicta talleres sobre pintura, historieta e ilustración para chicos (CCRecoleta, CCK, Refugio Literario-Tigre, taller propio, etc.). Actualmente también se desempeña como educadora en el Museo de Arte Tigre (MAT), dando talleres y visitas. Estudió Dibujo de Historieta con Alberto Breccia, Técnicas de Acuarela y Pastel con Carlos Nine, charlas sobre Historieta con José Muñoz, Curso de Color con Carlos Gorriarena, Clínica de Pintura con Mariano Sapia y Tulio de Sagastizábal, Sumi- e en el Centro Okinawense. Recientemente participo con su historieta en el libro de promoción turística de Buenos Aires, generado desde el Ministerio de Deporte y Turismo de la Nación (2023). Autora de novelas gráficas como Mujer Primeriza (Ed Burlesque, 2014) y Aprendiza (2019), Marilyn (Tren en movimiento, 2019), participo en Dis- Tinta (Ed Sudamericana, catálogo de historietas coordinado por Liniers y Martin Perez). Dibujo para Las moradas de Santa Teresa de Jesús en historietas (Ed. Loco rabia + CCE-BA Centro Cultural de España en Buenos Aires). Es miembro de la revista de historietas El Tripero fundada en 1993 junto al grupo de alumnos de Alberto Breccia. Su trabajo trata temas como la Naturaleza, lo femenino, la maternidad, identidad personal, sexualidad, familia.

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PALABRAS DE BIENVENIDA
DE LOS PRESIDENTES DE LAS SOCIEDADES

Queridos amigos, amigas y colegas,

con gran alegría les doy la bienvenida a esta sexagésima octava Reunión de la Sociedad Argentina de Investigación Clínica (SAIC), que este año se realiza en conjunto con la Sociedad Argentina de Biología (SAB), la Asociación Argentina de Farmacología Experimental (AAFE) y la Asociación Argentina de Ciencia y Tecnología de Animales de Laboratorio (AACYTAL). De hecho, ya tuvieron oportunidad de participar de varias actividades en el día de hoy.

Ante todo, los agradecimientos. Primero al Dr. Daniel Alonso, que, con su generosidad habitual, me permitió participar de numerosas actividades durante su presidencia, preparándome para encarar este año. A las Dras. Caroline Lamb y Victoria Fabris que realizaron un trabajo maravilloso como secretaria y tesorera respectivamente. Al Dr. Rodolfo Rey, quien participó activamente de todas las actividades. A la Dra. Mariana Tellechea, encargada de la confección del suplemento del Congreso y quien nos organizó el llamado a evaluadores y coordinadores. Al conjunto del Consejo Directivo, que se involucró con gran entusiasmo y eficacia en las numerosas actividades de SAIC y en la activa organización de los simposios. A la secretaria administrativa, María Florencia Rodríguez, que debió comenzar este año luego de 12 de gestión de Ivana y lo hizo con gran eficiencia y entusiasmo. Y al contador Carlos Resnik por su permanente acompañamiento profesional. Al personal de G2, especialmente Julián García y Patricio Golato, que estuvieron siempre presentes, asesorándonos y encargándose de todos los aspectos que tan bien conocen y que a nosotros nos resultan difíciles. Un agradecimiento también a las Comisiones Directivas de las demás sociedades que participan de esta Reunión, especialmente la Dra. María Eugenia Matzkin, secretaria de SAB.

Nuestro agradecimiento a CONICET y Agencia por sus valiosos aportes. A todas las empresas que nos permiten año a año realizar este congreso y a las organizaciones que tan generosamente otorgan los premios: Fundación Cherny, Fundación Bigand, Fundación Gador, el premio Irene Faryna y Roberto Raveglia otorgado por la empresa ETC, el Eugenia Sacerdote de Lustig, por la empresa mABxience y la Asociación Civil Eduardo Wilde por el premio de Genética Humana. Agradezco especialmente a los socios y socias que se ofrecieron para evaluar los trabajos y coordinar las diferentes sesiones y a los y las jurados de premios.

Este año el tema económico resultó muy complicado por la inflación y devaluación constante. Sin embargo, contamos con 3 conferencistas internacionales: uno de ellos, un destacado joven investigador de la European Molecular Biology Organization (EMBO), cuyo pasaje internacional fue cubierto con el generoso aporte de la European Society for Medical Oncology (ESMO). Contamos además con destacadas personalidades nacionales para otras conferencias. Este año inauguramos la Conferencia Christiane Dosne de Pasqualini, que esperamos se mantenga en el futuro como las conferencias Lanari y Taquini. Hay 11 simposios de SAIC, algunos de ellos compartidos con otras Sociedades.

Hay además una conferencia, un simposio y un minicurso organizados y financiados por el Consejo de Genética, que mostró una muy valiosa actividad durante todo el año, como es habitual en el Consejo. El Comité de Docencia también realizó un workshop.

Contamos también con otros dos minicursos, sobre Bioinformática y Biobancos.

Desde las diferentes Sociedades buscamos realizar actividades que involucren un amplio abanico de intereses de todas ellas. La SAIC, fundada en 1960 por una serie de investigadores clínicos entre los que se destaca el Dr. Alfredo Lanari, pretendió nuclear los trabajos de investigación, esencialmente clínicos, referidos a la patología de las enfermedades y su tratamiento. Los grandes avances de biología celular y molecular hicieron que los investigadores básicos en diferentes campos biomédicos encontraran en la SAIC un am-

biente propicio para discutir sus resultados. Es así como, siguiendo las temáticas de los últimos congresos y sin descuidar la excelente calidad de las exposiciones de investigadores e investigadoras básicas, se puso énfasis en la incorporación de numerosas ponencias traslacionales, como lo atestiguan la mayoría de los simposios. Se realiza un simposio en conjunto con la Asociación Argentina de Oncología Clínica y otro con la Sociedad Argentina de Pediatría, ambos con un enfoque clínico además de traslacional. Hay un convenio firmado en años anteriores con esta última Sociedad. Por otro lado, dos simposios se realizan en conjunto con la Sociedad Argentina de Biología y otro con la Asociación Argentina de Farmacología Experimental en temas comunes con estas Sociedades.

Retomamos este año la presentación en formato de miniorales, que eran una tradición de SAIC. Antiguamente todas las presentaciones eran miniorales. Por la enorme cantidad de asistentes y presentantes, se incorporaron los pósters, que pasaron a ser la forma exclusiva de presentación de resúmenes. Debido al interés de los y las participantes y a los problemas logísticos que genera la enorme cantidad de pósters, decidimos incorporar algunos de ellos como miniorales.

Otra innovación que realizamos es que, debido al ballotage, debimos comprimir la enorme cantidad habitual de actividades en tres días. El éxito de esta compresión es mérito de la Dra. Caroline Lamb.

Pusimos especial cuidado en la participación federal en los simposios, con participación de destacadas personalidades de diferentes zonas del país. Este año tuvimos la particularidad de contar con 19 mujeres entre los 20 miembros del Consejo Directivo. Y se buscó en la gran mayoría de los simposios y conferencias mantener un equilibrio de género. La mesa de género incluye a una disidencia y a una investigadora que nos contará cómo lograron obtener una guardería en su Instituto, un problema que aqueja principalmente, aunque no exclusivamente a las mujeres jóvenes.

Estamos viviendo un momento de deserción de becarios, becarias, investigadores e investigadoras especialmente jóvenes. Está resultando muy difícil encontrar becarios, becarias y residentes médicos. Un aspecto obvio es que los sueldos recibidos no están acordes con la preparación y exigencia. Pero otro aspecto que también ayudaría sería eliminar la dedicación exclusiva, para no coartar la posibilidad de desarrollar otras tareas durante los fines de semana, por ejemplo.

En la preparación de las diferentes actividades, conferencias, simposios y minicursos, tuvimos en cuenta temas de interés para las diferentes especialidades mayoritarias de la Sociedad.

En cuanto a la oficina, está adecuadamente equipada con mobiliario, internet y una computadora. Se realizan las reuniones presenciales del Consejo Directivo en la misma y la secretaria asiste presencialmente dos veces por semana. El Consejo de Genética ha realizado también algunas reuniones presenciales en la sede. Se irán agregando algunos otros elementos importantes en el futuro.

Esperando que esta Reunión Anual de Sociedades de Biociencias sea tan productiva e interesante como todas las anteriores, y con la esperanza de motivar a los y las jóvenes a seguir trabajando activamente en ciencia tanto básica como traslacional, dejo formalmente inaugurada la sexagésima octava Reunión de la Sociedad Argentina de Investigación Clínica (SAIC).

Isabel Alicia Lüthy
Presidenta SAIC

Es para mí un placer darles la bienvenida a todas y todos a la Reunión Anual de Sociedades de Biociencias 2023 y en particular a la XXV Reunión Anual de la Sociedad Argentina de Biología (SAB). Quisiera comenzar estas palabras agradeciendo al Comité Organizador de esta reunión conjunta, en particular a las autoridades de SAIC, AAFE y AACyTAL y al personal de G2 ya que un congreso como este hubiera sido imposible de organizar, sin un verdadero trabajo en equipo. Además, al Consejo Nacional de Investigaciones Científicas y Técnicas, a la Agencia Nacional de Promoción Científica y Tecnológica, y al Ministerio de Ciencia y Tecnología e Innovación por el financiamiento para el desarrollo de nuestras actividades. Gracias a todos los participantes y también a los asistentes que amablemente aceptaron actividades como la coordinación de sesiones.

Este año realizamos la XXV Reunión Anual de la SAB, reuniones que se vienen implementando desde 1998 sin interrupciones con el objeto de difundir las investigaciones realizadas en distintas ramas de la biología y de la medicina, y generar un ámbito de interacción y discusión científica, desde los estudiantes de grado en sus primeras etapas de formación hasta investigadores de larga y reconocida trayectoria.

Quisiera agradecer y destacar especialmente el trabajo de la Comisión Directiva de la SAB no solo por su participación en el desarrollo y organización de las actividades relacionadas con este congreso sino también por el esfuerzo y la dedicación que han brindado cada uno de los miembros para llevar a cabo las actividades SAB que nos propusimos para este año. Ejemplo de ellas son la difusión y la coordinación de los 10 Cursos y Talleres de postgrado de la Sociedad que se han dictado en el 2023, la organización y evaluación del "Subsidio Dr. Eduardo Charreau", que es entregado desde el año 2021 por la SAB para apoyar proyectos liderados por jóvenes científicas/os, y la participación en las actividades relacionadas con las Jornadas de las Sociedades de Biología del país y otras Sociedades de investigación como la Sociedad Argentina de Medicina Reproductiva (SAMER) y la Sociedad Argentina de Embriología Clínica (SAEC).

Para las Jornadas SAB de este año hemos elegido enfocarnos en la temática de la "Influencia del ambiente en la fisiología y en la patología de las células y los organismos", un tema amplio que será abordado en forma interdisciplinaria desde la biología básica hasta la salud pública. En este sentido, se han incluido temas relacionados con el ambiente, hábitos y el estrés, sobre el comportamiento y fisiología reproductiva, el impacto del medioambiente/microambiente en el desarrollo de anomalías celulares o tisulares, o los posibles beneficios de la exposición a factores ambientales en las secuelas del estrés o desórdenes del neurodesarrollo. También, habrá

presentaciones relacionadas con el cambio climático sobre el desarrollo de la leshmaniasis en la población argentina, y la influencia del microambiente celular, el metabolismo celular, y epigenética en el desarrollo de enfermedades como el cáncer. Como todos los años, incluimos también el Simposio de jóvenes SAB, donde se invita a quienes se encuentran en las primeras etapas de su carrera de investigación a compartir sus hallazgos con la comunidad científica. Asimismo, hemos otorgado becas a jóvenes investigadores en formación provenientes de distintos lugares del país para que puedan trasladarse hasta aquí para presentar y discutir sus trabajos en la sección de comunicaciones libres.

Uno de los objetivos de esta Reunión Conjunta es ofrecer a los asistentes un espacio propicio para el encuentro entre pares de distintas regiones que investigan en numerosas áreas de las biociencias, alentando a la discusión y formación científica en un clima de intercambio cordial y multidisciplinario.

Esperamos que disfruten esta jornada desde lo académico y científico, y también desde lo social, aprovechando esta hermosa ciudad turística en esta etapa del año.

Silvina Pérez Martínez
Presidenta SAB

Estimados amigos,

Para mí es un honor dar inicio al congreso que compartimos en esta ocasión, como ha sido muchas veces, con SAIC, SAB y AACYTAL. El trabajo conjunto de nuestras sociedades para llegar hasta este momento ha sido muy fructífero y en estas épocas de tanta incertidumbre económica claramente un desafío mayor.

En febrero de 2023 en nuestra asamblea se modificó nuestro estatuto, el cual permitió de manera formal algo que se empezó a dar desde hace varios años: la incorporación de farmacología clínica como uno de los ejes de la asociación. Esto trajo aparejado una decisión de crear una comisión para la farmacología básica y otra para la clínica, ambas unidas y necesarias para poder llevar tratamientos a la población.

Como reflejo de esto en nuestro congreso tendremos oportunidad de entender otras maneras de canalizar proyectos de investigación, a través por ejemplo de creación de emprendimientos, conferencias como el descubrimiento de medicamentos para enfermedades desatendidas o el desafío de la investigación traslacional en pediatría entre otras de las actividades que tendremos

Las presentaciones tanto en forma de póster como orales son una de las actividades más queridas por todos los miembros, dar una devolución constructiva y pensar con los becarios y directores otras maneras de enriquecer un trabajo de alta calidad también es un desafío para todos los evaluadores. Año a año ese desafío hermoso se concreta, mientras vemos cómo van creciendo no solamente los nuevos miembros sino también su producción científica.

En un mundo cambiante nosotros como Asociación también debemos hacerlo para poder dar respuesta a nuestra misión de acercar, establecer vínculos y generar espacios para los actuales investigadores en farmacología, pero a su vez atraer a otros a este campo maravilloso que mostró en momentos muy difíciles que serán recordados por futuras generaciones como la investigación no solo salva vidas, sino también nos permite pensar nuevos desafíos para poder tener un mejor futuro como sociedad.

Para terminar, quiero hacer un agradecimiento a toda la comisión directiva de AAFE, en particular a Guillermina Hernando, a Jeronimo Laiolo y a Susana Gorzalczany quienes permitieron llegar a este momento de manera no traumática. Para finalizar quiero dejar un recuerdo por el fallecimiento de quien fuera el primer presidente de la asociación, el Doctor Luis María Zieher. Su legado como docente, investigador y creador de uno de los primeros comités de ética para estudios clínicos de nuestro país esperamos honrarlo con esta reunión.

¡Bienvenidos!

Ventura A. Simonovich
Presidente AAFE

Estimados participantes de la Reunión de Sociedades de Biociencias:

Es un enorme placer inaugurar la Reunión Anual de Sociedades de Biociencias 2023 junto con las sociedades SAIC, SAB y AAFE.

Aquellas personas involucradas con los animales en laboratorios se preocupan por su bienestar. Hay numerosos grupos profesionales que participan activamente en el bienestar de los animales de laboratorio. Técnicos para Bioterios, veterinarios especializados e investigadores se dedican a velar por el bienestar de los animales a su cuidado. Estos animales son tratados con compasión y respeto por los profesionales que cuidan de sus necesidades físicas y conductuales diarias. En otros países como EEUU, Brasil, Uruguay y en la Unión Europea, el uso de animales está altamente regulado con numerosas leyes, reglamentos, políticas y directrices nacionales, regionales y locales establecidas para garantizar la supervisión de los estudios. El bienestar de los animales es de extrema importancia para los profesionales altamente capacitados que se ocupan de estos animales y es su deber informar cualquier situación concerniente a los mismos.

El principio ético de la investigación con animales requiere que los científicos «reduzcan, refinen y reemplacen» (las 3Rs) el uso de animales en investigación, y esto se hace en la medida de lo posible. En cada universidad o institución de investigación existe algún tipo de junta revisora que debe aprobar nuevos proyectos de investigación, asegurando que se adhieran al principio de las 3Rs. Esta junta revisora es conocida como Comité Institucional de Cuidado y Uso de Animales de Laboratorio (CICUAL) que supervisa todos los protocolos de investigación y asegura que se cumplan normas de bienestar animal y uso ético de los animales. Actualmente no es posible eliminar totalmente la investigación con animales sin comprometer la totalidad de la investigación biomédica. La simulación por computadoras, la micro- dosificación, la exploración por resonancia magnética y las pruebas in vitro suelen presentarse como alternativas al uso de animales vivos. Sin embargo, aún es muy difícil que reemplacen completamente el uso de los animales en la investigación. La razón de esto es que todo método científico está diseñado para responder a un tipo particular de pregunta, de modo que los métodos que utilizan animales, cultivos celulares, modelos informáticos o imágenes del cuerpo humano se complementan, pero no pueden reemplazarse. No hay otra forma de adquirir esta información que obtenerla de un organismo vivo. Los experimentos in vitro, donde se estudian moléculas (como proteínas o ADN) o cultivos celulares, son muy buenos para descubrir los mecanismos que suceden dentro de la célula, pero no siempre pueden emplearse para determinar cómo interactúan los diferentes tipos de tejidos, órganos y sistemas dentro del organismo. Es por eso que, a corto plazo, deberemos seguir utilizando animales vivos para responder a algunas de las preguntas científicas más importantes relacionadas con la salud humana y animal.

El reemplazo, la reducción y el refinamiento guían el uso ético de los animales en la ciencia. Los investigadores deben reemplazar o evitar el uso de animales donde de otra manera hubieran sido usados, emplear estrategias que reducirán el número de animales utilizados y continuamente refinan y modifican los procedimientos experimentales y de cría para minimizar el dolor y el estrés.

Es por ello que como investigadores y usuarios de animales de laboratorio tomemos consciencia de que el uso de animales debe ser considerado un PRIVILEGIO, agotando previamente la posibilidad de métodos alternativos. Para poder enmarcar legalmente este uso de animales de laboratorio es necesario el apoyo de todos los actores científicos y de la docencia del proyecto ya aprobado en la Cámara de Diputados 'Ley de protección para los animales de experimentación utilizados con fines científicos y educativos'.

Saludos cordiales.

Gabriel B. Pinto
Presidente AACyTAL

CONFERENCE SAIC I - opening conference - EMBO Young Investigator lecture.
Wednesday 15th November 12:00 – 12:50
Chair: Camila Martínez Calejman

THE NUTRIENT - RAG GTPASE SIGNALING PATHWAY: A METABOLIC ENGINE FOR CANCER AND AGING

Alejo Efeyan
Metabolism and Cell Signaling Lab, Spanish National Cancer Research Center, Madrid, Spain.

The mechanistic target of rapamycin complex 1 (mTORC1) is a master regulator of anabolic and energetically demanding cellular processes in response to growth factor signaling and nutrient availability across eukaryotes. The Rag family of GTPases links cellular nutrient sufficiency and mTORC1. When certain amino acids, lipids, and glucose levels are plentiful, the heterodimeric Rag GTPase complex, comprising RagA and RagC, interacts with and recruits mTORC1 to the outer lysosomal surface. At the outer lysosomal surface mTORC1 can undergo a growth-factor-dependent kinase activation switch. Mutations in components of the Rag GTPase pathway, particularly RagC, have been identified in B-cell lymphomas. To gain insight into their impact on B-cell functions and lymphomagenesis, we engineered mice with point mutations in the RagC locus, mimicking activating variants found in human lymphoma. Cells from heterozygous RagC-mutant mice exhibited only a mild increase in mTORC1 activity, but when bred to a lymphoma-prone strain, RagC-mutant mice displayed massive B-cell activation and accelerated lymphomagenesis. Notably, murine lymphomas expressing active RagC variants showed selective and remarkable sensitivity to pharmacological inhibition of mTORC1. Additionally, suppressing Rag GTPase signaling in mice, achieved through the expression of a hypomorphic RagC variant, compromised B-cell functions and delayed lymphomagenesis, suggesting a potential therapeutic avenue for inhibiting nutrient-Rag GTPase signaling. Without a lymphoma-prone genetic background, full-body RagC-mutant mice exhibited a 25% reduction in lifespan, with an unexpected suppression of spontaneous tumor development, and manifested multiple features associated with premature aging. This outcome underscores both the underlying commonalities and the antagonistic relationship between cancer and aging. RagC-mutant mice constitute the first genetic system with an increased nutrient signaling – mTORC1 axis in mice to understand cellular and molecular underpinnings that link this pathway to aging. The shortened lifespan of RagC-mutant mice occurred with prominent senescence in peripheral organs, parenchymal expression of inflammatory and chemo-attractant molecules, increased myeloid inflammation, and extensive signs of inflammaging. Transplantation experiments revealed that myeloid cells were not intrinsically dysfunctional but instead caused inflammatory damage secondary to their extravasation and accumulation in response to signals evoked from RagC-mutant organs. Acute control of myeloid inflammation reversed some of the premature aging features, while sustained suppression of myeloid cells extended the survival of mice with increased nutrient-Rag GTPase signaling. Our findings establish the first genetic link between elevated nutrient-mTORC1 activity and accelerated aging, supporting a two-component model: parenchymal dysfunction and inflammatory damage, through which increased nutrient signaling drives mammalian aging.

CONFERENCE AAFE I - opening conference.
Wednesday 15th November 16:00-16:50
Chair: Paula Scibona

DEVELOPMENT OF PEDIATRIC FORMULATIONS OF AVAILABLE DRUGS FOR CHAGAS DISEASE TREATMENT, BENZNIDAZOLE AND NIFURTIMOX

Jaime Altcheh
Parasitología, Hospital de Niños Ricardo Gutiérrez, Instituto Multidisciplinario de Investigaciones en Patologías Pediátricas (IMIPP), CONICET-GCBA.

For the treatment of Chagas disease only two drugs are available: benznidazole and nifurtimox. Both drugs have shown adequate efficacy in eliminating the disease-producing parasite Trypanosoma cruzi. Most infections are acquired during childhood either by the vectorial route or by transplacental transmission from an infected wom-

an. This makes early diagnosis and treatment of children essential to avoid progression of the disease with the appearance of cardiac complications after several years. In recent years, our group at the Ricardo Gutierrez Children's Hospital, Buenos Aires, through different collaborations with public and private institutions, has developed baseline population pharmacology studies of benznidazole and nifurtimox that allowed the development of their pediatric formulations For the development of these clinical studies, a pediatric clinical research network (PEDCHAGAS Network) was created involving pediatric centers in Argentina, Bolivia and Colombia Another area explored was the study of the passage into breast milk, demonstrating the safety of these drugs during lactation. We will present the relevant data from these clinical studies that allowed the registration of the pediatric formulations of benznidazole and nifurtimox by the FDA.

CONFERENCE SAIC II - Dr. Christiane Dosne de Pasqualini.
Wednesday 15th November 16:00-16:50
Chair: Omar Pignataro

ANTIPROGESTINS FOR BREAST CANCER TREATMENT: RESULTS FROM THE MIPRA TRIAL

Claudia Lanari
Instituto de Biología y Medicina Experimental (IBYME-CONICET), Buenos Aires, Argentina.

Progesterone receptors are currently evaluated in breast cancer samples as prognostic and predictive factors, however, they are not used as therapeutic targets in clinical practice. Attempts had been performed using progestins such as medroxyprogesterone acetate (MPA) or megestrol acetate or using antiprogestins such as mifepristone, onapristone and more recently telapristone acetate. Mifepristone has been used in three clinical trials and it was administered to patients with metastatic disease that failed other treatments. Although some partial responses were observed, the enthusiasm decreased since responses were only observed in a few cases. Preclinical evidence suggests that antiprogestins exert therapeutic effects only in tumors with higher levels of PR isoform A (PRA) than isoform B (PRB). This has been assessed using tumors from the murine MPA-induced breast cancer model, xenografts of human breast cancer cells expressing PRA or PRB, and in ex vivo tissue cultures from breast cancer patients. To move to a clinical scenario, we designed a single-arm window of opportunity trial at the Magdalena V. de Martínez Hospital from General Pacheco to explore the benefit of administering mifepristone (200 mg per os, daily during 14 days) to 20 breast cancer patients, naïve from previous treatment, selected for expressing PR (≥50%) and PRA/PRB≥1.5 as determined in western blot studies. The primary endpoint was to evaluate the change in Ki67 expression assessed by immunohistochemistry comparing the core needle biopsy and the surgical samples after treatment. It was pre specified that a difference higher than 30% between both samples would be considered a positive response. Secondary endpoints included transcriptomic and proteomic studies to confirm Ki67 data, the evaluation of morphological changes and apoptotic pathways, the evaluation of mifepristone plasma levels and other possible biomarkers. Seventy percent of tumors showed ≥30% relative reduction in Ki67 expression. RNA-Seq and proteomics data supported the cytostatic effects and showed increases in pathways related to the immune response and extracellular matrix remodeling. Morphological evaluation confirmed an increase in TILs, as well as a modest increase in apoptosis. An increase in cortisol precursors was confirmed, and seems to be a compensatory mechanism for the antiglucocorticoid effects of mifepristone. Only mild side effects were observed. Our data demonstrated the benefit of mifepristone treatment in a subgroup of patients, and opened new avenues of research with the idea of evaluating the possible scenarios in which this treatment may be exploited. Preclinical data anticipates the benefit of combining tamoxifen and mifepristone, or palbociclib and mifepristone as adjuvant treatments, or even administering mifepristone as a neoadjuvant therapy to prime tumors for immune therapies in patients with high PRA/PRB ratios.

CONFERENCE SAIC III - Genetics.
Wednesday 15th November 17:00-17:50
Chairs: Liliana Dain; Carlos David Bruque

GENETICS OF OSTEOPOROSIS (AND OTHER BONE PHENOTYPES), BEFORE AND AFTER GWAS

Daniel Grinberg1, Natalia García-Giralt2, Neus Roca-Ayats1, Núria Martínez-Gil1, Juan David Patiño-Salazar1, Diana Ovejero2, Carlos David Bruque3, Leonardo Mellibovsky2, Xavier Nogués2, Adolfo Díez-Pérez2, Raquel Rabionet1, Susanna Balcells1
1Universitat de Barcelona, Facultad de Biología, Departamento de Genética, Microbiología y Estadística, IBUB, IRSJD, CIBERER, Barcelona, España. 2Instituto de Investigación Médica del Hospital del Mar, Grupo de Investigación músculo-esquelética, CIBERFES, Barcelona, España. 3Unidad de Conocimiento Traslacional Hospitalaria Patagónica, Hospital de Alta Complejidad Servicio de Atención Médica Integral para la Comunidad (SAMIC) - El Calafate, El Calafate, Argentina.

We started the search of genes responsible for the susceptibility to osteoporosis by the end of last century. SNPs for association studies needed to be discovered first, since this was before the sequencing of the human genome. We found SNPs in a regulatory region of different genes associated with bone mineral density (BMD) and they proved to be functional. Then, we moved to whole-gene studies, in which we analysed SNPs covering the full length of genes and performed haplotype analyses. The next step was to participate in very large consortia, which allowed us to discover highly significant associations for SNPs in candidate genes and, afterwards, to identify novel genes involved in the disease through GWAS. At this point, we focused on the demonstration of the functionality of some of the GWAS hits. Some of these results are still unpublished. As related projects we studied the genetic bases of some monogenic bone diseases and two other bone phenotypes: atypical femoral fractures (AFF) and high bone mass (HBM). AFFs are a rare event, often but not always linked to bisphosphonate (BP) therapy. Our first study was that of three sisters who had atypical femoral fractures after receiving various oral bisphosphonates for 6 years. We performed whole-exome sequencing and we detected several gene variants shared by the three sisters. One of them was a novel missense mutation in the gene GGPS1, which codes for the enzyme geranylgeranyl pyrophosphate synthase (GGPPS), which is a target for inhibition by bisphosphonates in the mevalonate pathway. Functional studies showed the pathogenicity of this mutation. We then studied several unrelated cases and found mutations in several genes, including CYP1A1, which was replicated by another group. High bone mass is a phenotype which can be considered opposite to osteoporosis. There are genes, such as LRP5, that may bear gain-of-function mutations that cause high bone mass and other loss-of-function mutations responsible for a particular type of osteoporosis. Thus, the discovery of genes for HBM may help finding genes for osteoporosis. Same as in osteoporosis, there are HBM cases in which the aetiology is mainly multifactorial, in which the genetic bases are associated with many variants in the genome, each with small effect size, and cases in which there is a particular mutation with a large effect, as in monogenic diseases. We have analyzed both types of HBM patients and families. Finally, we have recently studied the evolution of the typical HBM gene, LRP5, across Neanderthals, Denisovans and anatomically modern humans. We have observed private mutations in the archaic genomes that we experimentally validated as putatively increasing high bone mineral density and showed that there was a very limited archaic introgression of this gene in modern human, consistent with a selection in favour of the skeleton gracilization of the human lineage compared with other primates and archaic populations such as Neanderthals.

CONFERENCE SAB I - opening conference.
Wednesday 15th November 18:00 – 18:50
Chair: Silvina Pérez Martínez

LINKING EPIGENETICS, METABOLISM AND CANCER: LESSONS FROM SIRT6

Raul Mostoslavsky
The Massachusetts General Hospital Cancer Center, Harvard Medical School, Boston, MA USA.

In recent years, chromatin regulators have emerged as key modulators in cancer. In past work, we discovered that the mammalian histone deacetylase SIRT6 is a key chromatin factor, modulating expression of metabolic, developmental, and ribosomal protein genes. Particularly in the context of cancer, we found SIRT6 to act as a robust tumor suppressor, by modulating glucose metabolism. As Otto Warburg described decades ago, cancer cells exhibit glycolytic metabolism, where pyruvate, instead of contributing to ATP production in the mitochondria, is converted to lactate even under normoxia conditions. We found SIRT6 as the first chromatin factor in charge of suppressing the Warburg effect in multiple cancers, including colon, pancreas and skin. At the cellular level, SIRT6 directly regulates expression of several key glycolytic and ribosomal genes, co-repressing Hif1a and Myc, respectively, and acting as a histone H3 lysine9 (H3K9) and lysine 56 (H3K56) deacetylase. Importantly, SIRT6 specifically inhibits transcriptional elongation, rather than initiation, the first such enzyme to work through this mechanism. Strikingly, we determined in new studies that such glycolytic switch provides an advantage even at the early initiating cancer stem cells stage, in what we identified as the cell-of-origin for the Warburg effect, and we further demonstrated that such adaptations occur in only a fraction of tumor cells, defining metabolism as an heterogeneous hallmark in cancer. Our studies highlight the important role epigenetic factors, such as SIRT6, play in protecting against tumor progression by providing “epigenetic plasticity”, inhibiting adaptive responses in transformed cells. In our latest set of studies, we started investigating epigenetic and metabolic adaptations in metastatic disease, and identified novel metastasis-specific drivers that will be discussed in this meeting.

CONFERENCE SAIC IV.
Thursday 16th November 12:00 – 12:50
Chair: Verónica Marignac

LIQUID BIOPSY IN WOMEN'S HEALTH, ONCOLOGY AND ORGAN TRANSPLANTATION

Lili Li
Founder, Acrux Fleeting-code

Liquid biopsy is a non-invasive diagnostic method of detecting and monitoring health status by analyzing bodily fluids, primarily blood. It offers a significant advantage over traditional tissue biopsy by reducing the risk, pain, and discomfort for patients. Non-invasive prenatal test (NIPT) has revolutionized prenatal care by offering screening for chromosomal abnormalities as early as the 10th week of pregnancy. It analyzes the cell-free fetal DNA circulating in the mother's bloodstream to assess the risk of Down syndrome (trisomy 21), Edwards syndrome (trisomy 18), Patau syndrome (trisomy 13). This offers parents-to-be timely and valuable information, aiding in informed decision-making regarding the pregnancy and preparation for potential outcomes. Technological advancements have improved the sensitivity and specificity, enabling the detection of subchromosomal copy number variants. 22q11.2 deletion syndrome (or DiGeorge syndrome) is the most common microdeletion and a leading cause of congenital heart defects and neurodevelopmental delay. Classical deletion and nested deletions that are ≥500 kb in the 22q11.2 low-copy repeat A-D region can be detected by SNP-based NIPT. Real-world population data showed 0.2% cases as high risk. As an emerging and promising tool for monitoring graft health and early detection of rejection, cfDNA (cell free DNA) assay can improve post-transplant rejection assessments by more than 50% than traditional methods, leading to better patient outcomes. It is now available for kidney, lung and heart transplantation. The clinical utility of ctDNA in patients with solid tumors has increased, aiding in risk stratification, prognosis, and treatment planning. Postoperative ctDNA-positive status indicates a higher risk of recurrence. Implementation of ctDNA testing can inform prognosis and assist in determining the level of treatment that may be needed to clear residue disease, prevent relapse and improve chances of long-term survival.

CONFERENCE SAIC V - Dr. Alfredo Lanari.
Thursday 16th November 11:00 – 11:50
Chair: Ariana Bruzzone

PENTAMERIC LIGAND-GATED ION CHANNELS: FROM MOLECULE TO MEDICINE

Cecilia Bouzat
Instituto de Investigaciones Bioquímicas de Bahía Blanca- CONICET- Departamento de Biología, Bioquímica y Farmacia, Universidad Nacional del Sur.

Pentameric ligand-gated ion channels (pLGICs) mediate ionotropic responses in vertebrates and invertebrates. These receptors are vital for converting neurotransmitter recognition into electrical impulses, contributing to essential physiological processes such as movement, memory, cognition, and plasticity. They are found in the central and peripheral nervous systems, as well as in various non-neuronal cells, and are associated with a wide range of disorders, making them significant pharmacological targets for clinically relevant drugs. In vertebrates, the pLGIC family includes the cation-selective channels, nicotinic acetylcholine receptors (nAChRs) and 5-hydroxytryptamine type 3 receptors, and the anion-selective channels, glycine and gamma-aminobutyric acid type A receptors. In invertebrates, the repertoire of pLGICs is even more diverse, encompassing anionic channels activated by glutamate, acetylcholine, and biogenic amines. Remarkably, the free-living nematode Caenorhabditis elegans, which serves as a model for human diseases and anthelmintic drug discovery, possesses one of the largest and most diverse receptor families. As a result, C. elegans is an ideal organism for investigating the biology and pharmacology of pLGICs and exploring their potential as targets for novel therapeutic interventions. Through the use of heterologous expression systems and patch clamp recordings of wild-type and mutant pLGICs, particularly α7 nAChRs and 5-HT3A receptors, we have elucidated the molecular mechanisms of their operation. Our studies have deciphered the kinetics and pharmacological peculiarities that enable these receptors to adapt to their physiological roles and have identified new compounds with therapeutic potential for neurological and neurodegenerative disorders. In C. elegans, our studies ranging from the molecular to the organism level have provided insights into novel aspects of pLGIC pharmacology and function as well as their physiological roles. Furthermore, these studies have identified novel receptor targets and attractive lead compounds for anthelmintic drug therapy. Overall, our studies lay the foundation for the design and development of therapies that can effectively target and modulate pLGICs for improved clinical outcomes.

CONFERENCE AAFE II.
Thursday 16th November 16:00-16:50
Chair: Ventura Simonovich

CHALLENGES OF TRANSLATIONAL RESEARCH IN PEDIATRICS

Paula Schaiquevich
Unit of Innovative Treatments, Hospital de Pediatría Juan P. Garrahan, CABA, Argentina.

Translation research entails translating basic investigations into patient treatment (commonly referred to as bench-to-bedside and back again) alongside the translation of clinical studies findings into routine clinical and community practice. It also involves translating novel knowledge into disease prevention or global health strategies. Hence, translational research offers a unique opportunity to investigate childhood disease to uncover new drugs, treatment strategies, and diagnostic modalities to improve children's health. Several aspects should be considered in order to conduct such studies including the interaction between basic and clinical scientists, the availability of an animal facility, the existence of a multi-omics facility and of a validated tissue and cell bank, the correct sample procurement, the establishment of human capacities trained in translational research, and proper funding support. In this presentation I will outline key issues of the aspects abovementioned and how our research group managed to find practical solutions. For the last decades, important breakthroughs in cancer treatment have been attained with the introduction of personalized medicine. Molecular diagnosis and predictive biomarkers allows the stratification of patients so as to tailor the best treatment according to the individual requirements. Nonetheless, it is usually disregarded that the concept of precision medicine also entails precision dosing recommendations. Suboptimal drug exposure can lead to poor efficacy while overexposure in safety concerns. Tailoring drug therapy according to the pharmacokinetics, disease state, patient population specific factors and the drug product characteristics is of significant importance to achieve efficacy and minimize toxicity. In this context, I will guide the audience through the studies conducted by our research group. In the pediatric population we can mention at least two aspects that may affect the adequate translation of research findings. Firstly, the availability of commercial formulations intended only for adult patients restricts the possibility of dosing pediatrics. Secondly, the off-label use of biologic drugs and the introduction of biosimilars to the treatment of complex disease carries a new problematic about the interchangeability between biologic drug products in terms of safety and effectiveness. I will also discuss about the results obtained in real-world patients intended to evaluate the impact of schemes of treatment, drug formulations, and interchangeability between biologic drugs on drug efficacy and safety. Lastly, I will present a novel preclinical platform of primary cell lines and cell-derived xenograft models derived from metastatic sites of patients with retinoblastoma. I will explain how these models enabled us to gain insights into tumor biology and conduct large pharmacological screening to prioritize hit candidates, ultimately aiming to enhance patient survival.

CONFERENCE SAIC VI.
Thursday 16th November 16:00-16:50
Chair: Flavia Saravia

NEURO DEGENERATION AND AGING, A PROBLEM OF THE SPECIES

Gustavo Sevlever
Departamento de Neuropatología, Fleni, Buenos Aires, Argentina.

The increase in life expectancy has brought about changes from the social, political, cultural and health point of view. The appearance at a large scale, as a political subject, of those over 65 years of age generates an unprecedented anthropological impact. A critical associated aspect is cognitive disorder and neurodegenerative disease, associated with aging. The clinical consequence is usually dementia. There has always been a critical debate about healthy and pathological aging. The evolution of ideas about Alzheimer's disease (AD), from its original description to the present, summarizes the different understandings of the aging process. Currently, AD is defined by the extracellular presence of cerebral amyloid and the accumulation of hyperphosphorylated tau in the neuronal soma. These proteins, in a probably insufficient form, are those that define the disease. There are different technologies that allow these two proteins to be detected in vivo, creating new, more objective, and quantifiable diagnostic paradigms. However, the detection of these biomarkers adds complexity to the diagnostic process and, ultimately, to the understanding of aging. There are many immunological therapeutic attempts to extract the abnormal proteins from the brain. The performance of these clinical trials also provides interesting information on pathogenesis. An important aspect is preventive since the risk factors coincide with those of

cardiovascular disease. In this presentation we will try to synthesize the complex biological phenomenon that exists behind these data, an example of contemporary biomedical research that includes clinical, imaging, cellular, molecular, genomic, social aspects, and clinical trials, among others.

CONFERENCE SAB II.
Thursday 16th November 16:00-16:50
Chair: Leandro Miranda

LEISHMANIASIS IN ARGENTINA AND CLIMATE CHANGE: INTERACTION BETWEEN BIOLOGY, CLIMATE AND SOCIOCULTURAL FACTORS

Oscar Daniel Salomón[1,2,3], María Gabriela Quintana[3,4,5]
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The impact of climate change, in relation to insect-borne diseases in general, and leishmaniasis in particular, involves multiple biological, physical and sociocultural aspects, which must be integrated into the frame of the eco-epidemiological analysis, generating complex conditions and modulators for modeling predictive projections. Previous categories to be considered for these analyses are the differences between cutaneous leishmaniasis (CL) and visceral leishmaniasis (VL), and the characterization of the climate effects, discriminating between those expected in the medium or long term and those produced by an increase in the frequency of extraordinary events, from extreme precipitation patterns to fires. Among the medium and long-term effects, in the processes of tropicalization of the environment and increase in mean temperature, the increase in the dispersion of vectors in geographical space and time has already been recorded, besides the potential for colonization of vector insects at higher latitudes for both LC and LV. However, predictive models should also consider the impact of changes in nocturnal and diurnal temperature-humidity, seasonal pattern and frequency and duration of extreme temperatures on the insect life cycles, its survival as requires a second feeding to be infective, on population size and number of annual cycles, on distribution of reservoirs, on changes in land use due to in the distribution of agriculture profitable lands, on human habits related to exposure and on climate-economic migrations. In relation to the increase of climate related extraordinary events, the impact is associated with to the probability of epidemic outbreaks. In LC, this effect has been recorded due to alteration of environmental interfaces (edge effect) by inundation, ecotone growth with time lags of up to one year, human occupation, and change of land use for exploitation or housing, sheltering or urbanization as changes the costs of land, infrastructure building or reparation. In relation to VL, mainly urban in Argentina, the risks are related both to the availability of potential breeding sites due to excess organic matter in yards as fallen fruit and intense rainfall, and the permanence of humans in unprotected open areas of the domestic space during peak vector activity hours. Thus, predictive health models for vector-borne diseases such as leishmaniasis, which use climate projections, will have to integrate other biological, economic and socio-cultural variables, which will increase complexity and uncertainty, with phenomena far from normality and closer to chaos functions, but so it will make possible to design more effective surveillance, prevention and mitigation strategies.

CONFERENCE SAIC VII.
Thursday 16th November 17:00-17:50
Chair: Fernanda Parborell

CONTROL OF INFLAMMATORY RESPONSES AND LUNG CANCER DISSEMINATION BY PHOSPHATIDIC ACID AND CERAMIDE 1-PHOSPHATE

Antonio Gómez-Muñoz1, Ana Gómez-Larrauri1,2, Patricia Gangoiti1, Laura Camacho1, and César Martín1
1Department of Biochemistry and Molecular Biology, Faculty of Science and Technology, University of the Basque Country (UPV/EHU). Bilbao, Spain. 2Respiratory Department. Cruces University Hospital. Bizkaia, Spain.

Ceramide 1-phosphate is a bioactive sphingolipid that regulates key biological cell functions, including proliferation, differentiation, survival and cell migration. Initially, we showed that C1P stimulates cell proliferation through mechanisms involving activation of the mitogen-activated protein kinase kinase (MEK)/Extracellularly regulated kinases (ERK)1-2, sphingomyelin synthase-derived diacylglycerol (DAG)/protein kinase C (PKC), mammalian target of rapamycin (mTOR), or vascular endothelial growth factor (VEGF) release. In subsequent studies we showed that C1P also increased cell number by interfering with programmed cell death. Specifically, C1P inhib-

ited apoptosis through mechanisms involving depletion of proapoptotic ceramides through inhibition of sphingomyelinase and serine palmitoyl transferase. Also, although C1P was first reported to induce proinflammatory processes, mounting evidence suggests that it has anti-inflammatory properties. C1P inhibited the production of pro-inflammatory cytokines that were stimulated by the bacterial toxin lipopolysaccharide (LPS) in peripheral blood mononuclear cells. In subsequent studies in collaboration with Dr. Marko Idzko from the Medical University of Vienna (Austria) we showed that C1P inhibits cigarette smoke (CS)-induced airway inflammation. In particular, mice exposed to CS showed increased number of macrophages and neutrophils in bronchoalveolar lavage fluid, and increased the levels of the proinflammatory cytokines interleukin (IL)-1b, IL-6, keratinocyte chemoattractant (KC), or macrophage inflammatory protein (MIP-2), and treatment with C1P blocked these actions. Also, C1P substantially reduced emphysema in mice exposed to CS. More recently, we found that C1P blocks cell migration in different types of lung cells, including alveolar macrophages (NR8383) and lung adenocarcinoma cells (A549), a type of non-small cell lung cancer (NSCLC), pointing to relevant roles of C1P at counteracting lung inflammation and cancer cell dissemination. Specifically, C1P completely inhibited both spontaneous and silica-induced alveolar macrophage migration through mechanisms involving blockade of ERK1-2 and/or Akt phosphorylation, and potently inhibited the stimulation of lung adenocarcinoma cell migration by phosphatidic acid (PA), a potent proinflammatory phospholipid that is involved in many pro-tumorigenic and metastatic processes. PA-stimulated lung cancer cell migration involved binding of the phospholipid to the lysophosphatidic acid (LPA)1 receptor and subsequent activation of the MEK/ERK1-2, PI3K/Akt and JAK-2/STAT-3 pathways, which were all inhibited by C1P.

SYMPOSIUM SAIC I. *Wednesday 15th November 11:00 – 12:30*
SYMPOSIUM IN HONOR OF DR. HORACIO A. REPETTO: EMERGING AND RE-EMERGING PEDIATRIC INFECTIOUS DISEASES
Chairs: Rodolfo Rey; Dr. Laura García Chervo

PATHOPHYSIOLOGY AND CLINICAL ASPECTS OF BRONCHIOLITIS

Gonzalo Pérez Marc
Departamento Materno-Infantil, Hospital Militar Central de Buenos Aires, CABA, Argentina.

Bronchiolitis (BQL) is an acute inflammatory disease of viral origin, which mainly affects the lower respiratory tract and causes obstruction of the bronchioles. It is one of the most frequent reasons for hospitalization of infants and young children. It is characterized by the presence of cough, wheezing, fever, tachycardia, tachypnea, and respiratory distress. For epidemiological purposes, it is defined as a first episode of small airway obstruction with clinical evidence of viral infection, which occurs in children under 2 years of age. The main cause of BQL is respiratory syncytial virus (RSV) infection. Less frequently, it can be caused by rhinoviruses, parainfluenza viruses, human metapneumoviruses, influenza viruses, adenoviruses, coronaviruses, and human bocaviruses. Inflammation of the small airway generates wall edema, increased respiratory secretions, cellular destruction, and spasms of bronchial smooth muscles. All these mechanisms come together to increase resistance to air flow. Bronchiolar obstruction produces air trapping through a valvular mechanism, generating hyperinflation and increased functional residual capacity. This phenomenon has two consequences. As there is a larger lung volume, the increase in pulmonary elastic force increases elastic resistance, so greater muscular work is necessary to mobilize the Vc. Secondly, hyperinflation further flattens the diaphragm's curvature and makes its fibers' contraction less effective in generating transdiaphragmatic pressure differences. The sum of these effects generates obstructive ventilatory incapacity. If diaphragmatic function decreases significantly, restriction is added to bronchiolar obstruction. The reduced volume of air entering the alveoli with each inspiration produces a decoupling between ventilation and perfusion. This mechanism constitutes the main cause of hypoxemia in this entity. If bronchiolar obstruction is complete, segmental or subsegmental atelectasis occurs. These generate respiratory restriction and increase venous admission. As a result, hypoxemia deepens, and the development of hypercapnia is favored. In young children, especially those under 3 months of age, the poor functional reserve of the diaphragm and the rest of the respiratory muscles can lead to muscle exhaustion and thus develop hypoventilation due to a pump defect. This is related to the poor development of type I muscle fibers, linked to resistance to muscle fatigue. Finally, decoupling in the V/Q ratio, increased venous intake, and hypoventilation due to muscle pump failure significantly alter O2 uptake and CO2 elimination, resulting in acute and potentially severe respiratory failure.

CHALLENGES AND OPPORTUNITIES IN PEDIATRIC HOSPITAL RESEARCH

Silvina Ruvinsky
Investigación Clínica y Sanitaria, Unidad de Conocimiento Traslacional, Hospital de Pediatría Juan P. Garrahan, CABA, Argentina.

New advances in science have generated new tools in basic and clinical research. Currently, science and technological innovation have led to new challenges and required an approach from multidisciplinary teams (basic researchers, pediatricians, bioinformatics, biotechnologists, biochemists, pharmacists, bioengineers, sociologists, and others). In Argentina, Pediatric Hospitals have upheld the importance of generating knowledge and training human resources as complementary and integrated activities to the sustained task of care in our country and Latin-American region. The final objective of integrating and generating a space for constant exchange between groups of researchers is to improve the quality of life of patients and their families. The mission is to promote and sustain hospital research activities, the development of scientific advances and innovations, promoting translational knowledge applied to children health based on the values of equity, ethics, excellence and diversity. The discovery of new diagnostic strategies, identification of prognostic factors, development of prevention measures and precision medicine have motivated the need to integrate these links with the inclusion of other sciences

(technological and social, among others) in the so-called translational research. Translational research is a paradigm shift related to a dynamic vision, transforming individual efforts into collectives, strengthening the system and providing high-impact responses in public health. The development of hospital research requires a particular institutional approach based on trans-discipline, a proactive process where different fields of knowledge come together to develop new theoretical, methodological, and conceptual frameworks in order to produce diverse approaches, appropriate to national and international guidelines. It's need of permanent link with external academic organizations, universities, institutes, Ministries of Health and Science and Technology. Clinical and other hospital areas related to research are fundamental for the proper development and execution of projects and subsidies. In addition, hospital Programs have a principal role to facilitate related to ethical aspects (pediatric patients are vulnerable population), methodological challenges (to assess and train educational program in hospital research), logistical difficulties, Data collection and analysis, financial support, to facilitate the procedures for identification, presentation and execution of national as international research grants, intellectual property and processes for scientific publication. Over the years, Pediatric hospitals scientific production has been increased and extended to new groups, generating and answering questions of interest and impact in pediatrics and public health in Argentina and Latin American region.

EXPERIMENTAL STRATEGIES FOR DIAGNOSIS, PREVENTION AND TREATMENT OF HEMOLYTIC UREMIC SYNDROME

Fernando Gómez[1,2], Daniela Luz[3], Melina Porporato[4], Daniel Girón[1,2], Alejandro Balestracci[5], Laura Alconcher[6], Cristina Ibarra[1,2], Roxane Piazza[3], Flavia Sacerdoti[1,2], María Marta Amaral[1,2]

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Shiga toxin-producing *E. coli* (STEC*)* is responsible for different clinical conditions including a serious systemic disease known as hemolytic uremic syndrome (HUS). HUS is characterized by microangiopathic hemolytic anemia, thrombocytopenia and acute kidney injury (AKI). Argentina has the highest incidence worldwide with 8–12 cases/100000 per year. Shiga toxin (Stx) is the main virulence factor of STEC that circulates in the bloodstream bound to different cells or within blood-cell-derived microvesicles (MVs). Stx binds to the globotriaosylceramide (Gb3) receptor on the cell membrane of target cells and develops apoptosis. Stx cytotoxic effects, and the clinical features that follow, are a result of damage in the endothelial cells of small vessels mainly localized in the colon, kidney, and central nervous system. Shiga toxin type 2 (Stx2) seriously affects the kidney because of the presence of especially Stx sensitive cells that express considerable amounts of Gb3 receptors. The early diagnosis of STEC infection is important to avoid chronic sequelae and long internment periods. MVs containing Stx (MVs-Stx) were reported to contribute to HUS physiopathology and disease. In this sense, we developed the detection of circulating MVs-Stx2 in a rat model of HUS, proposing them as a new biomarker that may help the early diagnosis. We were able to detect, by flow cytometry, circulating MVs-Stx2 in blood samples, 96 h after Stx2 injection. Following, we analyzed circulating MVs-Stx2 in HUS patients. From the controls, a *cut-off* point for MVs-Stx2 was established (1.02-1.90 %, n = 5). We found a significantly higher percentage of MVs-Stx2 in HUS patients with respect to healthy controls (P1: 3.63%, P2: 5.20%, $p<0.05$). On the other hand, we assayed the action of a Gb3 synthesis inhibitor, Eliglustat (EG), to prevent the damage caused by Stx2 on primary cultures of human glomerular endothelial cells (HGEC). EG was able to avoid Stx2 cytotoxicity since pre-incubation with EG (5 μM) for only 2 h was enough to protect the HGEC viability in about 73%. A hundred percent of protection was obtained after 24 h of pre-treatment. Furthermore, EG prevented the cell detachment (80%), swelling (81%), and necrosis (86%) after EG (1μM, 24 h). Finally, we analyzed Stx2 neutralization properties of recombinant anti-Stx2 Fab fragments. FabF8:Stx2 and FabC11:Stx2 protected HGEC against Stx2 cytotoxicity. For FabF8:Stx2 protection was: cell viability (90%), cell detachment (65%), swelling (95%) and apoptosis (90%). For FabC11:Stx2: cell viability (52%), cell detachment (82%) and swelling (82%). In conclusion, we proposed the detection of circulating MVs-Stx2 as an additional clinical biomarker for the early diagnosis of HUS. Furthermore, EG and FabF8:Stx2/FabC11:Stx2 could be promising therapeutic strategies to prevent kidney damage and the subsequent development of HUS. Future studies will be focused on analyzing the efficacy of these molecules in *in vivo* models.

SYMPOSIUM SAIC/SAB II. *Wednesday 15th November 11:00 – 12:40*
BEYOND THE RAT AND MOUSE: ENDOCRINOLOGY IN NON-TRADITIONAL MODEL ORGANISMS
Chairs: Lourdes Posadas Martínez; Paula Vissio

NEUROENDOCRINOLOGY OF REPRODUCTION IN THE FEMALE PLAINS VIZCACHA, A RODENT WITH A STRIKING ENDOCRINE BEHAVIOUR

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The South American plains vizcacha, Lagostomus maximus, is a hystricomorph rodent native from Argentina, Bolivia, and Paraguay. Female plains vizcachas show several peculiar reproductive features. They show the highest polyovulation rate for a mammal with a record of 800 oocytes per reproductive cycle. However, just 10 or 12 embryos will get implanted, which will suffer from a sequential and progressive reabsorption process triggered around the 50-embryonic day. This process will be only avoided by the two most caudally located embryos. From the 70-embryonic day, progesterone (P4) gradually decreases as a result of the decay in the steroidogenic activity of the primary corpora lutea and the inability of the placenta for steroidogenic activity, reaching its minimum level around the 90-embryonic day. At this moment, a true reactivation of the hypothalamic - pituitary - ovarian (HPO) axis occurs. Follicular recruitment and maturation enable a pseudo-ovulation event resulting in the formation of a new set of accessory corpora lutea and the recovery of the remaining primary corpora lutea, both with steroidogenic activity. This restores the P4 levels and allows the survival of the two remaining well-developed newborns after 155 days of gestation. This reproductive axis reactivation is enabled by the precise regulation of the hypothalamic gonadotropin-releasing hormone (GnRH) neurons allowing the pulsatile delivery of GnRH at mid-gestation. Such an event is followed by the sequential secretion of follicle-stimulating hormone (FSH), estradiol (E2), and luteinizing hormone (LH), ultimately leading to a reboot of luteal steroidogenesis and a consequent boost of P4 that enables the successful delivery. The regulation of the hypothalamic GnRH neurons results from the combination of different endocrine pathways. GnRH neurons in the preoptic area express progesterone and estrogens receptors, and aromatase, the estradiol-synthesizing enzyme. This local expression points to a mechanism of direct hormonal regulation of GnRH neurons that would synergize with the classic feedback pathways driven from the ovaries. Moreover, the presence of the kisspeptin system (with Kiss and KiNDy neurons) and the neuromodulators prolactin and dopamine (with prolactin receptor and tyrosine hydroxylase neurons), strongly suggests their involvement in the modulation of GnRH neurons. In addition, the photoperiod information is transduced by variations of the melatonin hormone throughout the gestation period. All these hormones, receptors, enzymes, and neurotransmitters coordinately regulate the functioning of this distinctive mechanism of which many details remain to be elucidated. In conclusion, the reactivation of the HPO axis during pregnancy is probably related to a reproductive strategy of the plains vizcacha that allows the maintenance of the pregnancy and guarantees birth at the most favourable season for offspring survival.

PHOTO-NEURODOCRINE MECHANISM OF REPRODUCTION IN AN OPPORTUNISTIC BIRD, THE EARED DOVE

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Bird species living at high latitudes show strong photoperiodic control of their reproductive activities. These activities start when the photoperiod is long (spring-summer), which is perceived by deep brain photoreceptors located in the hypothalamus. This initiates a photo-neuroendocrine response that leads to increased hypothalamic-pituitary-gonadal axis activity. As a result, gonadal growth and development occur (gonadal recrudescence/regression cycle) with the production of sexual hormones (testosterone and estradiol). This process is widely accepted in the scientific community as the classical model of seasonal reproduction in birds. However, there are other bird species that do not seem to follow this model. This is the case for the Columbiformes order (pigeons and doves), a reproductively successful group widely distributed worldwide. In our country, eight genera (*Columba, Zenaida, Scardafella, Columbina, Metriopelia, Claravis, Leptotila, Geotrygon*) are represented. Some of these genera (*Columba and Zenaida*) have species that have been considered pests for several decades. In the *Zenaida* genus, the Eared Dove (*Z. auriculata*) does not seem to conform to the photoperiodic control proposed by the classical model of seasonal reproduction. The Eared Dove reproduces throughout the year. Its reproductive success probably lies in the fact that, in addition to its opportunistic nature, it does

not exhibit a marked seasonal variation in the size of its gonads (gonadal recrudescence/regression cycle), although its plasma testosterone levels do. Low levels of plasma testosterone during autumn-winter appear to be sufficient to keep the gonads active at 40%. Additionally, the Eared Dove possesses at least three different opsins (Opn2, Opn3, and Opn5) as deep brain photoreceptors expressed in the septal region (Opn2 and Opn5) and the hypothalamic region (Opn3 and Opn5). Opn3 and Opn5 exhibit clear seasonal variation, with higher expression levels during the winter and lower levels in spring. On the other hand, the expression of Opn2 in the septal area appears to be constant across seasons. Multiple factors such as the ad libitum food availability (generated by human activities) and the constant expression of deep brain photoreceptors (Opn2) could interact to modulate reproduction in this opportunistic Dove.

INFLUENCE OF THE ENVIRONMENT ON FEEDING FROM A FISH POINT OF VIEW

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Teleost fish represent more than half of the extant vertebrate species. This group is characterized by a remarkable diversity in terms of genome, ecology, behavior, and anatomy. Besides, their feeding habits range from herbivore to carnivore, resulting in high variability of the gastrointestinal tract (GIT) morphology and hormone profiles. Food intake and feeding behavior are regulated by the interaction between the central nervous system and the GIT in conjunction with the associated organs (pancreas, liver, gall bladder), which synthesize neuropeptides and hormones similar to those that control this function in other groups of vertebrates. Although these neuropeptides and peptides are found throughout the vertebrate lineage, some functions would be conserved while others depend on the group studied. This fact makes them attractive for studying the evolution of appetite-regulating systems. At the central level, the main center that regulates food intake is in the hypothalamus, where different populations of neurons produce important neuropeptides: neuropeptide Y (Npy) / agouti-related peptide (Agrp), proopiomelanocortin (POMC), melanocyte-stimulating hormone (Mch), orexins, and cocaine- and amphetamine-regulated transcript (Cart). Besides, the main hormones produced by the GIT are ghrelin (secreted by the stomach or its equivalent in fish species that do not have it); cholecystokinin (Cck, synthesized by cells of the gastrointestinal tract); neuropeptide Y (Npy), and peptide yy (Pyy) (produced by endocrine cells of the intestine); insulin, and glucagon (produced by pancreatic cells) and leptin (secreted mainly by the liver). These peptides exert different actions at the local level, promoting the secretion of digestive enzymes, motility, or absorptive capacity, and in turn, enter the bloodstream and modulate the expression of neuropeptides at the central level. Besides, this network, which involves the neuroendocrine and endocrine cells mentioned above sense internal (such as reproductive and nutritional status, or stress) and external factors (such as temperature, photoperiod, light, hypoxia, salinity, social status, nutrients, or GIT microbiota/parasites), which in turn modulate food intake and the characteristics of the GIT, “adjusting” these functions to the demands of the organism. Thus, knowing how these neuropeptides and peptides act is essential to understand how different internal and external factors impact on the regulation of food intake in teleost fish, and it will also help maximize production efficiency in aquaculture. Considering that new actors have been included in this network, this presentation will summarize the current knowledge of the hypothalamic-GIT network, discuss the impact of external and internal factors, and the gaps that are necessary to access this complex and dynamic system. In this frame, we will present results obtained in the cichlid fish *Cichlasoma dimerus* about a new factor and the impact of tank color.

SYMPOSIUM AAFE I. *Wednesday 15th November 11:00 – 12:40*
EXPLORING THE LATEST TRENDS IN DRUG TREATMENTS FOR CANCER: FUTURE PERSPECTIVES
Chair: Mariano Nuñez

GLOBAL TRENDS IN CANCER THERAPEUTICS RESEARCH: TECHNOLOGIES, TARGETS AND INDICATIONS

Mariano Nuñez
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The development of new molecules is the main driver to gain access to new treatments for prevalent diseases, but also to cover unmet medical needs. The pipeline decision related to this development is based on two pillars: the incidence and prevalence of the diseases but, fundamentally, the potential market targeted by the new drugs, bearing in mind that once launched on the market, sales must cover the development costs. In this regard, when looking at the therapeutic areas with the largest number of molecules in development, of the more than 36,000 molecules currently in different phases of development, molecules with oncological pathologies as active indica-

tions rank first, representing 38% of the total number of molecules in active development. In addition, the therapeutic area of oncology is the fastest growing in the last five years. Looking at the specific oncology therapeutic area market, global spending on cancer drugs amounted to USD 185 billion in 2021 and is expected to reach more than USD 300 billion by 2026. Within the concept of targeted therapy, this segment is the fastest growing within the area of new developments in oncology. Between 2020 and 2031, the size of the global precision medicine oncology market is expected to increase nearly threefold to almost USD 130 billion. Looking at the performance of developments with next-generation biotherapeutic molecules, more than 40% of these developments were directed towards the oncology area. This makes the development of advanced drugs and treatments in this therapeutic area one of the major drivers of pharmacological research. The aim of this dissertation is to present the new technologies, targets and indications that are in the pipeline for oncological drugs and therapies.

ADVANCEMENTS IN GENITOURINARY CANCER THERAPY: EXPLORING NEW TECHNOLOGIES, TARGETS AND INDICATIONS

Juan Pablo Sade
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Urogynaecological malignancies are one of the areas where new drug developments are most prevalent. This is based on the fact that prostate cancer is the most common cancer in men, while kidney, bladder, uterine and ovarian cancer are also frequent causes of mortality in the population of developed countries. The behaviour of many of these neoplasms, and the low diagnostic yield of current studies, means that many are diagnosed at advanced stages. This fact means that new technologies and treatments are needed to prolong survival and response rates in patients. The aim of the presentation is to present the new molecular trends, new targets and new technologies in development, which establish the pipeline of drugs and treatments in this area of urogynecological neoplasms.

TRENDS IN ONCOHEMATOLOGICAL THERAPY: TECHNOLOGIES, TARGETS AND INDICATIONS

Mariano Berro
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Leukaemias and lymphomas cause life-threatening disease in both adult and paediatric patients. In recent years, significant advances have been made in complete response and cure rates in many subtypes of oncohaematological malignancies, and oncohaematology has been one of the first areas where advanced cell-based therapies have been applied. Likewise, there has been a great advance in the knowledge of the genetic and molecular alterations that lead to the appearance of the different subtypes of leukaemia, lymphomas and other oncohaematological neoplasms that allow the study of new pathways and new targets to improve the response and cure rates of the varieties of neoplasms that are resistant to current treatments. The aim of the presentation is to present the new molecular trends, new targets and new technologies in development, which establish the pipeline of drugs and treatments in Oncohaematology, with a focus on new advanced therapies.

SYMPOSIUM SAB I. *Wednesday 15th November 16:00-17:40*
INFLUENCE OF THE ENVIRONMENT ON THE PATHOPHYSIOLOGY OF CELLS AND ORGANISMS
Chairs: Paula Vissio; Juan Ignacio Fernandino

THE JUVENILE PERIOD AS A CRITICAL PERIOD FOR THE ACTION OF ENVIRONMENTAL FACTORS IN NEURODEVELOPMENTAL DISORDERS

Araceli Seiffe
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Neurodevelopmental disorders arise from the interaction of genetic predisposition and environmental factors. Animal models have contributed to our understanding of the genetic and environmental risk factors involved in the etiology and pathophysiology of these disorders. Autism spectrum disorder (ASD) affects almost 3% of the population and is characterized by social impairments, communication difficulties, restricted interests, and repetitive behaviors. This neurodevelopmental disorder is more prevalent in boys than girls. Maternal inflammation and immune activation are associated with an increased risk of ASD, while early social stimulation improves the behavioral performance of individuals with ASD. In our research, we utilize a mouse model of ASD induced by prenatal exposure to valproic acid (VPA). Our findings indicate that prenatal exposure to VPA leads to reduced

sociability and increased repetitive behaviors in male mice, while females are unaffected. Furthermore, we observed signs of neuroinflammation in VPA-exposed animals during both the juvenile period and in adulthood. Notably, at postnatal day (PD) 21, both male and female mice prenatally exposed to VPA exhibit reduced social play, but by adulthood (PD60), only male VPA mice show decreased sociability. To examine the influence of environmental factors on the progression of ASD-related behaviors in the VPA model, we focused on the post-weaning period (PD 21 to 35). During this period, mice undergo active synaptic pruning in the cortex and hippocampus, as well as neurogenesis and neurodifferentiation in several brain regions, including the cerebellum. Therefore, our aim was to investigate the impact of environmental factors on this critical period. We sought to identify environmental interventions capable of restoring sociability deficits observed in VPA-exposed males and those capable of breaking the resilience observed in adult VPA-exposed females. By identifying these factors, we aimed to elucidate the mechanisms underlying male susceptibility to VPA effects on adult behavior and understand how female resilience is achieved. Our results demonstrate that social enrichment following weaning can reverse sociability deficits in VPA-exposed males. Additionally, handling mice between PD21 and 35 restores sociability levels in adult VPA-exposed males. Conversely, inducing inflammation during this juvenile period in female mice leads to reduced sociability in adult VPA-exposed females. These findings highlight the existence of a juvenile critical period when social behaviors become consolidated. This period is particularly sensitive to environmental factors, including environmental enrichment, stress, inflammatory molecules, and gonadal hormones. Our data support the role of inflammatory molecules and cells in mediating these effects. Understanding the mechanisms involved in this juvenile determination of later behavior may contribute to the development of treatment strategies for ASD.

FISH SEX DETERMINATION: THE INTERACTION AMONG GENES AND ENVIRONMENT

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Even though in most vertebrates gonadal sex is determined genetically (a phenomenon known as Genotic Sex Determination, GSD), the gene that determines sex is not always the same. However, in some ectothermic animals gonadal differentiation can be modulated by the environment, being the influence of temperature the best known, although not the only one. This phenomenon is called Temperature Sex Determination (TSD). In this sense, Atheriniformes have become interesting vertebrate models to study. In two native species of Argentina, Odontesthes hatcheri (Patagonian pejerrey) and Odontesthes bonariensis (pejerrey), a Y-linked gene, amhy, has been identified, which is the determinant of testicular morphogenesis. It has also been seen that the expression of this gene is differentially regulated by water temperature, demonstrating that there is an interaction between both mechanisms of sexual determination (GSD-TSD). This fact makes possible to evaluate the relationship between genotypic sex (evidenced by PCR) and phenotypic sex (evidenced both by visual observation and histology) to study possible mismatches among them influenced by the environment revealing sexual inversions. Our studies with pejerrey show that, even though the presence of the amhy gene is important for the differentiation of males, we found sexual inversions (both in the environment, as well as in experimental conditions in the laboratory or in semi-controlled conditions in the field) that can be explained by the influence of the environment, not only by effects of natural environmental factors but also by anthropic influence. In the present work we will not only present a theoretical introduction of the phenomena of determination and sexual differentiation in pejerrey but also the comparison between experiments carried out in laboratory conditions and experiments carried out in the field.

ENVIRONMENTAL INFLUENCE ON *GIARDIA LAMBLIA* PHYSIOLOGY AND PATHOGENESIS: ROLE OF THE EXOSOME-LIKE VESICLES AND SMALL RNA CARGO

María C. Touz, Lautaro Natali, Gabriel Luna Pizarro, Andrea S. Rópolo, Melina M. Musri and Constanza Feliziani
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Giardia lamblia, a prevalent intestinal protozoan parasite, exhibits morphological indistinguishability among genetically related Assemblages closely associated with specific hosts. These assemblages display notable genetic divergence, contributing to their distinct biological and pathogenic characteristics. In this study, our focus was to investigate the RNA cargo carried by exosomal-like vesicles (ElVs) released by assemblages A and B, known to infect humans, as well as assemblage E, primarily affecting hoofed animals. Through comprehensive RNA sequencing analysis, we discovered that ElVs from each assemblage contained unique small RNA (sRNA) biotypes, indicating a preference for specific packaging within each assemblage. The most prev-

alent sRNA biotypes identified included ribosomal-small RNAs (rsRNAs), messenger-small RNAs (msRNAs), and transfer-small RNAs (tsRNAs), which likely play regulatory roles in parasite communication, host-specificity, and pathogenesis. Notably, novel uptake experiments revealed the successful internalization of EIVs by parasite trophozoites, providing evidence for their involvement in intercellular communication. Furthermore, we observed the localization of RNAs within EIVs beneath the plasma membrane, followed by their distribution throughout the cytoplasm. Overall, this study provides valuable insights into the molecular mechanisms governing host-specificity and pathogenesis in *G. lamblia*, shedding light on the significance of sRNAs in parasite communication and regulation.

SYMPOSIUM SAIC III. *Wednesday 15th November 17:00-18:40*
A TRANSLATIONAL VIEW OF CARDIOVASCULAR DISEASE: FROM ITS ORIGIN TO ITS TREATMENT
Chairs: Alberto Crottogini; Analía Tomat

PRENATAL AND EARLY POSNATAL ORIGIN OF CARDIOVASCULAR DESEASE

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An injury during fetal life leads to restricted intrauterine growth that not only results in low birth weight but also induce an adaptive response that can induce the loss of structural units (nephrons, cardiomyocytes, β pancreatic cells, skeletal muscle cells) and alteration in function of different organs to maintain the development of other systems. David Barker in 1980th postulated the *programming hypothesis*, suggesting that intrauterine growth restriction (IUGR) due to poor nutrition, oxygen restriction, obesity, stress in pregnancy not only results in low birth weight (LBW) but also causes an adaptive responses. Epigenetic changes have been postulated as the most important mechanism driving fetal programming. This new circumstance can bring immediate advantages by increasing perinatal survival in a poor nutritional environment. However, these adaptive responses decrease morphological and functional capacity in later life. Moreover, it has been proposed that could be more harmful when these individuals face a postnatal environment with greater metabolic demands. These findings are supported by clinical studies suggesting that LBW is associated with an increased risk of cardiovascular, renal, and metabolic diseases in adult life. WHO and UNICEF have defined as hidden hunger: a set of specific micronutrient deficiencies that are highly prevalent and important in children's growth and development, including zinc, iron, vitamin A, group B vitamins, folates, and/or essential fatty acids. Micronutrient deficiencies coexist with overweight and obesity or diet related diseases in all social classes. Different groups of pregnant women and many children eat high calorie but micronutrient-poor diets, with the result of hidden malnutrition. Besides, studies performed in rats revealed that moderate Zinc deficiency during prenatal and/or early postnatal growth is a risk factor for the fetal programming of hypertension, cardiovascular and renal alterations, obesity, and diabetes in adult life. An adequate zinc diet during postweaning life does not always prevent these diseases induced by zinc restriction during fetal and lactation periods. Some of the mechanisms involved include alterations in organogenesis, activation of oxidative, apoptotic, and inflammatory processes, endothelial and renin-angiotensin system dysfunction, changes in glucose and lipid metabolism, and adipose tissue dysfunction. Interestingly it was observed that male rats are more susceptible to this injury than females. Preserving body nutritional requirements during pregnancy, lactation, and growth periods could become a new target in the prevention and treatment of cardiovascular and metabolic disorders.

TRANSLATIONAL RESEARCH FOR SEX DIFFERENCES INVASCULAR FUNCTION.IS THE PERIMENOPAUSE THE TIME FOR PREVENTION?

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In Argentina, cardiovascular disease is the leading cause of death in women, surpassing gynecological cancer and appearing ten years later than in men. Women of fertile age (premenopausal) have a lower cardiovascular risk than postmenopausal women and matched-age men. Differences in vascular function between men and women couldcontribute to cardiovascular events in women. While several factors (genetics, epigenetics, hormones, and environment) can influence sex differences, some of these effects may be related to differences in vascular structure and function and the expressionand function of various proteins in endothelium and vascular smooth muscle cells (VSMC). Evidence suggests that estrogens and progestogens play a cardiovascular protective role;

however, the results of different clinical trials with hormone replacement therapy (HRT) showed that it does not provide primary or secondary prevention against cardiovascular events in postmenopausal women. However, during the transition through perimenopause, hormone levels fluctuate significantly and may play a role in vasculardysfunction and the development of high blood pressure. Recent evidence has suggested that initiating hormone replacement therapy in women early after menopause reduces the risk of myocardial infarction, or heart failure. Importantly, early initiation and prolonged hormone replacement therapy did not increase the risk of breast cancer or stroke. Nevertheless, today the cardiovascular effects of HRT are controversial. In men, low levels of endogenous androgens are associated with the progression of atherosclerosis, endothelial dysfunction, and hypertension in older men. Carotid artery thickness of intima-media, a common marker of clinical and subclinical atherosclerosis, is inversely correlated with testosterone levels. Similarly, the Rotterdam study population has demonstrated that bioavailable and total testosterone levels were negatively associated with calcified deposits in the abdominal aorta in men over 55, leading to vascular dysfunction. On the other hand, high testosterone was associated with increased cardiovascular risk factors in fertile age men and women. In hyperandrogenemia, such as polycystic ovary syndrome or in the postmenopausal period, increase the risk for metabolic syndrome, obesity, and hypertension.Due to the lack of evidence on this topic, and given that women have historically been relegated from both basic and clinical studies, it becomes imperative to increase the evidence n female cardiovascular health. Reinforcing this concept, the Lancet Commission has stated that cardiovascular diseases in women remain understudied, underrecognized, underdiagnosed, and undertreated.

GENE THERAPY AS A TRANSLATIONAL RESEARCH STRATEGY IN CARDIAC REGENERATION

Fernanda Daniela Olea

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Cardiovascular diseases are the leading cause of mortality worldwide. Within them, the most frequent and with the highest mortality is ischemic heart disease, which includes acute myocardial infarction (AMI). After AMI, the initial extent of it is directly related to the severity of the infarction, and can even lead to heart failure. In view of this situation, in the past decades, there have been increasing efforts in the search for novel strategies to reduce or limit the infarct size by minimizing cell death and improving cardioprotection or even regenerate the injured myocardium by promoting the generation of new vessels and inducing CM proliferation.

Gene therapy is one promising strategy to regenerate injured myocardium, through the inhibition or overexpression of sequences that promote the angiogenesis, the proliferation of cardiomyocytes or cellular reprogramming to cardiomyocytes. There are currently several gene therapy strategies that could have regenerative potential for the treatment of experimental ischemic heart disease with a view to future gene therapy clinical trials. Therefore, the proposed target, together with the selection of the vector used, administration route, and the experimental animal model used in the development of the therapy would determine the success in the clinical field.

CARDIAC HYPERTROPHY AND FAILURE: NEW THERAPEUTHICAL TARGETS

Alejandro Aiello

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Cardiac hypertrophy (CH) is one of the main factors associated with cardiovascular disease in hypertensive individuals. CH is characterized by abnormal thickening of the left ventricular wall and implies a compensatory response to sustained high blood pressure or cardiac insult. Furthermore, ventricular enlargement is accompanied by fibrosis and metabolic changes and, in case of persistence over time, it could progress to heart failure and malignant arrhythmia. Intracellular pH (pH_i) maintenance is required for any physiological process. Specifically, in cardiomyocytes is crucial for the correct functioning of many key features, such as electrical activity and contractility, which cannot be guaranteed under acidic conditions. Thus, cardiac cells rely on two sarcolemmal alkalinizing transporters: the Na^+/H^+ exchanger or NHE1, which catalyzes the influx of one Na^+ per H^+ removed from the cytosolic space, and the Na^+/HCO_3^- cotransporter or NBC, which drives the co-influx of Na^+ and HCO_3^-. There are currently two known cardiac isoforms of NBC, electroneutral (NBCn1 of stoichiometry $1Na^+$: $1HCO_3^-$) and electrogenic (NBCe1 of stoichiometry $1Na^+$:$2HCO_3^-$). There is compelling evidence that over-activation of NHE1 leads to elevated intracellular Na^+ concentration, increasing Ca^{2+} influx through reduced Ca^{2+} extrusion via Na^+/Ca^{2+} exchanger (NCX) forward mode or activation of its reverse mode. This mechanism could promote the stimulation of the calcineurin/NFAT pathway and contribute to cardiac hypertrophy development. Since NBC also promotes Na^+ uptake, it is reasonable to hypothesize the same mechanism as that previously proposed for NHE1. However, each NBC isoform contributes differently to the Na^+ uptake. Due to its stoichiometry, NBCn1 contributes one Na^+ per HCO_3^- in each transport cycle, while NBCe1 uses only half of the Na^+ per HCO_3^-. This differential

contribution of Na^+ may be related to the role of each isoform in the development of CH. Our laboratory and others have demonstrated differential activity and/or expression of cardiac NBC isoforms in different *in vivo* hypertrophy models. Moreover, we showed that the activity of NBCe1 is decreased while NBCn1 is over-expressed and over-activated in hypertrophied myocytes of spontaneously hypertensive rats (SHR). We have recently designed and developed an shRNA against NBCe1. shNBCe1 was cloned into a recombinant adeno-associated viral vector (AAV9-shNBCe1) to specifically inhibit NBCe1 in rat hearts. We demonstrated that downregulation of cardiac NBCe1 is sufficient to induce CH. Thus, we were able to reduce the expression and activity of NBCe1 in rats through a specific shRNA, which caused an increase in the size of the heart, modifications in the electrical properties, and homeostasis of Ca^{2+}. Increasing knowledge about the role of each of the NBC isoforms could be of great importance as possible therapeutic targets for treating cardiac hypertrophy.

SYMPOSIUM SAIC IV. *Wednesday 15th November 17:00-18:40*
DEVELOPMENT OF VACCINES IN ARGENTINA
Chairs: Osvaldo Podhajcer; Daniela Hozbor

SET UP OF AN ADENOVIRAL VECTOR-BASED PLATFORM FOR VACCINES PRODUCTION IN INFECTIOUS DISEASES: THE COVID-19 EXPERIENCE

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Zoonotic viral diseases present significant threats to public health and economies worldwide, surpassing any other time in human history. The recent COVID-19 pandemic serves as a clear example of the unpredictable danger that a pandemic can pose to public health, causing the loss of over 130,000 lives in Argentina alone. Massive vaccination has been the primary defense against a catastrophic development of the pandemic, with a positive impact on reducing the strain on healthcare systems worldwide. Based on available evidence, the World Health Organization (WHO) advisory group recommends that high-risk groups receive a booster shot every 6 months. Unfortunately, as of June 2023, only one out of three people in low-income countries has received at least one dose of the vaccine. Consequently, these nations continue to suffer from the severe consequences inflicted by the pandemic. Adenoviral vectors offer several advantages for vaccine development, including quick and cost-effective manufacturing, safety, high immunogenicity in humans, and no requirement for ultra-cold chain storage. These qualities make adenoviral vectors an ideal platform for equitable distribution and storage. In fact, four out of the eleven COVID-19 vaccines approved by the WHO for emergency use are based on adenoviral vectors, making it the most widely used technology. In order to select a candidate with broad coverage against SARS-CoV-2 variants, we have designed improved adenoviral vector-based vaccines. These vaccines retargeted to muscle and dendritic cells, express Variants of Concern (VOCs)-matched, prefusion-stabilized, membrane-bound Spike proteins with an active or mutated furin-cleavage motif. The efficacy of different vaccine variants has been extensively evaluated in preclinical models, including Balb/C mice, at the Instituto Leloir facilities. Additionally, a colony of K18-transgenic mice expressing human ACE2 obtained from Jackson Labs was established at the Center for Comparative Medicine in Santa Fe. Sera from vaccinated Balb/C mice were tested for their cross-neutralizing capacity against a wide range of in-house engineered pseudoviruses (PsV) expressing matched and mismatched Spike proteins. In collaboration with Malbran groups, we conducted comprehensive cross-protection studies in transgenic mice, challenging them with authentic VOCs to determine the vaccines' ability to restrict VOC replication and minimize lung, brain, and upper airway damage. Thanks to the support of the National Agency of I+D+i, a small-scale manufacturing facility with a GLP-like production capacity of up to 5 L has been established at Instituto Leloir and is nearly fully operational. Vaccine stocks have been generated in batches of up to 15 L through an agreement with INTA. A vaccine candidate has been selected for GMP scaling up, and a clinical trial designed by the staff at Hospital Italiano is underway. During the presentation, we will discuss COVID-19 as a paradigm for vaccine design, production, and preclinical assessment.

DEVELOPMENT AND CLINICAL STUDIES OF AN ADAPTED RECOMBINANT BOOSTER VACCINE AGAINST SARS-COV-2

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Our group has been working on the development of adjuvants for vaccines against infectious diseases. When pandemic started, in May 2020 we focused on the development of a vaccine against SARS-CoV-2 that can be produced in Argentina, can be adapted for new emerging variants of concern (VOC) of the SARS-CoV-2 virus and that can be used as primary or booster vaccine. The project is currently being developed in Argentina by our group in the University of San Martin together with the Pablo Cassará Foundation and the pharmaceutical company Cassará. To date we have performed preclinical studies in different animal species to evaluate the toxicity and immunogenicity of the developed vaccine. The vaccine formulation elicited high levels of neutralizing antibodies of the virus and induced a specific T cell response in line with the current requirements for vaccines against COVID19. Vaccine-induced antibodies can neutralize different VOCs. In addition, in an animal model of severe disease, the vaccine induced protection against the experimental challenge with SARS-CoV-2. Preclinical toxicological studies of the prototype vaccine have been completed in December 2021. In March 2022 we received the regulatory approval to start a phase I clinical trial in 80 vaccinated individuals. Results of this phase I study showed that the vaccine was safe and was able to significantly boost the neutralizing antibody response against different VOCs including Omicron BA1 and BA5 irrespective of the primary vaccination platform of the study participants. Moreover, ARVAC was able to boost antigen specific IgG and IFN-γ cellular immune responses in volunteers that have different primary vaccine platforms. Based on these results we started phase II/III trial in 2023. Phase II stage has already finish and Phase III is in course.

USE OF A NANOTECHNOLOGY-BASED VACCINE FOR INFECTIOUS AND NON-INFECTIOUS DISEASES

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Poly allylamine-based nanoparticles (Np) are self-assembling structures with a diameter of 200 nm, capable of loading different proteins, that showed stability over time and at different temperatures, and their synthesis is very simple, reproducible, and low-cost. They function as a vehicle for systemic and mucosal administrations, but they have also shown adjuvant power, which has not led to their use in different types of vaccines and immunotherapies. We have used them to control and reverse non-infectious processes (allergies and tumor diseases) and for the induction of humoral and cellular immunity in bacterial (brucellosis) and viral (SARS-CoV-2) processes. In particular, ARGENVAC has been studied in a pre-clinical model for the induction of specific immunity against SARS-CoV-2 infections. It has been shown to be non-toxic and immunogenic with the induction of systemic and mucosal specific antibodies and IFN-γ-dependent cellular immunity. Protection trials have shown partial protection and it is being studied as a booster dose and as an intranasal vaccine.

THE DEVELOPMENT OF A MUCOSAL VACCINE AGAINST *TRYPANOSOMA CRUZI*

Ana Rosa Pérez
Instituto de Inmunología Clínica y Experimental de Rosario, Consejo Nacional de Investigaciones Científicas y Técnicas (IDICER-CONICET). Centro de Investigación y Producción de Reactivos Biológicos (CIPREB), Facultad de Ciencias Médicas (FCM), UNR.

The route of entry of *T. cruzi* in natural infections occurs through the skin or diverse mucosa (oral, gastric and conjunctival). Since vaccines administered at the mucosal level can induce both a mucosal and systemic specific response, we assessed the immunogenicity and prophylactic effectiveness of intranasal administered experimental vaccines against *T. cruzi*. The Group-I of Trans-sialidase (TS-GI) proteins are highly conserved among *T. cruzi* linages and has recognized as auspicious immunogens in experimental vaccines against the parasite. Previously, we reported that a vaccine based on a TS-GI protect mice against *T. cruzi* infection when vaccinated by subcutaneous route. Now, we tested vaccine formulations consisted of recombinant fragments of TS formulated in different adjuvants. TS-GI fragments plus the mucosal STING agonist c-di-AMP were intranasal administered in 3 doses separated by 15 days each other. This vaccine schedule showed promissory results since induced an evident immunogenicity reflected by the increase of TS-specific IgG2a and mucosal IgA levels with neutralizing capacity. Interestingly, intranasal vaccine also triggers a mixed cytokine profile in the nasopharynx-associated lymphoid tissue, paralleled by an evident cellular response against TS with IFN-γ and IL-17 secretion by specific splenic T lymphocytes. Since human oral outbreaks caused by the ingestion of food or

beverages contaminated with *T. cruzi* are characterized by high mortality rates compared to the vector route of transmission, we tested the protective efficacy of the TS-GI+c-di-AMP after a sub-lethal oral *T. cruzi*-challenge. Vaccinated animals reflected a significant reduction of parasite load during the acute phase, a marked diminution of clinical manifestations, an evident attenuation of cardiac tissue inflammation and fibrosis, and diminution of electrocardiogram alterations. Therefore, TS-GI+c-di-AMP vaccine appears as a promising strategy for prophylaxis of Chagas disease.

SYMPOSIUM SAIC V. *Thursday 16th November 11:00 – 12:40*
NEURODEGENERATIVE DISEASES: A JOURNEY FROM THE BENCH TO THE BEDSIDE
Chairs: Gisela Mazaira; Valeria Roca

THE ROLE OF INNATE IMMUNITY IN AN ANIMAL RESEMBLING FEATURES OF CORTICAL PATHOLOGY OF THE PROGRESSIVE FORMS OF MULTIPLE SCLEROSIS

Carina Ferrari
Fundación Instituto Leloir, CABA, Argentina.

Multiple Sclerosis (MS) is a neuroinflammatory disease affecting both white and grey matter, is characterized by demyelination, axonal degeneration along with loss of motor, sensitive and cognitive functions. MS is a heterogeneous disease that displays different clinical courses: relapsing/remitting MS (RRMS), and MS progressive forms: primary progressive (PPMS) and secondary progressive (SPMS). Cortical damage in the progressive MS forms has considerable clinical relevance due to its association with cognitive impairment and disability progression in patients. We overexpressed interleukin 1 beta (IL-1b) in the cortex to develop an animal model reflecting the main pathological hallmarks of MS. The treated animals presented with neuroinflammation, demyelination, glial activation, blood brain barrier (BBB) breakdown and neurodegeneration along with cognitive symptoms and MRI images consistent with MS pathology. We also demonstrated the presence of meningeal inflammation close to cortical lesions, with characteristics similar to those described in MS patients. Systemic pro-inflammatory stimulation caused a flare-up of the cortical lesions and behavioural symptoms, including impairment of working memory and the appearance of anxiety-like symptoms. The effects of the chronic expression of IL-1β in the cortex resolved within 56 days. However, peripheral and sustained inflammation re-opened BBB, allowing the reappearance of the neuroinflammatory processes within the cortical lesions, increased demyelination and neurodegeneration, and an increase of the behavioral symptoms, such as cognitive impairment and anxiety-like symptoms. Additionally, we studied the effect of Environmental enrichment (EE) on the cortical lesions. EE has been demonstrated to exert positive effects on cognitive domains, such as learning and memory, and improving anxiety-like symptoms. We demonstrated that EE: 1) reduces the peripheral inflammatory response to the stimulus, 2) ameliorates cognitive deficits and anxiety-like symptoms, 3) modulates neurodegeneration, demyelination and glial activation, 4) regulates neuroinflammation by reducing the expression of pro-inflammatory cytokines and enhancing the expression of anti-inflammatory ones. The animal model that we have developed, reflected the main histopathological hallmarks and cognitive impairments characterizing the cortical pathology described in MS patients with progressive forms of the disease. Besides, EE housing could be considered an effective non-pharmacological therapeutic agent that can synergistically aid in the rehabilitation of the disease.

MICROVASCULAR ALTERATIONS IN THE HIPPOCAMPUS OF ALZHEIMER'S DISEASE PATIENTS AND A TRANSGENIC MOUSE MODEL. FOCUS ON ENDOTHELIAL EARLY INVOLVEMENT

Flavia Saravia, Jessica Presa, Juan Beauquis, Carlos Pomilio
Laboratorio de Neurobiología del Envejecimiento, Departamento de Química Biológica, FCEN, UBA. IByME. CONICET.

Alzheimer's disease (AD) is characterized by the accumulation of aggregated amyloid peptides in the brain parenchyma and vasculature. Although vascular alterations are intrinsic to the progression of AD, it is still debated whether these changes are a cause or a consequence of amyloid pathology. In addition, there is limited information regarding vascular changes during the neurodegenerative process of AD in the hippocampus, a crucial structure for learning and memory functions with unique vascular characteristics and, also, an important target in AD. In this symposium, we will present data on hippocampal vascular alterations in AD patients and PDAPP-J20 mice -model of AD- and define the impact of amyloid peptides Aβ40 and Aβ42 on in vitro endothelial activation. We found decreased vascular density and reduced physical astrocyte-endothelium interaction in the hippocampus of AD subjects as compared to age-matched controls. Astrocyte-endothelial interactions and levels of the tight junction protein occludin were altered at an early age in PDAPP-J20 mice, before vascular morphological changes or blood-brain barrier (BBB) disruption. At later stages, PDAPP-J20 mice showed decreased hippocampal

vascular density and extravasation of fluorescent tracers, indicating vascular and BBB compromise. *In vitro* studies showed that exposure of human brain microvascular endothelial cells (HBMEC) to soluble Aβ40 was sufficient to a) prompt a decrease in the transendothelial electrical resistance as assessed in a cell monolayer cultured in a transwell assay; b) promote NFκB translocation to the nucleus, leading to a reduction in occludin levels. These changes were prevented by treatment of HBMEC with an inhibitor of the membrane receptor for advanced glycation endproducts (RAGE). Conditioned media obtained from astrocytes exposed to Aβ42 had a similar effect on endothelial cells, indicating that Aβ42 acts indirectly on the endothelium by inducing astrocytic factors; c) induce a dysregulated proteostasis and activation of endoplasmic reticulum stress pathway. In summary, our results from human and mouse brain samples provide original evidence for the critical involvement of the hippocampal vasculature in Alzheimer's disease. Our *in vitro* data contribute to elucidate the molecular pathways implicated and shed light on new therapeutic strategies.

POTENTIAL BIOMARKERs FOR ALZHEIMER'S DISEASE: A TRANSLATIONAL STUDY BASED ON METABOLOMICS

Laura Morelli

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Alzheimer's disease (AD) is a progressive neurodegenerative proteinopathy characterized by deposition of amyloid β (Aβ) and hyperphosphorylated tau protein in the brain of patients. The pathology observed in AD begins years, or even decades, before the appearance of clinical symptoms. Thus, identification of biomarkers reporting on pathways modulating AD pathology in asymptomatic individuals at-risk is of paramount importance to define target groups for early prevention strategies once these become available. This, however, has been proven to be a major challenge as several, partially unknown, pathways contribute to the pathology leading to neurodegeneration, cognitive decline and finally dementia. Unfortunately, current validated biomarkers inform on the neuropathological hallmarks of the disease following the amyloid cascade hypothesis leaving other pathways (vascular changes, neuroinflammation and age-related factors relevant for reserve and resilience of the brain) uncovered. Given the difficulty linked to the search for biomarkers informing on these pathways in humans, research has turned into model organisms to identify and to characterize conserved pathogenic pathways and molecules that could serve as biomarkers for AD. Herein, a promising animal model is the McGill-R-Thy1-APP rat expressing the human amyloid precursor protein (APP) with the Swedish and Indiana mutations responsible for familial AD in humans. Recent developments in sensitivity and specificity of proteomics and metabolomics technologies have made it possible to identify different molecules targeting these additional pathological pathways. Consequently, we aimed to characterize metabolic abnormalities in the hippocampus of McGill-R-Thy1-APP rats by using Nuclear Magnetic Resonance (^{1}H-NMR) spectroscopy. Promising findings in the rat were followed up in human plasma by Gas Chromatography Electron Impact Mass Spectrometry (GC-EI-MS) to explore their potential utility as AD biomarkers. In rat hippocampus 26 metabolites were identified, 9 showed differences between rat genotypes that were nominally significant. Two of them presented partial least square-discriminant analysis loadings with the larger absolute weights and the highest Variable Importance in Projection scores and were specifically assigned to nicotinamide adenine dinucleotide (NAD) and nicotinamide (Nam). NAD levels were significantly decreased in Tg rat brains as compared to controls. In agreement with these results, plasma of AD patients showed significantly reduced levels of Nam in respect to cognitively normal participants. In addition, high plasma levels of Nam showed a 27% risk reduction of progressing to AD dementia within the following 2.5 years, this hazard ratio is lost afterwards. To our knowledge, this is the first report showing that a decrease of Nam plasma levels is observed couple of years before conversion to AD, thereby suggesting its potential use as biomarker for AD progression.

GENETICS OF MITOCHONDRIAL DISEASES: CURRENT APPROACHES FOR THE MOLECULAR DIAGNOSIS IN A PUBLIC ARGENTINIAN HOSPITAL

Verónica H. Aráoz [1,2], Mariana A. Loos[2,3]

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Mitochondrial diseases are a group of genetic disorders characterized by defects in oxidative phosphorylation. They are caused by mutations in genes found in both nuclear DNA (nDNA) and mitochondrial DNA (mtDNA), which encode proteins involved in mitochondrial function and structure. They represent the most common group of inherited metabolic disorders and among the most prevalent inherited neurological disorders. The clinical pre-

sentation of mitochondrial diseases varies significantly, which can lead to delay in diagnosis. To date, over 250 single nucleotide variants and numerous large rearrangements have been identified in mtDNA, along with more than 400 nuclear genes associated with mitochondrial diseases. In children, approximately 70% of cases are attributed to variants in nDNA. Traditionally, the diagnostic process relied on clinical characterization, followed by biochemical and histochemical analyses, as well as targeted genetic testing. Nevertheless, advancements in sequencing technologies have led to modifications in the diagnostic algorithm. The objective of this study was to develop molecular biology strategies for diagnosing mitochondrial diseases within a public healthcare institution. To achieve this, an interdisciplinary team was assembled to comprehensively characterize patients, and a database was established to guide the diagnostic approach tailored to each individual. Initially, conventional techniques such as Sanger sequencing for single nucleotide variant analysis, LONG-PCR, and MLPA for detecting large rearrangements were employed to study mtDNA. The choice of appropriate biological samples was determined based on each patient's phenotype and age. More recently, Next-Generation Sequencing (NGS) was incorporated for mtDNA analysis. For the nuclear genome, an 80-gene panel was designed, with a focus on pathologies with available treatment options. Exome sequencing studies were only conducted in a limited number of cases. Since 2014, molecular diagnoses have been performed on a cohort of 91 patients selected from a database of 208. The clinical phenotypes observed were diverse, with Leigh syndrome, non-specific encephalopathies, and MELAS being the most frequently encountered. Among the 91 patients, variants in mtDNA were identified in 53 cases, with mitochondrial tRNAs being the most commonly affected. Furthermore, nuclear genome variants were detected in 38 cases, primarily involving genes related to mtDNA replication and maintenance. These results allow to understand the complex causes and molecular mechanisms underlying mitochondrial diseases, and also provide the basis to perform phenotype-genotype correlation studies. Furthermore, obtaining a specific diagnosis enables improved clinical management and evaluate potential therapies for affected patients. Finally, these findings contribute to the understanding of the epidemiological landscape of mitochondrial diseases in Argentina, offering valuable insights for public health initiatives.

SYMPOSIUM SAIC-SAB VI. *Thursday 16th November 11:00 – 12:40*
IMPACT OF THE PLACENTAL ENVIRONMENT ON FETO-MATERNAL HEALTH
Chairs: Gabriela Jaita; María Laura Ribeiro

CALCIUM INTAKE IN THE PREVENTION OF HYPERTENSIVE DISORDERS OF PREGNANCY

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From the observation of the low frequency of hypertensive disorders of pregnancy (HDP) in the indigenous population of Guatemala and the high calcium intake derived from the Mayan custom of softening corn with water and lime, the hypothesis of a possible association between calcium intake and HDP emerged. A series of studies in laboratory animals and humans showed a reduction of blood pressure with calcium supplementation. A randomized clinical trial showed a reduction in the frequency of HDP in pregnant women supplemented with calcium compared to those receiving a placebo. This study was replicated by 27 similar studies involving 18,064 pregnant women showing a 64% (35 to 80%) reduction with calcium supplementation in women with previous low calcium intake. All this resulted in the World Health Organization making the strong recommendation that women with low calcium intake should be supplemented with calcium for the prevention of HDP. The effect of calcium intake would be mediated by parathyroid hormone since in parathyroidectomized rats no increase in blood pressure was seen in those on calcium-free diet. Follow-up studies of the randomized clinical trial showed that the offspring of women supplemented with calcium had a lower frequency of high blood pressure values and a lower frequency of caries than the offspring of women who received a placebo during pregnancy. A study in laboratory animals replicated the effect of maternal diet on blood pressure in the progeny, as at 52 weeks of life the progeny of rats whose mothers received a calcium-free diet showed higher values (12.1 (8.8-15.4 mm Hg)) of blood pressure compared to rats whose mothers received a normal calcium diet.

MOLECULAR PATHWAYS INVOLVED IN THE REGULATION OF THE HYPOXIA INDUCIBLE FACTOR HIF-1α IN THE PLACENTA

Ana C. Racca
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During pregnancy oxygen concentration is tightly regulated and alterations in its physiological levels are associat-

ed to pregnancy disorders such as preeclampsia. HIF-1α protein, the master regulator of the processes that occur under low oxygen tension, is increased in placentas from preeclamptic pregnancies and HIF-1α knockout mice do not survive post day E10.5 due to severe placental defects suggesting that a fine-tunned HIF-1α expression level is required by placental cells for proper placental development. HIF-1α protein is rapidly degraded in the presence of oxygen. However, it is stabilized when oxygen levels decrease, and translocates to the nucleus to heterodimerize with HIF-1β, regulating more than 100 genes in mammals, thus influencing cell behavior. Herein, we identified a novel regulatory pathway of HIF-1α under hypoxia triggered by the tumor suppressor gene Krüppel-Like Factor 6 (KLF6). Interestingly, not only KLF6 regulates HIF-1α, but also, HIF-1α regulates KLF6 increase under hypoxia creating a regulatory loop between these two transcription factors. Using the trophoblast-derived HTR8/SVneo cell line cultured in an atmosphere of 1% oxygen, we determined that HIF-1α silencing with specific siRNAs hinders KLF6 protein increase, while KLF6 downregulation further increases HIF-1α transcript and protein levels under hypoxia, as evaluated through qPCR and western blot. However, although KLF6 overexpression under hypoxia resulted in HIF-1α protein decrease, it did not modify HIF-1α mRNA level. Treatment with antioxidants and transfection of negative dominant variants or specific siRNAs suggest that the NFκB pathway and reactive oxygen species are involved in this regulatory loop. Moreover, treatment of KLF6-overexpressing HTR8/Svneo cells with 250 μM of cobalt chloride (an inhibitor of HIF-1α degradation) prevented HIF-1α protein decrease indicating that KLF6 also regulates HIF-1α protein stability. Finally, in HTR8/SVneo cells grown in 20% oxygen in a 3D culture system, where a hypoxic environment is generated in the center of the sphere, KLF6 silencing further increased HIF-1α protein levels. These results suggest a complex regulatory loop between HIF-1α and KLF6 that may be involved in the pathophysiology of pregnancy diseases such as preeclampsia and other disorders in which oxygen plays an essential role. The search for novel molecules or repositioning of drugs that target this pathway could provide new approaches to improve maternal and fetal outcome among pregnant women with preeclampsia.

HYPOXIA-REOXYGENATION IN THE PHYSIOPATHOLOGY OF THE PLACENTA: ALTERATION OF THE ENDOCANNABINOID SYSTEM AND TROPHOBLAST SYNCYTIALIZATION

Tomás Etcheverry[1], Nora Alicia Martinez[2], Mariana Farina[1]

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The placenta is a highly specialized transient organ. Within this tissue, different types of cells interact and function in synergy//a coordinated manner, providing a unique microenvironment. The cytotrophoblast (CTB) is a mononucleated cell capable of proliferating, differentiating, and fusing, giving rise to the syncytiotrophoblast (STB), the outer multinucleated layer that covers the chorionic villi. This process, known as syncytialization, begins from implantation and continues until the end of pregnancy, as the STB is continuously and highly relatedly renewed by the underlying CTB. Disruption of this process can lead to pregnancy-associated diseases. Preeclampsia (PE) is a severe pregnancy complication characterized by arterial hypertension, proteinuria, and damage to several organs. It is postulated that placental ischemia-reperfusion contributes to its development, triggering inflammation, oxidative stress, and endothelial dysfunction. The STB, responsible for maternal-fetal exchange in the placenta, is affected in PE, with alterations detected in the syncytialization process. //and abnormalities in the syncytialization process were detected. In this pathological condition, we have demonstrated alterations in several components of the endocannabinoid system (ECS). Endocannabinoids are a family of lipid signaling molecules that regulate physiological processes, being anandamide (AEA) one of the most relevant endocannabinoids. In our laboratory, we have shown an increase in cannabinoid receptor 1 (CB1) and the enzyme NAPE-PLD, the main pathway for anandamide synthesis, as well as a decrease in fatty acid amide hydrolase (FAAH), the enzyme responsible for anandamide hydrolysis, in preeclamptic placentas. We investigated the role of hypoxia-reoxygenation (HR) as a model of damage in PE and demonstrated that normal placentas subjected to HR show alterations in ECS components similar to those observed in pathological conditions, suggesting that changes in oxygen tension could be responsible for the detected alterations in pathological conditions. We evaluated the syncytialization process and demonstrated that both HR and stabilization of hypoxia inducible factor (HIF-1α) disrupt trophoblast fusion and differentiation. Additionally, we assessed the impact of the ECS and observed that an increase in AEA tone deregulates syncytialization. Taken together, the results obtained allow us to postulate that HR events, which placentas may undergo during pregnancy complications such as preeclampsia, could alter ECS expression and deregulate the syncytialization process, contributing to a deficiency in placental functionality.

SYMPOSIUM SAIC/AAOC VII. *Thursday 16th November 11:00 – 12:40*
TRANSLATIONAL ONCOLOGY
Chairs: Claudia Lanari; Eugenia Fermento

DEVELOPMENT OF A TECHNOLOGICAL PLATFORM FOR EARLY CANCER DETECTION IN LIQUID BIOPSIES

Clara García Samartino 1,4, Victoria Bocanegra 1,4,7, Cintia Celina Vaquer 1, Sebastián Arbona 1,2, Rodrigo Luis Ongay 3, Erika Gudiño 3, Paula Valdemoros 4, Guillermo Sanguinetti 5, Agustín Correa 5, Pablo Agustín Pellegrini 5, Rodrigo Damián Militello 1,4,7, Melani Carlen 5, Walter Ramon Minatti 5, Emanuel Martín Campoy 1,4,7
1epiliquid SAS. 2Facultad de Ingeniería, Universidad Nacional de Cuyo. 3Higea, Centro de Diagnóstico y Tratamiento Gastroenterológico y Hepatológico. 4Facultad de Ciencias Médicas, Universidad Nacional de Cuyo. 5Hospital Español de Mendoza. 6Hospital Italiano de Mendoza. 7Instituto de Histología y Embriología de Mendoza (IHEM-CONICET).

Cancer comprises a heterogeneous group of diseases characterized by specific diagnostic, prognostic, and treatment variations depending on the site of origin within the body. However, these diseases share a common molecular foundation marked by genetic and epigenetic alterations. Epigenetic modifications have been shown to contribute significantly to tumor development. Notably, distinct DNA methylation signatures have been observed in different tumor types, including tumor subtypes. Tumors typically exhibit global DNA hypomethylation throughout the genome, accompanied by aberrant hypermethylation primarily affecting the promoters of tumor suppressor genes. These modifications are acquired early in the tumorigenic process, suggesting their potential as diagnostic biomarkers. Precision medicine encompasses various fields, with liquid biopsies playing a prominent role. Despite numerous advancements in biomarker research for liquid biopsies, early cancer diagnosis remains a major challenge. The complexity of this approach stems from the consideration of multiple variables that impact the sensitivity and specificity of diagnostic tests. In 2021, we established epiliquid a Technology-Based Company recognized by CONICET with the aim of developing a technological platform for early cancer diagnosis. Our proposal is based on two fundamental pillars. Firstly, a bioinformatic platform capable of identifying sensitive and specific methylation-based biomarkers for different tumor types. Secondly, our own molecular technology enables the detection of multiple biomarkers in a single PCR reaction. Our development initially focused on validating the detection technology in colorectal cancer (CRC) since FDA-approved tests already exist for this tumor type. Initially, we detected a well-described biomarker, SEPT9, and subsequently proceeded to the individual and simultaneous detection of specific biomarkers identified through our bioinformatics platform. We validated the system using DNA derived from cell lines and different tissue samples obtained from CRC patients. Subsequently, we selected a panel of the most promising biomarkers for liquid biopsy detection. We utilized circulating DNA extracted from plasma samples of two distinct cohorts: healthy control patients (negative colonoscopy) and pre-surgical blood samples from CRC patients. The initial validation results of our early prototype were highly encouraging, as we successfully detected our biomarker panel in patients, even at early stages of the disease. Finally, to further validate the technological detection platform, we identified a sensitive and specific panel of biomarkers for hepatocellular carcinoma, which were corroborated using tissue samples from patients diagnosed with this disease. Given the nature of our technology, it is feasible to detect both biomarker panels in a single reaction, thus aiming to validate the first targeted multi-cancer detection test.

CLINICAL ASPECTS OF BREAST CANCER CARE IN LATIN AMERICA: AN ANALYSIS OF THE MOLECULAR PROFILE OF BREAST CANCER STUDY (MPBCS)

Andrea S Llera[1] and the Investigators of the Latin American Cancer Research
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Breast cancer mortality rates in Latin America (LA) are higher compared to Western countries, possibly due to factors such as advanced disease presentation, healthcare disparities or more aggressive molecular subtypes. To address these challenges and foster collaborative clinical research, the Latin American Cancer Research Network (LACRN) was established. The Molecular Profile of Breast Cancer Study (MPBCS) aimed to assess the clinical characteristics and treatment outcomes of Latin American patients with locally advanced breast cancer. The MPBCS enrolled more than 1400 patients from Argentina, Brazil, Chile, Mexico and Uruguay between 2011 and 2013. By implementing standardized procedures and quality assurance measures, the study was able to examine clinicopathological characteristics, response to neoadjuvant chemotherapy, and survival outcomes based on residual cancer burden (RCB) and the type of surgery performed. Moreover, it was possible to record intervals between diagnosis and surgery, intervals between diagnosis and the beginning of neoadjuvant therapy and other parameters of care quality. For the current analysis, a total of 1191 patients with treatment and survival data were eligible. Evaluation of the American Society of Clinical Oncology Quality Oncology Practice Initiative (ASCO-QOPI) breast module indicated a good compliance with pathological standards but

lower adherence to treatment administration standards. Notably, there was wide variation in compliance with trastuzumab administration across different countries (33.3-88.7%), although there was across-country consensus regarding its use. Moreover, only 35.5% of HR+ patients received adjuvant hormone therapy within one year. As expected, neoadjuvant treatment prolonged the time to surgery; therefore, patients in this arm of the study were more prone to non-compliance with timely access to adjuvant hormone therapy. Overall survival was found to be independently associated with RCB after neoadjuvant treatment (i.e. more than 8 times higher probability of death among RCB III (that is, no response to treatment) than in patients with complete pathological response, ($p<0.001$) regardless of subtype and other confounders). Additionally, the type of surgery influenced survival, with worse outcomes observed in mastectomized patients compared to patients that underwent breast-conserving surgery ($p=0.001$), regardless of other variables that are taken into account for the treatment decision in real-world cohorts. The MPBCS data yielded valuable insights into the clinical characteristics and treatment outcomes of locally advanced breast cancer in Latin America. The study underscores the significance of the multidisciplinary approach adopted by LACRN and the implementation of standardized diagnostic and treatment protocols. Access to standard-of-care therapies in LA countries, being the foundation of oncology practice, should be carefully addressed as they have impact on survival.

TRANSLATIONAL MEDICINE AND ITS GREATEST EXPONENT, ONCOLOGY, THROUGH PRECISION MEDICINE

Ignacio Casarini

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SYMPOSIUM SAIC VIII. *Thursday 16th November 16:00-17:40*
GENOMICS IN INTELLECTUAL DISABILITY AND INTERPRETATION OF GENETIC VARIANTS
Chairs: Paula Buonfiglio; María Eugenia Foncuberta

CLINVAR AND CLINGEN: GLOBAL RESOURCES FOR CLINICALLY RELEVANT GENES AND VARIANTS

Marina DiStefano[1] on behalf of the ClinGen Consortium[2]

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Monogenic diseases, though individually rare, collectively represent a substantial burden of morbidity and mortality as they are associated with a substantial risk of disease. Individuals have thousands of variants across their genomes, and differentiating those that are causal for monogenic disease (as opposed to normal population variation) has proved challenging. In order to fully realize the promise of precision medicine, clinicians and researchers need to be able to quickly and accurately identify the genes and variants that may be of clinical relevance to a given individual. The ClinVar database is a publicly available repository of human variation and its relationship to disease that is maintained by the National Center for Biotechnology Information (NCBI). ClinVar currently contains over 3.37 million records from over 2500 submitters, and has become one of the most frequently utilized resources to support variant classification around the world. Since 2013, the National Institutes of Health-funded Clinical Genome Resource (ClinGen) has been building and supporting authoritative, publicly-available resources to support the clinical annotation and interpretation of genes and variants. The ClinGen consortium, powered by the publicly available data submitted to ClinVar, provides extensively curated and expertly adjudicated knowledge through a process of 1) developing and implementing standards to support clinical classification of genes and variants; 2) sharing genomic and phenotypic data between clinicians, researchers, and patients through enhanced knowledge bases for clinical and research use; 3) enhancing and accelerating expert review of the clinical relevance of genes and variants; and 4) disseminating and integrating ClinGen knowledge and resources to the broader community. The community of experts contributing to the resource represent a range of specialties across all areas of pediatric and adult medicine (currently over 2,100 individuals from 59 countries). Our resources have been recognized and utilized by clinical laboratories, regulatory agencies (e.g., first human variant database recognized by the Food and Drug Administration), and payer organizations. ClinGen resources are used by clinical laboratories to decide which genes to include in clinical tests, and by clinicians to determine which results to utilize for patient care. In addition, the research community has used ClinGen data resources to validate new tools/methods for variant effect prediction, and to drive analyses of the functional impact of genomic variation. This resource is essential to the practice of genomic medicine; in its entirety ClinGen will improve, scale, and disseminate expert curation of the human genome to the global genomics community, with the goal of improving health care for all.

FROM OPITZ C TO SCHAAF-YANG AND TRAF7 SYNDROMES

Daniel Grinberg[1], Aina Prat-Planas[1], Mónica Centeno-Pla[1,2], Laura Castilla-Vallmanya[1], Mercedes Serrano[3], Juan Diego Gutiérrez-Ávila[1], Sophie Gibson[1], Isaac Canals[4], Raquel Rabionet[1], Roser Urreizti[2], Susanna Balcells[1]

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In 1969 John Opitz described a new syndrome called C syndrome, Opitz C Syndrome (OCS) or Opitz Trigonocephaly Syndrome. OCS is clinically very variable, with a broad range of severity. It is characterized by developmental delay that is usually severe. Trigonocephaly, due to the premature fusion of the metopic suture, is one of its main characteristics and, while it is not exclusive, it has become mandatory and definitional of OCS. The father of an OCS patient, diagnosed by John Opitz, came to us and prompted us to start the search for the gene responsible for the syndrome. Professors John Opitz and Giovanni Neri sent us samples from several patients and we performed whole exome sequencing. We identified MAGEL2 as the gene responsible for Opitz C syndrome in this first patient. In the rest of patients, we identified a different gene as responsible for the disease in each of them: TRAF7, FOXP1, DPH1, PIGT, KAT6A, PORCN, ZIC1. The conclusion was that Opitz C syndrome is genetically heterogeneous. We decided to concentrate our efforts on the study of the syndromes caused by the genes we found in the first two Spanish patients we studied: MAGEL2 and TRAF7. The MAGEL2 gene maps to the Prader-Willi genomic region in chromosome 15. Truncating mutations in this gene are the cause of a syndrome named as Schaaf-Yang syndrome (SYS). This chromosome region is imprinted and only the paternal allele is expressed. Thus, the affected individuals bear a de novo mutation in the paternal chromosome or a mutation inherited from their father, who must have carried the mutation in his maternal chromosome. The MAGEL2 protein plays a role in the retrograde-endosomal transport. A dysfunction of the retrograde transport could be disturbing for many cellular processes. We performed a transcriptomic and metabolomic characterisation of fibroblasts derived from patients with SYS. We were able to find biomarkers useful for diagnosis and follow-up of potential treatments. Additionally, we showed that the mutant proteins localize mainly to the nucleus. We are currently developing iPS-derived neurons and astrocytes and we are generating brain organoids to try to understand the pathophysiology of the disease in the affected cells and tissue. Somatic variants in TRAF7 (tumor necrosis factor receptor–associated factor 7) cause meningioma, while we and others identified a large series of patients with TRAF7 germline variants who presented with developmental delay and cardiac, facial, and digital anomalies. We published the first large-scale analysis of the clinical and mutational spectrum associated with the TRAF7 developmental syndrome, and we studied its molecular aetiology through transcriptome analyses. Through transfection in HEK293T of WT TRAF7, and constructs bearing cancer and syndromic TRAF7 mutations, we were able to study the differences in expression, protein aggregation and colocalization of the different mutants. For TRAF7 a therapeutic approach is our next goal.

SYMPOSIUM AAFE II. *Thursday 16th November 17:00-18:40*
UNMASKING NEURO ADAPTATIONS THAT INCREASE VULNERABILITY TO SUBSTANCE USE DISORDER: CONTRIBUTIONS FROM BASIC SCIENCE
Chair: Daniela Quinteros

COCAINE-INDUCED HIPPOCAMPAL NEUROADAPTATIONS: UNMASKING PHOSPHODIESTERASE 5 ROLE IN THE VULNERABILITY TO SUBSTANCE USE DISORDERS

Mariela F. Pérez, Emilce Artur de la Villarmois, María Fernanda Ponce Beti, Laura Gabach and Fernando Nasif

Departamento de Farmacología Otto Orsingher, IFEC-CONICET, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba. Córdoba, Argentina.

Behavioral sensitization to psychostimulants hyperlocomotor effect is a useful model to study addiction, the severe type of substance use disorders (SUD). Hippocampus (HP) and medial prefrontal cortex (mPFC) have been implicated in the neuropathological mechanisms underlying addiction. Nitric oxide (NO) is a neurotransmitter involved in neuronal excitability, synaptic plasticity and in psychostimulants sensitization. Furthermore, phosphodiesterase 5 inhibitors (PDE5i) enhance NO activated signaling pathways. The aim of our work is to characterize the role of NO in the development and expression of cocaine (COC) sensitization, analyzing the contribution of NO signaling in different brain areas and repursuing the angiotensin-1 receptors (AT1) antagonists, such as candesartan, as a potential pharmacological alternative to treat SUD. We have demonstrated that COC sensitization in rats (induced by repeated COC administration - 15 mg/kg/day i.p. for 5 days) enhanced synaptic

plasticity, increased NO synthase type 1 (NOS1) protein levels and activity within the HP. The proportion of sensitized rats was reduced by inactivation of NOS1/NO/sGC/cGMP pathway (administering NOS1 or sGC selective inhibitors systemically), as well as HP synaptic plasticity and NOS1 activity. Oppositely, its activation with the PDE5i, sildenafil (administered systemically), significantly increased proportion of sensitized animals and HP synaptic plasticity. On the other hand, Angiotensin II modulates different central neurotransmitter systems, and modifies HP activity via NO-dependent mechanisms. Also, AT1 antagonists prevented several neuroadaptive changes related to amphetamine sensitization. In our work, systemic candesartan administration after expression of COC sensitization seems to reverse sensitization. When the mPFC was analyzed, activity of the pyramidal neurons was increased after COC sensitization, and systemic NOS1 inhibition during sensitization development prevented the observed increments. These results support the possible involvement of NOS1/NO/sGC/cGMP pathway in the vulnerability to develop COC sensitization by its activation in HP and mPFC. Then, to characterize the contribution of NO signaling within HP or mPFC to the expression of COC sensitization, we infused a selective NOS1 inhibitor intra HP or mPFC in animals expressing COC sensitization. Intra HP inhibition reversed COC sensitization, while inhibition within mPFC did not affect expression of this behavior. Considering the results presented, we can speculate that upregulation of the NOS1/NO/sGC/cGMP signaling pathway in different brain areas could initiate, contribute, or exacerbate SUD in humans. Here, the participation of PDE5i in these actions were unmasked showing that they may increase vulnerability to drug abuse. Interestingly, AT1 receptors antagonists may be useful to treat SUD and more studies need to be performed to understand their role in down regulation of NO-activated pathways.

NEUROADAPTATIONS INDUCED BY PSYCHOACTIVE DRUGS: BRAIN AT1-R AS A NEUROMODULATOR TARGET

Claudia Bregonzio

Departamento de Farmacología Otto Orsingher, Facultad de Ciencias Químicas, Instituto de Farmacología Experimental Córdoba (IFEC-CONICET), Universidad Nacional de Córdoba, Córdoba, Argentina.

Mental disorders are frequently associated with imbalance in many neurotransmitter systems such as dopamine, glutamate and GABA. Angiotensin II, through its AT1 receptors (AT1-R), located in the neurovascular unit, is in close relationship with the aforementioned neurotransmitter systems. To better understand the physiopathology and to explore potential treatment advancements, it is crucial to consider the components of the neurovascular unit, given the intricate nature of brain functioning. In pathological conditions, AT1-R expressed in astrocytes, microglia and brain endothelial cells are key mediators in the development of an oxidative/inflammatory microenvironment. Therefore, pharmacological intervention targeting AT1-R provides a holistic and moderated approach to modulate neurotransmission systems, as aside from the glial and vascular responses. In rodents, D-amphetamine is commonly employed as a pharmacological tool to promote dopamine-imbalance modifying future responses to environmental stimuli. In line with this, our findings support the AT1-R involvement in the development of amphetamine-induced neuroadaptations at neurochemical, structural and behavioral levels. We observed a long-lasting overexpression of functional AT1-R in dopamine-innervated areas after amphetamine exposure, together with an altered AT1-R functionality regarding the classical angiotensin II-elicited actions. Using a repeated amphetamine administration protocol, we described an attentional deficit-like behavior in rats involving AT1-R along with morphological and structural changes in the non-neuronal cell types in the prefrontal cortex. Ketamine, an N-methyl-D-aspartate (NMDA) receptor antagonist, is another pharmacological tool used to induce glutamate-dopamine imbalance and to modify the responses to environmental stimuli. Acute administration of ketamine resembles the behavioral aspects observed in the early stages of schizophrenia, such as the first psychotic episode. Repeated administration of ketamine mimics the behavioral and neurochemical features of the pathology supporting the validity of ketamine administration as a preclinical model of schizophrenia. Our findings stand out a critical role for AT1-R in the long-term schizophrenia-related behavioral alterations and neuroadaptive changes induced by repeated ketamine administration. In dopamine-imbalance pathology like schizophrenia, nearly 30% of patients are refractory to the positive signs treatment and current treatments are ineffective for negative signs and cognitive deficits. Furthermore, the high incidence of side effects attempts to the patient's treatment adherence, leading to its discontinuation and the symptomatology relapse. The AT1-R blockers are already available for clinical use and our results support the AT1-R as a possible pharmacological target. These pieces of evidence strengthen the necessity of further studies focused on AT1-R in dopamine-glutamate imbalance disorders.

EARLY PROTEIN RESTRICTION INDUCES REWARDING DEFICITS IN ADULT OFFSPRING: A KEY ROLE IN ADDICTION AND ANHEDONIA.

Analía Valdomero, María C. Gutiérrez, María C. Perondi, Gabriel R. Cuadra and Otto A. Orsingher
Departamento de Farmacología Otto Orsingher, Facultad de Ciencias Químicas, UNC. Instituto de Farmacología Experimental de Córdoba (IFEC- CONICET).

Early malnutrition (i.e., maternal and child undernutrition) and substance abuse are serious problems worldwide with a high socioeconomic and health impact. In this regard, the figures related to these major injuries are alarming in our country. According to the World Health Organization, global trends reflect a disturbing increase in the use of illicit substances. It has been proposed that early onset and prolonged use of drugs of abuse is more likely among youth from communities that have a low life quality, no emotional support, and low educational aspirations. All of these factors are frequently associated with poverty and malnutrition. It is widely known that the central nervous system (CNS) is particularly vulnerable to nutritional deficiencies during its development. Substantial evidence indicates that protein undernutrition, coinciding with the CNS ontogenesis process, induce anatomical, neurochemical, and behavioral alterations persisting throughout life, that cannot be reversed after prolonged periods of nutritional recovery. Indeed, retrospective studies in humans suggest that protein restriction during pregnancy and early life increases the risk of developing psychiatric disorders later in life. In line with this, the findings of our laboratory showed that animals which suffered perinatal protein undernutrition are vulnerable to acquire addictive behaviors and molecular changes during withdrawal in adulthood. These changes are strongly related to craving and drug seeking. Moreover, the nutritional insult facilitates the onset of anhedonia, proposed as a predictor of increased drug craving leading to relapse. This talk will be focused on the neurobiological mechanisms underlying behavioral abnormality in undernourished animals, highlighting the molecular and morphological neuroadaptive changes in the brain reward circuit induced by nutritional injury.

SYMPOSIUM SAB II. *Thursday 16th November 17:00-18:40*
YOUNG SCIENTISTS SYMPOSIUM
Chairs: Clara I. Marin Briggiler; María Eugenia Matzkin

DOPAMINERGIC SYSTEM CONTRIBUTES TO COCAINE EPIGENETIC REPROGRAMMING IN MALE GERM CELLS: IMPLICATIONS IN PATERNAL EPIGENOME TRANSITIONS AND INHERITANCE MECHANISMS

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Accumulating research has documented that spermatogenesis presents windows of vulnerability for epigenetic reprogramming by environmental stressors that affect fertility and even transmit developmental, metabolic and behavioral traits to offspring. This type of non-genetic “Lamarckian” transmission has been established for paternal lifestyle and different forms of chronic stress, drug abuse and diet, involving changes in non-coding RNAs, DNA methylation, and histone posttranslational modifications (PTMs). Our laboratory studies the effect of cocaine and the role of the local dopaminergic system on the paternal epigenome, analyzing specific H3/H4 PTMs and key epigenetic enzymes responsible for their balance. We have characterized the expression profiles of all known histone acetylation and methylation writers and erasers in mice germ cells, and provide the spermatogenic phases when the transcription of these enzymes may be more susceptible to environmental disruption. We also characterized the epigenetic enzymes signature in the mature sperm RNA cargo, showing most of them positive translation at pre-cleavage zygote, suggesting that paternally-derived enzymes mRNA cooperate with maternal factors to embryo chromatin assembly. Importantly, we reported that cocaine-treated mice showed altered levels of H3K4/K9me2 LSD1 demethylase, H3K9me2 G9A methyltransferase, H4K16ac MOF acetylase, and deacetylases HDAC1, HDAC2, and SIRT1 in germ cells. Cocaine also increased H3K9me3/H3K27me3 silenced chromatin marks and decreased H3K27ac/H3K4me3 active transcription marks. In addition, cocaine increased H3K9ac/H4K16ac, involved in the replacement of histones by protamines that induce the compaction of the paternal genome. Moreover, cocaine increased tyrosine hydroxylase enzyme and downregulated dopamine receptor 1 (DRD1) expression in germ cells, showing a similar mechanism to that previously reported for this drug in the CNS, and pretreatment with a DRD1 antagonist (SCH23390) reversed the effects of cocaine on H3K4me3, H3K27me3, and H4K16ac marks, and restored HDAC1/2, SIRT1, and MOF levels. These results suggest a key role of DRD1 on cocaine-induced epigenetic modifications, directed towards gene transcription silencing and alterations in histone to protamine replacement during spermatogenesis. Then, we examined behavioral responses in male and female offspring of male mice treated with DRD1 agonist (SFK38393) and

observed increased anxiety, and altered social behavior compared to control offspring. Only male DRD1 agonist offspring showed increased repetitive, compulsive–like behaviors compared to control offspring. Paternal exposure to cocaine, together with alterations of the dopaminergic system, results in epigenetic reprogramming of the male germline through histone PTMs and can represent an epigenetic mechanism that may underlie the transmission of cocaine-associated information from male progenitors to their offspring.

BY THE MOUTH THE FISH DIES: RISK FOR HUMAN CONSUMPTION?

Sabina Llamazares Vegh[1,2], Esteban Avigliano[1,2], Alejandra Volpedo[1,2]

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Several coastal areas of the world are impacted by anthropogenic activities, which significantly affect marine ecosystems, biodiversity, and fisheries. The role of metals and metalloids as contaminants has been widely recognized as they constitute a serious problem due to their negative effect on ecosystems, particularly in aquatic food chains where they can bioaccumulate and transfer between different trophic levels. Fish meat is considered a high-quality source of proteins and vitamins, rich in essential amino acids, omega-3 type fats, and several micro-and macro elements. Nevertheless, fish has been shown to contain variable amounts of metals and metalloids, often in concentrations that exceed the recommended maximum levels, which generate a controversial debate on the health benefits and risks associated with its consumption. In the last decade, the global per capita fish consumption has increased, reaching values higher than 20 kg/year/person in 2019. Furthermore, the average consumption per capita varies according to the population of each country. For example, in Argentina it ranged between 4.8 to 8 kg/year/person. One of the difficulties that exists to estimate the risk generated by the presence of trace elements in fish for human consumption is that in Argentina there is no detailed information on which species are mainly consumed in each region of the country. In other words, the average value of fish consumption at the national level is not adequate to estimate the risk for different sectors of society. This is due to the fact that the consumption of fish is heterogeneous in the country, there being areas where its consumption is greater, for example in the Mesopotamian provinces, or that the profile of the consumers is different, for example the people associated with to fishing activity (fishermen and their families) consume a greater amount of this type of protein. For this reason, the consumption risk calculation should be differentiated in order to take early warning measures in the event that the concentrations of trace elements are high in some of the species consumed. The assessment of potential consumption risks associated with levels of potentially toxic metals in fish is essential for health and sanitary surveillance bodies to develop guidelines and warnings for the general population on the safe consumption of the target species, particularly for vulnerable groups such as fishermen.

IN THE SEARCH OF STRATEGIES TO MODULATE THE INTERACTIONS BETWEEN THE LEUKEMIC CLONE AND THE TUMOR MICROENVIRONMENT IN B CELL CHRONIC LYMPHOCYTIC LEUKEMIA (CLL)

Mercedes Borge

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Chronic lymphocytic leukemia (CLL) is the most common type of leukemia in adults and is characterized by the accumulation of clonal B cells in the blood and lymphoid organs. Leukemic cell proliferation and resistance to therapy occur within lymphoid tissues driven by B-cell receptor (BCR) stimulation and signals provided by stromal, myeloid, and T cells, supporting the idea that leukemic cell retention within this tumor microenvironment contributes to disease progression and relapse. G protein-coupled receptor kinase-2 (GRK2) plays a central role in B cell homing to lymphoid organs by inducing Sphingosine-1 phosphate receptor-1 (S1PR1) downregulation, which allows lymphocytes to overcome the S1P-mediated retention in the blood and to enter into lymphoid tissues. Migration towards the high concentration of S1P in the vascular compartment also mediates B cell exit from lymphoid tissues. We have previously reported that signals from the tumor microenvironment reduce CLL cell response to S1P. Others have reported a reduced expression of S1PR1 in leukemic cells from patients with poor prognosis and lymphadenopathies, suggesting that the S1P-axis is involved in the retention of leukemic cells in the pro-survival niches within lymphoid organs. In addition, GRK2 has been implicated in signaling pathways related to cancer progression in other tumors. The role of GRK2 in CLL has not been explored yet and is the focus of an on-going project of our Laboratory. Under this project we have studied the role of GRK2 on leukemic cell survival, activation and migration, using primary samples from CLL patients and also with the mouse model of CLL, Eμ-TCL1. We found that in vitro pharmacological inhibition of GRK2 does not affect spontaneous or drug-induced apoptosis on resting leukemic cells from CLL patients, while it decreases leukemic cell activation induced by co-culture with autologous activated T cells. Moreover, GRK2 inhibition increases in vitro leukemic cell migration towards S1P, without affecting the response to chemokines, on both leukemic cells from CLL patients and from

Eμ-TCL1 mice. We also have established a GRK2-deficient murine leukemic cell line (TCL1-GRK2KO) with the CRISPR/Cas9 system, which allowed us to show that GRK2 deletion increases the in vitro response of leukemic cells to S1P and also that it decreases their spontaneous and LPS-induced proliferation. In addition, we found that when injected into wild-type mice, TCL1-GRK-2KO cells have an altered in vivo localization, with a higher presence in peripheral blood and spleen and a lower presence in the bone marrow compared to TCL1-control cells. These results suggest that GRK2 inhibition could be explored as a strategy to induce leukemic cell retention in the blood, thereby increasing their exposure to therapeutic agents and overcoming interactions with the protective microenvironment.

SYMPOSIUM SAIC IX. *Thursday 16th November 17:00-18:40*
BRIDGING THE GAP: TRANSLATIONAL KNOWLEDGE TO IMPROVE HEALTH ACCESS
Chairs: Valeria Roca; Eugenia Fermento

CONCEPTUALIZING TRANSLATIONAL RESEARCH IN THE CONTEXT OF ARGENTINA

Zulma Ortíz
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Health problems are not necessarily just diseases, and social determinants play a key role if we want to understand or solve these problems. Twenty years ago, translational research focused on translating basic science discoveries into clinical practice more quickly and efficiently. This concept has evolved and currently prioritizes the translation and use of scientific knowledge in clinical and public or population health decisions. It also emphasizes the need to integrate the discourse of various and diverse disciplines and collaboration between researchers, politicians and entrepreneurs, among others. Some authors use the term "effectiveness research" to include all efforts to implement an intervention in the community. Instead of "translation," it is usual to find terms such as utilization research, knowledge transfer, community engagement and implementation. In other words, applying a translational approach requires prioritizing what is translated and how this translation is achieved so that it represents more health for our societies. As one would expect from any novel research approach involving professionals from diverse disciplines, one finds little agreement in the use of terminology, definitions of terms, and strategies for moving interventions into the community. However, almost all agree on the importance of having a common goal that goes beyond their own, mostly aimed at solving problems rather than contributing to scientific findings. In other words, the main contribution of the translational approach refers to incorporating a social perspective into the biomedical and clinical one. This incorporation implies escaping from the limitations of the positivist paradigm, typical of biomedical and clinical research that is predominant in health, in which randomized clinical trials are usually considered the "gold" standard. The Ministry of Science, Technology and Innovation in Argentina has promoted a translational research approach through different instruments (plans, programs, networks, centres, units, master's degrees, scholarships, and others). For fifteen years, there has been a sustained political construction that places translational knowledge as the axis for social, productive, economic and, more recently, health development with the participation of the National Ministry of Health and the creation of translational knowledge units in public hospitals. Translational research is still unsettled, but we are on the way. Much remains to be done to understand how existing epistemic, material, institutional, and political practices are co-produced. We have to sustain the efforts, share the roadmap and unite wills so that this approach becomes a paradigm.

WHY IT IS WORTH HELPING TO CREATE A CULTURE OF COMMITMENT TO CLINICAL RESEARCH IN RESOURCE-CONSTRAINED HEALTH SETTINGS AND HOW TO MAKE IT FEASIBLE

Laura Antonietti
Hospital El Cruce SAMIC Néstor Carlos Kirchner, Universidad Nacional Arturo Jauretche.

Clinical research from a translational approach implies (i) linking with basic and preclinical research and its application to real-world practice, (ii) the analysis of the health system environment to carry out clinical research, (iii) the timely incorporation into clinical practice of its results, and (iv) the availability of information to assess the improvements in health outcomes, among others. The translational approach allows incorporating innovative strategies for a more efficient process -such as phase 0 studies, "co-clinical trials", adaptive designs or clinical trial platforms- and adapting implementation science frameworks to improve clinical research development. It also makes it possible to assess its impact on health outcomes beyond bibliometric measures of scientific productivity. The preeminence of clinical trials as the gold standard in clinical research led to, in some

contexts, their being considered synonymous. However, it implies a much wider and continually evolving variety of designs and strategies that must be incorporated from this translational approach to finding solutions to health problems that are appropriate to the context. The clinical research process involves many stakeholders and is directly affected by the dynamics and complexity of the health system. In resource-constrained settings, it is a big challenge to carry out clinical research for all the reasons mentioned above, but developing it is not only possible but essential. For this reason, improving research capabilities and building a culture committed to clinical research in healthcare organizations is crucial. By doing this, even clinical care standards can be improved due to the rigorous quality standards in procedures required by clinical research. During the pandemic of COVID-19, many lessons were learned about challenges and opportunities to do clinical research in Argentina. A key factor identified was the need to strengthen capacities in public hospitals as clinical research sites. An example was the national multicenter clinical trial CARED - financed with public funds - which was carried out through interdisciplinary, collaborative work between researchers from the scientific system and from public hospitals. Twenty-two health centres – public and private hospitals- from four provinces were involved. The researchers trained even more than one hundred health professionals, many of whom participated in clinical research for the first time. In conclusion, creating a culture of commitment to clinical research in resource-constrained health settings requires a research question aligned with healthcare practitioners' interests and needs. It is not only about producing evidence. It is about strengthening the capacities and investing within the health system.

SEARCHING CONTEXT-TAILORED STRATEGIES TO ENHANCE ACCESSIBILITY TO COLORECTAL CANCER SCREENING THROUGH THE USE OF DIFFERENT TYPES OF EVIDENCE

Ariana Bruzzone[1], María Eugenia Esandi[2,3], Yamila Schenfeld[3], Iñaki Manuel Dopazo Danieli[3], María Belén Bellando[3], Gonzalo Picardi[3], Joel Schernenco[3], Manuel Robles[3], María Ernestina Reig[4]

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Although colorectal cancer (CRC) ranks second in both incidence and mortality, screening coverage in Argentina is very low, particularly among individuals with low socioeconomic status and public health coverage. Screening programs are cost-effective and successful in reducing disease-specific mortality. However, their implementation by health systems is complex and sub-optimal, partly attributed to difficulties in strategies design. Using methods and tools from dissemination and implementation sciences would contribute to designing evidence-informed strategies tailored to the specific context of the program. Our work aimed to explore how different types of evidence, stakeholder involvement, and evidence-informed deliberation contribute to the design of implementation strategies that address accessibility barriers to CRC screening. The study objectives were: 1) to generate local evidence on accessibility barriers in a programmatic area of Bahía Blanca, 2) to produce empirical evidence on effective strategies for increasing users and health professional adoption and adherence to CRC screening, 3) to design implementation strategies through stakeholder involvement and deliberation, incorporating theoretical, empirical, and experiential evidence, 4) to analyze how these stakeholder-driven strategies address the local barriers to CRC. This research was part of a broader project conducted by an interdisciplinary team from UNS, with the aim of bridging the knowledge-action gap on priority health issues. It consisted in a 4-stage sequential study, using a mixed-method design. 1: Barriers' assessment through key stakeholders' interviews based on the consolidated meta-framework of Implementation Research and Michie's theoretical framework. 2: Systematic review on the effectiveness of implementation strategies targeting to the uptake and adherence of CRC screening. 3: Evidence-informed deliberative dialogue involving key stakeholders aimed at designing contextualized implementation strategies. 4: Mapping exercise linking proposed strategies and barriers, considering Garbus' accessibility dimensions (technical, economic, political or symbolic). Over 80% of the proposed strategies focused on the technical accessibility dimension (network organization), while the remaining strategies addressed symbolic barriers (knowledge gaps, fear, underappreciated health issue). However, no strategies targeted to barriers related to the political-economic dimensions were proposed, despite being perceived among the most critical ones (limited videocolonoscopy access, no screening policy, fragmented health system). In low-resource contexts, it is crucial to include political-economic dimensions in the design of strategies in order to advance effective and sustainable guidelines for CRC prevention. While a participatory approach helped lay the groundwork for new strategies, it cannot formalize a screening policy in AP2 alone, as some barriers and accessibility dimensions remain unaddressed.

ANALYZING PRIORITIES IN BIOMEDICAL RESEARCH AGENDAS: FROM TRANSLATIONAL KNOWLEDGE TO SOCIO-ENVIRONMENTAL HEALTH

Matías Blaustein

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Conflicts of interest and commercial interests in biomedical research can influence research results and drive research agendas away from public health priorities. During the last years, our group has quantitatively analyzed the key actors and contents of the prevailing health and biomedical sciences (HBMS) research agenda, and how they influence research in non-core countries like Argentina. We found that: (a) the HBMS research agendas of large private firms and leading academic institutions are intertwined, (b) The prevailing HBMS agenda is mostly based on terms related to molecular biology, (c) studies on pathogens and biological vectors related to recent epidemics were marginal in the prevailing HBMS research agenda before the COVID-19 pandemic, and (d) the content of the prevailing HBMS research agenda prioritizes research on pharmacological intervention over research on socio-environmental factors influencing disease. In relation to CONICET's agenda, we found (e) similarities: terms linked to molecular biology research hegemonize CONICET's HBMS research agenda, whereas terms connecting HBMS research with socio-environmental cues are marginal; and (f) differences: CONICET's HBMS agenda shows a marginal presence of terms linked to translational medicine, while terms associated with molecular biology, plant research, agrobiotechnology, and food industry are more represented than in the prevailing agenda. CONICET's HBMS research agenda is internally heterogeneous, appearing to be mostly driven by a combination of elements that not only reflect academic dependency but also local economic determinants. In conclusion, predominant health research agendas, usually in line with existing financial incentives for obtaining lucrative research results, tend to focus on therapeutic and pharmacological intervention, prioritizing innovative therapies based on molecular biology and biotechnology approaches. The prevalence of health and biomedical research agendas often neglect not only the less lucrative diseases but also the study of the social and environmental determinants of health and disease, even when addressing these aspects could significantly improve population health at much lower costs. Some examples of absent studies in the health research agendas are the analysis of non-medical factors influencing health outcomes (social determinants of health), the analysis of the relationship between people and their environment (environmental health), or the evaluation of the socio-environmental factors that influence the deterioration of bodies and territories (such as the One Health approach). A translational knowledge production that takes these aspects into account would benefit from adopting an approach such as One Health, also associated with the concept of Socio-environmental Health, which implies human, animal, and environmental health as an intrinsically connected and interdependent triad.

SYMPOSIUM SAIC/AAFE X. *Thursday 16th November 18:00-19:40*
CHALLENGES FOR ACHIEVING A HEALTHIER ENVIRONMENT: REDUCING POLLUTION FOR A BETTER LIFE
Chair: Claudia Cocca; Andrea Randi

INDUCTION OF EPIGENETIC MODIFICATIONS BY ENDOCRINE-DISRUPTING CHEMICALS

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Endocrine-disrupting chemicals (EDCs) are widespread due to their numerous and frequent use throughout the world, so they become a global issue. Some pesticides are an escalating concern worldwide because of their ability to cause reproductive disorders, alterations in endocrine system and cancer. Growing evidence implies that during early life-sensitive stages, the risk of progression of acute and chronic diseases depends on epigenetic changes by the influence of environmental cues. Several reports deciphered the relationship between exposure to environmental chemicals and epigenetics, and have shown the mechanism that alter the epigenetic states. Changes in global DNA methylation and methylation of specific genes have been described. Because DNA methylation influences the binding of transcription factors to DNA, it usually results in a reduction in gene expression. The structure and function of chromatin may be altered by post-translational changes of histone proteins at certain amino acid residues, such as lysine. It is generally believed that acetylation of histones causes transcription to be activated as a result of chromatin relaxation, whereas deacetylation causes gene silencing

and transcriptional repression. We performed studies in experimental animals showing that different pesticides can disrupt the expression of early genes in neonates with long term reproductive consequences. We identified that abnormal uterine development was associated with effects on implantation process and epigenetic modifications of critical hormone-dependent genes. Disruption of Hoxa10 and estrogen receptor alpha gene expression was detected in association to changes on DNA methylation and post-translational histone modifications. The evidence indicated that epigenetic modifications are mechanism of action of some EDCs, like pesticides, associated with long term consequences on reproductive health.

LITHIUM, A RESOURCE OF STRATEGIC VALUE FOR THE REGION. ANALYSIS OF THE ENVIRONMENTAL AND HEALTH IMPLICATIONS. PERSPECTIVES AND PROPOSALS

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Lithium mining is an opportunity for the “lithium triangle” countries: Argentina, Bolivia and Chile. However, this opportunity can lead to negative externalities of the physical-natural medium and the inhabitants of the Andean areas, if the delicate hydrological and hydrogeological balance is not taking into account, in a mining production of salt water (brine) and fresh water reserves. The condition of development and high added value requires strengthening productive linkages, but also protect the environment. The role of governments is decisive, and requires basic information and institutional agreements to assess the implications of the different stages of these projects (prospecting exploration, exploitation and abandonment of production). In this sense, the analysis of environmental risk is proposed, which incorporates the vulnerability (ecosystem and inhabitants) and the dangerousness of the actions (impacts) throughout the mining project. Lithium mining will be an expanding activity in the region, so critical thinking, a systemic and holistic vision are essential, reinforcing the imaginary of the State and the social value of mining so that it contributes not only to growth but also to regional development.

GLYPHOSATE TOXICITY ON DEVELOPMENTAL SYNAPSES: INVOLVEMENT OF WNT SIGNALLING PATHWAYS

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Glyphosate (Gly) [N-(phosphonomethyl) glycine] is the active ingredient of many broad-spectrum herbicide formulations and constitutes one of the most commonly worldwide used organophosphate (OP) pesticides. Even though, numerous studies demonstrate the effects of Glyphosate based herbicide on developing mammals, the use of these formulations keeps expanding. The nervous system is highly vulnerable to the effects of many environmental pollutants including pesticides. Exposure to Glyph formulations during development could be related to the etiology of many neurodegenerative disorders. In this context, we study the effects of glyphosate exposure on brain development and maturation in rats by in vivo and in vitro assays. Our aim is to describe the Glyph effect on synapse assembly and functioning in the hippocampus of neonates exposed to the herbicide and in purified cultured neurons. Our results reveal that the Glyph exposure during a critical period of synaptogenesis, decreases dendritic spine density as well as their maturation. Furthermore, we observe that Glyph affects the expression of synaptic proteins, such as PSD-95 and Synapsin-I, in a dose-dependent manner. In this line, the exposure of juvenile rats to Glyph reduces the PSD-95 protein levels and alters the postsynaptic organization in the hippocampus. To evaluate the possible correlation between synaptic protein deficits and cognitive function, we also analyze learning and spatial memory by behavioral tests. We find that Glyph treatments induce memory deficits. Together, these findings suggest that Glyph exposure alters neuronal maturation and synaptic organization impairing normal brain connectivity and complex cognitive behavior. To go further, we attempt to identify the cellular mechanism underling the Glyph neurotoxicity during development. We evaluate the contribution of Wnt signaling pathways. Wnt factors are secreted proteins which activate, through their specific transmembrane receptors, three different signaling cascades involving cellular effectors. Wnts function as essential modulators of neuronal development, maturation and connectivity. In this context, we evaluate Wnt effectors after Glyph treatment. Results indicate that Glyph affects synaptic function through a downregulation of Wnt signaling pathways. Thus, activation of Wnt signaling by a therapeutic agent could function as a neuroprotective tool to countered Glyph induced neurotoxicity.

PLASTICS: A LIFE CYCLE THAT THREATENS SUSTAINABILITY

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During the last decades, plastics have changed almost all aspects of our society, encompassing with their presence all aspects of our lives; however, the growing evidence of cumulative processes and impacts on biota, human health, economic and social processes requires that its entire life cycle be examined to address all possible mitigation measures. About 400 million tons of virgin plastic produced mainly from fossil fuels (>98%) are produced annually and this production is on an annual trend of exponential growth, projected to triple by 2050. During its manufacture, a large amount of Chemical compounds known as additives are added to provide different functionalities to the resins (34% plasticizers, 28% fillers, 13% flame retardants, 25% others such as dyes, antioxidants, light and heat stabilizers, lubricants, biocides, etc.). In total, more than 13,000 chemicals are used in the manufacture of plastics, of which only 7,000 have so far been tested for their toxicological and/or endocrine-disrupting properties, and of these 3,200 present a potential risk. These include some flame retardants and UV stabilizers, perfluoroalkyl substances (PFAS), phthalates, bisphenols, alkyl phenols and alkyl phenol ethoxylates, polycyclic aromatic hydrocarbons, metals and metalloids, and other unintentionally added substances. These compounds have dangerous properties and can circulate both in the environment and within organisms, accumulating, blocking or altering the action of hormones, reducing fertility, damaging the nervous system and causing uncontrolled cell proliferation. Some groups are more susceptible to such risks and women and children are more exposed to the serious and/or long-lasting adverse effects of these substances, although recently a decrease in male fertility has also been shown. Plastics can release these substances throughout their life cycle, not only during the extraction of raw materials, polymerization and manufacturing of plastic products, but also during the use of the products and at the end of their life cycle during deposition, collection and, if they reach the environment, on their way to the atmosphere, water and soils. The presence of these compounds compromises the recycling processes and the true circularity of the product. Urgent action is needed to establish a sustainable process of production, manufacturing, use and recycling, which must certainly include simplification (less resins, fewer additives), standardization and the safety and traceability of chemicals in plastics.

SYMPOSIUM SAIC XI. *Thursday 16th November 18:00-19:40*
RECENT ADVANCES IN METABOLIC RESEARCH
Chairs: Silvina Álvarez; Lourdes Posadas Martínez

OFFSPRING FROM MALNOURISHED PARENTS ARE PREDISPOSED TO DEVELOPING METABOLIC DISEASES

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Intrauterine growth restriction (IUGR) affects 3-10% of pregnancies and is recognized as the leading cause of intrauterine fetal death and the second leading cause of neonatal death. Numerous epidemiological studies have shown an association between low birth weight and the development of the metabolic syndrome. Studies in animal models are consistent with the concepts that adverse events in utero predispose offspring to further development of the metabolic syndrome. Several studies in rats have reported gender differences in the development of obesity associated with feeding a high-fat diet, in which males showed greater resistance to gaining body weight than females. However, women appear to be more protected from disorders associated with obesity, such as hypertension and sucrose-induced insulin resistance, and this protection has been attributed to the sex hormone environment. Gender differences in mitochondrial function and oxidative stress parameters have also been observed in different tissues such as muscle, liver, and brown adipose tissue. We have shown that altered ROS homeostasis is an essential process that fundamentally contributes to mammalian vulnerability to disease. Our Laboratory has developed a strong line of research studying the liver function of children of malnourished parents. In all the studies, we found hepatocellular damage. The liver plays a preponderant role in the development of metabolic diseases. Very relevant data is that the oxidative stress parameters give very

high. Moreover, pro inflammatory cytokines production, Serum IL-6, TGF-β and TNF-α levels were higher in the malnourished animals. Mitochondria play a key role in cellular metabolic functions. Dysfunctional mitochondria contribute to oxidative stress, insulin resistance and inflammation. Epigenetic mechanisms have been related to alterations in genes involved in lipid metabolism, fibrogenesis, inflammation and tumorigenesis. The main enzymes corresponding to lipogenesis and gluconeogenesis expressions such as AcetylCoA carboxylase (AAC) and Fatty acid synthetase (FAS).We observed that the of both complexes are significantly increased in malnourished group. And that, the cellular pathway of the insulin response is significantly activated, observing a higher expression of IRS-2 and a higher expression of the GLUT-2 channel in the MM groups. On the other hand, it is very interesting that the expression of the enzyme Glycogen synthase was lower, the former being a fundamental enzyme in the regulation of glycogen synthesis. In accordance, studies have reported that mitochondrial dysfunction and epigenetics linked to early-life nutrition can be important contributing factors in the pathogenesis of NAFLD. We summarize the current understanding of the interplay between mitochondrial dysfunction, epigenetics and nutrition during early life, which is relevant to developmental programming of metabolic disease.

EPICARDIAL ADIPOSE TISSUE: A HIDDEN AND EVIL FAT

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Cardiovascular disease (CVD) is a condition affecting millions of individuals and the main cause of morbi-mortality all over the world. Obesity is one of the many risk factors associated with CVD, however, organ specific adiposity seems to play a stronger role in the development of CVD than obesity itself. Epicardial Adipose Tissue (EAT) is a visceral AT (VAT) directly adjacent to the myocardium and coronary arteries, with no fascia separating them. As a VAT, epicardial fat is metabolically active, producing and secreting a huge variety of adipokines that could be protective or harmful, according to the local microenvironment. Given the lack of animal models that develop EAT and the difficulty in obtaining it from humans, most studies have evaluated its volume through images, indicating an increase in EAT volume associated with CVD. Our Laboratory has developed a strong research line studying EAT from coronary patients. We showed that EAT presents a higher adipocyte density with lower adipocyte size than subcutaneous AT (SAT) and cellular infiltration with T lymphocytes and M1 macrophages. These characteristics could support the fact that EAT expansion is mainly dependent on hyperplasia. Among the main actors involved in the fatty acids (FA) influx to the tissue, it is worth mentioning very low-density lipoprotein receptor (VLDL-R), which expression is higher in EAT compared to SAT in patients with coronary artery disease (CAD). Besides, lipoprotein lipase (LPL) activity, which would also contribute to the uptake of FA, hydrolyzing triglycerides from VLDL and chylomicrons, was increased in EAT of these patients, even in those with diabetes, which is a paradoxical finding. A striking characteristic of EAT is its expression of proteins present in brown AT, indicating that EAT displays brown-like characteristics in healthy humans. We performed the study of the complete lipidome of the EAT in coronary patients, demonstrating a characteristic lipidomic signature in these patients, and an enrichment of EAT in plasmalogens, which could be implicated in brown activation of EAT through the regulation of mitochondrial dynamics. Supporting our previous finding, we also demonstrated the importance of endoplasmic reticulum and mitochondrion pathways in EAT, both organelles implicated in plasmalogens synthesis and activity. Besides, EAT showed an enrichment in bioactive lipids, with higher levels of ceramides [Cerd18:2/18:0, Cerd18:1/18:0, and Cerd18:1/24:1] and sphingomyelin, directly associated with LPL activity. The importance of our results lies in the fact that they add metabolic information to previous GWAS, in which Cers metabolism and LPL-associated genes were related to CVD risk. Up to date, EAT presents itself as a challenging tissue, full of paradoxes and unknown features. Understanding the metabolism of EAT and its possible dialogue with the myocardium will make it possible to elucidate eventual mechanisms involved in the development of CVD.

MASS SPECTROMETRY-BASED STRATEGIES FOR UNDERSTANDING CLEAR CELL RENAL CELL CARCINOMA, AND NEW TOOLS FOR DATA QUALITY ASSESSMENT IN UNTARGETED METABOLOMICS WORKFLOWS

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Clear cell renal cell carcinoma (ccRCC) is the most common histological subtype of RCC that is the main type of kidney cancer with more than 50% of cases incidentally diagnosed; and is characterized for being a glycolytic and lipogenic tumor. Our research group has developed a metabolic footprinting approach to profile the exometab-

olome of human renal cell lines using ultraperformance liquid chromatography coupled to quadrupole-time-of-flight mass spectrometry. The secretome from a ccRCC cell line (786-O, n=22) was differentiated from a non-tumor renal cell line (HEK-293, n=22) through a 21-feature panel. A subset of 9 features, including the identified metabolites isoleucine/leucine, phenylalanine, N-lactoyl-leucine, and N-acetyl-phenylalanine, provided a metabolic footprint capable of differentiating serum samples from stage IV ccRCC patients from controls (n=10). In addition, using a UPLC-ESI-HRMS-based untargeted lipidomics-machine learning strategy, we found a 16-lipid panel, which allowed discriminating ccRCC patients from controls with 77.1% accuracy in an independent test set. This panel was further evaluated in paired-serum samples collected from patients (n=41) before and after nephrectomy. Lipid and metabolite fingerprints were compared with those from healthy controls to evaluate metabolic restoration. The lipid panel differentiated phenotypes associated with metabolic restoration after surgery, representing a serum signature of phenoreversion to a healthy metabolic state. In particular, PC16:0/0:0, PC18:2/18:2 and linoleic acid discriminated serum samples from ccRCC patients with poor prognosis from those with an improved outcome during the follow-up period. In an attempt to increase analysis throughput, we have additionally evaluated the feasibility of detecting the 16-lipid panel using DART-HRMS. PC16:0/0:0 and PC18:2/18:2 in addition to cholesterol, allowed differentiating ccRCC patients from controls in a pilot sub-cohort (n=18) with 83.3% accuracy in training sets under cross-validation. Cholesterol, which is only detected by means of chemical ionization mechanisms operating in DART and is known to be altered in ccRCC, improved the classification performance. Results are promissory for translating these fingerprints to the clinical setting after developing targeted assays in larger cohorts, including subjects with different ethnicities, life style, and diets. Our research group has also focused on developing tools for preprocessing LC-HRMS-based data. In a recent effort, we provided a model to describe the sources of variation in LC–MS-based untargeted measurements, and used it to build a comprehensive data curation pipeline including new tools for data quality assessment, which are freely available in TidyMS.

HUMAN MICROBIOME STUDIES: BEHIND A WELL-MADE RESEARCH LOOKING FOR CLINICAL APPLICATIONS

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Microbiome research around the world is exponentially increasing. Studies in this field are based on a broad spectrum of possible applications, covering soil, air, water, and all living beings on our planet. Particularly, there is a clear interest in human microbiome studies mainly focused on uncovering and shed light on their potential clinical applications to better understand the health/disease balance. The final goal along this is to get actionable insights to improve diagnosis and treatment for almost all diseases and, in the best scenario, get actions for prevention. Over the last decade, more than 115,000 human microbiome studies have been published, many of which having very different experimental designs, with no standardization processes behind them, making them inappropriate for comparisons between related articles, as well as looking for capabilities setting reproducibility and traceability of their experiments. Then, subsequent review articles lack accurate comparisons and analysis and, at the end, they present no fair conclusions. Sample's representativity, technical bias at lab procedures, technical bias at bioinformatic processing, technical bias at data analyzing, robustness of results, fair comparisons and concluding remarks according to sample's nature are main factors not always taken into account when doing human microbiome research focusing into clinical applications. In this study we present the current status of the whole situation, looking to tackle these challenges through cutting-edge robust proposals, sharing cases both from the academy but also from experiences at the international market.

SYMPOSIUM SAB III. *Friday 17th November 11:00-12:40*
BIOLOGY SOCIETIES OF ARGENTINA
Chairs: Evelin Elia; Pablo Cetica

EFFECT OF MATERNAL CA INTAKE DURING PREGNANCY ON OFFSPRING HEALTH

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Calcium (Ca) is an important micronutrient, not only for bone health, but also to reduce the risk of other diseases such as hypertension, pre-eclampsia, colon cancer and metabolic syndrome. In Argentina, Ca intake is below the recommendations, which are 1000 mg/day for adults and 1200 mg/day for pregnant women. The National Nutri-

tion and Health Survey found an medians intake of 446 mg/day for pregnant women and 367 mg/day for women aged 19-49 years, and only 1% of women reported taking Ca supplements during pregnancy. Recent evidence from observational and experimental studies suggests that exposure to certain environmental conditions during pregnancy may have significant effects on foetal growth and the development of susceptibility to chronic diseases in adulthood. Epigenetic changes triggered in the foetal genome during certain time windows of pregnancy development appear to be one of the possible mechanisms for such effects beyond genomic changes. Among environmental factors, it is highlighted that maternal nutrition can induce persistent changes in the genome/epigenome of the offspring that may predispose them to potential diseases and risks in adulthood. Maternal Ca deficiency during pregnancy can cause epigenetic changes that affect the regulation of gene expression and lead to different metabolic phenotypes in the offspring, such as insulin resistance and changes in lipid metabolism. In addition, Ca intake in pregnant women may be inversely related with high blood pressure (BP) in their child. The World Health Organisation recommends that women with low Ca intakes take Ca supplementation during pregnancy to reduce the risk of hypertension in pregnancy. Elevated levels BP in pregnancy are associated with prenatal complications and maternal and neonatal mortality. In animals, a low Ca prenatal diet is associated with higher BP in offspring. It is possible that prenatal programming occurs through effects on Ca-regulating hormones. However, the effects of Ca supplementation during pregnancy on bone quality and offspring growth have been poorly studied. Some studies have found an improvement in bone mineral density during the neonatal period, but these results are no longer observed in the first year of life. The effect of Ca supplementation during pregnancy on offspring bone mineral density is considered unknown because there are few clinical trials and they do not provide conclusive research results due to unknown or high risk of bias in some clinical trials.

CAFS DELAY THE DEVELOPMENT OF PATHOLOGICAL COMPLICATIONS IN DIABETIC RATS BY PREVENTING TUBULIN/ALDOSE REDUCTASE ASSOCIATION

Julieta Swedzky[1], Matias Sebastian Furlan[1], Antonio Echegaray[2], Verónica S Santander[1], Alexis Campetelli[1], Noelia E Monesterolo[1], Gabriela Previtali[1], Gustavo Caro[1], César H Casale[1], Juan F Rivelli Antonelli[1]

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IIn recent studies we have shown that two of the main pathogenic pathways of diabetes mellitus (aldose reductase enzyme activation and Na^+, K^+-ATPase enzyme inhibition) are regulated by their association with tubulin. We also show that compounds derived from phenolic acids (CAFs) prevent the formation of the tubulin/aldose reductase complex and consequently prevent tubulin / Na^+, K^+-ATPase complex formation which drives may decrease the occurrence or delay the development of secondary pathologies associated with aldose reductase activation and Na^+, K^+-ATPase inhibition in diabetes mellitus as: i-cataract formation, ii- erythrocyte deformability, iii- nephropathy, iv- blood pressure, v- diabetic dyslipidemia and vi-non-alcoholic fatty liver. Based on these results, the existence of a new physiological mechanism is proposed, in which tubulin is a key regulator of aldose reductase activity. This mechanism can explain the incorrect functioning of aldose reductase and Na^+, K^+-ATPase, two key enzymes in the pathogenesis of diabetes mellitus. Furthermore, we found that such alterations can be prevented by CAFs, which are able to dissociate tubulin/aldose reductase complex. It is for the aforementioned that we propose the use of CAFs as an adjuvant therapy of the current antidiabetic therapy that only aims at maintaining normoglycemia.

HYPOTHYROIDISM: A REGIONAL DISEASE ASSOCIATED TO MAMMARY EPIGENOME

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In the Cuyo region of Argentina, hypothyroidism (hypoT) is a hormonal disorder of high prevalence. The relationship between thyroid hormones (TH) and tissue differentiation has been described. However, few studies have focused on the link between TH and tissue epigenome. The epigenome is established during tissue development and differentiation. It defines the cellular lineage. The mechanisms involved include post-transcriptional histone modification, methylation of the CpG promoter island and expression of microRNAs. All of these mechanisms have been studied in relation to the pathological development of mammary gland (MG). The MG is a tissue whose differentiation is dependent on the reproductive cycle in adult life. The epigenetic landscape of differentiated MG tissue is established by reproductive hormones. This epigenome prevents pathological tissue

development and promotes milk synthesis for future lactations. In the last decade, we have shown that TH participate in the molecular mechanisms involved in lactation. Our results proved that TH regulate the expression of histone deacetylase enzymes and members of the prolactin signaling pathway. These studies in animal models can explain the functional deficits of hypothyroid lactating mothers suffering from hypogalactia to premature MG functional involution. Both the TH-lactation link and the epigenetic-lactation link have now been established. Our current research is focused on the link between TH and the epigenetic landscape of MG differentiation. We studied the postlactating MG of Sprague-Dawley rats, both euthyroid and hypothyroid (T4 levels= 3.5±0.1 and 0.5±0.1ug/dl respectively). HypoT was induced by administering 6-n-propyl-2-thiouracil in the drinking water (PTU = 0.1 ug/L). Both groups completed one reproductive cycle. 28 days after weaning, inguinal MG was extracted for the following analyses: CpG island methylation by MSRE, microRNAs expression by real-time PCR, gene expression by real-time PCR and protein expression by Westernblot and immunohistochemistry. All studies were performed on differentiation- or pathological development- related genes. Our results show that hypoT during the reproductive cycle alters methylation of TET2, STAT5 and STAT6 CpG promoters. These are differentiation-associated genes. Accordingly, hypoT reduces the long-term gene expression of stat5a, stat5b, stat6 and rno-miRNA200a-3p. The latter is a differentiation-associated miRNA. HypoT reduces methylation of the CD1 and Ki67 CpG island promoters and increases the long-term protein expression of both. These results agree with the histological signs of benign lesions such as florid hyperplasia and fibroadenosis induced by HypoT. Taken together, our results suggest that TH are key for establishing the epigenetic landscape of MG and that hypoT alters its long-term ability to perform future lactation or prevent pathological development.

AAFE I ROUNDTABLE. *Wednesday 15th November 17:00-18:40*
FOSTERING INNOVATIVE COMPANIES: STRATEGIES FOR TECHNOLOGY-BASED COMPANIES
Chair: Hugo H Ortega

DRUG DEVELOPMENT FOR BASIC SCIENTISTS: SOME THOUGHTS BEFORE THE JUMP TO THE PHASE 1 CLINICAL TRIAL FROM THE PERSPECTIVE OF CLINICAL PHARMACOLOGY

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Clinical Pharmacology Section, Internal Medicine Service, Hospital Italiano de Buenos Aires, Buenos Aires, Argentina. Department of Research, Hospital Italiano de Buenos Aires, Buenos Aires, Argentina.

Research projects that involve the transition from basic science to clinical trial imply a series of challenges that range from the economic aspects to the lack of a frame of reference due to its novelty. Each project needs a specific accompaniment of specialists and a project leader who will be the person who orders and allows the dialogue in a fruitful way to be able to safely reach the first-time study in humans. Some of the aspects to take into account are the following: Indication: It must be as consistent with preclinical information, knowing the mechanism of action is essential to be able to foresee future toxicities, and in this way not only be attentive to possible adverse events but also define if it is not possible to advance in a study with healthy volunteers. Clinical development is determined by indication, among other things. The commercial potential should not be in conflict with the available scientific evidence, in any case progress should be made in another indication or eventually achieve the same. The probability of failure of a development is associated, among other things, with the lack of solid information from preclinical studies. Strategic Vision: Look for similar projects. These can give us not only an insight into future developments, but also an understanding of what happens in terms of difficulties. A review of the clinicaltrials.gov site allows us to see if a study has not finished, if it has finished but there are no published reports, if it has been open for a long time, if the centers are expanding to enroll. All these indicators can speak of a lack of efficiency, of difficulty of enrolling or of financing. We should not think that it cannot happen to us, but that we can do to avoid it. Volunteers or patients? This question is not only answered by thinking about the toxicities, but also whether healthy volunteers will not be able to give an answer to the pharmacokinetics of the drug or product. Costs: The study budget must include not only the procedures, research team fees, costs of presentation before the ethics committee and regulatory authorities, consultancy for presentation in foreign regulatory bodies, among others, but also define the period of time for enrollment and conclusion of the study since costs in a country with high inflation skyrocket even if one makes the budget in dollars. Contemplating this is not easy and one must agree to a revision of the contract to the extent that it stretches beyond what was planned. Implementation: The implementation of these phase 1 studies must be done in Argentina in centers approved by ANMAT in accordance with current regulations. Understanding international regulatory framework also helps to avoid problems when they occur in those entities. Approved centers are on ANMAT page, and contact with them should begin before having the study design. Preclinical studies must be carried out in places with the appropriate quality programs to be taken into consideration by the regulatory authorities, that is, the type of work and the data must have international quality. Good laboratory practices and good manufacturing practices: This differentiation is crucial, preclinical

studies can be carried out with GLP but the clinical study must be carried out with GMP. Finding a suitable manufacturer is a challenge, contemplating this in advance also allows accommodating the financing of the same since it is usually somewhat more expensive than the study itself and takes more time according to the complexity of manufacturing and industrial scaling. Finally, understand that each project is a multidisciplinary challenge where each one of the members contributes solutions and views necessary to be able to move successfully towards new treatments with an impact on health at a global level. Argentina is in a position to give regional and global responses, both as initiators of projects and as part of projects in other countries.

ARGENTINA'S OPPORTUNITY: LIFE SCIENCES BASED STARTUPS

Francisco Buchara
SF500, Rosario, Santa Fe, Argentina.

Argentina's emergent biotech industry presents a significant opportunity for company building, leveraging diverse skills, profiles, and intuitive strategies. Over the past 6 years, Argentina has witnessed the establishment of over 100 deep tech companies, a substantial portion of which are within the biotech sector. This panel aims to explore the potential within this sector, shedding light on effective strategies for company formation and initial-year management through insightful case studies. Argentina's educational institutions hold substantial promise and offer a platform for collaboration between the public and private sectors. A robust scientific community, particularly in the field of life sciences, is a distinguishing feature. Underlining the nation's scientific talent are three Nobel Prizes in life sciences. The recent trend of biotech companies listing on Nasdaq underscores Argentina's growing prominence on the global stage. With BIOX and MLEC standing out as exemplars, the country showcases its potential for nurturing biotech enterprises that attain international recognition and success. As we collectively explore Argentina's biotech landscape, this panel offers a platform to exchange experiences and insights that illuminate the trajectory for effectively building these transformative ventures.

BUILDING UNIVERSITY SPIN OFF COMPANIES

Norberto Julián Maggini
Unidad de Vinculación Tecnológica, Universidad Austral.

Science and technology are essential for disruptive innovation, competitiveness, and sustainable economic growth. To transform scientific research into innovation that benefits society, technology needs to be transferred to a company for its development and commercialization. In the technology transfer model traditionally used in pharmacology, a compound, or the product of original research, is patented by the research institution and then the patent is licensed to a third party that will market it and pay royalties to the patent holders in exchange. An alternative model is the creation of scientific spin-off companies. In this case the technology is developed and validated up to the early stages of clinical trials with the active participation of the founding scientific team in the company. This enhances the chances of success of the venture, increases investment in regional research and development infrastructure, and builds capacities that are transcendent for the development of an ecosystem powerful in terms of research and development. This model is growing significantly, with more and more venture capital funds interested in participating in the investment in early stages, scientific teams excited to join, and entrepreneurs specializing in deep tech ventures. Argentina has extraordinary scientific capabilities, which are evident by the number and impact of research publications. This is an essential asset to drive innovation and competitiveness in today's economy. The development of an adequate policy for the creation of science-based companies, which generates the appropriate incentives for each of the necessary participants, is an urgent need for economic growth and the empowerment of the country's scientific and technological ecosystem. This work can only be approached in a collaborative way between government, research institutions, investors, and industry.

PLATFORM BIOTECH VENTURES: INVESTING WITHOUT GAMBLING

Matías Vidal
Co-Founder and CEO Securitas Biosciences Group

Securitas Biosciences is dedicated to fostering innovation in various scientific fields to enhance the overall quality of life and well-being for individuals and societies. Our approach centers on interdisciplinary collaboration, where brilliant scientists and professionals from diverse backgrounds join forces to generate pioneering solutions. As a part of Finvest, Securitas Biosciences actively creates, establishes, develops, and invests in companies that leverage disruptive technologies and pioneering research areas.

SAIC ROUNDTABLE. *Thursday 16th November 17:00-18:45*
RAISING AWARENESS ABOUT GENDER ISSUES IN THE SCIENTIFIC FIELD
Chair: Ana Quaglino

ADVANCES AND CHALLENGES IN THE MAINSTREAMING OF A GENDERS AND DIVERSITIES PERSPECTIVE IN PUBLIC POLICIES

María Angélica Pignatta
Instituto de Investigaciones, Facultad de Ciencias Políticas y Relaciones Internacionales, Universidad Nacional de Rosario, Rosario, Santa Fe, Argentina.

As part of the roundtable on gender issues in the scientific field, this paper proposes a broad discussion framework which aims to analyze the advances and challenges in the process of mainstreaming the genders and diversities perspective into public policies, problematizing their capacity to transform inequalities. We consider that public policies —as a way of visualizing the State "in action"— do not reduce inequalities per se; but they can also perpetuate or exacerbate them, as they structure them. Gender inequalities go through society as a whole, public policies in general and the scientific field in particular, in multiple dimensions, like sexual division of labor, status hierarchy, and heterocisnormativity. Indeed, public policies are not neutral; they still show strong gender biases as they support usually binary categorizations, and understandings about the value of femininity and masculinity, which makes inequalities visible and perpetuates them. The perspective of genders and diversities is presented as a matrix which transforms, interpellates and resignifies public policies regarding their sense, scope and logic, as it helps to make gender inequalities and injustices visible and to modify them. Although the adoption of this approach in agenda building has generated important changes in legal frameworks in Argentina, there is an evident gap between these regulatory advances and the implementation of public policies with a genders and diversities perspective that move forward so that rights are effectively guaranteed. In this context, gender mainstreaming policies become a tool that would transform gender inequalities which are naturalized and installed in our societies' culture, institutions and structures, and, particularly, in public policies, State organization and the scientific field. This paper proposes a transformative model of gender mainstreaming which presents some innovative dimensions by emphasizing relational difference, questioning the prevailing binarism, and acknowledging intersectionality among different axes of inequalities. To operationalize this model, it focuses on the analysis of two specific policies which allow us to identify advances and challenges in the gap between the legal framework and its effective execution: 1) the implementation of Ley Micaela, a law which establishes compulsory training on gender for all people who work in the three branches of State government, as an strategy to achieve mainstreaming of the genders and diversities perspective; and 2) the incorporation of care policies in public agenda, in the face of the prevailing familiarization and feminization, and the construction of an integral care system aimed at their acknowledgment and democratization. The analysis shows that mainstreaming implies defying systems and structures in order to move towards an equality which recognizes and values differences but, above all, which does not support hierarchies and gender power relations.

DIVERSITY IN STEM: BARRIERS, CHALLENGES AND OPPORTUNITIES

Fran Bubani
Centro Atómico Bariloche, CONICET, Argentina.

Science, technology, engineering, and mathematics (STEM) fields have historically been dominated by hegemonic men and, traditionally, little importance has been given to diversity in science. As a result, inclusion in STEM has progressed slowly and discrimination against sex and gender diverse people is common. Because of this bleak outlook, many people end up leaving STEM and changing careers. Those who choose to remain in STEM must overcome several additional barriers: women and feminized identities often face unwarranted assumptions about their lack of suitability for STEM. Moreover, the scarcity of female and sex and gender diverse role models in STEM sends the implicit message that only heterosexual cisgender males are suitable for STEM, reinforcing traditional gender roles and contributing to the status quo. Additionally, unconscious bias in evaluations (and, sometimes, conscious gender-based discrimination) in STEM can make it extremely difficult for women and sex and gender diverse people to build successful careers, let alone thrive. Harassment and discrimination are widespread and can be observed in many different forms, from microaggressions and verbal abuse mislabeled as "jokes", to symbolic, psychological, sexual and physical violence, which makes some STEM environments hostile and unsafe for sex and gender diverse people. Nevertheless, research published in the last decades shows that increasing diversity has significant benefits both in business settings and in research groups. Diverse teams consistently display more creativity, more radical innovation potential and better

problem-solving skills. Diversity has also been proposed as an effective approach to deal with complexity, and cultivating more inclusive STEM contexts increases engagement and productivity for all. However, in spite of all the potential benefits brought by diversity, little has been done to build psychologically safe environments for sex and gender diverse people in STEM. Summarizing, the visibility of sex and gender diversity in STEM challenges archaic hetero-cisgender norms as it presents opportunities to build inclusive workplaces. Embracing sex and gender diverse people in STEM can drive better results in business and in research, but it is essential to develop a culture of respect and to have zero tolerance for discrimination and all forms of gender-based violence.

GENDER, DIVERSITY AND INCLUSION: A PERSPECTIVE FROM PATAGONIA

María Soledad Leonardi
Instituto De Biología De Organismos Marinos, CONICET, Centro Nacional Patagónico, Puerto Madryn, Chubut, Argentina.

The resurgence and strengthening of feminist struggles in recent years has highlighted the multiple forms of violence and gender inequality that dominate our daily and working lives. As people working in science, we are immersed in a patriarchal system of inequalities. Although we are the majority, women are underrepresented in decision-making positions. The system also perpetuates, reproduces and naturalizes a variety of violent practices. The role and participation of women and dissidents in spaces of power and in the construction of knowledge is constantly debated in this latest wave of feminism. The relevance of gender mainstreaming is becoming increasingly evident in all fields, and the scientific system is no stranger to it. In this presentation, I would like to share our experiences in Patagonia. Experiences that have led us to strengthen the collective, the need to generate solidarity and horizontal practices and policies that transform, improve and trigger better institutional policies. In this way, we try to create fairer, more inclusive and egalitarian working spaces. Sharing these experiences allows us to create the necessary networks to support us in our struggles. In particular, I will focus on the experience of recovering institutional day care. Between 1990 and 2011, the premises of the National Patagonian Center, now CCT CONICET-CENPAT, housed a daycare center for the babies and children of the staff. Despite the resistance and organization of many colleagues, the day care center was closed in December 2011. The facilities were transformed into offices and the proposals and alternatives that would have allowed its continuity were ignored for many years. The advance of feminism has allowed us to understand this need as our right to breastfeed and to combine motherhood with work. Not only were we unable to meet the basic needs of our babies, but we were also unable to perform adequately at work. As a result, we were forced to choose between falling behind in our academic and professional development and breastfeeding our babies. This reduced our contact with them and also limited their right to breastfeed. This situation creates an obvious inequality compared to our male colleagues and those who do not breastfeed. Under these conditions, we made a proposal to reopen the nursery, which resulted in us having a nursery again, 11 years after it was closed.

ROUNDTABLE AACyTAL. *Friday 17th November 11:00-12:40*
ENVIRONMENTAL WELFARE IN EXPERIMENTAL ANIMALS
Chair: Gabriel Pinto

USE OF DIGITAL TECHNOLOGIES TO OPTIMIZE FIELD OPERATIONS AND ANIMAL WELFARE TO DO MORE FOR LESS

Massimo Ferrari
Tecniplast, Italia.

Recent advanced technologies offer ways to alleviate these problems while improving efficiency, animal welfare and safety at work. In this presentation we will review the use of digitally enhanced rodent cages for continuous monitoring and data capture. From how machine learning can simplify the vivarium tasks and decrease the workload and overall costs of a facility, to how artificial intelligence can improve welfare checks by prioritizing essential cages and enhancing the ability to identify potential welfare warnings in advance, such as aggression and wound generation by fighting. Finally, it will be discussed how automation can reduce ergonomic risks and increase cage processing productivity.

LATEST STRATEGIES FOR THE MAINTENANCE AND WELFARE OF THE ZEBRAFISH AQUATIC MODEL

Marco Brocca
Tecniplast, Italia.

The introduction of a new aquatic animal to the scientific community raised the need for a different housing

environment. Even though this small fish is quite hardy, in order to maintain it properly, a basic understanding of housing systems must be taken into account. At the beginning, standard aquariums were usually one of the preferred and must-have options for new installations. Nevertheless, as the programs grew and the research expanded, professional systems with obvious benefits and advantages for animal welfare appeared. The theory behind RAS (Recirculating Aquaculture Systems) has been well applied to zebrafish housing technology and is now the preferred choice worldwide. All over the world, zebrafish now swim in autonomous systems. Once everything is set up, populating the system with animals is one of the most critical steps. It is common to make major mistakes during this phase. Now the animals swim in multiple tanks and proper breeding is the next challenge. It must be said that although there are common efforts to define standards at this level, commonly accepted housing rules are not yet established. In this talk, we will discuss the most commonly used ones.

EVALUATING ENVIRONMENTAL ENRICHMENT – HOW DO YOU DETERMINE IF A SPECIFIC ENVIRONMENTAL ENRICHMENT IS GOOD FOR YOUR ANIMALS?

Khia Dobbinson
The National Centre for Replacement, Refinement and Reduction of Animals in Research (NC3Rs), London, United Kingdom.

Good environmental enrichment is essential to the welfare of captive animals. It helps meet behavioural needs and allows animals engage in species-specific behaviours. Evaluating environmental enrichment allows researchers and technicians to make welfare-focused decisions regarding appropriate enrichment. In this talk Dr Dobbinson will cover ways to investigate if environmental enrichment is beneficial, neutral or harmful/inappropriate for the species/strain/sex and age of animals. Throughout the talk Dr Dobbinson will refer to support available in the online resource for evaluating environmental enrichment created by the National Centre for Replacement, Refinement and Reduction of Animals in Research (NC3Rs) in collaboration with the Royal Society for the Prevention of Cruelty to Animals (RSPCA) and Institute of Animal Technology (IAT).

ROUNDTABLE AAFE II. *Friday 17th November 16:00-18:00*
DESIGN AND DEVELOPMENT OF DRUG DISCOVERY PLATFORMS
Chair: Jerónimo Laiolo

IDENTIFICATION OF MOLECULAR TARGETS TO MODULATE ANTIGEN PRESENTATION AND ENHANCE ANTITUMOR IMMUNITY

Gabriel Morón, Luz María Palacios, Cintia Araujo Furlán, Inés Crespo
Centro de Investigaciones en Bioquímica Clínica e Inmunología, CONICET, Departamento de Bioquímica Clínica, Facultad de Ciencias Químicas, UNC. Córdoba. Argentina

Dendritic cells (DCs) represent a heterogeneous group of innate immune cells that infiltrate tumors, process, and present tumor-derived antigens to naïve T cells. DCs play a critical role in priming anti-tumor T cell immunity and thereby represent a major therapeutic target for cancer immunotherapy. While DCs constitute a small population among tumor infiltrating immune cells, they have the ability to shape the immune response within tumors and draining lymph nodes. An growing concept in cancer therapy is that several clinically effective treatments have an immune-mediated anti-tumor component. Therefore, most new generation anti-cancer immunotherapies are evaluated in combination with conventional therapies, including chemotherapy and radiotherapy. One less explored therapeutic pathway is increasing antigen availability. It has been observed that tumor-infiltrating dendritic cells exhibit defective cross-presentation of tumor antigens, which can be reversed by chemotherapy. Cross-presentation of antigens is a specific form of antigen presentation crucial for the induction of CD8+ T lymphocytes, responsible for destroying tumor cells. It is now evident that enhancing tumor antigen cross-presentation by tumor resident or lymph node draining DCs to activate tumor-infiltrating CD8+ T cells is a process that can be exploited in the immunotherapy of cancer. However, antigen cross-presentation has received little attention in the development of immunotherapeutic strategies. To date, there are few known compounds that stimulate antigen cross-presentation, most of which have pleiotropic effects on multiple cellular populations of the immune system. Therefore, it is urgent to develop strategies that enhance antigen presentation by identifying molecular targets that can be exploited to achieve better cross-presentation of antigens and, consequently, induce CD8+ T lymphocytes with enhanced effector capabilities to destroy tumor cells. In our project, we propose to identify molecular targets that allow the modulation of cross-presentation of antigens through a comprehensive search for modulating compounds in drug libraries. To accomplish this goal, we have developed a pharmacological approach through a high-throughput screening platform that uses an in vitro antigen presentation assay. Through this assay, we aim to identify drugs and lead compounds that can increase antigen presentation to CD8+ T cells.

Over the past two years, we have optimized the sensitivity and reproducibility of our platform and tested a wide range of compounds. Currently, we have narrowed our focus to five compounds and are actively examining new compound libraries.

UNLOCKING THE BATTLE AGAINST CHIKUNGUNYA: FROM IN SILICO RESEARCH TO PRECLINICAL DEVELOPMENT OF ANTIVIRAL STRATEGIES

Mariela Bollini[1], Leandro Battini[1,2], Malena Tejerina Cibello[2], Tamara J. B. Vázquez[1], Eliana Castro[2], Daniela Fidalgo[1], Maximiliano Rey[1], María E. Monge[1], Virginia Tribulatti[2], Diego E. Álvarez[2]

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Chikungunya (CHIKV) is an alphavirus transmitted to humans by Aedes spp. mosquitoes. Over the past decade, CHIKV has rapidly spread to tropical and subtropical regions worldwide, creating a significant demand for vaccines and therapeutics. The E2-E1 envelope glycoprotein complex, exposed on the surface of the viral particle, plays a crucial role in virus binding to cell receptors and facilitating the fusion of virus and cell membranes, allowing the release of the CHIKV RNA genome into the host cell. In our pursuit of identifying an inhibitor targeting E2-E1 function, we conducted a structure-based virtual screening that focused on a druggable site situated behind the fusion domain in E1. Through this approach, we successfully identified a specific inhibitor of CHIKV infection. Following lead optimization efforts, we obtained a compound named LB16, which exhibited potent antiviral activity in the low micromolar range. Notably, LB16 selectively targeted the fusion step during CHIKV entry, and mutations associated with antiviral resistance were found to map to the druggable site in E2 and a secondary site in E1. Furthermore, LB16 demonstrated favorable solubility for oral administration and displayed stability across various chemical environments, including pH 1.2, 6.8, and 7.4, as well as in enzymatic media such as human and mouse plasma. Metabolic stability studies were conducted using human and murine microsomes, allowing the identification of metabolites. With its confirmed potency, safety, and appropriate in vitro pharmacological properties, LB16 underwent acute and repeated dose toxicity studies, along with evaluation of its in vivo metabolic profile. The murine model of chikungunya arthritis was established to assess the efficacy of LB16. In addition, we successfully scaled up the synthesis of LB16, resulting in a product with 99% purity. Detailed analysis of impurities stemming from organic synthesis was conducted. In summary, computer-assisted design played a pivotal role in identifying a potent candidate molecule, LB16, with a robust safety profile and favorable pharmacokinetic properties. Notably, LB16 has the potential for large-scale production, making it a promising candidate in the fight against CHIKV.

PATIENT MICRO AVATAR PLATFORM TO DETECT SENSITIVITY/RESISTANCE TO DRUGS IN ACUTE LEUKEMIA.

Gerardo Gatti

OncoPrecision, Córdoba, Argentina.

The treatment of Acute Leukemias worldwide predominantly relies on Standard of Care therapeutic schemes that have been established as the result of populational studies. However, such one-fits all approach is associated with a high rate of refractory/relapsed patients for which is even more challenging to select sequential lines of therapies. It has therefore become clear that personalized approaches for therapy selection are necessary to improve outcomes, even more so when exploring later lines of treatment. We have developed a triple co-culture platform that mimic the tumor microenvironment and promotes the ex-vivo survival of Patient-Derived Cells (PDCs), allowing for predictions regarding the performance of the complete array of FDA-approved drugs, as well as experimental treatments, within 7 days. This pioneering technology, which we have named Patient Micro Avatars (PMAs), consists in the co-culture of PDCs with engineered neoplastic (System Control) and stromal (Tox Control) heterologous cells in combination with a multi-tagging approach coupled to high-throughput flow cytometry. Our PMAs not only allow us to rank the activity of all approved treatments, but also unveil potential unspecific toxicities of novel treatments on non- tumoral cells, thus unlocking unique insights for early drug development programs. In this work, we introduce PMA technology by presenting the clinical validation results from of an Observational Study performed in collaboration with several healthcare centers from Argentina, which through approved IRBs have provided AML patient samples from peripheral blood and bone marrow, as well as clinical follow-up. We present ex-vivo testing results with a comprehensive AML drug matrix which includes Standard of Care therapies, as well as experimental drugs and label expansion candidates. We illustrate the potential of PMAs to identify differential activity from several chemotherapy regimens and targeted therapies in more than 30 patients. We have established clear cases of accurate predictions in patients who did not respond to

treatment, others who responded to treatment, and even cases where the resolution of our readouts allowed us to anticipate relapse after a complete clinical remission.

C. ELEGANS AS A SCREENING PLATFORM FOR DRUG DISCOVERY

Guillermina Hernando, Ornella Turani, Noelia Rodriguez Araujo, Cecilia Bouzat
Instituto de Investigaciones Bioquímicas de Bahía Blanca, Departamento de Biología, Bioquímica y Farmacia, Universidad Nacional del Sur (UNS)-CONICET, 8000 Bahía Blanca, Argentina.

The free-living nematode Caenorhabditis elegans is a widely utilized model organism in various fields of modern biology. Its advantages, such as its easy manipulability, low maintenance cost, short reproductive cycle of approximately three days, large brood size of 300 progenies per hermaphrodite worm, and transparent body, make it the ideal choice for drug discovery screenings. Notably, C. elegans possesses the largest known Cys-loop receptor family, along with unique receptors absent in vertebrates, which present attractive targets for anthelmintic drugs. Additionally, this nematode has shown promise in the search for new therapeutic compounds, including Essential Oils (EOs). EOs have found extensive applications in human and veterinary health, with their active agents being isolated and incorporated into numerous pharmaceutical preparations. Our hypothesis states that EOs derived from aromatic plants commonly used in aromatherapy or as food additives contain compounds with anthelmintic activity. To test this hypothesis, we utilize C. elegans as a model organism for parasitic nematodes. We have developed behavioral and molecular assays using both wild-type and mutant worms lacking Cys-loop receptors involved in locomotion. These assays aim to identify the primary compounds within EOs that mediate anthelmintic activities and their respective pharmacological targets. Furthermore, we explore the combination of current anthelmintics with active compounds from EOs as a strategy to mitigate drug resistance.

Our objective is to provide a robust platform for the discovery of novel antiparasitic compounds. Through our research, we have identified six distinct EOs that inhibit C. elegans locomotion and egg hatching, each exhibiting varying levels of potency, thereby indicating their anthelmintic capacity. Moreover, we have successfully identified the key bioactive compounds and receptor targets associated with these EOs using single-channel and whole-cell recordings from cultured C. elegans muscle cells. These findings have allowed us to elucidate the molecular mechanisms behind the modulation of C. elegans nicotinic and GABA receptors by these compounds. In conclusion, our results highlight EOs as valuable sources of natural compounds with promising polypharmacological profiles for anthelmintic therapies. We have deciphered the molecular basis of their actions and provided insights into the efficacy of drug combinations as strategies to combat drug resistance in nematodes.

COURSES AND WORKSHOPS

COURSE SAIC I- Genetics. *Wednesday 15th November 8:00 – 11:00*
Chair: Viviana Dalamon

AN INTRODUCTION TO EXOME AND GENOME ANALYSIS

Dr. Marina DiStefano
The Broad Institute of MIT and Harvard. Cambridge, MA, USA.

With the falling cost of sequencing, exome and genome sequencing are becoming more common. Genome sequencing has future potential to investigate noncoding regions of the genome but as of this time, both types of sequencing usually target the protein coding parts of the genome. While this approach can increase the likelihood of a diagnosis, particularly in patients with heterogenous phenotypes, it places a high interpretation burden on laboratory staff. In this course, participants will learn the steps for performing analysis on exome or genome sequencing. It will begin with an overview of the analysis process and a discussion of potential workflows for the analysis and variant prioritization. A deep curation dive will follow this discussion where participants will learn how to curate gene-disease relationships and briefly review variant curation standards from the American College of Medical Genetics/Association for molecular pathology sequence variant interpretation guidelines. Resources to aid in curation will also be discussed (e.g. ClinGen, GenCC, ClinVar). The workshop will end with some concrete gene and variant curation examples.

COURSE SAIC II. *Thursday 16th November 8:00 – 9:00*
Chair: Mariana Tellechea

BIOINFORMATICS OF TRANSCRIPTOMES AND METATRANSCRIPTOMES IN ONCOIMMUNOLOGY

Martín C. Abba
Centro de Investigaciones Inmunológicas Básicas y Aplicadas, Facultad de Ciencias Médicas – Universidad Nacional de La Plata (CINIBA, FCM-UNLP)

Advances in cancer and immunogenomics have been propelled by the development of next-generation sequencing technologies (NGS) and bioinformatics pipelines for the analysis of functional genomics data from single-cell (scRNA-seq to tumor tissue (bulk RNA-seq) levels. Transcriptomic profiling allows comprehensive exploration of gene expression patterns, revealing key insights into the molecular mechanisms underlying cancer development, progression, and response to therapy. Bulk RNA-seq methods provide a global view of the tumor transcriptome and have revolutionized our understanding of tumor heterogeneity by identifying distinct intrinsic tumor subtypes and revealing the clinical implications of tumor-infiltrating immune cells. By capturing the transcriptomes of individual cells, scRNA-seq provides unprecedented resolution, enabling the characterization of rare cell populations, identification of novel cell types, and the detection of dynamic cellular states during tumor progression. While metatranscriptomics profiling allows researchers to examine the gene expression levels of multiple organisms within a microbial community providing insights into their metabolic processes and functional activities in cancer and immune-related diseases. Although many applications and software focused on data retrieval, visualization, and analyses of functional genomics data have been developed, their implementations require specialized knowledge and resources avoiding their massive use. This presentation discusses several Python and R/Bioconductor-based computational approaches, providing a comprehensive insight into the oncogenomics bioinformatics field.

WORKSHOP SAIC – Teaching Committee. *Thursday 16th November 13:00-14:00*
Chairs: Sandra Zárate; Gloria Cerrone; Maria Laura Ruiz

WHAT ARE WE TALKING ABOUT WHEN WE TALK ABOUT SCIENCE POPULARIZATION?

Gabriel Stekolschik
Facultad de Ciencias Exactas y Naturales - Universidad de Buenos Aires, CABA, Argentina.
María Florencia Labombarda
Instituto de Biología y Medicina Experimental – CONICET, CABA, Argentina.

In an era marked by rapid scientific advancements and an increasing need for informed decision-making, the role of science popularization has gained paramount importance. This thought-provoking presentation explores the very essence of science popularization, dissecting its underlying objectives, methods, and challenges. Stekolschik and Labombarda inquire whether the term itself warrants deeper scrutiny. Is science popularization merely about simplifying scientific concepts for mass consumption, or does it entail a broader mission to foster scientific literacy, critical thinking, and public engagement with science? This talk encourages us to reflect on the evolving role of science popularization in our increasingly interconnected world. It prompts us to critically assess how we discuss and disseminate scientific knowledge and how these conversations shape our perceptions, decisions, and the very fabric of society. By engaging in this dialogue, we gain insights into the complex interplay between science, communication, and society, paving the way for a more informed and enlightened future.

WORKSHOP AACYTAL. *Thursday 16th November 15:00-16:50*
Chair: Marina Snitcofsky

THE NC3RS AND THE EXPERIMENTAL DESIGN ASSISTANT: AN INTERACTIVE WEB-BASED TOOL TO PROVIDE BESPOKE FEEDBACK ON EXPERIMENTAL PLANS

Esther J. Pearl and Nathalie Percie du Sert
The National Centre for Replacement, Refinement and Reduction of Animals in Research (NC3Rs), London, United Kingdom.

The National Centre for Replacement, Refinement and Reduction of Animals in Research (NC3Rs) is an independent scientific organisation that provides guidance and resources to support the international biosciences research community, including researchers, animal technicians, funders, regulators and policy makers, implement the 3Rs. The NC3Rs has developed resources to assist researchers in designing more robust experiments, selecting the appropriate analysis method and reporting the experiment thoroughly. One such resource is the Experimental Design Assistant (EDA; https://eda.nc3rs.org.uk). The EDA is free online software with a supporting website to help researchers design more robust *in vivo* experiments. During this workshop Dr Pearl will demonstrate how to use the EDA, including how to improve experimental design based on feedback from the software.

COURSE SAIC III. *Friday 17th November 8:00 – 9:00*
Chair: Valeria Roca

BIOBANKS: A PLATFORM FOR BIOMEDICAL RESEARCH

Cecilia Gamba
Centro Regional de Hemoterapia & Banco Público de Referencia Nacional de Sangre de Cordón Umbilical, Hospital de Pediatría Garrahan, CABA, Argentina.

The field of biobanking plays a crucial role in driving advancements in biomedical research by providing scientists with access to high-quality biological samples and associated data. This short course offers a comprehensive overview of biobanking, its infrastructure, and its significance in the research community. The course begins with an introduction to biobanking, defining its purpose and emphasizing its importance as a valuable resource for researchers. Participants will gain an understanding of the diverse types of biological samples commonly stored in biobanks, including tissues, fluids, cell lines, and genetic material. During the presentation we will explore the essential components of biobanking infrastructure, sample collection and storage facilities, highlighting the importance of adhering to standardized operating procedures (SOPs) and implementing rigorous quality control measures. Participants will learn about the critical role of ethical considerations and informed consent in biobanking practices. Additionally, the course addresses the management of data and information systems, emphasizing the need for efficient tracking and retrieval of samples and associated data. Participants will be in-

troduced to various collection methods and techniques, including best practices for sample handling and storage to maintain sample integrity. Attention will be given to pre-analytical variables that can influence sample quality and how to implement quality assurance measures to mitigate potential issues. Furthermore, the course will cover processing and preparation techniques for long-term storage and discuss the significance of cryopreservation in ensuring sample viability. Quality standards and governance policies will also be addressed in the course. Participants will gain insights into the establishment of policies and procedures, encompassing considerations of confidentiality, intellectual property, and participant privacy. Legal and regulatory aspects of biobanking, including compliance with applicable laws and regulations, will be explored. The course will feature case studies and real-world examples to illustrate the impact of biobanking on biomedical research and healthcare. Participants will gain valuable insights into successful biobanking initiatives and understand how biobanks have contributed to advancements in personalized medicine. The course will also discuss potential future directions and emerging advancements in the field of biobanking. In conclusion, this mini course on biobanking equips participants with a comprehensive understanding of the field, its infrastructure, and its critical role in advancing biomedical research. This course serves as a foundation for individuals seeking to expand their knowledge in the field of biobanking and its contributions to cutting-edge biomedical research.

AAFE AWARD - Annual award to the best work in pharmacology.
Thursday 16th November 11:00-12:40
Chair: Guillermina Hernando
Juries: Ventura Alejandro Simonovich, Susana Gorzalczany, Hugo Héctor Ortega, Gabriél Morón

ADVANCING GASTROINTESTINAL NEMATODE TREATMENT THROUGH PHYTOCHEMICALS ADMINISTRATION TO LAMBS

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In the last 50 years, few antiparasitic drugs with new mechanisms of action have been introduced; leading to a global increase of drug resistance. Therefore, the search for alternative pharmacological tools is a priority in ruminant production systems. While numerous phytochemicals demonstrated efficacy against parasites *in vitro*, the transition to *in vivo* characterization poses a growing challenge. The drug concentration attained in the target parasites and, the resulting pharmacological effect are directly influenced by the administration route and pharmaceutical formulation. The aim of the current work was to analyze the pharmacokinetic-pharmacodynamic relationship of the combined administration of carvone (CNE) and ivermectin (IVM) to lambs. Three trials were conducted to evaluate the pharmacological interaction between CNE and IVM in lambs infected with nematodes. Drug concentrations were measured in plasma, target tissues and *H. contortus* by HPLC with fluorescent (IVM) and ultraviolet (CNE) detection. The decrease in fecal egg count was used as an indicator for estimating the efficacy of both compounds. CNE significantly enhanced the plasma bioavailability of IVM. CNE showed a moderate anthelmintic effect, which was greater on the susceptible isolate of *H. contortus*. After the combination of CNE and IVM as an oral emulsion, both compounds were quantified in *H. contortus* recovered from infected lambs. Although the coadministration of CNE and IVM in lambs demonstrated a moderate *in vivo* anthelmintic effect and enhanced systemic availability of IVM, the concentrations achieved in both target tissues and parasites remained notably lower compared to those documented to induce anthelmintic effects in the *in vitro* assays. Consequently, these levels were insufficient to achieve the desired optimal efficacy. Innovative pharmaceutical formulations are required to establish phytochemicals as a useful pharmacological tool for controlling nematodes in ruminants.

ANTIHYPERGLYCAEMIC ACTIVITY OF TWO SPECIES OF *PHYLLANTHUS*

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Phyllanthus niruri L. (*rompepiedra*) and *P. sellowianus* (*sarandí blanco*), are native species of Central and South America. In ethnopharmacology, *P. niruri* is used as a diuretic, while *P. sellowianus* is used as a hypoglycemic. The aim of the works was to evaluate the inhibitory potential of these extracts on α-glucosidase enzymes (APG) from different sources. Aerial Parts (APn) and Roots (Rn) of *P. niruri* collected in Corrientes-Capital, along with Leaves (Ls), Stem Bark (SBs), and Roots (Rs) of *P. sellowianus* from Corrientes-Monte Caseros, were employed for this research. The extracts were prepared with methanol: ethyl acetate (7:3) and dried using a rotary evaporator. Antihyperglycemic efficacy was studied based on the ability of the extracts to inhibit APG activity. Results were quantified in terms of inhibition ratios (Ir), with acarbose as positive control. Additionally, phytochemistry and the HPLC-DAD profiles of the extracts were evaluated. In the *Saccharomyces cerevisiae* APG inhibition assay, extracts from both species outperformed the acarbose. The most active extracts were SBs:

Ir=318.69, Ls: Ir=277.93 and APn: Ir=251.67. However, during the pig APG inhibition assay, all extracts exhibited lower potency (Ir<1). Leaf extract (L) from *P. sello*wianus had the greatest inhibitory activity (Ir=0.0875), followed by SB and AP extracts from *P. niruri* (Ir=0.0819). All extracts showed a high polyphenolic content, while only in R and SB condensed tannins were detected. Chromatographic analysis of active extracts revealed a major peak with a retention time of 25min and λ_{max}=220 and 276 nm. Our findings highlight the higher inhibitory activity of *P. sellowianus* compared to *P. niruri* extracts and emphasize the inhibitory influence of extracts from both species on alpha-glucosidases, with a higher affinity towards the yeast enzyme. These results have important implications for the design of future screening protocols to assess the antihyperglycaemic potential.

MULTITARGET THERAPEUTIC POTENTIAL OF *VALERIANA CLARIONIFOLIA* EXTRACT FOR ALZHEIMER'S DISEASE AND COMORBIDITIES: *IN VITRO* AND *IN VIVO* EVALUATIONS

Carolina Marcucci[1], Marina Rademacher[1], Valentina Pastore[1], Victoria Suarez[1], Fabiola Kamecki[1], Hernán Gerónimo Bach[2], Rafael Alejandro Ricco[2], Natalia Colettis[1], Mariel Marder[1]

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Alzheimer's disease (AD) is a neurodegenerative disorder characterized by abnormal protein accumulation (β-amyloid, tau), oxidative stress, and neurotransmitter imbalances, particularly acetylcholine. In this study, the hydroalcoholic extract of the underground parts of *Valeriana clarionifolia*, a native valerian species from Patagonia Argentina known as "Ñamkulawen" in Mapuzungum language, was evaluated. The extract's ability to inhibit acetylcholinesterase/butyrylcholinesterase (AChE/BChE) in mouse brain/plasma homogenates (Ellman's method), human recombinant monoamine oxidases A and B (MAO A and MAO B) (Amplex Red method), β-amyloid peptide aggregation (thioflavin T method), and its antioxidant effects (TBARS, DPPH and ABTS assays) were assessed *in vitro*. Furthermore, *in vivo* behavioral and cognitive evaluations were conducted on male Swiss mice using chronic treatments with 50 mg/kg/day of the extract in drinking water for 30 days. The hydroalcoholic extract inhibited murine AChE (IC_{50}($IC_{95\%}$) 1.29 (0.81-2.05) mg/ml) but displayed stronger inhibition of BChE in both mice (IC_{50} 1.86 (1.43-2.43) μg/ml) and humans (IC_{50} 0.44 (0.36-0.54) mg/ml). Additionally, it exhibited significant inhibition of $A\beta_{1-42}$ aggregation (82% at 0.1 mg/ml), but was unable to inhibit human MAO-A or MAO-B. The chronic treatment of mice with *V. clarionifolia* extract induced anxiolytic/sedative effects (plus maze and open field assays), improved spatial working memory (y-maze test), and antidepressant-like effects (tail suspension test), along with a significant decrease in AChE activity in their brains (*ex vivo*) compared to mice that drank water. In conclusion, *V. clarionifolia* demonstrates multitarget therapeutic potential for the treatment of Alzheimer's disease and its comorbidities.

MECHANISMS INVOLVED IN CARDIOPROTECTION BY DRONEDARONE IN ISCHEMIC RAT HEARTS: COMPARISON WITH AMIODARONE

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Previously we showed that perfusion of dronedarone (Dnd) in an isolated rat heart before ischemia/reperfusion (I/R) increased the post-ischemic contractile recovery (PICR) and energetical recovery. Contrarily, perfused amiodarone (Amd) did not improve PICR. The present aim was to evaluate the role of nitric oxide (NO) pathway and the mitochondrial ATP-dependent potassium channels (mKATP) in the cardioprotection induced by Dnd and the participation of NO-synthases (NOS) in the effects of Amd. Isolated perfused Wistar rat hearts were exposed to 30 min I/45 min R inside a flow calorimeter. Left intraventricular pressure and total heat rate (Ht) were continuously measured. Maximum developed pressure (P, mmHg) and total muscle economy (Eco=P/Ht, mmHg.g/mW) were calculated. Direct perfusion of Dnd 1μg/ml improved PICR up to P= 62.6 ± 6.6 % of pre-I ($p<0.05$ vs 13.1 ± 5.9 % in control (C), n=5,5) and Eco up to 86.2 ± 18.3 % of pre-I ($p<0.05$ vs 18.9 ± 8.5 % in C , n=5,5). L-NAME 30 μM completely reversed the cardioprotection induced by Dnd (P up to 6.0 ± 3.8 % and Eco up to 4.5 ± 3.7 %, both $p<0.05$ vs Dnd, n= 5-5). Contrarily, L-NAME did not affect the low contractile and energetic recovery induced by perfusion of Amd 5 μg/ml (P up to 17.9 ± 9.3 % and Eco up to 15.1 ± 7.0%, both $p<0.05$ vs Amd, n= 5-4). To evaluate the role of mKATP channels, 5-HD 100 μM was perfused before I. 5-HD partially inhibited the Dnd cardioprotection (up to P= 43.0 ± 7.3 % and Eco to 44.2 ± 7.1 %, both $p<0.01$ vs Dnd, n= 5-6). Results suggest that cardioprotective effects of Dnd in ischemic hearts are due to a beneficial NO production and to the

opening of the mKATP, which prevent mitochondrial dysfunction. Moreover, the NO pathway does not participate in the lack of cardioprotection when perfusing Amd.

IN-FEED DRUG MEDICATION IN PIG PRODUCTION: EFFECTS ON XENOBIOTIC METABOLIZING ENZYMES

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In-feed medication with the anthelmintic fenbendazole (FBZ) is routinary in pig husbandry. This drug undergoes hepatic metabolism through cytochrome P450 (CYP) and flavin-monooxygenase (FMO) enzyme families. Also, FBZ and its metabolite oxfendazole (OFZ) may induce the CYP1A subfamily. This work aimed to evaluate the effect of FBZ administration on i) CYP1A-dependent enzyme activities; ii) its own pattern of hepatic S-oxidation; iii) the metabolism of enrofloxacin (ERF) and aflatoxin B1 (AFB1). Female Landrace piglets remained untreated (n=5) or received a pre-mix of FBZ in feed as usually is recommended for 9 days (n=6). Liver microsomes from control and FBZ-treated animals were used for i) CYP content determination; ii) monitoring CYP1A-dependent enzyme activities, 7-ethoxyresorufin O-deethylase (EROD) and methoxyresorufin O-demethylase (MROD); iii) measurement of FBZ (50 μM) S-oxidation, ERF (50 μM) conversion into ciprofloxacin (CPF) and AFB1 (16 nM) disappearance. In liver microsomes from treated animals, EROD and MROD increased 20-fold (p=0.002) and 19-fold (p=0.001), respectively. An enhanced (3-fold, p=0.0037) participation of the CYP pathway in the hepatic S-oxidation of FBZ into OFZ was observed in the liver of piglets receiving FBZ compared to controls. ERF conversion into CPF increased (p=0.014) from 26.5±8.4 pmol/min.nmol CYP (controls) to 139.4±60.1 pmol/min. nmol CYP (FBZ-treated). The rate of disappearance of AFB1 in FBZ-treated pigs was 79% higher (p=0.036) compared to control animals. An auto-induction of the CYP1A-dependent S-oxidation of FBZ towards its active metabolite OFZ was observed. The in-feed medication with FBZ may cause potential metabolic interactions with the antimicrobial ERF and the mycotoxin AFB1. Enzyme induction caused by the anthelmintic may modify the pharmacokinetic behaviour of ERF and CPF. In addition, induction of the CYP1A-dependent metabolism of AFB1 may increase the production of a hepatotoxic AFB1-derived epoxide.

ANTIMICROBIAL ACTIVITY OF THE NOVEL BACTERIOCIN AP7121: FROM *IN VITRO ASSAYS* TO AN *IN VIVO PRELIMINARY* INTRANASAL TREATMENT APPROACH

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The impact of staphylococci infections on human and animal health increased as result of its ability to become resistant to antimicrobials. Most *Staphylococcus* spp. (*Staph*) infections are endogenously acquired, and treatment of nasal carriage is one strategy for prevention. AP7121 bacteriocin, showed *in vitro* bactericidal activity against *Staph*. The main goals of this study were: a) to assess the nasal carriage of *Staphylococcus* species in asymptomatic dogs, and b) to test the effect of intranasal administration of AP7121 in the staphylococci population. Dogs were randomly allocated in two groups (n=3) and two intranasal administration protocols were evaluated as follows: *protocol A:* canines received one dose of either 150 μL of sterile saline solution (SSS) (*control group)* or 150 μL of AP7121 solution (330 μg/mL) (*AP7121 group*); *protocol B:* dogs received 100 μL every 24 h for 3 days of SSS or AP7121 (330 μg/mL). Previous to each treatment (*T0)* and 24 h after the topical treatment (*T1*), standardized nasal swabs samples of both nasal vestibules were taken. Swabs were systematically inoculated and cultured in Mannitol salt agar and Blood agar. *Staph* isolates were phenotypically characterized according to antimicrobial sensibility using the disk diffusion method and VITEK® 2 system. Fischer exact Test was used for statistical analysis of treatments. Variability in *Staphylococcus* species was found in many cases among individual dogs in *T0* and *T1*. Fifty *Staph* strains were isolated, being 58% (29/50) resistant to Penicillin and 4% (2/50) showing inducible macrolide and lincosamide resistance. *Protocol A* treatment failed to reduce the viability of the nasal staphylococci population. However, *protocol B* (3 doses of AP7121) showed a significant 50% of decolonization *(p<0.01)*. In conclusion, the antimicrobial effect of AP7121 against *Staph* could be explored as a potential

alternative for nasal decolonization therapy in dogs, as in previous clinical humans´ studies.

GEMCITABINE AS A POTENTIAL NEW DRUG FOR RETINOBLASTOMA TREATMENT

Milagros Dinardi[1], María Belén Cancela[1,2], Santiago Zugbi[1], Po-Jen Tseng[3], Paul J. Dyson[3], Christina Stathopoulos[4], Francis L. Munier[4], Paula Schaiquevich[1,2]

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Retinoblastoma (Rb) treatment has changed over the years, leading to an improvement in ocular survival. Among these treatments, intravitreal melphalan is highly effective for vitreous seeds but causes retinal toxicity. In addition to toxicity, relapsed eyes remain difficult to treat and few second-line drugs are available. Therefore, new active agents against Rb are urgently needed. In a preliminary large-scale pharmacological screening study, we identified gemcitabine (GEM) as a promising hit for further investigations. Thus, this study aimed to determine the pharmacological sensitivity of GEM in commercial cell lines and primary cell cultures derived from human tumors and assess the efficacy after intravitreal injection in a xenograft model. Commercial Rb cell line Y79 and two patient-derived primary cell cultures, one established from an intraocular tumor (HPG-RBT-12L) and the other from cerebrospinal fluid (HPG-CSF-1) were used. GEM IC50 was determined using the MTT assay. For the efficacy study, Y79 cells were intravitreally injected in athymic BALBcnu/numice and after tumor engraftment, mice received two weekly doses of vehicle, GEM, melphalan, or GEM plus systemic carboplatin. Mice were monitored and eyes were enucleated upon achievement of the experimental endpoint (eyes reaching 3 times the normal size). GEM mean (range) IC50 for HPG-RBT-12 L, HPG-CSF-1 and Y79 was 1.66 nM (1.20-2.89), 1.39 nM (0.78-3.13) and 9.62 nM (8.61-10.74), respectively. The median (range) eye survival treated with vehicle, GEM, melphalan, or GEM plus carboplatin was 31 days (23-38), 37 days (34-49), 40 days (36-51) and 41 days (35-49), respectively. Thus, a significant increase in eye survival was observed after GEM and GEM plus carboplatin with respect to control animals ($p<0.05$). GEM is a promising drug for Rb treatment. Further efficacy evaluation and safety studies in preclinical models are undergoing to support the potential translation into the clinics.

SAIC AWARD - Fundación CHERNY - Multidisciplinary call.
Friday 17th November 10:30-13:00
Juries: Alejandro De Nicola; Omar Pignataro; Pablo Azurmendi; Cecilia Bouzat

BALANCING IMMUNE FORCES: EXPLORING IFN-γ YIN-YANG IN CORONAVIRUS DEFENSE

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Interferon gamma (IFN-γ) holds promise as a potential adjuvant immunotherapy for individuals with COVID-19. This study explored the gene expression patterns related to the IFN-γ pathway in reaction to Coronavirus infection. Through a case-control investigation involving samples of nasopharyngeal swabs of SARS-CoV-2-positive and -negative patients, we identified enriched IFN-γ-associated pathways among the positive cases. Bioinformatics analyses revealed an upregulation of key genes including *MAP2K6*, *CBL*, *RUNX3*, *STAT1*, and *JAK2* in COVID-19-positive patients compared to non-COVID patients. Notably, a positive correlation emerged between *STAT1* and *JAK2*, varying alongside the patient's viral load. Moreover, the expression of well-established IFN-stimulated genes (ISGs) such as *MX1, MX2, ISG15*, and *OAS1* displayed significant upregulation in COVID-19-positive patients. Integrative analyses further demonstrated elevated ISGs levels associated with higher viral loads and increased *STAT1/JAK2* expression. To validate these findings, we conducted *in vitro* experiments utilizing the A549 lung cell line treated with Poly (I:C), a synthetic double-stranded RNA analog, as well as bioinformatics analyses of transcriptomics data from pulmonary human cell lines and ferret tracheal biopsies infected with SARS-CoV-2. Consistent results were obtained from our pre-clinical murine model of Coronavi-

rus infection where BALB/cJ mice were infected with Murine Hepatitis Virus (MHV-A59, 6000 PFU), showing heightened ISGs expression in the liver and lungs of infected mice. Additionally, we evaluated the expression of Type I IFN; however, we did not observe changes in *Infa* expression with MHV infection in liver, suggesting that ISGs upregulation is triggered by IFN-γ signaling. These results extend the current knowledge about the role of IFN-γ in Coronavirus infection and provide biological basis for new therapies.

FILAMIN A EXPRESSION LEVELS MODULATE PATHOLOGICAL MARKERS OF PITUITARY NEUROENDOCRINE TUMORS

Jonathan Toledo[1], Pablo Aníbal Pérez[2], Graciela Díaz-Torga[1], Jorge Humberto Mukdsi[1], Silvina Gutiérrez[1]

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Aggressive pituitary neuroendocrine tumors (PitNETs) frequently prove resistant to treatment and pose a significant challenge due to the absence of predictive markers for their behavior. Over the last decade, the actin-binding protein Filamin A (FLNA) has been proposed as a pivotal player in tumor development due to its variety of functions. However, the role in PitNETs remains unknown. Thus, we aimed to analyze FLNA expression levels and its impact on aggressive markers of pituitary cells, using an integrative approach of in vivo and in vitro models and human samples. We utilized an *in vivo* model of hyperplastic adenomatous pituitary, an *in vitro* model for overexpression of FLNA in somatolactotropic cells (GH3), and a cohort of 20 PitNET human samples. Our techniques included flow cytometry; western blot; immunofluorescence, immunochemistry, and immunogold labeling; clonogenic and transwell assay. Statistical analysis: Tukey post-test, T-test, and Kruskal-Wallis test. An increase in FLNA expression was observed in the advanced tumoral stages of the *in vivo* model, concomitant with a decrease in cell proliferation and an increment in the nuclear localization. Similarly, overexpression of FLNA in GH3 cells induced a decrease in cell proliferation, colony formation, and the Ki-67 index. This overexpression promoted a fusiform phenotype with enhanced cell migration and decreased the prolactin secretion. Both models exhibited an increase in cyclin D1 and cyclin-dependent kinase 4 expression, correlating with the increase in FLNA levels, implying potential non-canonical functions for these proteins. In human samples a significant increase in FLNA was observed in tumors compared to normal pituitary, with heterogeneous intracellular localization. Interestingly, higher levels of FLNA expression were observed in invasive tumors. These results underline the crucial roles of FLNA as a modulator of pathological markers and as a potential prognostic marker in pituitary tumors.

CARDIOPROTECTIVE EFFECT OF NOVEL INHIBITORS OF G-PROTEIN-COUPLED RECEPTOR KINASE 2 (GRK2)

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GRK2 activity has been validated as a promising target for cardiovascular diseases, the major global causes of death. Via virtual screening and cell-based assays, we identified commercial molecules that inhibit GRK2's RGS homology domain (RH). Based on that, we developed novel analogues, L96B and L94C, that were synthesized and tested as GRK2 inhibitors. This study aimed to preclinically characterize their mechanism of action and potential therapeutic efficacy. L94C and L96B inhibited interaction of GRK2 with G protein as observed in co-immunoprecipitation assay (61.3±0.4% and 49.9±0.6% respect to vehicle control (VC) respectively). They also inhibited adrenergic receptors desensitization in HEK transfected cells in the nM range (EC50L94C= 250nM, EC50L96B= 20nM), and consistently, in primary cultures of cardiomyocytes obtained from neonatal rats, we observed an enhanced response to isoproterenol (42.2±1.4% of vc for L94C, 64.3±1.4% of vc for L96B). Cardiac ischemia-reperfusion experiments in isolated male Wistar rat's hearts showed that both inhibitors at 100nM improved post-ischemic myocardial function recovery where systolic and diastolic parameters reached 40% to 60% of baseline values after reperfusion in comparison with 10-20% recovery in vc ($p<0.01$). Daily administration of L96B or L94C in male spontaneously hypertensive rats (12 weeks old), significantly decreased the systolic blood pressure after a week and persisted for the 6 weeks of treatment (SBPvc= 194±4.4mmHg n= 12, SBPL94C= 158.8±11.3mmHg n= 13, SBPL96B= 148.9±9.3mmHg n= 12, SBPnormotensive= 100±3

mmHg n= 3 all values are at age of 18 weeks old). L94C showed potential in preventing ventricular mass increase and hypertrophy since LVMI and DPW values did not differ significantly from the normotensive group. Also, systolic function indicators MV% and FS improved with L94C. These results postulate L94C and L96B as promising GRK2 inhibitors with plausible application in clinical management of cardiovascular diseases.

TUMOR MICROENVIRONMENT IN BREAST CANCER: ANALYSIS AND CHARACTERIZATION OF ADIPOSE TISSUE

Priscila Ayelén Pagnotta[1,2], Tomás Gonzalez Garello[3], Rubén Dreszman[4], María Luján Crosbie[5], Natalia Santiso[5], Anabela Ursino[5], Celeste Frascarolli[5], Alicia Amato[5], Juan Carlos Calvo[1] and Judith Toneatto[1]

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Adipose tissue (AT) exerts influence on cancer progression. Our goal was to investigate the modulation of tumor-associated adipocytes (TAA), located within breast AT explants from cancer patients. These samples were gathered from two sites: immediate tumor adjacency (AC) and a 2 cm distance (BC), alongside normal controls. Human AT sections were paraffin-embedded and examined using immunofluorescence, quantified by automated intensity assessment. Tissue lysates underwent Western Blot analysis. Variance assessment utilized linear regression models with covariates, signifying $p<0.05$. In AC, perilipin 1, indicating lipid droplet interaction, surged notably in patients with: IIB over IIA/I, grade 1 over grades 2/3, and pre-menopause vs. post-menopause (obese patients). Lipases HSL and ATGL elevated within BC versus AC (lobular cancer), smaller tumors, and pre-menopause over post-menopause. HSL surged with grade 3 vs. grades 1/2, BC over AC (obese patients), and age advancement. Conversely, HSL dropped in obese vs. normal/overweight (invasive ductal cancer), AC region. ATGL surged in normal weight vs. overweight/obese, stage II vs. I, and grade 3 vs. grades 1/2.

UCP1, TBX1, mature adipocyte markers (FABP4, adiponectin, CAV-1) were constant; vimentin, CD44 rose in TAA. Glut4, LDH fell; MCT4, GAPDH hardly varied. MCT1 and browning markers (UCP1, TBX1) spiked solely in a specific subgroup. In sum, breast AT encounters metabolic shifts in cancer context, marked by lipid metabolic protein expression changes, adverse prognosis markers, and sporadic browning. Tumor traits and patient attributes drive these shifts, potentially fueling cancer advancement.

AN ORPHAN LIPID LIGAND ACTIVATES RESOLUTION PATHWAYS IN NEURON-GLIA CROSSTALK

Oriana Nicole Benzi Juncos[1,2], Natalia Paola Alza[1,3], José Cordero[4], Nelson Patricio Barrera[4], Gabriela Alejandra Salvador[1,2]

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Environmental neurotoxicants, such as Maneb (MB) and other dithiocarbamate pesticides, trigger chronic neuroinflammation probably due to defective resolution mechanisms, leading to neurodegeneration. The inflammation/resolution balance is governed by a plethora of specialized pro-resolving lipid mediators (SPM) that act as ligands of the GPCR receptor FPR2/ALX. SPM are mainly synthetized by lipoxygenases from arachidonic acid (AA) and docosahexaenoic acid (DHA). Thus, our aim was to study the resolution pathway modulated by FPR2/ALX in response to MB challenge in a context of neuro-glial communication. By metabolomics we detected significant changes in 11 metabolites in neurons and 27 metabolites in astrocytes as a response to MB treatment ($p<0.05$). In both cell types, phosphatidylcholine was reduced with a simultaneous increase in lysophosphatidylcholine. IPA software's Path Explorer, Connect and MAP functions revealed the upregulation of a secretory phospholipase A2, PLA2G2D. GC-MS fatty acid profile showed increased neuronal DHA content and decreased AA and DHA levels in astrocytes ($p<0.05$). In addition, increased phosphatidylcholine (DHA/16:0) content in neurons exposed to MB was confirmed by metabolomics. To evaluate resolution events under MB injury in neuron-glia crosstalk, cell-derived secretomes and their lipid extracts were used. Astrocyte secretome and its lipid extract were able to revert MB-induced neurotoxicity. This neuroprotective effect was abolished by blocking AA and DHA oxygenation as well as by the FPR2/ALX antagonist Quin-C7. Neurons secreted ERK1/2 -dependent glial proliferation signals, also inhibited by Quin-C7. The role of lipidome obtained from conditioned media in neuro-glia responses to MB injury confirmed the lipid nature of mediators involved in resolution.

Our results show that neurons and astrocytes secrete lipid ligands for FPR2/ALX -mediated resolution in response to MB toxicity.

SAIC AWARD - Consejo de Genética – Human genetics.
Friday 17th November 11:30-13:00
Juries: Mariano Gabri; Javier Cotignola; Liliana Rossetti

HIGH PRECISION CHARACTERIZATION OF RCCX REARRANGEMENTS IN 21- HYDROXYLASE ARGENTINE PATIENTS USING OXFORD NANOPORE LONG READ SEQUENCING

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Long read (LR) third-generation sequencing has taken a more prominent role in recent years, especially for allowing a better study of genome rearrangements, repetitive sequences and regions with high sequence identity. Objective: to leverage LR sequencing using Oxford Nanopore Technology (ONT) to analyze arrangements of the RCCX module (RP-C4-CYP21-TNX) and genetic variants (GVs) in a group of selected 21-hydroxylase deficiency patients previously studied in our laboratory by Sanger, MLPA and/or Southern blot. Two 8.5 Kb fragments from 11 patients and 1 control were sequenced with MinION spanning the module either with CYP21A2-TNXB (exon 32-44), or CYP21A1-TNXARP2 (amplicons A and B, respectively). We developed a set of custom scripts in Python based on the recommended ONT pipelines for the analysis of LR. The reads were aligned to both reference sequences and the human genome (hg38). We also generated de novo sequences. We only obtained amplicon A product in a sample genotyped chimeric/chimeric, as expected. For the remaining samples, read depth ranges between 490-11550 and 7050-35000 for amplicons A and B, respectively. Range of GVs were 17-106 in amplicon A and 3-66 for B. We successfully haplotyped and phased 11/12 samples for amplicon A and 8/11 samples for B. Six samples had bimodular organization and the number of reads with different GVs helped us to validate 1 sample having a duplicated CYP21A2 gene, 3 with duplicated CYP21A1, and 2 samples having monomodular arrangements. We were able to narrow the breakpoints in 5 samples having macroconversions and 3 with small-scale conversions of the 5′ end of the CYP21A1P sequence. We found all of the GVs previously known with Sanger sequencing. In conclusion, we were able to confirm the results obtained by other methods, adding valuable information for the characterization of the RCCX module. The use LR would replace 8 Sanger sequences for each sample and the use of MLPA, reducing the cost and time of analysis.

IDENTIFICATION OF THE GENETIC AETIOLOGY IN A COHORT OF PATIENTS WITH DISORDERS OF SEXUAL DEVELOPMENT (DSD): DIAGNOSTIC YIELD OF NEXT GENERATION SEQUENCING (NGS)

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In patients with DSD, an aetiologic diagnosis may not be reached even after deep phenotyping, i.e., clinical, hormonal and imaging assessment. The application of NGS technology is promising in ascertaining the genetic diagnosis in suspected DSD. Our aim was to determine the diagnostic yield of NGS studies to establish the aetiologic diagnosis in a cohort of patients with DSD. We performed a cross-sectional study including patients with DSD in whom an aetiologic diagnosis had not been reached after phenotypic characterisation. Patients with chromosomal DSD were excluded. Patients and/or parents consented to participate. NGS data from targeted gene panel sequencing (Agilent or Twist) or Whole Exome Sequencing (WES) were processed by in house bioinformatic pipeline. Variant filtering and prioritisation were performed using B_platform (Bitgenia). Copy Number Variants (CNVs) were screened using DECoN. The variants were classified according to their potential pathogenicity using the ACMG/AMP guidelines and following the SVI WG recommendations. A molecular diagnosis was obtained in 7 of the 22 included patients. Five of them, who presented non-syndromic DSD, had (likely) pathogenic variants in *AR, HSD17B3, NR5A1* or *SRD5A2*. A novel *MYRF* variant was identified in 1 patient with syndromic DSD, allowing us to identify a clinically inapparent congenital heart defect by reverse phenotyping. A clinically relevant deletion involving 40 genes (2,6 Mb in 3q27.1-3q27.2) was found in another patient with syndromic DSD. In conclusion, the diagnostic yield of NGS studies was 32% in patients with DSD in whom an aetiologic diagnosis had

not been reached after deep phenotyping. Furthermore, reverse phenotyping allowed the identification of a clinically inapparent congenital heart defect in one case.

COMPREHENSIVE APPROACH FOR THE GENETIC DIAGNOSIS OF PATIENTS WITH WAARDENBURG SYNDROME

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Waardenburg syndrome (WS) is one of the most common syndromic forms of genetic hearing loss (HL), accounting nearly for 2-5% of congenital HL. It is characterized by the presence of hearing impairment in association with pigmentation abnormalities that may affect the skin, hair, and/or eyes. WS is divided into four subtypes according to different concomitant phenotypes and its generally of autosomal dominant inheritance. Up to date, seven genes are related to WS: *PAX*3*, MITF, EDNRB, ENDR, SOX*10*, KITLG and SNAI2.* Disease-causing variants are mainly single nucleotide variants (SNVs), though copy number variants (CNVs) have also been reported. The aim of this work is to identify the genetic causes of WS in four family cases with a dominant mode of inheritance.
As the first step Whole Exome Sequencing (WES) was performed for SNVs screening, filtering out the target genes. When negative, CNVs were analyzed using DECoN tool on WES raw data. Multiplex ligation-dependent probe amplification (MLPA) was carried out to confirm and segregate CNVs identified in the family members.
Three of the 4 families analyzed carried heterozygous pathogenic variants: one SNV and two CNVs in the WS target genes. In family #1 a stop variant (NM_001354604.2:c.1198C>T p.Arg400*) was detected in *MITF* and segregated in one affected son of the family. In family #2 a deletion of 1 exon in *PAX*3 gene was detected and segregated also in the affected mother. In family #3, remarkably, a large novel deletion comprising 7 genes including *SOX*10 was detected in the exome CNVs analysis. The complete loss of *SOX*10 was confirmed and also segregated in the affected family members by MLPA. The combination of techniques and bioinformatic analysis resulted in a better diagnostic rate and in a substantial improvement in the molecular diagnosis of patients. These results highlight the importance of combining different strategies to achieve diagnosis leading to an accurate genetic counseling.

SAIC AWARD - Fundación BIGAND - Multidisciplinary call for young investigators.
Friday 17th November 16:00-18:30
Juries: Rodolfo Rey, Marta Tesone, Edith Kordon

PROTEIN KINASE D1 ACTIVITY IS ASSOCIATED TO MOUSE SPERM CAPACITATION

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Sperm are specialized and transcriptionally inactive cells dependent on post-translational modifications, such as protein phosphorylation, to carry out their functions. These changes occur during the process called sperm capacitation. The protein kinase D family (PKDs) includes three serine/threonine kinases (PKD1, PKD2 and PKD3) being PKD1 the most studied and ubiquitous one. This family of kinases are involved in fundamental biological process including signal transduction, cytoskeleton remodeling, golgi transport and oxidative stress among other functions. Recent results from our laboratory indicate that PKD1 is present in the sperm of human, mice, equine and bovine. Since there are no previous reports about the presence and function(s) of any PKD in mammal sperm, we examined its distribution and function(s).
We observed that: 1) PKD1 is present and catalytically active (p-PKD) in bovine, equine, mice, and human sperm (IIF/WB). 2) The capacitation process induces changes in kinase localization and activation levels, evaluated by super resolution microscopy in mouse sperm. 3) Mouse sperm incubated in the presence of specific PKD inhibitors (Kb142-70 and CRT0066101) before spermatozoa ca-

pacitation enhanced tyrosine-phosphorylation and phospho-PKA substrates proteins levels ($p<0.01$; n=3). 4) PKD inhibitors also promoted an increase in motile ($p<0.01$), progressive ($p<0.05$) and hyperactivated ($p<0.01$; n=6) mouse sperm population (SCA, Microptic system). 5) The inhibition of PKD1 before mouse sperm capacitation rises the percentage of reacted spermatozoa ($p<0.05$; n=7), induced with progesterone. 6) PKD1 inhibition prior capacitation improves IVF rates in mice ($p<0,05$; n=3). Therefore, these results indicate -for first time- that PKD1 is present in mammalian sperm and that its activation is associated to the regulation of the sperm capacitation.

CONTROL MECHANISMS OF SECURIN ABUNDANCE DURING CELL CYCLE

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The vertebrate securin *Pituitary tumor transforming gene* (PTTG) is a sister chromatid separation inhibitor and a transcription factor modulating the expression of genes involved in cell cycle progression. PTTG is timely ubiquitinated on the onset of anaphase and degraded allowing cell division. PTTG is also phosphorylated by a variety of kinases. Increased expression of PTTG in tumors has been associated with enhanced cell growth, aneuploidy and malignancy. We reported PTTG protein increase and stabilization by RWD-containing SUMOylation enhancer (RSUME or RWDD3) in pituitary tumor cells, which impacts on its securin and transcriptional activities, enhancing its oncogenic potential. This work aims to identify the molecular mechanisms of control of PTTG protein stability and its dependency of cell cycle. Analyzing stability of PTTG along the cell cycle by flow cytometry and western blot in COS-7 cells, we found that RSUME increases PTTG protein stability only in G1 and M phases, but the regulation does not occur when PTTG levels are reduced in S and G2. Focusing on post-translational modifications of PTTG protein, we observed by nickel affinity chromatography in COS-7 that RSUME promotes a decrease on ubiquitin conjugation to PTTG, stepping up its protein abundance. In AtT-20 pituitary cells incubated with a PKC kinase inhibitor (6uM Bisindolylmaleimide I), PTTG lost its stabilization by RSUME. However, the action of RSUME was not affected when a MAPK p38 inhibitor (5uM SB203580) was added. PTTG SUMOylation mutants (K25R and K168R) had a shorter half-life but still maintained their regulation by RSUME. By immunoprecipitation and co-localization assays we observed that the interaction of PTTG and RSUME is retained along the cell cycle and does not depend of SUMO site-mutations. Our results show that the mechanism of increasing PTTG stability and abundance involves post-translational modifications and depends on the cell cycle phase.

MRP4/ABCC4 IN PANCREATIC DUCTAL ADENOCARCINOMA (PDAC) ASSOCIATES WITH THE EMT PROGRAM AND CONTRIBUTES TO THE ESTABLISHMENT OF A PRO-TUMORAL MICROENVIRONMENT

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Pancreatic Ductal Adenocarcinoma (PDAC) is a lethal disease with no effective therapies. MRP4 is a xenobiotic transporter highly expressed in PDAC and is linked to increased proliferation, metastasis, and a mesenchymal phenotype, emphasizing its potential as a prognostic marker and therapeutic target. In this study, we investigated the epigenetic and molecular mechanisms regulating MRP4 expression in PDAC, and its participation in the parenchyma-stroma crosstalk that shapes the fibrotic stroma and determines tumor progression and chemoresistance. We applied bioinformatics to analyse PDAC *cis*trome and transcriptome datasets, and developed *in vitro* and *in vivo* models, such as MRP4 up and down-regulation in HPAF-II, BxPC3, and PANC-1 cell lines and xenografts. We found that modulation of MRP4 levels significantly associate with the *Epithelial Mesenchymal Transition* and *Collagen-Containing Extracellular Matrix* signatures. We applied bioinformatics to dissect the human parenchyma and mouse stroma transcriptome in xenografts and found that MRP4 high levels also affect the stromal populations, such as Cancer Associated Fibroblasts (CAFs). BxPC3 MRP4+ xenografts portrayed an upregulation of stromal genes related with collagen biosynthetic processes and adenosine receptor signaling. This matched the higher collagen content and αSMA staining in these tumors. Analysis of PDAC transcriptomic databases evidenced a positive correlation of MRP4 with marker-genes of CAFs subtypes, and fibroblasts exposed to conditioned medium from BxPC3 MRP4+ cells showed increased migration and expression of CAF markers and adenosine receptors, suggesting fibroblast activation. Altogether, our findings highlight the idea that the pro-tumoral roles of MRP4 in PDAC are not restricted to neoplasic cells, but also cooperate with the establishment of a pro-tumoral microenvironment. Hence, pharmacological inhibition of MRP4 could be a promising tool

impacting both the parenchyma and stroma compartments.

CORONAVIRUS BLOODLUST: HEME COMPLICITY DURING VIRAL INFECTION

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SARS-CoV-2 infection causes a multisystemic disease and various hematological abnormalities, yet the mechanism of viral dissemination remains elusive. Here, we present findings from our comparative study using a preclinical model of coronavirus infection and real-world data from COVID-19 patients. BALB/cJ mice were infected with murine hepatitis virus (MHV-A59, 6000 PFU, *i.p.*), an RNA coronavirus similar to SARS-CoV-2. Five days post-infection, mice were euthanized and multi-organ dissection was performed for histological and molecular analyses. Peripheral blood samples were collected to determine biochemical and hematological parameters. We demonstrate the presence of viral RNA and infectious particles in multiple tissues, including the blood, in infected mice. Surprisingly, when comparing plasma and RBCs, higher viral load levels were detected in RBCs ($p<0.001$), with decreased RBC count and hematocrit levels in infected mice ($p<0.05$, and $p<0.01$). Notably, transmission electron microscopy confirms the presence of viral particles within RBCs. Next, to explore the impact of heme on infection, we employed hemin (10 mg/kg, *i.p.*), a pharmacological analogue of the heme group, and chloroquine (CQ, 30 mg/kg, *i.p.*), a compound that interacts with the heme group. We found that hemin administration triggered more aggressive symptoms in infected mice. Strikingly, when combining hemin treatment with CQ, the infection and its clinical manifestations were distinctly attenuated. Computational docking further substantiates the potential of the heme group to facilitate infection via binding to the MHV Spike protein. Collectively, our findings elucidate the multi-organ involvement of coronavirus infection, with specific interactions occurring between viral components and RBC hemoproteins. These insights offer valuable implications for guiding therapeutic COVID-19 interventions.

THYROID HORMONES MODULATE JAK/STAT ONCOGENIC PATHWAYS VIA INTEGRIN AVB3 ACTIVATION: IMPLICATIONS ON BEXAROTENE TREATMENT FOR T CELL LYMPHOMAS

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T-cell lymphomas (TCL) are a heterogeneous group of lymphoproliferative disorders with an aggressive clinical course and dismal prognosis since the available therapeutic regimens have poor results. Activation of JAK/STAT pathways is associated with a bad prognosis in TCL patients. We previously showed that thyroid hormones (THs) are physiological factors that induce TCL proliferation and activate these oncogenic pathways through their membrane receptor, integrin αVβ3. Here we study the mechanisms underlying TH´s effects on JAK/STAT activation and on bexarotene therapy.

Using TCL cells from different subtypes, we found that THs-induced STATs phosphorylation is followed by an increase of MMP2 and MMP9 activity ($p<0.05$). The increment of MMPs activity is inhibited by the pharmacological inhibitor of integrin αVβ3, cilengitide, and by the STAT3 inhibitor, cryptotanshinone ($p<0.05$). In addition, preincubation with ruxolitinib (FDA-approved JAK1/2 inhibitor) diminished THs-induced STATs phosphorylation ($p<0.05$). Since ruxolitinib reported significant toxic effects in TCL patients, we evaluated bexarotene's anti-neoplastic activity on TCL. We found that bexarotene significantly decreases TCL *in vitro* cell viability and THs-induced STATs phosphorylation ($p<0.05$). Interestingly, ruxolitinib decreases TCL cell viability but to a lesser extent when compared with bexarotene and cilengitide. Moreover, we found using a murine syngeneic model that the combination of bexarotene and cilengitide significantly decreases the *in vivo* growth of TCL tumors ($p<0.01$) and the generation of kidney and liver experimental metastasis when compared to the vehicle group of treatment ($p<0.05$). We also found that this combination diminished STATs phosphorylation and metalloprotease activity in tumor cells ($p<0.05$). Our results provide a mechanistic rationale for evaluating bexarotene and cilengitide as therapeutic options for TCL treatment with no cytotoxic side effects.

SAIC AWARD - Fundación Gador - Metabolic syndrome and related disorders.
Friday 17th November 16:00-18:00
Juries: Cristina Arranz; María del Rosario Ferreira Cordoneda; Ana Genaro

DAM'S FRUCTOSE INDUCED-METABOLIC SYNDROME PROMOTES LONG-TERM NEUROLOGICAL ALTERATIONS IN OFFSPRING

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Objective: The aim of this work was to determine if maternal metabolic syndrome (MetS), induced by a high fructose supplementation, induces long-term neurological and metabolic alterations in offspring. Material & Methods: 2 months old female Wistar rats were fed a standard diet and drunk either tap water alone or supplemented with 20% fructose, for 10 weeks, to induce MetS. Then they were mated with healthy males to generate litters (OC: offspring from control dams n=6; OF: offspring from fructose dams, n=6). So as to analyze only the prenatal effects of maternal MetS, all the pups were breastfed by control nurse dams, that had access to a standard diet and water *ad libitum* until weaning. Cognitive and social performance were evaluated between postnatal day (PN) 22 and 90. Animals were sacrificed on PN100 and metabolic parameters were analyzed. Normality of the data was analyzed by Shapiro-Wilk´s test, homoscedasticity by Bartlett´s test and then parametric (*t-test*) or non-parametric (Mann-Whitney) tests were performed on the data. Results: The elevated plus maze, the open field and the marble burying tests revealed an increased anxiety-like phenotype in females OF. On the contrary, the novel object recognition test showed that only the males from the OF group had long-term memory impairment. In the reciprocal social interaction test, both male and female OF presented lower number of social interactions, while only females showed significant increments in "socially inactive" behavior. Furthermore, in the Three Chamber Test, only females OF had lower social preference and social novelty indexes. In regards to metabolic parameters, females OF had increased levels of serum triglycerides and higher visceral fat percentage. Conclusions: maternal MetS has long-term adverse effects on the neurological and metabolic status of offspring rats with sexual dimorphism.

HEPATIC ALTERATIONS IN EXPERIMENTAL METABOLIC SYNDROME: METFORMIN AND NARINGIN REVERSION

María Agustina Rizzi[1], Tamara Mazo[2], Nori Tolosa de Talamoni[1] y Valeria Rodríguez[1]
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Fructose-rich diets (FRD) are responsible for an increase in obesity and metabolic syndrome (MS) cases, many of which may develop into non-alcoholic fatty liver disease (NAFLD). Metformin (Met) is used for the treatment of insulin resistance associated with MS but some clinical studies showed little effect of Met on the histological characteristics of the liver. Naringin (NAR) is a flavonoid with antioxidant, antiapoptotic, and anti-inflammatory properties. Our purpose was to evaluate the effect of co-administration of Met+NAR on systemic and metabolic alterations leading to NAFLD in animals with MS. Male Wistar rats were divided in 5 groups: 1) controls (C); 2) FRD 10% (w/v) in drinking water, 60 days; 3) FRD+Met (100 mg/kg b.w.); 4) FRD+NAR (40 mg/kg b.w.); 5) FRD+Met+NAR. Treatments started on day 21 of FRD administration. Biometric, serum biochemical and liver structure parameters were measured. Fatty acid (FA) profile and gene expression of acetyl COA carboxylase (ACAC) and stearoyl-CoA desaturase 1 (SCD1) were determined in the liver. ANOVA/Bonferroni ($p<0.05$) was used for statistical analysis. Body weight and waist circumference were significantly higher in FRD rats compared to C rats. All treatments decreased waist circumference. Adiposity, hepatosomatic index, and epididymal fat increased in FRD animals, effects that were reversed with Met+NAR. FRD animals had higher levels of TG/HDL ratio, AST, and LDH enzyme activities; NAR and combined treatment reduced these parameters. Also, liver fibrosis was attenuated by Met+NAR. Palmitic acid, monounsaturated FA and ω6/ω3 ratio were higher in FRD rats compared to C rats, while Met+NAR improved these parameters. ACAC and SCD1 gene expression increased in FRD rats compared to C group and decreased with the treatments. In conclusion, Met+NAR could be used as a new therapeutic alternative for the treatment of NAFLD, as it reverses biochemical and histological alterations and liver fibrosis, altered in this pathology.

HORMESIS MEDIATED BY IL-1b PROTECTS PANCREATIC b-CELLS FROM DYSFUNCTION AND DEATH INDUCED BY INFLAMMATORY CYTOKINES

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Both type 1 and type 2 diabetes share pancreatic islet inflammation. Hormesis is a phenomenon by which a harmful substance administered to an organism in small doses provides resistance to subsequent contacts with higher doses. We aimed to assess if physiological concentrations of IL-1β induce hormesis leading to adaptive mechanisms, safeguarding β-cells against the characteristic inflammatory environment of diabetes. We used INS-1E rat cells and mouse pancreatic islets and measured NO by Griess, viability (MTT), death (Hoechst/PI, microscopic fluorescence; Annexin V-PE/7-AAD, flow cytometry), mRNA by RT-qPCR, NF-κB (immunofluorescence) and insulin (ELISA). GSIS (glucose-stimulated insulin secretion) index was calculated as the ratio of insulin released during 1h under stimuli of 20mM/2mM glucose. Hormesis was induced by incubation with 10 pg/ml IL-1β for 72h (IL-1β^{low}). Cytokine-induced damage was triggered by 100 pg/ml IL-1β + 5 ng/ml IFNγ for 16h (CYT). Hormesis induced by IL-1β^{low} protects INS-1E from the decrease in mitochondrial reduction potential triggered by CYT, diminishes cell death ($p<0.05$ vs CYT) and improves GSIS ($p<0.05$ vs CYT), the latter in pancreatic islets as well ($p<0.05$ vs CYT). IL-1β^{low} reduces NO production ($p<0.001$ vs CYT) through a decrease in *iNOS* mRNA ($p<0.01$ vs CYT) and its protein expression ($p<0.01$ vs CYT). IL-1β^{low} reduces CYT-triggered NF-κB nuclear translocation ($p<0.05$ vs CYT). IL-1β^{low} hampers the increase in CHOP expression ($p<0.001$ vs CYT), decreases the mRNA expression of *DP5* ($p<0.05$ vs CYT), *PUMA* ($p<0.05$ vs CYT), *Bax/Bcl-2* ratio ($p<0.01$ vs CYT) and caspase-3 ($p<0.001$ vs CYT; by WB). We demonstrate, for the first time, that β-cells are capable of exhibiting IL-1β-mediated hormesis. Interventions aimed at enhancing the hormetic response present a novel therapeutic avenue to strengthen β-cell functionality and viability, thereby mitigating the detrimental inflammatory conditions linked to metabolic disorders such as diabetes.

SAIC AWARD - Eugenia Sacerdote de Lustig – mAbxience - Neurosciences.
Friday 17th November 16:00-18:30
Juries: Diego Gelman; Laura Morelli; Carina Ferrari

LIPID DROPLETS AS YING-YANG MARKERS OF NEURODEGENERATION IN MODELS OF SYNUCLEINOPATHIES

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Alpha-synuclein (aSyn) pathology is a hallmark in the onset and progression of several synucleinopathies, including Parkinson's disease. It has been demonstrated that lipid disturbances are associated with aSyn pathology. Our aim was to study neutral lipid metabolism in several *in vivo* and *in vitro* models of aSyn accumulation. To this end, different forms of aSyn overexpressed in neurons and toxicant-induced animal models related with synucleinopathies were used. In neuronal cultures, we demonstrated that the overexpression of aSyn (WT and mutant A53T) induced the accumulation of lipid droplets (LD) and free cholesterol ($p<0.01$). We found that LD biogenesis is a "hormesis mechanism" for preventing neuronal death induced by proteostasis impairment. Neuron-glia crosstalk was evaluated using neuronal secretomes, demonstrating that WT and A53T neurons exacerbated LD accumulation in glial cells. Moreover, the pesticide maneb triggered ferroptosis associated with aSyn overexpression in neurons with a rise in neutral lipid content ($p<0.05$). These results suggest that altered lipidostasis could be a hallmark of early aSyn-induced neurodegeneration. Mice exposed to neurotoxicants, as maneb and iron overload, showed aSyn upregulation ($p<0.05$) in whole brain and midbrain associated with motor impairment. In addition, the loss of tyrosine hydroxylase neurons in midbrain ($p<0.001$) was related to ferroptosis markers. Midbrain lipid profiles revealed that injured mice presented lipolysis as a consequence of diminished neutral lipid acylation ($p<0.01$) and lipogenesis, and higher cholesteryl ester deacylation rendering cholesterol accumulation ($p<0.05$). Neuronal death and movement disorders are linked with active lipolysis in *in vivo* models. Thus, indicating that marked injury in synucleinopathies is accompanied by impaired LD formation with cholesterol accumulation. Taken together, our results postulate LD as ying/yang markers of different stages of aSyn-induced neurodegeneration.

MITOCHONDRIAL QUALITY CONTROL IN NON-EXUDATIVE AGE-RELATED MACULAR DEGENERATION: FROM MOLECULAR MECHANISMS TO STRUCTURAL AND FUNCTIONAL RECOVERY

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Non-exudative age-related macular degeneration (NE-AMD) is the leading blindness cause in the elderly. Clinical and experimental evidence supports that early alterations in macular retinal pigment epithelium (RPE) mitochondria play a key role in NE-AMD-induced damage. Mitochondrial dynamics (biogenesis, fusion, fission, and mitophagy) determines mitochondrial quality, which is, in turn, under a central control of AMP-activated kinase (AMPK). We have developed a NE-AMD model in C57BL/6J mice induced by unilateral superior cervical ganglionectomy (SCGx), which reproduces the disease hallmarks only at the macula-like (temporal) region of the RPE/outer retina. Our aim was studying RPE mitochondria structure, dynamics, function, and AMPK involvement on these parameters at an early stage of experimental NE-AMD. Histological, ultrastructural, and biochemical parameters of the nasal and temporal RPE were studied at 4 and 10 weeks post-SCGx. RPE mitochondria mass was preserved, but their function (by TMRE fluorescence) at the temporal RPE, which was higher than at the nasal RPE in sham eyes, was significantly decreased at 4 weeks post-SCGx (**P<0.01 vs. sham, by Tukey). At structural level, mitochondria were bigger, more elongated and with denser cristae at the temporal RPE from sham eyes. SCGx drastically affected mitochondrial morphology, together with the levels of phosphorylated AMPK (pAMPK), only at the temporal RPE (**P<0.01 vs. sham, by Tukey). Moreover, SCGx induced a decrease in mitochondria dynamics at the temporal RPE (**P<0.01 vs. sham, by Tukey). A group of animals was treated with 100 mg/kg metformin (an AMPK activator) for 10 weeks. Metformin restored the levels of pAMPK, as well as mitochondrial dynamics, structure, and functionality at 4 weeks post-SCGx, and visual function and RPE/outer retina structure at 10 weeks post-SCGx. These results demonstrate AMPK activation role in RPE mitochondria homeostasis and the protective effect of metformin in NE-AMD.

UNRAVELING THE INTERPLAY BETWEEN EPILEPSY AND EPIGENETICS: MODULATION OF ASTROCYTIC HOMEOSTATIC CAPACITY THROUGH EPIGENETIC CHANGES

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Epilepsy, a chronic neurological disorder with recurring seizures, is treated with anti-epileptic drugs that are ineffective in 30% of patients. Temporal Lobe Epilepsy (TLE) patients refer an initial early seizure event (IPE) followed by a silent period where epileptogenesis occurs. We here studied the epigenetic events that underlie the astroglial alterations observed in the silent period to analyze the pathophysiological mechanisms that may support epileptogenesis to identify potential intervention targets. Intially, we examined DNA methylation in epilepsy patient brain samples, detecting increased astroglial DNA methylation. Using an animal model of lithium-pilocarpine-induced TLE, significative astroglial DNA hypermethylation and decreased levels of key astroglial proteins, Kir4.1 and AQP4 were observed at various latency stages (7, 21, 35 days post-IPE). Primary astroglial cultures exposed to HMGB1, a molecule released from stressed neurons after seizures, resulted in astroglial DNA hypermethylation. This was linked to higher expression of proinflammatory cytokines IL-1B and IL-6. Additionally, there was an increase in the expression of DNA methyltransferases (Dnmt1, Dnmt3a) and MAFG. These lasting changes, seen for up to 7 days in culture, coincided with downregulation of key astrocytic homeostatic genes including LDHA, GS, Kcnj10, Slc16a1, and Aqp4. Methylation-sensitive PCR showed increased promoter methylation of their respective promoters. Normal gene expression was restored in astroglial cultures treated with 100 uM decitabine DNMT1 inhibitor. Gene expression was measured through qRT-PCR and immunohistochemistry followed by image analysis (FIJI). Statistical significance of the findings was done by one- or two-way ANOVA as necessary. We conclude that astrocytes suffer epigenetic changes during the latency period that impair their ability to support brain homeostasis. DNMT1i presents a promising avenue for mitigating these detrimental effects.

GALECTIN 1 RESCUES MICROVASCULAR ALTERATIONS IN ALZHEIMER'S DISEASE IN *IN VIVO* AND *IN VITRO* MODELS INVOLVING ENDOPLASMIC RETICULUM STRESS PATHWAYS

Jessica Presa[1], Carlos Pomilio[1], Soledad Gori[3], Eugenia Matzkin[2], Agustina Alaimo[3], Mariano Soiza-Relly[4], Oscar Perez[3], Rossana Ramhorst[3], Juan Beauquis[1], Gabriel Rabinovich[1], Flavia Saravia[1]

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As a multifactorial pathology, Alzheimer's disease (AD) exhibits marked vascular alterations that correlate with disease progression. The immense impact of AD requires new and effective therapeutics. Galectins, a family of proteins that bind to outer membrane oligosaccharides, trigger various cell responses involving immune and vascular modulation. Galectin-1 (Gal1) may have beneficial effects on neurological disorders. We treated PDAPPJ20 (Tg) mice -an AD model- with Gal1(3 weekly i.p. injections of 100 ug/dose, 9 injections). Tg-Gal1 mice showed a 50% reduction in perivascular Aβ compared to vehicle-treated littermates (Tg-Veh) ($p<0.05$) without altering vessel density and morphology. Tg-Gal1 mice had lower brain permeability to Evans blue dye than Tg-Veh mice ($p<0.05$) in addition to a reduced number of microbleeds. While Tg-Veh showed a decrease in ensheathment of the vasculature in contrast to NTg-Veh mice ($p<0.001$), Tg-Gal1 presented a recovery of this astrocyte feet-vessel contact ($p<0.05$ vs Tg-Veh). The significant recovery of astrocyte-feet Aquaporin-4 adjacent to vessels in Tg-Gal1 also supports the rescue of functional communication. Human brain endothelial cells (HBMEC) exposed to subtoxic concentrations Aβ1-40 exhibited nuclear translocation of NFκB, which was prevented by Gal1. In a BBB model on a transwell membrane, monitored by transendothelial electrical resistance, we found a decreased barrier resistance with Aβ1-40, which was counteracted by Gal1($p<0.05$). We linked Aβ activation of HBMEC to an altered unfolded protein response characterized by increased BIP protein expression, elevated IRE1α and PERK mRNA levels, and XBP1 protein activation leading to increased levels of the inflammasome marker NLRP3, parameters normalized by Gal1 ($p<0.05/0.01$). Gal1 emerges as a potential breakthrough treatment for the vascular consequences of AD. Our results suggest that Gal1 could alleviate Aβ-associated ER stress in brain endothelial cells.

GENE-IMMUNO-THERAPY TARGETING Aβ OLIGOMERS WITH A SELECTIVE SINGLE-CHAIN ANTIBODY FRAGMENT IN A RAT MODEL OF ALZHEIMER'S DISEASE

Natalia Claudia Colettis[1]; María Victoria Oberholzer[1], Martin Habif[1], Mauro Exequiel Alfaro[1], María Belén González[1]; Luis Ignacio Brusco[2]; Sergio Ferreira3; Augusto Claudio Cuello[4], Ricardo Francisco Allegri[5], Diana Alicia Jerusalinsky[1]

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β-Amyloid oligomers (AβOs) were reported to early contribute to synaptic impairment and memory deficits in animal models of Alzheimer's Disease (AD). A single-chain antibody fragment NUsc1 selectively targeting a subpopulation of AβOs rescued short-term memory (STM) in mice AD models. To neutralize AβOs and enhance therapeutic efficacy, we developed an Adeno-Associated virus-derived vector for expressing NUsc1 (AAV-NUsc1) in neurons. The McGill-R-Thy1-hAPP heterozygous transgenic (Tg+/–) rat bears a copy of *hAPP* with two mutations (Swedish e Indiana) from familial AD. This Tg rat displays progressive amyloid pathology, impairing the formation/recall of novel object recognition (NOR) long-term memory (LTM). We evaluated whether an early and late AAV-NUsc1 treatments could rescue NOR LTM in (Tg+/–) rats. Both Tg and wild-type (wt) male rats 10-12 weeks old and 15-month-old, were i.c.v. infused with AAV-NUsc1 or saline (control). After two months, exploration of an open field (OF), new object discrimination and recognition (NOR) performance, as well as STM and LTM were assessed. AAV-NUsc1-treated and control, wt and Tg+/– 5-month-old and 17-month-old rats showed similar exploratory behavior and habituation to the OF. At both ages, Tg+/– rats failed to express LTM for NOR ($P>0.05$, paired T-test, preference index comparison between training and test session). However, prior AAV-NUsc1 treatment restored this capacity in 5-month-old ($P<0.05$, paired T-test), though not in 17-month-old Tg rats ($P>0.05$, paired T-test). Current AD treatments underutilize genetic tools. In contrast with the recently FDA-approved ADUCANUMAB and LECANEMAB, expensive antibodies targeting higher-order Aβ aggregates, AAV-NUsc1 represents a viable leap in vector-mediated genetic-immunotherapy, leading to a sustained NUsc1 neuronal expression to neutralize neurotoxic AβOs in early AD.

SAIC AWARD - Irene Faryna y Roberto Raveglia - Oncology.
Friday 17th November 16:00-18:30
Juries: Daniel Alonso; Alejandro Curino; Virginia Novaro

SPARC AS A PROMISING BIOMARKER OF EARLY BREAST CANCER PROGRESSION

Marianela Sciacca[1,4], María del Pilar Carballo[2], Ezequiel Lacunza[3], Martin Abba[3], Lina Marino[2], Érica Rojas Bilbao[2], Ana María Eiján[1,4] and Catalina Lodillinsky[1,4].

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Ductal carcinoma in situ (DCIS) is a non-invasive type of breast cancer (BC) considered a non-obligate precursor to invasive tumor (IDC). There is no way to identify which DCIS lesions will progress to IDC. Thus, we search for promising biomarkers that can predict early progression and improve the treatment. MT1-MMP is required for DCIS to IDC transition in BC. Based on the differential MT1-MMP tumor expression after MCF10DCIS.com cells intraductal injection in mice, two cell populations were isolated. Transcriptome analyses was performed and compared against a set of human high-grade DCIS. We found SPARC, PTGS2, and CLCA2 up-regulated in both, worse prognosis-human DCIS and MT1-MMP^{high} population, highlighting these proteins as promising targets in early BC progression. SPARC expression correlates best with MT1-MMP in BC samples (from TCGA, p< 2.2e-16). SPARC protein levels in BC were assayed by IHC 3 different cohorts of patients (n=190). SPARC expression was higher in DCIS vs to normal tissue, particularly in nuclear grade III-DCIS (X^2 p=0.024), in IDC vs DCIS (X^2 p=0.035) and in grade III vs lower-grade IDC (X^2 p=0.016). Furthermore, SPARC expression was higher in triple negative BC (X^2 <0,0001). Lymph node metastases do not express SPARC, suggesting that SPARC is involved only in early progression of BC. In KO SPARC LM38-LP cell line, both, MT1-MMP expression and degradative capacity are reduced (p<0.05), showing a SPARC pro-invasive role, MT1-MMP-dependent. An interaction between SPARC and TGF-β1 was observed (STRING platform). The LM38-LP viability increased 40% by TGF-β1 treatment (p<0.05). The TGF-β RI inhibitor SB431542 (p<0.001) reduced cell viability (p<0.001) and SPARC mRNA levels. Also, exogenous SPARC increased p-SMAD 2/3 while in KO SPARC cells p-SMAD 2/3 was reduced. Finally, SPARC is involved in early tumor progression through a TGF-β1-dependent feedback mechanism, proposing TGFRI as a possible molecular target for SPARC positive tumors.

EXPRESSION OF GRANULOCYTE-MACROPHAGE COLONY-STIMULATING FACTOR BY MESENCHYMAL STROMAL CELLS BY *IN VITRO* TRANSCRIBED mRNA. A THERAPEUTIC STRATEGY FOR HEPATOCELLULAR CARCINOMA

María José Cantero[1], Barbara Bueloni[1], Esteban Fiore[1], Lucia Lameroli[1] Catalina Atorrasagasti[1], Guillermo Mazzolini[1], Mariana Malvicini[2], Juan Bayo[1], Mariana García[1]

1 Laboratorio de Terapia Génica, Instituto de Investigaciones en Medicina Traslacional (IIMT), Facultad de Ciencias Biomédicas, CONICET-Universidad Austral, Buenos Aires, Argentina. 2 Laboratorio de Inmunobiología del Cáncer, IIMT, Facultad de Ciencias Biomédicas, CONICET-Universidad Austral, Buenos Aires, Argentina.

Mesenchymal stromal cells (MSCs) are considered potential vehicles of therapeutic factors. Transfection with in vitro transcribed (IVT) mRNA is a promising tool for gene therapy. Our goal was to generate engineered MSCs by IVT mRNA transfection overexpressing granulocyte-macrophage colony-stimulating factor (MSC-GM) and determine their therapeutic effect alone or in combination with doxorubicin (dox) on a murine model of hepatocellular carcinoma (HCC). Methods: Ds-Red or GM-CSF IVT mRNAs were transfected using lipofectamine. Gene expression and cell surface markers were determined by qPCR and flow cytometry respectively. GM-CSF secretion was determined by ELISA. HCC model was developed by subcutaneous inoculation (SC) of Hepa129 in C3H mice. Tumor size and mice survival were evaluated after SC injection of MSC-GM, dox or their combination. Tumor samples were collected for mRNA analysis and flow cytometry. Results: DS-Red expression was observed from 2h to 15 days after IVT transfection and did not affect tumor growth in vivo. MSC-GM maintains the surface markers unmodified, secret GM-CSF, induces differentiation of dendritic cells and a pro-inflammatory phenotype of macrophages (J774 cells, p>0.05 t test), and reduces HCC tumor growth in vivo (p>0.05 two-way ANOVA). Even more, combination of the MSC-GM and dox treatments strongly reduces HCC tumor growth in C3H mice (p>0.05 two-way ANOVA) and extended mice survival in comparison with individual treatments. Notably, dox treatment results in Hepa129 cells immunogenic cell death which in turns increases the macrophages pro-inflammatory phenotype induced by MSC-GM in vitro (p>0.05 ANOVA). Besides, tumors of the MSC-GM+dox treated group present higher levels of TNF-α, IL1-β and IFN-γ gene expression and have an increased CD8+ T

cells and macrophages infiltration. Conclusions: Our results demonstrate that transfection with IVT mRNA is a suitable strategy to obtain engineered MSCs with therapeutic purposes for HCC.

CELL MIGRATION INDUCED BY HEREGULIN INVOLVES TGFB SIGNALING ACTIVATION IN LUMINAL BREAST CANCER CELLS

Angela Lara Montero[1], Mercedes Montani[1], Agustin Gonzalez[2], Julieta Aisemberg[2], Eva Wertheimer[2], Andrea De Laurentiis[2], Omar Coso[1], Edith Kordon[1]

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Previous results indicated that in luminal breast cancer (BC) cells, Heregulin (HRG) increased cell migration by ErbB3/P-Rex/Rac1 signaling activation and that this pathway also induced TGFβ2 expression. Therefore, our goal was to determine whether the TGFβ signaling pathway was involved in Hrg-induced BC cell migration. To that end, we performed experiments using specific pharmacological inhibitors and transient gene silencing for blocking mediators of the involved pathways. Analysis of mRNA (by RT-qPCR) and protein (Western Blot and immunofluorescence) levels as well as wound-healing assays in luminal BC cells, T47D and MCF-7, were performed. Our results show that Hrg induces not only TGFβ2, but also TGFβ1, TGFBR1, TGFBR2, SMAD3, and SMAD4 expression, all members of the canonical TGFβ signaling pathway. Supporting these data, we found positive correlations between HRG and TGFβ1, TGFβ2, TGFβ3, and TGFBR3 protein content in proteomic data sets from breast cancer samples. In addition, HRG treatment induced phosphorylation and nuclear translocation of SMAD-3, but this activation was blocked by adding SB525334 a TGFBR1 inhibitor. Furthermore, this treatment also inhibited HRG-induced cell migration. On the other hand, upon HRG treatment, Smad3 phosphorylation was not affected by P-REX1 silencing and Rac1 activation increases when TGFBR1 is inhibited. Taken together, our results indicate that HRG induces cell migration of luminal breast cancer cells not only by activating the ErbB3/P-Rex/Rac1 signaling, but also by inducing TGFβ ligands and SMAD-dependent pathways. However, the two signaling cascades seem not to synergize, but to compete with each other. In summary, we propose that our findings may contribute to the quest for new combination therapies that may help to develop more successful treatments for luminal breast cancer.

P-3RS
FRIDAY 17TH NOVEMBER 9:00 - 10:30
CHAIRS: PINTO, GABRIEL BERNARDO
SALVETTI, NATALIA
GINEVRO, PAULA

1. 156. **COMPARATIVE EFFECTS OF KETAMINE/XYLAZINE AND KETAMINE/MEDETOMIDINE ANESTHESIA FOR OVARIECTOMY IN WISTAR RATS**

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Refinement involves modifications of husbandry or experimental procedures that minimize or eliminate animals' pain and distress and improve their welfare. Balanced anesthesia and analgesia promote refinement in surgical procedures. This study aims to compare ketamine 75 mg/kg + xylazine 10 mg/kg (KX) combination with ketamine 60 mg/kg + medetomidine 0.25 mg/kg (KM) in clinical monitoring and time-related anesthesia parameters for ovariectomy in Wistar rats. Protocol was approved by the Faculty of Medicine IACUC (RESCD-2022-3545). Twenty-four three-month-old female Wistar rats were submitted to ovariectomy by dorsal approach. The anesthetic combination was assigned randomly, and treatment was blinded for the anesthesiologists up to data analysis. The complete protocol included pre-surgical meloxicam (1 mg/kg; sc) and yohimbine (2.1 mg/kg; ip) when procedure finished. Thermal support and ophthalmic ointment were provided during surgery and recovery.
After anesthesia administration, the animal was observed up to the loss-of-righting reflex (LRR) and the loss-of-pedal withdrawal reflex (PWR-). The PWR was tested until returned positive. Time points for consciousness and exploratory behavior recovery was also recorded. Intra-surgical rectal temperature, respiratory rate and cardiac frequency were recorded. The time to achieve LRR was not significantly different between anesthetic combinations, but PWR- was significantly faster for KM than KX combination ($p=0.0173$). Time to recover PWR, consciousness and exploratory behavior was significantly longer in rats receiving KM than those who received KX ($p<0.05$). Clinical parameters did not significantly differ between treatments. One rat from each anesthesia protocol did not recover from the procedure. KM combination resulted in a safe anesthesia surgical plane at lower doses than classical KX protocol. Still, considering the prolonged recovery times, other dosage schemes should be explored until it becomes a superior anesthetic option for ovariectomy by dorsal approach in rats.

2. 230. **NUTRITIONAL IMPACT ANALYSIS: COMPARISON OF TWO NATIONAL DIETS IN WISTAR RATS TO REFINE NUTRITION AND OPTIMIZE THE NUMBER OF ANIMALS USED IN PRODUCTION STOCKS.**

Mercedes Olivera[1], Marianela Ceol Retamal[1], Lourdes Lloret[1], Andrea Pecile[1], Solana Pesca Alba[2]
1 Instituto de Biología Celular y Neurociencias Prof. E de Robertis. (UBA-CONICET)
2 FeedVax-Oral Vaccines

Objectives: Evaluate the impact of feeding on the variables weight, consumption and excreta, during growth in Wistar rats, with two open formula diets and a mixture of both. To evaluate physical development, reproductive performance, digestibility and preference.
Materials and methods: Male and female Wistar rats (outbred) were used, divided into three groups of 9 animals in each (5 males and 4 females).
Group 1 fed with diet C, 2 with diet G and 3 with the mixture of both diets 50/50. The composition of each diet was identified with the information provided by the supplier. A weight curve, the comparison of the consumption and amount of excreta was made from the 17th day of age, also a macroscopic analysis of feces morphology in animals 60 days onwards. The productive and reproductive characteristics of the females of each group from the 9th week of age were analyzed, and finally the food preference.
Conclusions: It was observed that composition is similar in both formulations, but with differences in the origin of the proteins: C uses cereals as the main protein source, while G uses animal protein. When analyzing the feces, it was observed that those belonging to diet C contained pieces of whole ingredients, indicating that it was not digested completely with a size of 0.5-0.8 mm. In the feces of diet G, a homogeneous consistency and color was observed, measuring 0.9-1.3 mm. There was 15% more excreta with diet C and 11% more with mixed diet over diet G. There was 25% more intake with diet c and 11% more with the mixture over diet G. Reproductive outcomes: Pregnancy - C: 66.6%, mixed: 78%, G: 88%. Productivity (pups/month/female) - C: 8, mixed: 10, G: 11. Litter size at birth - C: 12, mixed: 15, G: 17. Last pup rate - C: 8/9, mixed: 11/13, G: 13/14 months. Mortality rate at birth and weaning: C: 35%, G/mixed: 10%. The weight curves elaborated for each group showed differences among themselves. It was verified that the animals prefer diet G, over diet C and the mixture.

3. 267. **3 RS APPROACHES FOR THE STUDY OF SIGNALING EVENTS TRIGGERED BY PESTICIDE INDUCED NEUROTOXICITY.**

Melisa Conde[1,2], Athina Maniscalchi[1], Leticia Nicasio[1], Oriana Benzi Juncos[1,2], Melania Funk[1], Natalia Alza[13], Gabriela Salvador[1,2].
[1] Instituto de Investigaciones Bioquímicas de Bahía Blanca, Universidad Nacional del Sur (UNS), Consejo Nacional de Investigaciones Científicas y Técnicas. [2] Departamento de Biología, Bioquímica y Farmacia, UNS. [3] Departamento de Química, UNS

Maneb (MB), a dithiocarbamate pesticide, can cross the blood-brain barrier and is considered an environmental risk factor associated with Parkinsonism. Our aim was to characterize redox signaling during MB-induced neurotoxicity in different experimental models according to the 3Rs principle.
Taking into account full Replacement we firstly exposed neuronal cell line cultures to MB and we detected increased expression levels of the redox sensitive transcription factor Nrf2 and its regulator Sirt1. Neuronal MB exposure also triggered the increase of lipid peroxidation, GSH depletion, GpX4 downregulation and mitochondrial alterations, indicating that ferroptosis is involved in the mechanism of cell death. We confirmed these results in a model of relative Replacement by using glial mixed primary cultures. We also demonstrate that Nrf2/Sirt1 signaling is a protective strategy against neurotoxicity. Based on these results and with the aim of evaluating phenotypical aspects for establishing a preclinical model, we design an experimental paradigm with C57BL/6 mice. Following Refinement criteria, treatments were performed with the supervision of a veterinarian who care the animal welfare during the entire procedure. C57BL/6 mice (18-20g) were intraperitoneally injected with MB (100 mg/kg) for 6 weeks. Behavioral tests (Open Field and Rotarod) were performed on week 3 and 6 of treatment. One day after the last administration, animals were sacrificed by cervical dislocation. Forebrain,

midbrain and hindbrain were separated for molecular determinations. To apply the Reduction criteria all other animal organs were used for related investigations. MB-treated animals showed a decrease in total distance travelled, supported and unsupported rears and in falling latency. These results indicate that MB neurotoxicity triggers a parkinsonian phenotype in C57BL/6.
The results presented here constitute a platform for toxicological and preclinical studies according to 3Rs principles.

4. 271. **DEVELOPMENT OF THREE-DIMENSIONAL (3D) ENGINEERED TISSUE MODEL FOR IN VITRO STUDY OF HUMAN ENDOMETRIOSIS (EDT)**

del Valle Sofía[1], Ruiz Ignacio[2], Oppenheimer Florencia[2], Leiros Gustavo[2], Ricci Analía[3], Meresman Gabriela[1].
1.Laboratorio de Fisiopatología Endometrial, IBYME-CONICET; 2.Instituto de Ciencia y Tecnología César Milstein; 3.Instituto de Ciencia y Tecnología Dr. César Milstein (FPC-CONICET)

EDT is a gynecological disease characterized by the presence of endometrial tissue outside the uterine cavity affecting 10-15% of menstruating people. The available in vitro models for studying EDT are insufficient to clarify the complex epithelium-stroma interactions within tissues. Therefore, our aim was to develop and characterize 3D endometrial constructs that could be used as experimental models for studying this disease. We generated 3D constructs using type I collagen (5 mg/ml) as extracellular matrix, and genipin (120 μM), a cross-linking reagent. First, 1x106 t-HESC endometrial stromal cells were embedded in the hydrogel, then 5x105 ECC-1 endometrial epithelial cells were seeded onto the collagen scaffold. They were incubated for 9 days at 37 °C with 5% CO2 in DMEM-F12 medium. We examined how the construct's percentage of contraction changed depending on whether genipin was present or not, as well as how toxic it was using the LIVE/DEAD Viability/Cytotoxicity kit. We also performed histological analysis by using Periodic Acid-Schiff (PAS) and hematoxylin-eosin (H&E) staining and characterized the cellular organization by immunodetection of epithelial and stromal cells with cytokeratin and vimentin, respectively. 3D constructs without genipin formed glandular structures positive for cytokeratin which shared morphological similarities with endometrial tissue. These structures were also positive for PAS staining, suggesting active secretory activity. The stromal phenotype was confirmed with positive immunodetection for vimentin. Genipin did not cause cytotoxicity since cell viability was equivalent to basal (95%). Furthermore, it significantly reduced collagen contraction improving the mechanical handling of the constructs. H&E staining revealed the formation of a partially columnar continuous epithelium over the stroma. This innovative model will allow us to study the complex interactions of different cell types within a relevant biological microenvironment.

5. 471. **GENETIC CONTAMINATION IN A COLONY OF BALB/cJ AND THE REFRESH OF THE COLONY AS A CRITICAL POINT**

María Alfonsina Lizárraga, Diego Manuel Posik, Guillermo Giovambattista.
IGEVET - Institute of Veterinary Genetics "Eng. Fernando N. Dulout" (UNLP - CONICET LA PLATA). Faculty of Veterinary Sciences, National University of La Plata, Argentina

The genetic quality of laboratory animals is essential for the reproducibility of scientific research. There are many genetically defined lines of mice, such as inbred strains and congenic. These lines have important characteristics, such as genetic isogenicity, phenotypic uniformity, which is the axis of the reproducibility of the experiments. The control and preservation of the genetic quality of the laboratory animal should be a priority. The aim of this work was to evaluate the authenticity the of mouse strain BALB/cJ from of colony from an experimental conventional animal care facility of the region and highlight the importance of genetic monitoring and show the consequences of an absence of refresh of the colony periodically. This strain BALB/cJ arrived at this animal care facility ground in the year 2000.Thereafter polygamous mating were made, until 2020.For twenty years a refresh of the colony of animals was not made. It is important to highlight that the periodicity of the colony refresh should be made from F5 to F10 generations, to have an optimal management of the colony. Microsatellite analysis by PCR is one of the most widely used methods in genetic quality controls of inbred lines. Tail samples(3-5mm) were taken from female(N=4), and genomic DNA was obtained by organic extraction. We analyzed 12 microsatellite loci that were located on twelve different autosomal chromosomes and polymorphic. Selected from the information database; www.informatics.jax.org . This panel allows the discrimination of the main strains present in Argentina: BALB/c, C3H/He and DBA/2J, C57BL/6J. The results showed evidence of genetic contamination with the positive control DBA/2J and C3H/HeJ strains; 10 of the 12 microsatellites analyzed were homozygous for the expected alleles and genotypes compatible with the positive control the BALB/cJ strain. On the contrary, the remaining two microsatellites presented the following genotypes: D2Mit493(97pb), compatible alleles for the DBA/2J strain and D12Mit12(164pb), for the C3H/HeJ strain. The results were reported to the experimental conventional animal care facility of the region, and based on this report the entire colony was renewed with new genetically controlled animals.

It is necessary to encourage routine genetic checks, every two years, to ensure that the results obtained are reproducible and have scientific validity and to encourage, technicians and professionals to continue training in the science of laboratory animals.

6. 513. **ASSESSMENT OF DEVELOPMENT AND REPRODUCTIVE TOXICOLOGY (DART): AN *IN VITRO* APPROACH**

Ernesto Gulin[1], María Soledad Lorenzo[1], Alejandro Maruri[1], Pablo Torres[2], Daniel Lombardo[1]
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Reproductive toxicity can be studied by a holistic approach on experimental animals in extensive and expensive trials. However, using an integrated *in vitro* approach, the reproductive cycle can be split into its main biological components integrating results and providing comprehensive information on the potential effects of chemicals on gametes and embryonic development, decreasing the number of animal studies and providing a more detailed toxicological profile.
This work aims to introduce the setting up of an *in vitro* platform for DART testing, applying a battery of assays based on standardized reproductive biotechnologies. To evaluate the toxicity on male gametes, bovine spermatozoa were exposed to increasing concentrations of the test substance, studying viability with vital stains along with motility and velocity parameters assessed by computer-assisted sperm analysis (CASA). The bovine oocyte *in vitro* (bIVM) was applied to assess the effect of substances during oocyte maturation. Cumulus-oocyte complexes (COCs) obtained from ovaries of slaughter cows were selected and incubated in IVM medium with increasing compound concentrations. After 22 h, the viability and nuclear maturation were evaluated. The obtaining results allowed determining the concentration that reduced the IVM of oocytes by 50% compared to the untreated control. In vitro fertilization of bovine oocyte (bFIV) and early embryonic development were used as a model to evaluate the toxic effect of the fertilization process. Bovine gametes were co-incubated with increasing concentrations of the test substance. After 18 h, pronucleus formation was registered to determine the concentration that reduced the bFIV by 50% compared to the untreated control. This initial battery of *in vitro* models is part of developing a biotechnological platform for DART testing, promoting the technological cooperation between academia and industry partners within a 3Rs approach.

7. 514. **POSACONAZOLE AS A TRYPANOCIDAL DRUG: GLOBAL EFFICACY AND THEIR IMPACT IN ANIMAL MODEL OF CHAGAS DISEASE**

Margarita Bisio[1], Laura Jurado Medina[2], Facundo García-Bournissen[3], Ernesto Gulin[4]
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[2] *Dipartimento di Scienze Mediche e Chirurgiche, ALMA MATER STUDIORUM - Università di Bologna, Italia.*
[3] *Division of Paediatric Clinical Pharmacology, Department of Paediatrics, Schulich School of Medicine & Dentistry, University of Western Ontario. London, Ontario, Canadá.*
[4] Instituto de Investigaciones Biomédicas (INBIOMED), Universidad de Buenos Aires (UBA), Facultad de Medicina – CONICET. Buenos Aires, Argentina.

Systematic reviews (SR) and meta-analysis (MA) contribute to the 3Rs in animal research by formally summarizing existing data without requiring new animal experiments. After promising results in *in vitro* and *in vivo* models, posaconazole (POS) was evaluated in human patients with indeterminate phase of Chagas disease. However, POS therapeutic trials were unexpected failures. Previously, we conducted an SR (PROSPERO #205000) to summarize animal models and assess the risk of bias. In this work, we analyzed the overall efficacy of POS in *Trypanosoma cruzi* infection, using survival, cure rate and maximum parasitemia as endpoints. MA was performed by comparing outcomes from POS, infected non-treated (INT) and positive control (i.e., benznidazole (BZ) or nifurtimox (NFX)) animals. Anticipating a high heterogeneity, the analysis was performed as a random effects model. Survival was compared with risk ratios (RR), and cure rates were compared with odds ratios (OR). Subgroup analysis considered the phase of the disease treatment dose and schemes and *T. cruzi* strain. POS treatment, compared to INT, led to significantly higher survival (RR=6.14[3.76, 10.02], P<0.00001) and cure rates (OR=15.70[10.02, 24.60], P<0.00001). Overall analysis showed that POS was unlikely to achieve higher parasitological cures when compared with BZ or NFX treatment (OR=0.73[0.37, 1.42], P=0.35). Subgroup analysis revealed no significant effect favouring POS cure rates during the chronic phase (OR=1.74[0.40, 7.65], P=0.46). POS 20 mg/kg od or bid did not have a statistically significant benefit over BZ or NFX (OR=0.47[0.08, 2.83] and 0.43[0.11, 1.71], respectively). There was no significant effect favouring POS cure rates with Y strain infection (OR=1.20[0.50, 2.86], P=0.68). Maximum parasitemia could not be included due to insufficient data reported. This SR/MA can contribute to understanding the reasons behind POS failure in human patients and promote a more stringent revision of preclinical studies before moving to clinical trials.

8. 540. **EUGENOL, MENTHOL AND BENZOCAINE AS ANESTHETICS AGENTS FOR *CHEIRODON INTERRUPTUS* (OSTARIOPHYSI: CHARACIDAE)**

Vercellini, María Clara[1]; Rearte, Ramiro[2]; García, Ignacio[3]; Ayala, Miguel Ángel[4]; Montes, Martin Miguel[1]
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Anesthetics are commonly used to facilitate capture, handling, transport in aquaculture practices and research. There are many anesthetic agents used in fish. Benzocaine is an anesthetic frequently used in aquaculture. Eugenol and menthol are anesthetics essential oils which are increasing attention in sustainable aquaculture practices. *Cheirodon interruptus* (Jenyns, 1842) is a small native freshwater baitfish widely used in Argentina. The simplicity to obtain and their tolerance to handling makes them a suitable model for bioassays. However, there is limited literature on the anesthetic effects in *C. interruptus*. The aim of the present study was to evaluate the response of benzocaine, eugenol and menthol anesthetics effects in *C. interruptus*. A total of 165 adults of *C. interruptus* (standard length 40.6 ± 2.9 mm; body weight 1.31 ± 0.33 g, and age 1+) were collected from a natural stream of the Salado River basin, Argentina. The tests are carried out with water at a temperature of 21±1 °C, pH = 7.54, dissolved oxygen 7.93 mg/L, conductivity 602 μS.cm^{-1}. The median time to induction and recovery from anesthesia (stage VI) depending on anesthetic agents were assessed with Kaplan-Meier survival analysis. Results indicated that eugenol 20 mgL^{-1} , menthol 125 mg^{L-1} and benzocaine 80 mgL^{-1} meet the requirements for a good anesthetic for use in fish (induction time <3 min with a recovery of 5 min or less). Eugenol induction time and recovery time were 785 sec (628-1050) and 548 (340-806) sec. Benzocaine were 62 sec (33-75) and 76,5 (21-360), and also menthol were 184,5 sec (130-278) and 354 sec (247-464) respectively. Our results indicate that the anesthetics administered to adults of *C. interruptus* in this study resulted in an effective concentration suitable for laboratory settings.

9. 651. **APPLICATION OF REFINEMENT IN UNIVERSITY TEACHING PRACTICE AS AN ALTERNATIVE TO THE USE OF ANIMALS**

María Carla Greco[1], Verónica Casanova[1], Marianela Ceol Retamal[1], Sofía Gimenez[1] Eduardo Caturini[1] , Eliana Cicale[1].
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Objective: To generate dexterity and manual ability in the student to perform the cervical dislocation technique in rats and mice, replacing and reducing the number of live animals used for the practice. Materials and Methods: A refinement technique, implemented in the Veterinary Sciences Faculty's Animal Husbandry School by the teaching team in charge of the supervised practices, is the use of an alternative resource as a didactic tool. An inanimate object was designed simulating the vertebral column of rats and mice, for teaching the cervical dislocation technique, an accepted method of euthanasia in rodents, prior to contact with live animals. APM Polyethylene (high molecular weight) was used, which was molded with a Turri T190 lathe, achieving a similarity in length with the vertebral column of these species, making removable what imitates the atlantoaxial joint (1st and 2nd. vertebra) granting a resistance similar to that exerted by traction on a dislocation. Which was placed inside an inanimate rat and mouse model occupying the same space as the spine. Conclusion: With the implementation of the use of this model, the students were encouraged to repeat the maneuver the necessary number of times until they acquired skill and confidence, recognizing the anatomical structures reinforcing the theory. Based on the principle of the 3 R's replacement, reduction and refinement and considering that acquiring the ability to perform euthanasia techniques is a specific competence of the technical professional, the chair developed and implemented this alternative, within the framework of supervised practices, using inanimate models as resources that allow students to carry out the learning process and acquire skills, significantly reducing the number of live animals used. It was verified that when performing the cervical dislocation technique in live rodents they do it with firmness, confidence and knowledge of the technique.

P1-BIOINFORMATICS & THERAPEUTIC TARGETS

WEDNESDAY 15TH NOVEMBER 14:00-15:30
CHAIRS: JUAN MIGUEL BAYO FINA
JUAN BIZZOTTO

10. 310. **BIOINFORMATIC ANALYSIS OF POSSIBLE NEW BORON CARRIERS FOR THE APPLICATION OF BORON NEUTRON CAPTURE THERAPY (BNCT) TO THE ANAPLASTIC THYROID CANCER (ATC)**

, [1]Luciano Rossich, [2]Antonella Pastini, [2]Tomas Peralta, [3]Susana Nievas, [1]Marina Carpano,
[1,4]Marina Perona, [1,4]Guillermo Juvenal, [1,4]Lisa Thomasz, [1,4]María Alejandra Dagrosa.

[1]Departamento de Radiobiología [3]Departamento de Coordinación de BNCT [1y3] Comisión Nacional de Energía Atómica [2]Universidad Favaloro.
4Consejo Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET),

Boron neutron capture therapy (BNCT) is a binary treatment modality for malignant tumors based on the administration of boron compounds and subsequent irradiation of the tumor area with thermal neutrons (<0.4 eV). In our laboratory we have previously shown that BNCT is a possible alternative for the treatment of anaplastic thyroid cancer (ATC). Taking into account recent studies on transcriptomes from ATC patients, we performed a bioinformatic analysis to identify solute transporter molecules with property of being enriched with 10B atoms to delivery boron at high concentrations. Sequencing data deposited in Gene Expression Omnibus (GSE123868) from ATC (n=10) and healthy thyroid tissue (n=6) were analyzed using the UseGalaxy platform. Quality control was performed with FASTQC, genomic alignment with HISAT2 (hg38) and read counting with featurecount. Differential expression analysis was carried out with the R edgeR package, considering a logFC>1 or logFC<-1 and FDR < 0.01 as significant expression. The gene ontology was checked with the R package pathfindR. The bioinformatic analysis identified 131 solute transporter genes with differential expression, of which 96 had higher expression in tumor tissue than in healthy tissue. Six genes with high tumor expression and their specific solutions were detected: LAT1 (logFC 7.09, neutral amino acids), MCT4 (logFC 4.41, monocarboxylates), OAT1 (logFC 3.71, p- aminohipurate), RFVT2 (logFC 3.67, riboflavin), SMIT2 (logFC 3.10, myo-inositol) and SMIT2 (logFC 3.06, pyrimidines). The identified transporters in this work offer new opportunities for the development of new boron compounds for clinical use such as p-aminohipurate, riboflavin and myoinositol.

11. 328. **CHARACTERIZATION OF CD20 ISOFORM DIVERSITY IN PEDIATRIC ACUTE LYMPHOBLASTIC LEUKEMIA: POTENTIAL IMPLICATIONS FOR RITUXIMAB TREATMENT**

Avendaño Daniel*[1,2], Gomez Mercado Ignacio*[1,2], María Cecilia Riccheri[3], Elba Vazquez[1,2], Geraldine Gueron[1,2], Javier Cotignola[1,2], María Sol Ruiz[1,2]
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**Estos autores contribuyeron en partes iguales al trabajo.*

Acute lymphoblastic leukemia (ALL) is characterized by the overproduction of immature lymphoid blasts in the bone marrow and is the most incident pediatric cancer worldwide. Numerous advances have been achieved in the treatment. Rituximab, an anti-CD20 monoclonal antibody, will be incorporated into the multinational clinical protocol ALL IC-BFM 2020, of which Argentina is a participating member. However, the existence of different *MS4A1* (CD20 coding gene) transcripts might have potential implications in treatment response. Our study aimed to characterize and quantify the CD20 isoforms in ALL patients. To accomplish this goal, we performed RNA-seq on bone marrow aspirates collected at diagnosis from Argentinian pediatric ALL patients (n=31). The analysis of CD20 transcript expression revealed high heterogeneity among patients, both in terms of total gene levels as well as in the absolute and relative abundance of transcripts. We found high levels of *ENST00000532073* transcript were associated with a lower chance of event-free survival (Hazard Ratio=6.45, Cox p=0.01; Log-rank=0.02). We also performed a bioinformatics analysis using bone marrow samples of ALL patients (TARGET, n=207) and healthy donors (GTEx, n=70). Of all CD20 transcripts, only *ENST00000532073* was detected in healthy donors, and with lower levels (TPM<1) than in ALL patients where this isoform was the most abundant. An *in silico* structural analysis of *ENST00000532073* showed that this transcript has a shorter exon 5, which is part of the epitope of rituximab binding. Overall, ALL patients displayed diverse CD20 isoform expression patterns, mainly with changes in *ENST00000532073* expression that impacted event-free survival. In addition, this CD20 isoform might have lower/null binding of rituximab. Further studies into the CD20 isoforms might provide valuable insights into the prediction of rituximab treatment efficacy.

12. 337. **PEPTIDES DERIVED FROM GENOMIC VARIANTS IN ALLOGENEIC MELANOMA VACCINE INDUCE T CELL ACTIVATION**

Ibel Carri[1], Erika Schwab[2], Morten Nielsen[1,3], José Mordoh[2], María Marcela Barrio[2]
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[3] Section of Bioinformatics, Department of Health Technology, Technical University of Denmark, Lyngby, Denmark

VACCIMEL is a recently approved cancer vaccine composed of four allogeneic human cell lines rationally selected to cover a wide range of tumor-associated antigens (TAA) in melanoma. T-cells reactive to TAA and private neoantigens increased during vaccination. However, the immune response (IR) to vaccine antigens arising from somatic mutations and polymorphisms remains unexplored. To study this, we performed whole-exome sequencing of paired tumor/normal samples from a responsive patient (pt#32) and the vaccine cells; also RNAseq and MHC typing were carried out. Variant calling was performed by comparing the vaccine and patient's exomes with MuTect2, and annotation with VEP. Non-synonymous coding variants were used to predict T-cell epitope candidates using MuPeXI. Similarly, we predicted pt#32 tumor neoantigens. In both cases, immunogen candidates were ranked based on allele and expression in the vaccine, predicted peptide-MHC (pMHC) affinity, and stability. IR to 103 candidates was evaluated with pt#32 PBMCs post-treatment by ELISPOT. Comparison of the vaccine and pt#32's germinal exomes revealed 10,566 coding variants, 62% of which were reported in COSMIC and 20% were shared with pt#32's tumor. After vaccination, we found 18 ELISPOT^{+}/46 candidate peptides expressed in pt#32's tumor; 20 ELISPOT^{+}/44 not expressed in the pt#32's tumor, and 4 ELISPOT^{+}/11 only expressed in the patient's tumor. This analysis reveals that VACCIMEL offers a large panel of antigen candidates that may stimulate a therapeutic antitumor IR in melanoma. The computational approach successfully identified antigens in allogeneic cell lines, evidencing the relevance of protein expression and pMHC stability in immunogenicity. Besides, our results demonstrate that the immune system simultaneously responds to a high number of antigens, either vaccinal or private, proving that the IR against epitopes not expressed in the patient's tumor, was not detrimental to the relevant IR against neoantigens and TAA.

13. 383. **BIOINFORMATIC ANALYSIS OF TCAB1 AS A MOLECULAR TARGET IN GLIOMA DATA SETS FROM PAN-CANCER-ATLAS**

Vilarullo Roman Nicolas, Casco María del Pilar, Mengual Gómez Diego Luis, Balcone Lara, Maggio Julian, Armando Romina Gabriela*, Gomez Daniel Eduardo*
Molecular Oncology Unit, Center of Molecular and Translational Oncology, Quilmes National University, Bernal B1876BXD, Argentina
**Contributed equally*

Gliomas represent the most prevalent tumors of the CNS in adult patients. The WHO classifies them into four grades based on their aggressiveness and malignancy, where glioblastoma (GBM) is considered the most lethal. Notably, 90% of these tumors are positive for telomerase activity, absent in adjacent tissue. The holoenzyme telomerase primarily comprises a catalytic subunit and an RNA template but requires multiple other proteins for assembly, trafficking, and telomere association. One of these is TCAB1, which is involved in directing factors to Cajal Bodies and telomeres, and also plays

a role in dsDNA repair, becoming an important target in developing new strategies to treat GBM. From this, we analyzed CbioPortal data sets and observed that patients with reduced TCAB1 expression showed better prognoses than those with higher levels. This tendency increases in patients that received TMZ as chemotherapy, demonstrating that low levels of TCAB1 expression are associated with improved patient survival. Moreover, we found that TCAB1 is overexpressed in GBM compared to normal tissue, and its expression correlates with MMR proteins. These findings allow us to postulate TCAB1 as a potential prognostic marker and molecular target in GBM. Once demonstrated the role of TCAB1, we decided to start the development of novel inhibitors by modeling its structure and selecting the nonstructural mutations described in dyskeratosis congenita (F164, H376 and R398). In order to address a docking-based virtual screening, we explored adjacent druggable cavities and identified target sites comprising these residues. In conclusion, these findings allow us to postulate TCAB1 as a prognostic marker and molecular target in GBM. Moreover, we obtained relevant information for developing compounds capable of interacting with TCAB1 and regulating both its telomeric and DNA-repairing functions. A drug with these characteristics could be a novel and valuable therapy for GBM

P2-BIOINFORMATICS & THERAPEUTIC TARGETS

THURSDAY 16TH NOVEMBER 9:00 - 10:30
CHAIRS: CAROLINA CANIFFI
MARÍA SOL RUIZ

14. 96. **IDENTIFICATION AND CHARACTERIZATION OF DIGESTIVE ENZYMES IN BLACK FLOUNDER USING BIOINFORMATIC TOOLS**

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Black flounder, *Paralichthys orbignyanus*, is a flatfish found in the coastal waters of Argentina, Brazil, and Uruguay and is an important regional fishery resource. Despite its potential for aquaculture, one of the obstacles to its large-scale commercial farming in South America is the lack of nutritionally suitable pellets for this carnivorous fish. Understanding digestive enzymes is critical because they play a central role in nutrient processing, particularly carbohydrate and protein digestion. Studying the key digestive enzymes of *P. orbignyanus* could lead to improved feed design that increases nutritional value and sustainably reduces waste. Therefore, integrating different bioinformatics approaches together with phylogenetic and comparative genomic analyzes can be of great help in studying the physiology of digestive enzymes. Thus, one of our goals was to perform a curated gene annotation of α-amylases (*amy2*) and alanine aminopeptidases (*anpep*) in the genome of *P. orbignyanus*. This may help us better understand the evolution of these digestive enzymes and the dietary preferences of the fish. In this work, we identified two different copies of the *amy2* gene and one copy of the *anpep* gene. The predicted protein products were analyzed and found to contain important residues and domains that contribute to enzymatic activity and stability. The genes were modeled using AlphaFold, and we were able to identify key residues involved in catalysis when compared to the resolved 3D structures. Consistent with other studies, these results suggest that this flatfish has the genetic capacity for initial carbohydrate digestion and final protein digestion. In addition, phylogenetic reconstruction analyses were performed for these two gene families and fish with diverse dietary preferences. Phylogenetic and comparative genomic analyzes revealed no relationship between gene copy number and dietary preference in fish. Through the use of comparative genomics, structural biology, and computational biology, this work provides valuable insights into the genetic basis, evolutionary relationships, and functional properties of these enzymes in black flounder.

15. 217. **PREDICTION OF DIABETES MODY TYPES USING MEDICAL VARIABLES AND MACHINE LEARNING**

Daniela Mennickent[1,2], Alejandro De Dios[3], María Silvia Pérez[4], Gustavo Daniel Frechtel[3], Ariel Pablo López[3,5]
[1] Departamento de Análisis Instrumental, Facultad de Farmacia, Universidad de Concepción, Chile.
[2] Machine Learning Applied in Biomedicine (MLAB), Concepción, Chile.
[3] División Nutrición, Hospital de Clínicas "José de San Martín", Universidad de Buenos Aires, Argentina.
[4] Laboratorio Manlab, Buenos Aires, Argentina.
[5] Cátedra de Genética Molecular, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Argentina.

Objective: MODY type diabetes is characterized by pathogenic variations in different genes and dominant autosomal inheritance. These patients differ from classical diabetics because of their pathogenesis, treatment, and prognosis. Despite that, they are often misdiagnosed and, as they require specific treatments, they are poorly treated with the consequent deterioration of their quality of life and prognosis. Since the clinical characterization of type of Diabetes and possible MODY type is usually done through molecular diagnosis by Sanger sequencing, it is often difficult to establish which gene to study first. Therefore, we aimed to apply machine learning (ML) on routine medical variables to evaluate if we could predict which is the gene with higher probabilities of having the MODY causal alteration. Materials and methods: 83 patients (MODY2=36, MODY3=7, MODYX=40) and 7 medical variables were considered: sex, age at diagnosis, body mass index, diabetes family history, fasting glycemia, post load glycemia (75g, 2h) and HbA1c. Data were preprocessed by autoscaling and analyzed by the classification ML technique partial least squares discriminant analysis. Every model was subjected to leave-one-out cross-validation (CV). Results: The simultaneous analysis of MODY2, 3 and X data allows to predict them with CV areas under the receiver operating characteristic curve (AUROC) of 0.7074, 0.9060 and 0.6576, respectively. The exclusion of MODYX allows to predict MODY2 (*GCK* gene mutation) and 3 (*HNF1A* gene mutation) with a CV AUROC of 1.0000. The most important medical variables for the latter differentiation are post load glycemia (75g, 2 h) and fasting glycemia. Conclusion: We established a first approach on how to apply ML techniques to try to predict the most probable gen altered in MODY patients. Our results show a significant difference between the medical variable profiles of MODY2 and 3 patients, which could help to focus their further study with molecular techniques.

16. 227. **DISCOVERY OF DIFFERENTIAL ISOFORM EXPRESSION DUE TO ALTERNATIVE SPLICING AFTER EX VIVO AND IN VIVO IRRADIATION AT 4 HOURS**

Jerónimo Leberle[1], Vanesa Biolatti[2], Soledad Ausas[2], Lara Negrin[2], Laura Mazzitelli-Fuentes[2], Adriana Cascón[2], Rocío Brezan[2], Julieta Irazoqui[2], Alejandro Álvarez[3], Romina Ventimiglia[3], Marina Perona[4], Irene L. Ibañez[5], Nicolás Bellora[1].
[1]Comisión Nacional de Energía Atómica (CNEA) - INTECNUS - Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), [2]CNEA - INTECNUS, [3]Fundación INTECNUS, [4]Gerencia de Área de Aplicaciones de la Tecnología Nuclear - CNEA - CONICET, [5]Instituto de Nanociencia y Nanotecnología - CNEA - CONICET

Introduction and objectives: The molecular study of the radioinduced response by the massive analysis of the radiomodulated transcriptome has gained great relevance in recent years, both in the field of radioprotection and radiotherapy (RT). The aim of this study was to evaluate the transcriptome of irradiated human leukocytes in order to identify differential isoform expression at different doses of ionizing radiation (IR). Materials and methods: Leukocytes from healthy individuals were X-ray irradiated *ex vivo* at 25, 100 and 200 cGy and cultured for 4 hours at 37°C and 5% CO_2 for RNA extraction. For *in vivo* studies, leukocytes were collected from cancer patients who received a single RT fraction, equivalent to a 1.6 cGy blood absorbed dose. Con-

trol samples were non-irradiated *ex vivo* or collected prior to the RT session. The transcriptome was sequenced by RNA-seq (Illumina Platform, 150 bp paired-end). Sequencing reads were mapped to the human genome hg38 (STAR software). A comparative analysis of isoforms was performed using SUPPA2 and BANDITS software. Results: We identified 39 genes which express different isoforms across the doses compared with the non-irradiated samples with SUPPA2. The most relevant were TNFSF4, KHNYN, MRPL36, CYCS, TRAPPC5, NR6A1 and AK6. BANDITS revealed 55 genes undergoing alternative splicing (AS) *ex vivo*. The most significant and biologically relevant genes in the radioinduced response were DDB2, DDIAS and TNFSF4. The latter showed an up-regulation of TNFSF4-201 transcript after IR, whereas TNFSF4-202 was down-regulated. This shows that there is an effect in the AS machinery in the cell when it is exposed to IR. Meanwhile, neither of the analyses could predict isoform changes *in vivo*, probably related to the low doses used. Conclusion: We determined a switch of isoforms of genes post-irradiation with potential as biomarkers of IR exposure or biodosimeters.

17. 295. ***IN SILICO* STUDY OF ECHINOCOCCUS GRANULOSUS GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE ASSOCIATED WITH BISPHOSPHONATES**

Facundo Ariel Aguero[1,2], Andrea Maglioco[1,2], Margot Paulino[3], Alejandra J Juaréz Valdés[1], Emilio AJ Roldán[1], Alicia G Fuchs[1,4]

1-centro de altos estudios en ciencias humanas y de la salud-universidad abierta interamericana. 2-consejo nacional de ciencia y tecnología. 3-departamento de experimentación, teoría de la estructura de la materia y sus aplicaciones, facultad de química, universidad de la república, uruguay. 4-instituto nacional de parasitología "dr. mario fatala chaben"-administración nacional de laboratorios e institutos de salud "dr. carlos malbrán"

The *Echinococcus granulosus* (Eg) produces cystic echinococcosis, a zoonotic disease, in ungulate animals and in humans. Argentina reported 630 human cases in 2018-2019. In our laboratory we work using a cellular line from Eg G1 protoscolices, EGPE. The bisphosphonates (BF) on EGPE decreased cell growth and ATP, increased the total calcium but decreased the Ca^{2+}. The ethidronate (EHDP) was effective and the alendronate (AL) had no effect. We identified the Eg's glyceraldehyde-3-phosphate-dehydrogenase (GAPDH), a tetramer, UniProt W6UJ19 by MS/MS. We investigated whether GAPDH, could be druggable with EHDP and AL. Materials and Methods: A validated GAPDH model bound to NAD^+, substrate, Pi and BF was subjected to molecular dynamics simulation (MD) for 100 ns in NAMD2. Structures were analyzed and visualized with software VMD 1.9.3 and MOE2022. Potential energy, RMSD and RMSF from the system with or without BF (control) were calculated. Binding free energy with ligands was calculated by MMPBSA in the last 100 frames of the MD. T-test was used to compare control and samples. Results: The EHDP was placed close to the Pi site and did not allow the Pi binding and the AL interacted with Thr154, Gln211, Thr 212, Gly213 and Arg235 impeding the substrate binding. Binding free energy for EHDP and GAPDH was favorable only in monomers A and B. The control showed favorable interaction with substrate only in monomer A (-10.7±5.1 Kcal/mol), while in presence of EHDP, the substrate had favorable interaction with 3 monomers (A, C, D). Significant differences were found between the binding free energy estimated for the substrate with each monomer with or without EHDP ($p<0.05$). Binding free energy for AL and GAPDH was favorable only in monomer B (-1.3±2.2 Kcal/mol) and binding free energy for NAD^+ increased in the monomers C and D ($p<0.01$) in presence of AL. Conclusion: Both BF affected the enzyme in different manner but if the enzymatic activity is modified by BF must be study.

18. 511. **STRUCTURE-BASED VIRTUAL SCREENING STRATEGY APPLIED TO THE SEARCH FOR NEW MODULATING AGENTS OF THE hHv1 PROTON CHANNEL**

María V. Chaulet[1,2], Melisa E. Gantner[1], Manuel A. Llanos[1], Clara Ventura[2], Verónica Milesi[2], Luciana Gavernet[1]

*[1]Laboratorio de Investigación y Desarrollo de Bioactivos (**LIDeB**). Facultad de Ciencias Exactas, UNLP, La Plata, Argentina.*

[2]Instituto de Estudios Inmunológicos y Fisiopatológicos (CONICET-UNLP-CIC), La Plata, Argentina.

The human voltage-gated proton channel (hHv1) is a highly selective ion channel that plays a fundamental role in various physiological processes such as innate and adaptive immunity, insulin secretion, and sperm capacitation. Recently we have demonstrated that ATP exerts an activating effect on hHV1, mainly mediated through the interaction with the ATP phosphate groups. With the aim of finding new modulatory agents through the novel ATP binding site, a structure-based virtual screening strategy was developed. First, a database defined as "ATP-like compounds" was built. For this purpose, the ATP scaffold was used as the query structure in Pubmed and GoogleScholar for literature searching and the 70% ATP -and ADP- similarity structure searching was conducted on the ZINC15 database. Then, molecules that present 3 or 2 phosphate groups were selected. Secondly, different hHv1 conformations were sampled to adequately represent the ATP binding site. Four target conformations were extracted from the production results of several Gaussian accelerated molecular dynamics simulations of our dimeric homology model of the channel, with and without ATP. The binding site volume and druggability score calculated from FPocket and DoGSiteScorer software were considered for the target conformation selection. Finally, docking simulations with AutoDock Vina were run over the ATP-like database of 601 compounds in each of the 4 channel conformations. 68 compounds with better docking score than ATP -and ADP- in at least 2 conformations were visually inspected, selecting 5 candidates with similar interactions to those observed for ATP. All of them will be evaluated *in vitro* on the hHv1 channel expressed in HEK293 cells by the patch-clamp technique. Finding new ATP site modulators would increase the actual understanding of the physiological and pathophysiological characteristics of the channel, and would provide new ATP-related structures with therapeutic potential.

O-BIOINFORMATICS & THERAPEUTIC TARGETS

FRIDAY 17TH NOVEMBER 14:00-15:30

CHAIRS: CAROLINA CANIFFI
VERÓNICA MARTÍNEZ MARIGNAC

19. 122. **MODELING A RISK SCORE BASED ON THE EXPRESSION OF LONG NON-CODING RNAs (lncRNAs) AND ISUP GRADE GROUP FOR THE PREDICTION OF PROSTATE CANCER PROGRESSION**

Sabrina Ledesma-Bazan[1,2], Florencia Cascardo[1,2], Juan Bizzotto[2], Elba Vazquez[1,2], Geraldine Gueron[1,2], Javier Cotignola[1,2]

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[2]Universidad de Buenos Aires, Facultad de Ciencias Exactas y Naturales, Departamento de Química Biológica, Laboratorio de Inflamación y Cáncer, Buenos Aires, Argentina.

Prostate cancer is a highly heterogeneous disease; hence, estimating patient prognosis accurately is challenging due to the lack of biomarkers with sufficient specificity and sensitivity. Therefore, we aimed to model a risk score containing transcriptomic and clinico-pathological data to better predict the probability of prostate cancer progression. We conducted bioinformatics analyses to identify lncRNAs differentially expressed across various prostate cancer stages and associated with progression-free survival. This information was then integrated into a prognostic risk score and nomogram including clinico-pathological features as covariates. We used RNA-seq data from 5 datasets from public repositories (total n=178) comprising pre-treatment primary prostate adenocarcinomas, post-treatment tumors and metastatic castration resistant prostate cancer. Our analyses revealed 30 lncRNAs differentially expressed in all com-

parisons made using two R packages. Multivariate progression-free survival analysis including the ISUP group as a covariate, showed that 7/30 lncRNAs were significantly associated with time-to-progression. We then combined the expression of these 7 lncRNAs to create a multi-lncRNA score, and subsequently categorized patients into low- or high-score groups. Patients with a high-score showed a 4-fold risk of disease progression (HR=4.30, 95%CI=2.66-6.97, p=1e-10). Furthermore, we modelled a transcriptomic/clinical risk-score that integrated the information of the multi-lncRNA score and ISUP group. Patients with a high-risk score had more than 7-fold risk of progression (HR=7.65, 95%CI=4.05-14.44, p=1e-13). Finally, we developed a nomogram to assist clinicians to better predict patient's risk of progression at 3 and 5 years post-diagnosis. Thus, integrating lncRNAs expression data and clinico-pathological features of prostate tumors into predictive models may improve disease risk assessment and enable personalized treatments for each individual patient.

20. 223. **DEFINING DISTINCT LNCRNA SIGNATURES FOR PRECANCEROUS LESIONS AND TUMORS IN COLORECTAL CANCER SCREENING**

Marcela Astiz[1], Mariano Di Tommaso[2], Melissa Hidalgo[2], Natividad Perlo[2], Rocío Laurini[2], Leandro Michelena[2], Leandro Spaletti[2], Malena Solis[2], Federico Campomenosi[2], Carla Salmon[2], Romina Canzoneri[1], Eugenia Salas[1], Martín Rabassa[1], Eduardo Alach[2], Martin Abba[1], Ezequiel Lacunza[1].

[1] CINIBA Facultad de Ciencias Médicas UNLP, La Plata, Buenos Aires, Argentina.

[2] Servicio de Gastroenterología, Hospital Interzonal General de Agudos San Roque, Gonnet, La Plata, Buenos Aires. Argentina.

Colorectal cancer (CRC) screening via video-colonoscopy is recommended for individuals aged 45 and above to meticulously examine the colon and rectum, aiding early polyp detection and prevention of CRC. Colonoscopy's preventive integration has significantly led to identifying precancerous adenomas and latent cancers, emphasizing the challenge of comprehending adenoma and tumor molecular diversity, driving precision medicine approaches. LncRNAs dysregulation contributes to colorectal tumorigenesis and affects clinical manifestations, such as tumor size, stage, patient survival, and treatment outcomes. These findings position LncRNAs as biomarkers and therapeutic targets in cancer. Currently, distinctive LncRNA signatures for precancerous CRC lesions or molecular subtypes (CMS) defining tumors are lacking. To address this, through comprehensive bioinformatics analysis from RNA-seq and microarrays data, we defined distinct LncRNA signatures for CMS1-4 subtypes in tumors and colorectal adenomas with classificatory (AUC>0.8), prognostic (p<0.05) and predictive value (p adj.<0.01, treatment response). These signatures are being validated by qRT-PCR in preoperative biopsy and plasma samples from individuals with adenomas or CRC, and in cell line models with proliferation and chemoresistance variations. We have refined protocols for patient inclusion, sample collection, RNA extraction, and LncRNA detection. Thus far, samples derived from twelve patients and cellular models have undergone testing with ten LncRNAs. Although preliminary, we identified CRNDE as an early progression biomarker and HAND2-AS1 as a predictive marker, aligning with bioinformatics analysis. Presently in its clinical validation pilot phase, this study presents a quantitative approach for screening distinct LncRNA in polyps and colorectal tumors, utilizing minimally invasive techniques (blood extraction) or standard preventive procedures (colonoscopy), facilitated by an accessible qRT-PCR methodology.

21. 264. **COMPUTATIONAL MODELING OF SOLUPLUS® NANOMICELLES AND IgG INTERACTIONS: A POTENTIAL APPROACH FOR TARGETING SHIGA TOXIN TYPE 2 AND PREVENT HEMOLYTIC UREMIC SYNDROME**

Casal Juan José[1,3], Girón Reyes Claudio Daniel[2,3], Amaral, Maria Marta[2,3], Sacerdoti Flavia[2,3]

[1] Universidad de Buenos Aires (UBA), Facultad de Ciencias Médicas, Departamento de Ciencias Fisiológicas, Buenos Aires, Argentina.

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[3] CONICET - UBA. Instituto de Fisiología y Biofísica Bernardo Houssay (IFIBIO Houssay), Buenos Aires, Argentina.

Shiga toxin type 2 (Stx2) is secreted by Shiga toxin producing *Escherichia coli* during infection and is responsible for developing Hemolytic Uremic Syndrome (HUS), a systemic disease characterized by hemolytic anemia, thrombocytopenia and acute kidney injury. Nowadays, there is no specific treatment available for HUS. Soluplus® is an amphiphilic polymer that spontaneously assembles in nanomicelles (NM) in aqueous solutions and is commonly used for solubilization and administration of poorly soluble drugs. We propose that Soluplus® nanomicelles associated with anti Stx2 IgG may improve IgG properties of toxin neutralization and propose them as a treatment for HUS. In this study we aimed to investigate potential interactions between Soluplus® and IgG by computational modeling to better understand the association between both molecules. For that, the crystal structure of Soluplus® was designed using Marvin Chemsketch 23.10, based on information from the manufacturer. A reduced version of Soluplus® was generated, preserving the monomer proportions and resulting in a molecule with a molecular weight of 42 kDa. Docking calculations were performed using AutoDock Vina 1.2.5 with the IgG antibody (PDB code 1IGY) as the model protein. The search box, centered on amino acid GLY236 of the B chain, had dimensions of 126 Å in each direction (x, y, z). The docking results were analyzed based on the scoring function, which implicitly incorporated the solvent effect. The pose with the lowest binding energy was selected for further analysis. Interactions between Soluplus® and the IgG antibody were examined using LigPlot+ 2.2.8. Our computational modeling indicates that Soluplus® interacts with IgG mostly by the Fc portion of the antibody, thus suggesting that IgG may perform a corona in the nanomicelles and give a better solubilization of the IgG in aqueous solutions. These results are in concordance with the biological studies that indicate that NM improve the neutralization capacity of anti Stx2 IgG *in vitro,* suggesting that coupling IgG to Soluplus® could be a potential strategy to prevent HUS.

22. 606. **GANING INSIGHT FROM CLINICAL, FREQUENCY AND PROTEIN STRUCTURE TO IMPROVE PATHOGENICITY PREDICTION IN SHORT LINEAR MOTIFS**

Franco Gino Brunello[1], Lorenzo Erra[1], Mariano Martin[2], Marcelo Marti[1]

[1]Facultad de Ciencias Exactas y Naturales, UBA. [2]Instituto de Bioingeniería de Cataluña, Barcelona, España.

The aim of our work is to better characterize variation on short linear motifs (SLiMs), amino acid sequences implicated in various physiological cellular processes, and also utilize this information in order to improve prediction of true functional SLiMs. We used sequence variant information from ClinVar and GnomAD, crystallographic structures of motif-domain interactions from PDB and performed protein stability predictions using FoldX. When a protein structure representative from the interaction was not present in PDB, we made use of AlphaFold2 in order to generate a prediction of it. We considered SLiMs true positives from the ELM (Eukaryotic Lineal Motif) database and expanded the universe of motif classes present by constructing an amino acid substitution matrix with variants from ClinVar and the energy change values from FoldX. We thought of creative novel ways of considering information coming from them and designed a system based on confidence scores whereby we made data coming from these three sources compete for a place in the matrix. When using AlphaFold2, we implemented a Random Forest (RF) approach in order to retain biologically plausible models from the interaction and study that motif class. We also included secondary structure, exposure, cellular compartments, and conservation filters in order to only retain sound SLiMs candidates. The generation of an amino acid substitution matrix collaborates both with the identification of new SLiMs candidate instances and also with the interpretation variant protocols given that ACMG standards

consider evidence from the variant occurring in a functional site of the protein. Combining both scopes, we provide a list of variant coordinates in order to include in automatic annotation pipelines, in an aim to improve molecular diagnosis yields.

23. 657. **EVOLUTION OF IZUMO1-JUNO PROTEINS FUSION PAIR IN *CARNIVORA***

Clara Campos[1], Francisco Pisciottano[1], Carlos David Bruque[2], Patricia Saragüeta[1]
[1]IBYME-CONICET [2]

JUNO and IZUMO1 are the only gamete fusion pair identified to enable fertilization in mammal.Studying their evolution could lead to elucidate some aspects of oocyte-spermatozoa interaction species-specificity. In this work, IZUMO1-JUNO protein positive selection signatures were studied in the context of *Carnivora* order, *Caniformia-Feliformia* suborders and *Pantherinae* subfamily. Sequence identity analyses of a comprehensive and carefully curated aligned sequence collection was conducted to characterize and compare amino acids diversity within these proteins. Subsequently, site-lineage-specific positive selection analyses were run in order to detect signals of molecular adaptation and diversifying evolution. Positive selected sites were mapped onto protein jaguar structural models. IZUMO1 was higher significant variable than its counterpart JUNO. Both proteins exhibited a significantly lower diversity in *Feliformia* suborder compared to *Caniformia* suborder. Signatures of adaptive evolution were detected in IZUMO1, showing eight sites under positive selection that are in the JUNO interaction region. On the contrary, JUNO does not show positive selection signatures, indicating neutral or negative evolution.
IZUMO1-JUNO protein evolution pattern suggests that prezygotic isolation barrier could emerge due to gametic incompatibility, even lacking positive selection driven solely by neutral changes.

O-CARDIOVASCULAR & RESPIRATORY

WEDNESDAY 15TH NOVEMBER 9:00 - 10:30
CHAIRS: ANDREA FELLET
SILVIA GARCÍA

24. 207. **SEX DIFFERENCES IN THE EFFECTS OF AGING OR HORMONE DEFICIENCY IN CARDIAC STUNNING MODELS**

Germán Andrés Colareda[1], Patricia Bonazzola[2], Juan Manuel Lofeudo[3], Romina Gisel Diaz[3], Alicia Elvira Consolini[1]
[1]Cátedra de Farmacología de Farmacia, GFEYEC, Departamento de Ciencias Biológicas, Facultad de Ciencias Exactas, Universidad Nacional de La Plata. 47 y 115, La Plata, CP: 1900, Buenos Aires; [2] IATIMET-UBA-CONICET. [3]Centro de Investigaciones Cardiovasculares (CIC) CONICET-UNLP.

Age is a cardiac risk factor, and the post-menopausal stage equals the incidence of heart attack between both sexes. Although the estrogenic cardioprotective effect is known, especially for reducing cardiovascular risk factors, the studies carried out to date suggest that protection against the ischemic event occurs specifically after a longer ischemic time. On the other hand, whether testosterone is involved is uncertain, and whether age modifies responses to I/R is unclear. We investigated sex- and age-dependent differences in cardiac stunning. Methods: Hearts of female (F) and male (M) young rats (5 months old) gonadectomized (OVX, GDX) or not (YF, YM) and aging rats (over 20 months old, AgF, AgM) were isolated and perfused with Krebs solution under stimulation in a flow calorimeter. Once stabilized, a period of 20 min I and 45 min R was generated. Intraventricular pressure (LVP, mmHg) and heat rate (Ht, mW/g) were simultaneously measured. Maximum developed pressure (P), diastolic pressure (LVEDP) and muscle economy (ECO=P/Ht) were calculated. Serum testosterone and estradiol levels were measured with ELISA assays. Results: AgF had less postischaemic contractile recovery (PICR) (48.02±4.6% of initial P) than YF (78.2±2.8%, p<0.05), as well as P/Ht, and higher LVEDP. Surprisingly, OVX have the same PICR than YF, despite they reduced estradiol levels but not testosterone. There was a sex difference, since PICR were higher in AgM (77.4±12.6%) and GDX (88.1±13.3%) than in YM (45.3±6.2%, p<0.05) and P/Ht improved similarly (100.3±12.5% and 103.2±7.6 vs 57.2±8.1). Estradiol levels were higher and testosterone levels lower in GDX than YM. Conclusions: Although gonadectomy had no effect on susceptibility to I/R in female rat hearts, it protected against stunning in males. Our findings indicate that low testosterone may be protective against I/R injury since the reduction of estrogen due to ovariectomy did not affect the PICR.

25. 322. **COBALT CHLORIDE ACTS A CARDIPROTECTOR AFTER AN ISCHEMIA/REPERFUSION INJURY IN INFANT RATS**

Christopher Gutierrez[1], Magdalena Peirone[1], Tomás Little[1], Matías Ramirez[1], Patricia Bonazzola[1], Rocío Castilla[1]
[1] Universidad de Buenos Aires. CONICET. Instituto Alberto C. Taquini de Investigaciones en Medicina Traslacional (IATIMET)

The damage induced by Ischemia-reperfusion (I/R) involves alterations in Ca^{2+} homeostasis and is reduced by ischemic postconditioning. $CoCl_2$ can trigger changes resembling the response to a hypoxic event in normoxia and blocks Ca^{2+} current in heart muscle. Previously we have demonstrated in adult rat heart that $CoCl_2$ administrated in reperfusion can reduce damage induced by I/R consistent with improved cellular calcium handling. Since the hearts of infant rats differ from those of adults in the dependence on plasma Ca^{2+} for myocyte function, we evaluated whether $CoCl_2$ can also protect infant heart from I/R damage. For that proposal, ventricles from 6-8 days old Wistar rats were isolated, arterially perfused, suspended and connected to an isometric force transducer. Ventricles were introduced in an organ bath at 37 °C, stimulated at 3Hz and subjected or not to 45 min of ischemia followed by reperfusion in the presence of 0.23 mmol/L $CoCl_2$. Mechanic parameters were analyzed.
The presence of $CoCl_2$ increased post ischemic contractile recovery (developed tension at 45 min: control: 26.4 ± 1.7 %, $CoCl_2$: 51.8 ± 8.3 % p <0.05) but did not affect diastolic tension, neither both, maximum speed of contraction and relaxation. At the onset of reperfusion, $CoCl_2$ decreased both, the increase in contracture (control: 1.24 ± 0.16 g, CoCl2: 0.66 ± 0.12 g, p <0.01) and the mean velocity of its increase (control: 1.00 ± 0.18 g/s, $CoCl_2$: 0.52 ± 0.08 g/s, p <0.05). In addition, the ventricular damaged area was also decreased by $CoCl_2$ (control: 65.15 ± 3.15%, $CoCl_2$:52.6 ± 3.41%, p <0.05). On the other hand, $CoCl_2$ acted as a negative inotropic agent in not ischemic hearts and its effect was eliminated by $CoCl_2$ washing. Conclusion**:** The presence of $CoCl_2$ in reperfusion induces cardioprotection in infant heart. The use of $CoCl_2$ constitute a potential cardioprotective tool of clinical relevance.

26. 367. **EVALUATION OF PULMONARY TOMOGRAPHIC PATTERNS IN PATIENTS POSITIVE TO SARS-COV- 2 DURING THE YEAR 2021**

Maria Sol Lovato[1], Magdalena Schneider[2], Maria Rosana Ramirez [1,3]
[1]Instituto Universitario de Ciencias de la Salud, Fundación Barceló. [2] Hospital San Juan Bautista, Santo Tome, Corrientes, [1,3] Consejo Nacional de Investigaciones Científicas Técnicas (CONICET).

To describe the pulmonary tomographic findings in patients with covid-19 evaluated by chest computed tomography (CT) and to establish the predominant pattern. For this purpose, COVID-19 positive cases (n=300) were retrospectively selected and classified according to age by reviewing the existing data in an anonymised computerised spreadsheet of these patients at the University Hospital in the year 2021. The inclusion criteria were: patients with a diagnosis of COVID-19 who required hospital admission and who had an initial and/or subsequent follow-up CT scan. Patients who could not be followed up due to death, and those who did not have a CT scan were discarded. The review of the images was done separately, resolving discrepancies by consensus. For this, the patterns established in the consensus endorsed by the American College of

Radiology and the Radiological Society of North America were used, classified as typical, atypical, indeterminate and negative. The results showed that the typical tomographic manifestations in patients with CoV-2 consist of ground glass opacities with or without consolidation or cobblestone pattern, and often with bilateral, peripheral, multilobar involvement and with inverted halo sign or other findings of organised pneumonia (n=16). In eight patients, the negative pattern was found where many of them presented with this pattern but evolved to a typical pattern or vice versa. In six patients, the intermediate pattern was detected, but the images did not show specific features; however, no images with the atypical pattern were found. The clinical course was favourable in all cases. Although this work describes in a simple way the pulmonary tomographic findings in patients with COVID-19, it is necessary to study more cases to know its prognostic significance in order to know if it can become a marker of disease progression, and consequently to establish specific therapeutic measures or recommendations.

27. 531. **ENHANCING LONG-TERM LEFT VENTRICULAR FUNCTION AND MITIGATING FIBROSIS IN ISCHEMIC HEARTS THROUGH PRE-ISCHEMIC VAGUS NERVE STIMULATION**

Verena B. Franco-Riveros[1,2], Nahuel Méndez Diodati[1,2], Beatriz González Peña[1], Eduardo Bernatené[1,2], Eliana Cicale[3], María Celina Morales[1], Martín Donato[1,2], Ricardo J. Gelpi[1,2], Bruno Buchholz [1,2]

[1] *Universidad de Buenos Aires. Facultad de Medicina. Departamento de Patología. Instituto de Fisiopatología Cardiovascular.* [2] *Consejo Nacional de Investigaciones Científicas y Técnicas – CONICET.* [3] *Universidad de Buenos Aires, Facultad de Ciencias Veterinarias.*

Objective: To evaluate the effects and mechanisms of brief pre-ischemic electric vagus nerve stimulation (VS) on acute myocardial infarction (MI), and remodeling and function of chronic infarction with and without reperfusion. Materials and Methods: FVB mice underwent 45min regional MI, followed by 2 hours (2hR), or 28 days of reperfusion (28dR), or permanent ischemia (28dI), with or without a 10-min pre-ischemic VS. Left ventricular function (LVF) was assessed via LV catheterization and echocardiography. Histological measurements examined chronic IS and heart remodeling. Results: VS reduction of the acute IS was blocked by de muscarinic blocker atropine (ATR). VS improved LVF after 28dR evidenced by a lower LVEDP(mmHg) (Sham-28d:3.8±0.2; IR-28d:6.8±0.5; VS+IR-28d:3.7±1; p<0.01), higher EF% (Sham-28d:77.3±11.7%; IR-28d:59.7±2.8%; VS+IR-28d:69.6±2.4%; p<0.05), and lower TRIV (Sham-28d:19.4±1.4; IR-28d:30.3±1.2; VS+IR-28d:25±0.9; p<0.05). ATR did not reverse the positive effect of VS on LVF. VS did not affect CSAm (Sham-28d:271±12um; IR-28d:341,3±11um; VS+IR-28d:374,3±20um; p<0.05), but it significantly reduced collagen ventricular fraction (CVF%) on the infarcted (IA) and non-infarcted areas (n-IA) (IA: IR-28d:45.9±3%; VS+IR-28d:19.6±3.5%; ATR+VS+IR-28d:16.1±1.4; p<0.05 and, n-IA: IR-28d:4.3±0.7%; VS+IR-28d:2.75±0.3%; ATR+VS+IR-28d:1.26±0.2 p<0.05). Moreover, after 28d of MI without reperfusion VS improved LVF (LVDP: I28d:8.4±1mmHg; VS+I28d:3.1±0.8; p<0.05; EF%:I28d: 50.6±4; VS+I28d:69.5±1; p<0.05) and reduced CVF% on IA and n-IA (IA: I-28d:82.8±5%; VS+I-28d:64.9±6%; p<0.05 & n-IA:I-28d:3.9±0.7%; VS+I-28d:0.82±0.3%; p<0.05) without changes in neither CSAm nor on the IS. Conclusion: short-term pre-ischemic VS decreased acute IS, improved long-term LVF and extracellular matrix remodeling regardless of infarct size and muscarinic receptors.

28. 620. **REMOTE ISCHEMIC PRECONDITIONING ATTENUATES EARLY AND LATE MYOCARDIAL POSTINFARCTION VENTRICULAR REMODELING**

Eliana P. Bin[1], Mariana Garces[3], Federico Penas[2], Camila Marquez Roa[1], Luciana Wilensky[1], Maria Celina Morales[1], Pablo P. Evelson[3], Ricardo J. Gelpi[1], Martín Donato[1]

1. Institute of Cardiovascular Pathophysiology, School of Medicine, University of Buenos Aires

2. Institute of biomedical research in retroviruses and AIDS

3 Institute for Biochemistry and Molecular Medicine (IBIMOL)

Remote ischemic preconditioning (rIPC) reduces infarct size in a models of myocardial infarction (MI) with reperfusion, however the effect of rIPC on post-infarction ventricular remodelling is controversial. The aim was to evaluate the effect of rIPC on early (7days) and late ventricular remodelling (28 days) in a model of MI without reperfusion. Male FVB mice were underwent to MI without reperfusion and recovered for 7 or 28 days. In other group, a rIPC protocol (3 cycles of 5 min ischemia/reperfusion in the left lower limb) was performed before MI. The sham group were undergoing a thoracotomy without MI. The same procedures were performed in thioredoxin-1 dominant negative mutant mice, which were recovered for 7 days post-MI. We measured infarct size, ventricular function, MMPs 9 activity and Trx-1 expression in ventricular tissue and calculated the expansion index. While oxidative damage to macromolecules, TNF-α, IL-6 and IL-10 were measured in serum. At 28 days we evaluated ventricular function and collagen volume. At 7 days, MI and rIPC groups had the same infarct size, however rIPC improved systolic function (p≤0,05) and attenuated ventricular expansion (p≤0,05). rIPC significantly reduced MMP-9 activity, proteins and lipids oxidation, as well as TNF-α and IL-6 levels (p≤0,05). IL-10 was increased in rIPC compared with MI group (p≤0,05). Additionally, rIPC increased the Trx-1 expression in the MI remote area (p≤0,05). Meanwhile, in the transgenic mice the rIPC cardioprotection was completely abolished, in terms of ventricular function, oxidative stress and inflammatory response. Finally, at 28 days, rIPC decreased collagen volume in the MI remote zone and improved ejection fraction (p≤0,05). In conclusion, rIPC increases Trx-1 ventricular expression, prevents the development of ventricular early and late remodeling and congestive heart failure through a mechanism related to a reduced oxidative stress and inflammatory response, independently of the infarct size.

29. 648. **DIFFERENTIAL RESPONSE TO BRADYKININ OF THE YOUNG FEMALE RAT MESENTERIC ARTERIAL BED**

Luis López Fernández[1], Rodrigo O. Marañón[2,3]

Cátedra de Oftalmología de la Facultad de Medicina de la Universidad Nacional de Tucumán[1].Departamento de Morfofisiología, Instituto de Fisiología, Facultad de Medicina, Universidad Nacional de Tucumán-INSIBIO-CONICET[2].

Bradykinin is a peptide that plays a significant role in vascular function regulation. It is a potent vasodilator, meaning it relaxes blood vessels and increases blood flow. Regarding its effect on the mesenteric arterial bed in female rats, there is limited direct information available. Consequently, in a study of the interaction of the adrenergic and kallikrein-kinin systems, we studied the effects of bradykinin (BK) on the response of the rat mesenteric arterial bed of young female rats to vasoconstrictor agents. The isolated mesenteric bed was connected to a peristaltic pump, recording the mean perfusion pressure (PPM) by a transducer connected to a paper recorder. Perfusion with high concentrations of K+ (K) and noradrenaline (NA) gave vasoconstrictor responses directly related to the concentration of the agent. BK infusion, in contrast, had no effect at concentrations greater than 5.0×10^{-7} M, while at lower concentrations (<2.5×10^{-7} M), the response tended to be vasoconstrictive without reaching statistical significance. In addition, continuous infusion of BK significantly inhibited the response to K but, in contrast, did not affect the action of NA, with no clear explanation for the phenomenon. The response to bradykinin can vary based on factors such as the concentration of bradykinin applied, the specific receptors that are activated, and the overall health and condition of the rats being studied. Additionally, hormonal fluctuations and the reproductive cycle of female rats may also influence the mesenteric arterial bed response to bradykinin. Therefore, further investigations are needed to contribute to our understanding of cardiovascular physiology and its implications for vascular dysfunction conditions, such as hypertension and ischemic disorders.

P-CARDIOVASCULAR & RESPIRATORY
THURSDAY 16TH NOVEMBER 14:00-15:30
CHAIRS: GRACIELA CALABRESE
MARÍA GABRIELA MARINA PRENDES
ANA MARÍA PUYÓ

30. 31. **HYPERTHYROIDISM REDUCES THE CARDIOPROTECTION OF SUBACUTE ORAL TREATMENT WITH NEBIVOLOL IN HEARTS EXPOSED TO ISCHEMIA-REPERFUSION: MECHANISMS INVOLVED**

María Inés Ragone[1,3], Matías Bayley[1], Romina G. Díaz[2,3], Alicia E. Consolini[1]
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The third generation beta-blocker nebivolol could be useful to prevent stunning in patients suffering myocardial ischemia. However, influence of hyperthyroidism is not known. The consequences and mechanisms of subacute oral treatment with nebivolol in hearts from euthyroid (EuT) and hyperthyroid (HpT) rats exposed to ischemia-reperfusion were investigated. Its effects were compared with those of subacute oral atenolol, a first-generation β1 antagonist. EuT and HpT rats were orally treated during 1 week with 20 mg/kg/day nebivolol (Neb-O), 30 mg/kg/day atenolol (Ate-O) or not treated (C). Isolated perfused hearts were exposed to 30 min ischemia and 45 min reperfusion (I/R) inside a flow calorimeter. Left intraventricular pressure and total heat rate (Ht) were continuously measured. Maximum developed pressure (P, mmHg), diastolic pressure (LVEDP) and total muscle economy (Eco=P/Ht, mmHg.g/mW) were calculated. In EuT, Neb-O significantly increased post-ischemic contractile recovery (PICR) (110.5±17.7% vs 19.8±4.6% of initial, n=5-9, p<0.05) and Eco (5.10±0.72 vs 1.55±0.41 mmHg.g/mW, p<0.05) without changes in ΔLVEDP. In HpT, Neb-O induced a slight increase in PICR (47.7±7.4% vs 23.5±3.4%; n=6-5, NS) and Eco (3.5±0.2 vs 1.6±0.2 mmHg.g/mW, NS), but increased ΔLVEDP. Neb-O effects were reversed by perfusing L-NAME (non-selective inhibitor of NOS) in both EuT and HpT. Aminoguanidin (selective iNOS inhibitor) reversed effects in EuT but partially in HpT. Neb-O does not affect the expression stablished differences in eNOS/iNos between EuT and HpT, but also showed a tendency to increase iNOS in EuT. Ate-O totally prevented stunning in HpT and partially in EuT. Results suggest that oral subacute nebivolol prevented stunning in EuT hearts due to adrenergic β1 blockade and activation of eNOS/iNOS, but in HpT hearts this effect was attenuated by excessive iNOS-dependent nitrosative pathways.

31. 116. **MITOCHONDRIAL PATHWAYS IN ENDOTOXEMIA: BIOENERGETICS AND ROS PRODUCTION IN H9c2 CARDIOMYOCYTES**

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Alterations in energy production, redox balance and active mitochondrial dynamics in cardiac tissue during endotoxemia have been previously demonstrated by our group. The aim was to elucidate the role of mitochondria in energy and ROS production during endotoxemia in a cardiomyocytes cell model. Rat myoblast cell line H9c2 was cultured under standard conditions. Myoblasts were differentiated into cardiomyocytes with 100 nM retinoic acid and treated for 1, 3, or 6 h with serum from animals with severe endotoxemia (SE, 8 mg kg^{-1} LPS) or low-grade endotoxemia (LE, 0.5 mg kg^{-1} LPS). ATP production and mitochondrial membrane potential were measured to study mitochondrial function. ATP production was found decreased by 38% at 1 h and 19% at 3 h in LE. Moreover, in SE the decrease was 50% at 1 or 3 h and 84% at 6 h (control: 161 ± 17 nmol ATP min^{-1} mg $protein^{-1}$, p<0.05). Mitochondrial inner membrane potential was increased by 43% at 1 h in LE and 93% at 1 h or 50% at 3 h in SE (control: 100 ± 29, p<0.05). ROS production and redox state were assessed through different approaches. ROS production was found increased by 55% at 1 or 3 h in SE (p<0.05). In particular, O_2^- production was increased by 56% at 1 h in SE (p<0.05). An increase of NO production by 37% was found at 6 h in LE while in SE a 40% increment was found at 1 or 3 h (p<0.05). Also, H_2O_2 production presented an increase of 76% at 1 or 6 h and 90% at 3 h for SE (control: 0.153 ± 0.034 nmol H_2O_2 min^{-1} mg $protein^{-1}$, p<0.01). These data were complemented with an analysis of mitochondrial dynamics. Our results highlight the role of mitochondria and their pathways in endotoxemia. This observation is mainly defined by ATP metabolism and mitochondrial structure. It is important to note that these results are dependent on the time and degree of inflammatory insult in this model of endotoxemia.

32. 163. **LEPTIN-THYROLIBERIN PATHWAY IN LEFT VENTRICLE HYPERTROPHY: DIRECT ACTION ON CARDIAC CELL CULTURE.**

Micaela Landro Geada[1], Maia Aisicovich[1], Ludmila S Peres Diaz[1], María Fernanda Rodriguez[1], Maria S Landa[1], Mariano L Schuman[1], Silvia I García[1,2].
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Cardiac TRH (cTRH) induces LVH and fibrosis in the heart. As the adiponectin Leptin induces TRH in the CNS, we hypothesized that in obesity, the increase of cTRH induced by hyperleptinemia was responsible for the LVH, mostly attributed to pressure load. In accordance, Agouti hyperleptinemic mice presented higher cTRH expression accompanied with a marked fibrosis and LVH. LVH and fibrosis were prevented with the cTRH system blockade pointing out that cTRH mediates LVH development induced by hyperleptinemia. To confirm that TRH´s Leptin induction was direct, we used cardiomyocytes cell line H9C2 stimulated with Leptin (100 ng/ml) and evaluated TRH expression by RT-PCR and peptide by WB. TRH expression and precursor content increased (p<0.05) at 30min and 4h post leptin treatment. With the purpose of studying if TRH mediates the action of leptin on cardiac cells, we assayed leptin stimulus (100ng/ml) on H9C2 culture with (control-siRNA) or without TRH (TRH-siRNA) gene silencing and evaluated hypertrophic markers. A group with no siRNA was used as lipofectamine control. We harvested cells (30min, 4h and 8h) post leptin stimulus and evaluated (TRH, BNP, βMHC, TGF-β). RNA was extracted for gene expression by RT-PCR and also we analyzed nucleus to evaluate hypertrophy by microscopy. SiRNA treatment was successful as we observed leptin-induced increase of TRH expression only in the group with the native TRH system, no increase was observed in the siRNA-TRH+leptin treated cells. We found significant increases on TGF-β, βMHC and BNP (ANOVA, n=7, p<0.05) expression and nucleus diameter (ANOVA, n=172, p<0.05) only in the group stimulated with leptin carrying the native system and no difference was observed in the cells with the TRH knocked down, suggesting that TRH is essential for the leptin induce hypertrophic effect. Our results indicate the direct effect of leptin on cardiac cells, placing TRH as the direct mediator of the leptin-induced hypertrophic effect.

33. 442. **BACULOVIRAL THERAPY ENCODING REPROGRAMMING MOLECULES TO INDUCE CARDIOMYOCYTE REPROGRAMMING**

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2-Universidad Nacional de Quilmes, Argentina

Objectives: Ischemic heart failure is one of the leading causes of death worldwide, being heart transplant the only known treatment. An arising therapeutic alternative is cardiac reprogramming through gene therapy. Therefore, we designed a baculoviral vector encoding

reprograming molecules to evaluate its functionality on the conversion of myofibroblasts into cardiomyocytes in vitro. Materials and Methods: Ovine myofibroblasts were transduced with a baculovirus encoding Hand2, Gata4, Tbx5, Myocd, VegfA, miR-133a-3p and miR-29a-3p (Bv-HGTMV-133-29 group) or a null baculovirus (Bv-Null group) at 0 and 5 days of culture. To characterize the reprogramming of myofibroblasts, gene expression of cardiomyocyte genes (TPM1 and TNNT1) and profibrotic genes (Col1A1, Col1A2, Col3A1 and FSP1) was measured by RT-qPCR at 5 days post transduction. Results: Bv-HGTMV-133-29 group showed higher expression of cardiomyocyte genes than Bv-Null group (TPM1: 2.1 vs 1.1 fold increase, p<0.05; and TNNT1: 1.2 vs 0.7, p=0.26). At the same time, Bv-HGTMV-133-29 group showed decreased expression of profibrotic genes compared to Bv-Null group (Col1A1: 0.5 vs 1.0, p<0.05; Col1A2: 0.5 vs 1.1, p=0.9; Col3A1: 0.9 vs 1, p=0.48 and FSP1: 0.7 vs 1, p<0.01). Conclusions: myofibroblasts transduced with Bv-HGTMV-133-29 increased the expression of cardiomyocyte genes and decreased the expression of profibrotic genes. These preliminary results suggest that Bv-HGTMV-133-29 would induce cardiomyocyte like changes, making it a potentially useful treatment for heart failure.

34. 560. **THIOREDOXIN-1 IS INVOLVED IN THE CARDIOPROTECTION MECHANISM OF VOLUNTARY EXERCISE IN MICE**

Eugenia Godoy Olazar[1], Virginia Perez[1,2], Verena Franco Riveros[1,2], Jorge Godoy Olazar[1], Aurora Lee[1], Bruno Buchholz[1,2], Verónica Casanova[3], Carla Greco[3], Eliana Cicale[3], Tamara Zaobornyj[4], Verónica D´Annunzio[1,2].

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Voluntary exercise reduces myocardial injury caused by acute ischemia/reperfusion (I/R). However, whether thioredoxin1 overexpression (Trx1) is involved in the cardioprotection mechanism of voluntary exercise has not been studied. The aim was to study if Trx1 and exercise share cardioprotection mechanisms. Wild type mice hearts (Wt), transgenic mice hearts overexpressing Trx1, and a dominant negative mutant (DN) of Trx1 were used and divided in exercise (E) and sedentary group (S). Mice hearts were subjected to 30 min of I and 120 min of R (Langendorff technique). We measured infarct size, and transverse sections of quadriceps, gastrocnemius, soleus and myocardium muscles (H&E) and the cross-sectional area (CSA) of myofibers were measured. Also, western blotting was performed to study GSK3β phosphorylation. Data were expressed as mean±SEM and p<0.05 was considered statistically significant. n= 4 each group. As we previously showed, training was confirmed by heart rate variation and exercise reduced infarct size in Wt but not in DN mice. The changes in body weight and running distance at the fourth week of training in transgenic mice were comparable with Wt mice. Nevertheless, caloric intake was higher in E groups compared to S groups. Heart weight increased significantly with exercise and soleus weight was greater in Wt-E and DN-E groups but were similar in S groups. There were no differences in quadriceps and gastrocnemius weight between E and S groups. CSA results showed a similar behavior as muscles weight (soleus: Wt-S: 1164±89, Trx1-S: 1274±166, DN-S: 1102±144, Wt-E: 1665±174, Trx1-E: 1169±170, DN-E: 1326±86).Finally, GSK3β phosphorylation was increased in E groups (Wt-I/R: 1.28±0.14, Trx1-I/R: 1.28±0.08, DN-I/R: 1.08±0.14) compared with S groups (Wt-I/R: 0.87±0.12, Trx1-I/R: 1.02±0.06, DN-I/R: 1.04±0.17). In conclusion, we found that Trx1 and exercise could share cardioprotection mechanisms.

35. 626. **ISCHEMIC STROKE INDUCES MILD ACUTE LEFT VENTRICULAR DYSFUNCTION AND ECG ABNORMALITIES IN A MOUSE MODEL OF BRAIN ISCHEMIA/REPERFUSION**

Ignacio P. Barbieri[1], Verena B. Franco-Riveros[1,2], Verónica Casanova[3], Bruno Buchholz[1,2].

[1] Universidad de Buenos Aires. Facultad de Medicina. Departamento de Patología. Instituto de Fisiopatología Cardiovascular. [2] Consejo Cacional de Investigaciones Científicas y Técnicas – CONICET. [3] Universidad de Buenos Aires, Facultad de Ciencias Veterinarias.

Objective: The aim was to determine if a transient ischemic stroke induces cardiac dysfunction through autonomic imbalance, due to sympathetic overactivity. Materials and Methods: Mice underwent transient middle cerebral artery occlusion (tMCAo) for 60 min and 24h reperfusion. Gsalpha transgenic mice (TG) were used as a model of basal sympathetic hyperactivity and were also exposed to tMCAo (I/R-TG). Left ventricular function (LVF) was assessed by echocardiography: ejection fraction (EF), shortening fraction (SF) and isovolumetric relaxation time (IVRT). Electrocardiography (ECG) was performed throughout the ischemia and after 24h of reperfusion, to assess electric abnormalities. Infarct size (IS) was measured by TTC stain after 24h reperfusion. Neurological deficit was assessed 4h and 24h after tMCAo using Longa's scale (LS). Mortality was also assessed and compared between groups. Results: tMCAo produced similar IS in both experimental groups (I/R-NTG: 30.0 ± 4.3 %, I/R-TG: 26.2 ± 5.0 %; p=NS). We observed LV dysfunction, characterized by a mild decrease in EF in NTG mice (I/R-NTG: 72.5±1.8% vs SHAM NTG: 87.4±1.0% p<0.05) and TG mice (I/R-TG: 81.4±1.5% vs SHAM TG: 87.3±1.3% p<0.05) and SF in NTG mice (I/R-NTG: 42.6±1.9% vs SHAM NTG: 51.3±1.8% p<0.05) and TG mice (I/R-TG: 44.8±1.4% vs SHAM TG: 49.8±1.5% p=NS). IVRT significantly decreased in the TG group (I/R-TG: 18.1±0.7ms vs I/R-NTG: 20.8±0.7ms; p<0.05). Prolonged QTc interval was observed after 60 min of ischemia (157.4±5.8ms) and persisted after 24h reperfusion (158.9±2.9ms) respect to basal (129.2±5.2ms; p<0.05) in the I/R-TG group. After 24h of reperfusion, 42.9% of the NTG mice survived, compared to 26.7% of TG mice (p<0.05). Conclusion: tMCAo induces an acute mild LV dysfunction and ECG abnormalities after 24h reperfusion. A significant increase in the mortality rate was observed in TG mice.

P1-CELLULAR AND MOLECULAR BIOLOGY

WEDNESDAY 15TH NOVEMBER 9:00 - 10:30

CHAIRS: CLARA MARÍN BRIGGILER
MARÍA LAURA RIBEIRO

36. 14. **PROTUMORIGENIC GALECTINS 1 AND 3 ARE UPREGULATED IN THE LIVER OF MICE WITH GROWTH HORMONE DEFICIENT ACTION**

Santiago De La Fuente [1], María Lorena Bacigalupo [1], Luciana Sarrias [1], Lorena Rocío García [1], Ana Isabel Sotelo [1], Andrzej Bartke [2], María Fernanda Troncoso [1], Johanna Gabriela Miquet [1]

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Studies in humans and animals revealed a link between growth hormone (GH) and cancer risk. Transgenic mice overexpressing GH (GH-Tg) exhibit increased incidence of spontaneous and carcinogen-induced hepatocellular carcinoma. Galectins (GAL) 1 and 3 are involved in liver tumorigenesis in humans. Their expression is very low in normal hepatocytes but increase in liver tumors. We previously found that GAL1 and GAL3 are upregulated in GH-Tg mice liver, suggesting that GH positively modulates the hepatic expression of these protumorigenic galectins. The aim of this study was to evaluate if mice deficient in GH action, either due to GH deficiency or to

absence of GH receptor, exhibit alterations in GAL1 and GAL3 liver expression. Hepatic GAL1 and GAL3 were analyzed by immunoblotting and RT-qPCR in GH receptor knockout (GHR-KO) mice, and in Ames dwarf mice deficient in GH, prolactin and thyroid-stimulating hormone. Young adult mice (2-3 months old) were used, normal littermates served as controls. Female and male mice were analyzed in independent experiments. Statistical analysis was performed by unpaired Student´s t-test (P<0.05, significant; at least 6 animals/experimental group). Unexpectedly, GHR-KO and Ames dwarf mice displayed significantly higher GAL1 liver expression compared with normal controls in both sexes. GAL3 was also elevated in GHR-KO males, but displayed no significant changes in GHR-KO females and in Ames dwarf mice of both sexes. Therefore, lack of GH action in mice is associated with hepatic upregulation of GAL1 in both sexes, while the regulation of GAL3 seems to be more complex and exhibit sexual dimorphism. Considering that the hepatic expression of these galectins was also increased in mice overexpressing GH, we postulate that exposure to normal pulsatile GH secretion is implicated in the maintenance of normally low expression of these galectins in the adult liver. The relevance of these changes in liver health and disease remains to be elucidated.

37. 175. **UVB INCREASES OXIDATIVE STRESS AND INDUCES SENESCENCE IN DERMAL PAPILLA SPHEROIDS ALTERING EPITHELIAL-MESENCHYMAL INTERACTIONS THAT AFFECT HAIR FOLLICLE AGING**

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Instituto de Ciencia y Tecnología César Milstein- Fundación Pablo Cassará- CONICET- Argentina.

The hair follicle is a compelling model in aging research, as its miniaturization and the hair loss are significant phenotypic traits of aging. Dermal papilla cells (DPC) regulate cyclic hair growth by inducing differentiation of hair follicle stem cells (HFSC). DPC from bald patients have reduced proliferative capacity associated with premature cell senescence. We previously established a 3D model of UVB-induced senescence in human DPC spheroids and observed that senescent DPC lose their inductivity on HFSC differentiation. We further characterized this model and explored the impact of senescent DPC on paracrine senescence in keratinocytes. Repeated UVB exposure (4mJ/cm^2, six times) upregulated expression of senescence associated secretory phenotype (SASP) factors (IL-1α, IL-1β, IL-6, MMP-1 and PAI) accentuating the pro-inflammatory milieu and diminished laminB1 marker in DPC spheroids. Moreover, catalase enzyme activity increased in a time dependent manner (UVB 2.11-fold vs Control at 24h) and percentage of ROS positive cells was significantly higher in UVB irradiated DPC, indicating elevated oxidative damage. Also, Dkk-1, an inhibitor of HFSC differentiation was upregulated, whereas Wnt10b, known inducer of differentiation, was downregulated by UVB. Keratinocytes derived from HFSC stop proliferating and upregulate SASP expression when cultured with conditioned medium from irradiated DPC spheroids. We conclude that oxidative damage generated by UVB is a major factor that induces senescence, which in turn, diminishes inductivity in DPC spheroids. Moreover, keratinocytes showed paracrine senescence from irradiated DPC, that would impair their differentiation to hair lineage. These findings underscore the disruptive influence of UVB-induced senescence on the crosstalk between epithelial (HFSCs) and mesenchymal (DPCs) compartments and shed light on potential targets for interventions aimed at mitigating the deleterious effects of senescence in hair follicle aging.

38. 232. **THE ROLE OF GLUCOCORTICOID RECEPTOR IN PANCREATIC PROGENITOR CELL DIFFERENTIATION AND β CELL GENESIS**

Lucas Bacigalupo[1,2], Silvio A. Traba[1,2], Ana C. Heidenreich[1,2], Daniele Muraro[3], Jose Garcia-Bernardo[3], Chris Gribbens[3], Fatima Lugtu[3], Juan Ignacio Burgos[1,2], Agustín Romero[1,2], Mariya Chhatriwala[3], Adalí Pecci[1,4], Ludovic Vallier[3,5†], Santiago A. Rodríguez-Seguí [1,2†]
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The *in vitro* production of functional β-cells for transplantation in type 1 diabetic patients is a long standing challenge that could suppress insulin administration. It has been shown that while conditional deletion of glucocorticoid receptor (GR) in Pdx1+ progenitors (an thus from the beginning of pancreas specification) increases β-cell mass, its deletion from the β-cell stage does not have the same effect. To gain insights into this mechanism, here, we profiled the transcriptomic changes of *in vitro* derived pancreatic multipotent progenitor cells (MPCs) treated with Dexamethasone (Dex, a known GR agonist) or control (Ethanol) at the single-cell level (scRNA-seq). Analysis of these data, combined with the MPC epigenomic profile and scRNA-seq profiled in the human embryonic pancreas, revealed the induction of genes with a known role in pancreas development, as well as other transcripts with yet unknown functions. Taken together, our results suggest that activation of the GR in this model induces MPC differentiation to endocrine progenitors (EPs). In sharp contrast, a similar treatment with Dex in E11.5 embryonic mouse pancreatic explants (composed mostly of MPCs at this stage) induced cell commitment to the acinar fate. These results were supported by immunofluorescence and RT-qPCR ($p<0.05$) for key genes which were regulated by Dex in the human pancreatic differentiation model. To assess the potential mechanisms underlying this difference, we performed E11.5 mouse pancreatic explants culture in the presence of Dex with or without Dkk-1 (an inhibitor of the Wnt pathway, known to induce acinar differentiation in MPCs), as well as their corresponding controls. Our preliminary results show that treatment with Dkk-1 enhances the expression of genes involved in EP differentiation. Future work aims at evaluating in more detail the effects of the combined treatment, ultimately revealing how the GR might tune MPC differentiation towards the endocrine fate.

39. 234. **ANTIOXIDANT CAPACITY OF A PLANT-BASED BEVERAGE IN CELLS EXPOSED TO THE HERBICIDE PARAQUAT**

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Normal aerobic metabolism in eukaryotes through cell respiration involves mitochondrial activity and oxygen consumption to generate the necessary energy for life. This creates an excess of free radicals that can easily react with biomolecules, causing damage. Cells have an endogenous enzymatic defense system (i.e. catalase, CAT, and gluthathione-s-transferase, GST) that is triggered in presence of free radicals generators, such as the herbicide paraquat. Food like plant-based beverages are a novel source of antioxidants, and the consumption of foods of plant origin emerges as an alternative that satisfies the demands conditioned by health, ethical, environmental or dietary issues. The aim of this work was the *in vitro* assessment of the antioxidant capacity from a plant-based fermented beverage previously developed in UADE. For this, CHO cells were treated either with DMEM/F-12 (control, CTL), paraquat 100uM (PQ100)

for 24hs, an 1:10 dilution of the beverage antioxidant extract (B) or with paraquat 100 uM for 22hs and then for 2hs with the extract (PQ100+B). Cell viability (trypan exclusion assay) and antioxidant enzymatic defense (CAT, GST) activities were determined *in vitro*. Statistical analysis was performed using an ANOVA followed by Tukey's multiple-comparison post hoc test. Results showed that CAT activity was higher in PQ100 group, but remarkably, PQ100+B treatment significantly reduced enzymatic activity, near to CTL values. On the other hand, GST activity was also significantly increased in cells treated with paraquat, but post-treatment with the antioxidant extract changed this pattern. Trypan blue exclusion assay revealed that neither PQ100 treatment, B, nor both combined affected cell viability. These results suggest that the CAT is the main antioxidant defense system that is triggered by paraquat, and that the antioxidant content of the beverage can modulate its activity without altering cell viability in a context of oxidative stress.

40. 257. **HYPOCHLOROUS ACID AS POTENTIAL ENDODONTIC IRRIGANT IN YOUNG PERMANENT TEETH. PRELIMINARY STUDY**

Romina Loiacono[1], Lorena Cabirta[1], Pablo Rodríguez[1], Ariel Gualtieri[1], Gisela Soledad Gualdoni[2], Romina De Lucca[2].
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Sodium hypochlorite (NaClO) is the most commonly used irrigant in endodontic therapy. It shows acceptable clinical and radiographic results; however, its high toxicity should not be discarded, especially in young patients where underdeveloped apices have higher permeability towards periapical tissues requested in regeneration. Hypochlorous acid (HOCl) is the bactericidal fraction from NaOCl solution.The aim was to evaluate the effect of HOCl as endodontic irrigant on apical periapical tissues in young permanent teeth in an experimental rat model. Wistar rats (4 weeks) were intraperitoneal injected with ketamine/xylazine to open the occlusal face of lower first molars using a carbide bur. Dental pulp was disrupted followed by irrigation with saline (S) in the left molar and 2.5% NaOCl (T1) or 0.05% HOCl (T2) in the right molar. Control group received no treatment. Animals were euthanized with EUthanyl at 2 or 7 days to dissected jaws for microCT scanning and histology (H&E). MicroCT revealed that at 2d, interradicular bone volume (IBV) tended to be lower in S and T1 compared to T2 (S 1,28±0,46 T1 1,24 ±0,38 T2 1,52±0,29) with no significant differences (NS). The thickness of the periodontal ligament (LP) showed apparent compression in T1 compared to T2 (T1 1,76 ±0,50 T2 2,83±0,85) at 2 and 7d. At 7d post-treatment, the IBV seems to be lower in T1 compared to others (S 1,97± 0,53 T1 1,42±0,44, T2 2,11±0,37), NS. Histologically, S group showed exacerbated inflammatory processes at 2d due to an extended lymphomonocytic infiltrate in the canal, coronal pulp, LP and medullary spaces of IBV, extending up to a third of the total molar height, in contrast to the controls which showed no infiltrate. In T1 and T2, the inflammatory infiltration was confined to the periapex and lower third of intraradicular bone marrow. Based on these results, we could conclude that periapical tissue response to irrigation with HOCl is biologically acceptable and similar to hypochlorite action.

41. 298. **EXTRACELLULAR VESICLES FROM BACILLUS SUBTILIS 168 AND THEIR EFFECT ON HUMAN HCT-116 CELLS**

Barnech Carolina[1,2],, Gaona Bruno[1], Malamud Florencia[1,2], Coluccio-Leskow Federico[1,2] and Cimolai María Cecilia[1,3]
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Bacillus subtilis is a Gram-positive bacterium that has demonstrated probiotic properties and can be isolated from various environments with many applications. Bacterial extracellular vesicles (EVs) are nanoscale structures composed of lipids, proteins, and nucleic acids that are released into the extracellular medium by membrane blebbing. Our hypothesis is that secreted EVs may serve as communication particles. Our aim was to characterize *Bacillus subtilis* EV in detail and to evaluate their possible role in inter-kingdom-related responses. EV isolation was performed by several steps of centrifugation, filtration and ultracentrifugation of culture supernatants obtained from *Bacillus subtilis 168*. The resulting EV samples were extensively characterized by TEM, DLS and macromolecules content. The EV protein load was qualitatively analyzed by SDS-PAGE and proteomics (Orbitrap nanoHPLC). Gene ontology analysis revealed the presence of proteins related to metabolic processes, biological regulation, localization and response to stimulus. Furthermore, EV generated under oxidative stress (H_2O_2 58 µM, non-lethal dose) were enriched in stress response related proteins . Therefore, the ability of these bacterial EV to modify the eukaryotic cellular response to stress was analyzed by treating human colon cancer cells (HCT-116). EV pre-treatment (0.5 µg/well; 2 h) showed protection when cells were loaded with DCFDA (2',7'-dichlorofluorescin diacetate) and challenged with H_2O_2 to detect intracellular reactive oxygen species (ROS). In summary, we could demonstrate that EV are secreted into the extracellular medium and that their *cargo* is modified by environmental conditions. Considering that bacterial EV could protect cells from external insults, it is plausible that EV represents a new cell-cell communication medium that needs to be further investigated.

42. 445. **NUCLEAR DISTRIBUTION OF SOX2 IN EMBRYONIC STEM CELLS**

María Candelaria Diaz[1], Camila Oses[1], Paula Verneri[1], Marcos Francia[1], Martin Stortz[1], María Cecilia De Rossi[1], Alejandra Guberman[1,2], Valeria Levi[1,3]
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The transcription factors (TFs) OCT4, SOX2 and NANOG maintain the fundamental properties of embryonic stem cells (ESCs): unlimited capability of self-renewal and pluripotency. In mouse ESCs these TFs distribute heterogeneously forming condensates or foci. It was proposed that OCT4 condensates are involved in the reorganization of genome topologically associated domains required for cell reprogramming. This evidence suggests that TFs condensates could be relevant to cell fate decisions. However, it remains elusive whether SOX2 or NANOG incorporate in these condensates in live cells and their impact in pluripotency. Here, we studied the nuclear distribution of SOX2 by using confocal microscopy in living mouse ESCs expressing this TF fused to the fluorescent protein YPet. We analyzed the biophysical behavior of the foci formed by SOX2-YPet to assess if they present properties compatible with those expected for liquid condensates and studied their response to osmolarity changes, to perturbations in the chromatin structure, and to the disruption of the nucleus-cytoskeleton mechanical communication. Finally, we tested SOX2-OCT4 interactions at condensates and analyzed the dynamics of TFs exchange by performing FCS (Fluorescence correlation spectroscopy) and FRAP (Fluorescence recovery after photobleaching) experiments. Our results suggest that SOX2 dynamically partitions in OCT4 condensates although this recruitment does not require OCT4-SOX2 interactions. In addition, chemical agents that perturb the epigenetic landscape of chromatin affected SOX2 condensates suggesting that these structures depend on the chromatinic environment. These results could contribute to unveiling the molecular mechanisms involved in the interplay between pluripotency TFs, impacting in gene regulation programs that ultimately define cell identity.

43. 495. **ROLE OF VMP1 IN THE REGULATION OF THE INFLAMMASOME ACTIVATION IN ACUTE PANCREATITIS**

Lourdes Torasso, Candelaria Santorun, Felipe Javier Renna, María Inés Vaccaro, Alejandro Ropolo.
Instituto de Bioquímica y Medicina Molecular Prof. Alberto Boveris (IBIMOL), Universidad de Buenos Aires, CONICET.

Acute pancreatitis (AP) is a local inflammation that induces systemic inflammatory response syndrome in severe presentations. VMP1 is an autophagy-related protein induced in pancreas during AP. Previously, we demonstrated that VMP1-mediated autophagy is involved in selective degradation of damaged mitochondria, mitophagy, and degradation of zymogen granules activated during AP, zymophagy. Thus, VMP1-mediated autophagy prevents disease severity. The Inflammasome is a multimeric protein complex responsible for the activation of inflammatory responses leading to IL-1β production. NLRP3 is a sensor protein of inflammasome involved in the activation of monocytes and neutrophils that infiltrate pancreas in the AP context. Activation of the inflammasome has been associated with disease severity. Autophagy regulates inflammasome removing NLRP3 components, activators, and cytokines. In this context, we hypothesize that VMP1-mediated autophagy modulates the inflammatory process by regulating inflammasome activity. To assess if VMP1 is implicated in inflammasome activation we used monocyte cell lines. We worked with THP-1 and THP-1-ASC-GFP cells activated by LPS+ATP. We found out that NLRP3-inflammasome activation increased VMP1 expression and IL-1β production analyzed by western blot. The adaptor molecule ASC links inflammasome sensors to IL-1β production. In THP-1-ASC-GFP cells with no stimulation, no GFP signal was detected. Upon inflammasome activation, ASC-GFP polymerizes, and a green spot was observed under the fluorescence microscope. The expression of ASC-GFP analyzed by western blot was also increased. Interestingly, inhibition of NLRP3 with butyrate induced strong expression of VMP1 and autophagy, evidenced by LC3-II expression by western blot. All these results suggest that VMP1-mediated autophagy regulates NLRP3-inflammasome activation. This is another possible mechanism by which VMP1-mediated autophagy would reduce the severity of AP.

44. 501. **IMPACT OF AGE-ASSOCIATED CHANGES IN RET-INDUCED MAMMARY TUMOR DEVELOPMENT**

Clara de los Santos[1], Roberto P. Meiss[2], Edith C. Kordon[1], Sabrina A. Vallone[1]*, Albana Gattelli[1]*.
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Most cancers arise in individuals over the age of 60-years old. As the world population is living longer, cancer is becoming a substantial public health problem. Yet, the contribution of aging to oncogenic signals is largely ignored, with most preclinical studies designed in 2-month-old mice rather than older mice reflecting an age-appropriate to the disease being modeled.
We study the oncogenic function of RET receptor tyrosine kinase in breast cancer. RET is overexpressed in 40% of breast tumors respect to normal tissue and high RET correlate with decreased survival. Using a doxycycline (DOX)-induced transgenic mouse system (RET/MTB), we previously demonstrated that RET expression in the mammary epithelium induced estrogen receptor (ER) positive tumors, representing the human luminal subtype. Here, we aim to address the impact of aged microenvironment in the development of neoplastic lesions induced by RET. Firstly, we analyze mammary gland tissue from aged-virgin female mice. In addition to the reported morphological changes (histological analysis), we observe that aged glands (10 to 12-month-old, none cycling) express endogenous RET protein which is generally absent in young counterparts (2 to 4-month-old). Interestingly, phosphorylation (p) pattern of RET as well as ER, is differential: aged mammary gland displays high levels of the pY1062RET fully glycosylated isoform and an increase in both pS167ER and pS118ER (Western blot) respect to young mammary tissue. Then, we chronically DOX-induced RET overexpression in RET/MTB aged- vs. young-female's groups. Surprisingly, we found that tumor incidence is reduced in older females (20%, 1/5) respect to younger females (62,5%, 5/8), suggesting an aged-related protective role against RET oncogene. Histology and signalling pathways are being analyzed in both epithelial tumor cells and adjacent mammary tissue microenvironment.

45. 592. **FIRST EVIDENCE OF THE N-RULE MECHANISM IN TRYPANOSOMA CRUZI**

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Introduction: The N-rule mechanism is a proteolytic system in which target proteins are tagged at their N-terminal residues for degradation. This system has been observed in all eukaryotes and prokaryotes studied so far, regulating the homeostasis of various physiological processes crucial for the cell. The target proteins are marked at their N-terminal residues by the action of the aminoacyl-tRNA:protein transferase enzyme, an enzyme present in all cell types. On the other hand, in *Trypanosoma cruzi*, the causative agent of Chagas disease, there are no records about this mechanism or its components in. Methods: Using the Tritryps database and *in silico* prediction tools, we identified the gene TcCLB.506977.40 as a candidate encoding an aminoacyl-tRNA:protein transferase-like enzyme. The gene was amplified by PCR and cloned into a vector (pET22 b+) compatible with a bacterial expression system. The recombinant vector was sequenced to confirm the cloned fragment identity. Additionally, the product of the TcCLB.506977.40 gene was studied through RT-PCR. Results and conclusions: *In silico* analysis of the hypothetical protein from TcCLB.506977.40 gene showed the presence of a complete transferase domain, which shares 96% similarity with transferase domains reported in other species. The PCR-amplified had a length of 1080 bp, and its sequencing indicated similarity of 98% to the sequence reported in TriTryps. This sequence was *in silico* translated and the protein product obtained showed 97% of similarity with the described transferase domain. RT-PCR revealed the presence of a band of the expected size and a second band 200 bp smaller. This observation would suggest that the gene is being expressed and has two isoforms, possibly obtained through alternative sites to trans-splicing. In conclusion, our studies suggest that *T. cruzi* would have an active aminoacyl-tRNA:protein transferase-like protein, and therefore, the N-rule mechanism might be functional in this parasite.

46. 663. **CELL ADHESION MODULATION BY A CONDUCTIVE POLYMER-BASED PLATFORM**

Miguel Pasquale[1], Marcos Carballido[1], Omar Azzaroni[1]
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Conductive polymers are promising materials for tissue engineering as well as the differentiation of neural stem cells. In this contribution, the viability and adhesion of murine pre-osteoblast (MC-3T3) and myoblast (C2C12) cells are modulated by a conductive polymer poly-3,4-ethylenedioxythiophene (PEDOT)-based thin film and the application of electrical perturbations. The film was fabricated employing PEDOT doped with either synthetic or natural polyelectrolytes (PE) and deposited on a glass substrate by spin-coating. Films with different thickness are achieved by changing the depositing conditions. Poly(allylamine hydrochloride) (PAH) was used as synthetic PE, and polysaccharide-based PEs as natural ones. The electrical perturbation consisted in a constant potential applied by (a) a three-electrode configuration with the contact points inside the cell culture medium, and (b) by a configuration with contacts outside the medium, both employing a potentiostat. Results indicated that cell proliferation and cell adhesion are significant larger in a PEDOT/natural PE-based films than that observed for PEDOT/synthetic PE-based films. These observations were independent of the film thickness, at least up to 120 nm. Potential application experiments indicate that low negative potential applied employing the configuration (a) favors MC-3T3 cell adhesion measured by the average

spreading area of cell cytoplasm. In contrast no significant effect was observed for C2C12 cells. In the other hand, configuration (b) for applying a potential with contact outside the culture medium, indicated that film regions at relative negative potentials were more appropriate for either MC-3T3 and C2C12 cells. Results can be rationalized by basic characteristics of the double layer modulated by the applied electric potential. Results from this contribution may have implications in cell adhesion and differentiation processes on conducting polymer.

O1-CELLULAR & MOLECULAR BIOLOGY

THURSDAY 16TH NOVEMBER 9:00 - 10:30
CHAIRS: DÉBORA COHEN
GABRIELA JAITA

47. 69. **DIFERENTIAL EXPRESSION OF LONG NON-CODING RNAs (LNC-RNAs) TRANSCRIBED FROM TELOMERES DURING THE PROCESSES OF MIOGENESIS AND ADIPOGENESIS**

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Lnc-RNAs transcribed from telomeres, known as TERRA (*telomeric repeat-containing RNA*), are associated with telomere and genome stability. Telomeres are particularly prone to be damaged by oxidative stress and, their protection is relevant to avoid their dysfunction. We showed that the level of TERRAs is increased in response to oxidative stress as a mechanism of telomere protection. However, ROS have proven to be signaling molecules, and as such we found that a physiologic ROS increase required for adipogenesis to proceed induces TERRA expression. Thus, we asked whether TERRAs are regulated during other cell differentiation processes, such as myogenesis. Murine C2C12 myoblasts were induced to differentiate, and a bifasic expression of TERRAs was observed. They increased at day 2 post induction of C2C12 cell differentiation but dramatically decreased at day 7 in fully differentiated myotubes. Thus, TERRAs exhibit a high expression in adipocytes but a negligible expression in myotubes. Next, we tested whether C2C12 myoblasts and particularly myotubes could induce TERRAs expression in response to oxidative stress. In C2C12 myoblast or myotubes incubated with 500µM H_2O_2, TERRAs level dramatically increased, event prevented in the presence of the antioxidant N-acetyl-L-cysteine. To test whether oxidative stress induced DNA damage at telomeres, we. evaluated the effects of H_2O_2 exposure on the formation of persistent telomere-associated DNA damage foci (TAF) by IIF labelling TRF1, a component of the sheltering complex in telomeres and gH2AX, the phosphorylated histone variant found at sites of DNA damage. We found an increased in TAFs in both myoblasts and myotubes incubated in the presence of H_2O_2. In summary, TERRAs expression is differentially regulated in myogenesis compared to adipogenesis, possibly dependent on the action of ROS as signaling molecules. However, C2C12 myoblasts and myotubes respond to oxidatve stress inducing TERRAs possibly to protect telomeres.

48. 129. **AMPLIFICATION OF PITUITARY TUMOR SENESCENCE BY IL-6: A MECHANISM TO CONTROL TUMOR EXPANSION**

Florencia Herbstein[1], Melanie Sapochnik [1], David Gonilski-Pacin[1], Nicolas Ciancio del Giudice[1], Manuel Fiz[1], Alejandra Attorresi[1], Cora Pollak[1], Sergio Senin[1], Belén Elguero[1], Mariana Fuertes[1,2], Lucas B. Pontel[1] and Eduardo Arzt[1,2]
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IL-6 is a pleiotropic cytokine of the senescence-associated secretory phenotype (SASP) that has antagonistic actions. IL-6 canonical signaling via STAT3 induces proliferation when it interacts with the membrane receptor, IL-6R. Pituitary adenomas are common, benign tumors characterized by premature proliferative arrest, in which the activation of the cellular senescence program emerges as a mechanism to explain these features. In previous studies, we demonstrated that IL-6 increases senescence in a senescent pituitary cell line, indicating that the origin of IL-6 is crucial for its action. To analyze this mechanism of action of IL-6, we studied the secretion-independent effects of IL-6 in two cellular models of senescence: Pituitary MtT/S cells and doxorubicin-induced senescent A549 cells. In both we observed an increase in senescence biomarkers ($p16^{Ink4}$/$p21^{Cip1}$ and pRb, $p<0.001$) by western blot (WB) when IL-6 accumulates intracellularly with brefeldin A (100 ng/ml), a pharmacological secretion inhibitor. By immunofluorescence, we analyzed the spatial locations of IL-6, IL-6R, and lamin B1 to assess the nuclear membrane integrity, commonly affected by senescence. We investigated the involvement of the cyclic GMP–AMP synthase (cGAS)-stimulator of interferon gene (STING) pathway. We detected nuclear blebbing and overlapping signals of cytosolic DNA with those of IL-6/IL-6R and cGAS-STING in the perinuclear regions when IL-6 was concentrated in the cytoplasm. Furthermore, we observed an increase in STING expression levels by WB in this condition, which when blocked with a STING siRNA, reverses the IL-6 senescence signal. We propose that by this new mechanism IL-6 acts as an amplifier of the cellular senescence in an intracrine manner coupling IL-6R to cytosolic DNA and the cGAS-STING pathway with downstream activators involved in the maintenance of the SASP. Thus, IL-6 contributes to inhibit tumors growth and expansion and to maintain pituitary adenomas benign nature.

49. 405. **PURA AND GLUT1: SWEET SYNERGY FOR BRAIN HEALTH?**

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Introduction: Recent advancements have identified Pur-alpha(PURA) as a key player in normal brain development. With its purine-rich binding capabilities, PURA modulates gene transcription and mRNA transport. It also interacts with other proteins, within the PUR family and beyond, to exert its effects. Intriguingly, hypoglycorrhachia, typically linked to GLUT1 deficiency, emerged in a PURA-deficient patient (Mayorga,et.al.2018), hinting for shared functions between the proteins. We explore PURA's potential influence on GLUT1, hypothesizing regulatory effects. Methods: Hela cells were manipulated to modify PURA and GLUT1 levels (mRNA plasmids and inducible shRNA). mRNA quantification was explored with qPCR. Protein content was measured via Western Blot(WB). Glucose uptake was assessed using *2*-NBDG fluorescent glucose and measured through fluorescent microscopy and flow cytometry. Indirect immunofluorescence+ confocal microscopy was used to unveil spatial relationships, and co-immunoprecipitation (protein A agarose bead PURA immunoprecipitation + GLUT1 WB of the pull-down) explored PURA-GLUT1 complex formation. Bioinformatical protein-protein docking insights were used to predict PURA-GLUT1 interaction and atomistic molecular dynamics to refine it. Results: Overexpressed PURA increased glucose uptake($p<0.05$), though GLUT1 mRNA and protein expression saw minimal shifts. Confocal microscopy displayed proximate PURA-GLUT1 localization supported by a higher Pearson´s coefficient($p<0.05$). PURA immunoprecipitation dragged GLUT1 in the pull-down fraction, suggesting a protein complex. Molecular dynamics simulations showed that PURA-GLUT1 interactions are plausible, supported by the prediction that when PURA is truncated and/or mutated the complex´s stability is jeopardized. Conclusions: PURA enhances GLUT1's function, possibly by forming a regulatory complex. These findings bridge PURA's role with brain glucose control, unraveling paths to decipher neurodevelopmental anomalies.

50. 488. **EVALUATING AUTOPHAGY LEVELS IN PANCREATIC CANCER CELLS USING LC3 IMMUNOFLUORESCENCE**

Felipe Javier Renna, Malena Herrera Lopez, Alejandro Ropolo, María Inés Vaccaro
Instituto de Bioquímica y Medicina Molecular Prof Alberto Boveris (IBIMOL), Universidad de Buenos Aires, CONICET

Autophagy is a specialized catabolic process that selectively breaks down cytoplasmic components, such as proteins and damaged organelles. Autophagy enables cells to respond physiologically to stress stimuli, thus maintaining cellular balance. Cancer cells may adjust their autophagy levels to cope with adverse conditions triggered by chemotherapy. Among the deadliest cancers is ductal pancreatic adenocarcinoma. Pancreatic cancer cells exhibit heightened autophagy activity due to the upregulation of autophagy proteins through transcriptional and post-translational mechanisms. In this study, the PANC-1 cell line served as a model for human pancreatic cancer cells, while the AR42J pancreatic acinar cell line represented highly differentiated mammalian cells. Immunofluorescence of microtubule-associated protein light chain 3 (LC3) served as an indicator of autophagy activation status. LC3, an autophagy-related protein, exhibits a diffuse cytoplasmic distribution under basal conditions (LC3-I). Autophagy initiation prompts the conjugation of LC3 to phosphatidylethanolamine on forming autophagosome surfaces, producing LC3-II, a membrane-bound protein that aids in autophagosome formation and expansion. For quantifying labeled autophagic structures, open-source software FIJI was utilized alongside the "3D Objects Counter" tool. Method validation involved quantifying LC3 dots in two models. Firstly, PANC-1 cells treated with 20μM Gemcitabine for 24 hours displayed a significant increase in LC3 dots (97.67 vs 24.30, p=0.001 by Mann-Whitney). Secondly, AR42J cells, after differentiation with dexamethasone, underwent pharmacologically induced autophagy via PP242, resulting in a significant increase in LC3 dots (5.96 vs 3.14, p=0.0071 by Student's t-test). Assessing autophagic levels, both under physiological conditions and in cancer cells, facilitates the study of autophagy modulation across varied scenarios, including hypoxia, chemotherapy, or targeted protein knockdown.

51. 504. **MAGE-C2 AND ITS ROLE ON VEMURAFENIB RESISTANCE IN A375 HUMAN MELANOMA CELL**

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Resistance to the BRAF(V600E) kinase inhibitor Vemurafenib (PLX4032) limits its therapeutic efficacy in patients with advanced melanoma. Therefore understanding the underlying mechanism of PLX4032 (PLX) resistance is crucial for advanced melanoma treatment. Previously, we established a link between the BRAF(V600E)/MEK/ERK pathway and p53 suppression on the A375 melanoma cell line, through MAPK-driven MAGE-C2 stability (a powerful p53 inhibitor). MAGE-C2 belongs to the superfamily of tumor-specific expression proteins, which correlate with worse cancer outcomes and resistance to chemotherapy and poor melanoma prognosis. To continue with our characterization of the role of MAGE-C2 in BRAF(V600E)/p53 WT melanomas, we analyzed cell viability after treating A375 and A375 MAGE-C2 KO cell lines with PLX for 24, 48, and 72 h. We observed a lower percentage of viable cells in A375-MAGE-C2 KO at 72 h (3 %) in comparison to WT (33 %). To understand the behavior of MAGE-C2 in an inhibited BRAF(V600E) cellular environment, we treated A375 cells with 5 uM PLX overnight. We observed a strong increase in MAGE-C2 protein levels that could play a role in the resistance to PLX. Then, we analyzed the p53 transactivation activity in A375 WT and MAGE-C2-KO cells treated with PLX 5 uM overnight, measuring the mRNA levels of p21 and BAX. We observed that when MAGE-C2 is present, the induction of p53 by PLX is repressed, while in its absence it increases significantly (3.5 times for BAX and 5.2 times for p21). We propose a novel model of Vemurafenib resistance in melanoma, involving MAGE-C2 stabilization and the consequent inhibition of the p53 response

52. 546. **HIGH-RESOLUTION FLUORESCENCE MICROSCOPY REVEALS PROGESTERONE RECEPTOR INTERFERENCE WITH GLUCOCORTICOID RECEPTOR OLIGOMERIZATION *IN VIVO***

Norma Roxana Carina Alves [1,2], Agustina Laura Lafuente [2], Lautaro Damián Álvarez [1,3], Diego Martín Presman [2,4], Adali Pecci [1,2]
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The Glucocorticoid Receptor (GR) and Progesterone Receptor (PR) are ligand-activated transcription factors part of the Steroid Receptor (SR) family. SRs share conserved sequences and tertiary structures, exhibiting promiscuous ligand-receptor and inter-receptor interactions. Activated SRs translocate to the nucleus, oligomerize, and bind to specific DNA sequences named Hormone Response Elements (HRE) to exert their functions. Previously, our group reported an antagonistic GR effect on PR-dependent activity in mammary epithelial cells. In turn, multiple reporter gene assays on single HRE sequences confirmed a transcriptional PR interference on GR actions. Here, we aimed to assess a potential GR/PR interaction. The Number and Brightness (N&B) technique allows the characterization of protein oligomerization state *in vivo* through high-resolution fluorescence microscopy in live cells. To assess GR oligomerization in the presence of PR, we conducted N&B experiments on D4-GR-KO cells, which lack GR expression and have a tandem gene array with ~200 copies of the SR-responsive promoter structure mouse mammary tumor virus (MMTV array). Cells were transfected with GR, or GR and PR, and stimulated with GR agonist dexamethasone [10nM] alone or combined with the synthetic progestin R5020 [10nM]. Previous studies demonstrated that GR forms dimers within the nucleoplasm and tetramers on enhancers. Our results indicate that PR and GR interact in live cells, and PR alters GR stoichiometry both in the nucleoplasm and on MMTV enhancers, particularly with the combined treatment. We observed decreased mean ε brightness values of active GR in the presence of PR, indicating a mixed population of GR complexes with different stoichiometry, possibly due to the formation of GR/PR heterocomplexes. These results might have potential pharmacological implications.

P2-CELLULAR AND MOLECULAR BIOLOGY

THURSDAY 16TH NOVEMBER 14:00-15:30
CHAIRS: NOELIA DI GIORGIO
VERÓNICA WHITE

53. 653. **STARD7 MODULATES LIPID METABOLISM IN HEPATIC CELLS**

Jesica Flores-Martín [1], María Laura Rojas [1], Pilar Cerminato[1], Candela Vega-Rodriguez[1] Ana Cristina Racca [1], Graciela Panzetta-Dutari [1], Susana Genti-Raimondi [1].
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StarD7 is a ubiquitous phosphatidylcholine transfer protein that belongs to the START superfamily, which are involved in metabolism, transport and intracellular signaling of lipids. Previous laboratory reports indicate that cellular StarD7 deficiency alters mitochondrial morphology, functionality, and dynamics. Numerous evidences demonstrate that liver mitochondria play a critical role in the de-

velopment of the pathogenesis of non-alcoholic fatty liver disease (NAFLD). NAFLD affects one quarter of the world's population and may progress to non-alcoholic steatohepatitis (NASH), with risk of developing liver fibrosis and cancer. Here we evaluated the role of StarD7 in lipid metabolism in hepatic cell line HepG2. Stable HepG2 silenced of StarD7 (shD7), and its control (shC) were generated. A significant accumulation of lipid droplets (LDs) was observed in shD7 cells respect to shC, analyzed by fluorescence microscopy and flow cytometry. qRT-PCR and Western blot experiments demonstrated increased levels of transcripts and proteins of enzymes involved in *de novo* lipogenesis (FASN, ACLY, ACC, SCD and DGAT1) and increased CPT-1 protein involved in fatty acid transport across the mitochondrial inner membrane, in shD7 vs. shC. In addition, increased endoplasmic reticulum stress markers Ire1 and BIP/GRP78 were observed, while the antioxidant enzymes catalase and hemoxygenase 1 (HMOX1) were significantly decreased. Also, the levels of reactive oxygen species (ROS) were raised in shD7 vs. shC cells, measured by flow cytometry. To determine the mechanism by which StarD7 modifies the regulation of *de novo* lipogenesis, the transcription factor Srebp1 were measured. Increased expression of Srebp1 transcript and protein was demonstrated in shD7 relative to shC. Collectively, these results indicates that StarD7 depletion in HepG2 cells generates a model of metabolic syndrome similar to that described in NAFLD, contributing to the regulation of hepatic lipogenesis.

54. 41. ***IN VITRO* AND *IN VIVO* EVIDENCE OF THE ANTINEOPLASTIC ACTIVITY OF QUERCETIN AGAINST KAPOSI'S SARCOMA**

Gabriel Principe[1,2], Silvina Tiburzi[1,2], Virginia Lezcano[1,2], Betina N. García[2,3], Fernanda Gumilar[1,2], Verónica González-Pardo[1,2]

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Quercetin (QUE) is a natural flavonoid known to exhibit anticancer properties. Kaposi's sarcoma is a viral cancer caused by the human herpesvirus-8, which expresses a constitutively activated G protein-coupled receptor (vGPCR) during its lytic phase, able to induce oncogenic modifications that lead to tumor development. The goal of this work was to explore the antineoplastic effect of QUE in an *in vitro* and *in vivo* model of Kaposi's sarcoma. Firstly, the antiproliferative effect of QUE was determined by crystal violet technique at different concentrations of QUE (1 - 100 μM) or vehicle (DMSO) for 48 h in endothelial cells stably expressing the vGPCR (vGPCR cells), with an IC50 of 30.078 μM. In addition, a significant decrease in cell viability was observed by neutral red staining (QUE 30 - 100 μM, $p < 0.05$). At a molecular level, F-actin stained with phalloidin showed disorganized actin filaments in the presence of QUE (30 μM), resembling an apoptotic event. Then, apoptosis induced by QUE (30 μM, 24 h) was revealed by annexin V/PI analysis ($p < 0.01$) and caspase-3 activity ($p < 0.001$). Secondly, tumors from vGPCR cells were induced in nude mice. After 15 days of tumor development, mice were treated with QUE (50 or 100 mg/kg/d) or PBS (as control) administered by IP injection three times a week for 30 days. The results showed that tumor progression was retarded in mice treated with QUE (100 mg/kg/d) compared to control ($p < 0.001$); whereas tumor weight was reduced by QUE (50 or 100 mg/kg/d) at the end of the test ($p < 0.05$ and $p < 0.01$, respectively). Neither kidney nor liver damage was observed by analyzing biochemical parameters in serum. In conclusion, this study suggests that QUE exhibits antineoplastic activity in both, an *in vitro* and *in vivo* model of Kaposi's sarcoma.

55. 52. **INCREASED α/β HYDROLASE DOMAIN-CONTAINING 6 (ABHD6) PROTEIN EXPRESSION IN SH-SY5Y CELLS OVEREXPRESSING THE P5-ATP13A2**

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The P-type ion pumps are membrane transporters energized by ATP-hydrolysis which are classified into five subfamilies termed P_1-P_5. *ATP13A1-ATP13A5* genes that belong to this group have been identified in humans. Mutations of the *ATP13A2* gene were associated with neurodegenerative diseases like Parkinson's Disease, Neuronal Ceroid Llipofuscinosis (CNL12), Hereditary Spastic Paraplegia (SPG78), Amyotrophic Lateral Sclerosis and most recently with colorectal cancer. ATP13A2 is localized in lysosomes and late endosomes. Dysfunction of this protein diminishes the lysosomal protein degradation, the autophagic flux and the exosome externalization. We have previously shown that ATP13A2 expression diminishes the bis (monoacylglyceryl) phosphate (BMP) content, which is an essential phospholipid for lipid degradation and exosome biogenesis inside acidic compartments. By using SH-SY5Y cells overexpressing the human P5-ATP13A2 (ATP13A2) or an inactive mutant (ATP13A2-D508N), we investigated the expression and distribution of ABHD6 protein, which is responsible for BMP degradation. Through immunodetection analysis we found that ATP13A2 cells showed an increased expression of ABHD6. Moreover, treatment of cells with spermine -the recently found substrate of ATP13A2- increased the relocalization of ABHD6 towards the cytoplasm when analyzed by immunofluorescence microscopy. Preliminary results show that ATP13A2 cells showed a higher activity of glucocerebrosidase measured with the fluorogenic substrate 4-Methylumbelliferyl β-glucophyranoside. Altogether these results suggest that ATP13A2 overexpression may be altering the lipid digestion process inside acidic organelles.

56. 82. **STUDING THE ROLE OF FLUOXETINE AS A GLOBAL SUMOYLATION INHIBITOR AND ITS POSSIBLE USE IN THE TREATMENT OF HUNTINGTON'S DISEASE**

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SUMOylation is a post-translational modification that involves the covalent attachment of a SUMO peptide to a target protein. Huntington's disease (HD) is caused by a mutation in the CAG repeat of the huntingtin (htt) gene. When an expansion of the CAG repeat occurs in the htt gene, a mutant htt protein (mHTT) is transcribed. mHTT forms insoluble aggregates that disrupt neuronal homeostasis. Preliminary results from our group suggest that fluoxetine (FLX) is a global SUMOylation inhibitor. Based on these results and considering the pathological role of SUMOylation in HD, our aim is to elucidate the molecular mechanism by which FLX inhibits global SUMOylation and its potential use as a treatment for HD. To this end, HEK293 cell clones that stably express endogenous levels of His-SUMO1 and 2 were treated with vehicle (-) or FLX (10 µM). Global SUMO1 and SUMO2 conjugation was analysed by Ni2+ affinity purification followed by Western blotting using a monoclonal antibody against SUMO. We observed that FLX reduced overall SUMOylation levels in SUMO1 and SUMO2 clones (SUMO1=0.42±0.3 $p<0.05$ and SUMO2=0.75±0.2 $p<0.05$). We then performed molecular Docking assays to model the interaction between FLX and Ubc9 using spectomycin B1 as a reference inhibitor. Both compounds interact with the same group of amino acids relevant for the formation of the thioester bond between SUMO and Ubc9 with close binding energies. To confirm the effect of SUMOylation on the formation of mHTT aggregates, we transfected HEK293T with the SUMO2 and HttQ25-mCherry (Q25) or HttQ74-mCherry (Q74) plasmids. After

48 hours, the cells were treated with vehicle (-) or FLX (10 μM). Confocal microscopy analysis revealed that SUMO overexpression increased the amount of mHTT aggregates (Q25:0.25±0.3, Q74:9.5±0.5, p<0.05). On the contrary, FLX treatment strongly reduced the formation of these aggregates (vehicle= 12.75±0.4, FLX=11±0.4, p<0.05), suggesting its potential therapeutic use.

57. 121. **STUDY OF PLACENTAL CELLS BEHAVIOR UNDER PTHrP EFFECT AND OTHER FACTORS OF THE CELLULAR ENVIRONMENT**

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Through paracrine signals, trophoblast cells invade the endometrium in early placentation. Thus, an unbalanced influence of environmental, maternal, and fetal factors on these cells can lead to gestational diseases. Parathyroid hormone-related peptide (PTHrP) expressed in the placenta may be one of these factors. It promotes epithelial-mesenchymal transition (EMT) and invasion, phenomena associated with physiological and pathological events. Other factors that could influence on placental structure and function are those derived from gut microbiota; in fact, *Bifidobacterium* is an attractive genus for its study in the gestational stage. This work aims to investigate if PTHrP influences trophoblastic cells phenotype and if so, whether human milk-derived *Bifidobacterium animalis* subsp. *lactis* INL1 *(B. lactis* INL1) is able to participate in its effect. We used HTR-8 and Bewo cells from normal placenta and choriocarcinoma, respectively, whose express PTHrP receptor, as revealed an *in silico* analysis. PTHrP at physiological concentration (10^{-12}M) induces EMT-associated phenotype in HTR-8 cells (p<0,05) with even greater changes at higher concentration (10^{-8}M) (p<0,05). According to choriocarcinoma behavior, which has a low probability of metastasis, we did not find changes in Bewo cells. In line with these findings, an *in silico* analysis showed elevated expression of epithelial phenotype markers in Bewo cells concerning HTR-8 cells. Also, by western blot and immunocytochemistry we found that the expression of mesenchymal and epithelial markers was modulated in HTR-8 cells exposed to PTHrP (10^{-8}M). This effect was reversed when the cells were pre-incubated with *B. lactis* INL1 supernatant. These findings suggest that PTHrP concentrations higher than physiological promote changes associated with a migratory-invasive phenotype, events that could relate to pathologies development. Interestingly, these effects could be reversed through metabolites derived from *B. lactis* INL1.

58. 154. **TUMOR PROTEIN P53 METHYLATION IS NOT INVOLVE IN DNA DAMAGE PRODUCED BY LOW IONIZING RADIATION DOSES (<0.5Gy) IN MICE MODEL**

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Low ionizing radiation (IR) such as X rays can influence the chemistry of cell and DNA and its repair selectivity due to it genomic oxidative stress. DNA is oxidized by endogenous reactive oxygen species(ROS) in vivo, or by reactive species as a result of IR, metabolic processes, and as consequences of other sources of oxidative stress (OS) such as xenobiotic chemicals. Not only, IR and OS oxidized DNA, it creates DNA damage and changes in methylation patterns which results in more frequent mutations in human p53. In order to detect plausible pathways influenced by low IR in the presence of DNA damage on methylation profile; we performed MSP PCR on (exon 5 and 6) of TP53 and on transcription region of IL1r1 and cadherin 8 -CDH8 gene in a BALB/c model validated to study effect of acute exposure to low doses of IR (20-50, 100 and 250mSv).By Alkaline Comet Assay (ACA) on peripheral blood we confirmed that all X ray doses produced DNA damaged. Our results on methylation pattern did not evidence significant changes in the methylation state of exon 5-6 of TP53 and IL1r1, been all samples hypermethylated or 30% semimethylated, respectively. On the other hand, CDH8 gene showed methylation significant changes profiling from controls under low doses (<100mSV) while not at high doses (250mSV).It's known that Cadherin's are involved in calcium-dependent cell adhesion proteins, as well with Cell junction organization and ERK Signaling. Gene Ontology related to this gene includes *calcium ion binding*. We suggest that low doses and low rate dose of IR affect extracellular matrix environment and suffer from reactive species from water electrolysis. However, the DNA comet % was significant different from controls (p Value <0.01) it did not affect methylation on exon 5-6 of TP53 in mouse. Briefly, 3 genes from different non reported related pathways were reported as undergoing significantly different methylation profiling under very low doses of IR.

59. 279. **EFFECTS OF THE CFTR MODULATORS LUMACAFTOR AND IVACAFTOR ON THE MITOCHONDRIAL DYNAMICS**

Camila Dib, Javier Kamida, Tatiana Limpias del Valle, Nadia Nuñez, Bianca Serrano Soto, Pilar Predassi. Pablo Iglesias, Tomás A. Santa Coloma, Ángel G. Valdivieso

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Mutations in the CFTR gene, encoding a cAMP-regulated channel, cause cystic fibrosis (CF). Impaired CFTR function has been linked to mitochondrial abnormalities, including changes in mitochondrial dynamics. Previous findings showed increased mitochondrial fission when stimulating CFTR activity via cAMP in IB3-1 (CF) cells. This study delves into the impact of lumacaftor (VX-809) and ivacaftor (VX-770), the initial CFTR modulators approved for CF therapy, on mitochondrial dynamics. Particularly, VX-809 is known to augment CFTR localization at the cellular membrane, whereas VX-770 amplifies CFTR activation. For this study, IB3-1 (CF) cells (ΔF508/W1282X) were exposed to VX-809 (10 μM) and VX-770 (0.1 μM) for 48 h, followed by assessment of mitochondrial morphology by confocal microscopy. Combining VX-809 and VX-770 for 48 h increased several indicators of mitochondrial fission (p<0.05, n=7), measured via MiNA plugin for Fiji and Micro-P software. While individual drug treatments had no effect on IB3-1 cell mitochondrial morphology, lumacaftor raised cellular ROS levels (p<0.05, n=7). Interestingly, higher concentrations of VX-770 heightened Ψm at 1 μM in C38 and IB3-1 cells (p<0.05, n=4), suggesting Ivacaftor's potential mitochondrial influence irrespective of CFTR expression. Furthermore, we examined MFN1 and DRP1 levels in IB3-1 (CF), S9 (IB3-1 expressing wt-CFTR), and C38 (IB3-1 expressing a truncated functional CFTR) cells. CFTR presence and modulator effects distinctly influenced DRP1 and MFN1 regulation. This emphasizes the potential of CFTR modulators to bring about CFTR-dependent and non-target effects, impacting mitochondrial function. Further investigation is needed to unveil the underlying mechanisms and enhance drug optimization.

60. 364. **DYNAMICS OF PRE-OSTEOBLAST AND TUMORAL CELLS INTERACTIONS IN QUASI-LINEAR COLONY FRONTS**

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In this contribution the physical interaction process between pre-osteoblasts and tumoral cells, both located at the front region of quasi-linear (q-linear) opposed fronts is studied. The q-linear fronts of murine pre-osteoblast MC-3T3 or tumoral T-47D cells from human breast are generated employing a device provided with two compartments for cell seeding separated by a central partition of micrometric dimensions. This device is located on a polystyrene patterned substrate with channels 3,3 μm in period, fixed to the bottom of a Petri dish. After the formation of congruent cell layers in both compartments, the device is withdrawn and the evolution of the fronts are followed by optical microscopy and a time-lapse system in a chamber that resembles the conditions of an incubator. Individual cell trajectories and the velocity field are determined. Results indicate that although, both MC-3T3 and T-47D cells are oriented by the channels, the former exhibit a significant increase in the velocity magnitude while for the latter it remains almost constant. Furthermore, during the "collision" of MC-3T3 and T-47D fronts, the average cell displacement perpendicular to the colony fronts decreases for MC-3T3 cells approaching the value of the average cell displacement perpendicular to the colony fronts, while it increases for T-47 cells. The parallel component of the average cell displacement increases for both type of cells and the overall result is the engulfment of T-47D agglomerates and the disruption of the pre-osteoblast monolayer. On the other hand, for two fronts of MC-3T3 cells, the perpendicular component of the displacements remains higher than the parallel one. These observations are not affected by soluble factors in the range of time of our experiments. The presented model could be useful for the design of new strategies for treating certain invading tumors based on controlling the alteration of bone microstructure originated by the disorganization of osteoblasts.

61. 438. **OXIDATIVE STRESS MODULATES GSK3β SIGNALING ASSOCIATED WITH LIPOLYSIS IN FAT CELLS**

Melania Funk[1], Athina Maniscalchi[1], Oriana Benzi Juncos[1,2], Natalia Alza[1,3], Melisa Conde[1,2], Gabriela Salvador[1,2], and Romina Uranga[1,2].

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Oxidative stress (OS) modulates fat metabolism and triggers chronic inflammation, promoting the onset of metabolic diseases such as type 2 diabetes and obesity. We have previously demonstrated that iron-induced OS increases lipolysis in mouse white adipose tissue as well as in *in vitro* differentiated adipocytes. We found that iron treatment triggered β-catenin upregulation and exacerbated lipolysis by ATGL activation. In addition, adipocytes where β-catenin had been deleted showed no ATGL upregulation by OS. Our aim was to study the role of GSK3β kinase in the modulation of β-catenin cascade in adipocytes exposed to OS. For this purpose, we used differentiated 3T3-L1 adipocytes (0.5 mM IBMX, 1 μM dexamethasone, 6 μg/ml insulin, and 5 μM rosiglitazone) exposed to 500 μM ferric ammonium citrate (FAC) for 24 h. We also analyzed adipose tissue and liver isolated from mice challenged with iron overload. As previously reported in adipose tissue, OS enhanced lipolysis in liver ($p<0.05$). To unravel the role of GSK3β pathway in OS-activated lipolysis, we performed experiments using LiCl (20 mM), a pharmacological inhibitor of the kinase. FAC and LiCl concentrations used in cell culture experiments had no effect on cell viability. A very well-known mechanism of β-catenin regulation and subcellular localization is its phosphorylation by GSK3β. We performed subcellular fractionation in 3T3-L1 adipocytes exposed to iron overload to study the localization of GSK3β/β-catenin. In nuclear fractions, GSK3β expression showed no significant difference between adipocytes treated with FAC, LiCl, and FAC plus LiCl. However, β-catenin expression was increased in LiCl-exposed adipocytes compared to controls ($p<0.001$) indicating that GSK3β inhibition favors β-catenin translocation to the nucleus. Our results show that nuclear translocation of β-catenin is promoted by its dephosphorylated form and that this mechanism is involved in the lipolytic response of the adipocyte to iron-triggered OS.

62. 521. **FUNCTIONAL ANALYSIS OF GLUCOCORTICOID AND PROGESTERONE RECEPTOR CROSSTALK**

Maximiliano Gutierrez [1], Norma Roxana Carina Alves [2], Adali Pecci [1,2], María Florencia Ogara [1]

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The glucocorticoid and progesterone receptors (GR and PR, respectively) are closely related members of the steroid receptor family of transcription factors. Despite they share similar structural and functional properties, as their DNA sequence recognition motif, the cognate hormones display very distinct physiological responses and even in tissues expressing both receptors they exert opposite biological actions in proliferation, differentiation and cell death. Results from our group demonstrated an antagonistic effect of activated GR on PR-dependent features in mammary epithelial cells. To evaluate whether GR activation could affect PR function, we analyzed the expression of several progestin target genes in MCF-7L cells which express both PR and GR. RT-qPCRs of selected genes show that GR activation by Dexamethasone (DEX) [10 nM] inhibited the R5020 [10 nM]-dependent induction of STAT5A, SNAI1A and EGFR, wherein potentiated R5020-mediated GREB1 and ELF5 expression induction. These results were confirmed by siRNA-mediated GR knockdown, where the progestin-dependent expression of those genes was restored. Moreover, cell cycle analyses performed in cells treated for 18 h with R5020 show that the percentage of cells accumulated in S phase was significantly higher compared to untreated cells (13.7±0.7% vs 10.2±0.3%). DEX alone did not affect S phase accumulation (10.3±0.6%) but inhibited R5020-mediated action (10.9±0.9%). To assess whether the presence of GR affects proliferation, survival and cell migration induced by progestin, clonogenic and wound healing assays were performed in MCF-7L cells. Clonogenic assay shows that treatment with R5020+DEX decreases the proportion of colonies by half compared to R5020 alone. In the same way, wound closure decreased by 20% when treated with both ligands compared to R5020 alone. These results seem to indicate that activated GR modulates PR-dependent cell proliferation and migration in mammary tumor epithelial cells.

63. 558. **PULMONARY NEUTROPHILIC INFLAMMATION CAUSES MUTATIONS IN GENOMIC DNA**

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Pulmonary neutrophilic inflammation (PNI) is caused by the homing and activation of neutrophils in the lung microvasculature exposed to environmental irritants. Upon activation, neutrophils release myeloperoxidase (MPO), the only enzyme that can, under physiological pH, use H_2O_2 to oxidize chloride ions to hypochlorous acid (HOCl). Released MPO can be taken up by surrounding lung epithelial cells, where it can produce HOCl. Genomic DNA oxidation by intracellularly produced HOCl can lead to mutagenesis and further cell transformation. The hypoxanthine phosphoribosyl transferase (*hprt*) gene is one of the most sensitive genes to oxidative mutagenesis. The *hrpt* mutated cells survive in the presence of 6-thioguanine (6-TG). Herein we used an *in vitro* experimental model to test whether intracellularly produced HOCl can damage the genome, and whether the nitrone spin trap 5,5-dimethyl-1-pyrroline *N*-oxide (DMPO) can protect the genome. To accomplish our aim we used human lung epithelial cells (A549 cell line) and incubate them to human MPO or human neutrophils activated with a phorbol ester (PMA) a well-characterized model of PNI. After these incubations, MPO was traced inside A549 epithelial cells very close to the cell nuclei (Confocal). Upon exposure of MPO-loaded A549 cells to H_2O_2, HOCl was intracellularly generated (luminol assay), 8-oxo-deoxyguanosine (8-oxo-dG) was formed (ELISA in isolated DNA), and 6-TG-resistant cells

increased (*hrpt*-mutagenesis assay). The addition of DMPO 30 min after incubation with H_2O_2 resulted in DNA nitrone adducts, but 8-oxo-dG and 6-TG-resistant cells were reduced. Taken together, our data show that intracellularly produced HOCl can damage DNA causing *hrpt*-gene mutations. Furthermore, our study suggests that trapping HOCl-induced DNA radicals with the nitrone DMPO can reduce genomic damage and consequent mutagenesis in the lung exposed to irritants found in the air. PICT-2018-03435, PICT-2021-0147 and PUE013

64. 575. **REGULATION OF IMMEDIATE EARLY GENES MEDIATED BY CRHR1 ACTIVITY**

Ignacio Dellavalle[1], Luciana Ant[2], Karen Lindl[1], Paula dos Santos Claro[1], Patricia Saragüeta[2], Micaela Silbermins[1,*], Susana Silberstein[1,3,*]

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The main goal of our work is the identification and characterization of cellular mechanisms and molecular components involved in corticotropin releasing hormone (CRH) signaling downstream of its type 1 receptor (CRHR1), which will be instrumental for understanding the physiological function of CRH in the central nervous system. We previously demonstrated that AKT kinase is phosphorylated by activated CRHR1 from endocytic compartments. We now performed an RNA-seq assay on the neuronal hippocampal cell line HT22 stably expressing CRHR1, after 1 hour treatment with CRH and/or inhibiting endocytosis (Dyngo4a) or AKT signaling (MK2206). 372 genes were found to be regulated by CRH, most of which were up-regulated (299/372). Among them were known immediate early genes (IEGs) as Nr4a2, Ddit4, Fosl2 and Rgs2, and previously undescribed CRH-IEGs as Midn, Epha2 and Slc3a2. Analysis of the transcriptional profiling showed that Dyngo4a antagonized the effect of CRH on 195 genes, while MK2206 did so on 22 genes. Additionally, most of MK2206 antagonized genes (19/22) are encompassed within Dyngo4a antagonized genes. As expected, immediate early genes (IEGs) up-regulated by CRH are enriched in regulation of cell differentiation ($p\text{-val}=4.10^{-35}$) and neurogenesis ($p\text{-val}=2.10^{-16}$) GO terms. Moreover, MAPK cascade ($p\text{-val}=10^{-16}$), AP1 ($p\text{-val}=9.10^{-13}$) and ATF ($p\text{-val}=10^{-10}$) pathways and ATF ($p\text{-val}=6.10^{-26}$) and CREB ($p\text{-val}=2.10^{-20}$) target genes are also enriched. Dyngo4a antagonized genes were also involved in regulation of cell differentiation ($p\text{-val}=10^{-22}$), neurogenesis ($p\text{-val}=5.10^{-13}$), AP1 ($p\text{-val}=2.10^{-4}$) and ATF ($p\text{-val}=5.10^{-6}$) pathways and ATF ($p\text{-val}=3.10^{-13}$) and CREB ($p\text{-val}=2.10^{-6}$) target genes. We validated some of the most regulated IEGs by RT-qPCR, explored their time profile and their dependency on other pathways activated by CRH. To the best of our knowledge this is the first description of genome wide IEGs regulated by CRH in hippocampus or elsewhere.

O2-CELLULAR & MOLECULAR BIOLOGY

FRIDAY 17TH NOVEMBER 9:00 - 10:30

CHAIRS: ARACELI SEIFFE
CORA CYMERYNG

65. 172. **INTERTWINED DOMAIN REQUIREMENTS FOR DIMERIZATION OF THE GLUCOCORTICOID RECEPTOR**

Agustina L. Lafuente[1], Alba Jiménez-Panizo[2], Theophilus T. Tettey[2], R. Louis Schiltz[2], Adali Pecci[1], Pablo Fuentes-Prior[3], Eva Estébanez-Perpiñá[4,5], Gordon L. Hager[2], María Florencia Ogara[1*] and Diego M. Presman[1*]

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Glucocorticoids (GCs) are steroid hormones that have a major role in the clinic due to their powerful anti-inflammatory and immunosuppressive actions. Unfortunately, their chronic use is linked to severe metabolic side effects. GCs act through the glucocorticoid receptor (GR), a ubiquitous transcription factor organized into three structural and functional domains: the NTD (*N-terminal domain*), DBD (*DNA binding domain*), and LBD (*ligand binding domain*). A relationship between the quaternary structure of GR and its transcriptional activity is still accepted. This paradigm establishes that monomeric GR is unable to bind DNA directly and is responsible for its anti-inflammatory effects. On the other hand, GR dimers can bind to DNA, and promote the transactivation of genes associated with metabolic side effects. This monomer-dimer dichotomy encouraged the search for dissociated GCs that could favor a monomeric state of GR. However, up to date, there is no synthetic glucocorticoid exempt from serious side effects. Using the microscopy technique *Number and Brightness* (*N&B*), our lab has previously characterized the oligomerization state of full-length GR in living cells. Our results indicate that, after stimulation, GR is mostly dimeric in the nucleoplasm, and DNA binding triggers tetramerization of the receptor, which we proposed as the final active form. We found no evidence to support a functional role for the monomeric GR. Here, we performed *N&B* assays on selected GR mutants designed from new crystal structures of GR LBD. Our data suggest that several topologically distinct GR dimers co-exist within the nucleoplasm of live cells and that GR dimerization depends, at least in part, on the allosteric communication between the DBD and LBD domains. These results open new venues in the search for safer glucocorticoid treatments.

66. 222. **ROLE OF GLUCOCORTICOID RECEPTOR CONDENSATES IN TRANSCRIPTIONAL MODULATION**

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The cell nucleus is organized into a variety of membrane less compartments that concentrate certain biomolecules with specific functions, including those involved in transcriptional regulation. It has been proposed that many of these compartments are formed by a liquid-liquid phase separation process (LLPS). The glucocorticoid receptor (GR) is a pharmacologically relevant ligand-dependent transcription factor that translocates to the nucleus upon hormone binding and partitions between the nucleoplasm and multiple discrete nuclear foci or condensates. Our group has previously shown that GR foci present properties compatible with condensates formed by LLPS in the context of living cells. However, the biological role of these structures remains unclear. Here, we explore whether intranuclear GR condensates have a role in transcriptional activity. By using advanced fluorescence microscopy techniques in living cells, we characterized the behavior of GR foci and other proteins involved in the transcriptional response. Consistently with a positive role in transcription, GR foci redistribute and colocalize with a subunit of the Mediator complex (Med1), a key player in RNApol II transcrip-

tion. Interestingly, we observed at least two sub-populations of GR condensates that differ in their size, relative intensity, and respond differently to Med1 overexpression. We also observed an anti-correlation between foci formation and local chromatin condensation wherein GR foci are excluded from heterochromatin regions. Finally, we show that pharmacological inhibition of RNApol II elongation leads to a decrease in foci density, while increasing their relative intensity and sizes. Taken together, our results suggest that at least a subpopulation of GR condensates are involved in GR's transcriptional activity by recruiting proteins related to this process. A further exploration of this new layer of transcriptional regulation might open new venues for pharmacological control of GR action.

67. 474. **EFFECT OF GSH IN THE DISCHARGE MECHANISM OF THE CNIDOCYST: TRASNDUCTIONAL PATHWAY AND ROLE OF CALCIUM**

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Hydra is a freshwater member of phylum Cnidaria defined by the presence of a unique cell type named cnidocyte, involved in several functions. It presents two types of cnidocysts participating in prey capture and ingestion: stenoteles and desmonemes. In the present work we analysed the discharge mechanism of the cnidocysts. First, we test the effect of natural stimuli (i.e. larvae of *Artemia salina*, its homogenate, and reduced glutathione (GSH)). The next step was to study and elucidate the role of Ca^{2+}, and the signaling pathways activated by GSH in the discharge of desmonemes. We use two chelators: EDTA and BAPTA/AM. In addition, we carried out assays using inhibitors and blockers of different proteins involved in intracellular signaling: Nifedipine, Ryanodine (Ry); Xetospongine-C (Xe-C), U73122 and SCH202676- hydrobromide. Differences between groups were analyzed by one way ANOVA. Single post hoc comparisons were tested by the LSD method. Only differences ≤0.05 were considered significant. Results and conclusions: all the stimuli tested increased the number of discharged desmonemes in comparison to control group. Regarding the signalling pathway activated by GSH, it was unable to induce the discharge of desmonemes in the presence of BAPTA/AM, confirming the relevance of the Ca^{2+} in its way of action. The results obtained using EDTA and Nifedipine suggest that GSH stimulates the entry of Ca^{2+} from the extracellular medium by L-type VGCC. On the other hand, assays using Ry and Xe-C suggest that Ca^{2+} would be released from the endoplasmic reticulum through both, IP3R, and RYR. Finally, the experiments performed using SCH202676 allow us to propose that GSH receptor is a GPCR type, coupled with a protein Gq. In the best of our knowledge, this work represents the first *in vivo* study showing the signal transduction pathway, and the intracellular events caused by GSH on cnidocyst discharge.

68. 489. **UBIQUITINATION MODULATES VMP1-MEDIATED AUTOPHAGY IN PANCREATIC CANCER CELLS**

Felipe Javier Renna, Juliana Enriqué Steinberg, Mariana Tadic, Tamara Orquera, Carolina Vecino, Alejandro Ropolo, Mario Rossi, María Inés Vaccaro
Instituto de Bioquímica y Medicina Molecular Prof Alberto Boveris (IBIMOL), Universidad de Buenos Aires, CONICET

Autophagy constitutes a tightly controlled breakdown process engaged in the degradation and renewal of proteins and cellular components. The orchestration of autophagy heavily relies on ubiquitination. A fundamental participant in autophagy is Vacuole Membrane Protein 1 (VMP1). In instances of pancreatic cancer stem cells bearing the activated Kirsten rat sarcoma viral oncogene homolog (KRAS), VMP1 expression sparks autophagy and fosters resilience against therapeutic interventions. Through a combination of biochemical and cellular methods, we have pinpointed ubiquitination as a post-translational adjustment occurring in the initial stages of autophagosome generation for VMP1. The ubiquitination of VMP1 endures throughout the autophagic process, enveloping VMP1 within the autophagosome membrane until the creation of autolysosomes. Notably, this ubiquitination doesn't prompt VMP1 degradation, whether through autophagy or the ubiquitin-proteasomal system. By employing mass spectrometry and immunoprecipitation, we've established that the cell division cycle protein cdt2 (Cdt2), a key component of the E3 ligase complex named cullin-RING ubiquitin ligase complex 4 (CRL4) linked with cancer, interacts with VMP1 in an unprecedented manner, thus participating in VMP1's ubiquitination process. This ubiquitination of VMP1 wanes when exposed to the CRL inhibitor MLN4924 but intensifies when Cdt2 is overexpressed. Furthermore, the inhibition of CRL significantly impacts VMP1's recruitment and the formation of autophagosomes. These findings underscore the innovative nature of ubiquitination as a post-translational alteration of VMP1 during autophagy in pancreatic cancer cells. The ubiquitination of VMP1 might hold clinical significance in the context of tumor cell therapy resistance.

69. 658. **EFFECT OF SERUM FROM PATIENTS WITH ACUTE INTERMITTENT PORPHYRIA ON DELTA-AMINOLEVULINIC ACID SYNTHASE 1 PROTEIN EXPRESSION IN C3A HEPATOMA CELLS**

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Acute Intermittent Porphyria (AIP) is a hereditary disorder of heme biosynthesis, characterized by a decreased activity of the enzyme porphobilinogen deaminase, associated with an induced expression of delta-aminolevulinic acid synthetase 1 (ALA-S1), first enzyme and regulator of this pathway. *In vitro* investigations performed in our laboratory in C3A hepatoma cells, demonstrated that the addition of human serum (HS) to culture medium caused a significant increase in ALA-S1 through Akt/mTOR/4EBP pathway. Based on these results, our aim was to extend the study using AIP patient's serum (PHS) in order to elucidate the mechanisms underlying the deregulation of ALA-S1 in this pathology. The serums used belonged to patients classified according to symptomatology: Latent (PHS-L), without symptoms; Manifest (PHS-M), at least one attack and biochemical values that returned to normal levels; and Subclinical manifests (PHS-MS), which suffered attack and their biochemical values remain elevated. Cells were cultured in low glucose (5 mM) DMEM medium, 10% fetal bovine serum (FBS). Subsequently, they were starved of FBS for 18 hours and then incubated for 2 hours with 2% control HS (CHS), PHS-L, PHS-M or PHS-MS. All PSHs caused a significant increase in ALA-S1 protein levels (45%) and a decrease in its mRNA expression (50%). In the different treatments with PHS, the phosphorylation of Akt (Ser473) was not increased, unlike that observed when CHS was used. 4EBP-1 was phosphorylated in all treatments. According to these results, the protein tyrosine kinase c-Src that activates mTORC1 pathway was also analyzed. It was found that c-Src was phosphorylated in PHS groups suggesting that this protein would be responsible for the increase in the translation of ALA-S1 by activation of c-Src/mTOR/4EBP-1 independent of Akt/mTOR pathway.

P3-CELLULAR & MOLECULAR BIOLOGY

FRIDAY 17TH NOVEMBER 14:00-15:30
CHAIRS: ANDREINA CESARI
MARIANA FARINA

70. 40. **QUERCETIN-LOADED MAGNETIC NANOPARTICLES: A PROMISING TOOL FOR ANTITUMOR TREATMENT IN BREAST CANCER CELLS**

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Quercetin (QUE) is a phytoestrogen with known antitumor effects. Its hydrophobicity and low bioavailability are a limitation to be used as an anticancer drug. One strategy to overcome this difficulty is loading QUE in a non-toxic nanocarrier. The aim of this study was to investigate the biological activity of magnetic iron oxide nanoparticles coated with polyethylene glycol (Mag@PEG) loaded with QUE in a human breast cancer cell line (MCF-7). Our previous research has shown that unloaded Mag@PEG (0-150 μg mL-1) are not cytotoxic for MCF-7 cells and can be targeted to a specific area when an external magnetic field is applied. To continue these studies, further analysis of MCF-7 3D cultures evidenced the presence of Mag@PEG inside the spheroid by histological techniques. Then, Mag@PEG were loaded with QUE by adding an alcoholic solution of the polyphenol to an aqueous dispersion of Mag@PEG under stirring inducing the surface adsorption of the drug. The incorporation of QUE was confirmed by FTIR spectroscopy and quantified by UV-visible spectroscopy. Cytotoxic studies in MCF-7 cells incubated with Mag@PEG@QUE at a concentration equivalent to the IC50 of free QUE (75 μM) for 48 h showed that Mag@PEG@QUE significantly decreased the cell proliferation by crystal violet staining ($p<0.05$). This result was accompanied by an increase in cell apoptosis as evidenced by Annexin V/IP labeling ($p<0.05$). Furthermore, MCF-7 cells incubated with Mag@PEG@QUE exhibited changes in actin cytoskeleton reorganization, characteristic of apoptotic cells, revealed by F-actin staining with phalloidin. In conclusion, these findings suggest that Mag@PEG@QUE could be an effective nanosystem for QUE delivery increasing its bioavailability for breast cancer treatment. In addition, these results provide evidence of the potential use of Mag@PEG as a carrier for other bioactive compounds as well as other biomedical applications.

71. 128. **INSIGHTS INTO THE INTERPLAY BETWEEN RETINOIC ACID AND GLUCOCORTICOID RECEPTORS IN AML CELL DIFFERENTIATION**

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In Acute Myeloid Leukemia (AML) cell differentiation is arrested and retinoic acid (RA) therapy is not encouraging. Our previous results showed that synthetic glucocorticoid dexamethasone (DEX) significantly increases RA-induced cell differentiation in several subtypes of AML and primary blasts. Concomitantly, RNAseq analysis revealed that expression regulation of genes associated with myeloid differentiation and inhibition of stemness was markedly enhanced upon the combined addition of DEX+RA, though in ATACseq experiments chromatin accessibility was mainly determined by RA. In view of these results, we hypothesize that interaction and functional effects between glucocorticoid (GR) and RA (RAR) receptors on chromatin are required for maximal induction of the myeloid differentiation program in AML. In this sense, to understand the molecular mechanisms underlying this scenario to the detriment of tumor progression, the general aim of this study is to gain functional insights into the interplay between GR and RAR in the human myeloid leukemia cell differentiation process. We first conducted immunofluorescence assays in wild type APL NB4 human cells to study the subcellular localization of both nuclear receptors upon activation. We observed that both GR and RAR significantly change their localization in the nucleus upon 24 h in the presence of either DEX 0.5 µM, RA 0.1 µM or combined DEX+RA ligands (student's t-test $p<0.05$). At the molecular level, we plan to assess whether transcriptional cooperation with RAR during cell differentiation implies GR binding to DNA. Then, we successfully designed retroviral shRNA interference strategies to initially silence endogenous GR expression and performed site-directed mutagenesis in GR DNA binding domain (C421G) for further rescue studies. Overall, we acquired essential molecular cloning tools to contribute to the understanding of GR's role as a key regulatory partner of RAR in the myeloid cellular differentiation process.

72. 314. **PANCREAS-SPECIFIC GαS DEFICIENCY TRIGGERS EXOCRINE AND ENDOCRINE TISSUE ARCHITECTURE DEFECTS AND A DEFICIT IN β-CELL MASS**

Juan I. Burgos[1,2], Martina Rossotti[1,2], Dana Steffen[3], Agustín Romero[1,2], Ana C. Heidenreich[1,2], Silvio A. Traba[1,2], Silvio Gutkind[3], Santiago A. Rodríguez-Seguí[1,2]
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Heterotrimeric G-protein coupled receptor (GPCR) signaling pathway mediated by G protein α-subunit (Gαs) plays a key role in the control of pancreatic β-cells function and proliferation. When deleted in β-cells, it has been shown to be required for β-cell growth and maturation. However, the effects of deleting Gαs during pancreas development still remain largely unexplored; therefore, we sought to investigate the role of Gαs-dependent signaling during β-cell development. Conditional knockout mice in which the α-subunit of the Gs protein was ablated only and specifically from the pancreatic compartment since the specification of this organ were generated by crossing $Gαs^{fl/fl}$ mice with Pdx1-Cre transgenic mice, obtaining PGsKO mice. Mice were characterized in detail from the beginning of pancreatic specification to the stage of the adult organ, including *in vivo* glucose tolerance tests. The pancreas of PGsKO and $Gαs^{fl/fl}$ mice (control) were further characterized by immunofluorescence using different combinations of pancreatic cell type markers, as well as other markers revealing tissue architecture organization. PGsKO were found to be hyperglycemic from 4 weeks postnatal as a result of having fewer β-cells, which also show maturation defects. Furthermore, PGsKO islets show an increased number of α-cells, with unusual distribution, in expense of the β- cell pool. We also see disorganization of the exocrine pancreatic tissue. Despite having smaller islets, PGsKO have a larger pancreas than control, presenting ductal enlargements and a malabsorption phenotype. We conclude that Gαs is required for the correct establishment of the endocrine composition of pancreatic islets and for proper pancreatic exocrine tissue architecture and function. Understanding the mechanisms involved holds great interest, and claims to be explored in detail in future work.

73. 348. **INSIGHTS INTO LYSOSOMAL, AUTOPHAGIC AND MITOCHONDRIAL ALTERATIONS IN NOVEL NEURONAL CELL MODELS FOR MUCOPOLYSACCHARIDOSIS IIIA**

María Colonna[1], Marcos Gabriel Francia[2], Alejandra Sonia Guberman[2], Mónica Lidia Kotler[1], Roxana Mayra Gorojod[1], Soledad Porte Alcon[1].
[1]Laboratorio de disfunción celular en enfermedades neurodegenerativas y nanomedicina.[2]Laboratorio de regulación de la expresión génica en células madre. QB-FCEN-UBA. IQUIBICEN- CONICET.

Mucopolysaccharidosis type III (MPSIII), also known as Sanfilippo syndrome, is a rare lysosomal storage disorder (LSD) characterized by early childhood neurodegeneration. MPSIIIA arises from mutations in the gene coding N-sulfoglucosamine sulfohydrolase (SGSH), involved in heparan sulphate degradation in lysosomes. Currently, no nervous system-based MPSIIIA cellular models are

available. Our lab developed SGSH-deficient HT22 neuronal cell lines, named 12, 124 and 15. In this study, we aim to characterize these models, elucidating the impact of SGSH deficiency on lysosomal/autophagic pathways and mitochondrial integrity. Our previous findings displayed an expansion of the lysosomal compartment together with lysosomal membrane permeabilization in MPSIIIA lines 12 and 124. However, the autophagic flux remained functional and possibly increased compared to the control line. MPSIIIA cells displayed fragmented mitochondrial networks, likely related to cellular stress. Yet, here we show total mitochondrial mass remained unchanged, as determined by MitoSpy FM staining. ROS production decreased in all three MPSIIIA lines (12: 30.8% $p<0.05$; 124: 43.6% $p<0.001$; 15: 54% $p<0.001$), with lower Cytochrome C expression in line 124 (56% $p<0.01$). Moreover, our research on mitophagy suggests higher mitochondria-LC3 colocalization in lines 12 and 124, implying enhanced mitochondrial elimination by mitophagy. MPSIIIA cell lines´ viability is not affected under basal conditions, but it is lower than the control line under exposure to an oxidizing agent (H202 24hs; 124- 600uM: 32% $p<0.05$; 15- 500uM: 28% $p<0.05$, 600uM: 39% $p<0.01$). Initial studies suggest MPSIIIA lines exhibit increased glycosaminoglicans accumulation, as expected. These results contribute to our MPSIIIA models´ characterization, providing new evidence of lysosomal, autophagic and mitochondrial alterations. We expect our models, together with our findings, to be useful in the design of future therapies for this disease.

74. 536. **THE EFFECT OF AHR MODULATION ON AXL-MEDIATED DENV-2 IN VITRO INFECTION**

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The main objective of this work was to evaluate the interaction between the Aryl Hydrocarbon Receptor (AHR) and the Axl receptor in cell cultures infected with dengue virus (DENV), while exposing their potential as pharmacological targets. First, to determine whether there is a relationship between the AHR and Axl activation pathways, reporter gene assays were performed in Hek293T cells transfected with a plasmid encoding the Axl promoter upstream of the luciferase gene (Axl-Promoter-Luc) and treated with AHR modulators. It was observed that treatment with an AHR agonist (kynurenine) generated a significant increase in Axl promoter activity with respect to the untreated control. On the other hand, treatment with an AHR antagonist (CH223191, 10μM) generated a significant 65% decrease in Axl promoter activity (one-way ANOVA followed by Tukey's post-test; $p<0.05$ was considered significant). These results were confirmed by measuring Axl transcript levels using RT-qPCR. Treatment of A549 cells infected with DENV (serotype 2, strain 16681; multiplicity of infection: 0.5) with another AHR agonist (10μM I3S) or with CH223191 generated a 4-fold change increase in Axl transcript levels and a 0.6-fold change decrease respectively measured by RT-qPCR. Similarly, a 17% increase in Axl protein levels was observed in kynurenine-treated and infected cells relative to the untreated infected control as measured by flow cytometry. Finally, assays were carried out to determine the effect of the AHR/Axl signaling pathways modulation on the DENV-2 infectious cycle. It was observed that the pretreatment with a specific inhibitor of Axl (R428, 10μM) decreased the viral titer by 95% determined by plaque forming unit assay. In turn, treatment with R428, CH223191 and a combination of both, generated a decrease in viral RNA levels, showing a synergistic effect between the drugs. In conclusion, this work proposes the AHR/Axl pathways as a focal point during DENV infections.

75. 550. **HIGHLIGHTING THE MULTIDISCIPLINARY COLLABORATION BETWEEN CLINICIANS AND BASIC RESEARCHERS ACCOMPANYING THE DIAGNOSIS AND TREATMENT OF INHERITED METABOLIC DISEASES TOWARDS A PERSONALIZED MEDICINE IN LATIN AMERICAN POPULATIONS**

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In Pediatrics, the diagnosis of inborn errors of metabolism is complex due to the need of a multidisciplinary approach and the complexity of the studies. Clinicians face an heterogeneous group of diseases caused by mutations in human genes. Congenital disorders of glycosylation (CDGs) are one of these metabolic pathways that require complex studies of carbohydrate structures such as N- and O-glycoproteins, glycosaminoglycans, and other glycoconjugates. These structures are involved in multiple biological functions, and glycan synthesis disruption results in multisystemic diseases with neurological involvement. CDGs include more than 150 affected genes, that remain widely under- or miss-diagnosed in Latin America. Our objective is to highlight the complexity of the clinical and research collaboration needed for a specific CDG diagnosis and treatment when available. The understanding of the CDG physiopathology is required to detect novel biomarkers and new genes to support diagnosis and treatments. This study includes patients from Argentinean medical centers who present multisystem phenotypes with mild to severe psychomotor disability, hypotonia, seizures, failure to thrive, hormonal anomalies, coagulopathy, and cerebellar hypoplasia. Ethical permissions and informed consents were obtained. The technical approaches include the study of glycoproteins (transferrin or Apo C-III) by isoelectric focusing, capillary electrophoresis or HPLC. The CDG diagnosis was confirmed by genetic testing through massive sequencing. Results: We were able to diagnose 15 patients more with these CDGs that are very rare worldwide: 8 PMM2-CDG, 3 ALG2-CDG, 1 ALG13-CDG, 1 COG1-CDG, 1 ATP6AP2-CDG, 1 MAN1B1-CDG. Clinicians and researchers have been working with a more effective collaboration in the last years, and encouraging families to get involved and participate. This advance represents an excellent opportunity to collaborate in a CDG Latam Consortium for professionals and families.

76. 581. **CYTOTOXICITY ARISING FROM IRON ACCUMULATION AND BETA AMYLOID PEPTIDE: EXPLORING PO-**

TENTIAL NEUROPROTECTIVE ROLES OF ERYTHROPOIETIN AND ITS CARBAMYLATED DERIVATIVE

Juan Ignacio Maciel Paccini, Romina Maltaneri, Diana Wetzler, Alcira Nesse, María Eugenia Chamorro, Daniela Vittori.
Universidad de Buenos Aires. Facultad de Ciencias Exactas y Naturales. Departamento de Química Biológica. CONICET - Instituto de Química Biológica de la Facultad de Ciencias Exactas y Naturales. Buenos Aires, Argentina.

Evidence suggests the accumulation of iron (Fe) in connection with senescence and neurodegenerative diseases, alongside the aggregation of the β-amyloid (βA) peptide. Numerous aspects of Alzheimer's disease (AD) pathogenesis align with ferroptosis indicators, such as the generation of oxidative stress due to Fe overload. The potential neuroprotective impact of erythropoietin (Epo) against these detriments prompts inquiries regarding its role within the Central Nervous System (CNS). Our goal was to probe into whether the cytoprotective effects of Epo and its non-erythropoietic carbamylated derivative, cEpo, could counter these AD signs. Evaluation of Epo's structural alteration revealed that cEpo exhibited altered mobility due to the inhibition of positive charges (SDS-PAGE and Zone Capillary Electrophoresis), yet its secondary structure remained unaffected (Circular Dichroism). In Epo-dependent UT-7 cell line cultures (MTT assay), cEpo displayed no erythropoietic potential; however, it retained its protective influence against staurosporine-triggered apoptosis in the SH-SY5Y neuroblastoma cell line (Flow Cytometry: increase in annexin-positive cells). The addition of βA aggregates (preincubated at 37°C, 1 μM) to SH-SY5Y cell cultures induced apoptosis. Both Epo and cEpo (50 U/mL) prevented this outcome (Annexin C 1.00; **βA 2.9±0.80, Epo-βA 1.8±0.32, cEpo-βA 1.0±0.21 a.u. Mean±SEM (n=4), **significant differences vs. C, $P<0.01$). In contrast, erythropoietins were ineffective in mitigating the consequences of Fe accumulation. Various Fe concentrations (150-300 μM) induced significant increases in both ROS levels (DCFDA assay) and apoptotic cell counts (*significant differences Fe; Epo-Fe and cEpo-Fe vs. C, $P<0.05$). Further research is needed so as to identify the mechanisms of the Epo action in the CNS which may contribute to future development of appropriate therapeutics.

77. 621. **USE OF FLUORESCENT REPORTERS TO STUDY CROSSTALK BETWEEN AKT AND UPR**

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The ability of cells to sense, integrate, and respond to stimuli is crucial for their survival and proliferation. Of particular relevance to this topic is to define and quantify the role of crosstalk between the different cell signaling pathways determining the final physiological outcome. Akt and the Unfolded Protein Response (UPR) are prototypical signaling cascades regulating cell survival and death, two opposite cell programs whose control is lost in different health disorders. Akt is a serine/threonine kinase that plays a central role in proliferation, differentiation, glucose uptake, metabolism, protein translation, cell survival and apoptosis. UPR is a control system that activates during different endoplasmic reticulum (ER) stress. Three different signaling UPR pathways have been described, the main players of which are ER transmembrane proteins: IRE1, ATF6 and PERK. Recent evidence revealed two-way crosstalk mechanisms between the AKT and the UPR pathways, suggesting that they might constitute a unified homeostatic control system. The aim of this work was to explore the crosstalk between Akt and UPR, specifically on Akt activity and localization. Combining the design and use of fluorescent reporters for both pathways and different molecular biology techniques, we show that inducing ER stress in HeLa cells increases the phosphorylation and subcellular re-localization of different Akt substrates as well as Akt itself. In addition, by using antibodies and fluorescent reporters for each UPR pathway, we also evaluated the effect of Akt inhibitors and activators in the regulation of UPR. Understanding how Akt activity and localization varies in a context of ER stress and how Akt regulates the UPR gives us more detail about the crosstalk between PI3K/Akt and UPR pathways, which may be crucial in key cellular processes (both under normal and tumor conditions), such as apoptosis, survival and proliferation.

78. 634. **ENDOMETRIAL TUMOR CELLS OF TAMOXIFEN USERS CHANGE PROGESTIN RECEPTOR CHROMATIN POSITIONING**

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The DNA-binding sites of ERα are key genomic regions under hormone control and endocrine therapy. Cell proliferation and tumor progression of 75% of breast cancer depend on ERα. Consequently, most breast cancer treatments are aimed to inhibit ERα activity. Tamoxifen is an effective and widely applied therapy in breast cancer that acts through E2 competitive inhibition, affecting ERα interactions with other nuclear proteins. Although tamoxifen inhibits the progression of breast cancer, it increases the endometrial cancer risk. Chromatin landscape of endometrial tumor cells from tamoxifen-users is different from those of non-users. Here, endometrial tumors ERα cistromes of both tamoxifen users and non-users were compared with adenocarcinoma cell ERα and PR cistromes generated in our laboratory. Ishikawa ER binding sites were enriched in cells of tamoxifen users while Ishikawa PR binding sites were enriched in ER binding sites of endometrial cancers of tamoxifen non-users. Our data indicate that tamoxifen treatment could change the chromatin landscape of endometrial tumor cells that can be occupied by PR in response to progestins. These results propose a possible mechanism to explain a context dependent response and genomic regions critical to be taken into account to prevent a deregulation of endometrial cells under tamoxifen treatment.

79. 640. **ENDOTHELIAL DIFFERENTIATION FROM INDUCED PLURIPOTENT STEM CELLS AND ITS TIME EXPRESSION OF ENDOTHELIAL MARKERS**

Martire-Greco Daiana[1,2], Guadalupe Amin[1], Carolina Colli[1], Federico Birnberg–Weiss[2], Jose Ramón Pittaluga[2], Verónica Landoni[2], Gabriela Fernández[2], Santiago Miriuka[1].
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Differentiation of induced pluripotent (iPSCs) cells into highly specialized cell types, such as endothelial cells (iECs), neurons, cardiomyocytes, fibroblasts, lung and intestinal cells are technologies related to stem cells that has been growing for many years. Blood vessels are distributed within all tissues of the body and perform various functions. Therefore, the derivation of mature vascular endothelial cells, which restore the lumens of blood vessels, from human pluripotent stem cells is crucial for a multitude of tissue engineering and regeneration applications The aim of this work was to established a protocol to derived induced pluripotent stem cells (FN2.1) to iECS cells and observed the temporary expression of endothelial markers during its differentiation. 250.000 iPSCs cells were cultured with 6uM CHIR at day 0 and B27 (-) and on day 3 we added 10 ng/ml VEGF for 8 days. We observed that on day 5 endothelial markers began to increased their expression per cell respect to day 0, measured by flow cytometry (percentage (%) CD144: 6 ±0,5; CD34: 4±0,2; CD31: 2±0,1, $p<0,05$) and on day 11, iECs reached the maximum of expression of these markers compared to day 0(percentage (%) CD144: 12 ±0,8; CD34: 15±0,5; CD31: 17±0,6, $p<0,05$) while on day 19 expression of CD34 and CD144 significantly decreased and CD31 remained the same respect to day 0 (percentage (%) CD144: 9 ±0,1; CD34: 6±0,1; CD31: 15±0,1, $p<0,05$). Moreover iECs expressed ICAM-1, another endothelial surface protein (percentage

(%) ICAM-1 Day 0: 0; Day11: 15±0,5, p<0,05). Also, we could purify iECS using magnetic beads reaching about 80 % of purity. These cells could grow in EGM-2 medium and maintain iECs markers after several passages in cultured dishes. In conclusion, we could differentiate iECS from iPSC and observed that CD144 and CD34 are endothelial proteins that are the first to be expressed and decreased its expression, while CD31 appears later and maintain its expression for more time.

80. 671. **S-PALMITOYLATION OF AKT: EFFECTS ON PROTEIN LOCALIZATION, ACTIVITY AND CELL FATE**

Analía Amante[1,2], Antonella Vila[1,2], María Corvi[4], Catalina Sierra[1,2], Alejandro Colman-Lerner[2,3] and Matías Blaustein[1,2]
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Akt/PKB protein kinase is involved in a wide variety of physiological processes, including cell metabolism, proliferation, and survival, as well as pathological processes such as malignant transformation. Thus, it is not surprising that different clinical trials are underway to test the efficacy of a variety of inhibitors of the Akt pathway as anticancer treatments. In recent years, new Akt post-translational modifications (PTMs) have been found, which have been reported to affect its activity. However, it is not fully understood how these modification patterns affect certain key features of Akt. Our hypothesis is that the profile of Akt PTMs, can determine its subcellular localization and vice versa, regulating Akt function. We have recently demonstrated that Akt1 can undergo S- palmitoylation, a PTM related to protein sorting through subcellular membranes, at C344 and recently, another group independently discovered that Akt1 is also palmitoylated at C60. Therefore, we have developed fluorescent reporters for WT as well as different Akt versions that cannot be palmitoylated at residues 60 (C60S), 344 (C344S) and at both sites (C60S/C344S). Using a strategy that combines the use of palmitoylation-deficient Akt mutants, palmitoylation inhibitors, fluorescence imaging and flow cytometric techniques, we show that C60S/C344S that the alteration of palmitoylation in Akt significantly affects its cellular localization and affects essential processes such as cell death and survival in different human cell lines. Finally, using the CSS-Palm predictive software, we performed a prediction of possible Akt palmitoylation sites in C.elegans, D. discoideum, S. cerevisiae and D. melanogaster to extend the study of palmitoylation of Akt in these model organisms. Understanding the relationship between protein kinases molecular codes and cell's decision-making brings us closer to understanding how these PTMs influence the development and progression of diseases such as cancer.

P1-ENDOCRINOLOGY

WEDNESDAY 15TH NOVEMBER 14:00-15:30
CHAIRS: ANA LUCIA DE PAUL
SILVIA BIANCHI
VIRGINIA MASSHEIMER

81. 102. **EXPOSURE TO AIR POLLUTION FINE PARTICULATE MATTER (PM2.5) ALTERS EXPRESSION OF SYSTEMIC AND TISSUE-SPECIFIC COMPONENTS OF THE RENIN-ANGIOTENSIN SYSTEM**

Jorge A. Narváez Pardo[1], Agustina Freire[2], Santiago De La Fuente[1], Benjamín Barrales[1], Marina C. Muñoz[1], Mariela M Gironacci[1], Bruno Buchholz[3,] Natalia Magnani[2], Pablo A. Evelson[2], Fernando P. Dominici[1].
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Previous studies have implicated air pollution fine particulate matter (PM2.5) in various cardiovascular and cardiometabolic disease states. However, the molecular mechanisms by which these pollutants mediate these comorbidities have not been fully elucidated. Dysregulation of the renin-angiotensin system (RAS) may be one potential mechanism. To study the impact of PM2.5 on systemic and tissue components of the RAS, male 8-week-old Balb/C mice were exposed to filtered air (FA) or urban air (UA) from Buenos Aires City, in whole-body exposure chambers for 14 weeks. Levels of main RAS components including angiotensin converting enzyme (ACE) and ACE2, as well as AT_1, AT_2 and Mas receptors (R) abundance were determined in kidney, heart and lung tissue by Western Blotting (WB) and their corresponding mRNA expression was detected by RT-qPCR. Circulating angiotensin (Ang) II levels were determined by radioimmunoassay. Exposure to air pollution resulted in increased mRNA levels of ACE and MasR, and increased protein levels of ACE in the kidney; upregulated mRNA and protein abundance of cardiac and pulmonary AT_2R and MasR, together with increased levels of proteins nitrated at Tyr residues in both kidney and lung homogenates, indicative of nitrosative stress in these tissues. In addition, exposure to PM2.5 was associated with increased levels of circulating Ang II, indicative of an exacerbation of the RAS. Our findings indicate that chronic exposure to air pollution induces an altered expression of both tissue and systemic components of the RAS. Given that both the AT2R and the MasR have been ascribed to participate in tissue-repair mechanisms, the upregulation of these receptors detected in mice heart and lung after chronic exposure to polluted air could represent a mechanism of tissue protection against damage induced by PM2.5.

82. 167. **ADENOSIN RECEPTORS COULD INTERACT WITH DOPAMINE RECEPTORS DISTURBING DOPAMINE SIGNALING IN PROLACTINOMAS**

Dana Bornancini[1], Milagros Peña Zanoni[1], Susana Rulli[2], Jimena Ferraris[3], Daniel Pisera[4], Graciela Díaz-Torga[1].
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Most prolactinomas are effectively treated with dopamine D2 receptors (D2R) agonists. Nevertheless, a subset (~20%) became resistant to the treatment and require extirpation. The molecular mechanisms underlying the escape from dopamine inhibition include alterations in D2R signalling. D2R belongs to the family of G protein-coupled receptors (GPCR). Previous studies found that D2R can homo- or hetero-dimerize with different GPCRs. This interaction can alter the affinity of one receptor for its own agonist and the intracellular signaling. It is known that adenosine receptors (AR), form complex with D2R in the brain, modifying D2R affinity for dopamine through allosteric modulation. ARs (A1R and A2R) are expressed in the pituitary. After activation, A1R recruits the Gi protein, decreasing prolactin (PRL) secretion. On the contrary, the activation of A2R recruits Gs, inducing an increase in cAMP and PRL release. As adenosine synthesis increases in the tumoral microenvironment, we postulate that an increased expression of ARs facilitates AR-D2R dimerization, disturbing D2R signaling in prolactinomas. In the present study, we proposed to assess: 1- the expression of A1R y A2R comparing normal pituitaries and prolactinomas from two transgenic mice models, 2- whether D2R co-localizes with ARs in lactotrophs, 3- genotype and sexual differences. Mice models: a- mice overexpressing the β subunit of the human chorionic gonadotrophin (hCGβ+); b- mice lacking dopamine type 2 receptor (D2RKO). Results: A1R and A2R are expressed in lactotrophs (doble confocal

microscopy). A1R and A2R gene expression (RTqPCR) are higher in males than females without genotype differences. A1R is reduced in females with prolactinomas from both experimental models compared to normal pituitaries, while A2R is increased. Conclusions: While preliminary, these results suggest that an increased A2R expression in prolactinomas could disturb D2R signalling.

83. 458. **CONTRIBUTION OF MITOCHONDRIAL FUNCTIONALITY IN GONADOTROPH PITUITARY NEUROENDOCRINE TUMORS**

Silvia Fernández1*, Clara Bertetti1*, Jesica Ramírez1,2, Celina Bernhardt3, Liliana Sosa1,2; Ana Clara Venier1,2, Favio Pesaola4, Vanesa Palla5, Alexandra Latini6, Ezequiel Grondona1,2, Ana Lucía De Paul1,2.

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Gonadotroph Pituitary Neuroendocrine Tumors (PitNETs) comprise one-third of all pituitary tumors and are categorized as non-functioning PitNETs. Depending on the degree of invasion, they often cannot be completely removed through surgery. Metabolic reprogramming also plays a significant role in tumorigenesis.

Mitochondria are pivotal in bioenergetics for tumor progression. Mitochondrial ultrastructure, composition, and functionality significantly vary in tumor contexts. In this context, we aimed to assess mitochondrial functionality in gonadotropin PitNETs and analyze its implication in pituitary cell senescence.

Samples from gonadotroph PitNETs (n=9) were immunolabeled for FSH, LH, and Ki-67; cell lineage was confirmed by ERα and GATA3. Tumor invasiveness was determined based on the Knosp classification. Protein fusion levels (Mfn1-2 and Opa-1) were assessed by western blot. Senescence-associated β-galactosidase (SA-β-Gal) expression was analyzed by colorimetric method in cryosections in cryosections. Mitochondrial number and morphology were evaluated through the analysis of 25 micrographs obtained by transmission electron microscope. Statistical and data analysis: Mann-Whitney test followed by Fisher's test (post hoc); Image J and SPSS 23.0 software.

In invasive gonadotroph, a higher number of mitochondria with altered morphology (swelling), increased organelle area and perimeter, alongside elevated levels of Mfn1-2 and Opa-1 were observed. Conversely, expression of these fusion proteins and ultrastructural mitochondrial parameters were lower in non-invasive gonadotroph. SA-β-Gal expression was negative in the analyzed proliferative lesions.

Our results suggest that mitochondrial dynamics could be affected in invasive gonadotroph PitNETs. The increase in the expression of fusion proteins would reveal a strategy aimed at ensuring the energy supply to sustain pituitary tumor growth. The absence of SA-β-Gal expression, a hallmark of the senescent process, would indicate the evasion of this mechanism for controlling tumor growth.

84. 500. **FGFR1 EXPRESSION AND DNA METHYLATION LEVELS IN PitNETs ASSOCIATED WITH TUMOR PROLIFERATION AND INVASION**

Melina Rocio Bravo[1], Abril Turina[1], Laura Cecenaro[1], Patricia Calafat[2], Juan De Batista[2], Juan Pablo Petiti[1], Liliana del Valle Sosa[1].

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The genesis of pituitary neuroendocrine tumors (PitNETs) could be associated with numerous molecules. A common alteration involves the basic fibroblast growth factor receptor 1 (FGFR1), whose dysregulation was observed in numerous neoplasms. PitNETs are sporadic with no known alterations driving somatic mutations. Hence, epigenetic mechanisms have become more relevant, yet inducing signaling pathways have not been fully defined. In this context, FGFR1 signaling could modulate DNA methylation (5mC), to lead to differential gene expression associated with proliferation and invasion. The objective was to determine FGFR1 expression and DNA methylation levels in functioning (F) and nonfunctioning (NF) PitNETs and their association with proliferation and invasion. The FGFR1 and 5mC expression were evaluated in F- (n=16) and NF- PitNETs (n=22) by IHC and compared with clinical-pathological parameters. Expression of LINE-1, DNMT1, MMP9, B-integrin and Cdh2 were determined in control pituitary gland, primary tumor cell culture of somatotroph (H4) and silent somatotroph (R1) and a cell line (GH3), by PCR and FGFR1 by WB. Cell viability in GH3 stimulated with FGF2 (100 ng/ml), with or without FGFR1 inhibitor AZD4745 (AZD; 0.1-10 μM) for 24h was analyzed by MTT. Statistics: Pearson, Kruskal-Wallis or ANOVA-Tukey. FGFR1 expression was higher in NF *vs* F PitNETs (p=0,043), with a positive correlation between tumor volume and FGFR1 (p=0,04). *In vitro,* DNA hypomethylation was observed in tumor cells. FGFR1, MMP9 and Cdh2 expression was higher in R1 and GH3, with lower levels of DNMT1 and B-integrin *vs* control. FGF2 increased GH3 cell viability, which was reverted with AZD (p<0,05). In summary, PitNETs exhibit differential expression of FGFR1, being higher in NF tumors and correlated with tumor volume. *In vitro*, lower levels of DNMT1 could be associated with DNA hypomethylation, whereas higher FGFR1 expression would be with MMP9 and Cdh2 levels as well as proliferation.

85. 510. **LONG-TERM EFFECTS OF POLYCYSTIC OVARY SYNDROME ON THE ENDOCRINE STATUS OF THE RAT UTERUS**

Inri Iñiguez[1], Gisela Soledad Bracho[1], María Virginia Acosta[1], Verónica Lis Bosquiazzo[1]

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Women with polycystic ovary syndrome (PCOS) have a high risk of endometrial hyperplasia and cancer. These effects could be associated with alterations in the tissue metabolism of steroid hormones. The objective of this study was to investigate how long-term PCOS affects the uterus of rats. Female Wistar rats were treated subcutaneously with sesame oil (CONTROL group) or dehydroepiandrosterone 6 mg/100 g body weight (PCOS group) from 21 to 40 days of age. Then, the rats remained without any treatment. At 24 months, blood and uterine horns were collected. To analyze the endocrine environment in these animals we evaluated: a) serum levels of estradiol (E2), progesterone (P4) and testosterone (T), b) mRNA expression levels of uterine steroidogenic enzymes: steroidogenic acute regulatory protein (StAR), 17b-hydroxysteroid dehydrogenase isoform 1-3 (17b-HSD1, 17b-HSD2 and 17b-HSD3), 5a-reductase isoform 1 (SRD5A1), aromatase (P450arom) and steroid sulfatase (STS), and c) mRNA expression of uterine steroid receptors: estrogen alpha (ESR1) and beta (ESR2), progesterone (PR) and androgen (AR) receptors. The PCOS group showed no differences in serum E2 and T levels compared to CONTROL, however, a decrease in P4 levels was observed. The uterine expression of StAR, 17b-HSD1, 17b-HSD2, 17b-HSD3 and P450arom was similar between groups, but an increase of SRD5A1 and STS mRNA expression was demonstrated in PCOS. Regarding steroid receptors, no changes were observed in ESR1, ESR2 and PR, however, AR decreased in PCOS rats. These results suggest that the uterus of PCOS rats is exposed to higher estrogenic effects due to increased STS that increase active estrogen levels and decreased P4 levels that do not oppose estrogen stimuli. This endocrine state could be contributing to the development of uterine lesions.

86. 512. **HEMIN TREATMENT IMPROVES GLUCOCORTICOID**

RESPONSE TO EXPERIMENTAL SEPSIS IN DIET-INDUCED INSULIN RESISTANT RATS

Lilian Caldareri[2], Franco Meisner[1], Morena Wiszniewski[2, 4], Camila Martínez Calejman[2], Esteban M. Repetto[2, 3] y Cora B. Cymeryng[1, 2]

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An exacerbated response to infection, resulting in systemic inflammation, is observed during sepsis. Under these conditions, an adequate production of glucocorticoids is critical since they prevent an excessive and unbalanced immune response and for its effects on the cardiovascular system and metabolism. We have previously demonstrated the onset of adrenocortical insufficiency in rats made insulin resistant by the administration of a sucrose rich diet (SRD). An increase in oxidative stress and inflammation parameters were detected in the adrenal cortex of SRD-treated rats. The goal of the present study was to evaluate the production of GCs in SRD-treated rats in a model of sepsis by cecal ligation and puncture (CLP) and to assess the effects of hemin treatment, an inductor of an antioxidant and anti-inflammatory response, on the adrenal function. Adult male Wistar rats were fed a standard diet (controls, C) or a SRD, and then treated with/without hemin (SRDH, 15 mg/kg every 48 hours) for the last 2 weeks before surgery. At the end of the 12th week rats underwent CLP and were sacrificed 24 or 72 hours later (survival curve). Hormone determinations and RT-PCR were performed. Our results show higher plasma ACTH levels in the SRD-group 24 h after CLP ($p<0.05$ *vs.* C). These animals exhibited a diminished response to exogenous ACTH ($p<0.01$ *vs.* C), an effect that was not observed in the DRSH group. A significant increase in StAR protein levels was also observed in DRSH treated rats ($p<0.01$ vs. DRS). Lower serum corticosterone levels were detected 72 h after CLP in DRS-treated rats ($p<0.01$ vs. C or DRSH). The rate of survival to sepsis was higher in SRDH group ($p<0.05$ *vs.* DRS). In summary, administration of a SRD for several weeks affects the steroidogenic function of the adrenal cortex. Hemin treatment appears to prevent this adrenal dysfunction observed in DRS treated rats in sepsis and improves its survival rate.

87. 570. **EXPRESSION OF ITPR ISOFORMS IN SECRETING PITUITARY TUMOR CELLS**

Gilda Florencia Mezger[1], Facundo Garcia Barberá[1], Natacha Zlocowski[1], Juan De Batista[2], Laura Cecenarro[2], Verónica Andreoli[2], Liliana Sosa[1], Juan Pablo Petiti[1]

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Large-scale chromosomal alterations and mutations in Ca^{2+} signaling genes, which are involved in regulating hormone secretion and cell proliferation, have been detected in hyper-secreting pituitary neuroendocrine tumors (PitNET). Inositol 1,4,5 trisphosphate receptors (ITPR1-3) are a family of endoplasmic reticulum Ca^{2+} channels essential for the control of intracellular Ca^{2+} levels in different mammalian cell type, but its role in the behavior of pituitary tumors has not been full described. The objective of the study was to analyze the gene expression and subcelluar localization of ITPR1-3 in pituitary tumor cells with linage PIT1(GH- and PRL-secreting). We used primary cultures from patients with somatoroph, silent somatotroph and lactotroph tumors from Hospital Privado Universitario of Córdoba, Argentina, and GH3 cell line that secret GH and PRL. This project was approved by the Ethics Committee (RepisN° HP 4-342). The RNAm expression levels were calculated by quantitative real-time PCR analysis and the GAPDH gene was used as a reference gene. The ΔΔCT method was used for relative quantification. The subcellular localization of ITPRs was analyzed by immunofluorescence. In all PitNETs and also in the GH3 cell line we observed a very strong expression in ITPR3, followed by ITPR1 with no expression in ITPR2. In somatotroph cells the fluorescence of ITPR1-3 was strongly and widely distributed within the cell with a higher intensity in the nucleus and plasma membrane, while in silent somatotroph cells the immunostaining was principally in the perinuclear region and absent from the cell nucleus. These results suggest that ITPR3 may be the isoform more linked with the Ca^{2+} release, and consequently modulate the hormone secretion in GH and PRL tumor cells. In addition, the subcellular localization of ITPRs could indicate that PitNETs possess mechanisms able to produce selective increases of Ca^{2+} in different compartment according functional necessity.

88. 587. **EXPLORING HEART FUNCTION IN MALE SPRAGUE-DAWLEY RATS WITH CONGENITAL HYPOTHYROIDISM**

Fiorella Lista[1, 2], Sofía Epíscopo[1, 2], Kiara Peloso[1, 2], María Belén Ces[1, 2], Eugenia Pietronave[1, 2], Juan Manuel Gaetani[1,2], Gabriela Noceti[1, 2], Noelia Arreche[1, 2], Mónica Navarro[1, 2], Celeste Ferrari[1, 2], Andrea Fellet[1, 2]

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Objectives The objective of this study was to evaluate whether congenital hypothyroidism induced by methimazole (MMZ) during gestation or gestation plus lactation would affect cardiovascular function in rats. Materials and methods Sprague-Dawley cubs were divided into the following groups: Group A (cubs from mother that had free access to water with 0.02% MMZ during gestation), Group B (cubs from mother that had free access to water with 0.02% MMZ during gestation plus lactation), Group C (cubs from mother that had free access to tap water), n = 8/each group. Treatment efficacy was determined by measuring total triiodothyronine (T3) and thyroxin (T4) using radioimmunoassay at the beginning and the end of the experimental period. During all this time, body mass, length of tibia and tail were measured weekly from 21 days after birth until four weeks of life. At this time r 90 days, systolic arterial pressure (PAS, mmHg) was evaluated by indirect tail cuff method and left ventricular function was evaluated by echocardiography. Left ventricle internal diameter (LVID), left ventricle posterior wall thickness (PWT) and anterior wall thickness (AWT) were measured in both systole and diastole. Ejection fraction (EF), fractional shortening (FS) and systolic volume were measured from ventricular internal diameters by the echocardiography system. All determinations were made according to the guidelines of the American Society of Echocardiography. Results are mean values ± SEM. All statistical procedures were performed using the SPSS statistical software package version 23. Statistical significance was set at $P < 0.05$ versus C group. Results Hypothyroidism induced during gestation and lactation (groups A and B) decreased growth parameters compared with group C. No significant differences in these parameters were observed between group A and C. Animals from A and C groups had similar values of PAS (mmHg: 115 ± 2 and 111 ± 2, respectively). However, this parameter was increased in rats from B group (122 ± 3* mmHg) in comparison with C group. All rats had similar values of heart rate. Echocardiographic measurements showed that all groups had similar EF, SF and H/R. No differences were observed between groups regarding PWT in both systole and diastole. Animals from A and B groups had lower AWT (mm) values in both systole and diastole compared with C group (group A: 2,42 ± 0,11*; 1,20 ± 0,08*, group B: 2,53 ± 0,13*; 1,32 ± 0,12*, group C: 3,00 ± 0,19; 1,61 ± 0,13, respectively). LVID in systole and diastole were similar in all groups of animals. Conclusion A deficiency of thyroid hormones during intrauterine life and lactation did not modify linear growth rate. No changes in body mass and linear growth was seen when hypothyroidism was induced during fetal life. Inadequate levels of thyroid hormones during intrauterine life and lactation did not affect EF and SF. How-

ever, congenital hypothyroidism reduced AWT without altering PWT and LVID. In conclusion, this study suggests that hypothyroidism induced in fetal life appears not to affect cardiovascular function in adulthood. These results suggest that the heart would have compensatory mechanisms that would exacerbate its performance to maintain adequate cardiovascular function despite having low levels of thyroid hormones.

89. 614. **FUNCTIONAL CHARACTERIZATION OF HUMAN INDUCED PLURIPOTENT STEM CELLS-DERIVED PITUITARY CELLS AFTER 60 DAYS OF MONOLAYER CULTURE**

Gonzalo Tomás Chirino Felker[1], Carolina Romano Florit[1], Melina Pelanda[2], Juan Manuel Lazzati[2], Guadalupe Amin[1], Sheila Castañeda[1], Ariel Waisman[1], Santiago Gabriel Miriuka[1], María Inés Pérez Millán[3], Lucía Natalia Moro[1], Maria Andrea Camilletti[1,3]

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Human induced pluripotent stem cells (hiPSCs) are able to differentiate into any cell type of the body. Ongoing research with hiPSCs-derived pituitary cells in 2D and 3D organoids holds promise for the modeling of hormonal deficiencies. In an earlier study, we adjusted the 2D protocol, guiding iPSCs into pituitary progenitors over 40 days. To cut costs, we optimized by switching to SB4, an affordable analog of BMP4, and substituting FGF8 and FGF10 with recombinant FGF2. Both hiPSCs cultures treated according to the original protocol and those following the adapted version exhibited a pattern of gene expression consistent with adenohypophyseal lineage differentiation and loss of pluripotency. Based on these findings, this study aimed to continue characterizing the adapted protocol over the days previously studied and extending differentiation to day 60, testing various cell culture strategies to replating cells on day 15. At the end of the 60-day protocol, RNA samples were collected for qRT-PCRs and secreted media was separated for hormonal determinations. Preliminary results show that day 60 differentiated cells express the hormonal transcripts *GH1*, *PRL*, *LHB*, and *POMC* in both protocols. Also, human GH and ACTH levels were measured in Immulite 2000 XPi - Siemens by the chemiluminescence immunoassay and found detected in 60-day cultures media. Furthermore, the gene expression of the hypothalamic development marker RAX was studied during cell differentiation using qRT-PCR. The expression of *RAX* was increased between days 4 and 15 of the conducted differentiation. In summary, while efficiency is moderate, our results suggest that the adapted protocol may enable the generation of functional pituitary cells *in vitro*. This model has potential for research in human pituitary development along with the possibility of studying novel disease genes and genetic variants involved in pituitary disorders.

90. 641. **MATERNAL CORTISOL NEGATIVELY AFFECTS CHILD GROWTH AND REGULATES SALIVARY CORTISOL LEVELS AT 3 MONTHS OF AGE**

Ana Luz Kruger[1,3], Agustina Malpeli[1], Laura Orellano[2], Lucía Mazziota[2], Daniela Rocha[2], Lucrecia Fotia[1], Ignacio Méndez[1], Marisa Sala[1], Andrea Tournier[2], Liliana Disalvo[1], Ana Varea[1], María Florencia Andreoli[1,3]

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Objective: to explore the relationships of plasma and milk cortisol concentrations with maternal characteristics and infant growth.
Material and Methods: A cross-sectional study was conducted. Mother-child dyads were recruited at 3 months postpartum (n=34). In mothers, anthropometric measurements and cortisol levels in plasma and milk were assessed. In children, cortisol was assessed in saliva and anthropometric measurements were measured. Multiple linear regression analysis was used to test the relationship of hormone concentrations with maternal and child characteristics, adjusting for maternal age, parity, adequacy of gestational weight gain, pre-pregnancy maternal body mass index and birthweight for gestational age z-score when appropriate. Regression coefficients (β) are presented with 95% confidence interval and significant a value p<0.05. *Findings*: A positive relationship was observed between maternal plasma and milk cortisol levels [*β*:0.84(0.52, 1.16), p<0.001]. No association was observed between plasma or milk cortisol and maternal BMI [*β*:-0.05(-0.39, 0.29), p=0.752; *β*:0.01(-0.03, 0.04), p=0.622] or fat mass percentage [*β*:-0.05(-0.35, 0.26), p=0.745; *β*:0.01(-0.4, 0.02), p=0.635]. A negative relationship was observed between maternal plasma cortisol and child BMI [*β*:-0.13(-0.21, -0.05), p=0.003] and weight z-scores [*β*:-0.07(-0.14, -0.01), p=0.034]. A positive relationship was observed between maternal plasma and milk cortisol and child saliva cortisol [*β*:0.05(0.01, 0.10), p=0.023; *β*:0.74(0.31, 1.17), p=0.001]. No association was observed between child salivary cortisol and BMI [*β*:0.04(-0.41, 0.49), p=0.861] and weight z-score [*β*:-0.11 (-0.71, 0.48), p=0.707] *Conclusions:* Maternal plasma cortisol influences child growth. A potential regulation on salivary cortisol levels may be involved in this effect. Future studies are necessary to show the regulatory role of salivary cortisol on child growth.

O-ENDOCRINOLOGY

THURSDAY 16TH NOVEMBER 14:00-15:30
CHAIRS: CORA CYMERYNG
VICTORIA LUX

91. 71. **BIOCHEMICAL ACTION OF MEDROXYPROGESTERONE ACETATE ON OVARIECTOMIZED OBESE RATS**

Pablo Cutini, Sabrina Cepeda, Marisa Sandoval, Virginia Massheimer

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Obesity, cardiovascular and bone diseases are multifactorial clinical entities that often coexist in postmenopausal women. Medroxyprogesterone acetate (MPA) is usually employed in hormone replacement therapy. Previous studies carried out in our laboratory at vascular level, demonstrated a divergent action between native progesterone (Pg) and its synthetic analogue MPA, evidenced by an oxidative, proatherogenic and prothrombotic effect, and an impairment in aortic recovery elicited by MPA in contrast to Pg action. VEGF is a key mediator in vascular and bone repair. The aim of this work was to evaluate the effect of MPA on VEGF production on vascular and bone tissue, as wells as the impact on adiposity oxidative stress in a model of obesity and hypoestrogenism. To that end, aortic endothelial cells (EC), retroperitoneal adipose tissue (AT) and femoral diaphysis (FD) were isolated from ovariectomized Wistar rats fed with a high-fat diet (27%) for 10 weeks. For *in vitro* treatments, AT explants, FD and EC cultures were incubated with 10 nM MPA for 20 h. Control group received vehicle only (isopropanol). Treatment of EC culture with MPA induced a significant reduction in VEGF synthesis (34% vs control, *P*<0.0001, ELISA Kit). This inhibition of growth factor synthesis was also observed in FD after exposure to the synthetic progestin (33% vs control, *P*<0.01). Both results suggest an impair action on tissue repair capability. When AT was incubated with MPA, reduction in oxidative stress was detected. Significant decrease in H_2O_2 release from AT isolated form obese rats was observed (2725 ± 432 vs 2043 ± 69 nmol H_2O_2/g of tissue, control vs MPA respectively, *P*<0.05, fluorometric assay). In addition, after treatment of AT with MPA, leptin levels increased in the culture medium (28% vs control, *P*<0.025, ELISA kit). The results presented suggest that, under hypoestrogenism and obesity, MPA could exert negative or positive effects according to the target

tissue involved.

92. 86. **EFFECT OF ESTROGEN ESTRONE ON UTERINE AND ADIPOSE TISSUE UNDER OBESITY**

María I. Valle, Sabrina B. Cepeda, Pablo H. Cutini, Marisa J. Sandoval, Virginia L. Massheimer
Instituto de Ciencias Biológicas y Biomédicas del Sur (INBIO-SUR), CONICET-Universidad Nacional del Sur (UNS). Departamento de Biología, Bioquímica y Farmacia. UNS. Bahía Blanca. Argentina

Previously we reported that, estrone (E_1) counteracts uterine oxidative stress induced by an inflammatory environment. In this work, using an experimental model of obesity, the direct action of E_1 on uterus (Ut) and adipose tissue (AT) was evaluated. In order to rule out the influence of the hormonal impact during estrous cycle, animals were bilaterally ovariectomized (OVX). Rats were fed with high-fat diet (OVX-Ob) for 10 weeks. After isolation, Ut and retroperitoneal AT explants were in vitro expose to 10 nM E_1. Histological analysis of Ut explants (H&E staining) showed that in non-OVX rats exhibited a thicker endometrial layer with great amount of uterine glands compared to uterus sections from OVX rats, verifying the absence of estrous cycles in OVX group. The serum biochemical evaluation showed that, Ob rats exhibited higher levels of serum H_2O_2; TBARS, and leptin than non-obese (8; 65; 122% above non-Ob, respectively, $p< 0.05$), profile compatible with obesity. After E1 treatment (4h), Ut slices exhibited an enhancement in nitric oxide synthesis (33% above control $P<0.001$) and reduction in ROS production (30% below control, $P<0.01$). To assess whether the effect of E_1 on H_2O_2 generation depends on its ability to enhance NO synthesis, an irreversible NOS inhibitor (L-NAME) was employed. In the presence of L-NAME, the reduction on ROS production elicited by E1 was blocked, suggesting NO dependence. Indeed, in OVX-Ob rats the steroid treatment stimulates uterus angiogenesis. Concomitantly, on AT explants isolated from OVX-Ob rats, E_1 prompted an antioxidant action. E_1 reduced H_20_2 secretion (2375±263 vs 1978±241 nmol/g AT, C vs E1 $p<0.05$), and TBARS released (502±195 vs 353±30 nmol/g AT, C vs E_1 $p<0.05$). Indeed, a 14% reduction on leptin secretion was also detected ($p<0.05$). The results presented evidenced that adipose tissue is targeted of E_1 action, and that under obesity the hormone exerts a protective action on Ut and AT reducing oxidative stress.

93. 168. **OVARIECTOMY PREVENTS THE LOSS OF NUCLEAR MENIN EXPRESSION IN LACTOTROPHS PREVENTING PROLACTINOMA DEVELOPMENT**

Milagros Peña-Zanoni, Alejandra Abeledo-Machado, Dana Bornancini, Agustina Marcial-López, Susana Rulli, Graciela Díaz-Torga.
Instituto de Biología y Medicina Experimental, Buenos Aires.

Among its multiple functions, menin plays a key role in activins signalling in the pituitary. We previously showed the importance of activin inhibitory function on lactotrophs: decreased activin biological function is involved in prolactinoma development. Moreover, the loss of gonadal inhibins after a prepuberal ovariectomy (OVX) recovers the inhibitory function of pituitary activins preventing prolactinoma development. In this work, we analysed the effect induced by a prepuberal OVX on menin expression and cellular localization in lactotrophs, and its impact on menin targets as: *Inhbb* gene (which encodes activin subunit βb), $p27^{Kip1}$ and pAKT. We used a mouse model of prolactinoma: females overexpressing the β subunit of the human chorionic gonadotrophin (hCGβ+). Data was analysed by One-way ANOVA comparing groups: WT (normal pituitaries) vs hCGβ+ (prolactinomas) vs hCGβ+OVX (hCGβ+ with prepuberal OVX). We found nuclear and cytoplasmatic menin expression in lactotrophs from WT female mice (confocal microscopy). The nuclear expression of menin was lost in prolactinomas (hCGβ+ females). Accordingly, hCGβ+ prolactinomas presented lower levels of *Inhbb* (RTqPCR), reduced p27 expression in lactotrophs, with a concomitant sharp increase in the percentage of lactotrophs pAKT+ compared to WTs. Interestingly, after OVX, the nuclear localization of menin is recovered (hCGβ+OVX group). Accordingly, pituitary *Inhbb* levels sharply increases, the percentage of lactotrophs p27+ was recovered, with concomitant reduction in the percentage of lactotroph pAKT+ in hCGβ+OVX pituitaries. The present results demonstrate that a prepuberal OVX not only recovers activin inhibitory action but also prevents the loss of nuclear menin expression in lactotrophs, avoiding p27 dysregulation and pAKT increase, preventing prolactinoma development.

94. 197. **DEXAMETHASONE ACCELERATES WHITENING AFTER BROWNING OF WHITE ADIPOSE TISSUE IN MICE**

Alejandra Giordano[1], Patricia Castro[1], María Amanda Rey[1], María Guillermina Zubiría[1], Andrés Giovambattista[1]
[1]*Instituto Multidisciplinario de Biología Celular (IMBICE) CONICET-CICPBA-UNLP*

Browning is the emergence of thermogenic beige adipocytes in white adipose tissue (WAT), mainly in response to cold or B-adrenergic stimuli. Previously, we demonstrated that Dexamethasone (Dxm) inhibits browning in WAT depots from rats. However, it remains unexplored the Dxm effect in beige to white adipocyte transition (Whitening, wtng) and its relationship with mitophagy activation. Our aim was to study if Dxm affect this process in mouse inguinal AT (IAT), using in vivo and in vitro models. First, male mice were kept at 4°C during 7 days (CF) and then two groups were housed during 8h or 2 days at RT and treated daily with or without Dxm (Sc. injection: 0.03 mg/kg; experimental groups: DW8h, CW8h, DW2d and CW2d, respectively). IAT pads were dissected and processed for quantification (qPCR) of thermogenic (Ucp1) and mitophagy (Pink1 and Parkn2) marker expressions. We evaluated the effect of the variables Time (T) and Dxm (2-way ANOVA). First, we found that Dxm diminished Ucp-1 mRNA levels ($p<0.05$) at both times, supporting a stimulatory effect of Dxm in IAT wtng. On the other hand, Dxm caused differential effect in Pink1 and Parkn2 expressions: at 8h both markers were increased while at 2 days both were similar to control (Interaction TxDxm $p<0.05$), indicating an accelerate activation of mitophagy by Dxm. We also evaluated the in vitro Dxm effect in wtng process, using differentiated beige adipocytes. During the last 12h or 24h of culture T3 and rosiglitazone were withdrawn to allow wtng process occurs, and cells were incubated with or without Dxm 0,25 uM. We found that Dxm decreased Ucp1 and increased pink1 mRNA levels, in both times ($p<0.05$). While, Prkn2 and Resistin (white adipocyte marker) expressions were increased only at 24h, indicating a higher effect of Dxm at longer times (Interaction TxDxm $p<0.05$). Overall, here we described for the first time that Dxm accelerates the wtng of IAT, which could be due to an increase in mitophagy-related genes expression

95. 241. **SPEXIN PROMOTES WHITENING OF WHITE ADIPOSE TISSUE THROUGH MODULATION OF AUTOPHAGY**

Patricia Castro[1], Catalina Latina[1], Agustina Castro[1], Sabrina Gambaro[1,2], Andrés Giovambattista[1,2]
[1] *Instituto Multidisciplinario de Biología Celular (IMBICE) CONICET-CICPBA-UNLP*
[2]*Facultad de Ciencias Exactas, UNLP*

Spexin (SPX), a peptide adipokine, has been involved in many metabolic processes such as body weight regulation, food intake, energy balance and glucose and lipid metabolism. Recently, it was also related to disrupt the thermogenic profile of brown and white adipose tissue (AT), but no information was found about its role during white adipose tissue whitening. For this purpose, C57BL/6J male mice were exposed 7 days at 4°C and then returned to room temperature for 8h or 24h where spx was administered (ip. 29 μg/kg; 8SPXw and 24SPXw) or PBS (8CTRw and 24CTRw). Body weight and caloric intake were recorded daily. At the end of the experiment, plasma was collected for Triglycerides (TG) measurement and Epididymal AT (EAT) and Inguinal AT (IAT) was dissected and weighted. IAT was also processed for qPCR gene expression (Ucp1; thermogenic marker and Pink, Parkin, Atg12 and Atg7; autophagic markers) and for mitochondrial DNA (mitDNA) quantification. Two-way ANOVA was used to determine variable (SPX and Time) and interaction (SPX x Time) effects. No differences in total caloric intake were ob-

served but body weight change revealed that both SPX groups gain more weight than CTR ones (P<0,01). For TG levels we observed an increased after 24h whitening (Time P<0,05). SPX increased IAT mass percentage (P<0,01) whereas EAT mass percentage was not affected. IAT Ucp1 gene expression showed a decreased (SPX x Time P<0,05) only after 24h of SPX treatment and for Pink and Atg7 an increase was observed (SPX x Time P<0,05). Parkin gene expression was increased in both SPX groups (8SPXw and 24SPXw; SPX P<0,01). mitDNA was analyzed in 24h groups where DNAmit in 24SPXw was decreased vs. 24CTRw (P<0,01). Overall, just after 24h of SPX treatment (SPXw) Ucp1 was decreased vs. CTRw together with the increase in the autophagic gene expression. We can conclude that Spx treatment may favored whitening process through increasing autophagic markers.

96. 339. **ROLE OF SPHINGOSINE KINASE 1 (SK1) AND SPHINGOSINE-1-PHOSPHATE (S1P) RECEPTOR (S1PR) IN TRIIODOTHYRONINE (T3) ACTION IN DENDRITIC CELLS (DCS) AND IN THE DRIVEN ADAPTIVE IMMUNITY**

Dana M. Negretti-Borga[1], Mariana P. Teixeira [1], Antonella Blanco [1], Elida N. Puentes[1], Ana C. Donadio [1], María del Mar Montesinos[1], Christopher J. Clarke[2], Yusuf A. Hannun [2], Claudia G. Pellizas[1]

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We demonstrated that T3 induces Th1 and Th17 proinflammatory and cytotoxic responses through DC activation (T3-DC), restraining tolerogenic signals. T3 effects are mainly triggered by a mechanism that involves Akt and NF-kB activation. S1P is a bioactive sphingolipid implicated in many proinflammatory conditions. Preliminary results from our group suggested that SK1 (enzyme that produces S1P from sphingosine) participates in the actions of T3 in DCs. Herein, we aimed to investigate the role of SK1 and S1PR in these effects, and in the driven adaptive immune response. Bone marrow-derived DCs were obtained from C57BL/6 WT mice and stimulated (or not) with T3 (10nM). PF-543 (SK1 inhibitor, 100nM) or FTY720 (S1PR antagonist, 1uM) were added, and 30 min later, the T3 stimuli. p-Akt/total Akt and p-STAT3/total STAT3 were analyzed by Western Blot in DCs. Allogenic splenocytes isolated from BALB/c mice were co-cultured for 3 days with T3-DC (inhibited or not with PF543 or FTY720 and exposed to T3 for 18h). Cell viability, proliferation, lineage, maturation, and activation markers were measured by FACS. Cytokines were measured in supernatants by ELISA. Statistical analysis: Two-way ANOVA/Tukey test, and paired t test. p<0.05, statistically significant. Results showed that DC viability was not modified by PF-543 or FTY720. Both inhibitors reduced p-Akt (30min, p<0.005) and p-STAT3 (3h, p<0.05) in T3-DC. IL-12 production and secretion were higher in T3-DC in the presence of PF-543 or FTY720 (p<0.005). FTY720, but not PF5-43, increased IL-6 secretion over T3-DC values (p< 0.002). In turn, IFN-γ secretion and splenocyte proliferation were not significantly modified in the co-cultures with T3-DC exposed to PF-543 or FTY720, but IL-17 was significantly higher (p<0.005). Our results showed that SK1 and S1PR inhibition exacerbate certain proinflammatory responses induced by T3-DC, and highlight an immunomodulatory role of the sphingolipid pathway in the underlying mechanism.

97. 584. **NEW THERAPEUTIC TARGETS IN SOMATOTROPINOMAS: SHP2 AND FGFR4**

Facundo Garcia Barberá[1], Liliana Sosa[1], Gilda Florencia Mezger[1], Florencia Picech[1], Juan De Batista[2], Laura Cecenarro[1], Jorge Mukdsi[1], Juan Pablo Petiti[1]

1 Centro de Microscopía Electrónica-INICSA-CONICET. Facultad de Medicina-Universidad Nacional de Córdoba, Córdoba-Argentina. 2 Hospital Privado Universitario de Córdoba, Córdoba-Argentina

Octreotide (OCT), a somatostatin analog, binds SSTR2 to inhibit proliferation via SHP1, SHP2 in somatotropinomas. In these tumors, FGFR4 receptor has been described as a prognostic and therapeutic biomarker. Studies suggest that SHP2 is a key mediator in the signaling of SSTR2 and FGFR4, but its role in tumor growth and therapeutic response in GH-tumors is still unknown. Our aim was to assess whether SHP2 and FGFR4 modulate OCT effect. SHP2 and FGFR4 expression was evaluated in 41 human samples: 36 Pit-NETs (GHx9, ACTHx8, PRLx2, NFx16), 6 controls, and PDX NUDE mice tumors post-11-day OCT treatment (IHQ, WB).GH3 and patient-derived cells were treated with OCT, SHP2 (SHP099,15 μM) or FGFR4 inhibitor (Blu99931, 50-100 nM). pSTAT3-Tyr705, pERK1/2, pAKT were analyzed by WB and IF. Viability (MTT) and proliferation (BrdU) were examined. Python analyzed RNA-seq (GSE209903). Stats: Kruskal-Wallis, ANOVA, t-test, Chi^2, Pearson. Bioinformatic analysis showed lower FGFR4 expression in GH tumors compared to ACTH and NF tumors, while SHP2 levels were similar in all phenotypes. Gene expression data demonstrated a negative correlation between SHP2 and FGFR4, but positive with STAT3, particularly in GH tumors. In our cohort, GH-tumors exhibited elevated SHP2 expression in contrast to non-tumor tissue, unrelated to clinical factors. OCT-pre-treated patients displayed higher FGFR4 H-score than non-treated (p<0.01). In PDX mice (GH tumor), 11-day OCT treatment inhibited growth, lowered SHP2, and raised FGFR4 expression (p<0.001). *In vitro*, SHP2 inhibitor decreased GH-secreting cells proliferation reducing pSTAT3 levels. Also, OCT induced translocation of SHP2 to the plasma membrane and pSTAT3 to the nucleus. Moreover, Blu99931 enhanced the anti-proliferative effect of OCT (p<0.001). The results suggest the therapeutic potential of SHP2 and the use of FGFR4 inhibitors to enhance OCT treatment, particularly in patients with elevated FGFR4 expression or somatostatin analog resistance.

P2-ENDOCRINOLOGY

FRIDAY 17TH NOVEMBER 9:00 - 10:30

CHAIRS: SUSANA VALDEZ
MARÍA MERCEDES MILESI
RICARDO CABRERA

98. 138. **MISSENSE *IGF1* VARIANTS IN TWO SEPARATED DOMAINS OF IGF1 ARE ASSOCIATED TO PHENOTYPIC DIFFERENCES IN VITRO**

Fernandez Maria Celia, Rampi María Gabriela, Martin Ayelen, Clément Florencia, Pennisi Patricia.

Centro de Investigaciones Endocrinológicas Dr. César Bergadá (CEDIE) CONICET- FEI - División de Endocrinología, Hospital de Niños Ricardo Gutiérrez.

Insulin-like growth factor 1 (IGF1) gene mutations in humans are extremely rare causes of pre- and post-natal growth retardation. We have described two patients with novel homozygous *IGF1* variants (c.247A>T,pSer83Cys-IGF1; c.322T>C,p.Tyr108His-IGF1) presenting a wide variability in the clinical presentation and biochemical profiles. *In silico* modelling predicts that variant pSer83Cys-IGF1 could interfere with IR signalling. Aim: To evaluate *in vitro* the effect of the *IGF1* variants in bioactivity, proliferation, cellular differentiation and matrix deposition compared to WT-IGF1. Methods: Site-directed mutagenesis was performed on the RG 21 2527 vector. Punctual changes of A>T at position 247 or of T>C at position 322 were performed. HEK293 and SaOS-2 cells were transfected with WT or mutated vectors and stable clones selected. HEK293 cells were used for phosphorylation, viability and apoptosis assays. SaOS-2 cells were used for osteocyte differentiation and bone matrix deposition assays. Gene expression was quantified by rqPCR;IGF1R phosphorylation was assessed by western blotting. Results: HEK293 cells expressing p.Ser83Cys-IGF1 (HEK247) and p.Tyr108His-IGF1(HEK322) showed a significant decrease in IGF1R autocrine phosphorylation compared to WT-IGF1 expressing cells (HEKWT). Also, cells carrying the variants had a pronounced decreased in viability by day 3. On day 6 viability of HEK322 clone became differ-

ent from HEK247 reaching values similar to HEK293 parental cells treated with rhIGF1. On day 3 HEK247 clone showed a significant increase in cell death compared to HEK-WT cells. SaOS-2 cell differentiation from osteoblasts to osteocytes resulted accelerated for cells expressing WT-IGF1 and delayed in time for p.Tyr108His-IGF1 variant. SaOS-2-pSer83Cys-IGF1 cells behaved like parental cells treated with insulin. Conclusion: In vitro studies of c.247A>T,pSer-83Cys-IGF1 and c.322T>C,p.Tyr108His-IGF1 demonstrated that both variants impaired cell viability and increased cell apoptosis when expressed in HEK293 cells, as well as delayed bone cell differentiation and matrix deposition in SaOS-2 cells, with a particular phenotype for the pSer83Cys-IGF1. Whether this effect is related to activation/interference of the IR by pSer83Cys-IGF1 or not remains to be elucidated.

99. 157. **LACK OF GABAB RECEPTORS IN KISS1 CELLS AFFECTS CENTRAL AND PERIPHERAL REGULATION OF REPRODUCTION AND METABOLISM IN FEMALE MICE**

Rocío Mastropierro[1], Candela Velazquez[2], Juan M. Riaño Gómez[1], Victoria A. Lux[1], Noelia P. Di Giorgio[1].
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Kisspeptin (Kp), encoded by *Kiss1*, and GABA play a key role in the regulation of reproduction and metabolism. Arcuate nucleus (ARC) *Kiss1* neurons co-express neurokinin B (*Tac2*) and dynophin (*Pdyn*) and interact with *Npy/Agrp/Pomc* neurons linking metabolic signals and reproduction. Adult females lacking GABAB receptors (GABABR) exclusively from *Kiss1* cells/neurons (*Kiss1*-GABAB1KO or KO) were fertile but had clear metabolic disorders: increased body weight (BW), non-fasted glycemia, insulin secretion, HOMA-beta-cell index and reduced insulin sensitivity. Here we studied the impact of GABA, through GABABR, in Kp physiology at neuroendocrine-reproductive and metabolic levels in control (WT) and KO females (Statistics: Student´s t Test). Compared to WTs, KO females showed early vaginal opening (VO, $p<0.05$), similar BW at VO and the day of first estrous tended to decrease ($p=0.08$). Adult diestrous females: ARC *Kiss1/Tac2* ($p<0.05$) increased in KOs, while *Pdyn* did not differ (qPCR). HMB Kp content (ELISA) increased in KOs ($p<0.05$), coincident with ARC *Kiss1* expression. AH Kp content (ELISA), serum LH (RIA), Estradiol (E2) and Kp (ELISA) were similar between genotypes, in line with normal cyclicity. Antral follicles decreased ($p<0.05$) and corpora lutea increased ($p=0.05$) in KOs ovaries (H&E). Regarding metabolic genes, KOs had increased ARC *Npy* ($p<0.05$), *Agrp* ($p<0.05$) and *Pomc* ($p<0.01$) expression (qPCR). Visceral plus gonadal white adipose tissue (WAT) weight tended to increase ($p=0.09$) and Kp content in WAT was higher in KOs ($p<0.05$) compared to WTs. MBH and WAT increased Kp contents were not due to E2 levels. In sum, lack of GABABR specifically in *Kiss1* neurons/cells has a clear impact on puberty onset, follicular development as well as central and peripheral regulation of reproduction and metabolism. Our results highlight the impact of GABABR in ARC Kp and non-Kp neurons, peripheral WAT and ovary in females, which will be further studied.

100. 190. **VASCULAR CALCIFICATIONS INDUCED BY AN EXPERIMENTAL METABOLIC SYNDROME CAN BE PREVENTED BY ORAL METFORMIN**

Lucas Pablo Streckwall[1], Nancy Martini[1], Claudia Sedlinsky[1], León Schurman[1], Antonio Desmond McCarthy[1], María Virginia Gangoiti[1]
[1]Laboratorio de Investigaciones en Osteopatías y Metabolismo Mineral, Fac Cs Exactas, UNLP, La Plata, Argentina

Metabolic Syndrome (MetS) is associated with accumulation of arterial calcifications (AC) due to osteogenic transdifferentiation of vascular smooth muscle cells (VSMC). Metformin (MET) can inhibit in vitro transdifferentiation of VSMC. Here we evaluate the efficacy of oral MET in preventing AC in an experimental model of MetS. 20 young male Wistar rats were initially divided into 2 groups: one received water, the other 20% Fructose solution as drinking source. After 14 days and for an additional 4 weeks, MET 100 mg/kg/day was added to half of each groups drinking water, thus: C (only water), F (20% Fructose), M (100 mg/kg/day MET in water) and FM (Fructose+MET). Before sacrifice, rats were weighed and blood samples obtained. After sacrifice, adipose tissue was dissected to calculate indices. Aortic arches were dissected and placed in an osteogenic medium for 7 days to evaluate *ex vivo* calcification (for groups C and F, with or without MET in the medium), after which they were weighed, calcium was extracted and quantitated. VSMC were isolated from thoracic and abdominal aorta and cultured to evaluate markers of osteogenic transdifferentiation: type 1 Collagen secretion (COL), Mineralization (MIN) and *Runx2* expression. 5um sections of the aorta were evaluated by histologic analysis. One-way ANOVA with Tukey's post-test was performed. Increased glycemia, triglyceridemia (TG), TG/HDL and adiposity were observed in animals exposed to Fructose (vs C). Animals from group F showed higher *ex vivo* calcification of the aortic arch (186% vs. C) that could be prevented by MET (in vivo or in vitro); and their VSMC had increased COL (200% vs. C), MIN (146% vs. C) and *Runx2* (195% vs. C). No significant differences were found for overall tunica media (TM) thickness; however, TM elastic to muscular ratio decreased in group F (74% vs. C), and was partially prevented in FM. In our model, MetS induces osteogenic transdifferentiation of VSMC that can be prevented by oral treatment with MET.

101. 285. **SPEXIN ALTERS THE ADIPOGENIC POTENCIAL OF ADIPOCYTE PRECURSOR CELLS**

Carolina Carla Garraza[1], Catalina Latina[1], Sabrina Eliana Gambaro[1], María Guillermina Zubiría[1], Andrés Giovambattista[1]
[1]Instituto Multidisciplinario de Biología Celular (IMBICE) CONICET-CICPBA-UNLP

Spexin (SPX) is an adipokine involved in the regulation of adipose tissue (AT) metabolism and biology. Among its effects, SPX inhibits terminal adipogenesis, lipogenesis and glucose uptake. Here, we study the effect of SPX on adipogenic potential of adipocyte precursor cells (APC). For this aim, male mice were treated (SPX) or not (CTR) with SPX for ten days (ip. 29 μg/kg/day), body weight and caloric intake were recorded daily. Stromal Vascular Fraction (SVF) cells, containing APC, from Inguinal and Epididymal AT (IAT and EAT, respectively) were isolated. APC number was quantified (Flow Cytometry, APC markers: CD34+/CD31-/CD45-) and gene expression in SVF cells was evaluated (qPCR). Proadipogenic (PPARg2, Zfp423) and antiadipogenic markers (Pref-1 and Wnt10b) were assessed. SPX induced weight loss ($p<0.05$), but no changes in caloric intake. SPX treatment caused a decrease in proadipogenic markers in SVF cells from IAT and EAT (PPARg2, $p<0.05$) and an increase in Wnt10b in SVF cells from EAT ($p<0.05$). Moreover, gene expression of SPX receptor (GALR2) was measured and cells from both AT depots from SPX mice showed diminished levels ($p<0.05$). Regarding APC number, no differences were found between CTR and SPX mice. Additionally, we evaluated if SPX effects in SVF cells were dependent on the AT depot. For that, we performed Two-way ANOVA test to determine variable (SPX and AT Depot) and interaction (SPX x AT Depot) effects. GALR2 expression was lower in SVF cells from IAT than from EAT (AT Depot $p<0.05$), but SPX decreased GALR2 expression in the same extent in both depots (SPX $p<0.05$). On the other hand, we found that SVF cells from IAT were more susceptible to SPX actions in PPARg2 mRNA level, showing a more marked decrease than cells from EAT (SPX x AT Depot $p<0.05$). Overall, our results support the inhibitory effect of SPX on the adipogenic potential of APCs, mainly downregulating key proadipogenic genes, being this effect dependent on the AT distribution.

102. 454. **PHENOTYPIC SPECTRUM IN TYPE 2 3B-HYDROXYSTEROID DEHYDROGENASE (3BHSD2) DEFICIENCY**

María Celeste Mattone[1,2], Natalia Perez Garrido[1], María Sonia Baquedano[1,2], Pablo Ramírez[1], Marta Ciaccio[1], Alicia Belgorosky[1,2], Roxana Marino[1], Mariana Costanzo[1], Gabriela Guercio[1,2]
[1]Servicio de Endocrinología, Hospital de Pediatría "Prof. Dr. Juan P. Garrahan"; [2]CONICET. Buenos Aires, Argentina

Introduction: Pathogenic biallelic variants in HSD*3B2* gene cause a rare form of congenital adrenal hyperplasia (CAH) and compromise genital development in both sexes. Aim: To report the clinical, biochemical, and molecular findings in 6 affected patients with 3βHSD2 deficiency (3β-D). Methods: Genital phenotype at birth, CAH neonatal screening (NS), adrenal function, and pubertal milestones was evaluated in 5 46,XY and one 46,XX 3β-D patients. Results: All 46,XY subjects presented atypical genitalia at birth, 3 with positive NS and salt waste. The 46,XX patient was evaluated for postnatal pubarche, clitoromegaly. Pseudoprecious puberty was found in one non treated 46,XY patient. Spontaneous pubertal onset was observed. Testicular adrenal rest tumors were found in 3 male patients. Glucocorticoid deficiency along with high serum DHEAS (8918,3±7814,1 ng/ml) and 17OHP (70,2±92,4 ng/ml) levels was found in all patients. Salt wasting was evident in 3 patients, and compensated mineralocorticoid deficiency in 2. Homozigocity in 5 patients suggest consanguinity. The variant p.Val228Met found in 3 unrelated patients was classified as likely pathogenic by ACMG. Conclusion: In 3β-D the phenotype is variable and complex. A detailed biochemical profile is useful in the identification of patients, in the differential diagnosis with other forms of CAH, and to individualize medical care. The presence of the p.Val228Met variant in three unrelated patients could be due to a founder effect in our population. Pathological NS in a 46,XY DSD suggest a no 21-hidroxilase CAH. A milder genital phenotype in 46,XX patients could delay diagnosis and/or lead to unrecognized infant death and reinforce the importance of the adrenal *backdoor* pathway in the virilization of other forms of 46,XX CAH. Spontaneous puberty might be related to the expression of gonadal Type1 3βHSD.3β-D is infrequent but should be actively considered in the differential diagnosis for timely identification and management.

103. 459. **STEROID HORMONE RECEPTORS AND NUCLEAR RECEPTOR COREGULATORS IN A POLYCYSTIC OVARY SYNDROME RAT MODEL**

María Virginia Acosta, Gisela Soledad Bracho, Inri Iñiguez, Laura Kass, Verónica Lis Bosquiazzo
Instituto de Salud y Ambiente del Litoral (ISAL, UNL-CONICET), Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral.

In a polycystic ovary syndrome (PCOS) rat model, we previously demonstrated an increased incidence of uterine epithelial and glandular lesions. The aim of this study was to investigate the expression of steroid hormone receptors and nuclear receptor coregulators in the uterus of PCOS rats. Female Wistar rats were treated subcutaneously with sesame oil (control) or dehydroepiandrosterone 6mg/100g bw (PCOS) from 21 to 40 days of age. After 24 hours, uterine horns were collected. Uterine expression of estrogen alpha (ESR1), estrogen beta (ESR2), progesterone (PR) and androgen (AR) receptors was analyzed by real-time PCR and immunohistochemistry. Also, real-time PCR was used to measure mRNA expression levels of coregulatory molecules essential for transcriptional regulation by these receptors, such as nuclear receptor coactivators (NCOA1 and 3) and corepressors (NCOR1 and 2). ESR1 mRNA expression was similar among groups, whereas ERS2 mRNA expression was decreased in PCOS animals. Notably, PCOS animals showed decreased ESR1 protein expression in the epithelium, but no difference in both the subepithelial stroma (SS) and myometrium (M) was observed between experimental groups. No changes in PR mRNA and protein expression were observed among the study groups. The expression of AR mRNA increased in the PCOS group, and protein expression followed the same pattern of change, mainly in SS and M. In control epithelial cells, the AR protein was only localized in the cytoplasm, whereas in PCOS animals, its expression was observed in both the cytoplasm and nucleus. In addition, the transcriptional expression of nuclear receptor coregulators was not significantly different between experimental groups. The results show that PCOS modifies the expression levels of steroid hormone receptors in the uterus. This suggests that PCOS alters AR and ESR-mediated mechanisms, which could promote the development of uterine abnormalities found in PCOS animals.

104. 520. **POLYCYSTIC OVARY SYNDROME: POTENTIAL TARGETS IN BLOOD LEUKOCYTES**

Cardozo María Alejandra[1,2,4], Acosta María Virginia[1], Bracho Gisela Soledad[1], Andreoli María Florencia[3], Bosquiazzo Verónica Lis [1,2]
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4. BLUT Laboratorios, Santa Fe, Argentina.

Polycystic ovary syndrome (PCOS) is the most common hormonal disorder in reproductive-aged women. In these patients, obesity, insulin and leptin resistance and inflammation are prevalent findings and are associated with comorbidities that include development of conditions like type 2 diabetes mellitus, hypertension, and metabolic syndrome. The aim of this study was to evaluate the expression of different molecules, leptin receptor (LEPR), insulin receptor (INSR), insulin receptor substrate 1 and 2 (IRS1, IRS2) and suppressor of cytokine signaling 3 (SOCS3) in blood leukocytes as potential biomarkers of metabolic and inflammatory status in PCOS women. Women in reproductive age (20 to 40 years) with and without PCOS were recruited and these were matched for body mass index (range 20.7-39.3). The mRNA expression of the mentioned molecules was evaluated by real-time RT-PCR. Similar expression of LEPR, INSR, IRS1 and IRS2 were observed between women with and without PCOS ($p>0.05$). However, SOCS3 increased in PCOS women ($p<0.05$). The results suggest that the expression of SOCS3 in blood leukocytes could be a probably early biomarker of leptin resistance related to the metabolic and/or inflammatory status of PCOS women. This could contribute to prevent the development of comorbidities.

542. **TYPE 2 DIABETES-ASSOCIATED FATTY LIVER DISEASE: EFFECTS OF OLIGONUCLEOTIDE IMT504**

Ayelén Coverti, Juan Manuel Riaño Gómez, Luis Mendez, Damasia Becú-Villalobos, Eleonora Sorianello, Victoria Lux-Lantos, María Silvia Bianchi
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The association between non-alcoholic fatty liver disease (NAFLD) and type 2 diabetes is strong. Up to 70% of obese patients with type 2 diabetes (T2D) have NAFLD. The spectrum of NAFLD ranges from simple steatosis to non-alcoholic steatohepatitis with variable degrees of fibrosis and cirrhosis. Aim: the present work was designed to evaluate alterations in the liver in a model of T2D induced by high fat diet in mice and the possible recovery by treatment with the oligonucleotide IMT504. Materials and Methods: male C57BL/6LP mice were fed either a standard diet (SD) or a high-fat diet (HFD: ResearchDiet, D12492, 60% fat content) for 12 weeks. HFD animals showed higher non-fasting glycemia (Gly: $p<0.01$) and body weight (BW: $p<0.01$). SD mice received one daily dose of IMT504 for 16 consecutive days (SD-IMT: 6mg/kg, sc) or saline (SD-sal). HFD mice received one daily dose of IMT504 for 16 consecutive days (HFD-IMT: 6mg/kg, sc), a control polyC oligonucleotide (HFD-PolyC: 6mg/kg, sc, to evaluate the specificity of the IMT504 effects), or saline (HFD-sal). Intraperitoneal glucose tolerance test (ipGTT, day 10) and insulin tolerance test (ITT, day 12) were performed. On day 16, after 3 hours fasting, mice were weighed (BW), sacrificed, and blood, white adipose tissue (WAT) and liver samples were collected. Results: Alterations GTT, ITT and BW induced by HFD were all reversed by IMT treatment but not by PolyC ($p<0.05$ or less for all). Liver weight decreased in HFD animals ($p<0.05$) and tended to normalize with IMT ($p=0.07$) but not with PolyC. WAT and serum cholesterol were increased in HFD animals ($p<0.05$) but not modified by

IMT or PolyC. qPCR analysis of inflammatory, metabolism and ER stress markers of hepatic tissue are underway. Preliminary studies showed that *Pparγ* was not modified by treatments. These results show that IMT504 is effective in improving metabolic state in a T2D animal model; effects of IMT504 on liver and WAT in this model will be further analyzed.

O-GASTROENTEROLOGY & NEPHROLOGY

WEDNESDAY 15TH NOVEMBER 14:00-15:30

CHAIRS: ENRIQUE SÁNCHEZ POZZI
VALERIA RIVAROLA

105. 11. **BASELINE SEVERITY AND INFLAMMATION WOULD INFLUENCE THE EFFECT OF SIMVASTATIN ON CLINICAL OUTCOMES IN CIRRHOSIS PATIENTS**

Alberto E. Muñoz[1,3], Florencia Pollarsky[1], Mónica Marino[1], Mariano Cartier[1], Carlos Míguez[1], Enrique G. Rodger[1], Horacio Vázquez[2], Pablo Salgado[3], Daniel Álvarez[4], Gustavo Romero[1]

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Background: Simvastatin administration to decompensated cirrhosis patients improved Child-Pugh (CP) at the end of a safety trial (EST). Aim: To evaluate whether simvastatin reduces cirrhosis severity through a secondary analysis of the safety trial. Methods: Thirty patients CP class (CPc) CPc A (n = 6), CPc B (n = 22), and CPc C (n = 2) received simvastatin for one year. Primary endpoint: cirrhosis severity. Secondary endpoints: health-related quality of life (HRQoL) and hospitalizations for cirrhosis complications. Statistical analysis: Student's *t*, Wilcoxon, x^2, and Wilcoxon rank-sum tests. Results: Cirrhosis severity decreased baseline versus EST only across CP score (7.3 ± 1.3 versus 6.7 ± 1.7, P = 0.041), and CPc: 12 patients lessened from CPc B to CPc A, and three patients increased from CPc A to CPc B (P = 0.029). Due to cirrhosis severity changes and differences in clinical outcomes, 15 patients completed the trial as CPc A_{EST} and another 15 as CPc B/C. At baseline, CPc A_{EST} showed greater albumin and high-density lipoprotein cholesterol concentrations than CPc B/C (P = 0.036 and P = 0.028, respectively). Comparing EST versus baseline, only in CPc A_{EST}, there was a reduction in white blood cells (P = 0.012), neutrophils (P = 0.029), monocytes (P = 0.035), and C-reactive protein (P = 0.046); an increase in albumin (P = 0.011); and recovery in HRQoL in the physical component summary and its domains (P < 0.030). Finally, admissions for cirrhosis complications decreased in CPc A_{EST} (9%) versus CPc B/C (69%) (P = 0.017). Conclusions: Simvastatin would reduce cirrhosis severity only in CPc B at baseline in a suitable protein and lipid milieu, possibly due to its anti-inflammatory effects. Furthermore, only in CPc A_{EST} would improve HRQoL and reduce admissions by cirrhosis complications.

106. 149. **QUANTIFICATION OF RENAL IONIC CONTENT IN SALT-SENSITIVE HYPERTENSION USING NEUTRON ACTIVATION ANALISIS. EFFECT OF OVARIECTOMY AND SODIUM INTAKE**

Sandra Vlachovsky[1,2], Pablo Azurmendi[1,2], Marcelo Olmedo[3], Rodrigo Invernizzi[3], Romina Rodríguez[1], Claudia Silberstein[4], Elisabet Oddo[1,2], Raquel Jasan[3], Fernando Ibarra[1,2,4].

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We worked in a model of salt-sensitive hypertension (SSH) in which female Wistar rats undergoing ovariectomy (oVx) have high blood pressure under high sodium intake (HS) for 5 days or more. We have previously described that oVx rats show differences in renal function respect to intact female rats (IF), leading them to excrete less Na upon saline overload, even when filtered Na load is similar. Thus, we propose that oVx can change renal elements composition. The aim of the present work was to evaluate the influence of female gonads and sodium intake on total renal content of eight elements. IF and oVx rats with normal (NS) or HS intake were used. At 60 days of life, half of the rats were oVx, and at 145 days IF and oVx rats were divided into NS (0.24% NaCl) or HS (1% NaCl in drinking water) intake. After 2 weeks, the rats were sacrificed, and the kidneys were extracted. Total renal content of Na, K, Fe, Zn, Se, As, Rb, and Br was measured by neutron activation analysis. In IF NS rats, the abundance was (MEAN±SEM, µmol): Na (747±28) > K (493±14) > Fe (21.3±1.2) > Zn (3.3±0.08)> Br (1.6±0.09) > Rb (0.4±0.05) > Se (0.1±0.008) > As (0.009±0.001). A linear regression between the content of Na and both Zn (R = 0.61, p = 0.013) and Br (R = 0.59, p = 0.016), Zn and Br (R = 0.85, p < 0.0001) and Fe and Rb (R = -0.58, p = 0.019) was found among groups. Factorial MANOVA showed that oVx reduced total Na by ~20%, whereas HS intake decreased Fe levels. Hence, an interaction between oVx and HS intake was seen in Zn and Br. No differences were found in K, As, Se, and Rb. The reduction of Na in oVx rats could be due to the movement of ions from parenchyma to blood and/or to other organs, secondary to enhanced activity of renal Na^+ transporters that we have already observed in this model. Results also showed changes in the content of other elements than Na according to the presence/absence of sex hormones and/or amount of Na intake, indicating a complex regulation leading to SSH.

107. 256. **ELIGLUSTAT PROTECTS AGAINST THE EFFECTS OF SHIGA TOXIN 2 INEXPERIMENTAL MODELS OF HEMOLYTIC UREMIC SYNDROME IN RATS**

Daiana S. Sanchez[1], Lilian K. Fischer Sigel[1], Claudia Silberstein[1].

1. *Universidad de Buenos Aires - CONICET. Instituto de Fisiología y Biofísica Bernardo Houssay (IFIBIO Houssay), Facultad de Medicina, Departamento de Ciencias Fisiológicas. Laboratorio de Fisiología Renal. Buenos Aires, Argentina.*

Shiga toxin (Stx) binds to the globotriaosylceramide (Gb3) receptor on target renal cells, causing Hemolytic Uremic Syndrome (HUS) and acute kidney injury (AKI). Previously, we demonstrated that the treatment of human renal cells with Eliglustat (EG, Sanofi), an inhibitor of glucosylceramide synthase, significantly decreased Gb3 expression and totally prevented the cytotoxic effect of Stx2. Our aim was to evaluate the protective effect of EG against renal damage caused by Stx2 in experimental models of HUS in male Sprague-Dawley rats (100 g body weight (bw)). Treatments: Rats were intraperitoneally (ip) injected with Stx2 lethal (5 ng/g bw) or sublethal (1 ng/g bw) dose, or with eluent (Controls), and orally treated with or without EG (25 mg/d), starting 1 or 2 days prior until 2 days after Stx2 injection. Food and water intake, and rat survival were daily monitored. Renal function and histopathology were evaluated at 3 days post-injection (dpi). Gb3 expression was measured in kidneys of rats treated with EG. Stx2 lethal dose produced significant decrease in food and water intake, extended renal tubular necrosis, significant increase in creatinemia and uremia, decreased creatinine clearance, compared to control rats (n=6, p<0.01), and caused rat death at 3-4 dpi. Stx2 sublethal dose also produced a significant renal damage compared to control rats (p<0.05), but significantly lower than that caused by Stx2-lethal dose. However, EG pretreatment for 2 days prior injection, significantly reduced renal Gb3 levels and totally prevented the effects of both lethal and sublethal

Stx2 doses at 3 dpi, and prevented rat mortality (100%) caused by the lethal Stx2 dose. Hence, EG pretreatment for only 1 day was enough to prevent Stx2 sublethal effects on creatinemia, uremia, and creatinine clearance. No significant alterations were shown by rats treated with only EG. In conclusion, EG could be used as a therapeutic strategy to prevent AKI caused by Stx2 in patients with HUS.

108. 292. **EFFECTS OF L-NAME ON RENAL DAMAGE CAUSED BY A SUBLETHAL DOSE OF SHIGA TOXIN 2 AND TUBULAR EPITHELIAL REGENERATION**

Lilian K. Fischer Sigel[1], Daiana S. Sánchez [1], Agostina Presta [1], Elsa Zotta [1,2], Claudia Silberstein[1].

[1] Universidad de Buenos Aires - CONICET. Instituto de Fisiología y Biofísica Bernardo Houssay (IFIBIO Houssay), Facultad de Medicina, Departamento de Ciencias Fisiológicas. Laboratorio de Fisiología Renal. Buenos Aires, Argentina. [2] Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Departamento de Ciencias Biológicas. Cátedra de Fisiopatología.

Shiga toxin (Stx)-producing *Escherichia coli* causes Hemolytic Uremic Syndrome and acute renal injury. It was reported that nitric oxide (NO) inhibition by L-NAME increased Stx2-induced renal damage in mice. Hence, adaptive mechanisms during pregnancy involve increased NO production. We have previously shown that Stx2 caused lower renal damage and faster renal recovery in pregnant rats than in non-pregnant rats. Our work aimed to study the effects of L-NAME on renal damage caused by Stx2 and on tubular epithelial regeneration in pregnant rats. Pregnant Sprague-Dawley rats, at day 8 of gestation, and non-pregnant rats were injected intraperitoneally with a sublethal dose of Stx2 (0.5ng/g body weight) (PS and NPS, respectively) or eluent for control pregnant (PC) and non-pregnant (NPC) rats. Some pregnant rats were treated with L-NAME (1 mg/ml) in drinking water from 1 day prior to 4 days post-injection (dpi) with Stx2 (PSL) or eluent (PCL). At 4dpi, renal histology, and tubular expression of Ki67 (proliferation marker) and Vimentin (Vim, mesenchymal marker) were evaluated. PC, NPC, and PCL rats showed conserved renal histology while PS, NPS, and PSL rats showed tubular necrosis. PSL rats showed significantly more renal damage than PS rats, but not as much as NPS rats. Loss of tubule basal membrane was observed only in PSL kidneys. PS and NPS rats significantly increased Ki67 and Vim expression in medullary tubules with respect to controls, being the Vim expression significantly higher in NPS than in PS rats ($p<0.01$). However, PSL rats neither expressed Vim in tubules nor increased Ki67 tubular expression with respect to controls. In conclusion, L-NAME treatment aggravates the Stx2-induced tubular damage in pregnant rats, approaching renal damage showed by NPS rats. We propose that increased vasodilatation during pregnancy, due to NO, may protect the maternal kidneys from Stx2 effects in rats.

109. 407. **PANCREATIC MITOCHONDRIA FUNCTIONS ARE AFFECTING DURING ACUTE ENDOTOXEMIA**

Virginia Vanasco[1,2], Bruno Hernan Pappalettera[2], Juan Santiago Adán Areán[1,2], María Ines Vaccaro[1,2], Silvia Alvarez[1,2]

[1] Instituto de Bioquímica y Medicina Molecular, Prof. Alberto Boveris, Facultad de Farmacia y Bioquímica (UBA-CONICET). [2]Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Cátedra de Fisicoquímica.

The molecular mechanisms involved in the development of sepsis and endotoxemia are multifactorial and have not yet been fully elucidated. The pancreas is one of the organs affected early during endotoxemia and sepsis, which could be relevant in the development of the disease at a systemic level. Given the inflammatory nature of this pathology, pancreatic mitochondria could be affected, compromising tissue bioenergetics. The objective of this work was to analyze the state of mitochondrial function, regarding ATP production and as a relevant source of active oxygen species, and the repair mechanisms that trigger experimental endotoxemia. Female Sprague Dawley rats (45 days old) were treated i.p. with: vehicle (control); LPS 0.5 mg/kg (LPS 0.5) and LPS 8 mg/kg (LPS 8). Mitochondrial function is assessed by O_2 consumption, ATP production, and mitochondrial membrane potential. LPS 0.5 group showed an ATP production decreased only at 6h after LPS injection. On the other hand, LPS 8 group presented a similar decrease in ATP production at 6h but this decrease being increased at 24h. Furthermore, LPS 8 animals also showed a significant drop (35%) in mitochondrial membrane potential (control value: 147 ± 20 mV) and O_2 production decreased 24h (control value: 62 ± 3 ng-at O/min mg protein). On the other hand, mitochondrial NOX4 activity was found significantly increased (5 times) only in LPS 8 group, from 6h after starting treatment. Finally, changes in protein expression related to mitochondrial dynamics, and structures compatible with mitophagy (assessed by TEM) were observed in both endotoxemia groups. Taken together, our results suggest a relevant role for mitochondria in the pancreatic conditions observed during endotoxemia. Greater knowledge of the mitochondrial mechanisms that are activated in the pancreas during endotoxemia could be of great relevance, since its possible modulation could allow the development of new therapeutic strategies.

110. 456. **REDUCED GLOMERULAR NUMBER AS A POTENTIAL MECHANISM UNDERLYING HYPERTENSION PROGRAMMING IN MALE OFFSPRING FROM MOTHERS WITH ADENINE-INDUCED CHRONIC KIDNEY DISEASE**

Lucas Humberto Oronel[1], Sofía Gayone[1], María Ángeles Magnanelli[1], Camila Jencquel[1], Ramiro Alonso[1], Gabriel Suchowolski[1], M. Florencia Albertoni Borghese[1,2], Mónica P. Majowicz[1]

[1]Cátedra de Biología Celular y Molecular, Facultad de Farmacia y Bioquímica, UBA, [2]CONICET

At least 3-4% of women in reproductive age present Chronic Kidney Disease (CKD) and pregnant women with CKD have an increased risk of perinatal complications. However, limited attention has been given to the potential effects of maternal CKD on offspring. The maternal Adenine-induced CKD (m-Ad-CKD) rat model is very recent and unexplored. It was reported that adult male rat offspring of m-Ad-CKD develop hypertension in adulthood. Our aim was to evaluate glomerular count in 21 days old rat offspring from m-Ad-CKD, bearing in mind that low glomeruli number is one of the mechanisms underlying hypertension programming. Adult female SD rats were treated for 3 weeks with Ad 0.5% in standard rat chow powder (CKD mothers=CKDM) or regular rat chow powder (Control mothers=CM). Then, they were mated with male SD rats. Offspring number from CKDM was lower than that from CM (8 vs 14). Litter size of the CM was fixed at 10 pups. The offspring was divided into 4 groups: male from CM (M_{CM};n=5); male from CKDM (M_{CKDM};n=3), female from CM (F_{CM};n=5), and female from CKDM (F_{CKDM};n=5). After weaning (day 21), pups were weighed and sacrificed. Blood and urine samples were obtained to determine plasma and urinary creatinine and proteinuria. The right kidney of each pup was used to count glomerular number by the acid maceration method. Results were expressed as media ± SD and 2-way ANOVA was used for statistics. We found a reduced glomerular number in M_{CKDM} (73881±34347) vs M_{CM} (105895±28111) and vs F_{CKDM} (96454±28520) with $p<0.01$, while no changes were observed between F_{CM} (95517±27368) vs F_{CKDM}. Offspring from CKDM had lower body weights vs those from CM [M_{CM}: 56,4±1,7; M_{CKDM}: 53,1±1,9; F_{CM}: 54,1±1,6; F_{CKDM}: 51,5±1,6 (g); $p<0.01$] with sex differences ($p<0.05$). Plasma and urinary creatinine and proteinuria did not differ among groups. Our results suggest that reduced glomerular number could be one of the potential mechanisms underlying hypertension programming in male offspring of m-Ad-CKD.

P-GASTROENTERLOGY & NEPHROLOGY

THURSDAY 16TH NOVEMBER 14:00-15:30

CHAIRS: ALEJANDRO ROPOLO
MÓNICA MAJOWICZ

111. 637. **EFFECTS OF THE INHIBITION OF RENAL DOPAMINERGIC SYSTEM ON BLOOD PRESSURE AND RENAL FUNCTION**

Nicolás M. Kouyoumdzian[1,2], Gustavo Risso[1], Carolina Kuhn[1], María J. Rudi[2], Beatriz Perazzi[3], Hyun J. Lee[2], Natalia L. Rukavina Mikusic[1,2], Marcelo R. Choi[1,2].
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Renal dopamine has natriuretic, diuretic, anti-inflammatory and antioxidant effects with an impact on blood pressure (BP) levels. Peripheral dopa decarboxylase (AADC) inhibition (together with L-dopa) represents one of the pharmacological strategies for the treatment of Parkinson's disease, but its effects on renal function and its possible impact on BP are unknown. We hypothesized that AADC inhibition with carbidope (Cb) would lead to a state of increased inflammation and kidney damage with increased BP levels. Our aim was to evaluate the effects of Cb treatment on BP levels, renal function and the expression of the Na+,K+-ATPase (NKA) pump and the anti-inflammatory marker Parkinson's protein 7 (PARK7) in normotensive rats and spontaneously hypertensive rats (SHR). 16 male Wistar Kyoto (WKY) and 16 SHR rats were divided into 4 groups (n=8/group): WKY Control (drinking water), WKY+Cb (25 mg/kg/d in drinking water), SHR Control (drinking water) and SHR+Cb (25 mg/kg/d in drinking water) during 3 weeks of treatment. Systolic blood pressure (SBP) was measured by the indirect method of tail-cuff. 24-hour diuresis was determined, as well as urinary and fractional excretion of sodium. Renal expression of NKA and PARK7 were determined by Western Blot. A $p<0.05$ (*) was considered statistically significant.
Treatment with Cb unaltered SBP levels, but it was associated with a significant reduction in fractional and urinary excretion of sodium (*), accompanied by an increase in renal expression of NKA, in both SHR and WKY rats (*).Cb treatment was associated with a significant increase in PARK7 expression only in SHR rats (*). In conclusion, inhibition of renal dopamine synthesis with Cb alters tubular handling of sodium, increasing NKA activity and promoting greater sodium reabsorption. In the context of hypertension, these changes would be accompanied by a greater inflammatory response in kidney.

112. 165. **URINARY TOTAL AND EXTRACELLULAR VESICLES CAMP CONTENT ARE ASSOCIATED WITH AUTOSOMAL DOMINANT POLYCYSTIC KIDNEY DISEASE (ADPKD) PROGRESSION**

María Lucía Rosenberg [1, 2], Agustin Yaneff [3], Nora Paula Goette [1, 2], Sandra Gabriela Vlachovsky [1, 2] , Ezequiel Branca [1,2], Natalia Riera [1,2], Jorge Toledo [1,2],Gonzalo Manuel Ferradas [1,2] , Carlos Alberto Davio [3] , Roxana Noemí Peroni [3, 4] , Elisabet Mónica Oddo [1, 2] , and Pablo Javier Azurmendi [1, 2]
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ADPKD is the most common genetic renal disease that is characterized by the presence of multiple cysts which, through slow and gradual growth, leads to glomerular filtration rate (GFR) decline and end-stage renal disease. Cystic growth is associated with increased intracellular levels of 3´,5´-cyclic adenosine monophosphate (cAMP). Extracellular vesicles (EVs) are proposed to participate in "remote-sensing" by transporting different cargoes, but their relevance in ADPKD progression is poorly understood. This study aims to determine whether cAMP is contained in urinary EVs and, if exists, how total and/or EVs cAMP content participates in disease progression. Fourteen ADPKD patients, naïve for V 2 receptor antagonism treatment, and 7 controls were studied. Progression was evaluated by estimated GFR (eGFR) and height-adjusted total kidney volume (htTKV). Fresh morning urine was collected to determine cAMP by competitive radioligand assay. Urine EVs were isolated by an adapted centrifugation method and characterized by electron microscopy, dynamic light scanning, flow cytometry with FIT-C CD63 labeling, protein, total RNA content, and AQP2 and GAPDH mRNA detection. Total and EVs cAMP was measurable in both control and patient urine samples. Total cAMP significantly correlated with eGFR and its annual change and inversely correlated to htTKV. The cAMP-EVs showed a bimodal pattern with htTKV, increasing to ≈1 L/m and falling at larger sizes. Our results demonstrate that urine cAMP correlates with ADPKD progression markers, and indicate that it´s extracellular delivery by EVs could reflect the architectural paramount of the organ.

113. 384. **PI3Kγ MEDIATES Mrp2 ACTIVITY IMPAIRMENT INDUCED BY IL-β IN SANDWICH CULTURED RAT HEPATOCYTES**

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In intrahepatic cholestasis, IL-1β is involved in altering canalicular secretion by causing changes in the localization and function of canalicular transporters such as Mrp2, this action promotes transporter disinsertion through activation of kinase-mediated signaling proteins. We have previously shown that the PI3K/AKT pathway is involved in this process. Therefore, our aim was to identify which isoform of PI3K is involved in Mrp2 alterations. Since there is evidence of a role of PI3Kγ in Mrp2 expression in sepsis, we analyzed whether this PI3K isoform participate in IL-1ß action in sandwich-cultured rat hepatocytes (SCRH). Methodology: SCRHs were transfected by adding 5 μL of lipofectamine with 70 nM of PI3Kγ siRNA per well or scrambled siRNA, followed by a 6 h incubation at 37 °C. After transfection, hepatocytes were washed and overlaid with gelled collagen for 1 h at 37 °C to obtain a collagen sandwich configuration. SCRHs were exposed to IL-1β (10ng/ml) for 20 min. After treatments, Mrp2 transport function was evaluated by the pseudocanalicular accumulation of its substrate glutathione-S-methylfluorescein (GSMF). Chloromethylfluorescein diacetate (CMFDA) 2,5 μM for 15 min, was added to the medium and time lapse imaging was done every minute during 8 min with a fluorescence microscope. Between 70 and 100 pseudocanaliculi were selected in each image, and the average of time fluorescence of CMFDA-metabolite GSMF was measured. The slope of GSMF fluorescence vs. time was used as an estimate of initial rate of transport (IRT). Result: (% of Control ± SEM; n=3-4) IL-1ß decreased the initial rate of Mrp2 transport (49%±1a). This was partially prevented by knockdown of the PI3Kγ protein (68%±2ab). *a different from control, b different from IL-1ß*. Conclusion: The cytokine IL-1ß produces a reduction in Mrp2 function, associated with internalization of the transporter, mediated in part by the activation of the PI3Kγ protein.

114. 432. **DETECTION OF RENAL DAMAGE BIOMARKERS ASSOCIATED WITH HEMOLYTIC UREMIC SYNDROME**

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The Shiga toxin (Stx), produced by E. coli, is the primary etiological agent responsible for causing hemolytic uremic syndrome (HUS), characterized by hemolytic anemia, thrombocytopenia, and acute renal failure. In previous reports, a decrease in the expression of Nephrin and Podocalyxin in the filtration diaphragm was shown in an acute HUS model at 48 hours. Proteinuria was also observed in the first week and at 3 months in a sublethal model. Currently, the disease diagnosis is based on the clinical presentation. Detecting biomarkers associated with renal damage during the early days of bloody diarrhea and prior to the onset of signs and symptoms could be crucial for initiating timely and appropriate treatment to improve prognosis. The aim of this study is to identify the presence of proteins originating from renal tissue in extracellular vesicles of urine (uEVs), which could be considered as renal damage biomarkers. For this purpose, Sprague-Dawley (SD) rats weighing between 150 and 200 g were intraperitoneally inoculated with a sublethal dose of Shiga toxin. Urine samples were collected at 96 hours post-inoculation for uEV isolation through differential centrifugation. The uEVs were characterized in terms of identity, quantity, and size using techniques such as Western blot (WB) with the Alix marker, BCA (bicinchoninic acid assay), and Dynamic Light Scattering (DLS). The renal origin of uEVs from the experimental group (n= 6 per each group) was identified through immunodetection of NEPH-1, whereas it was not detected in the control group. This is the first fine tune and methodological approach to describes, the use of the isolation of urine-derived uEVs from rats with HUS. Considering that NEPH-1 forms a complex with Nephrin, and since Nephrin decreases at 48 hours, this technique could serve as a tool for early detection of renal damage. Further studies with other glomerular markers are required to confirm their utility as biomarkers.

115. 627. **COMPARATIVE TRANSCRIPTIONAL ANALYSIS OF FREE FATTY ACID-TREATED HEPATOCYTE PRIMARY CULTURE UNCOVERS THE ROLE OF SPARC IN LIPOTOXITY**

Lucía Lameroli Mauriz[1], Esteban Fiore[1], Juan Bayo[1] María Jose Cantero[1], Barbara Bueloni[1], Mariana García[1], Josep María Arguemi[2], Guillermo Mazzolini[1]; Catalina Atorrasagasti[1].
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Introduction: Hepatic fat accumulation is a hallmark of non-alcoholic fatty liver disease (NAFLD). Loss of hepatocytes capability to keep lipid homeostasis leads to dysregulation of lipid metabolism creating a lipotoxic environment which promotes NAFLD. The matricellular protein SPARC is involved in several pathophysiological processes such as inflammation, tissue remodeling and metabolism regulation. Our aim was to study the role of SPARC on hepatocytes' response to saturated/unsaturated free fatty acids (FFA). Methods: Hepatocyte primary cultures from SPARC knockout ($SPARC^{-/-}$) and wild type ($SPARC^{+/+}$) mice were treated for 24h with 0.5 mM FFA (2:1 oleate and palmitic acid (PA) or only PA (0.2 mM) (n= 3/group). RNA was extracted and libraries were prepared to RNAseq analysis (Illumina). p-value<0,01 and |logFCl<0,585 were used to define differentially expressed genes (DEGs). Biological process related with DEGs were identified by gene ontology (GO) analyses of protein coding genes using a False Discovery Rates (FDR)<0.05. Data was analyzed using RStudio. Results: We found 70 and 68 DEGs between $SPARC^{+/+}$+FFA and $SPARC^{-/-}$+FFA vs. non-treated cells respectively. Gene ontology analysis of both sets of DEGs showed similar biological processes suggesting that FFA effect does not vary depending on the genotype. On the other hand, we found 174 and 186 DEGs between $SPARC^{+/+}$+PA and $SPARC^{-/-}$+PA vs non-treated hepatocytes. GO analysis of DEGs of $SPARC^{+/+}$ hepatocytes treated with PA are related with a down-regulation of cell cycle, DNA replication and DNA repair. Instead, GO analysis of DEGs of PA exposed-$SPARC^{-/-}$ cells were related with an increase of response to inflammation, response to lipids and oxygen reactive species. Conclusions: Overall, transcriptomic analysis suggests that SPARC played a major role in the response to PA lipotoxicity by exerting a cytoprotective role.

116. 667. **IDENTIFICATION AND CHARACTERIZATION OF BIOMARKERS OF PODOCYTE INJURY IN PROTEINURIC NEPHROPATHIES. FABRY DISEASE / FOCAL AND SEGMENTAL GLOMERULOSCLEROSIS POST KIDNEY TRANSPLANTATION RECURRENCE**

Costales-Collaguazo Cristian [1,2], Lago Néstor [3], Ochoa Federico [1,2], Zotta Elsa [1,2]
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Many human glomerular diseases are associated with a decrease in the number of podocytes (podocytopenia) due to the persistence of podocyturia during the course of the disease. Within this type of nephropathies is Fabry disease. In this disease there is a reduction or absence of the activity of the lysosomal enzyme α-galactosidase A (α-Gal A), which results in the accumulation of globotriaosylceramide (GL-3), with the characteristic lysosomal inclusions, associated with sloughing of podocyte processes and proteinuria. Focal segmental glomerulosclerosis (FSGS) is a common glomerular disorder that manifests clinically with nephrotic syndrome and >80% sloughing or loss of primary foot cells. After renal transplantation, recurrence of primary GSFS occurs in 30% to 40% of adults. The objective of our study is to analyze the presence of podocyturia in the evolution of proteinuric nephropathies and its characterization as an early marker of renal injury. Indirect immunofluorescence techniques with primary antibody labeling of podocyte proteins, both podocalyxin and synaptopodin, were performed in urine samples from patients. Our results suggest the presence of podocyturia as an early marker of kidney damage. Fabry patients showed higher podocyturia than controls. Fabry-treated subjects (n=33) had significantly higher UPCR compared to untreated subjects (n=15), podocyturia was significantly higher, and proteinuria was lower in colocalized subjects. Results are expressed as median and range. Variables were analyzed using the Wilcoxon Mann–Whitney test. Correlations between variables were obtained with the Spearman correlation coefficient. Results were considered significant when P< 0.05. Currently, there are still no validated biomarkers for the diagnosis and follow-up of primary GSFS or for recurrence in post-kidney transplantation or for Fabry disease.

P-GENERAL BIOLOGY

FRIDAY 17TH NOVEMBER 9:00 - 10:30
CHAIRS: SERGIO MORADO
GUILLERMINA BILBAO

117. 9. **DETECTION OF *PLEISTOPHORA HYPHESSOBRYCONIS* IN ARGENTINE ZEBRAFISH BIOTERIES**

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The use of fish as a biological reagent in biomedical research has increased in the last decade thanks to the zebrafish (Danio rerio).

Concern about infectious diseases in fish used in research is increasing due to the poor implementation of biosecurity measures and that it could lead to the spread of pathogens, which can cause disease and alter research results. Among the pathogens that affect zebrafish, Pleistophora hyphessobryconis has been detected in several research laboratories in our country. This microsporidium is characterized by causing skeletal muscle necrosis and inflammation with a high degree of mortality. The objective of this work was to detect the presence of P. hyphessobryconis using the polymerase chain reaction (PCR) molecular technique to determine the health status of zebrafish production and research colonies in our country. Fifty adult zebrafish from 6 vivariums that use them in our country were controlled. The animals were processed for sampling and were controlled by molecular techniques (conventional PCR) and also observations under OM for the detection of the pathogenic agent. The OM observation results did not find characteristic lesions while the PCR controls showed that 2 institutions out of a total of 6 were positive, showing that the fish colonies are contaminated with P. hyphessobryconis (33.3%). The conclusions in reference to the results obtained, in addition to preventing the spread of this bacterium between facilities, it is desirable to have fish free of pathogens for use in experiments. The implementation of a sanitary control program in the zebrafish facilities is also recommended, to mitigate the risks and ensure that the results obtained from the investigations are free of uncontrolled variables.

118. 244. **OSMO-IONIC PHYSIOLOGY OF THE EURYHALINE FRESHWATER SHRIMP *Macrobrachium borellii* AFTER TRANSFER TO SALINE MEDIA.**

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The freshwater shrimp *Macrobrachium borellii* (Caridea: Palaemonidae) is widely distributed in the La Plata basin (northern Argentina, Paraguay and southern Brazil), inhabiting continental aquatic habitats in which salinity seldom can reach 7‰. As a part of ongoing studies about saline tolerance in freshwater palaemonids, our laboratory experiment involved the abrupt transfer of shrimp from a control condition (2‰) to 7‰, 15‰, 20‰ and 25‰ for short- (24 h) and long- (> 21 days) acclimation periods. We measured osmolality of hemolymph and external media using a cryoscopic osmometer (Gonotec) and relevant inorganic ions (Na^+, K^+, Cl^-, Ca^{2+}) using an electrolyte analyzer (Diestro). Osmolality of hemolymph and external media as well as hemolymph and external medium ion concentration were tested by Student t tests (n= 3-9). Shrimp showed great osmo- ionic regulatory plasticity managing to survive for more than 21 days between 7‰ and 20‰, and up to 10 days in salinity 25‰. Shrimp hyper-regulated osmolality of hemolymph in ≈393 mOsm/kgH_2O at 2‰ and 7‰; in 512 mOsm/kgH_2O at 15‰ and osmolality was isosmotic in 595 and 684 mOsm/kgH_2O at 20‰ and 25‰, respectively. Hemolymph ion concentration (mM) of shrimp kept in the control condition were: [Na^+] 270±13; [K^+] 10±2; [Cl^-] 201±12; [Ca^{++}] 5±0.75. Hemolymph sodium and chloride were hyper-regulated at 2‰ and 7‰. Whilst hemolymph sodium was hyper-iso regulated at 15‰, 20‰ and 25‰, hemolymph chloride was iso ionic in those salinities. Hemolymph potassium was hyper- regulated between 2‰ and 25‰, while calcium was hyper-regulated between 2‰ and 15‰S but iso ionic in salinity 20‰ and 25‰. Our results showed that the freshwater shrimp *M. borelli* still retain a high degree of euryhalinity, being able to survive between 2‰ and up to 25 ‰. This species is a hyper osmo iono regulator between 2‰ and 15‰. Although shrimp osmoconform in salinity 20‰ and 25‰, status of iono regulation is ion depend.

119. 566. **EFFECT OF THE FIRST FEEDING ON SOMATIC GROWTH AND DEVELOPMENT OF THE ALIMENTARY TRACT OF THE PEJERREY BONAERENSE (*ODONTESTHES BONARIENSIS*)**

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The pejerrey bonaerense is a highly valued fish in Argentina for the quality of its meat and its importance in sport fishing; however, its culture is under development, presenting low larval growth and survival rates. Considering that the initial feeding is a determining factor for the animals´ survival and development and that feeding protocols for the early life stages of this species have not yet been established, the aim of this study is to evaluate the possible effects of different initial diets on the larval development and the maturation of the alimentary tract of the pejerrey bonaerense. From hatching to 40 days post-hatching (dph), animals were fed daily until satiation with unenriched *Artemia* nauplii (S), *Artemia* nauplii enriched with fish oil (P), or *Artemia* nauplii enriched with a mixture of fish and vegetable oil (M). The live food was then replaced by commercial dry feed in each tank until the end of the experiment. The degree of enrichment of the *Artemia* in fatty acid was assessed by GC/MS, the survival and growth of the fish were evaluated at 90 dph, and the development of their alimentary tracts was studied by histological sections at 9 dph, focusing on the number of taste buds, the width and height of the intestinal folds and the area of hepatocytes. The enriched *Artemia* showed an increase in their content of ω3 fatty acids. No significant differences were obtained in the survival of the pejerrey, while a tendency to a higher weight gain was observed in animals of treatment M (0.47 ± 0.05 g *vs.* S: 0.33 ± 0.03 g, P: 0.39 ± 0.03 g). Histological analysis showed a trend towards a difference in the number of taste buds between treatments and a significant increase in the area of hepatocytes in individuals of treatment M (135.73 ± 10.92 μm^2 *vs.* P: 101.32 ± 6.21 μm^2), suggesting a greater energy reserve at the beginning of their development. The results obtained provide additional knowledge for improving the larval culture of the pejerrey bonaerense.

120. 588. **INFLUENCE OF TANK COLOR ON SOMATIC GROWTH, BODY COLOR AND SOMATOLACTIN RESPONSE IN *CICHLASOMA DIMERUS* LARVAE**

Agustina Carla Beriotto [1,2], Julieta Emilse Sallemi [1,2], María Paula Di Yorio [1, 2], Andrés Breccia [1,2], Daniela Irina Pérez Sirkin [1,2], Paula Gabriela Vissio [1,2]

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Many studies indicate that certain species perform better in terms of growth, larval survival, stress levels, behavior, and body coloration, among other things, when kept in certain colored tanks. However, this variable has received little attention in most studies and is often not even reported. Therefore, the aim of this study was to examine how tank color affects somatic growth, body color, and the response of somatolactin (SL), which is a pituitary hormone in fish that is linked to growth and skin pigmentation, in the larvae of *Cichlasoma dimerus*. Fish were reared for 90 days after hatching in white, light blue or gray indoor tanks with two independent replicates. Somatic growth was estimated in terms of body weight and total length at the end of the study. Body weight was significantly higher ($p < 0.02$) in larvae reared in white tanks (147.8 ± 18. 6 mg) compared to those reared in light blue (84 ± 9.7 mg) or gray tanks (80.9 ± 9.7 mg). Larvae reared in white tanks showed a tendency toward larger sizes without any significant differences over those reared in light blue and gray tanks. Body color was evaluated by quantifying the number of head melanophores at 5, 12, and 25 days after hatching. In this case, no significant differences were observed among colors.

To study SL response, the number of SL-immunoreactive cells was counted at 60 days after hatching, which tended to be higher in larvae reared in light blue and gray tanks than in white-reared larvae. In conclusion, the color of the rearing tank appeared to influence the somatic growth and SL production of *C. dimerus*, but not their body color for the days analyzed. These findings should be taken into consideration when designing the physical environment of *C. dimerus* rearing tanks.

121. 629. **RELATIONSHIP BETWEEN GONADOTROPIN INHIBITORY HORMONE (GNIH) AND FEEDING RELATED HORMONES IN THE CICHLID FISH CICHLASOMA DIMERUS**

Andrés Breccia[1,2], Julieta Emilse Sallemi[1,2], Paula Gabriela Vissio[1,2], Gustavo Manuel Somoza[3], María Paula Di Yorio[1,2]

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Gonadotropin inhibitory hormone (Gnih) is a neuropeptide that inhibits the reproductive axis in birds and mammals. In recent years, Gnih has also been shown to be involved in the regulation of feeding and the control of energy homeostasis in these groups. In teleost fishes, a growing number of studies demonstrate that Gnih regulates reproductive function; however, there are few studies linking Gnih to feeding and nutritional status in this group. In our laboratory, preliminary results showed that four weeks of fasting conditions induced a significant decrease in Gnih mRNA levels in the hypothalamus of the cichlid fish *Cichlasoma dimerus*. Taking into account our results, we proposed that Gnih could act as a link between feeding and reproduction. Therefore, this work aimed to study the anatomical relationships between Gnih producing neurons and those that produced the main hypothalamic hormones involved in feeding control, proopiomelanocortin (Pomc), and neuropeptide Y (Npy) in *C. dimerus*. Adults were sampled, and the brains were dissected and processed for double-immunofluorescence. By double immunofluorescence, we found a close association between Npy-immunoreactive (-ir) somata and Gnih-ir fibers in the *nucleus posterior periventricularis* of the hypothalamus, as well as fiber-fiber close associations along the ventral hypothalamus. With respect to Pomc-ir neurons, we observed fiber-somatic and fiber-fiber contacts with Gnih-ir fibers in the *nucleus lateralis tuberis* as well as fiber-fiber contacts in the preoptic area. We also found that Npy-ir fibers are in close contact with Pomc-ir somata and its fibers. No differences were observed between males and females. In conclusion, to our knowledge, this is the first study performed in a teleost fish to show a morphological association between Gnih, Pomc, and Npy, providing evidence that this neuroendocrine network may be conserved along the vertebrate lineage.

122. 630. **CHARACTERIZATION OF LH AND TSH EXPRESSION IN THE SACCUS VASCULOSUS OF THE NEOTROPICAL CICHLID FISH *CICHLASOMA DIMERUS***

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The saccus vasculosus (SV) is a highly vascularized organ of gnathostome fishes, located ventral to the hypothalamus and posterior to the pituitary gland. It is characterized by a folded neuroepithelium composed mainly of coronet and supporting cells, but its function remains controversial. In 2013, Nakane et al. proposed that the SV could be homologous to the *pars tuberalis*, being involved in the control of seasonal reproduction since they detected the expression of genes involved in this function: thyrotropin (*tsh*) and its receptor (*tshr*) and rhodopsin family genes in the SV of *Oncorhynchus masou*. In addition, the authors observed that isolated SV of this species could respond to photoperiod and that their removal suppressed the gonadal response to these signals. Furthermore, in the last six years, studies in different fish species have shown the expression of neuropeptides and receptors related to reproduction. Previously, in our laboratory, we characterized the SV of *Cichlasoma dimerus* and detected the innervation of gonadotropin inhibitory hormone (Gnih) in adults and ontogeny, a neuropeptide whose role in reproduction has been widely discussed. This study aims to characterize the expression of Lh and Tsh in the SV of adults of *C. dimerus* and to evaluate the effect of Gnih on lh expression *in vitro*. Adult SV were sampled and processed for immunohistochemistry, PCR, and *in vitro* assays. β-Tsh immunoreactivity was observed in the vesicles of the apical processes of the coronet cells in both sexes, and we detected the mRNA expression of both β-tsh and β-lh by PCR. Regarding the in vitro assays, preliminary results indicate that the β-lh mRNA expression was stimulated by the presence of Gnih synthetic peptide, especially in females. The present work is the first report showing the expression of β-tsh and β-lh in a freshwater species and is the starting point for future experiments on the role of SV in reproductive function.

123. 654. **MULTI-YEAR SURVEY OF GENOTYPIC AND PHENOTYPIC SEX RATIOS AND FREQUENCIES OF GENOTYPE/PHENOTYPE MISMATCHES IN PEJERREY *ODONTESTHES BONARIENSIS* FROM LAKE CHASCOMÚS**

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In the pejerrey *Odontesthes bonariensis*, exposure to high and low temperatures during the critical period of sex determination (CPSD) induces testicular and ovarian differentiation, respectively, regardless of the presence or not of the sex-determining gene *amhy*, which is crucial for testis formation only at intermediate, sexually neutral temperatures. Therefore, the presence/absence of *amhy* serves as a marker of genotypic sex (XX/XY) and allows to monitor the changes in genotypic and phenotypic sex ratios of wild pejerrey populations as well as to analyze the impacts of anthropogenic and climatic factors on pejerrey resources. Nevertheless, studies on sex determination in natural populations are virtually inexistent. Our group has conducted yearly biological monitoring of the pejerrey population in Lake Chascomús since 2014 using the *amhy* as a genetic marker of genotypic sex. In this study, we performed otolith increment analysis and growth history reconstruction (back-calculation) in representative individuals from different size classes collected on each year between 2014 and 2019 to discriminate all possible year classes represented in the annual collections. The analysis classified individuals as born between 2012 and 2019 and as age 0, 1, or 2 years old. Marked year-to-year variations in genotypic and phenotypic sex ratios were observed. Genotypic sex ratios were generally balanced, but XY fish were notably abundant in the 2013 year-class whereas the opposite was true in 2019. Genotypic female-to-phenotypic male transitions were far more prevalent that its reciprocal, male-to-female transition, and this was reflected in markedly male-biased phenotypic sex ratios in at least three year-classes. Combining otolith analysis with genotypic and phenotypic sex discrimination provides important insights into the ecology and reproductive health of pejerrey populations.

124. 664. **UTERINE INVOLUTION DURING EARLY POSTPARTUM IN SUCKLED BEEF COWS IN PASTORAL CONDITIONS**

Victor Leavi De Asis[1,2], Karen D. Moran[1,2], Luisina Chapero[1,2], María Florencia Farcey[2], Juan Pablo Piccini[2], Evelin Mariel Elia[3], Valeria Analía Sander[4], Julián A. Bartolomé[2,5], María Guillermina Bilbao[1,2].

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Cow-calf profitability depends on maintaining one-year calving intervals, underscoring the value of calving-conception reduction. This study aimed to assess endometrial status in the initial 60 days post-calving. Fifteen cows were enrolled in this trial. Glucose (mg/dL), Urea (mg/dL), Progesterone (ng/mL), vaginal discharge (VD, scale from 0 to 3), and polymorphonuclear cell count (%PMN) were evaluated at 30, 45, and 60 days postpartum (DPP). Relative expression of antioxidant defenses (CAT, SOD, GPx) and inflammation markers (TNFα, IL-6, IL-1β) using RPL19 as a housekeeping gene was evaluated in a subset (n=5). Marginal models were employed to analyze repeated measures, with time as a fixed effect and the individual cow as a random effect. The association was evaluated using the Mcnemar´s test, and the Friedman test was used if normality was lacking. Antioxidant defenses correlation was assessed using the Pearson test. No variation was observed in Glucose (P=0.612) and Urea (P=0.235). A total of 73.34% (11/15) of cows resumed cyclicity. The proportion of cows with VD=0 was 73.33%, 80.00%, and 73.33% at 30, 45, and 60 DPP, respectively, without any association between these time points. No variation in %PMN was detected (P=0.983). SOD exhibited variation across DPP (P=0.010). Decreased expression was noted between 30 and 45 DPP (P=0.009), as well as between 30 and 60 DPP (P=0.041), with no difference between 45 and 60 DPP (P=0.950). No effect of DPP on the other two antioxidant defenses was observed (GPx: P=0.173; CAT: P=0.369). However, the expression of all three enzymes showed a strong correlation (SOD-CAT: r=0.564, P<0.05; CAT-GPx: r=0.794, P<0.001; GPx-SOD: r=0.642, P<0.01). In this preliminary analysis, no effect of DPP on inflammation markers was detected (TNFα: P=0.609; IL-6: P=0.226; IL-1β: P=0.409). These findings suggest that while uterine involution seems effective, there is a noticeable distinction in the endometrial status pre- and post- 45 DPP.

O-GENETICS

WEDNESDAY 15TH NOVEMBER 14:00-15:30
CHAIRS: ALEJANDRA DUARTE
ABEL CARCAGNO

125. 133. **THE CHARGE SYNDROME ORTHOLOG CHD-7 REGULATES TGF-β PATHWAYS IN *Caenorhabditis elegans***

Diego M. Jofré[1], Ailen S. Cervino[2], Luciana F. Godoy[3], M. Cecilia Cirio[2], Judith L. Yanowitz[4] and Daniel Hochbaum[3]
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2. IFIBYNE, Conicet
3. CEBBAD, Universidad Maimonides
4. University of Pittsburgh. Pittsburgh, PA, US

Dauers are long-lived larvae specialized in survival and dispersal that are induced when *Caenorhabditis elegans* encounters harsh environmental conditions in early development. In a RNAi screen looking for dauer suppressors, we identified the chromodomain helicase CHD-7. Loss of *chd-7* completely bypass dauer development or fail to complete morphogenesis, resulting in partial dauers. Mechanistically, our epistasis analysis placed CHD-7 as a TGF-β pleiotropic regulator, a pathway required for dauer development. CHD7 is the only gene associated with CHARGE syndrome, a neurodevelopmental disorder with a range of defects ranging from hearing loss and facial dysmorphism to heart defects. RNA-seq of *chd-7*-depleted worms, shows misexpression of collagen genes. To explore a potential conserved function in vertebrates, we used *Xenopus laevis* embryos, an established model to study craniofacial development. Morpholino-mediated knockdown of Chd7 led to a reduction in col2a1 messenger RNA (mRNA) levels, a collagen whose expression depends on TGF-β signaling. Both embryonic lethality and craniofacial defects in Chd7-depleted tadpoles were partially rescued by overexpression of col2a1 mRNA. We suggest that Chd7 has conserved roles in regulation of the TGF-β signaling pathway and pathogenic Chd7 could lead to a defective extracellular matrix deposition.

126. 334. **SKEWED X CHROMOSOME INACTIVATION AND MANIFESTING FEMALE CARRIERS OF DYSTROPHINOPATHY**

Micaela Carcione[1,2], Chiara Mazzanti[1,2], Leonela Luce[1,2], Macarena Bollana[1,2], Carmen Llames Massini[1,2], Triana Visconti[1,2], Florencia Giliberto[1,2].
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Dystrophinopathies are recessive X-linked neuromuscular diseases caused by disease associated variants in the *DMD* gene. These variants consist of CNVsin 80% of cases and SNVs in the remaining 20%. Generally, being an X-linked recessive disorder, males are primarily affected due to having a single X chromosome, while females are carriers of the disease. However, it's estimated that 2.5-7.8% of females with a heterozygous alteration in *DMD* will develop symptomatology, and one of the causes is believed to be skewed X chromosome inactivation. In other words, these females will have the X chromosome without the molecular alteration in the *DMD* gene inactivated. When manifesting female carriers exhibit heterozygous alterations, their X chromosome inactivation in peripheral blood is studied. The objective of this study is to determine if skewed X chromosome inactivation could explain the clinical presentation of women with dystrophinopathy. Sixteen manifesting female carriers were studied using MLPA, NGS, and HUMARA test techniques. Heterozygous alterations were found in 14 of the analyzed women, a hemizygous alteration in one of them, and a homozygous alteration in the remaining one. It was determined that 6 (43%) of the women with heterozygous alterations exhibited skewed X chromosome inactivation, while the remaining 8 did not. In conclusion, skewed inactivation could explain the symptoms in 6 out of the 14 patients. However, it cannot be ensured that what is observed in blood is being replicated in muscle. Regarding the remaining 8 women without skewed inactivation, we cannot rule out the existence of another variant not detected by the methodology used or the occurrence of other events such as autosomal:X translocations, Turner syndrome or uniparental disomy.

127. 365. **IN-DEPTH ANALYSIS OF NKX2-5 BINDING SITES AND ITS POTENTIAL FUNCTIONAL PATHWAYS**

Kolomenski, Jorge Emilio[1]; Dain, Liliana[1,2]; Nadra, Alejandro Daniel[1]
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NKX2-5 codes for a homeobox protein that plays a key role in heart development and function. It is a transcription factor (TF) known to interact with others and form complexes. Some of its genetic variants were found to be related with congenital heart disease. In this study, we aim to characterize NKX2-5 binding sites to study possible correlations between binding sequence, gene function and interaction with other TFs. We worked with the results of ChIP-seq studies from human and mouse identifying NKX2-5 binding regions. We complemented them with RNA-seq transactivation studies showing which genes were upregulated or downregulated by NKX2-5 expression when compared to knock-out or knock-down lines. We also analyzed binding sites from 8 TFs known to interact with NKX2-5. We processed these results with MEME-ChIP, ShinyGO and custom Python scripts and gathered them for analysis. We compiled 6362 binding sites consisting of 20 different unique sequences. We observed that binding sites are often in the 10 kbp upstream of gene +1, but they are regularly found up to 500 kbp upstream and downstream of the target genes, consistent with NKX2-5's function as an

enhancer. We found 369 downregulated and 510 upregulated genes near one of our compiled NKX2-5 binding sites, although we did not find an association between any of the binding site sequences and up- or down-regulation. We found 3294 binding sites for the other 8 TFs within 1000 bp of NKX2-5 binding sites. Among others, analyzing ShinyGO's results, we found that a significant proportion of the genes near both NKX2-5 and TBX20 binding sites were involved in heart development. In conclusion, we made an in-depth analysis of NKX2-5 binding sites and 8 potential co-regulators, which could help distinguish how and why NKX2-5 up- or down-regulates downstream genes in the path to build a complete NKX2-5 regulatory network.

128. 400. **CO-OCCURRENCE OF HOTSPOT GAIN-OF-FUNCTION AND NOVEL FGFR3 VARIANTS IN PATIENTS WITH DISPROPORTIONATE SHORT STATURE**

Paula A. Scaglia[1,2], Debora Braslavsky[1], Franco Brunello[3], María Esnaola Azcoiti[1,2], Agustín Izquierdo[1,2], Lourdes Correa Brito[1], Romina Armando[4], Florencia Villegas[4], María del Carmen Fernández[4], Hamilton Cassinelli[1], Ana Keselman[1], Nora Sanguineti[1], Claudia Arberas[4], Ignacio Bergadá[1], Rodolfo Rey[1,2], María Gabriela Ropelato[1,2]

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FGFR3 is a tyrosine kinase receptor involved in growth regulation. Gain-of-function (GOF) variants in this gene have been linked to diverse skeletal disorders. The aim of our work is to report the molecular findings and explore genotype-phenotype correlation in patients (pts) with *FGFR3* gene variants (SNVs). SNVs were detected by NGS in 4 (3M, 1F) disproportionate short stature (SS) pts. SNVs were classified according to ACMG/AMP and ClinGen recommendations. FoldX software was used for thermodynamic analysis of *FGFR* variants. RefSeq: NM_000142.5, NP_000133.1. Different known *FGFR3* GOF pathogenic SNVs were found: c.1950G>C (K650N), c.1950G>T (K650N), c.1948A>C (K650Q) and c.749C>G (P250R). SNVs arose *de novo* in 2 cases and were inherited from an affected parent in the other 2 with familial SS. Body disproportion and relative macrocephaly were observed in all the cases. Interestingly, a 2nd novel VUS on the same *FGFR3* allele was found in 3 cases. Thermodynamic analysis predicted no relevant impact for T651S and D709G, while G268D was predicted to disrupt a turn in an Immunoglobulin I-Set domain. The co-occurrence of both P250R, a well-studied GOF variant with increased affinity for its ligand, and the destabilizing loss-of-function G268D variant may explain the absence of the typical Muenke syndrome phenotype described in pts with the P250R variant. Clinical characteristics and radiological findings varied significantly, even in the 3 cases with variants located on the same hotspot codon 650. The spectrum included SS with mild skeletal anomalies, typical hypochondroplasia (HCH) and HCH associated with severe acanthosis nigricans. The identification of 2 *FGFR3* SNVs in *cis* in 3 of the pts highlights the importance of studying not only hotspots associated with classical phenotypes but the whole gene to expand our understanding of FGFR3-related disorders. Functional studies will be required to fully elucidate the impact of novel SNVs, especially the [P250R;G268D] combination.

129. 446. **ALTERATIONS IN DIFFERENT GENETIC REGIONS IN RETINOBLASTOMA**

Diana Parma[1], Daiana Ganiewich[2], Daniela Alves da Quinta[3], Miguel Martin Abelleyro[4], Irene Szijan[1]

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Retinoblastoma (RB) arises by inactivation of *RB1* tumor suppressor gene, inducing cell division in retinal precursors. RB, the prototype of developmental tumors, from neonatally to 5 years, has an incidence 1 in ~ 20,000. Identification of *RB1* variants allows detection of risk in patients´ relatives. Moreover, diagnosis of alterations in other genes and chromosomal regions enables RB prognosis. Large-scale copy number alterations reported in chromosomal regions were gains in 1q, 2p, 6p and loss in 16q, with low changes, they are typical for RB, not found in other cancers. The most recurrent are *MYCN* amplification (8%) and 6p gain (28%). Our aim was to study alterations in genes related to RB and gains/losses in specific chromosomal regions for additional aberrations affecting RB development and evolution. Exoma sequencing data of RB patients´ somatic and germline DNA were explored for genetic alterations in 2 steps: 1) Analysis of variants in ten genes, probably mutated in RB; 2) Specific gains/losses in chromosomal regions. 1) Ten genes, probably mutated in RB, were analyzed. The variants found were missense, intronic, synonymous and splice in 4 to 8 position of consensus site and 3´UTR. *BCOR*, the most mutated in RB, showed a splice variant c.4977-4G>T in 1 somatic and 1 germline DNA, with allele frequency (AF) 0.136689. *CREBBP* showed a variant c.3837-8C>T with AF 0.211062 in one somatic sample. Other genes, *TMEM135*, *IGSF3*, *ADGRB3*, *STOML2*, *TXK* and *CDH11* showed missense and similar splice variants in most samples with low AF. *CCNC* and *CRX* had only intronic or synonymous variants. 2) We are studying chromosomal alterations in our patients for their influence on RB progress and have not yet finished. In conclusion, genetic alterations found were moderate and low impact variants in *BCOR* in 2 samples and other genes in almost all samples. with low AF. Thus, the RB genome is relatively stable with mild, not frequent alterations in several genes apart *RB1*.

130. 451. **MOLECULAR DIAGNOSTIC STRATEGY IN PEDIATRIC PATIENTS WITH PRESUMPTIVE DIAGNOSIS OF MARFAN SYNDROME**

María Esnaola Azcoiti[1,2], Paula Alejandra Scaglia[1,2], Agustín Izquierdo[1,2], Bárbara Casali[1,2], Claudia Arberas[3], Romina Armando[3], María del Carmen Fernández[3], Florencia Villegas[3], Lourdes Correa-Brito[1], Rodolfo Rey[1,2], María Gabriela Ropelato[1,2].

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Marfan Syndrome (MFS) is produced by heterozygous variants along the 66 exons of the *FBN1* gene. The diagnosis is based on clinical criteria according to the number of major and minor systems affected following international Ghent criteria (GC). The precision molecular diagnosis of *FBN1* alterations is critical for the preventive management of thoracic aortic aneurysm rupture or dissection through follow-up and lifestyle modification of the patients. We aimed to analyze clinical features and *FBN1* sequencing results of pediatric patients (pts) with presumptive MFS to implement an appropriate molecular diagnostic strategy. We studied 9 pts (6 M, 3 F) with a median age of 10 y (range: 2 months-17 y). Five pts (G1) fulfilled GC for MFS; the other 4 pts (G2) only presented some MFS characteristics probably associated to *FBN1* variants. *FBN1* sequencing was performed by NGS (5 pts custom panel-Twist (TP), 4pts WES or clinical exome. Copy number variants (CNV) were screened using DECoN and confirmed by aCGH. The variants were classified using the ClinGen *FBN1* expert panel guideline for applying ACMG/AMP criteria. We found 6 causative variants (3 novel) in the 5 pts of G1 and in one pt of G2. Sequence variants (3 missense, 1 splicing and 1 frameshift) were distributed along the gene in exons 5, 16, 25, 58, and intron 14. The CNV found was a deletion of exons 7-16. The diagnostic yield in G1 was 100%, while 3 out of 4 G2 pts remain undiagnosed (2 studied with TP). NGS *FBN1* sequencing was a good approach for G1 given the extension of the gene and the diversity of variant types found. The inclusion of genes of MFS differential diagnosis in an NGS panel is particularly important at pediatric age when

clinical diagnosis is challenging because some pathognomonic features may still be absent. Precise molecular diagnosis is crucial in this group of pts to adjust treatment, personalize follow-up, and modify lifestyle to reduce cardiovascular associated morbimortality.

131. 665. **ASSESSMENT OF CARRIER FREQUENCIES OF AUTOSOMAL-RECESSIVE AND X-LINKED DISEASES IN OUR POPULATION**

Mónica Fabbro, Micaela Galain, Eloisa Caviglia, Yannina Díaz, Sebastián Menazzi, Sergio Papier, Cecilia Fernández
Novagen, Buenos Aires, Argentina

The carrier screening test allows the identification of carrier status of autosomal-recessive and X-linked pathologies. It is widely used among gamete donor, couples and individuals to know their reproductive risk of having an affected child. Our laboratory has been using different carrier screening panels since 2013 and has previously reported the carrier rates for these panels. Although many databases containing variant information have been created, our population is not well represented, and the carrier frequencies (CF) of rare genetic diseases is inferred from the international prevalence of the disorders. However, the carrier screening test provides us with a unique opportunity to estimate the CF in our population. The objective was to describe the CF of several recessive and X-linked disorders using the results from the carrier screening study. 2441 test results from individuals, couples or gamete donor with no history of recessive and X-linked conditions were included. 1477 individuals were evaluated using panel A (SNP array genotyping of 302 genes), 589 with panel B (NGS targeted genotyping of 299 genes) and 375 using panel C (full-exon sequencing of 302 genes). 855 clinically relevant variants (CRV) were identified in 123 genes in panel A, 720 CRV in 118 genes in panel B and 578 CRV in 142 genes in panel C. To estimate the most representative CF, we considered only the genes in common across all 3 panels. Among the 2441 samples, a total of 1222 CRV were identified in 40 genes. 17 genes had CF ≥1/100 (*CFTR*, *SERPINA1*, *CYP21A2*, *GJB2*, *MEFV*, *SMN1*, *PAH*, *ACADM*, *GALT*, *GAA*, *DHCR7*, *FMR1*, *PKHD1*, *ATP7B*, *HBB*, *MMACHC*, *PMM2*), 6 had CF <1/100 and >1/200, and the remaining genes were between 1/200 and 1/800. The CF of 40 genes were determined in over 2400 individuals. The CF calculated here could be used as an approximation of the CF in our population. Information regarding CF can influence health policies, such as access to genetic testing and counseling services.

P1-GENETICS

THURSDAY 16TH NOVEMBER 9:00 - 10:30
CHAIRS: MATÍAS VALENZUELA ÁLVAREZ
VIVIANA DALAMON
JUAN MIGUEL BAYO FINA

243. **DE NOVO DOUBLE RECIPROCAL TRANSLOCATIONS AND PARTIAL DELETION AT ANOTHER CHROMOSOME IN A CHILD WITH SEVERE GLOBAL DEVELOPMENTAL DELAY: CLINICAL, CYTOGENETIC AND CYTOGENOMIC CHARACTERIZATION**

Cynthia Bravo[1], Ramiro Luna[2], Lilian Franzi[1], Fabiana Guerrisi[1], Liliana Kreiman[3], Aldana Claps[1], Julieta Laiseca[1], Melisa Taboas[1], Tania Castro[1], Lucía Vago[3], Roxana Cerretini[1]
[1]Centro Nacional de Genética Médica, Administración Nacional de Laboratorios e Institutos de Salud (ANLIS) "Dr Carlos G. Malbrán", Buenos Aires, Argentina [2]Hospital Interzonal Dr. José Penna, Bahía Blanca, Buenos Aires, Argentina [3]Hospital de Niños Zona Norte Dr Roberto Carra, Rosario, Santa Fe, Argentina.

It is infrequent to observe more than two structural chromosome rearrangements in an individual. This case report describes a 11 year-old boy with severe global developmental delay, microcephaly, short stature among other dysmorphia and complex chromosome rearrangements (CCR) involving 5 abnormal chromosomes. The proband's mother had a perinatal history of exposure during the entire pregnancy to fumigation due to the proximity of the residence to the soybean field. The aim of this study was to identify these CCR using classical cytogenetic, FISH and CGH-array techniques and relate them to the patient's phenotype. Karyotype analysis using Giemsa banding showed 46 chromosomes in all cells counted, two reciprocal translocations, t(1;2)(q42;p12) and t(5;6)(p13;p25) and a interstitial deletion on chromosome del(12)(p11.1p12). The reciprocity of the translocations and the interstitial deletion were confirmed with FISH with subtelomeric probes and the extent of the deletion was studied with CGH-array which comprised a size of 11MB and 73 genes including the SOX5 gene. The monosomy of the SOX5 gene is associated with to Lamb-Shaffer Syndrome (OMIM # 616803) whose phenotype overlaps with the clinic of the purpose. Parental karyotypes were normal. Although it is theoretically possible that the two reciprocal translocations and the deletion occurred at different times, it is more plausible to consider them to be the result of a single event, arising either very early in the zygote or in a parental gamete during gametogenesis. Exposure to pesticides increases the frequency of chromosomal aberrations in exposed individuals. However, to establish an association between genotoxicity by pesticides and the anomalies found in the purpose, indirect biomarkers of genetic damage had to be studied in both parents exposed in the perinatal stage. Since these studies were not performed at the time of exposure, it is very difficult to establish a cause-effect relationship 11 years later.

132. 303. **FUNCTIONAL CHARACTERIZATION OF VARIANTS IN HUMAN TPO GENE USING BACULOVIRUS EXPRESSION SYSTEM**

Maricel F. Molina[1,2], Ezequiela Adrover[1,2], Sebastián R. González[1], Adriana V. Sabljic[4], Aldana Trabucchi[4], Silvina N. Valdez[4], María Victoria Miranda[3], Rodolfo M. González-Lebrero[5], Alexandra M. Targovnik[3], Carina M. Rivolta[1,2].
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[4]CONICET-Universidad de Buenos Aires. Instituto de Estudios de la Inmunidad Humoral Prof. Ricardo A. Margni (IDEHU). Buenos Aires, Argentina.
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The congenital hypothyroidism (CH) is the most common endocrine disease in children with a prevalence of 1 in 2000-3000 live births. Variants in Thyroid peroxidase (TPO) are more often associated with permanent CH by iodide organification defect and are commonly inherited in an autosomal recessive manner. TPO is a 933 amino acids long membrane-bound glycoprotein located at the apical membrane of the thyroid follicular cells that plays a key role in thyroid hormones biosynthesis. The TPO enzyme activity depends on both proper folding and membrane insertion, and an intact catalytic site. The aim of this study was to investigate the functional impact of TPO gene missense mutations previously identified in our laboratory (p.Ala576Glu, p.Arg595Lys and p.Val748Met) in patients with permanent CH. The baculovirus expression vector system (BEVS) was used to express the WT and mutated proteins. First, TPO cDNA obtained by RT PCR and sequential cloning strategy in pGEMT previously in our laboratory was cloned into the pFastBac™DUAL expression vector. Then, site-directed mutagenesis was performed, and recombinant virus were constructed using the Bac-to-Bac® Baculovirus Expression Systems (Invitrogen). Recombinant proteins were expressed in Sf9 insect cells, purified, and characterized by SDS-PAGE and Western blot. Enzyme activity was determined by monitoring the oxidation reaction of the substrate guaiacol spectrophotometrically.

The values of apparent Km and Vmax were determined using the Gauss–Newton algorithm. Two of three mutants (p.Arg595Lys and p.Val748Met) were absent in the enriched membrane fraction. The functional evaluation of the third mutant (p.Ala576Glu) showed decreased activity with respect to WT, with a higher Km value and lower reaction efficiency (Vmax). The identification and characterization of TPO variants is undoubtedly a valuable approach to study the TPO structure/function relations and for the elaboration of a clinical diagnosis and genetic counseling.

133. 420. **EVALUATION OF MITOCHONDRIAL FUNCTION IN DIFFERENT OBESITY PHENOTYPE**

Rojo Mailén[1,2], Millán Andrea Liliana[1,2], Pautasso Constanza[1], Guillermo Armando Blanco[3], Frechtel Gustavo Daniel[1,2], Cerrone Gloria Edith[1,2], Pérez Hernán[1].

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3- Universidad de Buenos Aires. CONICET Instituto de Estudios de la Inmunidad Humoral Prof. Ricardo a. Margni (IDEHU)

Obesity is a risk factor for developing mitochondrial dysfunction. Dysfunctional adipose tissue induces low-grade inflammation, disrupting insulin signaling, and altering mitochondrial function, leading to increased reactive oxygen species (ROS) production. Our objective was to evaluate aspects of mitochondrial function in relation to metabolic and inflammatory parameters in different obesity phenotypes: without metabolic syndrome (MS): Metabolically Healthy Obese (MHO), and with MS: Metabolically Unhealthy Obese (MUO). Methods: 65 MHO, 67 MUO and 28 normal weight (NW) individuals were studied. Fluorescent probes; MitoTracker green, DIOC6, and Mitosox were used to assess mitochondrial function in mononuclear cells by flow cytometry. mtDNA content in peripheral blood leukocytes was determined by real-time quantitative PCR (qPCR), by SYBR Green method. Results: MHO have an intermediate phenotype between MUO and NW, considering the lipid profile, the inflammatory state (hsCRP), blood pressure, and glycemia. A decreased mitochondrial mass and DNA content were detected in obese individuals compared to NW ($p=0.01$, $p=0.07$ respectively). A significant reduction in mitochondrial content was observed between NW vs. MHO ($p=0.02$) and NW vs. MUO ($p=0.02$); and in mtDNA content between NW vs. MUO ($p=0.05$). No significant differences were found between both obesity phenotypes. An increased O^{2-} production was observed in mononuclear cells from obese individuals (MHO and MUO) ($p<0.01$). No differences in mitochondrial membrane potential were observed between NW and obese individuals. hsCRP level showed a negative correlation with mtDNA content ($p=0.03$) and a positive correlation with O^{2-} production ($p=0.01$). Conclusion: The lower mitochondrial content and increased ROS production observed in obese individuals are related to chronic inflammation, leading to metabolic stress and mitochondrial homeostasis imbalance, accelerating the progression of metabolic diseases.

134. 424. **AN OVERVIEW OF PATHOGENIC VARIANTS IN ACUTE INTERMITTENT PORPHYRIA IN ARGENTINA**

Varela Laura[1], Caballero Alejandra[1], Guolo Marcelo[1], Buzaleh Ana[1,2], Parera Victoria[1]

[1]Centro de Investigaciones sobre Porfirinas y Porfirias (CIPYP), Hospital de Clínicas, UBA-CONICET. [2]Departamento De Química Biológica, Facultad De Ciencias Exactas Y Naturales, Universidad de Buenos Aires.

Acute Intermittent Porphyria (AIP), an autosomal dominant disorder resulting from hydroxymethylbilane synthase (HMBS) deficiency, presents with life-threatening neuroabdominal crises. More than 520 variants in the HMBS gene were identified worldwide. This study aimed to analyze patients with AIP from unrelated families, focusing on genetic heterogeneity and pathogenic variants. We examined 127 AIP patients diagnosed at CIPYP. Diagnosis involved biochemical measurements (urinary 5-aminolevulinic acid and porphobilinogen, urinary and plasma porphyrins, and blood HMBS activity) and genetic studies for identifying pathogenic variants. In the population analyzed, 49 different pathogenic variants were detected. Notably, p.G111R (49%), p.Q34P (5.5%), p.R173W (4%), and c.202_203delCT (1.6%) were prominent variants. Other variations were private to specific families. This study revealed the identification of 4 new pathogenic variants for the Argentinian population, among them, p.(Leu49Cysfs*49) has not been previously reported. Moreover, compound heterozygosity was evident in 3 families, and dual PAI/PCT (Porphyria Cutanea Tarda) was found in another family. This study provides insights into the genetic landscape of AIP, highlighting the significance of genetic analysis for accurate diagnosis and informed medical guidance. The identification of novel variants further enriches our understanding of the genetic basis of AIP in the Argentinean population. Genetic analysis is indispensable in some cases to confirm AIP diagnosis. It is also relevant for the identification of latent patients and for counseling about triggering factors, thereby avoiding the clinical manifestation of the disease.

135. 603. **ANALYSIS OF THE ROLE OF *NR1I2* GENE VARIANTS IN HEPATIC PORPHYRIAS ONSET**

Kiara Martínez Yucovsky[1], Isabella Moltrasio[1], Elizabeth Woo[1], Tomas Santillán[2], Sebastián Yun[2], Viviana Melito[2,3], Laura Varela[2], Victoria Parera[2], Ana Buzaleh[2,3], Johanna Zuccoli[2]

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Porphyrias are due to heme enzymes deficiencies: Porphobilinogen deaminase in Acute Intermittent Porphyria (AIP), and Uroporphyrinogen decarboxylase in Porphyria Cutanea Tarda (PCT). Several factors, as therapeutic drugs, are needed for the onset of these hepatic diseases. *NR1I2* gene encodes for *PXR* transporter; NM_022002.3:c.196C>T and NM_003889.4:c.-22-7659C>T variants affect the expression of many proteins like ABCB1 and CYP3A4. The aim was to evaluate the role of *PXR* SNVs in AIP and PCT triggering. Cohorts studied: Control, symptomatic AIP (S-AIP), asymptomatic AIP (L-AIP) and PCT. Individuals signed informed consent. PCR-RFLP was used for genotyping. S-AIP allelic frequency for c.196C>T (0.13, $p<0.05$) was lesser than control (0.29) and L-AIP (0.27). A different genotypic profile was observed: S-AIP showed a minor value in heterozygosis (26%, $p<0.05$) vs Control (45.5%) and L-AIP (48.5%); TT was in a very low/null value (Control= 6%, S-AIP=0%, L-AIP=3%). No differences were found for c.-22-7659C>T allelic frequency among groups, while TT genotypic frequency was less in L-AIP (15%, $p<0.05$) vs Control (43%) and S-AIP (30%). In PCT, T allele frequency for c.196C>T was low (0.02, $p<0.01$) vs Control (0.29), while data for c.-22-7659C>T were similar to PCT and Control. Genotypic profile showed less proportion ($p<0.01$) of CT (4%) in PCT vs Control (45.4%); TT was null in PCT and very low in Control (6%). Haplotype studies revealed that C/C and C/T were the most frequents; T/T was only found in L-AIP. A high prevalence of wild type alleles in symptomatic Porphyrias (S-AIP and PCT) would indicate PXR positive regulation for *ABCB1* and *CYP3A4*. We observed that *ABCB1* variants could influence in AIP and PCT trigger, but it would be independently of PXR variants here studied, without synergistic effect between SNVs. However, expression level of *NR1I2* genetic variants should be considered as a possible modulator in the pharmacological induction of Hepatic Porphyrias onset.

635. **A METATRANSCRIPTOMICS CHARACTERIZATION AND MONITORING OF KEY METABOLIC PATHWAYS IN PEOPLE WITH OBESITY, PREDIABETES AND TYPE 2 DIABETES UNDER A LIFESTYLE INTERVENTION**

Rosario Taussig[1], Rodrigo D. Peralta[1], Andrea L. Millán[3], M. Constanza Pautasso[3], Ignacio Cassol[1], Gloria E. Cerrone[3], Gustavo D. Frechtel[3], Juan P. Bustamante[1,2].

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The study of the metabolic pathways driven by the gut microbiota, and those associated with the production of specific compounds present in the intestinal microbiota plays a fundamental role in understanding its impact on human health. Particularly, for type 2 diabetes, the pathogenicity of this metabolic disorder in relation to key metabolic pathways driven by the microbiota and gut microbiome has not yet been fully addressed in literature. Herein, a metatranscriptomics characterization and monitoring of key metabolic pathways in people with the mentioned disorders under a lifestyle intervention is addressed, together with health indicators of these metabolic disorders. Data from 98 patients (40 people with obesity, 21 with prediabetes, 12 with T2D) and 25 control people from the undergoing MicrobiAr's clinical trial were analyzed (ClinicalTrials.gov ID NCT05372445). Microbial profiling was obtained from stool samples using the Bunny Wipe Fecal Sample Collector from Zymo Research. DNA extraction was achieved using *ZymoBIOMICS™ DNA Miniprep Kit*. Genomic libraries were constructed according to *Illumina DNA Prep Reference Guide*. Samples were sequenced with a NextSeqTM 500 sequencer according to Illumina's Protocol. Here, a deep analysis was achieved focusing on key functional groups abundances differences upon different food intakes (traditional diet according to American Diabetes Association recommendations and a plant-based diet). Particularly, a greater presence of functional groups such as regulation of inflammatory processes and production of SCFAs under plant-based diet in the three cohorts with metabolic diseases were observed, compared to the traditional diet. An analysis of stratified contributions to crucial expressed metabolic pathways under the previously mentioned functional groups was also achieved, providing evidence the synergistic mechanisms throughout our microbiota are shaping activities for our disease/health balance.

136. 645. **FOLLOW-UP OF THE MICROBIOME AND HEALTH INDICATORS IN COHORTS WITH OBESITY, PREDIABETES AND TYPE 2 DIABETES UNDER LIFESTYLE INTERVENTION**

Andrea L. Millán[1], M. Constanza Pautasso[1], Rosario Taussig[2], Rodrigo D. Peralta[2], Ingrid S. Feijoo, Mailén Rojo[1], Ignacio Cassol[2], Gloria E. Cerrone[1], Juan P. Bustamante[2,3], Gustavo D. Frechtel[1].
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The gut microbiota (GM) is defined as the community of microorganisms colonizing the gastrointestinal tract and its alterations are involved in the pathogenesis of metabolic syndrome, obesity and type 2 diabetes. A controlled randomized clinical trial was conducted at the Hospital de Clínicas "José de San Martín" to assess the intervention of physical activity and diet on lifestyle in 44 individuals with obesity (Ob), 23 with prediabetes (preD) and 13 with type 2 diabetes (T2D), in comparison with a reference group of 21 individuals, in which the composition of the GM and health indicators were followed-up for 6 months. Microbial DNA was obtained from stool samples using *ZymoBIOMICS DNA Miniprep Kit*, and genomic libraries were constructed according to Illumina's protocol for *NextSeq 500* platform sequencing. Quality control and processing of sequencing data were performed by using KneadData. The taxonomic profile was obtained with MetaPhlAn4. Alpha and beta diversity metrics were calculated with R's package: MicrobiotaProcess. Clinical, biochemical and anthropometric parameters were determined. The taxonomic analysis of bacterias from the GM revealed that Bacteroidetes (Bac) and Firmicutes (Fir) were the predominant phyla in all cohorts. A higher abundance in the phylum Fir along with a lower abundance in the phylum Bac, and a high ratio Fir/Bac were observed in the Ob, preD and T2D cohorts versus the reference cohort. Alpha diversity metric was lower in all metabolic disease cohorts than in the reference cohort, and beta diversity metric was different between all studied cohorts. Differences in several clinical, biochemical and anthropometric parameters were also found associated with microbiota changes. The impact of changes in lifestyle in the Ob, preD and T2D cohorts was studied after 6 months, finding a progressive trend to decrease the Fir/Bac ratio in correlation with an improvement in clinical, biochemical and anthropometric parameters.

P2-GENETICS

FRIDAY 17TH NOVEMBER 14:00-15:30
CHAIRS: LEANDRO MAINETTI
MARÍA JOSÉ RICO
IRMA SLAVUTSKY

137. 127. **HYPOXIA EFFECTS OVER RSUME LEVELS, WHITE BLOOD CELLS AND TISSUES ON A KNOCKOUT MOUSE MODEL**

Nicolas Ciancio Del Giudice[1], David Gonilski-Pacin[1], Florencia Herbstein, Manuel Fiz[1], Sergio Senin[1], Carol Fagundez[1], Mariana Fuertes[1], Eduardo Arzt[1].
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RWdd3 or RSUME (**R**WD-containing **SUM**Oylation **E**nhancer) was cloned in our laboratory, is highly expressed in heart and kidney and is induced under stress conditions like Hypoxia (HPX), and heat shock. RSUME enhances glucocorticoid receptor transcriptional activity and regulates the HIF-VHL pathway. The aim of this study is to characterize the knockout mouse model for RSUME (Tm1d) that we generated, in heart and kidney under normoxia or hypoxic conditions. Wild type (WT), heterozygous (Het) and Tm1d mice were exposed to HPX for 6h, 72h, or 168h. We obtained blood samples and collected kidney and heart samples. We found that RSUME mRNA levels in WT mice were higher than in Het, both in heart ($p=<0.0001$) and kidney ($p=0.008$) while Tm1d mice lacked RSUME expression. After exposing WT to HPX, we observed that RSUME mRNA levels increased after 6h ($p=0.015$), returned to baseline at 72h, and decreased its expression after 168h ($p=0.007$).We observed that both the percentage and quantity of neutrophils in Tm1d animals were lower compared to WT ($p=0.036$ and $p=0.030$). When exposed to 6h HPX, neutrophil count decreased and lymphocyte percentage increased in WT animals ($p=0.005$ and $p=0.027$), this effect was absent in Het and Tm1d animals. Eosinophils exhibited a reduction in percentage at 6 hours of HPX in Tm1d ($p=0.0487$). Heart VHL mRNA and Protein levels were found decreased ($p=0.013$ and $p=0.004$) in Tm1d. After 72h HPX, protein levels of VHL exhibited a reduction in Tm1d ($p=0.016$). A decrease in VEGF protein expression was evident in Tm1d ($p=0.020$), but no significant changes were observed in VEGF mRNA levels. A knockout model for RSUME was effectively established, enabling us to examine RSUME action in an *in vivo* model. We confirmed previous *in vitro* data that show that RSUME is an acute HPX-responsive gene. Tm1d KO mice exhibit differences in white blood cell series and their HPX response, in terms of regulation of key proteins (VHL and VEGF) associated with HIF pathways in heart.

138. 161. **DEVELOPMENT OF A PCR-BASED METHODOLOGY TO DIFFERENTIATE DONOR AND RECIPIENT-DERIVED CELL-FREE DNA FOR ITS APPLICATION IN PEDIATRIC LIVER TRANSPLANT PATIENTS**

Arrigone Agostina[1], Trezeguet Renatti Guido[1,2], Moragas Matias[1], Camacho Maria Fernanda[3], Gamba Cecilia[1], Imventarza Oscar[1], Halac Esteban[1], Belli Carolina[3], Schaiquevich

Paula[1,2].
1. Hospital de Pediatría Prof Dr Juan P Garrahan, Buenos Aires, Argentina.
2. Consejo Nacional de Investigaciones Científicas y Técnicas, Buenos Aires, Argentina.
3. Laboratorio de Genética Hematológica, Academia Nacional de Medicina, Buenos Aires, Argentina.

Cell-free DNA (cfDNA) has emerged as a promising biomarker due to its variable concentration in diverse physiological and pathological conditions. In transplant patients, cfDNA is shed to blood from both the transplanted organ and recipients tissues due to cellular turnover. Under certain clinical conditions cfDNA levels in blood can increase due to necrosis, being a promising biomarker to monitor allograft health. Genotyping both individuals is essential to differentiate alleles and quantify cfDNA from both origins. One approximation is the use of biallelic deletions and insertions polymorphisms (DIP). The aim of this study was to evaluate the capacity of different molecular techniques to distinguish 9 DIPs from two individuals in the same specimen for ultimately monitoring cfDNA levels of transplant patients. For that purpose, genomic DNA (gDNA) from healthy volunteers was used. To characterize each gDNA, two approaches were considered. First, real-time PCR (qPCR) reactions were performed using allele-specific primers (ASP) for each DIP. Then, a unique qPCR reaction using universal typing primers (UTP) followed by High-resolution melting analysis (HRMA) was performed per DIP. Initially, qPCR conditions (e.g., annealing temperature, primer concentration) were optimized. Both techniques allowed us to identify the 9 DIPs of each of 10 healthy volunteers´gDNA. Nonetheless, the ASP approach required 18 reactions while using UTP and HRMA to obtain the complete genotype for each individual required only 9 reactions. In silico calculations revealed that the developed techniques are able to discriminate DIPs among 44 of 45 possible individual pairs of gDNA out of the 10 genotyped individuals. This technique has the potential to act as a non-invasive biomarker for evaluating the transplant tissue health and drug efficacy in rejection treatment. The system could offer a method for regularly assessing post-transplant individuals after further refinement and validation.

139. 475. **UNVEILING EPIGENETIC STRATEGIES FOR HETEROPLASMY MODULATION IN MITOCHONDRIAL DISORDERS**

Lía Mayorga[1], Sergio R. Laurito[1], Jimena Pérez[2], Carlos T. Moraes[3], María Roqué[1]
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2. Facultad de Ciencias de la Nutrición, Universidad Juan Agustín Maza, Mendoza, Argentina.
3. Department of Neurology, University of Miami Miller School of Medicine, Miami, Florida, USA.

Introduction: In mitochondrial disorders that arise from mitochondrial DNA (mtDNA) mutations, pathogenic mtDNA coexists with wild-type molecules, and the proportion of mutated mtDNA (heteroplasmy) dictates disease beyond a threshold. Thus, lowering heteroplasmy holds therapeutic promise. Evidence suggests that varying degrees of mitochondrial stress exert an influence on the nuclear epigenome which is proposed to be an adaptive response to severe stress. We then hypothesize that high heteroplasmy cells activate this epigenetic mechanism. Disruption of the latter could jeopardize these cells and favor the less dysfunctional ones to promote a decrease in heteroplasmy. Methods: Nuclear DNA methylation was characterized using Infinium 850k Methylation EPIC array® in cybrid cells carrying the m.13513G>A and m.8344A>G disease-causing mutations across varying heteroplasmy levels. Proliferation(BN00566®) and apoptosis(Annexin V) were investigated in response to DNA methylation inhibitors(DMIs) 5-Azacytidine and Decitabine. Heteroplasmic mosaicism was simulated by mixing high and low heteroplasmy cybrids and the distinct populations were traced using Green CMFDA® when exposed to DMIs. Results: Nuclear DNA methylation profiles differed according to mutation load and type. High heteroplasmy cells displayed heightened proliferation rates relative to their low heteroplasmy counterparts ($p<0.05$), effect abolished upon treatment with DMIs. In m.13513G>A cybrids, high heteroplasmy cells exhibited reduced apoptosis ($p<0.05$), which increased upon exposure to DMIs ($p<0.05$). In a heteroplasmy mosaic scenario involving m.13513G>A cybrids, treatment with DMIs (Decitabine, $p< 0.05$) enhanced the proportion of low heteroplasmy cells. Conclusions: A discernible nuclear DNA methylation pattern correlates with heteroplasmy levels. Modulation of this nuclear epigenetic blueprint seems to be an option to selectively uphold low heteroplasmy cells, providing an approach for heteroplasmy shift.

140. 493. **SCREENING OF FREQUENT GENETIC VARIANTS FOR NON-SYNDROMIC HEARING LOSS PATIENTS IN A COHORT FROM ARGENTINA**

Mariela Pace[1], Paula Buonfiglio[1], Vanesa Lotersztein[2], Sebastián Menazzi[3], Bibiana Paoli[4], Ana Belén Elgoyhen[1,5], Viviana Dalamón[1].
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2- Servicio de Genética del Hospital Militar Central Cirujano Mayor "Dr. Cosme Argerich"
3- División Genética del Hospital de Clínicas "José de San Martín"
4- Servicio de Otorrinolaringología Infantil del Hospital de Clínicas "José de San Martín"
5- Tercera Cátedra de Farmacología, Facultad de Medicina, Universidad de Buenos Aires.

Hearing loss (HL) is the most common sensory disorder affecting approximately 460 million people worldwide. In more than half of the cases, the cause of HL is genetic and early detection results essential, since treatment impacts exponentially on the development of hearing and language skills. Thus, understanding the underlying causes of hereditary hearing loss becomes a major issue in health. Most of the patients present non-syndromic hearing loss (70%) with an autosomal recessive mode of inheritance (80%). In these cases, *GJB*2 and *GJB*6 are the most frequently altered genes and patients with pathogenic variants in those genes present excellent outcomes in speech perception/production skills after cochlear implantation. The aim of this work was to identify frequent genetic variants in the *GJB*2 and *GJB*6 genes in patients with non-syndromic hearing loss, using different molecular biology techniques. A total of 84 patients were tested by Sanger sequencing for *GJB*2 variants in the coding exon and splicing donor site, and GAP- Multiplex PCR for detecting large reported deletions in *GJB*6. Twelve of the 84 patients were diagnosed (14%): 10/12 patients carried both pathogenic variants in *GJB*2 (83%) including one of them in the splicing acceptor site and 2/12 patients had 1 variant in *GJB*2 and the second variant in *GJB*6 (17%). The most frequent pathogenic variant was c.35delG (p.Gly12Valfs*2) in *GJB*2 (58%), and then: p.Met34Thr, p.Ile20Thr, c.167delT (p.Leu56Argfs*81), p.Asn206Ser, p.Arg184Pro, c.-22-2A>C, Del(*GJB*6-D13S1830) and Del(*GJB*6-D13S1854). 47/84 (56%) patients resulted carriers (1 pathogenic variant in *GJB*2), and 25 patients did not carry pathogenic variants either in *GJB*2 or *GJB*6 (30%) so other genes should be studied in order to achieve diagnosis. These results are in accordance with reported frequencies worldwide, and illustrate the importance of an accurate molecular diagnosis that enables precise clinical and genetic counseling for the patient.

141. 497. **MOLECULAR ANALYSIS OF CONGENITAL MYASTHENIC SYNDROMES IN PEDIATRIC PATIENTS FROM ARGENTINA**

María Eugenia Foncuberta[1], Soledad Monges[2], Silvina Gómez Montoya[2], Constanza Aimi[2], Lourdes Nuñez Antello[2], Giovanna Aschettino[3], Victoria Huckstadt[4], Fabiana Lubieniecki[5], Luis Pablo Gravina[1]
[1]Laboratorio de Biología Molecular-Genética, [2]Servicio de Neurología, [3]Laboratorio de Bioinformática-Unidad de Genómica, [4]Servicio de Genética, [5]Servicio de Patología. Hospital de Pediatría Garrahan

Congenital Myasthenic Syndromes (CMS) are a heterogeneous group of neuromuscular disorders caused by pathogenic variants in genes expressed at the neuromuscular junction. To date, 35 genes associated with CMS have been identified. The majority of CMS subtypes are amenable to pharmacological treatment; however, the appropriate pharmacotherapy varies among subtypes, and a drug effective for one subtype might exacerbate symptoms in another. The aim of this study is to describe the molecular findings in nine unrelated patients (7 males and 2 females) and one affected sibling (1 male) who are followed up by the interdisciplinary neuromuscular group of our hospital. Molecular diagnosis was achieved using a customized NGS panel for neuromuscular disorders, which included 27 CMS-related genes. We have identified pathogenic or likely pathogenic variants in *COLQ* (3 patients and 1 sibling), *RAPSN* (2 patients), *DOK7* (1 patient), *MUSK* (1 patient) and *PLEC* (1 patient with epidermolysis bullosa simplex and CMS). One patient presented a pathogenic variant and a variant of uncertain significance in the *CHRNB1* gene. We identified a total of 14 variants, six of which had not been previously described. Interestingly, all patients with pathogenic variants in *COLQ* were homozygous for missense variants; consanguinity was corroborated in only one family. The median age for molecular diagnosis was 8.2 years (range: 4.4-20 yrs.) among patients born prior to 2019 (n=8), before the routine use of the NGS panel for neuromuscular disorders. In contrast, for individuals born after 2019, the median age at diagnosis was reduced to 0.9 years (n=2). In conclusion, early genetic diagnosis in CMS is crucial for selecting the specific pharmacological treatment according to the underlying molecular mechanism and is essential for providing genetic counselling to the families. Moreover, it allows differential diagnosis with congenital myopathies, that, in some cases, may exhibit overlapping phenotypes.

142. 596. **MOLECULAR DIAGNOSIS BY MS-MLPA IN COHORT OF PATIENTS WITH BECKWITH-WIEDEMANN AND SILVER RUSSELL SYNDROMES SELECTED BASED ON A CLINICAL STRINGENT SCORE**

Bárbara Casali [1,2,] Paula Scaglia [1,2], Maria Esnaola Azcoiti [1,2,] Debora Braslavsky [1], Nora Sanguineti [1], Romina Armando [3], Florencia Villegas [3], Ana Keselman [1,] Analia Freire [1], Romina Grinspon [1], Mariana Vilas [4], Sandra Rozental [1,5], Claudia Arberas [3], Ignacio Bergadá 1, María Gabriela Ropelato [1,2]

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Introduction: Beckwith-Wiedemann (BWS) and Silver Russell (SRS) syndromes are opposite growth disorders associated with molecular defects in the 11p15.5 imprinting locus. A small SRS subgroup may have methylation defects in chromosomes 7 and 14. Their clinical diagnosis is based on international consensus scoring systems. MS-MLPA is the first line diagnostic test for the detection of methylation defects and copy number variants (CNV). The molecular diagnostic yield reported ranges from 20 to 40% according to the stringency patient's selection criteria. Aim: To evaluate the detection rate of MS-MLPA in a cohort of patients with BWS and SRS based on stringent clinical criteria. Patients and Methods: Specific clinical scores adapted from the latest consensus scoring system for each syndrome were used. Patients with a score of ≥4 were included. All patients were tested with the ME030 probemix targeting IC1 and IC2 imprinting centers in 11p15.5 and for SRS the probemix ME032 with further probes for the imprinted loci on chromosomes 7 and 14. Results: 29 patients met the inclusion criteria. Positive results were obtained in 7/19 SRS and 7/10 BWS. All SRS positive cases showed loss of methylation in IC1 (IC1-LOM). BWS cases presented IC2-LOM in 4, paternal uniparental disomy in the remaining 3 (IC1-GOM and IC2-LOM). We found a mosaic methylation profile in 4 patients in total. Conclusions: MS-MLPA diagnostic yield (BWS 70%, SRS 37%) was higher than previously reported in literature, probably due to the stringent patient selection criteria used. Mosaicisms represent a challenge in the interpretation of results and could be the underlying cause of some undiagnosed cases. In other cases, specific genetic testing may be needed. A larger cohort is required to validate these preliminary results.

143. 650. **THE PATAGONIAN HUMAN GENETICS NETWORK: INITIAL OUTCOMES**

Mariela Paola Teresita Vilte[1], Cecilia Alvarado[2], Josefina Suils[2], Sabrina Soledad Fernandez[3], María Soledad Andersen[3], Facundo Pelorosso[3], Maria Victoria Freire[4], Mailén Costa[4], Pablo Almazan[4], Inés Navarro[4], Jaen Oliveri[5], Laura Barrientos[2], Maria Sofia Medrano[5], Gabriela Gauna[5], Ana Malvina Bravo[6], Laura Thouyaret[7], Fernanda Rodriguez[7], Vanina Sanchez[7], Romina Armando[7], Javier Lerena[3], Marisol Delea[3], Maria Silvina Juchniuk[6], Carlos David Bruque[3,8].

1. Hospital Zonal Bariloche "Dr. Ramón Carrillo", Bariloche, Rio Negro, Argenitna.

2. Hospital Regional de Comodoro Rivadavia "Dr. Víctor Manuel Sanguinetti", Comodoro Rivadavia, Chubut, Argentina.

3. Unidad de Conocimiento Traslacional Hospitalaria Patagónica - Hospital de Alta Complejidad El Calafate S.A.M.I.C., El Calafate, Santa Cruz, Argentina.

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Genetic healthcare in Argentina exhibits regional variability, with accessibility in major cities and inadequacy in other areas. Limited investment in medical genetics prompted the creation of the Patagonian Human Genetics Network to address access and resource disparities. The overarching aim is to ensure equitable genetic healthcare, particularly in underserved regions. The general objective is to implement the Patagonian Network, providing genetic care and diagnosis in Patagonian hospitals. Specific objectives involve establishing coordination protocols, remote evaluations, and translational research, involving a cohort of 200 patients and their families. The method involves collaboration with provincial experts in rare diseases, agreements with the Ministry of Health, and protocols for sample exchange between hospitals. Patient evaluation occurs remotely through telemedicine, encompassing various genomic and molecular biology studies. Patient inclusion criteria and informed consent are prerequisites. Results demonstrate successful integration of public hospital professionals in Patagonia to deliver genetic care. Participating hospitals include the Neuquén Provincial Hospital, Bariloche Zonal Hospital, Trelew Zonal Hospital, among others. Coordination was established with the Rare Diseases Program of the Ministry of Health, enabling interprovincial sample referrals. Training in genetic technologies is underway to enhance diagnostics. Furthermore, a laboratory needs assessment was conducted, with ongoing standardization of protocols. In conclusion, the Patagonian Human Genetics Network strives to bridge the genetic healthcare gap, particularly in marginalized regions, through professional collaboration, interprovincial coordination, and education.

144. 656. **UNDERSTANDING THE HUMAN GUT MICROBIOTA IN SYSTEMIC LUPUS ERYTHEMATOSUS**

Sofía Quesada[1,2,5], Ayelén D. Rosso[1,2,4,5], Valeria Soler Rivero[1,2], Sebastian N Mascuka[1,2], Pablo. N. Aguilera[2,5], Andrea Boiro[1], Evangelina Areniello[3], Marina Flavia Caputo[3], Alberto Penas-Steinhardt[1,2,5,6], Fiorella S. Belforte[1,2,4,5,6].

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BACKGROUND: Systemic lupus erythematosus (SLE) is a chronic autoimmune disease associated with genetic and environmental risk factors. Currently, emerging evidence suggests that intestinal dysbiosis would play a crucial role in the appearance and development of SLE. Possible mechanisms linking dysbiosis to SLE could include intestinal barrier dysfunction, bacterial biofilms, autophagy of intestinal epithelial cells, extracellular vesicles, and microRNA. METHODS: Clinical, biochemical, and molecular parameters of 23 controls without SLE and 23 patients with SLE from Hospital Alejandro Posadas were evaluated. Fecal RNA was extracted and Stem Loop-RTqPCR was performed to study hsa-miR-155-5p and hsa-miR-223-3p. Fecal microbial DNA was extracted and the V3-V4 regions of the 16SR gene were sequenced using a MiSeq platform and analyzed using the QIIME2 environment. Differential functional pathways were assessed using PICRUSt. The core microbiota was defined as the set of amplicon sequence variants detected in 50-100% of the samples with a relative abundance threshold value greater than 0.01% (CoreMicrobiome-R package). Logistic regression models were built. Global performance was assessed using the Area Under the Republic of China (AUROC). RESULTS: Beta diversity, differential abundances (ANCOM) and metabolic pathways were calculated, observing significant differences between groups (p-value < 0.05). The bacterium Lachnospiraceae NK4A136 and Odoribacter were differentially found within controls without SLE. Collinsella, Bifidobacterium, Lactobacillus, Streptococcus, Marvinbryantia, were found to be differential only in patients. Finally, in the logistic regression model, differential genera were found between cases and controls such as Bacteroides, Parabacteroides, among others. CONCLUSIONS: Overall, our study provides new insights into the gut microbiota composition of our population, allowing for the association of local changes in gut microbial diversity in SLE.

P-HEMATOLOGY

WEDNESDAY 15TH NOVEMBER 14:00-15:30

CHAIRS: MARÍA SOL RUIZ
MARISA SANDOVAL

145. 35. **THE ALTERNATIVE NF-KB PATHWAY INTERFERENCE INDUCES A SIGNIFICANT METABOLIC CHANGE IN CLASSICAL HODGKIN LYMPHOMA WITH POTENTIAL THERAPEUTIC OPPORTUNITIES**

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**Both authors contributed equally to this work.*

We previously shown the constitutive activation of the alternative NFkB arm (RelB/p52 mediated) role in classical Hodgkin Lymphoma (cHL) survival. It has been reported that the Hodgkin and Reed-Sternberg cells (malignant cHL cells) are OXPHOS (oxidative phosphorylation) dependent. We revisited our expression arrays (induced shRelB vs non-induced shRelB human UH01 cells) to evaluate RelB involvement in metabolism regulation. Differentially expressed genes (DEG) were explored with the limma R package, considering the top 10000 DEG with 5% FDR (adjusted p-value <0.05). We analysed the functional significance of DEG in shRelB vs. control by exploring gene ontology (GO) terms and signalling pathways from KEGG database using goanna and kegga functions respectively. We next conducted gene set enrichment analysis using GSEA software [based on leading edge-enrichment score] and the Hallmarks gene list. We further explored enrichment results clustering with the network software Cytoscape. The shRelB cells phenotype was deeply altered compared to control, with more than 10000 DEG at 5% FDR. Top ranked GO terms including DEG were related to metabolism, mitochondria and autophagy. Top-ranked Kegg terms included metabolic pathways such as OXPHOS, autophagy, carbon and fatty acid metabolism, citrate cycle and pentose phosphate pathway. The gene set enrichment results using Hallmark gene lists showed shRelB affected many UH01 cells metabolic functions (FDR q-val<0.05). The GSEA network clustering confirmed the shRelB significant impact on metabolism. We conclude that shRelB caused a massive change in UH01 gene expression profile compatible with a collapse in metabolism-related functions such as OXPHOS, redox-homeostasis, mitochondrial structure, respiratory complexes, glycolysis and lipid metabolism. We report for the first time that the alternative NFkB pathway interference may induce a cHL metabolic reprogramming and could be a potential therapeutic target for its treatment.

146. 36. **EPIDEMIOLOGICAL PERSPECTIVES OF AL AMYLOIDOSIS IN ARGENTINA: RESULTS OF AN ANALYSIS OF INCIDENCE AND MORTALITY IN A POPULATION AFFILIATED WITH A MEDICAL CARE PROGRAM**

María Lourdes Posadas Martínez[1], Delfina Cirelli[1], Florencia De Florio[2], Jimena Vicens[2], María Adela Aguirre[3], Elsa Mercedes Nucifora[4], Valeria Inés Aliperti[2], Marcelina Carretero[1].

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Agradecimientos: a todo el Grupo de Estudio de Amiloidosis, en especial: Eugenia Villanueva, Diego Pérez de Arenaza, Erika Bárbara Brulc, María Soledad Sáez, Patricia Beatriz Sorroche Objective: Estimate the incidence and mortality rates of systemic AL amyloidosis in individuals affiliated with a Medical Care Program (MCP) in Buenos Aires, Argentina. Methods: The study included MCP-affiliated patients at the Italian Hospital of Buenos Aires from January 2011 to December 2022. Participants were followed since AL amyloidosis diagnosis, death, loss to follow-up, program withdrawal, or study closure. Incident AL amyloidosis cases and deaths were recorded from the Institutional Amyloidosis Registry. Incidence and mortality rates were calculated for the entire study period (2011-2022) and by four-year intervals (2011-2014, 2015-2018, 2019-2022) with corresponding 95% confidence intervals (95%CI). Crude, age-standardized, and stratified rates by age and sex were determined. Results: The crude incidence rate of AL amyloidosis was 16 (95%CI 11-24) cases per million persons/year. Incidence remained stable throughout the study [2011-2014= 13 (95%CI 6-28); 2015-2018= 14 (95%CI 7-29); 2019-2022= 20 (95%CI 11-36)], with an IRR of 0.7 (95% CI 0.2-1.8) when comparing the first and last four-year intervals. The age-standardized incidence rate compared to the US population was 6 (95%CI 4-9) cases per million persons/year. Regarding mortality, the crude rate was 11 (95%CI 7-17) cases per million persons/year. No significant sex differences were found, except in the age group over 80 years, where mortality was higher in men (IRR: 4.6, 95%CI 2-12). Additionally, mortality increased over time [2011-2014= 4 (95%CI 1-15); 2015-2018= 11 (95%CI 5-24); 2019-2022= 17 (95%CI 9-31)], with an IRR of 0.2 (95%CI 0.02-1). Conclusion: This study provides detailed information on the incidence and mortality of AL amyloidosis in a specific population, confirming its rarity. The observed increase in mortality rates over the study period could be partly attributed to a survival bias, where variations in disease severity and the timing of diagnosis may have influenced the results. These findings underscore the need for further epidemiological research to better understand the disease.

147. 331. **OXIDATIVE STRESS AND INFLAMMATORY PATTERNS IN DIFFERENT SUBTYPES OF NEWLY DIAGNOSED ACUTE LEUKEMIA**

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The reactive oxygen species (ROS) plays in maintaining haemopoietic stem cells quiescence, self-renewal and long-term survival, but it is unclear how ROS would affect neoplastic disease onset and progression. The aim of this work was to evaluate at transcriptional and systemic level the redox and inflammatory profile in newly diagnosed acute leukemia (AL) patients. Antioxidant enzymes *catalase* (CAT), and *superoxide dismutase* (SOD), and cytokines *TNF-α* and *IL-6* gene expression were analyzed by qPCR in peripheral white blood cells. Malondialdehyde (MDA) and nitrites (NO_2^-) levels and antioxidant defenses [CAT, SOD, glutathione peroxidase (GPx), glutathione (GSH)] were determined in serum by spectrophotometric methods. TNF-α and IL-6 concentration were measured by ELISA. Statistical analyses were performed by SPSS V.25 statistical software and were considered significant at p<0,05. We analyzed 89 AL and 68 healthy individuals (CTRL) between 2018-2022. AL patients were classified according to their immunophenotype as: acute lymphoblastic leukemia (ALL) (n= 31), acute promyelocytic leukemia (APL) (n= 18) and acute myeloid leukemia (AML) (n= 40). SOD expression was down regulated in all AL groups and APL showed lower IL-6 mRNA expression than CTRL. MDA concentration and CAT activity were significantly higher in the AML group. AL groups revealed higher GPx and GSH concentration than CTRL [(GPx μmol/mg protein: AML=15,2±0,8; ALP=18,4±1,8; ALL=15,6±0,7 CTRL=11,2± 0,4) (GSH μmol/mg protein: AML=10,0±0,6; ALP=9,6±1,2; ALL=9,2±0,4; CTRL=7,3± 0,2)]. APL presented higher serum IL-6 level than CTRL; whereas SOD, TNF-α and NO2- levels were similar in all groups. These results show an asynchronism between gene expression and systemic parameters associated with the oxidative-inflammatory state in newly diagnosed AL, which could be a reflection of the pathological state and dysregulation of the redox balance present in these patients according to the AL subtype.

148. 338. **THE TRANSCRIPTION FACTORS C/EBP-α AND HIF-1α ARE INVOLVED IN THE REGULATION OF HEPCIDIN BY ERYTHROPOIETIN**

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Instituto del Departamento de Química Biológica de la Facultad de Ciencias Exactas y Naturales de la Universidad de Buenos Aires (IQUIBICEN) - CONICET

Administration of the erythroid survival factor erythropoietin (Epo) has been linked to the downregulation of hepcidin (Hep), a small peptide expressed mainly in the liver which impairs Fe release through the ferroportin exporter. We previously demonstrated that Epo (20 U/mL, 6 h) decreases Hep mRNA expression in the human liver HepG2 cell line, depending on the activation of the Epo receptor followed by the JAK2/PI3K/AKT/mTOR pathway. Hereby we aimed to determine which transcription factors downstream of this kinase cascade may play a role in Epo-induced Hep suppression. The transcription factor C/EBP-α is known to enhance Hep expression after a variety of stimuli. In HepG2 cells, Epo decreased C/EBP-α binding to the Hep promoter, as observed in electrophoretic mobility shift essays (EMSA, band densitometry: C=1, *E3h=0.7±0.1, n=4, *p<0.05). Preincubation with inhibitors AG490 (50 μM) and LY294002 (50 μM) allowed C/EBP-α to bind the Hep promoter despite the presence of Epo, thus demonstrating the participation of JAK2 and PI3K in this effect (n=4). In the same line, mRNA levels of CHOP, a factor that dimerizes with C/EBP-α thus impairing its binding to the Hep promoter, were found increased after Epo treatment (RT-PCR: C=0.5±0.1, *E6h=1.1±0.1, n=4, *p<0.05). On the other hand, protein levels of the transcription factor HIF-1α, which acts as a suppressor of Hep expression, were found increased after exposure of HepG2 cells to Epo (SDS-PAGE and Western blotting: C=1, *E3h=1.8±0.5, n=4, *p<0.05). Interestingly, nuclear levels of HIF-1α were significantly higher after 1 h of Epo treatment (SDS-PAGE and Western blotting: C=1, *E=1.4±0.2, n=3, *p<0.05). The present findings suggest that Epo impairs the binding of the transcriptional enhancer C/EBP-α to the Hep promoter and favors the activity of the transcriptional suppressor HIF-1α, thus regulating Hep expression and thereby Fe levels in liver cells.

149. 534. **RED BLOOD CELL SENESCENCE IN INFLAMMATORY CHRONIC DISEASES**

Rocío Stampone[1], Antonella Pacini[1], Alejandra Ensinck[2], Federico Tanno[3], Brenda Dinatale[1], Rodolfo Leiva[4], Karina Ramos[4], Fernando Bessone[3], M. Virginia Reggiardo[3], Ana Rosa Pérez[1], Carlos Cotorruelo[1], Silvina Villar[1]

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Chronic inflammatory diseases, regardless of their nature, could impact various physiological processes, including the senescence of blood cells. While it is known that the average lifespan of red blood cells (RBCs) is 120 days, there is evidence to support that chronic inflammatory processes could accelerate the erythrocyte aging. Thus, it is possible that RBCs may have a shorter half-life in pathologies like Chagas disease (CD) and autoimmune hepatitis (AIH). This study aims to identify changes in aging markers of RBCs, such as an increase in autologous IgG antibodies on the RBCs membrane that bind to band 3, stimulating C3b deposition and erythrophagocytosis, and a decrease in the expression of CD47, a transmembrane protein which acts as a “don’t eat me” signal. In both cases, the removal of RBCs by macrophages is promoted. For this, we recruited individuals infected with *Trypanosoma Cruzi* who developed Chronic Chagas Cardiomyopathy (CCC, n=7) and those without cardiac pathology (Indeterminate form (IND), n=18), as well as patients with AIH (n=10). Healthy volunteers were matched according to sex and age (Co, n=10). Flow cytometry has been applied for detection of CD47 and RBCs-bound IgG. CD47 expression was lower in both AIH, IND vs. Co, as indicated by the Median Fluorescence Intensity values (*p<0.05). There was no significant difference between CCC and Co. In addition, the detection of RBCs-bound IgG showed significant difference between the groups, IND and CCC vs. Co, with a higher amount of *membrane-bound IgG* in patients with CD (*p<0.05). However, there was no significant difference between AIH and Co. Regarding the two aging-associated mechanisms studied, in AIH the predominant process of RBCs clearance could be attributed to the CD47’s role as a molecular switch for controlling erythrocyte.

P-IMMUNOLOGY

THURSDAY 16TH NOVEMBER 9:00 - 10:30

CHAIRS: YANINA LANGLE
DANIELA MONTAGNA

150. 113. **DEVELOPMENT OF AN ALTERNATIVE METHOD TO DETERMINE THE PRESENCE OF ANTIBODIES TO DEAMIDATED GLIADIN BY SPR**

Gabriel Alejandro de Diego[1,2], Natacha Cerny[1,2,3], María Eugenia Díaz[1,2], Brian Martínez Ruiz[1,2], Rubén Francisco Iacono[1,4], Marisa Mariel Fernández[4], Mauricio César De Marzi[1,2]

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Celiac disease (CD) is a chronic autoimmune enteropathy with a reported prevalence of approximately 1%. The associated symptoms and the severity of the immune response triggered by gluten exposure vary between patients. Several antibodies are used as biomarkers for the diagnosis and monitoring of this pathology. The kinetic interaction and the affinity of autoantibodies for autoantigens could influence the evolution and clinical presentation of the pathology. In recent years, anti-deamidated gliadin antibodies (a-DGP) have become more relevant. Therefore, this work aims to develop surface plasmon resonance (SPR) assays to determine the presence of a-DGP and their avidity characteristics in patient sera. DGP was obtained by deamination of gliadin in acidic medium and separation of the products using an anion exchange column (Mono Q 5/50 GL) in FPLC. Sera samples were obtained from CD patients who completed a questionnaire with symptom and clinical data. Antibodies levels anti-transglutaminase, gliadin and DGP were determined by ELISA and expressed as a positivity index (mean of sample / (mean + 3 SD of controls)). In a preliminary qualitative assay a-DGP by SPR, DGP (1200 RU, active cell) were immobilized on a CM5 chip. Different dilutions of positive and negative sera in PBS (1:100, 1:200 and 1:400) were injected over the active surface and the binding compare with the reference surface. The results obtained were analyzed with BIAevaluation software. The 1:100 and 1:200 dilutions of positive sera showed a differential binding compare with the negative sera. These promising results aim us to develop an alternative method to determine the seroprevalence of a-DGP antibodies. In addition, it allows us to analyze the kinetic parameters of the antibodies and the humoral response, which would correlate with the severity of the pathology.

151. 290. **ANALYSIS OF PD1/PDL1 PATHWAY IN PRIMARY AND PERSISTENT EPSTEIN BARR VIRUS INFECTION**

Amarillo María Eugenia[1], Moyano Agustina[1], Ferressini Natalia[1], De Matteo Elena[2], Preciado M. Victoria[1], Chabay Paola[1].
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Our aim was to study CD4+ and PD1+ cells and their relationship with the contribution of PDL1 from macrophages in the tonsils of children infected with Epstein Barr infection (EBV). Thirty-eight patients undergoing tolsillectomy were studied. EBV infection status was defined by serology. Immunohistochemistry (IHC) was performed for IL-10, TGF-β, CD4, PD1, LMP1 and EBNA2 proteins. Double staining was performed to characterize both CD68/PDL1+ and CD163/PDL1+ macrophages. IL-10, TGF-β, CD4, PD1 and double staining counts were differentiated between germinal center (GC) and interfollicular (IF) regions of the tonsil. Results were expressed as positive cells/mm^2. Twelve patients were primary infected (PI), 8 with reactivation (R), 14 healthy carriers (HC) and 4 no infected (NI). Five patients presented a latency profile 0, 10 a latency profile I, 9 a latency profile II and 10 a latency profile III. As expected, the PD1+ cell count was higher in the GC than in the IF region ($p<0.0001$). There were no significant differences when comparing this count between the different stages of infection ($P>0.05$). There was a positive correlation between CD4+ and PD1+ count in the group of patients with a latency II profile only ($p=0.0345$; $r=0.7033$). There was a negative correlation between PD1 and TGF-β particularly in GC ($r=-0.3526$; $p=0.0478$) and no correlation of PD1 with IL10 was observed. There was a negative correlation between PD1 and CD163/PDL1 mostly in the tonsil GC ($r=-0.5539$; $P=0.0061$). In contrast, no correlation was observed between PD1 and CD68/PDL1 ($P>0.05$). When CD4 count was compared with CD68/PDL1 and CD163/PDL1 there was no correlation ($P>0.05$). Conclusion: EBV infection does not seems to induce an exhausted PD1/PDL1 environment. Furthermore, at the GC, PD1 might be inversely influenced by exhaustion ligands and anti-inflammatory cytokines. Only the expression of the main viral oncoprotein, LMP1, have influence on PD1+ cells expressed by CD4+ T cells.

152. 386. **ANALYSIS OF NK CELLS IN THE TUMOR INFILTRATE IN PATIENTS WITH BREAST CANCER**

María Belén Bordignon[1], Ayelén I. Pesce Viglietti[1], Luciana Sabatini[2], Azul Perazzolo[2], Verónica Fabiano[2], Federico Coló[2], Martín Loza[2], Florencia Cappuccio[2], Mora Amat[2], José Mordoh[1], Estrella M. Levy[1].
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Adaptive NK (adNK) cells are a subpopulation of CD3-CD56dim cells that proliferate after human cytomegalovirus (HCMV) infection. This subpopulation has a more robust production of cytokines via CD16 stimulation (ADCR), longer lifespan, and persistence compared to conventional NK cells (cNK) and is therefore interesting for cancer immunotherapy. We recently described peripheral blood (PB) adNK cells from breast cancer (BC) patients at a functional and phenotypic level. Furthermore, we observed an expansion of this cell population in vivo in patients under treatment with HER2-target therapy. Here, we studied adNK cells in the tumor immune infiltrate. For this, we evaluated a cohort of BC patients of the luminal and triple negative subtypes (n=12), without previous treatment, from whom a PB, tumor, and, in some cases, mammary tissue sample was taken on the day of surgery. Although no difference was observed in the percentage of NK cells (Live CD45+CD3-CD56+) with respect to the lymphocyte population in tumor and PB, there was a greater immune infiltrate in the tumor sample in terms of CD45+ cells compared to the normal tissue. From samples with an average mass of 620mg range (135-1461 mg), we obtained between 33000 and 160000 CD45+ cells and 500 to 10000 NK cells. In addition, NK cells from the tumor infiltrate exhibited a decrease in the expression of CD16 ($p<0.001$) and NKp46 and an increase in the expression of the inhibitory receptor NKG2A ($p<0.05$). Regarding adNK cells, defined as LiveCD45+CD3-CD56dimNKG2C+NKG2A-, the proportion in PB and tumor was equivalent (range 10-35%). As in PB, adNK cells in the tumor infiltrate have a lower expression of NKp46 than their cNK counterparts. Further research will require functional and proliferation assays that will allow us to expand our knowledge of the biology of these cells in tissues.

153. 399. **NATURAL ANTIOXIDANT EFFECTS ON ADIPOCYTES AND MACROPHAGES RELATED TO INFLAMMATION PROCESS**

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We obtained boldo (*Peumus boldus*), cassis (*Ribes nigrum*) and carqueja (*Baccharis articulata*) extracts with high antioxidant activity. We showed that boldo (B) and cassis (Cs) significantly inhibited lipid accumulation on mature adipocytes 3T3-L1 (AD), while carqueja (C) didn´t affect lipid content. Here, we aim to study the effects of these extracts on AD and macrophages (Mφ), cells related to obesity disease. Extracts (EXT) were made from B and C leaves, at 37°C for 1h and from Cs fruit by enzymatic extraction. 3T3-L1 cells were differentiated to AD and THP-1 monocytes were induced with PMA for 72 h for the development of Mφ. We performed 24 to 72 h treatments with EXT on Mφ and AD to determine nitrite production (NO) by Griess reagent method and secretion of IL-1β and IL-10 by ELISA technique. We observed a NO production increment in AD treated with Cs and B regarding controls (CCad) ($p<0.05$) (35.39 ± 2.92 μM [AD+Cs], 23.83 ± 3.19 μM [AD+B], 15.18 ± 0.60 μM [CCad]). In Mφ, only Cs increased NO secretion compared to controls (CCmφ) ($p<0,05$) (63.40 ± 32.34 μM [Mφ+Cs], 20.21 ± 6.17 μM [CCmφ]). Cs treatment increased IL-1β secretion in Mφ ($p<0,01$) (76.98 ± 1.41

ρg/ml [Cs+Mφ], 4.31 ± 0.62 ρg/ml [CCmφ].), C raised IL-10 secretion while Cs decreased it (p<0,01) (119.7 ± 0.92 ρg/ml [C+Mφ], 10.12 ± 1.26 ρg/ml [Cs+Mφ], 58.93 ± 3.86 ρg/ml [CCmφ]). In Mφ, NO and IL-1β increase and IL-10 decrease after Cs treatment could suggest an inflammatory effect of this EXT related to its acidity. C raised IL-10 in Mφ, suggesting an anti-inflammatory effect of this EXT related to its known digestive properties. Since it has been reported that similar concentration of NO inhibits terminal markers in AD, we could propose that NO produced by AD after Cs and B treatments is associated with lipid accumulation decrease, as we have previously shown in these cells and, a paracrine effect could be observed between them.

154. 441. **FENOFIBRATE INCREASES THE EFFEROCYTOSIS OF CARDIAC MACROPHAGES. IMPLICATIONS FOR TISSUE FIBROSIS IN AN ACUTE *T. cruzi* INFECTION MODEL**

Javier Ruiz Luque[1], Marcus V. Reis[1], Carolina Poncini[2], Fernando Erra[1], Ágata Cevey[1], Azul Pieralisi[1], Martín Donato[3], Gerardo Mirkin[2], Nora Goren[1] and Federico Penas[1]

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Monocyte-derived macrophages (Mφ) play a crucial role in the response to infection in Chagas disease in the heart. Due to their functional and phenotypic versatility, manipulation of specific Mφ subsets may be crucial to assist vital cardiovascular functions, such as tissue repair and defense against infection. PPARα are ligand-dependent transcription factors involved in lipid metabolism and regulation of inflammation. We previously demonstrated that fenofibrate (fen), a PPARα ligand, modulates the inflammatory response and improves left ventricle functionality in an acute model of infection. Here, our approach was to assess two aspects: first, the impact of fen on the functionality of cardiac Mφ (cMφ), and second, the influence of this ligand on the progression of tissue fibrosis. For this, C57BL/6 mice were infected with *T. cruzi* and treated with 100 mg/kg/day of fen for 14 days. cMφ (CD11b+LY6C+F4/80+) were examined from ficoll 1083 gradient purified leukocytes. We observed that fen reduces the M1 profile (CD206-), while increasing the M2 profile (CD206+), compared to untreated infected mice (FACS, p<0.05). Next, we performed functionality assays and evaluated cMφ efferocytosis. Fen increases the efferocytic activity of cMφ, thereby highlighting the resolution capacity of these cells (FACS, p<0.05). Finally, we evaluated the effects of fen, both on cardiac fibrosis and on mRNA expression of pro-fibrotic mediators. Fen effectively mitigates the increase in interstitial collagen accumulation (picrosirius red staining, p<0.05), while exerting a regulatory effect on the transcript levels (RT-qPCR, p<0.05). In conclusion, this study delves into Chagas disease's cardiac response, spotlighting the crucial role of cMφ. These findings position fenofibrate as a promising therapeutic strategy, together with an antiparasitic, for the management of cardiac complications associated with Chagas disease.

155. 465. **CHARACTERIZATION OF THE IMMUNE LANDSCAPE OF EPSTEIN-BARR VIRUS HARBOURING TONSILS**

Ignacio Ezequiel Rojas Campión[1], M. Eugenia Amarillo[2], Rocío A. Pastor[1], M. Paula de la Guardia[1], Bibiana Paoli[3], M. Elena Arabolaza[3], Andrea Paes de Lima[4], Isabel Aspe Scetti[5], Andrés Blanco[5], Paola A. Chabay[2] & Eloísa I. Arana[1,6]

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Palatine tonsils are secondary lymphoid organs crucial for B cell-mediated immunity. The Epstein-Barr virus (EBV) is a member of the herpesvirus family with tropism for B cells. Around 94% of all healthy individuals are seropositive for EBV. It has been recently shown that EBV is a cause of multiple sclerosis and it has been associated to other autoimmune diseases. Here, we report initial studies aimed to understand the immune mechanisms that contain the viral infection and their evolution at different stages of human life. Multiparametric flow cytometry (FACS) was used to identify lymphocyte subsets on tonsillar mononuclear cells (TMC). EBV infected cells were detected by immunohistochemistry for LMP1 (a viral antigen) on formalin fixed paraffin embedded biopsies from the same patients analysed by FACS. All patients analysed resulted EBV positive (n=12, children under 12 years old), with LMP1+ cells detected either in germinal centers or interfollicular zones. Their lymphoid compartment resulted as follows. While B cells (CD20+) represented 63,73% ± 11,08% of TMC, T cells (CD3+) represented 35,31% ± 12,91%. T helper cells (T_H, CD3+ CD4+) resulted roughly around 4x more than cytotoxic T cells (T_C, CD3+ CD8+, 75,84% ± 9,80% vs 16,09% ± 6,38%). Among T_H cells, follicular T_H cells (CD3+ CD4+ $PD1^{HIGH}$ $CXCR5^{HIGH}$ and CD3+ CD4+ $PD1^{INT}$ $CXCR5^{INT}$) represented 73,53% ± 5,61% of T_H cells, and the extrafollicular ones (CD3+ CD4+ $PD1^{LOW}$ $CXCR5^{LOW}$) represented 16,78% ± 4,72%. Finally, follicular T_C cells (CD3+ CD8+ $PD1^{HIGH}$ $CXCR5^{HIGH}$) made up for 32,12% ± 13,80% of all T_C cells. To conclude, we have not detected negative samples for EBV infection so far and the lymphocyte compartment at young ages seemed fairly consistent. Our perspective is to perform the same assays on samples of different ages to detect changes in viral expression associated with altered immune subsets, which might shed light on the initiation of immune-pathology by EBV.

156. 484. **IDENTIFICATION OF INFREQUENT IMMUNE CELLS IN TONSILS: SCORING THEIR PROPORTION AT DIFFERENT AGES**

M. Paula de la Guardia[1], Rocío A. Pastor[1], Ignacio E. Rojas Campión[1], M. Elena Arabolaza[2], Andrea Paes de Lima[3], M. Soledad Collado[1], Isabel Aspe Scetti[4], Andrés Blanco[4], Bibiana Paoli[2], Eloísa I. Arana[1,5]

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The effector immune responses that provide protection to the oro-pharyngeal mucosa are partly granted by the palatine tonsils. Considering that tonsils decrease in size from puberty on, we propose them as a model to study the accumulative impact of time and constant antigenic exposure on local immunity. From this perspective, we have analyzed infrequent subsets of immune cells at different ages, by FACS. We found an age-dependent increment in the percentage of the so called, Aged B Cells (ABC, CD19+ CD27+ CD11c+, n=43, 2 groups of age, means statistically different, Tukey test) relative to CD19+ cells. These are a particular type of memory B cells, which have been reported to accrue after repeated infections in other human tissues and in some mice models of chronic infections. Moreover, we found that double positive T cells (CD3+ CD4+ CD8+ HLA DR+), a mature T cell subset described in a number of peripheral tissue locations, showed a significant decrease in tonsils from adults (n=31, 2 groups of age, p<0,05) in comparison

to those from children. Finally, an age-associated regression was observed for double negative T cells (CD3+ CD4- CD8- HLA DR+, n=31, p<0,05) as well. The latter is a pro-inflammatory mature T cell population associated with inflammation and tissue damage. In addition, non-lymphocytic populations were also studied, finding a decrease in the amount of monocytes (CD45+ CD14+) as the patient's age increased (n=44, p<0,05). In conclusion, we demonstrated for the first time the presence of tonsillar ABC and their time-dependent accumulation. Furthermore, we characterized aged dependent changes in the proportion of unusual T cell populations and monocytes previously reported in tonsils. These findings shed light on the effects of the continued exposure to antigenic material that flows into the lymphoid tissue from air and food, causing changes in the proportion of some tissue specific immune subsets which remain to be functionally characterized.

157. 535. **APPLICATION OF MACS METHOD ON SIGA-COATED BACTERIA IN THE CONTEXT OF INFLAMMATORY BOWEL DISEASES (IBD)**

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BACKGROUND: Secretory immunoglobulin A (sIgA), the predominant mucosal isotype, is a mediator of intestinal immunity. It can coat the microorganisms they recognise, providing protection against infections through neutralization and exclusion, as well as discriminating commensal microorganisms. There is evidence that IgA coating allows identification of colitogenic bacteria in IBD using magnetic cell sorting (MACS) and IgA-Seq strategy. It was described that gut bacteria with high IgA coating conferred intestinal disorders in murine models, suggesting sIgA coating would identify proinflammatory commensals involved in disease. METHODS: Fecal samples were incubated with biotinylated anti-SIgA antibodies and coated commensals were magnetically separated with streptavidin-conjugated beads. sIgA-coated and uncoated bacteria were collected and evaluated by cytometry. MACS efficiency was evaluated by spectrofluorometry considering primary antibody concentration, incubation lapse, number of times the sample was passed through the magnet and the use of specific commercial columns for separation. Furthermore a fluorescence microscope was used to validate the binding between magnetic beads and bacteria in the positive fractions. In addition, different sampling strategies as well as initial mass of the starting sample was evaluated by bacterial DNA extraction and 16SR sequencing. RESULTS: We were able to efficiently separate sIgA-coated bacteria in the positive enriched fractions. High concentrations of anti-sIgA achieved a better separation of coated bacteria. Increasing the incubation time of biotin and beads-streptavidin also increased the separation efficiency. Starting from 250 mg of sample was considered for better performance in DNA extraction after MACs. CONCLUSIONS: Our study provides new knowledge on sIgA coated gut microbiota isolation from magnetic cell sorting.

158. 599. **CLINICO-IMMUNOLOGICAL PATTERNS OF CRITICAL OR FATAL DISEASE DUE TO *BORDETELLA PERTUSSIS* AND RESPIRATORY SYNCYTIAL VIRUS IN YOUNG INFANTS**

María Agustina Wirth[1], Pilar Goñi[1], Julia Dvorkin[1,2,3], Fernando P. Polack[2], Mauricio T. Caballero[1,2,3].

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Background: Respiratory syncytial virus (RSV) infection presents different types of clinical phenotypes including pertussis-like syndrome, which makes in some cases the differential clinical diagnosis with whooping cough more complex. The aim of this study is to determine clinical and pathological patterns of critical or fatal disease due to *B. pertussis* and RSV in infants with whooping cough or pertussis-like syndrome. Method: An analytical cross-sectional observational study of patients diagnosed with RSV pertussis-like syndrome and *B. pertussis* infection was conducted. The expression of toll-like pattern receptors 2, 3 and 4 and the production of inflammatory cytokines IL4, IL5, IL1, IFN-gamma, IL17, TNF-alpha and IL1 were analyzed in samples of nasopharyngeal secretions from hospitalized infants under 12 months. Results: A total of 106 hospitalized infants under 1 year old were enrolled in the study. No differences in the levels of inflammatory cytokines were observed between the groups of patients with RSV and *B. pertussis* illness. However, the mean value for leukocytes was 63,476/mm^3 in patients with *B. pertussis* in comparison with those with RSV that was 14,076/mm^3 and coinfected with 15,422/mm^3 (p=0.006). The case fatality rate due to *B. pertussis* was 2.8% while due to RSV was 0.9% (p<0.001). Individuals who died were older (p=0.043), lighter (p=0.048), with lower SpO2 (p<0.001), higher leucocytes and C-reactive protein levels (p=0.002, p<0.001) and longer hospital stay (p<0.001) than those who survived. Conclusions: Although there were no differences in the levels of biomarkers in the patients with RSV or *B. pertussis*, a higher mortality rate and hyperleukocytosis with neutrophilia due to *B. pertussis* infection was observed, and we found several differences in the severity, mortality, and biomarkers levels in patients with critical or fatal *B. pertussis* disease, compared with those who survived or had mild to moderate illness.

159. 617. **ROLE OF CYTOKINES AND RISK FACTORS ASSOCIATED WITH APNEA DURING ACUTE RESPIRATORY TRACT INFECTIONS**

Pilar Goñi[1], María Agustina Wirth[1], Julia Dvorkin[1,2,3], Adrián Ferreti BSc[2], Romina Libster[2], Fernando Polack[2], Mauricio Caballero[1,2,3].

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Background: Apnea is one of the most severe complications of acute respiratory tract infections (ARI) in young infants and it is a life-threatening event. Reports often associate apnea with viral respiratory infections, however the mechanisms and risk factors associated with this syndrome are poorly understood. Methods: Cross-sectional, multicenter study conducted in 12 pediatric departments of the southern region of Buenos Aires. Hospitalized infants under 12 months due to ARI were enrolled. Apnea was defined as an unexplained episode of cessation of breathing for 20 seconds or longer, or a shorter respiratory pause associated with bradycardia, cyanosis, pallor and/or marked hypotonia. Predictors in patients with apnea were compared to those with ARI and no apnea. Nasal aspirates were obtained from all patients and tested for respiratory pathogens by RT-PCR and for biomarkers (IL1β, TNFα, IFNγ, IL4, IL5, IL9, IL13). Results: We enrolled 4255 infants hospitalized with ARI, and 77 infants presented apnea (1.81% of infants hospitalized with ARI). Increased odds for developing apnea during ARI were associated with prematurity, age in months, birth weight, malnutrition, chronic underlying disease (neurological disease or immunodeficiency), and severe crowding (5 or more people per bedroom). Severe crowding (OR 1.02-4.97), age ≤1.5 months (OR 3.91-15.49), prematurity (OR 2.34-10.16), and weight Z score <-2 (OR 3.52-14.93) were the only variables independently associated with apnea. The level of production of IL13, IL9, IL5, and IL4 in the nasopharynx of the patients with apnea were significantly lower than those without this clinical sign. Conclusions: Apnea during ARI was associated with prematurity, age in months and weight during illness. We found lower levels of inflammatory cytokines in patients with apnea. Further investigation is needed to elucidate pathways related to apnea.

160. 628. **CYTOTOXICITY AND IMMUNOMODULATORY ACTIVITY OF CROTOXIN ISOLATED FROM *CROTALUS DURISSUS TERRIFICUS* ADSORBED TO SILICA NANOPARTICLES**

Florencia S. Conti[1], Exequiel Giorgi[1, 2], María Eugenia Díaz[1, 2], Mauricio De Marzi[1, 2], Juan Pablo Rodríguez[3], Federico G. Baudou[1, 2].

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In Argentina, snakes from the Viperidae family cause most accidents and so far the only medical treatment approved is serotherapy with antivenoms (AV). Particularly, *Crotalus durissus terrificus* (rattlesnake) has a neurotoxic venom (V) whose main component is Crotoxin (CTX) responsible for its high lethality. This toxin has immunosuppressive properties that hinder the production process of its AV. In addition, the use of nanoparticles (NP) opened a range of possibilities within the medical field given its properties. Thus, the possibility of using NP, with V toxins, would allow modifying its biological properties in order to use them in the production of new AV.
In this work, we isolated CTX by fast protein liquid chromatography (FPLC) and generate nanovenoms (NVs), complexes formed by CTX adsorbed to silica nanoparticles (SiNPs) of different sizes (150 and 330nm). For this, 1 mg/ml of CTX was mixed under stirring with 10 mg of SiNPs (+/-). By Transmission Electron Microscope (TEM) NVs were microphotographed and revealed CTX's presence in them. The protein profile of whole V, isolated CTX and CTX adsorbed to SiNPs was studied by SDS-PAGE (14%), showing CTX subunits bands post-NVs desorption. The enzymatic activity exerted by CTX of the NVs was studied by hemolysis radial test, and it was found that NVs retain their enzymatic activity (42.57 ± 3.18% NV+, 41.65 ± 3.89% NV-). In addition, some biological properties of these NVs were studied such as their cytotoxicity (MTT cell test over THP-1 cells), in which no significant differences between the NVs and the whole V and CTX were seen. The immunomodulatory activity (cytokines by ELISA) of NVs was also studied, and we observed that both V and CTX induce low levels of IL-1β (1.0 ± 0.4, 0.2 ± 0.1 pg/ml) but a proinflammatory effect can be observed with NVs +/- (12.5 ± 0.3, 12.4 ± 2.4 pg/ml).Thus, the results obtained in this work allow us to further refine the NVs for their possible use as adjuvants in the production of new generation AVs.

P-INFECTOLOGY

THURSDAY 16TH NOVEMBER 9:00 - 10:30
CHAIRS: MARÍA MARTA AMARAL
FLAVIA SACERDOTI

161. 10. **EXOTOXINS HLYA AND SHLA CONTROL CYTOTOXIC PHENOTYPES OF TARGET EUKARYOTIC CELLS THROUGH PURINERGIC SIGNALING**

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Aim: we characterized the effects of exotoxins from gram(-) bacteria, i.e., HlyA from uropathogenic strains of Escherichia coli and ShlA from Serratia marcescens, on eukaryotic cell models. Focus was given to the capacity of these toxins to induce intracellular ATP release from target cells, and consequent purinergic signaling. Human erythrocytes (RBCs) were exposed to HlyA, while CHO (chinese hamster ovary) cells were exposed to ShlA. Methods: luciferin-luciferase luminometry was used to quantify extracellular ATP (eATP). Electrical impedance was used to quantify cell volume of RBCs. Autophagy in CHO cells was revealed by an EGFP-LC3 green fluorescent punctate pattern. Significance level at $P<0.05$. Results: both exposure of RBCs to HlyA, and of CHO cells to ShlA, induced a significant increase of [eATP] (3-40 fold over 40 min exposure), indicative of intracellular ATP release from target cells. This release was partially inhibited (40-70%) by carbenoxolone and mefloquine, two inhibitors of the hemichannel pannexin 1. Toxin dependent eATP accumulation was partially opposed by nucleotide hydrolysis of endogenous ectonucleotidases from target cells. In HlyA treated RBCs, eATP activated P2X receptors leading to swelling (1.5-fold), while osmotically blocking this swelling reduced ATP release by 80%. In ShlA treated CHO cells, eATP activated a P2Y2 receptor, that in turn transactivated α5β1, leading to autophagy. Conclusions: exposure of exotoxins to target cells led to intracellular ATP release from target cells, partially mediated by pannexin 1. The resulting eATP, in spite of eATP hydrolysis by ectonucleotidases, was capable of activating purinergic signaling, so as to: 1 (for HlyA)- induce an osmotic imbalance leading to swelling in RBCs, a prerequisite for hemolysis; 2 (for ShlA)- induce P2Y2-α5β1 integrin activation, a prerequisite for autophagy in CHO cells. In the absence of purinergic signaling, the cytotoxic phenotypes induced by the toxins are highly reduced.

162. 42. **MODULATION OF C-TYPE LECTIN RECEPTORS IN DENDRITIC CELLS BY HYDATID FLUID FROM *ECHINOCOCCUS GRANULOSUS* INDUCES AUTOPHAGY AND POLYFUNCTIONAL T CELL RESPONSES**

Maia Chop[12], Julia Loos[13], María Celeste Nicolao[13], Camila Ledo[13], Andrea Cumino[13], Christian Rodriguez Rodrigues[12].

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Background: Echinococcosis is caused by *Echinococcus granulosus,* which develops cysts filled with hydatid fluid (HF). C-type lectin receptors (CLRs) recognize carbohydrate structures leading to phagocytosis, autophagy and cytokine production. Autophagy plays a key role in antigen presentation by DCs and promotes T-cell activation. The aim of this study was to analyze whether HF can be recognized by CLRs and induce autophagy to polarize the T cell response *in vitro.* Methods: HF was collected from infected cattle. Autophagy induction in BMDCs was evaluated by FACS, qPCR and confocal microscopy. The expression of CLRs were evaluated by FACS. Cytokine profiles were analyzed by qPCR. Results: HF-stimulated BMDCs significantly enhanced the number (**p<0,01) and MFI (****p<0,0001) of $LC3^+$ structures compared to unstimulated cells. This phenomenon was enhanced by the inhibition of lysosomal acidification. Autophagy related genes: *beclin-1, atg16l1 and atg12* were also upregulated upon HF stimulation (n=3, *p<0,5, ***p <0.001 vs control). Induction of transcription and nuclear translocation of

TFEB were not observed after HF stimulation. Then we evaluated the expression of CLRs in BMDCs. HF induced a downmodulation of CD205 and Clec9a in BMDCs (n=3 **p<0.01), even in presence of EDTA. Finally, we measured the expression of different cytokines in splenocytes co-cultured with HF-stimulated BMDCs by qPCR, and found that HF-stimulated BMDCs significantly increased the levels of il-6, il-10, il-12, tnf-α, tgf-β and inf-γ (n = 3, *p<0,05, **p<0,01, ***p<0,001 HF vs control). Conclusions: These results suggest that HF could be recognized by DEC205 and Clec9a in BMDCs, inducing autophagy and promoting antigen presentation. HF-stimulated BMDCs modulate gene expression of cytokines related to tTh1, Th17 and Treg profiles, and strongly inhibit Th2 response.

163. 270. **DEVELOPMENT AND STANDARDIZATION OF AN ELISA BASED ON LECHIGUANAS VIRUS RECOMBINANT PROTEIN FOR DETECTION OF ANTIBODIES AGAINST HANTAVIRUSES**

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Hantaviruses comprise a genus of enveloped viruses which are transmitted by rodents. Depending on the particular Hantavirus involved, infection in humans could cause Hantavirus Hemorrhagic Fever with Renal Syndrome or Hantavirus Cardiopulmonary Syndrome. (H.P.S.). The genome of these viruses consists of 3 RNA segments: small (S), medium and large. The S segment codes for the nucleoprotein (N), which induces an early; strong and long-lasting immune response. The objective of the work was to develop, evaluate and standardize an IgG ELISA for the serological detection of human hantavirus infections. The standardization of the assay was done using a recombinant antigen (rLECH 13) produced in bacterial and derived from the Hantavirus Lechiguanas. From RNA isolated from a rodent, RT-PCR was done to amplify the complete fragment of the N. The gene encoding the entire ORF was cloned into the pBAD/Thio-TOPO vector and transformed into *E. coli*. The protein was obtained from inclusion bodies, and purified using IMAC columns under denaturing conditions. To detect an antibody response to Hantavirus infections in patients, direct IgG Elisa test based on rLECH13 antigen was developed. The evaluation and standardization assay was carried out, analyzing a total of 50 patients with a confirmed diagnosis of HPS by the reference technique, 50 negative sera and 53 patients with other pathologies. The parameters: analytical sensitivity, analytical specificity, diagnostic sensitivity and cut-off value were determined using the software *Analyze it*. The assay data analyzed showed a diagnostic sensitivity value of 95% and a diagnostic specificity of 80%.The high sensitivity of the novel assay allows us to conclude that rLECH13 is feasible for use in the immunodiagnosis of hantavirus infection. Additionally it is very important to have antigen produced in conditions that do not require highly complex laboratories, also the new assay is economical, reproducible and shows very good performance.

164. 317. **THE MODULATION OF RNA GRANULES BY HEPATITIS B VIRUS REPLICATION IN HUMAN HEPATOCYTES IMPLIES A VARIABLE COLOCALIZATION WITH CORE ANTIGEN**

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More than 295 million people are chronically infected with hepatitis B virus (HBV) worldwide. The role of RNA granules (stress granules, SGs and processing bodies, PBs) in liver has been described and their disfunction could participate in the pathophysiological mechanisms of chronic B infections. We previously reported that RNA granules can be modulated by replication of genotype F1b and F4 HBV, and that surface antigen can colocalize in these structures. The aim of this study was to evaluate if HBV genotype D replication in a stable transfected hepatocyte cell line modulates RNA granules and if HBcAg colocalizes with them. Materials and methods: The simultaneous detection of core antigen (HBcAg) and SGs or PBs was performed by double indirect immunofluorescence using antibodies directed to HBcAg and SGs (TIA-1, TIA- 1/R, G3BP1) or PBs (DCP1a) in HepG2 (control) and HepG2 2.15 cell lines. The images obtained by confocal microscopy were analyzed with ImageJ. Results: HBV replication increased the number of SGs positive cells compared to the control when the granules were detected with TIA-1 (24%) or TIA-1/R (46%) ($p < 0.05$), but not with G3BP1. When the number of SGs/cell was studied, no differences were detected for any of the analyzed proteins in the cells with replication. On the other hand, the number of PBs positive cells were not significantly modified, nor the PBs/cell in transfected cells. When detecting HBcAg in SGs and PBs, a variable colocalization was observed in HepG2 2.15 cells, suggesting that the presence of HBcAg in the RNA granules may be necessary at any step of the viral cycle to generate HBV particles. In conclusion, HBV gt D replication can modulate RNA granules in human hepatocytes, in variable magnitudes according to the specific protein used for their detection. The changes in RNA granules induced by the virus may alter its normal functions, contributing to hepatocytes disfunction and participating in the pathogenesis of chronic infections.

165. 396. **DEVELOPMENT OF REAL TIME PCR BASED PROTOCOLS FOR SYPHILIS DIAGNOSIS BASED ON *TPP47* AND *POLA* GENS IN YOUNG ADULT'S PATIENTS**

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Syphilis is caused by Treponema pallidum pallidum (TPA) and continues afflicting 6 million people annually including 1 million pregnant women. Despite the existence of effective treatments, during recent decade's cases have increased affecting disproportionately young women causing impact on the congenital syphilis rates and direct costs. There are public initiatives addressing the serological diagnosis tests to reducing syphilis incidence but there are still insufficient. The use of molecular biology techniques (MBT) in the diagnosis of TPA is in the beginnings. We collaborated in an international study evaluating MBT for the diagnosis of syphilis in young adult's patients with syphilis. Our aim was the performance of real time PCR (qPCR) in blood and swab samples for the detection of *tpp47*and *pola* gens and explore TPA results among clinical stage and samples sources. We incorporated 95 cases of acquired syphilis transmitted through sexual contact and 272 samples were processed for DNA extraction (QIAGEN, Germany) followed by qPCR using Taqman probes in a CFX 96 equipment (Bio-Rad, USA). Among demographic, media (IQ_1-IQ_3) characteristics was: 28 years old (22-35) and VDRL results: 64 (32-128), previous syphilis diagnosis was 1(1-2) and the numbers of sexual partners were 2(1-3). The % of women as well as HIV patients was 23%. The qPCR efficiency per patient for *tpp47* in swabs was: Sensitivity (Se)=86.3, Specificity(Sp)=100, Positive predictive value(PPV)=100, Negative predictive value(NPV) = 60.5; with lowest values in blood samples (16/100/100/24). For *pola* the values were: Se=85.4,Sp= 100, PPV= 100,NPV= 62.2 and for blood samples (17/100/100/24.3). Notably, the Se in normal oral mucosa´ swabs from secondary stage patients was 70%. These preliminary data support the use of qPCR methods as confirmatory techniques in blood samples and for screening in swab samples in syphilis diagnosis algorithm. Which could improve prevention policies for congenital syphilis cases.

166. 450. **MENINGITIS Y SEPSIS POR BACILLUS GRUPO CEREUS EN UN RECIEN NACIDO PRETERMINO**

Moncecchi,Laura , Rossi,Paola , Reschia,Andreina , Capuchinelli,Agostina, Pessoa,Claudia , Sorribas,Aranza , Caprile,Luis , Rossignol,Gustavo , Scarafia,Leila , Maria Carolina Martinez

Hospital Provincial de Rosario

Introducción: Los microorganismos que integran el Bacillus grupo cereus son bacilos Gram positivos, anaerobios facultativos, formadores de esporas y con flagelos perítricos que pertenecen al phylum Firmicutes.Este grupo está compuesto por 16 especies relevantes desde el punto de vista clínico. Bacillus gr. cereus puede persistir y sobrevivir en condiciones ambientales adversas mediante la producción de endosporas y la formación de biopelículas.Su reservorio ambiental incluye suelo, cursos de agua, agua de mar y plantas. También se han descripto varias fuentes ambientales de infección nosocomial, incluyendo la ropa de hospital, las superficies de las unidades y las manos del personal sanitario. Bacillus gr. cereus es un microorganismo productor de intoxicación alimentaria y asociado a cuadros de diarrea y vómitos. También se lo considera como un patógeno oportunista que puede afectar a personas inmunocomprometidas y recién nacidos, causando infecciones tanto locales como sistémicas: meningitis, endocarditis, infecciones del tracto respiratorio, infecciones de heridas y sepsis. En los recién nacidos prematuros, las principales fuentes de infección sistémica son las contaminaciones aéreas debido a respiradores. Descripción del caso: Paciente de sexo femenino, II gemelar, nacido pretérmino de cesárea por preeclampsia materna. Ingresa al servicio de neonatología con prematurez y dificultad respiratoria. A partir del quinto día, cursa cuadro febril y desmejoramiento con sospecha de infección por lo que inicia tratamiento antibiótico con vancomicina y meropenem. Se diagnostica bacteriemia a Staphylococcus aureus 2/2 y Bacillus gr. cereus en una de las muestras. Se solicita estudio microbiológico de LCR donde se observan al exámen directo, bacilos gram positivos, desarrollando en el cultivo Bacillus gr, cereus. El panel sindrómico de meningoencefalitis dio negativo. Al sexto dia, ingresa a asistencia mecánica respiratoria con cuadro convulsivo y progreso del compromiso cardiovascular. Al octavo día, se produce el óbito por shock sépticodebido a meningitis por Bacillus gr. cereus. Discusión: Existiría una subestimación de la incidencia de Bacillus gr. cereus en humanos por la complejidad de poder asociarlo como el verdadero causante de la patología del paciente. El caso de sepsis / meningitis de un recién nacido que docu- mentamos, genera un alerta ante casos de hemocultivos positivos por este microorganismo, que con frecuencia es considerado como contaminante de las muestras

167. 481. **INFLAMMATORY AND OXIDATIVE MARKERS IN DIFFERENT CLINICAL STAGES OF CHAGAS CHRONIC CARDIOMYOPATHY: IMPLICATIONS FOR DISEASE PROGRESSION BIOMARKERS**

Pieralisi A.V.[1], Cevey Á.C[1], Vinuesa A.[2], Molina C.[2], Prado N.[2], Penas F.N.[1] Alba Soto C.[4], Mirkin G.A.[4], Gagliardi J.A.[2], Repetto S.[4], Goren N.B.[1]

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Chronic cardiomyopathy is the most important clinical manifestation of Chagas disease. It has been described that exacerbated inflammatory response is associated with increased cytokine and reactive oxygen species (ROS) production in patients with chronic Chagas disease. To characterize the inflammatory and oxidative status of seropositive individuals with different clinical forms of Chagas disease, patients in stages E0(no evidence of cardiac damage), E1(abnormal ECG), E2(abnormal ECG and chest X-ray), and E3(abnormal ECG, chest X-ray, and heart failure) were recruited, following Kuschnir's staging. Whole blood was collected, serum and plasma were isolated and peripheral blood mononuclear cells (PBMC) were purified. First, we analyzed the expression of inflammatory and oxidative mediators in CD14+ cells using flow cytometry. We observed an increased expression of TNFα in E1, E2, and E3 patients compared to healthy individuals (HI) ($p<0.05$). On the other hand, we evaluated inespecific ROS (DCFDA probe) and mitochondrial superoxide (Mitosox probe) and observed an increase in E0 compared to HI ($p<0.05$), with higher cytosolical superoxide (DHE probe) levels in E3 patients ($p=0,063$). Subsequently, we examined plasma cytokine expression. We observed increased IL-10 levels among E1 patients ($p<0.05$), as well as elevated IL-23 levels in both E0 and E3 patients($p<0.05$). Moreover, TNF-α and IP-10 plasma levels were significantly elevated in E3 versus HI patients ($p<0.05$), consistent with up-regulation of IFNγ. Additionally, IL-12p70 levels were elevated in E2 patients vs HI ($p<0.05$). Finally, as indicators of tissue injury, we measured CK-MB, GOT and LDH-P activity in plasma, and observed an increase in activity in patients at different stages of the disease. Our main focus is to examine inflammatory and oxidative markers in patients with Chagas disease, as a crucial basis for identifying new progression biomarkers of Chagas Chronic Cardiomyopathy.

168. 624. **EFFECT OF FENOFIBRATE AND BENZNIDAZOLE TREATMENT ON THE OXIDANT/ANTIOXIDANT BALANCE IN AN EXPERIMENTAL CHAGASIC CARDIOMYOPATHY**

Ágata Cevey[1], Azul Pieralisi[1], Federico Penas[1], Gerardo Mirkin[2], Nora Goren[1].

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Chagas disease stands as the primary cause of dilated cardiomyopathy across the entire American continent. Benznidazole (Bzl) is the primary parasite-fighting method due to scarce Nifurtimox. Chagas disease is seen as an inflammatory cardiomyopathic syndrome, rooted in inflammation. Evidence shows that infection-triggered ROS-RNS overproduction and disrupted antioxidants accelerate heart disease. Peroxisome proliferator-activated receptors (PPAR) regulate inflammation. We previously showed that combined treatment with Fenofibrate (Fen), a PPAR-α ligand, with Bzl, improves several aspects of cardiac pathology. To delve deeper into comprehending the mechanisms driving this enhancement in cardiac function, we aim to study the effect of combined treatment with low doses of Bzl and Fen on the modulation of redox homeostasis in *T. cruzi* (*Tc*) infection and its potential correlation with the improvement of cardiac functionality. For that, BALB/c mice were sequentially infected with a non-lethal strain of Tc (CA-I, K-98 clone, DTU TcI) for 42d, followed by reinfection with a lethal strain (RA, DTU TcVI), for 30d. Combined treatment turned the parasitological parameters negative ($p<0.0001$), restored to normal the ejection and shortening fractions, left ventricular end-diastolic and end-systolic diameter, and isovolumic relaxation time ($p<0.0001$). Here we showed that the treatment reduces both protein and mRNA cardiac iNOS expression (WB and RT-qPCR) ($p<0.001$) and restores to normal cardiac GSH levels. Furthermore, this both oxidative and nitrosative markers significatively correlates with cardiac dysfunction ($p<0.05$). Besides, combined treatment increases the Superoxide dismutase activity, an antioxidant enzyme. These preliminary findings present novel evidence of treatment effects of Bzl and fen, emphasizing their antioxidant efficacy in *Tc*-infected cardiac tissue.

169. 659. **SEROPREVALENCE OF TRYPANOSOMA CRUZI INFECTION AND ITS RELATIONSHIP WITH THE VECTORAL INFESTATION OF TRIATOMA INFESTANS IN PERIURBAN AND RURAL AREAS OF THE PROVINCE OF LA RIOJA**

Rodriguez Jimena [1,2], Abrahan Luciana[1,2], Diaz Ariza Maria [2]
Consejo Nacional de Investigaciones Científicas Técnicas (CONICET). [1] Instituto Universitario de Ciencias de la Salud, Fundación Barceló.[2]

Infection by the Trypanosoma cruzi parasite, which causes Chagas disease, is a major public health problem. This disease is transmitted to man, for the most part, vectorially through hemipterous blood-sucking insects belonging to the Triatominae subfamily (WHO, 2021). Currently, in the province of La Rioja, there are no studies carried out in peri-urban and rural areas carried out on blood samples from human patients and on samples of *T. infestans* obtained within the home and around the home, which allow detecting the presence of the parasite. These antecedents, added to the absence of official data and/or public access, lead us to consider as objective of this project, to know the prevalence of infection by *T. cruzi* in people and *T. infestans* captured from different peri-urban and rural areas of the province of La Rioja as a strategic instrument for planning and conducting the health system in the knowledge of profiles, risk factors, including living conditions, in population units-space for Chagas disease. 2009 participants were studied between the years 2019 and 2022 by serology for T. cruzi infection. The presence of intra- and peri-domicile triatoma infestans was evidenced in areas where seropositive patients live in the southeast and southwest of the province of La Rioja with the highest prevalence. Although the inspection of T. cruzi by optical microscopy in *triatoma infestans* was negative, they are found in coexistence. Through Epi INFO 7.2, a map was created showing the relationship between areas with seropositive patients and areas with the presence of *Triatoma infestans.* Paying attention only to the total number of cases observed or the general incidence observed in the population and verifying that it is within the expected limits may be insufficient. The use of maps to present data on the distribution of *Triatomas infestans* and seropositive patients facilitates the identification of clusters and provides important clues about the presence of common sources of infection and risk exposures (WHO,2021).

O1-INFECTOLOGY & IMMUNOLOGY

FRIDAY 17TH NOVEMBER 9:00 - 10:30
CHAIRS: ROXANA SCHILLACI
MARIANA MALVICINI
DOLORES CARRER

170. 29. **TONSILLAR IMMUNITY OVER TIME, FROM IMMUNE RESISTANCE TO IMMUNE REGULATION**

Rocío A. Pastor[1], Juliana Puyssegur[1], M. Paula de la Guardia[1], Ignacio E. Rojas Campión[1], Andrea Paes de Lima[5], Bibiana Paoli[2], M. Elena Arabolaza[2], Isabel Aspe Scetti[6], Mailén Rojo[1], M. Soledad Collado[1], Andrés Blanco[6], Fernando Chirdo[4], Eloísa I. Arana[1,3]
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The tonsils are mucosal lymphoid tissue located at the back of the mouth. They are thought to experience involution in adulthood. In this context, we have used tonsillar mononuclear cells isolated from patients at different stages of life, to study age-related changes in mucosal immunity. Likewise, we determined the most prevalent bacterial species within the cohort of patients. To do so, we combined flow cytometry, immunohistochemistry and bacterial culture with subsequent identification of the respective isolates by MALDI-TOF MS. We found an age-dependent reduction in the proportion of germinal center B cell population (BGC, n=76, 4 groups of age, means statistically different, t test) and its T cell counterpart (T follicular helper germinal center cells, TfhGC, n=54, 4 groups of age, means statistically different, t test). Also, we demonstrated an increment in the percentage of local memory B cells (BMEM, same statistics as BGC). Furthermore, younger tonsils rendered a statistically significant higher proportion of proliferative $CD4^+$ and $CD8^+$ T cells than those from older ones (n=37, 5 groups of age, means statistically different, t test). We detected the expansion of a B cell subset metabolically adapted to catabolize adenosine triphosphate (CD20+CD39+CD73+ cells), as patients get older (n=69, 2 groups of age, means statistically different, t test). Finally, the most prevalent bacterial species in tonsilar tissue were *S. aureus*, *H. influenzae* and *S. Pyogenes*, with no difference between age groups. To conclude, our data reflects a reduction in the proportion of effector cells (BGC and TfhGC), an increase in the fraction of BMEM, an enrichment in B cells with regulatory function and the concomitant decrease in proliferative immune cells as the patients age. This study may help predicting disparities in the immune responses to oro-naso-pharyngeal antigenic challenges in the life span of individuals.

171. 117. ***BIFIDOBACTERIUM ANIMALIS* SUBSP. *LACTIS* INL1 CELL-FREE SUPERNATANT ATTENUATES THE INFLAMMATORY RESPONSE OF LPS-STIMULATED MACROPHAGES**

Pedro Carriere[1], María Belén Novoa Díaz[1], Gabriela Sica[2,3], Gabriel Vinderola[4], Natalia Calvo[1], Claudia Gentili[1].
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The inflammatory response protects the body against pathogens; however its persistence can lead to inflammatory diseases. This work aimed to explore the effect of the cell-free supernatant (CFS) of the human milk-derived strain *Bifidobacterium animalis* subsp. *lactis* INL1 (*B. lactis* INL1) (transfer agreement UNS-UNL No REC-1092496-2) in an *in vitro* model of inflammation, specifically lipopolysaccharide (LPS)-stimulated RAW264.7 macrophages. To evaluate the mitochondrial integrity of these macrophages, they were pretreated with CFS for 3 hours and then exposed to LPS for 24 hours. By JC-1 assay we observed that LPS maintains mitochondrial health but it increases due CFS pretreatment followed LPS treatment ($p<0,05$), suggesting that CFS from *B. lactis* INL1 has beneficial properties for cell physiology. Next, an *in silico* analysis was performed to identify the signaling pathways associated with the documented effect of the *B. lactis* bacteria on macrophages. Using Cytoscape software, the gene interaction network was obtained. JUN, which encodes the c-jun transcription factor involved in the inflammatory response, was found to be the core gene of the network by betweenness and closeness. Functional enrichment showed that the genes obtained are associated with the inflammatory response, the LPS response, and inflammatory bowel diseases. In addition, we analyzed gene expression data from RAW264.7 macrophage microarrays exposed or not to LPS (GSE21548-GEO). Differentially expressed genes ($Log_2FC\geq1$ or ≤-1) showed that the JUN gene was overexpressed in LPS-exposed macrophages ($Log_2FC=1,435$; $padj<0,05$). Based on these data, we study c-jun protein levels status by Western blot in our experimental model. The expression of c-jun increases by LPS action but this effect is attenuated by CFS pretreatment of *B. lactis* INL1 in these cells ($p<0.05$), suggesting that c-jun pathway could be a potential mediator of the effects of *B. lactis* INL1 in the inflammatory context.

172. 245. **PROGNOSTIC SIGNIFICANCE OF THE NEUTROPHIL/LYMPHOCYTE RATIO IN PERPHERAL T-CELL LYM-**

PHOMA: A SYSTEMATIC REVIEW AND META-ANALYSIS

Rafael Pichardo-Rodriguez[1], Liz B Cordova-Cueva[1], Dante Quiñones-Laveriano[1], Susy Bazán-Ruiz[2], Brady E. Beltrán-Gárate[1], Jhony A De La Cruz-Vargas[1]

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OBJETIVE: Here we present a systematic review and meta-analysis on the prognostic value of the NLR in PTCL. METHODS: A systematic search encompassed PUBMED, EMBASE, and SCOPUS databases until July 2023. Prospective and retrospective cohorts were evaluated based on WHO criteria for TCL diagnosis. NLR denoted pre-therapy neutrophil-to-lymphocyte ratio. Two reviewers selected studies, extracted data, and performed quality assessments. Variables included clinical characterizes, NLR values, Hazard Ratio (HR), 95% CI. Quality-verified data underwent meta-analysis. Influence analysis employed leave-one-out. Meta-regression gauged impact of <200 sample size on heterogeneity. Primary endpoints: overall survival (OS) and Progression-Free Survival (PFS). Risk of bias was evaluated via Newcastle-Ottawa Scale. Data analyzed using R 4.2.3. RESULTS: Thirteen studies (1,825 patients) were identified, with 3 from Latin America. The median NLR was 3.8. NLR was associated with worse OS (HR: 2 [95% CI: 1.5-2.6, I2: 49%; P=0.02]) in 13 studies. No association between NLR and PFS was observed. Regionally, the NLR was linked to worsened OS in Latin America (HR: 3.4 [95% CI: 1.7-6.9, I2: 40%; P<0.01]) and Asia (HR: 1.8 [95% CI: 1.3-2.4, I2: 45%; P=0.05]). An NLR>4 worsened OS (HR: 1.8 [95% CI: 1.2-2.5, I2: 48%; P=0.06]), but was not observed in PFS. Excluding the study of Zhou et al reduced heterogeneity, while the effect size remained unchanged notable (HR: 1.9 [95% CI: 1.6-2.3, I2: 20%; p<0.01]). Excluding the study of Zhou et al for PFS reduced heterogeneity and increased the effect size (HR: 1.6 [95% CI: 1.2-2.1, I2: 0%; p>0.05]). For OS, there was no evidence of publication bias, and for PFS, the studies were limited in number. A sample size <200 did not have an impact on heterogeneity. CONCLUSION: The NLR is a relevant prognostic factor in PTCL for OS. Inconsistent NLR cutoffs across studies suggest standardization. Cut-off >4 could be a promising biomarker for LATAM patients.

173. 260. **COMBINED TREATMENT OF NIR RADIATION AND PHOTODYNAMIC INACTIVATION IN AN *IN VIVO* MODEL OF *S. AUREUS* INFECTION**

Roberto Tomás[1], Gabriela Di Venosa[1], Fernanda Buzzola[2], Adriana Casas[1], Leandro Mamone[1].

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Photodynamic Inactivation (PDI) combines a photosensitizer compound with visible light and molecular oxygen, to generate reactive species and kill microorganisms. 5-aminolevulinic acid (ALA) is a precursor in the biosynthesis of photosensitizing porphyrins.
Near-infrared therapy (NIRT) uses infrared light to deliver heat into tissues. NIRT can inactivate microorganisms and promote healing.
The aim of this work was to employ a combination of NIRT and ALA-PDI (visible light irradiation after topical ALA treatment) to reduce the progression of wounds caused by *Staphylococcus aureus* infection, in an *in vivo* model in mice. CF1 mice were injected subcutaneously with a suspension of *S. aureus* RN6390. After 48 h, 20 mg/ml ALA solution was applied to the skin. NIRT was performed with a 980 nm laser (96 J/cm^2). Porphyrins produced from ALA, and their localization, were determined by fluorescence spectroscopy and microscopy. The PDI was performed employing a 635 nm laser device (144 J/cm^2). The effect of light treatments and untreated controls was determined by measuring the area of the wound caused by infection during four weeks after treatments. Bacterial load at the infection site was measured by counting CFUs from skin homogenates. Wounds treated with ALA-PDI reduced area sooner than the untreated control. Differences between these two groups were significant every day after irradiation ($p<0.05$). Furthermore, the time required for complete wound closure in the ALA-PDI group was significantly less ($p<0.01$) than in the light and untreated controls (14 vs 21 and 27 days respectively). There was no difference in wound closure time when PDI was combined with NIRT, despite the results indicating that NIR treatment increases porphyrin levels at the site of infection. No statistically significant differences were detected in the bacterial load at the infection site between any of the treatments. Our results suggest that PDI is a promising option to treat superficial infections.

174. 265. **NOVEL COMPETITIVE ENZYME-LINKED IMMUNOSORBENT ASSAY FOR THE DETECTION OF THE HIGHRISK HUMAN PAPILLOMAVIRUS 18 E6 ONCOPROTEIN**

Natalia Estefania Contreras[1, 2], Julieta Suyay Roldán[1, 2], Daniela Susana Castillo[1, 2].

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[2]*Escuela de Bio y Nanotecnologías (EByN), Universidad Nacional de San Martín (UNSAM), San Martín, Buenos Aires, Argentina*

Cervical cancer represents a global concern with 604,000 new cases and 342,000 deaths reported annually, with the vast majority diagnosed in low income countries. Despite high-risk Human Papillomavirus (HR HPV)-induced cervical cancer has become highly preventable through prophylactic vaccines, screening programs are critical in the control of cervical carcinogenesis in populations with limited access to vaccination and in older generations of women who have already been exposed to HR HPV infection. In this context, the E6 oncoprotein from HR HPV types arises as a promising diagnostic marker for its overexpression in transformed HPV positive cancer cells. For this reason, the aim of this study consisted of obtaining monoclonal antibodies (mAbs) against the E6 oncoprotein of one of the most prevalent HR HPV types worldwide, HPV18, in order to develop a highly specific and sensitive indirect competitive ELISA (icELISA). We selected the 7D2 hybridoma clone, which enabled the development of a sensitive icELISA to detect and quantify small amounts (226 ng/ml) of E6 disease marker. To validate our icELISA, we performed spike-and-recovery tests in C-33 A HPV-negative cervical cancer cell lysates spiked with three concentrations of HPV18 E6 recombinant oncoprotein. The average recoveries were between the ideal range from 80 to 120%. Furthermore, we carried out linearity-of-dilution assays with cells extracts from HEK293T cells that stably express HPV18 E6. Results showed an average concentration of 4.5 ng/ml of HPV18 E6 oncoprotein per 1000 cells. Finally, we tested cells extracts of HPV18-positive cervical cancer-derived HeLa cell line, which gave high signal (632.5 ng/ml). In conclusion, the present study establishes a valid, sensitive, reliable and reproducible 7D2-based icELISA that constitutes a promising bioanalytical method for the early detection and quantification of HPV18 E6 oncoprotein in cervical swab samples and cancer prevention.

175. 363. **MICROVESICLES CARRYING SHIGA TOXIN TYPE 2 (MVS-STX2) AS A NEW CLINICAL BIOMARKER FOR THE RAPID DIAGNOSIS OF PATIENTS AT RISK OF DEVELOPING HEMOLYTIC UREMIC SYNDROME (HUS)**

Fernando Gómez[1,2], Flavia Sacerdoti[1,2], Daniel Girón Reyes[1,2], Carla Pascuale[3], Tomás Lombardo[4], Roxane Maria Fontes Piazza[5], Laura Alconcher[6], María Marta Amaral[1,2].

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In Argentina, Hemolytic Uremic Syndrome (HUS) caused by Shiga toxin (Stx)-producing *Escherichia coli* (STEC-HUS) infection is an endemic disease and one of the most common causes of acute kidney injury in children. Negligible amounts of free toxin are present in the circulation during HUS, and it circulates mainly bound to blood cells and microvesicles (MVs). Platelets and leukocytes derived MVs were detected in the plasma of STEC-HUS patients. In this sense, preliminary, we successfully identified circulating MVs carrying Stx2 (MVs-Stx2) in two STEC-HUS patients. In this study, our objective was to analyze the presence of MVs-Stx2 in blood samples of six STEC-HUS patients, between 3 and 17 years old, that were admitted to the Hospital Penna-Bahía Blanca. Blood samples were collected and underwent sequential ultracentrifugation to obtain a suspension enriched with MVs. Then, MVs were labeled with Annexin V-FITC and MVs-Stx2 were detected by a monoclonal anti-Stx2 antibody and a secondary antibody conjugated to Alexa Fluor 647. Finally, MVs were analyzed by flow cytometry. Data are expressed as the percentage of positives MVs-Stx2. Ten age-matched healthy controls were also recruited and a cut-off point for MVs-Stx2 was established. Medical history of patients indicated that to the day of hospitalization, three of them exhibited aqueous diarrhea, two showed bloody mucous diarrhea and the other had no diarrhea. For Stx2 PCR analysis, 50% of them were positive. Anti-LPS antibodies were positive for O145 IgM/IgG in three patients and for O157 IgG in one of them. All patients showed a significant percentage of MVs-Stx2 in circulation compared to controls (3.4%, 7%, 13.7%, 6%, 3.6% and 13.8% vs 1.76 ± 0.74 %, n=10, ($p<0.05$). Thus, these patients had MVs-Stx2 in their blood circulation on admission to the hospital. Detection of circulating MVs-Stx2 in patients infected with STEC could be useful for the early identification of patients at a high risk of developing HUS.

176. 462. **SIMULTANEOUS DETECTION OF THREE RESPIRATORY VIRUSES BY A SINGLE QPCR AMPLIFICATION FOLLOWED BY HIGH RESOLUTION MELTING ANALYSIS**

Mariela Caputo[1,2], Santiago Ginart[1], Daniel Corach[1,2], Lucía Garrigós[1,2], Federico Remes Lenicov[2,3], Andrea Sala[1,2]

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After pandemic confinement, other respiratory viruses, such as Influenza and RSV, have started to circulate again. Since acute symptoms, overlap with those of SARS-CoV-2, molecular methods are required to identify the infecting virus. Accordingly, many research groups worked on the development of specific, rapid and cost/efficient methods to detect these viral infections. Most methods were developed on probe based qPCR techniques, strategies that requires expensive reagents. The aim of this work was to develop a diagnostic method based on a multiplex qPCR-HRM to identify specific sequences of RSV, H1N1 and SARS-CoV-2. M&M: synthetic targets of DNA sequences were designed to detect SARS-CoV-2 (103bp); Influenza (H1N1) (98 bp) and RSV (111bp). Online tools were employed for primer design and melting temperature (Tm) simulations. Quantitative PCR was carried out in multiplex reaction, mixing RSV, H1N1 and SARS-CoV-2 primers and SYTO9™ intercalating dye to visualize the progress of the qPCR/HRM melting. Results: Based on the GC content and amplicons length it was possible to detect the tested viruses. Specific viral sequences displayed differential Tm: RSV 76.1°C, H1N1 80.7°C and SARS-CoV-2 83.8°C. The sensitivity was assessed with serial dilutions of synthetic targets. Detection limit was up to 10 copies/ul for SARS-CoV-2 and H1N1, and 100 copies/ul for RSV. Simulated co-infections were efficiently detected. A preliminary assay with cDNA from nasopharyngeal swabs of patients previously diagnosed were concordant. Conclusion: This technique was able to discriminate three respiratory viruses, with high sensitivity and reproducibility. Preliminary tests involving positive samples showed result consistent with our approach. The next steps will include testing an extended number of diagnosed patients and optimize the reaction by coupling with a previous RT-PCR step. This development constitutes a simple and cost-effective strategy for viral diagnosis.

177. 643. **METFORMIN ANTHELMINTHIC EFFECTS ASSOCIATED WITH A DECREASE IN PARASITIC GLUCOSE AND GLYCOGEN IN VIVO**

Julia A. Loos[1*], Facundo Salinas[2*], Paula Taborda[2], Ulises Notaro[2], Luciano Lausero[1], Hugo Ortega[2*], Andrea C. Cumino[1*]

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(*) equal contributions.

Most patients with cystic echinococcosis (CE) are diagnosed at advanced disease stages when the therapy with benzimidazoles is limited, being imperative new therapeutic approaches. Since in cestodes, glycogen is the main energy storage molecule and glucose the major fermentative substrate, we aim to target the energy production pathways in the parasite. Here, we assess the efficacy of metformin as an indirect AMPK agonist in experimental models of advanced CE, at 6 and 12 months post-infection, employing the highest dose of the drug tested (250 mg kg^{-1} day^{-1}) orally or intraperitoneally, respectively. Based on *in situ* detection of fluorescent glycogen with 2-NBDG, biochemical determinations of hydatid fluid, and biodistribution analysis of IRDye-800CW2-DG in mouse whole-body (using near-infrared fluorescent *in vivo* imaging combined with 3D optical tomography), we demonstrated that metformin increased glucose utilization in metacestodes through the glycolytic pathway, leading to the elevation of intracystic lactate levels (up to 7-10 mM), with a tendency to increase the glucose uptake in peritoneal cavity tissues of infected mice. Given that *Echinococcus* cells cannot oxidize lactate, it would diffuse to the surrounding tissue, activating signal transduction pathways involved in the differentiation of macrophages, fibroblasts and lymphocytes, and promoting the development of the cystic adventitial layer. By biochemistry and molecular approaches, we demonstrated intracystic accumulation of metformin at supra-pharmacological concentrations (>1 mM), which induced mitochondrial complex I inhibition and increased cellular Pi/ATP ratio, with AMPK activation and TOR inhibition (by dephosphorylation in Ser^{3122}) and, consequently, reduction of the Warburg effect and proliferation in germinal cells. In the context of normoglycemic mice, our results are in accordance with previous data showing glucose reduction in gut-liver crosstalk induced by metformin.

P1-METABOLISM & NUTRITION

WEDNESDAY 15TH NOVEMBER 9:00 - 10:30

CHAIRS: ROMINA HERMANN
ESTEBAN REPETTO

178. 61. **CHANGES IN BONE RESORPTION AND FORMATION MARKERS BY AGE AND DIFFERENT CONDITION THAT AFFECT BONE HEALTH. EXPERIMENTAL STUDY**

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Bone is constantly being remodeled by bone cells coordinated action. A delicate equilibrium between bone formation by osteoblasts and resorption by osteoclasts is needed to maintain mineral homeostasis and biomechanical integrity. Activity of these bone cells called bone remodeling is biochemically assessed by specific bone turnover markers of bone formation (osteocalcin or OCN) and bone resorption (CTX). Bone remodeling changes with age and is regulated by hormonal and nutritional factors. Objective: Evaluate bone remodeling in different life stages and conditions that impact bone metabolism: age, estrogenic status, dietary calcium (Ca) and/or vitamin D nutritional status (VD) in female Wistar rats. Animals were divided (n=8 per group) based on their age and estrogen deficiency: newborn (NB); weaning (RD); puberty (R45); young adult (R90 SHAM), late adulthood (R105SHAM), and estrogen deficiency (R90OVX, R105OVX) that fed a commercial diet. Effect of VD and/or Ca insufficiency was evaluated by feeding semi-synthetic diets formulated according to AIN'93-M modified in vitamin D (D): 100IU% or 0UI% (+D and -D, respectively) and/or calcium: 0.5% or 0.3% (0.5 and 0.3): R105OVX+D0.5; R105OVX-D0.5; R105OVX+D0.3, and R105OVX-D0.3. CTX and OCN were assessed by ELISA. ANOVA was used., p<0.05 was considered significant. Results (mean±SD) in the following order CTX (pg/mL) and OCN (ng/mL): RN: 83.9±3.5, and 2.49±0.19; RD: 70.5±10.6 and 3.06±0.05; R45: 52.5±7.4 and 3.39±0.15; R90SHAM: 58.8±5.6 and 4.00±0.98; R105SHAM: 56.7±11.1 and 2.68±0.06; R90OVX: 53.4±4.3 and 4.08±0.21; R105OVX:48.9±6.4 and 2.67±0.08; R105OVX+D0.5:38.7±5.4 and 2.05±0.12; R105OVX-D0.5: 67.3±12.3 and 2.24±0.18; R105OVX-+D0.3: 52.7±5.4 and 2.31±0.20; R105OVX-D0.3: 85.8±40.5 and 2.35±0.2. Under our experimental conditions, CTX decreased while OCN levels increased with age. Estrogen deficiency had no significant effect on bone markers, although VD-Ca insufficiency increased CTX and OCN levels considerably.

179. 98. **COMPARATIVE EFFECT OF GALACTOOLIGOSACCHARIDES (GOS) FROM TWO DIFFERENT DIETS MATRIX IN BONE HEATH OF GROWING RATS**

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In previous studies we demonstrated the effectiveness of GOS to increase calcium (Ca) absorption and bioavailability, and to improve bone retention. Objective: to compare the effect of galactooligosaccharides (GOS) from two different food matrix on bone formation and quality during normal growth in rats. Methods: Male weaning rats were divided in 3 groups and fed until 40 days of age: AIN-93G control diet (C), GOS mixed with fructooligosaccharides (FOS) in a proportion of GOS/FOS® 9:1 (PM) or GOS mixed with probiotic bacteria's containing in an experimental yogurt diet (EY). Femur length (LF) (cm) and its Ca and phosphate (Pi) content; bone mineral density (BMD) and (BMC) of the total skeleton (tSk), lumbar spine (lSp) and proximal tibia (pT) by densitometry; bone volume percentage (BV%) and epiphyseal (GPC. Th) and hypertrophic (HpZ.Th) cartilages (μm) by histology; maximum fracture strength (N), and bone stiffness (N/mm) by biomechanical method. Shapiro-Wilk and Levene normality tests were applied, and ANOVA was performed to determine differences between groups using SPSS 19.0. p<0.05 was considering significant. Results: tSk, lSp and pT BMDs were significantly higher in EY than in PM (p<0.001) while both presented higher values than C group (p<0.010). Conversely, the highest significant tSk BMC was observed in PM (p<0.05) while EY had higher value than C (p<0.05). The highest BV and Ca and Pi content in femur was observed in PM (p<0.01 and p<0.001, respectively) while EY and C groups showed similar values. The highest significant values of GPC.Th and HpZ.Th was observed in EY (p<0.05). PM and EY showed similar values in the studied biochemical parameters than C and no significant differences between them were observed. Conclusion: Although both sources of dietary GOS induced a greater retention that benefits bone health, the symbiotic effect of GOS in the EY, due to the presence of probiotic bacteria's, appears to further enhance its effect in bone retention.

180. 250. **ANTIOXIDANT EFFECT OF RESVERATROL ADMINISTERED WITH THE CONSUMPTION OF ENRICHED WINE OR AS A DIETARY SUPPLEMENT IN PATIENTS WITH RISK FACTORS FOR METABOLIC SYNDROME**

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Resveratrol (R) treatment improves the antioxidant response in cells and plasma of patients with risk factors of Metabolic Syndrome (MS) by protecting from inflammation and oxidative damage. Previous results indicated that R decreases oxygen consumption (ΔO_2) and peroxidation of lipids in PMN isolated of MS patients. The objective of this study is to compare the protective effect of R in patients with MS administered with resveratrol-enriched wine (REW) or as a dietary supplement. Voluntary patients with a diagnosis of MS (n=92) based on the diagnostic criteria of the National Cholesterol Education Program, Adult treatment Panel III, 2002 were supplemented 6 months with REW (n=30, age: 63 ± 10 years old, 150 mg/L), or 3 months with dietary R in tablets (n= 23, age: 68 ± 5 years old, 50 mg resveratrol, 25 mg alpha-tocopherol and 5 mg piperine) along with their usual treatment. Control was the patient himself in baseline conditions, avoiding interindividual variables and bioavailability of active principles. Venous blood was collected (53 patients), and biochemical markers were assessed at 0, 3 and 6 months of treatment in PMN, plasma and red blood cells: ΔO_2, lipid oxidation (TBARS), protein oxidation (carbonyl protein, CO), catalase (CAT) and superoxide dismutase (SOD) activities, and glutathione (GSH) content. At 6 months of treatment, ΔO_2 decreased with REW (56%, p<0.001). At 3 months, CO decreased 49% (p<0.01) with REW and 30% (p<0.05) with R treatments, TBARS increased 61% (p<0.05) with REW. CAT, SOD and GSH increased (30%, p<0.05; 100%, p<0.0001, and 58%, p<0.05, respectively) with R. These preliminary results indicate that R activates enzymatic and non-enzymatic intracellular antioxidant protection, reducing inflammatory processes and oxidative damage associated with MS, while REW protects against inflammatory processes and protein oxidation with the dose and treatment time evaluated.

181. 288. **ASTAXANTHIN-RICH EXTRACT DERIVED FROM CONTINENTAL NATURAL SOURCES HAS BENEFICIAL EFFECTS UPON THE ALTERED OXIDATIVE HEART STATUS AND COGNITIVE DECLINE DEVELOPED IN A DIET-INDUCED METABOLIC SYNDROME RODENT MODEL**

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The cluster of metabolic abnormalities included in the Metabolic Syndrome (MetS) are linked to a higher risk of cardiovascular diseases and -as reported recently- with poor performances in multiple cognitive domains. There is increasing evidence supporting that Astaxanthin (ASTX) –one of the most powerful natural antioxidants- could have beneficial effects upon several diseases. However, those few studies have focused on the effects of ASTX of marine origin. The aim of this work was to evaluate the effect of an ASTX-rich extract from freshwater decapod crustaceans- an alternative natural source- upon the altered cardiac redox status and memory deficits developed in an experimental model of MetS. Male Wistar rats (8 weeks old) were fed for 90 days with 1 of 3 experimental diets: 1-Reference group received a standard commercial rodent diet, 2- High-sucrose diet (HSD) group received a HSD, 3-HSD+ASTX group received a HSD plus ASTX-rich extract (10 mg/kg body weight/day oral dose). We evaluated biomarkers of heart oxidative status (reactive oxygen species-ROS-, lipid peroxidation -TBARS- and reduced glutathione levels -GSH). Novel Object recognition (NORT) and the T- maze tasks were performed in order to explore non-spatial and spatial memories, respectively. Heart and brain weights, bodyweight gain, energy intake, lipid serum levels and blood pressure were also determined. Data was statistically analyzed by one-way ANOVA and Newman Keuls post-hoc. Compared with HSD-fed rats, HSD+ASTX-fed animals showed reduced heart weight and ROS levels and increased cardiac GSH levels ($p<0.05$). No changes were observed in TBARS levels. These changes were accompanied by a better cognitive performance in both memory tasks performed and a higher brain weight. Besides, bodyweight gain, triglyceride and cholesterol serum levels and blood pressure were also reduced in HSD+ASTX-fed rats. The results show that this ASTX-rich extract has cardioprotective and neuroprotective effects.

182. 439. **ASSOCIATION BETWEEN NOVEL MARKERS OF CARDIOVASCULAR RISK AND FATTY ACID PROFILE IN APO B-DEPLETED PLASMA FROM OBESE CHILDREN AND ADOLESCENTS**

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Introduction: Childhood obesity is an important cardiovascular (CV) risk factor, closely related to lipid alterations. New markers of CV risk have been developed such as lipoprotein associated phospholipase A_2 (Lp-PLA_2), cholesteryl ester transfer protein (CETP) and paraoxonase 1 (PON 1). Moreover, plasma fatty acids display a complex network of both pro and antiatherogenic effects and could influence these markers. Our aim was to characterize novel markers of CV and their relation to fatty acids present in apo B-depleted plasma in pediatric obesity. Methods: Twenty obese children and adolescents and 20 healthy controls were studied. Anthropometric parameters and dietary inquiries were registered. Glucose, insulin, lipid and high sensitivity C reactive protein (hs-CRP) levels were measured by automated methods, Lp-PLA_2 and CETP activities by radiometric assays, PON 1 activities [PON and arylesterase (ARE)] by colorimetric assays and fatty acids in apo B-depleted plasma by gas chromatography. Results: The obese group showed a more atherogenic lipid profile, plus higher Lp-PLA_2 and lower ARE activities ($P<0.05$). This group also presented higher bakery product and lower cereal consumption. With respect to fatty acids, the obese group showed higher levels of myristic, palmitoleic, margaric and gamma-linoleic acids, in addition to lower concentrations of linoleic, arachidic, gadoleic, eicosatrienoic and eicosapentaenoic (EPA) acids ($p<0.05$). Hs-CRP correlated positively with palmitoleic and gamma-linoleic acids, plus negatively with linoleic acid and EPA. Lp-PLA_2 correlated positively with myristic acid, CETP with arachidic and lignoceric acids, and PON 1 with gadoleic and cis-octadecenoic acids. ARE correlated negatively with arachidonic acid. Conclusion: The obese group presented alterations in novel markers of CV risk, which were associated with modifications in fatty acid profile. These interactions could contribute to an increased cardiovascular risk.

183. 553. **EFFECT OF A DIET ENRICHED IN OLIVE OIL IN A KNOCKOUT MOUSE MODEL OF ACUTE INTERMITTENT PORPHYRIA**

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Acute intermittent porphyria (AIP) is a hereditary disorder due to a decrease of porphobilinogen deaminase (PBG-D) activity; secondarily, 5-aminolevulic synthetase (ALA-S) is induced. Fasting is one of the triggering factors of acute attacks. To elucidate the mechanisms that lead to AIP onset and considering that a regulated diet can attenuate or prevent the attacks, the aim was to investigate if a diet rich in lipids affects heme biosynthesis. Male and female knockout mice deficient in PBG-D activity (Pbgd-/-) were used. For each sex, animals were divided in 3 groups: groups 1 and 2 received standard diet (Purina 3) for 12 weeks; group 3 was fed with standard diet supplemented with 15% commercial olive oil (20% of total lipids) during the same period; groups 2 and 3 were fasted 16 hours prior sacrifice. Serum glucose levels and lipid profile parameters (high- and low-density lipoproteins, total cholesterol and triglycerides) were measured. Initially the effect was evaluated through ALA-S activity/protein expression. In both sexes, oil did not alter the amount of food eaten, liver weight/body weight ratio or lipid profile compared to controls starved or not. Fasting caused 60% ($p<0.05$) decrease of glucose levels in male mice receiving standard diet, while in females the reduction was more striking leading to undetectable values by the method used. In those animals fed with standard diet plus oil, starvation also provoked a similar reduction in males but in females, the drop was less. Due to fasting, ALA-S activity increased more than 200% ($p<0,01$) in both males and females independently of the diet. A similar profile was observed for protein expression. In conclusion, although high lipid diet improved glucose levels in fasting female mice, it was not reflected in a decrease of ALA-S, thus this type of diet is not able to protect against ALA-S induction in fasted mice.

184. 567. **EFECTS OF TWO LOW LIPID DIETS ON C57BL/6J MICE**

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The C57BL6J mouse strain is a widely used model to evaluate the effects of high-fat and low-fat diets. The intention of this study is to determine if the source of lipids modifies the metabolism of male mice C57BL6J. Bovine grease (Primer jugo bovino, SENASA category – Food code of Argentina) and extra virgin olive oil (EVOO, quality determine by olive oil tasting panel, agricultural school, UNCuyo – Argentine) was added to balanced standard diet (SD) for mice to obtain lipid diets, bovine grease diet (mG) and olive diet (mO). First at all the biochemical fed composition, after addition of lipids, was determined at INTI lab (national institute of industrial technology). Inti´s report indicate: kcal/100g = 341 (SD), 371 (mG) and 357 (mO); saturate grease (g/100g) = 0,43 (ND), 3,4 (mG), 1,33 (mO); cholesterol (mg/100g) = 18,4 (ND), 26,8 (mG), 18,5 (mO); percentage of proteins = 19 (ND), 17 (mG), 18 (mO) and total grease (%) 6 (ND), 19 (mG), 19 (mO). Then, male mice C57BL6J were feed with a con-

trol diet (SD), a low-fat diet derived of bovine fat (mG) and a low-fat diet derived of extra virgin olive oil (mO) for sixteen weeks. Body weight, food intake, body fat, liver weight and serum biochemical parameters (Glucose, cholesterol, HDL-col, LDL-col, triglycerides, atherogenic index) were measured. The food intake by mG group showed a significantly decreased (SD = 3,46 +/- 0,21, mG = 2,91 +/- 0,22 *, mO= 3,34 +/- 0,21 *p<0,01), but energy and cholesterol intake were significantly increased (data not shown, p<0,01). The body fat weight (SD = 1,62 +/- 0,32, mG = 1,64 +/- 0,58, mO = 0,47 +/- 0,03 ** p<0,01) was significantly decrease in mO. There was no significant difference in body weight, as well as biochemical parameters and liver weight, except the relation between HDL and LDL cholesterol that is in favor to HDL in mO. These findings indicate that a low-fat diet derived of bovine fat produces different effects than a low-fat diet of olive oil.

185. 585. **COMPARATIVE EFFECT OF FRIED SUNFLOWER OIL DIET INTAKE ON MORPHOMETRICS AND BIOMECHANICAL COMNPETENCE OF THE FEMUR AND MANDIBLE DURING GROWTH**

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Our previous studies demonstrated that growing rats fed fried sunflower oil (SFOx) diet presented diminished total skeleton bone mineral content, femur and tibiae. Objective: to compare the effect of a diet rich in SFOx on morphometrics and biomechanical competence of the femur (appendicular bone) and the mandible (axial bone) in growing rats. Methods: Male Wistar rats (21±1 days old) (n=21) were assigned at weaning to one of three diet groups for 8 weeks: control diet (C), a SFO diet or a SFO diet which was repeatedly heated (SFOx). At wk=8, animals were euthanized and femur and hemimandible were extracted. Femur and mandible biomechanical competence were assessed to estimate the structural properties of the bone; load bearing capacity (Wf) and stiffness (Wy/dy). Results: rats fed SFOx attained the lowest final body weight (SFOx<SFO=C; p=0.03) and length (SFOx< SFO=C; p=0.001) and mandibular weight (SFOx<SFO<C; p=0.001); femur weight was similar in SFOx and SFO groups. The mandibular growth was more affected on the posterior part of the bone in SFOx, being lower than SFO and C (p=0.001); the higher anterior/posterior ratio indicated that SFOx induced deformation of the mandible (p=0.004). In femur, Wf and Wy/dy of SFOx were significantly lower than SFO and C (p=0.001 and p=0.001, respectively); in hemimandible, SFOx induced a significant reduction in Wy/dy (p=0.007), though Wf was similar in SFOx and SFO. Conclusion: During growth, the frequent consumption of a SFOx diet showed different morphometric and biomechanical responses of the femur and the mandible. Although, the mandible exhibited alterations in the morphometric properties (deformation); in the femur, the biomechanical competence in terms of reduced bone stiffness was evident. These adaptations suggest alterations in the dynamics of the growth mandible; whereas in the femur, could denote changes in the quality of the mineralized tissue or in the architectural design. Acknowledgment: to Ricardo Orzuza.

186. 607. **DOES THE ENRICHMENT OF LIPOPOLYSACCHARIDE IN THE CHYLOMICRON PROMOTE ITS LIPOLYSIS BY LIPOPROTEIN LIPASE?**

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Introduction: previous studies from our laboratory have demonstrated an association among high fat-high sucrose diets (HFHS), an altered intestinal microbiota (dysbiosis) and metabolic endotoxemia. The enrichment of chylomicron (CM) with lipopolysaccharide (LPS) would impact in the Lipoprotein lipase (LPL) lipolytic action, determining its metabolic fate. Nevertheless, little is known about the interaction between CM and LPL in a dysbiotic context.
In diet-induced dysbiosis model, our aim was to assess the quality of a LPS-enriched CM, as a LPL substrate, through an *in vitro* lipolysis assay. Materials and methods: male Wistar rats (180-200g) were fed with standard diet (C, n=6) or standard diet +40% fat +15% sucrose (HFHS, n=6) throughout 14 weeks. In serum, lipid profile, free-fatty acids (FFA) and LPS were determined; CMs were isolated (ultracentrifugation d<0.95 g/ml) and characterized in their lipid composition and LPS content. Bovine LPL was incubated with CM containing different TG enrichment grades for the lipolysis assay (0.05-1.5mmol/L). FFA was determined and the Km was calculated as an enzymatic affinity indicator. Results: HFHS group exhibited an atherogenic lipoprotein profile and increased markers of insulin-resistance, along with increased LPS serum levels (HFHS=1.79 ± 0.18 vs. C=1.25 ± 0.32 EU/mL, p=0.01). CMs from HFHS showed higher TG content (238 ± 113 vs. 40 ± 15 mg/dL, p=0.03) and LPS (8.28 ± 3.55 vs. 3.05 ± 2.19 EU/mL, p<0.01) than C. The affinity of LPL for CM was higher in HFHS compared to C (Km= 0.91 ± 0 59 vs. 2.75 ± 1.11 mM, p=0.01) and was significantly correlated with CM-LPS (r=-0.681, p=0 04) and CM-TG (r=-0.817, p=0.03). However, the latter lost significance when adjusting for CM-LPS content. Conclusion: LPS-enriched CMs, in a gut dysbiosis context, contribute to LPL activity, promoting the generation of smaller CM-remnants and the release of FFA and LPS, both with pro-inflammatory action on the vascular wall, increasing the atherogenic risk.

187. 608. **VASCULAR LIPOLYSIS: CHYLOMICRON IMPACT ON AORTIC LIPOPROTEIN LIPASE ACTIVITY IN AN INSULIN RESISTANCE MODEL**

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Introduction: The chylomicron (CM) and its remnants (CMr) are increased in insulin resistance (IR) contributing to the atherogenic risk. Lipoprotein lipase (LPL), a key enzyme in triglycerides (TG) hydrolysis, presents a paradoxical and tissue-dependent behavior in IR. The activity of LPL in aortic tissue and its modulation by fatty acids (FA) from CM have not been explored and could contribute to explain the vascular lipolysis degree and the generation of atherogenic CMr.

Objectives: Measure the activity of LPL in aortic tissue and the effect of isolated CM from rats with diet-induced of IR. Evaluate the association between the FA presents in the CM and the enzymatic activity. Methods: male Wistar rats were fed with standard diet (C,n=6) or high-fat high-sucrose diet (HFHS,n=6), for 14 weeks. Thoracic aorta was isolated and LPL activity was measured. In serum, lipid profile was determined, CM were isolated by ultracentrifugation and their FA profile was assessed. In an isolated organ bath, aortic rings from Sprague-Dawley rats were incubated in presence of CM at 37°C-120 minutes. The activity of LPL in both cases was measured by radiometric assays. Results: CM from HFHS group were enriched in TG, myristic, palmitic and oleic acid (p<0.02) and presented less content of EPA, DHA, linoleic and alpha-linolenic acids (p<0.05). LPL activity in HFHS was increased in aortic tissue (p=0.04), and higher enzyme activity was also observed after ex-vivo incubation with CM from HFHS (p<0.01). Post-incubation LPL activity was directly associated with TG(r=0.729,p=0.02),myristic (r=0.921,p<0.01),palmitic (r=0.667,p=0.05)and oleic acid(r=0.967,p<0.01),and inversely with alpha linolenic(r=-0,750,p=0,02),EPA(r=-0.833,p<0.01) and DHA(r=-0.795,p<0.01),from isolated CM. Conclusions: HFHS diet modifies the FA composition of CM which differentially modulates the LPL activity. This favors the release of FA with harmful potential and the generation of remnant lipoproteins, with greater atherogenic potential.

188. 631. **LIPOPROTEIN(A) CONTRIBUTION TO TOTAL CIRCULATING APOB IN SEVERE HYPERCHOLESTEROLEMIC PATIENTS**

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Lp(a) is one of the atherogenic lipoproteins that contributes with its cholesterol (C) to the measured and/or calculated value of LDL-C, but so far there is no reliable commercial assay to determine Lp(a)-C neither conversion factors to estimate Lp(a)-C from particle number. Given that Lp(a) also contains one apoB molecule, is proposed to calculate the contribution of Lp(a) to total circulating apoB particles, which is more relevant when LDL-C is increased. Aim: to assess the contribution of Lp(a) to total apoB in severe hypercholesterolemic individuals. Methods: this descriptive study includes 151 adult patients of both sexes with LDL-C>190 mg/dL. A lipid panel, apoB and Lp(a) particle number (Lp(a)-P) were measured. apoB-P was calculated converting the mass to molar concentration using apoB100 molecular weight (512 Kg/mol) and the percentage (%) of Lp(a)-P relative to apoB-P. Statistical tests applied: Spearman, ANOVA (Tuckey, Bonferroni). Results: Median (range): Lp(a) 43 (1.3-618) nmol/L, % Lp(a) contribution to total apoB 1,6 (0.07-38.1) increasing as Lp(a) increases: r=0.85 p<0.001, adjusted by triglyceride concentrations. Subdividing Lp(a)-P into deciles it was observed that from 113 nmol/L -corresponding to the P80th lower value- the contribution of Lp(a)-P in total apoB become significant p<0.001. Conclusion: in hypercholesterolemic subjects it would be advisable to estimate the Lp(a)-apoB contribution when Lp(a) is above 113 nmol/L to handle more real values of apoB until having wide possibilities of measuring Lp(a)-C.

189. 646. **COENZYME Q10 (COQ10) SUPPLEMENTATION ON HIGH-FAT FED RATS**

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[#] Equally contribution

This work was focused on the effects of CoQ10 administration to ameliorate the negative effects of a high-fat on rats. Male wistar rats *were divided into 4* groups: control diet (10% calories from fat) (C), control diet+50 mg CoQ10/kg body weight (BW)/day (CoQ), HF diet (60% of calories from fat) (HF) and HF diet+50 mg CoQ10/kg BW/day (HFCoQ) for 12 w. Results: Food and caloric intake, and BW were evaluated throughout the treatment. CoQ10 supplementation did not affect neither the consumption of food nor that of energy, but final BW was higher in HF respect to C and CoQ (15%, p<0.05). In HFCoQ, BW was 5% lower respect to HF (p= 0.055, marginally significant). The epididymal adipose tissue (AT) showed an expansion of 52% (p<0.05) in HF respect to C. Plasma glucose, triglycerides and cholesterol were measured to correlate with CoQ9 and CoQ10 levels, that were detected by HPLC. Supplementation with CoQ10 increased the plasma values of CoQ9 and CoQ10. Plasma CoQ9 values were higher in CoQ respect to C (0.40±0.06 and 0.17±0.02 μM, respectively, p<0.05), and in HFCoQ respect to HF (0.180±0.010 μM and 0.088±0.007 μM, respectively, p<0.05). Plasma CoQ10 levels were non-detectable in C and HF, and 0.9±0.1 and 0.64±0.05 μM in CoQ and HFCoQ groups, respectively. In animals subjected to high-fat diet the detected levels of CoQ9 were lower than the found in animals subjected to control diet (48% and 55%, for non- CoQ10 supplemented and CoQ10-supplemented respectively, p<0.05). For plasma CoQ10 the decrease was 29%. Plasma levels of CoQ9 inversely correlated with the final BW of all the animals, and the expansion of epididymal AT and the plasma glucose and triglycerides of the high-fat fed groups. Taken as a whole, these results indicate that: i) CoQ10 supplementation was effective increasing CoQ9 and CoQ10 plasma levels, but with differences depending on the diet suggesting the establishment of conditions that disturb CoQ9 and CoQ10 distribution and/or metabolism in the high-fat fed animals, and ii) CoQ10 attenuated key aspects of the negative effects of the high-fat diet, probably associated with the AT dysfunction. Additional experiments are necessary to understand the mechanisms operative under our experimental conditions. PICT-2021-CAT-I-00082 and PICT-2021-CAT-II-00024. UBACYT 20020190100157BA and 20020170100586BA.

P2-METABOLISM & NUTRITION

WEDNESDAY 15TH NOVEMBER 14:00-15:30

CHAIRS: MAGALÍ BARCHUK
GUSTAVO HEIN
WOLFSON M

190. 159. **OMEGA-3 FORTIFIED YOGURT AND ITS INFLUENCE ON PLASMA LIPID PROFILE IN AN ANIMAL MODEL**

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Background and objectives: Regular intake of omega-3 fatty acids (O–3FAs) improves human health helping to reduce chronic non-communicable diseases among others. Enriched O–3FAs yogurts are a value-added strategy to ingest them. The objective was to analyze the effect of O–3FAs yogurt intake on plasma fatty acids profile of mice. Methods: Four-week-old mice were divided into 3 groups (6 for each group). The control group (I) were fed the basal diet only throughout the entire feeding period. The second group (II) were fed the basal diet added with plain yogurt, meanwhile the (III) were feed the basal diet added with fortified ω-3 PUFA yogurts (250 mg/200 g yogurt). Experimental feeding was continued for 4 weeks and provided with permitted ad libitum access to the diet and water. All the experiments were carried out as approved by Ethical Committee. After 28 days, blood was collected; plasma was obtained and stored at-80 °C. The plasma fatty acid profile was determined by gas chromatography (GC) after extraction of the lipids to obtain the fatty acid-derived methyl esters. Statistical analysis used ANOVA. Result (% Area) were expressed as Mean ± SE. Results: When analyzing the values of the plasma fatty acid profile, it was seen that Oleic acid (18:1): 17.94±0.64b,16.60±0.46b, 14.69±0.97a; Eicosapentaenoic acid (EPA, 20:5 n-3): 0.46±0.06a, 0.62±0.06ab, 0.74±0.02b were obtained for (I), (II) and (III) respectively. Means with no letters (a,b) in common for each fatty acid, are significantly different (p< 0,05). There were not statistically significant differences for the rest of the fatty acids evaluated. Conclusions: In the context of a balanced diet, the fortified yogurt is a good vehicle to improve plasma lipids profile by increasing EPA -which has beneficial effects such as anti-atherosclerotic and anti-inflammatory properties.

191. 231. **REDUCTIVE ENVIRONMENTS POTENTIATE IGG AGGREGATION INDUCED BY COPPER(II): THERAPEUTIC IMPLICATIONS**

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Free copper ions present in plasma react with H_2O_2 promoting oxidative damage. However, this metal also interacts with proteins causing misfolding and aggregation. Previously, we demonstrated that Cu(II) induces selective aggregation of IgG in human sera and pro-oxidant environments render these aggregates irreversible. Thus, it is possible that reducing environments decrease the pro-aggregating capacity of the metal by reducing it to Cu(I). The objectives of this research is to determine the effect of the reducing environment on Cu(II)-induced IgG aggregation. Human plasma (HP), purified protein (PP-IgG) and ascorbic acid (AA) solutions were employed, and optical density (OD), determination of proteins by Lowry, SDS-PAGE and silver staining were used. Cu(II) induces aggregation of PP-IgG (20 μM) determined by OD and increases the mass of aggregated proteins (p<0.01). However, contrary to expectations, the addition of 1 mM AA as a reducing agent significantly increased the mass of protein added by Cu(II) (p<0.01). EDTA addition leads to complete re-dissolution of the Cu(II)-induced aggregate (p<0.01) but remarkably, the aggregate obtained in the presence of AA is not reversible (p<0.01). The SDS-PAGE confirmed the presence of soluble high molecular weight aggregates after the incubation of the Cu(II)-induced IgG in the presence of AA with EDTA. The addition of AA to HP without the addition of Cu(II) also leads to the formation of an aggregate detectable by OD. However, contrary to what was observed with the addition of Cu(II), it is re-solubilized by EDTA (p<0.01). Therefore, AA potentiates Cu(II)-induced IgG aggregation. Considering that Cu(II) and AA with isolated IgG yield an irreversible aggregate whereas AA addition to HP yields a reversible aggregate, additional factors should modulate this process in complex samples. Due to the similarities of IgG aggregates and immune complexes, both may activate inflammatory pathways linked to AA supplementation.

192. 236. **NUTRITIONAL STATUS OF VITAMIN D STATUS OF PREGNANT WOMEN OF THE CITY OF BUENOS AIRES: IMPACT ON THE PREVALENCE OF CESAREAN SECTIONS AND IN THE LEVELS OF VITAMIN D IN THEIR NEONATES' CORD BLOOD**

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VD insufficiency in pregnancy is related to a higher incidence of cesarean section, preeclampsia and preterm delivery. Objective: To evaluate 1) the degree of VD inadequacy according to 25-hydroxyvitamin D (25OHD) levels 2) The correlation between 25OHD in the mother with those levels of their newborn cord blood. Women (n=128) were divided according to their 25OHD levels (ng/mL): G1:<20 (deficiency), G2:20-30 (insufficiency), G3:>30 (sufficiency). Age (years); gestational age (GA) (weeks); body mass index (BMI) (kg/m^2); systolic (SBT) and diastolic (DBT) blood pressure (mmHg); type of delivery and season of year were recorded. Calcemia (ng/ml); 25OHD; intact parathormone (pg/ml); bone alkaline phosphatase (IU/L); Crosslaps (βCTX) (pg/ml) were determined. ANOVA and Chi2 were used to determine differences between groups using SPSS 19.0, p< 0.05 was considering significant**.** Results: Average age was 26±6 and GA 35.8±2.7 without differences between groups. Cesarean sections % was higher in G1 vs. G2 and G3 (31.3%, 21.4% and 25%; p<0.05). Overweight % were: G1:18.3%, G2:25.0% and G3:37.5% moreover in G1 9.8% were obese. Percentage of women were G1 65; G2 22; G3 13; according to the year season these percentages were: summer G1 19. G2 10.7; G3 37.5; autumn: G1 12,0 G2 14.3 and G3 18.8; winter: G1 19.3, G2 25.0; G3 25; spring: G1 49.4; G2 50.0; G318.8. Spring showed differences. No differences in the other studied maternal variables were observed. Cord blood samples were obtained from 41 women. Mother 25HOD positively correlated with those of their new born cord blood (r=0.67; p<0.0001). Conclusion: Beyond the season of the year and BMI, a high percentage of the studied pregnant women showed low 25OHD levels. As reported in literature, we evidenced that low VD nutritional status in the mother had associated with cesarean sections number. But more important was that 25HOD levels in the mother highly correlated with those of its newborn.

193. 388. **REDUCED PDX1 LEVELS IN THE FETAL PANCREAS AND REDUCED PROLACTIN RECEPTOR LEVELS IN THE MATERNAL DECIDUA OF DIABETIC RATS ARE PREVENTED BY MATERNAL DIETS ENRICHED IN OLIVE OIL**

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Introduction: Maternal diabetes programs type 2 diabetes in the offspring. We previously found that an olive oil-enriched diet during pregnancy protects beta cell development but only in male offspring. We hypothesized that a preconceptional olive oil-enriched diet can improve pancreas development in female fetuses of diabetic rats. Pancreatic duodenal homeobox 2 (Pdx1) plays a central role in pancreatic beta cell differentiation. Prolactin receptor is relevant for decidual function, needed to support pancreatic development. Aim: To evaluate whether a preconceptional treatment with extra virgin olive oil can modulate Pdx1 levels in the pancreas of female fetuses and

prolactin receptor levels in the decidua of diabetic rats. Methods: A mild pregestational diabetic rat model was induced in F0 females by neonatal administration of streptozotocin (90 mg/kg sc). Control and diabetic females received a 6% olive oil-enriched diet or a standard diet from the preconceptional stage (one week before mating) and until the day of euthanasia. Control and diabetic females were mated with control males and evaluated on day 13.5 of pregnancy (decidua studies) and on day 20.5 of pregnancy (fetal pancreas study). Pdx-1 was evaluated by immunohistochemistry and prolactin receptor by Western Blot. Results: Pdx1 levels were reduced in 20.5-day-female fetuses of diabetic rats (34%, p<0.05 vs controls), an alteration not observed when the mothers received the preconceptional olive oil-enriched diet. Prolactin receptor levels were reduced in the decidua of 13.5-day-pregnant diabetic rats (28%, p<0.05 vs controls), an alteration not observed when the mothers received the preconceptional olive oil-enriched diet. Conclusions: The reduction of a marker of beta cell differentiation in pancreas of female fetuses of diabetic rats and of a marker of proper decidual function needed for proper pancreas development are likely related with fetal programming of metabolic diseases and are ameliorated by an olive oil-enriched preconceptional diet.

194. 410. **MODIFICATIONS IN TRADITIONAL AND NOVEL RISK FACTORS AND BIOMARKERS OF CARDIOVASCULAR DISEASE INDUCED BY BARIATRIC SURGERY**

Ezequiel Lozano Chiappe[1], Leonardo Gómez Rosso[1], Maximiliano Martín[1], Belén Davico[1], Graciela Jiménez[2], Gustavo Frechtel[3], María Soledad Sáez[2], Axel Beskow[2], Laura Boero[1], Susana Gutt[2], Dong Woo[2], Fernando Brites[1].

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Introduction: Morbid obesity is associated with different comorbidities, including dyslipidemia and cardiovascular disease (CVD), being bariatric surgery (BS) the most successful therapeutic option. The objective of this study was to evaluate the effect of BS on traditional and novel risk factors and biomarkers of CVD. Materials and methods: Seventeen patients with morbid obesity were recruited from the Italian Hospital of Buenos Aires and were evaluated before, 6 and 12 months after BS. Weight and height were recorded, and body mass index (BMI) calculated. Hemogram, lipid profile and glucose levels were determined. Total cholesterol (TC)/HDL-C, triglycerides (TG)/HDL-C and neutrophils/lymphocytes (N/L) ratios were estimated, as well as the TG glucose (TyG) index [Ln (TG x G/2)]. Paraoxonase (PON) and arylesterase (ARE) antioxidant activities of PON1 enzyme were evaluated by developed methods. Data were analyzed employing parametric and non-parametric tests for paired samples. Results: Six months after BS, weight and BMI significantly reduced. Moreover, decreases were detected in glucose and TG levels, as well as in TG/HDL-C ratio and TyG index (p<0.05). The N/L index decreased 12 months after BS (p<0.05) and TC/HDL-C ratio decreased both 6 and 12 months after the intervention (p<0.05). HDL-C levels significantly increased 6 and 12 months after BS (p<0.05). Regarding PON activity, an initial reduction was detected after 6 months (120±55 *vs.* 93±58 nmol/mL.min, p<0.05) and an increase was observed 6 months after BS, returning to values similar to basal ones (93±58 *vs.* 130±52 nmol/mL.min, p<0.05). Conclusions: BS produced improvements in anthropometric parameters, atherogenic potential of lipoprotein profile, inflammatory markers (N/L ratio) and in the insulin resistant status (TG/HDL-C ratio and TyG index). Although PON activity decreased 6 months after BS, it recovered after 12 months. These changes evidence different CV benefits of BS in morbidly obese individuals.

195. 435. **PROTECTIVE EFFECT OF COMPOUND A (CPDA) ON β-CELLS IN A GLUCO/LIPOTOXIC MICROENVIRONMENT: INITIAL EVIDENCE**

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Type 2 diabetes mellitus (T2DM) is a prevalent endocrine-metabolic disease characterized by hyperglycemia, peripheral insulin resistance and frequently linked to hyperlipidemia. Oxidative stress, coupled with the high demand for insulin leading to endoplasmic reticulum (ER) stress, contributes to dysfunction and death of pancreatic β-cells. We showed that Compound A (CpdA), a dissociative GR-ligand, mitigates oxidative and ER stress in β-cells under the inflammatory microenvironment of type 1 diabetes, improving their survival and function. We aimed to ascertain whether CpdA can also protect β-cells from the gluco/lipotoxic environment of T2DM. We used the rat β-cell line NS-1E. Gluco/lipotoxicity was induced with 30mM glucose and 100-500μM palmitate, and the effect of 10μM CpdA was evaluated. We analyzed nitric oxide (NO) by Griess, viability by MTT, mRNA by RT-qPCR and insulin by ELISA. GSIS (glucose-stimulated insulin secretion) index was calculated as the ratio of insulin released during 1h under stimuli of 20mM/2mM glucose. Results showed that exposure to 30mM glucose and 400μM palmitate (GLT) for 24h reduced viability by 50% (p<0.05 vs control) and impaired the ability of INS-1E cells to secrete insulin in response to increased glucose in the medium (GSIS). Unlike the response to cytokine injury, GLT did not induce NO production in INS-1E. CpdA treatment mitigated GLT's impact on β-cell viability and function. Under GLT exposure, CpdA stimulated the expression of genes involved in antioxidant response (e.g., HMOX-1, SOD-2, p<0.05 vs GLT) and favored the expression of key genes responsible for β-cell identity (e.g., PDX-1, p<0.05 vs GLT) in INS-1E. We present initial evidence regarding the potential protective effect of CpdA against oxidative stress induced by gluco/lipotoxicity, enhancing beta cell functionality. Ongoing *in vitro* and *in vivo* experiments will lead to a deeper understanding of the mechanisms underlying the beneficial effects of CpdA in β-cells.

196. 440. **MARKERS OF LIPOPROTEIN QUALITY AND FUNCTION IN CHILDHOOD OBESITY: INFLUENCE OF INSULIN RESISTANCE**

Belén Davico[1], Laura Gaete[3], Ezequiel Lozano Chiappe[1], Leonardo Gómez Rosso[1], María Soledad Sáez[2], Graciela Jimenez[2], Liliana Trifone[3], Walter Tetzlaff[1], María Gabriela Ballerini[1], Laura Boero[1], Miriam Tonietti[3], Fernando Brites[1], Maximiliano Martin[1].

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Introduction: Childhood obesity is associated with comorbidities such as insulin resistance (IR) and dyslipidemia characterized by: a) abnormal lipoprotein levels and composition, b) altered activities of associated enzymes and lipid transfer proteins, as lipoprotein associated phospholipase A_2 (Lp-PLA_2) and paraoxonase 1 (PON1), and c) impaired reverse cholesterol transport (RCT), constituted by cellular cholesterol efflux (CCE), and the activities of lecithin:cholesterol acyltransferase (LCAT) and cholesteryl ester transfer protein (CETP). IR may deepen these proatherogenic disturbances. We aim to characterize the effect of IR on lipoprotein quality and functionality in obese children and adolescents. Methods: The study included 25 obese children and adolescents with and 25 without IR. Anthropometric parameters and Tanner stage were registered. Glucose, insulin, lipid levels and high sensitivity C reactive protein (hsCRP) were measured by standardized methods. Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) was calculated and a cutoff point of 3.16 defined the presence of IR. Lp-PLA_2, LCAT and CETP activities were quantified by radiometric assays, PON1 activity and non-esterified fatty acid (NEFA) levels by colorimetric assays and CCE employing THP-1 cell line. Results: There were no differences in age, sex and Tanner stage. As expected, the obese group with IR showed higher levels of insulin and NEFA (p<0.05). They also showed higher Lp-PLA_2 and lower PON1 activities (p<0.05). No differences were observed in lipid profile, hsCRP or RCT. Lp-PLA_2

was positively associated with HOMA-IR, while PON1 negatively with HOMA-IR ($p<0.05$). Conclusion: The presence of IR in obese children and adolescents was associated not only with alterations in glucose metabolism, but also with vascular specific inflammation and reduction of PON1 activity, main responsible for HDL antioxidant capacity, thus increasing cardiovascular risk in adulthood.

197. 487. **NON-ALCOHOLIC STEATOHEPATITIS: REGULATION OF THE INTRACELLULAR CHOLESTEROL METABOLISM PATHWAY IN RABBITS FED WITH A FAT DIET. USE OF EXTRA VIRGIN OLIVE OIL TO CUSHION LIVER DAMAGE**

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Non-alcoholic steatohepatitis due to lipid overload is a global health concern. Lipotoxicity mediated by hepatic free (F) cholesterol (C) overload is a mechanistic driver for inflammation and fibrosis in many animal models. Diet, lifestyle, obesity, key genetic polymorphisms, and hyperinsulinemia secondary to insulin resistance are drivers leading to aberrant cholesterol signaling, which leads to the accumulation of FC (hepatic cholesterol, HC). High-fat diets (HFD), with over 30% of calories from fats, affect liver function, while extra virgin olive oil (EVOO), has known benefits, but its mechanism is unclear. Sterol regulatory element-binding protein 2 (SREBP2) influences intracellular C-metabolism and is diet-sensitive. Our study explored EVOO's impact on hepatic C-pathways using rabbits as hypercholesterolemic models. They received: control diet, an HFD (14% beef fat) for up to 6 (HFD≤6M) and more than 12 months (HFD≥12M), or HFD with EVOO (HFD 7% + EVOO 7%) for up to 6 months. Blood sampled, serum lipids, liver enzymes, and liver histological sections were analyzed. HC deposit was determined: using filipin III stain, and chromatography. SREBP2, HMGCR (3-hydroxy-3-methyl-glutaryl-coenzyme A reductase), and LDLR (low density lipoprotein receptor) expression were also studied by western blot and PCR. HC increased in HFD-fed rabbits but decreased with EVOO addition. No hepatic steatosis was observed, and liver enzyme values showed no significant differences. SREBP2 mRNA remained unchanged with HFD, but protein expression decreased (HFD≤6M) and increased (HFD≥12M). EVOO significantly raised the SREBP2 expression. HMGCR mRNA expression decreased significantly with HFD≤6M but increased with EVOO. Levels of HMGCR protein increased to HFD≥12M and decreased with EVOO. Both mRNA and LDLR protein increased with EVOO. Fat intake disrupted SREBP2 pathway, causing rabbit hepatocyte lipid build-up. Specific studies are needed for the protective EVOO mechanism's.

198. 547. **ZINC SUPPLEMENTATION DURING POSTNATAL GROWTH: IS IT BENEFICIAL FOR REDUCING CARDIOVASCULAR AND METABOLIC EFFECTS ASSOCIATED WITH METABOLIC SYNDROME?**

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Introduction: The metabolic syndrome represents the association of various risk factors such as obesity, dyslipidemia, hyperglycemia and hypertension. Zinc deficiency is a common phenomenon in diabetic and obese patients.

Objective: To evaluate the effects of zinc supplementation on retroperitoneal adipose tissue (RPAT), systolic blood pressure (SBP), and intermediate metabolism in male Wistar rats fed a high-fat and fructose diet during post-weaning growth.

Methods: Male Wistar rats received from weaning (21 days) to 81 days of life: C: control diet (Zn^{2+}: 30 ppm); CHF: high-fat diet (fat 60% kcal, Zn^{2+}: 30 ppm) and fructose 10% in drinking water; ZHF: high-fat diet supplemented with zinc (fat 60% kcal, Zn^{2+}: 190 ppm) and fructose 10% in drinking water. At day 81 SBP, plasma lipid profile, oral glucose tolerance test (OGTT) and RPAT morphology were evaluated. Values are mean±SEM. One-way ANOVA Bonferroni post-test: **p<0.01 and *p<0.05 vs C; $$p<0.01 and $p<0.05 Vs CHF. N=6 per group.

Results: CHF presented an increase in body weight (C: 460 ± 11; CHF: 505 ± 7**; ZHF: 472 ± 8$ g), SBP (C:133 ± 2; CHF:159 ± 2*; ZHF:144 ± 1$), area under OGTT curve (C: 25867± 824; CHF: 30012 ± 1643*; ZHF: 25650 ± 636$$), triglyceridemia (C: 115 ± 8; CHF: 156 ± 6**, ZHF: 154 ± 10), adipocytes area (C:4705 ± 193; CHF:7450 ± 838**; ZHF:7435 ± 484 μm^2), media layer area/light area (C:0.91 ± 0.03; CHF: 6.2 ± 1.3*; ZHF: 2.0 ± 0.5$) and the perivascular collagen area/light area (C: 1.7 ± 0.3 ; CHF:6.5 ± 0.4*; ZHF: 2.5 ± 0.4$) of RPAT vessels and in RPAT thiobarbituric acid reactive species concentration (C:0.25 ± 0.03; CHF: 0.38 ± 0.04*; ZHF: 0.23 ± 0.04$ nmol/mg protein) compared to C. ZHF group presented lower body weight, SBP, area under OGTT curve, lipid peroxidation and vascular morphological changes of RPAT compared to CHF .

Conclusion: Zinc supplementation during growth could reduce cardiovascular and metabolic damage associated with metabolic syndrome.

199. 556. **LUNG INFLAMMATION IN AN EXPERIMENTAL MODEL OF DIET-INDUCED OBESITY**

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Chronic dietary consumption of hypercaloric foods is linked to inflammation in several tissues. Excess energy as free fatty acids is known to activate TLR2 and cause gut dysbiosis. These events can lead to NF-kB activation, and therefore increased expression of inflammatory biomarkers, such as inducible nitric oxide iNOS expression (iNOS) and neutrophil retention in tissues. Neutrophils contain myeloperoxidase (MPO) which is a hemeprotein that uses H_2O_2 to oxidize chloride ions to HOCl which causes chlorinating stress. However, the impact of a chronic positive energy balance on lung inflammation profile is rarely reported. Herein we aimed at testing whether chronic consumption of a hypocaloric diet causes lung inflammation. To accomplish our aim, we fed C57 male mice with a control (COD, rodent chow, and tap water) or hypercaloric diet (HCD, 22% bovine fat + 10% fructose in the drinking water) for 26 weeks. Energetic value of different diets were measured. Food, water, body weight, caloric consumption, and insulin resistance were weekly measured. With respect to a COD, feeding a HCD caused increased weight gain, IL-6 (a marker of systemic inflammation), and insulin resistance. At the end of the dietary regime iNOS, nitrotyrosine, MPO, and chlorotyrosine content were measured by ELISA in homogenates of the lung parenchyma. Mice fed an HCD for 24 weeks showed a higher expression of iNOS, MPO, and markers de nitrosative and chlorinating stress. These data suggest that chron-

ic-low grade inflammation caused by an energy excess causes pulmonary neutrophilic inflammation. Data gathered from this study will provide mechanistic information for avoiding the impact of obesity and overweight on lung physiology. Supported by PICT2018-03435, PICT2021-0147 and PUE013

200. 557. **LIVER ADAPTIVE INFLAMMATORY RESPONSE TO DIETARY INTERVENTION WITH VIRGIN OLIVE OIL IN A DIET-INDUCED OBESITY TNFR1-DEFICIENT MOUSE MODEL**

Guzmán CF[1], Chacón, I del V[1], Barrera FS[2], López CM[2], Penna FO[3], Fornes MW[4], Ramirez DC[1] & Gomez Mejiba SE[2]
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The liver is a target of excessive dietary energetic balance-induced inflammation. Inducible nitric oxide synthase (iNOS) and myeloperoxidase (MPO) in the liver are known to cause nitrosative and chlorinating stress, respectively. Moreover, upon binding to its ubiquitous receptors (TNFR), TNFα can interfere with normal liver physiology. Virgin olive oil (VOO) is a complex mixture of nutrients, and an important component of the Mediterranean diet, which health benefits are well known, but the effect on the inflammatory profile of the liver is rarely reported. Herein we tested whether a short- or long-period of intervention with a VOO-supplemented diet (VOOSD, 11% bovine fat plus 11% VOO) can affect critical inflammatory parameters in the liver of diet-induced obese TNFR1-deficient mice. To accomplish our aim, we fed TNFR1$^{-/-}$ mice with a control diet (CD, rodent chow) or obesogenic diet (OD, 22% bovine fat plus 10% fructose in tap water) for 24 weeks and intervened with VOOSD every 6 weeks. A VOOSD reduced the impact of an OD on insulin resistance and dyslipidemia caused by an OB, but enhanced weight gain. Liver iNOS, TNFα, and MPO, three well-known inflammation markers were measured by using ELISA. In the liver of TNFR1-deficient mice fed a CD, iNOS increased in the groups intervened with VOOSD for 6 and 12 weeks, however, these changes were abolished when mice were intervened with VOOSD for 18 weeks. Compared to CD, MPO content in the liver was higher in the groups fed for 6, 12, and 18 weeks. Whereas no significant changes in hepatic TNFα content were found. Interestingly, in the liver of TNFR1-deficient mice fed an OD, iNOS increased in the group fed a VOOSD for 6 weeks, meanwhile MPO increased in the groups fed a VOOSD for 6 and 12 weeks, whereas TNFα was higher in 6 than in 18 weeks. Taken together these data suggest an adaptive liver response to inflammatory stress caused by intervention with a VOOSD a diet induced TNFR1-deficient obesity mouse model.

201. 572. **EFFECT OF HYPERCALORIC DIET AND HYPOANDROGENISM IN RAT LUNG**

Biaggio Veronica[1, 2,3], Aballay Federico[1], Salinas Marysol[1], Zelarayan Sarmiento Daniela[1], Piguillem Silvana[1], Ciminari Eugenia[1], Razzeto Gabriela[1], Salinas Eloy[1], Álvarez Silvina [1, 2,3], Pérez Chaca Veronica[1, 2], Gomez Nidia N. [1, 2 ,3]
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Obesity is a condition of oxidative stress and systemic inflammation that impairs respiratory function. In addition, the presence of androgen receptors in lung indicates that testosterone play a key role in lung physiology. Obesity-induced oxidative stress in adipose tissue is one of the main factors considered as a source of oxidants and inflammation mediator. The aim of this study was to analyse the effects of androgen deficiency and obesity on lung morphophysiology. Male Wistar rats (200 ± 20 g) were divided in four groups: Control on normal diet (CoN), castrated on normal diet (KN), Control with hypercaloric diet (CoOB), and castrated with hypercaloric diet (KOB) and they were sacrificed 30 days after castration. Biochemical parameters were analysed and the expression of antioxidant enzymes, NOX -2, FOXO, HO -1, and RA in lung. Histological sections were obtained for morphometric analysis and Mason trichrome staining. ANOVA and Tukey test were used for statistical analysis. Results demonstrated that TBARS levels were increased in KOB ($p< 0.001$) and KN ($p<0.01$) groups. CAT activity was increased in the KN group ($p<0.05$). Expression of CAT decreased in the groups CoOB and KN, and RA expression increased in KN group. Antioxidant enzymes NOX-2, SOD-2 ($p<0.01$) and GPx-1 ($p<0.05$) were increased in the KOB group. Morphometric analysis revealed large alveolar spaces, which may not have been functional, in the CoOB and KOB groups ($p<0.01$). Manson trichrome staining showed an increase in connective tissue especially in KOB group. Morphometric analysis revealed large alveolar spaces, possibly non-functional, in CoOB and KOB groups ($p<0.01$). We have previously demonstrated a condition of significant oxidative stress in castrated animal model. Obesity also produces a basic inflammatory situation. In our experimental model the lung present non-functional alveolar spaces and increase in some regions connective tissue. Added to this, obesity would contribute to a pro-inflammatory state that would aggravate the damage caused by androgen deficiency.

O1-METABOLISM & NUTRITION

THURSDAY 16TH NOVEMBER 9:00 - 10:30
CHAIRS: SILVINA VIDUEIROS
SILVINA ÁLVAREZ

202. 150. **CERAMIDES FROM CIRCULATING TRIGLYCERIDE-RICH LIPOPROTEINS IN SYSTEMIC AND EPICARDIAL ADIPOSE TISSUE METABOLIC PROFILE**

Magalí Barchuk[1,2], Jeanniel Campos[1], Patricia Ancel[3], Ljubica Svilar[3], Juan Patricio Nogueira[4], Anne Dutour[3], Jean Charles Martin[3], Bénédicte Gaborit[3], Marcelo Damonte[5], Gabriela Berg[1,2].
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Cardiovascular diseases (CVD) are still the leading cause of death worldwide. Identification of patients with increased CVD risk is mostly based on lipid profile measures, which sometimes fail to recognize a proportion of high-risk patients. Recently, new markers have been proposed to assist in CVD risk prediction, and ceramides (Cer) are promising candidates. Some indexes, including Cer18:1/24:1, are reported to be altered in plasma before the onset of coronary artery disease (CAD). Epicardial adipose tissue (EAT) is a visceral adipose tissue, surrounding myocardium and coronary arteries, which volume is considered a risk factor for CVD. Circulating lipoproteins could be a source of fatty acids to this tissue, thereby determining its expansion and composition. We have reported an increase in atherogenic Cer indexes in EAT from CAD patients, and in this opportunity we aim to evaluate Cer18:1/24:1 in isolated VLDL, in relation with EAT composition and patients metabolic profile. Methods: we studied patients undergoing coronary by-pass graft (CAD, n=18) and patients without CAD (noCAD, n=14). In serum, metabolic profile was assessed, and VLDL were isolated by ultracentrifugation. EAT and VLDL lipidomes were evaluated by

UHPLC-MS. Results: CAD patients presented a more deleterious metabolic profile, with increased insulin-resistance (IR) markers. Total Cer and Cer18:1/24:1 were higher in EAT from CAD (p<0.001). Although VLDL total Cer and Cer18:1/24:1 contents were not different between CAD and noCAD, they were directly associated with TG/HDL-C index (r=0.7,p<0.001 and r=0.5,p=0.01 respectively), No HDL-C (r=0.6,p=0.002 and r=0.5,p=0.01 respectively), triglyceride-rich lipoproteins (TRL) (r=0.7,p<0.001 and r=0.6,p=0.003 respectively) and glycaemia (r=0.6,p=0.002 and r=0.4,p=0.04 respectively). Conclusion: atherogenic Cer from EAT circulating TRL would determine a local and systemic deleterious metabolic profile, thereby increasing CVD risk.

203. 278. **EFFECTS OF PARENTERAL SUPPLEMENTATION OF ANTIOXIDANT MINERALS AND VITAMINS IN LIVER NF-KB ACTIVITY AND INSULIN-SIGNALING OF DAIRY CATTLE DURING THE TRANSITION PERIOD**

Emmanuel Angeli[1,2], Antonella Schneider[1], Valentina Matiller[1,2], Florencia Rey[1,2], Hugo H Ortega[1,2], Gustavo J Hein[1,3]
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The transition period (last 3 weeks of prepartum until 3 weeks postpartum) is the most critical stage in the lactation of dairy cows, characterized by a proinflammatory state, oxidative stress and insulin resistance. The aim of this study was to evaluate the effects of parenteral vitamin and mineral supplementation on the hepatic insulin signaling pathway, a relevant proinflammatory factor in the liver, and systemic antioxidant enzymes in cows during the transition period. The supplemented group (SG; n = 11) received injections of 5 ml of the vitamin supplement ADAPTADOR® Vit and 5 ml of the mineral supplement ADAPTADOR® Min (Biogenesis Bagó, Bs. As.; vitamin A palmitate 3.5% and vitamin E acetate 5%, copper edetate 1%, zinc edetate 4%, manganese edetate 1% and sodium selenite 0.5%) on -60, -30 and 7 days relative to calving. The control group (CG; n = 11) received two injections of 5 ml of 0.9 % sodium chloride solution. Whole blood with heparin was used to measure superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activities by commercial kits, following the manufacturer's instructions (RANSOD and RANSEL, Randox Laboratories Ltd., Crumlin, UK). The liver protein expression of insulin receptor (IR), insulin receptor substrate 1 (IRS-1), protein kinase B (also known as Akt), nuclear factor-κB (NF-κB), and their phosphorylated forms were analyzed by Western blot. Results showed that the GSH-Px activity was higher in the SG than in the CG ($P < 0.05$). No differences between groups were observed in SOD activity ($P > 0.05$). The activation of NF-κB was higher in SG than in CG ($P < 0.05$). No differences between groups were observed in the insulin signaling components ($P > 0.05$). These results suggest that the vitamin and mineral supplementation provided to dairy cows had beneficial effects on the antioxidant system and liver inflammatory state during the transition period.

204. 374. **EFFECT OF DIETARY SALVIA HISPANICA L. (CHIA) SEED ON GLUCOSE TOLERANCE, GLUCONEOGENIC PATHWAY AND HEPATIC INSULIN SIGNALING IN HIGH-SUCROSE DIET FED RATS**

Michelle Vega Joubert, María Eugenia Oliva, María Eugenia D'Alessandro
Laboratorio de Estudio de Enfermedades Metabólicas relacionadas con la Nutrición. Facultad de Bioquímica y Ciencias Biológicas. Universidad Nacional del Litoral.
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Liver tissue plays a crucial role in controlling glucose homeostasis and is one of the main sites of action of insulin. The aim of this study was to evaluate the effect of chia seed administration on glucose tolerance, gluconeogenic pathway, and hepatic insulin signaling in high-sucrose diet fed rats. Materials and methods: Male Wistar rats were fed a reference diet (RD) for -6 months- or a HSD (diet that mimics human Metabolic Syndrome) -3 months-. Then, the latter group was randomly divided into two subgroups. One subgroup continued receiving HSD for up to 6 months and the other was fed with HSD where whole chia seed was incorporated as a source of dietary fat for the next 3 months (HSD+CHIA). Three batches of animals groups were needed for: 1) basal samples, 2) intravenous glucose tolerance test, and 3) insulin- stimulated conditions We analyzed: a) Serum: triglycerides, cholesterol, glucose and insulin levels, b) intravenous glucose tolerance test, c) Liver: Fructose-1,6 bisphosphatase and phosphoenolpyruvate carboxykinase (PEPCK) enzyme activities and protein mass levels of: GLUT-2, AMPK and pAMPK. Also, the protein mass levels of pAKT in basal and insulin-stimulated conditions were determined. Results: Chia seed administration vs HSD: improved dyslipidemia and glucose levels without changes in insulin levels; decreased fructose-1,6 biphosphatase and PEPCK hepatic enzyme activities and improved glucose tolerance. It also increased AMPK and pAMPK protein mass levels, decreased GLUT-2 and pAKT protein mass levels. In basal state, HSD group showed higher levels of pAKT than the other groups. Under insulin stimulation: pAKT protein mass levels increased significantly in the HSD+-chia and RD groups compared to their respective values obtained under unstimulated conditions. No changes were observed in HSD group. Conclusion: Chia seed modulate different mechanisms that regulate gluconeogenesis and insulin signaling in liver and improve glucose tolerance.

205. 498. **EFFECTS OF METFORMIN ON THE RENAL DOPAMINERGIC SYSTEM IN AN EXPERIMENTAL MODEL OF METABOLIC SYNDROME**

Silvana M. Cantú[1,2], Hyun Jin Lee[1,2], Christian Höcht[3], Adriana S. Donoso[1,2], Ana María Puyó[1,2], Marcelo R. Choi[1,4]
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Chronic high fat diet (HFD) intake induces metabolic syndrome (MS), a complex entity that includes among other alterations, high blood pressure. Renal dopaminergic system regulates blood pressure through its natriuretic actions. Furthermore, metformin (M), an insulin sensitize drug, has been demonstrated to have pleiotropic effects. Then, the aim of this study is to evaluate the effects of M on the RDS in an experimental model of MS. Six weeks old male Sprague-Dawley rats were divided into four groups (n=4-6) and studied for 8 and 12 weeks. Control (C8, C12: standard diet and tap water to drink), HFD (HFD8, HFD12: C + 50% w/w fat added to C diet), Control+M (CM8, CM12: C + 500 mg/kg/day of M diluted in water), HFD+M (HFDM8, HFDM12: HFD+CM). Results: At 8 weeks of treatment, M improved fractional sodium excretion (FENa%, HFDM8: 0.343±0.094 vs HFD8: 0.148±0.020; p<0.05) and urinary sodium excretion (UNa.V, mEq/24hs, HFDM8: 0.962±0.125 vs HFD8: 0.405±0.050; p<0.05), without significant changes at 12 weeks. Systolic blood pressure (SBP) was significantly reduced from 8 weeks (mmHg, HFDM8: 128.6±1.7 vs HFD8: 139.0±2.5; p<0.01. HFDM12: 133.3±2.4 vs HFD12: 148.0±1.9; p<0.01), as well as body weight, triglyceridemia and glycemia (HFDM8 vs HFD8, p<0.05. HFDM12 vs HFD12, p<0.05). OCTN1,2,3 expression measured by Western blot at 8 weeks of treatment was significantly increased (HFDM8: 0.923±0.053 vs HFD8: 0.550±0.051, p<0.05), while L-dopa/dopamine index (index) was significantly improved at both times (HFDM8: 1.16±0.38 vs HFD8: 3.67±0.75, p<0.05. HFDM12: 2.09±0.13 vs HFD12: 4.82±0.18 p<0.05). Conclusion: In this experimental model, M improved RDS action, increasing natriuresis that lead to SBP reduction at 8 weeks of treatment. This effect persisted throughout 12 weeks of treatment, despite M effects on natriuresis.

206. 516. **AQUAGLYCEROPORINS IN RATS FED WITH HIGH FRUCTOSE: EFFECT OF (-)-EPICATECHIN ADMINISTRATION**

Ezequiel J. Hid [1,2], Fiorella Lista [3,4], Daniel Keber [3,4], Larisa Maidana [3,4], Hyung Jin Lee [5,6], Pablo Basile [1,2], Cesar G. Fraga [1,2] , Noelia Arreche [3,4], , Monica Galleano [1,2]

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Aquaglyceroporins are being studied as targets for therapeutic intervention in health conditions associated with dysfunction of adipose tissue (AT) and insulin resistance. Aquaporin (AQP) 7 and AQP9 are involved in glycerol transport and may play a role in the homeostasis of energy metabolism, in coordination with glucose transporters (GLUT). The aim of this study was to examine modifications in the most relevant AQPs in AT and liver by (-)-epicatechin (EC) administration in fructose fed rats. Male Sprague-Dawley rats were divided into 4 groups: C: control diet and tap water; CE: EC (20 mg/kg BW/d) in the diet and tap water; F: control diet and 10% (w/v) fructose in the water, and FE: EC in the diet and fructose in the water. After 8 w, animals were euthanized and blood (plasma), epididymal AT (EAT) and liver were obtained. Results: Final body weights were not different between experimental groups. Glycemia and insulinemia were increased in F vs all groups ($p<0.05$), and subsequently the HOMA-IR followed the same behavior (40%). Plasma triglycerides (TG) were increased by 74 and 66 % in F and FEC vs C and CEC ($p<0.05$), respectively. EAT showed a 4.2-increase in TG content in F respect to C ($p<0.05$) that was attenuated by EC. Levels of AQPs and GLUTs were determined by western blot in EAT and liver. AQP7 expression was found higher (32%, $p<0.05$) in F vs C and significantly lower in FEC vs F. GLUT4 levels were lower (21%, $p<0.05$) in F vs C and significantly higher in FEC vs F. Liver steatosis was determined by lipid droplet quantification in histological samples. Steatosis degree was 2-fold higher in F vs to C ($p<0.05$), and the increase was absent when EC was administrated in the diet. Expression of AQP9 was 20% higher in F vs C ($p<0.05$) and the increase was absent when EC was administrated in the diet. GLUT2 expression did not show differences among the experimental groups. EC administration to fructose fed rats was associated with: i) improvement in the HOMA-IR, and ii) the restoration of normal levels of EAT AQP7, liver AQP9, and EAT GLUT4, with no changes in liver GLUT2. Further experiments are necessary to dilucidated if EC effect is mediated by the insulin signaling pathway and/or alternative mechanisms. UBACYT 20020190100157BA, 20020170100087BA, 20020170100586BA. PICT-2021-CAT-I-00082 and PICT-2021-CAT-II-00024.

207. 551. **EFFECTS OF GADOLINUM CHLORIDE TREATMENT ON METABOLISM IN INSULIN RESISTANT RATS**

Morena Wiszniewski[1,2], Diego Mori[1], Cora B. Cymeryng[1,3], Esteban Martín Repetto[1,4].

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Non-Alcoholic fatty liver disease (NAFLD) is the most prevalent chronic liver disease. Although of yet unknown origin, several factors such as inflammation are postulated to contribute to its etiology. Previous results from our laboratory showed that rats fed a sucrose rich diet (SRD) for 12 weeks develop steatohepatitis and metabolic dysfunction. It has been described that inflammation perpetuates metabolic syndrome. Taking this into account, the purpose of the present study was to assess the effect of systemic macrophage inhibition in central tissues involved in energy metabolism: adipose tissue and the liver. Male Wistar rats were fed a normal diet and either tap water (control, n=12) or a SRD (n=12) for 12 weeks. A subgroup of SRD treated animals received gadolinium chloride ($GdCl_3$ 10mg/kg ip every 3 days) for the last two weeks of the dietary modification (SRD+Gd, n=8). Gd treatment had no effect on body weight, caloric consumption or triglyceridemia compared to the SRD group. However, it was associated with a reduction in glycemia and a restoration of peripheral insulin resistance (assessed by an insulin tolerance test, $p<0.01$ *vs* SRD, in both cases), with no changes in hepatic glucose production (evaluated by a pyruvate tolerance test). In the liver, $GdCl_3$ treatment was associated with less inflammatory tissue injury with no changes in steatosis or in the expression of lipogenic enzymes induced by SRD. Macrophage inhibition was associated with a restoration of insulin signaling in the epididymal adipose tissue (EAT), as assessed by the phosphorylation of AKT1 ($p<0.001$ *vs* SRD). As expected, Gd treatment attenuated tissular damage associated with inflammation. While we can't disregard possible effects of Gd treatment in other tissues, our results suggest that the restoration of peripheric insulin sensitivity could be, at least in part, due to an improvement in insulin signaling in the EAT.

P3-METABOLISM & NUTRITION

THURSDAY 15TH NOVEMBER 14:00-15:30

CHAIRS: HYUN LEE
VALERIA ZAGO

208. 72. **ANTIOXIDANT AND ANTIPROLIFERATIVE ACTIVITIES OF EXTRACTS FROM THE WASTES OF PECAN NUT WITH POTENTIAL NUTRACEUTIC APPLICATIONS**

Lucas Emanuel Ribas[1,2,3], Fátima Belén Gasser[2,3], Adriana Perello[1,2,3], Candela Simonetto[2,3], María Eugenia Baravalle[1,2,3], Graciela Hilda Savino[1], Hugo Héctor Ortega[2,3], Franco Van de Velde[3], Gustavo Juan Hein[1,2,3].

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The waste from the pecan nut industry contains high contents of phenolic compounds with proven biological activities. We aimed to evaluate the in vitro bioactivity of enriched extracts from pecan nut shells and husks on the prostate cancer cell line PC3. Extracts were obtained through an optimized aqueous extraction process and purified by using SPE C18 columns (PES: shell and PEH: husk). The content of total phenolics (TP) was determined by the Folin-Ciocalteu method (mg of gallic acid equivalent/g) and the antioxidant capacity by the DPPH method, determining the relative antioxidant activity (%AAR; IC50 Extract/IC50 Trolox). The cytotoxic activity was studied by evaluating cell viability through the MTT assay with increasing concentrations of extracts (10-300 mg/L) and Doxorubicin (DO) as the positive control, for 48 h. Lactate dehydrogenase (LDH) activity released in the damaged cells was evaluated using a commercial kit (Wiener Lab) in the supernatant of the cell culture incubated with 200 mg/L of each extract, DO, and triton as a positive control. In addition, the %AAR of extracts at 200 mg/L and their combination with DO was evaluated by flow cytometry, analyzing the production of reactive oxygen species (ROS). TP values were 80 mg/g and 3.8 mg/g, and the %AAR were 196.70 and 189.52, for the PES and PEH extracts, respectively. Both extracts demonstrated antioxidant activity ($p<0.05$) by reducing ROS levels in cells with oxidative stress

caused by DO. PES and PEH were cytotoxic for cells when the concentration was greater than 150 mg/L and 100 mg/L, respectively. Both extracts and DO significantly increased LDH activity in PC3 cells compared to the negative control, suggesting cell membrane damage as a possible mechanism of action. These results indicate a cytotoxic effect on the prostate cancer cell line, also presenting an antioxidant effect against DO, further enhancing its use as an antitumor agent and thus revaluing an agro-industrial waste.

209. 155. **NITRO OLEIC ACID DECREASES METALLOPROTEINASES ACTIVITY IN EPICARDIAL ADIPOSE TISSUE FROM CORONARY PATIENTS**

Jeanniel Campos[1], Andrés Trostchansky[2], Julio Baldi[3], Marcelo Damonte[3], Homero Rubbo[2], Magali Barchuk[1,4], Gabriela Berg[1,4].

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Over the past years, modified fatty acids have emerged as newly identified molecules that can either provide protective effects or induce potential changes on tissue metabolism. Among these, nitro-oleic acid (AONO2) has gained recognition as a compound with antioxidant and anti-inflammatory properties, along with a range of other protective functions in multiple tissues. Epicardial adipose tissue (EAT), which is a type of visceral AT surrounding the myocardium and coronary arteries, has been identified as a cardiovascular disease (CVD) risk factor when its volume is increased. Previous findings from our laboratory have revealed an increase in metalloproteinase (MMP) 2 and 9 activity in EAT from coronary patients, along with a pro-inflammatory profile. Our aim was to evaluate the modifications induced by AONO2 on MMP-2 and -9 activity in EAT and subcutaneous adipose tissue(SAT) from coronary and non-coronary patients. Materials and Methods: EAT and SAT biopsies from patients undergoing by-pass (CAD n=10) or valve replacement (NCAD n=6) surgery were incubated in DMEM or DMEM+AONO2 10 μmol/L during 3 hours. After each treatment, the samples were homogenized and MMPs activities were assessed by gelatinolytic zymography. Results: In SAT from the whole population we observed a decrease of MMP-2 activity when comparing DMEM vs DMEM+AONO2 (MMP-2: 0.14±0.05 vs 0.10±0.05 RU, p=0.05). In regard to EAT, we evidenced a reduction in MMPs activities under AONO2 treatment (DMEM vs DMEM+AONO2; MMP-2: 0.07±0.03 vs 0.06±0.03, RU p=0.06; MMP-9: 0.08±0.04 vs 0.05±0.01, RU p=0.002). This reduction remained significant for MMP-9 when considering CAD patients (DMEM=0.09±0.04 vs DMEM+AONO2=0.04±0.01, RU p=0.02). Conclusion: AONO2 treatment reduces MMPs activity in AT, with a more evident response of MMP-9 in EAT, potentially limiting the further progression of inflammatory processes.

210. 199. **SPEXIN IMPROVES METABOLIC ADIPOSE TISSUE PROFILE IN OBESE FEMALE MICE**

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Adipose Tissue (AT) plays a key role in the development and maintenance of metabolic alterations associated to obesity. Spexin (SPX) is a novel adipokine whose plasmatic levels are decreased in obesity, and is involved in several functions of AT, including glucose and lipid metabolism. However, most of SPX actions have been described in males. For this reason, our aim was to evaluate the effect of SPX in the metabolic profile of AT from female mice under an obesogenic diet. Four groups of female mice (C57BL/6J) were studied: CTR mice, FRD mice (10 weeks of 20% w/v fructose in drinking water), and two similar groups that were treated with SPX for ten days prior to the end of the protocol (ip. 29 μg/kg/day; CTR-SPX and FRD-SPX). Body weight and caloric intake were recorded every day. Plasma was collected for triglycerides (TG) and glucose (GLU) levels quantification. AT depots (Inguinal (IAT), retroperitoneal (RPAT) and Parametrial (PAT)) were dissected, weighted and PAT was used for quantification of Ob, Adiponectin, PPARγ2, IRS1 and GALR2 by qPCR. Two-way ANOVA was used to determine variable (SPX and FRD) and interaction (FRDxSPX) effects. SPX treatment caused weight loss, which seemed to be more marked in FRD females. FRD increased total caloric intake (P<0.002), while SPX showed a tendency to decrease it in FRD mice. TG and GLU showed no changes. FRD increased RPAT mass (P<0.05) and SPX generated a more marked decrease in RPAT mass from FRD mice (SPXxFRD=0.05). The same result was found when RPAT masses were normalized to body weight (SPXxFRD=0.05). Additionally, SPX treatment generated a beneficial lowering in mRNA expression of all PAT markers quantified, in greater extent in FRD obese mice (FRDx-SPX P<0.05). Overall, SPX caused weight loss and improved the expression of genes related to glucose metabolism and endocrine function of visceral AT in FRD mice. In conclusion, SPX showed a beneficial effect on PAT from female mice, especially in obese ones.

211. 237. **SECRETORY LEUKOCYTE PROTEASE INHIBITOR (SLPI), VITAMINA D (VD) AND OBESITY IN ELDER WOMEN. PRELIMINARY STUDY**

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Besides its role on bone calcium homeostasis, VD has anti-inflammatory actions, increasing antimicrobial peptides expression as cathelicidin. Adequacy in VD demonstrated anti-inflammatory activity while low 25hydroxyVD (25OHD) levels were linked to obesity. SLPI is a highly cationic non-glycosylated serine protease peptide with antimicrobial and anti-inflammatory activity that, among other function lowers the expression of inflammatory cytokines as TNFα and IL1β. Growing evidences suggest that there is a negative correlation between SLPI and risk factors of atherosclerosis, such as obesity and inflammation. Objective: to evaluate 25OHD correlation with SLPI in obesity a condition of chronic inflammation. Obese women (n=32), 69±6 years, without other conditions or medications that could affect inflammatory status (infectious diseases in the last 3 months; autoimmune diseases; renal insufficiency [i.e., creatinine clearance <60ml/min], cancer, glucocorticoid or immunosuppressive medication) were studied. Body weight (BW)(kg) and height (m) were measured and body mass index (BMI) calculated. SLPI (ELISA) and 25OHD (ng/mL) (RIA) levels were determined. Fat mass (densitometry) (gr) was normalized by BW to obtain fat mass percentage (%FM). ANOVA and Person correlation was determined. SPSS 19.0 for Windows was used, p<0.05 was considered significant. Results (X±SD): BMI was 30.3±6.9 and %FM 44.7± 7.8. SLIP ranged between 1.96 and 29.7 (13.38 ± 7.11) and 25HOD between 10 and 48 ng/mL (22.1 ± 13.1). %FM but no BMI negatively correlated with SLIP (r= -0.64, p=0.0001) and 25HOD (r= -0.69, p=0.0001). SLPI correlated with 25HOD only in women with adequate VD (25OHD >30 ng/mL) levels (r= 0.62, p=0.0001). Conclusions: Beside favoring the release of antimicrobial peptides, our results showed that, adequate VD levels positively correlate with SLIP in obesity. Additional studies in a larger population would be necessary to clarify if this correlation is clinically relevant.

212. 269. **INFLUENCE OF MATERNAL OBESITY ON OFFSPRING´S METABOLIC HEALTH**

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Maternal pre-conception obesity leads to high prevalence of obesity and metabolic anomalies in the offspring. Using cafeteria diet (CAF)-induced obesity as animal model, we previously showed that maternal obesity causes increased postnatal body weight in offspring. Aim: to evaluate the effect of maternal obesity on the metabolic state of their offspring. Experimental design: female CF1 mice (21-23 days old) were fed *ad libitum* with a standard diet. Mice were divided into 2 groups: control and CAF. CAF mice also offered with cafeteria diet until detecting a significant increase in their body weights compared to controls. Afterwards, mice were mated, and the subsequent offspring birth was allowed. Dams were euthanized after nursing and offspring were euthanized on postnatal days (P) 25, 45 and 75. In all cases blood was collected for metabolic characterization. Results: CAF mice showed a significantly higher body weight than controls after 75 days of diet protocol. They did not show alterations in basal glycemia nor GTT. After gestation and nursing, obese dams showed no alterations in serum cholesterol, triglycerides and glucose levels compared to controls. Offspring born to obese dams did not show alterations in serum glucose nor total cholesterol levels in P25, P45 nor P75, compared to those born to control dams. However, alterations in serum triglyceride were detected in offspring born to obese dams compared to the control group, with these discrepancies being age and sex dependent. Specifically, increased triglycerides were seen in P45 male offspring (p=0,0081) as well as in P75 females (p=0,0207). **Conclusion:** CAF induces obesity in CF1 female mice after 11 weeks of consumption, without altering the carbohydrate metabolism. Maternal obesity does not alter serum total cholesterol levels nor glycemia. However, the offspring born to obese dams showed a time-dependent alteration of serum triglyceride levels in both sexes.

213. 423. **SALVIA HISPANICA (CHIA SEED) PREVENTS SARCOPENIA AND SKELETAL MUSCLE FIBROSIS IN RATS FED FOR A SHORT TERM WITH A SUCROSE-RICH DIET**

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Lipid accumulation in skeletal muscle (SkM) is correlated with insulin resistance (RI). Previously we showed that the availability of circulating triglycerides (TG) and free fatty acids (FFA) leads to early (at 3 weeks of sucrose-rich diet -SRD- feeding) changes in SkM (gastrocnemius) lipid metabolism. These disturbances deepen over longer periods. Furthermore, we recently found that lipid accumulation in SkM was accompanied by alterations in redox status and collagen deposition (at 30 weeks of SRD feeding). Replacement of the dietary fat source with chia seed reversed or improved these disturbances. The aim of this study was to explore the early changes in the redox status and extracellular matrix of the SkM of SRD-fed rats and the potential preventive effects of chia seeds. Male Wistar rats were divided into two groups and received SRD or SRD where chia seed was incorporated as source of dietary fat for 3 weeks (SRD+Chia). A reference group consumed a control diet all the time. In SkM were analyzed: i) sarcopenia index (SI), ii) lipoperoxidation as thiobarbituric acid reactive substances (TBARS) content, iii) catalase activity (CAT) and iv) collagen deposition (Picrosirius red staining). Plasma TG, FFA and TBARS levels were also measured. Statistical analysis was performed using one-way ANOVA and Newman-Keuls test, $p<0.05$ was considered significant. SRD-fed rats exhibited elevated serum TG, FFA, and TBARS levels. In the SkM of SRD-fed rats, a lower SI and CAT activity and increased collagen deposition were observed; whereas the TBARS content remained unchanged. Chia seed prevented dyslipidemia and serum lipoperoxidation. Moreover, sarcopenia and fibrosis were prevented in the SkM of SRD-fed rats. The current study expands the knowledge on the harmful effects of sucrose in SkM and provides new information on the properties of chia seed with potential application in the prevention of metabolic diseases.

214. 434. **ADDED SUGAR INTAKE IN DYSPEPTIC PATIENTS AND ASYMPTOMATIC VOLUNTEERS**

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Objectives: To compare added sugar intake between patients with dyspeptic symptoms and asymptomatic volunteers. Materials and Methods: Adult dyspeptic patients referred to the Esophagus-Stomach Section of the Hospital de Gastroenterología "Dr. Carlos Bonorino Udaondo" and asymptomatic volunteers from the Universidad de Buenos Aires were included. A 24h-dietary recall and a sociodemographic questionnaire were administered. Weight, height and waist circumference were measured. Statistical analysis was performed using Chi-squared test, Mann-Whitney and multiple linear regression by the SPSS Software. Results: We included 24 dyspeptic patients (39.0±13.3y) and 22 asymptomatic volunteers (37.9±11.3y), which did not differ in terms of age (p=0.77), gender (p=0.86), crowding conditions (p=0.23), or nutritional status (p=0.60). There were no significant differences in mean energy intake between the dyspeptic and the asymptomatic group (1737.2 Kcal/d vs 1855.9 Kcal/d, p=0.50), nor in total sugar intake (82.6 g/d vs. 61.9 g/d, p=0.17). The percentage of patients exceeding the rate of added sugar intake to total energy intake recommended by the WHO was higher in dyspeptic patients than in asymptomatic volunteers (83.3% vs. 27.3%, p=0.0003). Added sugar intake differed significantly between both groups (59.1 g/d vs. 34.6 g/d, p=0.001); however, this association did not remain significant after adjusting by age, gender, nutritional status, energy intake, ethnicity and educational level (p=0.15). Conclusion: Added sugar intake did not differ significantly between dyspeptic patients and asymptomatic volunteers after adjusting by other variables; however, a significantly higher percentage of dyspeptic patients exceeded the WHO recommendation of added sugar intake.

215. 470. **ALTERED RESPONSE TO FLUOXETINE TREATMENT IN MICE EXPOSED TO CHRONIC STRESS FED A HIGH-FAT DIET. BEHAVIORAL AND METABOLIC CONSEQUENCES**

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In recent decades, overweight and obesity have become a growing health problem. Among the main factors involved in the etiopathology of this disease are the consumption of high-fat diets (HFD) and exposure to chronic stress (CS). In this context, the aim of this work was to investigate the effect of treatment with fluoxetine (FLX) -an antidepressant drug- in normal diet (ND) and HFD fed mice exposed to CS on anxious behavior and its possible metabolic consequences. Taking into account that HFD promotes alterations in lipid metabolism and considering the importance of the liver in this metabolism, we studied liver histopathology. In addition, translocation of intestinal bacteria is known to favor inflammation and hepatic fibrosis, therefore the presence of bacteria through colony forming unit (CFU) counts in the liver was determined. For these studies, C57Bl/6J mice were fed ND or homemade HFD for 6 months and

exposed or not to CS/FLX for 4 month. Animals exposed to CS with both diets show a higher level of anxiety in open field, tail suspension and marbel test ($p<0.05$). FLX treatment reversed this effect in ND-fed but not in HFD-fed mice ($p<0.05$). A significant increase in body weight and plasma cholesterol levels was observed with HFD feeding ($p<0.05$). Histological analysis of the liver with hematoxylin and eosin staining indicated a higher steatosis, inflammation and fibrosis score in HFD-fed mice. This score was aggravated by fluoxetine administration in HFD-fed mice and improved in ND-fed mice ($p<0.05$). Similar results were obtained for the frequency and number of CFU in the liver. In conclusion, HFD and CS induce metabolic and behavioral alterations that improved in ND-fed but not in HFD-fed mice. These results point to the need for further studies to evaluate the usefulness of treating anxiety with fluoxetine in patients with obesity and exposed to stressful events.

216. 505. **UTILITY OF A SPOT URINE SAMPLE TO ESTIMATE SODIUM INTAKE IN OLDER ADULTS: COMPARISON WITH 24-HOUR URINE SAMPLE**

María del Rosario Cueto[1], Verónica Chiaradía[2], Stefanía Díaz[1], Cristian Nápoli[1], Inés Fernandez[1], Gabriel Tarducci[3], Cristina Possidoni[4], Silvina Mariela Vidueiros[1], Anabel Pallaro[1].

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High sodium intake (NaI) is an understudied risk factor associated with not only high blood pressure but cardiovascular and kidney diseases which have become a huge burden for public health. In Argentina, NaI data is particularly scarce especially in older adults (OA). The aim of this work was to analyze possible differences between NaI estimated in spot urine samples and NaI determined in 24-h urine samples in OA. This study was conducted in 34 women (W, 73,2 ± 9,1 years) and 21 men (M, 74,7 ± 9,5 years) who had signed an informed consent. As inclusion criteria, participants were not institutionalized and had a similar lifestyle. These subjects, who came from different places, voluntarily agreed to participate in this study organized by the Cátedra de Nutrición (FFyB, UBA) as part of different activities for the community. Body weight (BW, kg) and height (H, m) were determined to calculate Body Mass Index (BMI= BW/H^2, kg/m^2). Sodium, potassium (direct ISE) and creatinine excretion (Jaffé method) were determined in spot urine and the 24-h urinary sodium excretion (24-hUNa) was estimated using the INTERSALT (I) prediction equation. In a subsample (n=10), 24h-UNa was determined in 24-h urine collections. The results showed that 74% of W and 67% of M were overweight or obese (BMI >25 kg/m^2). 24-hUNa was higher in M than in W (4,0 ± 1,0 vs 1,9 ± 0,6; $p<0.0001$). Moreover, 24-hUNa pointed out an increased sodium dietary intake (OMS guidelines >2g/d) in 62% of the subjects. In addition, Na/K ratio was >1 (cutoff 1:1) in 85% of the cases. In the subsample, the NaI determined in 24-h urine collections was associated to 24-hUNa estimated by I (r= 0,8761; p= 0,0004) and no differences were observed between them (Bland & Altmann test, r= -0,002; p<0,995). This study showed that sodium intake estimation by INTERSALT would be a good predictor of daily sodium intake; however, it is necessary to increase sample size in different age groups in future studies.

217. 506. **STUDY OF BODY COMPOSITION OF 6 – 23 MONTHS HEALTHY INFANTS**

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Malnutrition in early life could lead to the risk of non-communicable diseases by modifying growth patterns and body composition (BC). Nowadays, it is difficult to find validated equations based on anthropometric variables that can be applicable in primary health care (PHC) to assess BC in infants. Moreover, there is no local information of infants BC obtained by reference methods. The aim of this study was to evaluate the BC of infants between 6 and 23 months. 24 infants aged 6-23 months from Basavilbaso, Entre Ríos, Argentina, were studied, previous Protocol´s approval by the Ethics Committee and signature of informed consent. Weight (W,kg) and length (L,m) were measured and body mass index ($BMI/Age=W/L^2$) was calculated. Tricipital (TS;mm) and subscapular (SS;mm) skinfolds were assessed. Infant's fat mass (FM;%) was determined by isotopic dilution technique (D) through the oral ingestion of a dose of deuterated water and determination of deuterium in saliva by FTIR infrared spectrometry. FM was also estimated by the Slaughter prediction model, which uses TS and SS. FM by D was not significantly different among 6-12m (n=8;18.7±7.5), 12-18m (n=10;14.4±5.8) and 18-23m (n=6;14.3±7.8); however, FM tended to decrease with age. FM was associated with BMI (r=0.453;p=0.04), TS (r=0.448;p=0.01) and SS (r=0.513;p=0.02). Furthermore, FM by D was not significantly different from that estimated by the Slaughter equation (15.9±8.8 vs 15.6±3.1). Data of argentinean infants BC was obtained for the first time using a reference method like deuterium dilution technique. Slaughter's equation seems to be valid for this age group, but it is difficult to measure skinfolds in PHC, so it is necessary to validate equations that use easier anthropometric tools. Deuterium dilution technique allows assessing fat mass in field studies in a simple and accurate way, so the measurements of the present work were incorporated into a latin american database with the aim to obtain regional equations.

218. 622. **EVALUATION OF THE ANTIOXIDANT ACTIVITY OF NATURAL POLYPHENOLS EXTRACTED FROM FIBRE MICROPARTICLES OF SWEET CHERRIES (*PRUNUS AVIUM* L.)**

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Sweet cherries discarded by harvest can be a useful source of bioactive compounds, recovering valuable compounds such as polyphenols. Natural polyphenols were co-extracted in fiber microparticles (MPCs) obtained from sweet cherries (*Prunus avium* L.) discarded at harvest. These compounds may exert cytoprotective actions in the gastrointestinal tract by decreasing oxidative stress. This work aimed to evaluate the antioxidant capacity and cytotoxicity of polyphenols co-extracted in MPCs obtained from sweet cherries. MPCs obtained after saturated steam blanching of cherries, processing with deionized water and freeze-drying, retained polyphenolic compounds: tetrameric proanthocyanidins (631 ± 34 mg/100 g MPCs) and smaller amounts of anthocyanins, flavonoids, and hydroxycinnamates. Undifferentiated (ND: 3d culture) and differentiated (DIF: 21d) Caco-2 cells were used as a model of the intestinal epithelial cells. Cell viability was evaluated through MTT assay. Caco-2 cells were co-incubated 2h with polyphenol extract (8-752 µg polyphenols/mL). The range of concentrations tested did not present cytotoxicity. The antioxidant capacity of polyphenols was evaluated by dichlorofluorescence (DCF) assay. Oxidative stress was induced with tert-butyl-hydroperoxide 3mM for 1h; cells treated increased oxidation 50%, compared to control (untreated) cells. ND cells were co-incubated with 0.5-10.0 µg/mL of polyphenol extract during oxidation, obtaining 20% antioxidant protection ($p<0.05$) for extract concentration of 10.0 µg/mL, while for DIF cells 50% protection was observed for the same concentration. In conclusion, natural polyphenols extracted from fiber MPCs of sweet cherries showed a protective effect against oxidative stress, with low cytotoxicity. This

effect was greater on Caco-2 DIF cells. Therefore, fiber microparticles of sweet cherries constitute a natural source of source of polyphenols. We propose the upcycling of natural and healthy antioxidant, which could be considered as food additive.

O2-METABOLISM & NUTRITION

FRIDAY 17TH NOVEMBER 9:00 - 10:30
CHAIRS: MARISA REPETTO
PAOLA ILLESCA

219. 188. **IMPACT OF HIGH FAT DIET ON C-TYPE NATRIURETIC PEPTIDE SYSTEM IN SKELETAL MUSCLE**

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C-type natriuretic peptide plays a crucial role in cardiovascular balance, but their impact on skeletal muscle remains relatively unexplored. The aim of our study is to evaluate the morphology and expression of components of natriuretic peptide system in skeletal muscle of high-fat fed rats (HFD). Experimental design: Male wistar rats received HFD (60% of fat) or standard diet (SD) from weaning until the 14th week of life. At the end of the experimental period, body weight (BW) and systolic blood pressure (SBP) were measured, and oral glucose tolerance test was performed. Animals were sacrificed and SM morphology was evaluated. Natriuretic peptide receptor B (NPR-B) or C (NPR-C), neprilysin (NEP) and C-type natriuretic peptide (CNP) expression were evaluated (RT-qPCR) in SM. Also, CNP expression was studied by immunohistochemistry. The protocol was approved by Ethic Committee CICUAL-FFyB-UBA. Results are expressed as mean ± SEM. Statistical analysis: Student test (n=6 rats/group; *p<0.05; **p<0.01; ***p<0.001 vs SD). Results: BW was higher in the HFD group (SD=492.3±9.5; HFD=553.4±8.0*** g). No differences in SBP were observed. HFD´s glycemia (Gly) at 120 and 180 minutes was higher than SD´s (120 min Gly SD=137.9±6.9; HFD=157.7±4.9*; 180 min Gly SD=114.4±5.8 HFD=147.8±7.0** mg/dL). SM fibers of HFD were bigger than SD cells (SD=1851±44; HFD=2383±103**µm^2) and exhibited lower cell density (SD=30.7±0.6; HFD=24.8±0.8** number cells/field). The expression of NPR-C (SD=0.59±0.05; HFD=0.86±0.07**) and NEP (SD=1.32±0.20; HFD=0.59±0.11**) were higher in HFD group, and no differences in NPR-B and CNP expression were observed. However, CNP immunostain was lower in HFD (SD=27.7±2.4; HFD=18.0±2.2* %area/total area). Conclusion: HFD promotes an increase in BW and glycemia that is accompanied by changes on the SM morphology. Decrease in CNP expression and increase in the components involved in CNP degradation in SM could be a link between obesity and cardiovascular diseases.

220. 272. **PROTECTIVE ACTIONS OF GENISTEIN IN A MODEL OF HYPOESTROGENISM AND OBESITY**

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Menopause is associated with higher prevalence of chronic diseases such as obesity and osteoporosis. Genistein (Gen) has been proposed as a natural therapy to counteract these diseases. Previously in our lab we demonstrated that, in the absence of ovarian function, obesity exhibited a deleterious action on bone metabolism through an enhancement in bone oxidative stress and reducing mineralization. In this work, we studied the effect of Gen on bone stress induced by obesity and hypoestrogenism. Females Wistar rats were bilaterally ovariectomized (OVX) and fed with a high-fat diet (OVX-Ob) for 10 weeks. Experimental systems: diaphysis of femurs (DF) and retroperitoneal AT explants isolated from OVX-Ob rats, or primary culture of DF-osteoblast (OBL). DF, AT, OBL were *in vitro* exposed to Gen for different interval of treatment. In DF, Gen (18 h) reduced H_2O_2 (DC fluorescent probe) levels and stimulated VEGF (ELISA kit) synthesis respect to control (21.6±0.8vs16.9±1.1µmol/g tissue, p<0.0001 and 12.39±1.68 vs 14.25±1.58 pg/mg tissue, p<0.025, respectively). On OBL monolayer, Gen treatment (6 d) increased NO (Griess assay) production (82%, <p 0.0001) and alkaline phosphatase activity (ALP) (colorimetric assay) respect to control (90%, p<0.0001). These results suggest a protective action of Gen on OVX-Ob bone. In AT, Gen reduced ROS production (2.7±0.4 vs 1.8±0.1 µmol/g tissue p<0.001); TBARS secretion (0.455±0.09 vs 0.254±0.01 µmol MDA/g tissue, p<0.0001), and Leptin release (16% below control, p<0.025). These results suggest an antioxidant action of Gen on AT in obese animals. To assess whether Gen bone action depends the antioxidant effect on AT, conditioned medium obtained from AT exposed to Gen (CM-Gen) was employed for OBL proliferation assay. The presence CM-Gen enhanced OBL cell growth (32% p<0.0001) respect to OBL/CM-control. The results suggest that, Gen protects bone from the deleterious effects induced by obesity, through a down regulation of AT-oxidative stress.

221. 276. **UNBALANCED MATERNAL DIET DURING DEVELOPMENT ALTERS MIR27A EXPRESSION IN MALE ADULT MICE**

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It is accepted that diet quality supply during gestation and lactation programs pup´s development. Our aim was to evaluate the impact of maternal fructose rich diet (FRD; during pregnancy and lactation) intake on adipose tissue (AT) development in adult male mice, with special focus on thermogenesis and miRNA27a and miRNA30b factors barely studied in this model. On pregnancy day 1, dams were provided either with tap water alone or containing fructose (20% w/v; FRD) and fed *ad libitum* up to delivery. Six pups were left per mom and same diet was provided until pups were 21 days old. Males were separated and maintained until 120-days old (experimental day) with normal diet *ad libitum*. From weaning until experimental day, body weight and food intake were registered. C and F indicate pups born to control and FRD dams, respectively. On experimental day, trunk blood was collected, AT depots were dissected and weighted. Epididymal AT (EAT) was used for H&E staining and mRNA expression levels quantification. Our results show that F pups gain more weight due to a higher food intake. Nevertheless, no differences in AT mass were observed, despite a tendency towards a higher EAT adipocyte area. No differences were found in functional AT gene expression markers such as leptin and adiponectin, but a lower expression of ucp-1 was found in F EAT, maybe due to a lower thermogenic capacity. Considering miRNA biosynthesis, F pups showed significant lower expression levels of exportin 5. Finally, miR27a expression was significantly increased in F pups, without changes in miR30b. It is known that miR27a is negatively regulated during adipogenesis, and concomitantly a lower PPARγ tissue expression was observed. In conclusion, these results suggest that unbalanced maternal diet provided during a key period of the individual's development, can generate permanent alterations in miRNA27a levels, which condition AT expansion, causing alterations that persist into adulthood.

222. 483. **INTESTINAL EFFECTS OF A SUCROSE RICH DIET IN MALE WISTAR RATS**

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Cora Beatriz Cymeryng[1,3] y Esteban Martin Repetto[1,4].
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In recent years, the obesity epidemic has highlighted the importance of metabolic dysfunction-associated fatty liver disease (MAFLD). We have previously described a model of steatohepatitis associated with insulin-resistance developed in rats by the administration of a sucrose rich diet (SRD). Excessive carbohydrate consumption has been shown to alter the intestinal microbiota and affect intestinal permeability, leading to an increased passage of endotoxins into circulation, primarily reaching the liver. The objective of the current study was to assess the intestinal effects of the SRD. Male Wistar rats were divided into two groups: a control diet group (C, n=6) and a group fed 30% sucrose in the drinking water for 12 weeks (SRD, n=6). Fecal samples were collected weekly, and during the 7th week, insulin sensitivity was assessed by an insulin tolerance test. Histological analysis of the ileum showed epithelial changes (hyperplasia), along with a reduced expression of the junction proteins ZO-1 ($p<0.05$, Mann-Whitney test) and occludin ($p=0.05$, Mann-Whitney test) by qPCR. Changes in claudin distribution were evaluated by immunofluorescence analysis showing a significance decrease ($p<0.001$, Student's t-test). Additionally, significantly higher levels of circulating endotoxin were observed in SRD-treated rats ($p<0.001$, Mann-Whitney test). In line with these findings, in the SRD group we also detected an elevated amount of bacterial DNA in the liver ($p<0.05$, Mann-Whitney test), and a higher Firmicutes/Bacteroidetes ratio, a dysbiosis indicator (by qPCR) in cecal feces from SRD animals was only evident at the 12th week ($p<0.001$, Mann-Whitney test). In summary, our results indicate that consumption of a high sucrose diet is linked to dysbiosis and disruption of intestinal permeability, potentially fostering an inflammatory process in the liver and exacerbating the effects of insulin resistance in this tissue.

223. 577. **PARTICIPATION OF CHLORIDE CHANNELS IN CARDIOVASCULAR AND KIDNEY HEALTH. EFFECTS OF HIGH CHLORINE DIETS ON BLOOD PRESSURE**

María J. Rudi[1]; Nicolás M. Kouyoumdzian[1, 2]; Melanie Kim[1]; Natalia L. Rukavina Mikusic[1,2]; Mónica Galleano[3, 4]; Belisario Fernández[5]; Ana M. Puyó[1]; Marcelo R. Choi[1, 2, 5]
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The excessive consumption of sodium chloride (NaCl) in the diet leads to the development of high blood pressure (HBP) and target organ damage. The contribution of the chloride anion (Cl^-) on these deleterious effects is unknown. The ClC-K1 and ClC-5 channels are known to be essential regulators of Cl^-. The objective was to evaluate the participation of Cl^- and the expression of ClC-K1 and ClC-5 in the renal inflammatory and oxidative response and in the development of hypertension. Male Wistar rats (n=8/group) were fed with different equimolar diets for 3 and 6 weeks: control (group C); NaCl 8% (NaCl group); high Na^+ without Cl^-, $Na_3C_6H_5O_7$ 11.8% (Na group); high in Cl^- without Na^+, $CaCl_2$ 3.80%, KCl 3.06% and $MgCl_2$ 1.30% (Cl group). Systolic blood pressure (SBP), renal function, and oxidative parameters in the renal cortex were determined. Renal expression of p50-NFkB, the AT1, AT2 and D1 receptor, PARK7, and the ClC-K1 and ClC-5 chloride channels were also determined. Differences with a value of $p<0.05$ are considered statistically significant (*). An increase in SBP, glutathione peroxidase (GPx) enzyme activity, and p50-NFkB and AT1R expression in the NaCl and Cl groups compared to the other groups* were observed. No changes in AT2R expression was observed. The production of thiobarbituric acid reactive substances (TBARS) was increased in the experimental groups with respect to C. PARK7 expression was reduced in the Cl group compared to C*. Finally, the NaCl and Cl groups presented a higher expression of ClC-K1, while ClC-5 was reduced in the NaCl group compared to C*. In conclusion, Cl^- would be co-responsible, together with Na^+, in triggering renal oxidative and inflammatory damage and increasing blood pressure; thus, the importance of reducing the intake of both ions as a non-pharmacological preventive measure for the prevention and control of hypertension is deduced. The role of the ClC-K1 and ClC-5 channels as mediators of this process remains to be determined.

O1-NEUROSCIENCES

WEDNESDAY 15TH NOVEMBER 9:00 - 10:30
CHAIRS: JUAN BEAUQUIS
RAMIRO QUINTÁ

224. 79. **EFFECTS OF SHORT AND LONG-TERM HIGH-SUCROSE DIET CONSUMPTION UPON CEREBRAL CORTEX REDOX STATUS AND COGNITIVE PERFORMANCE OF MALE RATS**

María del Rosario Ferreira[1,2], María de los Milagros Scalzo[1], Silvia Rodríguez[1,2], María Eugenia D´Alessandro[1,2].
[1]Laboratorio de Estudio de Enfermedades Metabólicas relacionadas con la Nutrición, Facultad de Bioquímica y Cs. Biológicas, Universidad Nacional del Litoral, Santa Fe, Argentina. [2]Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET).

Several pieces of evidence suggest that the deleterious effect of high simple sugars consumption – a feature of the modern societies- is not only restricted to the development of cardiometabolic risk factors (CRF). It has been reported that these dietary patterns and the associated metabolic disorders may also promote the development of neurodegenerative disorders and cognitive decline. Although this has emerged as a relevant area of research, it has not been fully explored. The aim of this work was to evaluate whether the presence of a greater number of CRF could have a greater impact on the central nervous system (cerebral cortex) and/or cognitive performance. Male Wistar rats (8 weeks-wk-old) received a high-sucrose diet (HSD) or a control diet (CD) during 3 wk (short-term) or 15 wk (long-term). We previously shown that the metabolic and hormonal profile worsens as the time of consumption of HSD increase, being a suitable model for achieve our goal. We analyzed rats brain weight and in cerebral cortex: a-Oxidative damage markers: Reactive oxygen species (ROS), TBARS and advanced glycation end products (AGEs) levels, b-reduced glutathione levels (GSH), glutathione peroxidase (GPx), glutathione reductase (GR), glutathione -S-transferase (GST) and catalase (CAT) enzyme activities. Besides, Novel Object recognition and T- maze memory tasks were performed. Data were analyzed by two-way ANOVA. ROS, TBARS, AGEs, GPx and GR were increased ($P<0.05$) in 3 wk HSD-fed rats. Oxidative damage markers and GR were also increased ($P<0.05$) in the 15 wk HSD-fed group and values were similar to those observed in 3 wk HSD-fed rats. GSH, CAT and brain weight were reduced ($P<0.05$) in long-term HSD-fed animals. GST was similar in all dietary groups. A poor performance in memory tasks was observed in 3 wk and 15 wk HSD-fed rats in a similar extent. Our results add new evidence related to the association between an adverse peripheral metabolic environment and brain/cognitive dysfunction.

225. 283. **HISTONE METHYLATION CHANGES IN ASTROGLI-**

OSIS: H3K27ME3 AS SUPPRESSOR MARK OF ASTROCYTIC PRO-INFLAMMATORY GENES

Camila Vidos[1], Alberto Javier Ramos[1], Alejandro Villarreal[1,2]
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Astrocytes respond to brain injury through a phenomenon called reactive astrogliosis, in which a pro-inflammatory astrocytic phenotype was described. Astrocyte pathological conversion with a pro-inflammatory gain of function involves stable transcriptomic changes following activation of transcription factor NF-kB. We have shown that pro-inflammatory gain of function correlates with increased histone acetylation and that these molecular mechanisms are dependent on microglia abundance and microglia soluble factors.
We aimed here to establish a two-step treatment protocol that allows us to address molecular changes in astrocytes exposed to microglial soluble signals, in microglia-depleted cultures. We are currently focused on studying mechanisms involving histone 3 methylation at lysine 27 which is a repressive mark for gene expression.
We exposed primary cultures of mixed glial cells (containing astrocytes and microglia) to lipopolysaccharide (LPS) during 24hs to obtain a pro-inflammatory conditioned medium (PCM). We then exposed astrocyte enriched cultures (microglia-depleted) to the PCM from 1-6hs with a 1h interval to address NFkB activation, and during 24, 48 and 72 hs to address morphological changes and pro-inflammatory marker expression.
Using immunofluorescence, we observed after PCM treatment a time-dependent increase in NFkB (p65 subunit) nuclear translocation, an increase in complement 3 protein (C3) immunoreactivity and increased astrocyte reactive phenotype. We did not observe major changes in global H3K27me3 after PCM treatment but inhibition of the JMJD3 histone demethylase for H3K27me3 showed less C3 immunoreactivity at 48 hs.
These preliminary results validate the two-step treatment protocol as a paradigm to address pro-inflammatory phenotype of astrocytes in microglia-depleted cultures. We will further address changes in H3K27me3 enrichment at the promoters of pro-inflammatory gene (e.g. C3) using ChIP-qPCR using this two-step protocol.

226. 372. **EFFECTS OF THE EARLY GLUCOCORTICOIDS-LOADED NANOSYSTEMS IN MOTOR SEQUALAE AND NEUROINFLAMMATORY PROCESSES INDUCED BY MILD TRAUMATIC BRAIN INJURY**

Sofía de la Fuente[1], Aida Marcotti[1], María Lina Formica[2], Santiago Daniel Palma[2], Alicia L. Degano[3] and Mariela Fernanda Pérez[1]
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Mild traumatic brain injury (mTBI) causes a variety of neuropathological manifestations including cognitive, emotional, and physiological deficits, probably related to early neuroinflammatory processes. Despite the efforts to develop neuroprotective treatments, most preclinical studies did not report significant effects. We have previously shown that nanocarriers loaded with the synthetic glucocorticoid triamcinolone (NT) prevent oxidative stress processes and reduce cognitive and emotional sequelae induced by mTBI. Nevertheless, little is known about NT effects in mTBI-induced motor impairments nor neuroinflammation processes in deficits-related brain structures. The aim of the present work was to characterize mTBI-induced motor deficits and the underlying neuroinflammatory processes, and the impact of early NT treatment in behavioral nor biochemical alterations. mTBI was induced in anesthetized adult male Wistar rats, which 15 min and 24h later received a dose of NT (0,5 mg/Kg). Seven days after, they were tested for amphetamine (0.5 mg/kg)-induced locomotor activity (LA) or grip strength, and then sacrificed to assess pro-inflammatory cytokines (by RT-PCR) levels in dorsal and ventral striatum. Additional groups were sacrificed at 24h to assess pro-inflammatory cytokines or 7d post-mTBI to ascertain TBARS (by spectrophotometric method) in the same brain structures. Preliminary results indicate that mTBI induced significant increase in LA (RM-ANOVA) and IL-6 and IL-1β (one-way ANOVA) in dorsal striatum. We concluded that mTBI induced increases in neuroinflammatory mediators that could have detrimental actions on motor activity. More experiments are in process, to confirm alterations in TBARS levels and NT effects in behavioral and neurochemical alterations induced by mTBI.

227. 452. **HYPERTHERMIC SEIZURES CAUSE CHANGES IN ASTROCYTIC HOMEOSTATIC FUNCTIONS**

Alicia Rossi, Dante Gómez Cuautle, Florencia Rodríguez, Luca Sapia, Alberto Javier Ramos.
Instituto de Biología Celular y Neurociencia Dr. E. De Robertis. UBA-Conicet.

A significant percentage of patients suffering Temporal Lobe Epilepsy refer to a history of an Initial Precipitating Event (IPE) in childhood, of which Complex Febrile Seizures are the most frequent. The period between febrile seizures and chronic TLE is called the latency period where it is considered that the processes that lead to epileptogenesis have place. Using a Wistar rat Hyperthermic Seizure model we previously demonstrated that exposed male-HS have moderate reactive gliosis with an atypical astrocytic distribution at 15 days post-HS (DPHS), present lower convulsive threshold, and show a significant increase in Iba-1+ microglia while females do not. Having these results in mind here we investigate changes in the homeostatic function of astrocytes. Rat pups (10 days old) were placed in a glass chamber, and their core temperature was raised (39-42°C). Seizure onset was monitored behaviorally. At 15DPHS brains were rapidly dissected and RNA was extracted for real-time qPCR studies. Another group of rats was fixed at 15DPHS, and brains were removed and processed for immunohistochemistry. We observed that HS induces a significative reduction in the Kir4.1 (Kncj10) and AQP4 mRNA expression levels at 15DPHS. Furthermore, immunohistochemical studies have shown that AQP4 expression was not only reduced but also seems to be mobilized from the astroglial end-feet to the astroglial soma, especially in exposed males-HS in both the piriform cortex and the hippocampus. We conclude that HS acting as IPE induces alterations in some atroglial homeostatic functions in males that could contribute to epileptogenesis. Supported by: CONICET PIP 1992; PICT 2017-2203 and 2019-0851; UBACYT

228. 518. **ALTERATIONS IN MITOCHONDRIA-ENDOPLASMIC RETICULUM CONTACTS IN MANGANESE-INDUCED NEUROTOXICITY**

Soledad Porte Alcon, Valentina Saud, Roxana Mayra Gorojod, Mónica Lidia Kotler.
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Mitochondrial-associated membranes (MAMs) are regions of the endoplasmic reticulum (ER) in contact with mitochondria (M). These specialized cellular compartments participate in intracellular Ca2+ and lipid homeostasis, mitochondrial dynamics, autophagy, and other physiological functions. MAMs alterations are observed in diverse pathological settings, including neurodegenerative diseases. Manganese-induced neurodegeneration, caused by chronic exposure to Mn, is characterized by motor dysfunction and cognitive impairment. The mechanisms involved in metal neurotoxicity—reactive oxygen species (ROS) generation, mitochondrial dysfunction, and autophagy impairment—are closely related to MAMs disruption. However, to our knowledge, this topic has not been addressed yet. Our goal was to study the effect of Mn on MAMs. Exposure of HT22 hippocampal cells to 50 μM Mn for 24 hs reduced cell viability (Crystal Violet: 33%, $p<0.001$; MTT Reduction: 51%, $p<0.001$) and increased mitochondrial (MitoSOX Red: 1.5-fold, $p<0.05$) and total ROS pro-

duction (H2DCF-DA: 2-fold, p<0.05). Moreover, Mn-exposed HT22 cells exhibited shorter mitochondria (p<0.05) with fewer ramifications (p<0.05). The area (p<0.05), perimeter (p<0.05), and circularity (p<0.05) of mitochondria were also affected. Preliminary results showed an increment in LC3 levels after Mn exposure (western blot: 2.8-fold); however, bafilomycin A1-treated cells exhibited similar levels, suggesting autophagic flux impairment. Colocalization analysis of GFP-Sec61β (ER) and DsRed2Mito revealed that Mn increases ER-M contacts (M2: p<0.05). Furthermore, we detected an accumulation of lipid droplets in HT22 cells exposed to Mn (Red Nile staining). In summary, Mn-induced mitochondrial dysfunction is accompanied by alterations of ER-M contacts and lipid dyshomeostasis. Understanding the role of MAMs in Mn-induced neurodegeneration may contribute to discovering novel therapeutic targets to treat this and other neurodegenerative disorders.

P1-NEUROSCIENCES

WEDNESDAY 15TH NOVEMBER 14:00-15:30

CHAIRS: CARLOS POMILIO
SOLEDAD PORTE ALCÓN
MARIELA FERNADA PÉREZ

229. 33. **PHYTOCANNABINOIDS DECREASE NEUROINFALMMATAION AND IMPROVE LOCOMOTOR OUTCOME FOLLOWING SPINAL CORD INJURY**

Julián Del Core[1], Alejandro F De Nicola[1,2], Florencia Labombarda[1,2]

1 Laboratorio de Bioquímica Neuroendócrina, Instituto de Biología y Medicina Experimental, CONICET. 2 Departamento de Bioquímica Humana, Facultad de Medicina, UBA.

Neuroinflammation is involved both in secondary damage and functional deficits after traumatic spinal cord injury (SCI), so its regulation represents a therapeutic target. In this regard, Tetrahydrocannabinol (THC) and Cannabidiol (CBD), the main phytocannabinoids of *Cannabis Sativa*, emerge as anti-inflammatory molecules. In the present study we used a model of SCI in rats to evaluate the effects of oil extracted from a resin composed of THC: CBD 1:1 (CAN). SCI rats received an oromucosal dose (20mg/kg/day) during 15 days post-injury (dpi) and they were sacrificed in the acute (3dpi) and chronic phases (60dpi). By Real Time RT-PCR, pro-inflammatory (TNFα, IL-1β, IL-6) and anti-inflammatory (TGFβ, ARG-1, MRC) markers were determined during the acute phase. After SCI, the expression of all pro-inflammatory markers was increased compared to sham rats (p<0.001, ANOVA one-way), while the expression of anti-inflammatory molecules remained as sham values. Unlike, CAN treatment decreased the expression of pro-inflammatory molecules (p<0.05 vs SCI rats, ANOVA one-way) and increased the expression of anti-inflammatory ones (p<0.01 vs SCI rats, ANOVA one-way). Moreover, confocal analysis of a double immunohistochemistry (Iba-1 and ARG-1) showed that CAN treatment increased the number of microglial cells (Iba-1+ cells) which express ARG-1 (an anti-inflammatory marker, p<0.05 vs SCI rats, ANOVA one-way). Finally, we evaluate functional recovery at the chronic phase. Rotarod analysis showed that CAN treatment increase the latency to fall compared to SCI rats (p< 0.05, ANOVA one way). Regarding horizontal ladder, SCI rats increased the number of hindlimb foot misplacements compared to sham rats (p<0.05, ANOVA one way), while CAN treatment reduced the value of this parameter (p<0.05 vs SCI rats, ANOVA one way).These results suggest that THC and CBD offer a promising perspective in reducing acute neuroinflammation promoting functional recovery after SCI.

230. 59. **UPREGULATION OF CHOLESTEROL METABOLISM AS A HALLMARK OF MIDBRAIN NEURODEGENERATION INDUCED BY IRON OVERLOAD**

Athina Maniscalchi*[1], Oriana Benzi Juncos[1,2], Melisa Conde[1,2], Melania Funk[1,2], Natalia Alza[1,3] Gabriela Salvador[1,2]

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Iron (Fe) accumulation in specific brain areas and ferroptosis are associated with various neurodegenerative disorders. We have previously established an *in vivo* model of Fe overload (C57BL/6 mice treated with Fe 333 mg/kg) with midbrain neurodegeneration and lipid cacostasis. Our aim was to study the link between lipid metabolism alterations and ferroptosis taking into account neuroglial metabolism. In midbrain of Fe-overloaded animals, we detected a decrease in the expression of *SLC7A11* and an increase in *ACSL4* (p<0.001), both markers of ferroptosis. We found that cholesterol (chol) was elevated in midbrain of Fe-treated mice, coincidentally with *SREBP2* and *ABCA1* upregulation (p<0.001). In addition, increased levels of *CPT1c* (p<0.001) were observed after Fe overload, indicating an enhanced β-oxidation for the removal of fatty acid released by lipolysis. In the open field test, Fe-overloaded mice displayed motor impairment, with a lower rearing activity, a shorter time spent in the central square, and a longer time in the periphery (p<0.001). Next, we investigated chol metabolism in dopaminergic neurons and astrocytes exposed to Fe overload with ferric citrate ammonium (FAC). Chol content in neurons (N27), astrocytes (C6), and mouse primary glial culture was increased both in intracellular compartments as well as in secretomes after Fe treatment (p<0.001). This rise correlated with an increase in chol *de novo* synthesis and transport, respectively, by means of *HMGCR* and *ABCA1* upregulation (p<0.001). To study the link between chol accumulation and ferroptosis, cells were exposed to the inhibitor ferrostatin (FER). We found that FER reduced chol levels when cells were exposed to FAC (p<0.001). Our findings indicate that altered chol metabolism could be a biomarker of midbrain neurodegeneration triggered by ferroptosis, with motor impairment as a final outcome.

231. 65. **NEURONAL DIFFERENTIATION OF MURINE NEUROBLASTOMA N2A CELLS IS DIFFERENTIALLY INDUCED BY SELECTIVE INHIBITION OF CLASS I OR CLASS IIA HDACS**

Stephanie Junge[1], Alejandra Bernardi[1], María Sofía Villalba[1], Francisco J. Urbano[2] and Veronica Bisagno[1]

[1]Instituto de Investigaciones en Medicina Traslacional, Facultad de Ciencias Biomédicas, Universidad Austral, Buenos Aires, Argentina.

[2]IFIBYNE-CONICET, Universidad de Buenos Aires, Argentina.

Histone deacetylases (HDACs) are vital enzymes for regulating chromatin functions. Their primary role is to eliminate acetyl groups allowing histones to wrap the DNA more tightly. This study aims to investigate the in vitro application of selective inhibitors targeting class I/IIa HDACs. HDAC inhibitors (HDACi) are upcoming interesting targets due to their involvement in epigenetic/non-epigenetic regulation, and their potential as use as anti-cancer agents. All statistical differences were tested using ANOVAs. We used N2a cell cultures, a fast-growing mouse neuroblastoma cell line, capable of differentiating into neurons. They were cultured for 4 or 7 days in vitro (d.i.v.) using a 24-well plate in a DMEM with low serum (0.5%) condition and treated with HDACi MS275 (class I) and MC1568 (class IIa), at high or low concentrations. Equivalent DMSO concentrations were used as control. Whole cell patch-clamp recordings were performed to study neuron-like characteristics. Results showed a severe decrease in cell viability 4 d.i.v with MS275 (high), further corroborated by dapi staining. The same tendency was observed at 7 d.i.v (p<0.05). None of the inhibitors (low) affected cell viability. Cells showed a positive mark for tyrosine hydroxylase (TH) and MAP2. Moreover, at 4 d.i.v., patch-clamp recording showed an increase in voltage-gated ionic channel expression; 70% of the cells treated with Class I HDACi at low concentration showed positive voltage-gated ionic current versus only a 30% increase in DMSO (p<0.05). Both groups of HDACi at high concentrations showed lower levels of capacitance compared to DMSO. In summary, treatment with HDACi increased N2a differentiation at 4 days in vitro. Survival and voltage gated ionic channel were affected. These results suggest that HDACi are arresting tumoral growth, leading to a N2a

neuron-like differentiation and a specific role of class I HDACs on maintaining a tumor-like conformation.

232. 92. **PROLONGED EXPOSURE OF CORTICAL NEURONS TO DIAZEPAM INDUCES DOWNREGULATION OF $GABA_A$ RECEPTOR α1 SUBUNIT VIA A CALCIUM/ PROTEIN KINASE A SIGNALING PATHWAY**

L. Carolina González Gómez, Sara Sanz Blasco, María Clara Gravielle

Instituto de Investigaciones Farmacológicas (ININFA), Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires-CONICET

Benzodiazepines exhibit a high therapeutic index and low toxicity in short-term treatments, however, prolonged administration induces tolerance to most of their therapeutic actions. In previous studies, we demonstrated that the prolonged exposure of rat cortical neurons to diazepam (DZ) produces a transcriptional repression of the $GABA_A$ receptor α1 subunit gene. This regulatory mechanism depends on the activation of L-type voltage gated calcium channels (L-VGCC). The aim of this work was to investigate the signaling cascade triggered by the benzodiazepine-induced stimulation of calcium influx that leads to the regulation of the $GABA_A$ receptor expression. To this end, we exposed rat neuronal cultures to DZ (1 µM) for 48h in the presence or absence of different inhibitors. Results from this study indicated that the DZ- induced decrease in the mRNA and protein levels of the α1 subunit was inhibited in the presence of 1 µM H-89, a protein kinase A (PKA) inhibitor ($p<0.05$, one-way ANOVA and Tukey post-hoc test). This suggests that the DZ- induced downregulation of the α1 subunit depends on activation of a PKA cascade. In a previous report, we reported that the DZ-induced decrease in α1 expression is associated with the activation of the cAMP response element- binding protein (CREB) and the induction of the cAMP early repressor (ICER). We observed here that the increase in CREB phosphorylation and ICER expression produced by the DZ exposure was prevented in the presence of H-89, indicating that the activation of these transcription factors was mediated by the PKA activity ($p<0.05$, one-way ANOVA and Tukey post-hoc test). Taken together, our results suggest that sustained stimulation of $GABA_A$ receptors by DZ stimulates the calcium influx through L-VGCCs. The increase in intracellular calcium activates a PKA cascade that in turn activates two transcription factors, CREB and ICER, leading to the transcriptional downregulation of the $GABA_A$ receptor α1 subunit gene.

233. 139. **THE HIGH-FAT AND/OR HIGH-CARBOHYDRATE DIETS WORSENED THE BRAIN MICE SUSCEPTIBILITY TO DAMAGE PRODUCED BY ENTEROHEMORRHAGIC *E. COLI* (EHEC) SHIGA TOXIN 2**

David Arenas-Mosquera[1], Natacha Cerny[2], Adriana Cangelosi[3], Patricia A Geoghegan[3], Emilio L. Malchiodi[4], Mauricio De Marzi[5], Alipio Pinto[1], Jorge Goldstein[1].

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Shiga toxin 2 (Stx2), is responsible for producing hemolytic-uremic syndrome characterized by thrombocytopenia, microangiopathic hemolytic anemia and renal compromise, which primarily targets children up to 5 years of age. Encephalopathy produced by Stx2 is the mean predictor of death. Nutritional quality could be one of the reasons by which in the face of an EHEC outbreak, some patients experience more profoundly deleterious effects than others, including death. Thus, the aim of this study was to determine whether excessive high fat and/or high carbohydrate diets could negatively modulate the deleterious action of Stx2 on ventral anterior and ventral lateral thalamic nuclei, the neurological centers responsible for motor activity. Mice were fed with regular, high fat and/or high carbohydrate diets in the context of Stx2 or vehicle intravenous administrations to determine the alterations produced in the neurovascular unit at the cellular and functional levels. Statistical significance was performed using the One-way analysis of variance followed by Bonferroni *post hoc* test. The criterion for significance was $p<0.05$ for all the experiments. Mice fed with high fat and high carbohydrate diets under the Stx2 context significantly enhanced the deleterious effect of Stx2, while the combination of both diets yielded the worst results in comparison with mice fed with a regular diet under Stx2 context, as follows: the endothelial glycocalyx area and myelin basic protein expression were reduced 3.0 and 1.5 folds respectively, astrocyte and microglial reactivities, and neurodegeneration were increased 3.0, 2.0 and 8.0 folds respectively, TNFα expression was increased 4.0 folds, while motor performance and paw sensitivities were reduced 2.0 and 1.1 folds respectively. In view of the results presented here, poor nutritional quality could negatively influence patients affected by Stx2 at a neurological level, without ruling out its effect at a systemic level.

234. 140. **VOXEL- BASED MORPHOMETRY ANALYSIS OF BRAIN REGIONS IPSILATERAL AND CONTRALATERAL TO THE EPILEPTIC FOCUS IN PATIENTS WITH FOCAL EPILEPSY**

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Objectives: Voxel-based morphometry (VBM) is a technique for magnetic resonance image (MRI) analyses. In epilepsy, it has been used to investigate areas with reduction or increase of gray matter or white matter. The aim of this study was to analyze the gray matter concentration (GMC) of brain regions ipsilateral and contralateral to the epileptic focus in patients with focal epilepsy, and to correlate with clinical and behavioral variables. Methods: Patients admitted during 2019-2021 who signed the informed consent were included. Approval was obtained from the Ethics Committees of El Cruce Hospital. All subjects underwent a neurological examination including complementary studies (Video-EEG and MRI), to determine the epileptogenic zone and the laterality of the epileptic focus. Also, neuropsychiatric assessments using SCIDI-II of DSMIV, GAF (Global assessment of functionality) and Barrat scale for impulsivity were determined. MRI (T1) images were used to calculate the GMC using VBM with SPM12 and DARTEL. The individual z-scores values were calculated according to a control group. Nonparametric tests were used. Results: Thirty-six patients with temporal lobe epilepsy (TLE) and 7 patients with frontal lobe epilepsy (FLE) were included (27 women/ 16 males and 19 left focus /24 right focus). Patients

with TLE showed lower levels of GMC in ipsilateral amygdala, hippocampus, entorhinal cortex, and temporal pole, compared with contralateral homologue regions ($p<0.05$). In TLE group, GAF scores positively correlated with the GMC of ipsilateral planum polare ($r=0.44$, $p=0.007$) and ipsilateral temporal transverse gyrus ($r=0.43$, $p=0.009$). In patients with FLE, the GMC negatively correlated with impulsivity in ipsilateral temporal lobe ($r= -0.857$, $p=0.014$) and contralateral temporal transverse gyrus ($r=0.89$, $p =0.007$). Conclusion: In this exploratory study, reductions in GMC in temporal regions correlated with lower functionality in TLE and higher impulsivity in FLE.

235. 148. **OXIDATIVE STRESS IN zQ175 mouse model of HUNTINGTON'S DISEASE**

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Huntington's disease (HD) is a progressive neurodegenerative disorder affecting the brain's striatum and cortex, leading to motor, cognitive, and psychiatric impairments. Proposed pathological mechanisms include oxidative stress, mitochondrial dysfunction, and neurotoxicity. Oxidative stress arises from elevated reactive oxygen species (ROS), that can be countered by antioxidants like glutathione (GSH) and mitochondrial enzyme superoxide dismutase 2 (SOD2). Uncoupling proteins UCP2 and UCP4 are proposed to minimize mitochondrial ROS via proton gradient dissipation. We have previously shown that ROS levels were unchanged in the zQ175 knock-in mouse model of HD (HD mice), but reduced GSH levels were evident at 4 months (4m) and 8 months (8m) in the striatum of HD mice. Now, we evaluated UCP2, UCP4 and SOD2 expression by Western blot in the striatum and cortex of WT and HD mice at 4m and 8m. In HD mice's striatum, UCP2 decreased at 8m ($p<0.05$), UCP4 decreased at 4m and 8m ($p<0.05$), SOD2 dropped at 4m but rose at 8m ($p<0.05$). In HD mice's cortex, UCP2 increased at 8m ($p<0.05$), UCP4 increased at 4m and decreased at 8m ($p<0.05$), SOD2 showed no difference. We have previously shown that BDNF increased SOD2 expression and reduced ROS levels in rat astrocytes exposed to 3-nitropopionic acid, a toxic model of HD. We now tested BDNF's impact on ROS (DCFH-DA assay) and SOD2 expression (Western blot) in WT and HD mice striatal and cortical astrocytes. BDNF reduced ROS in HD striatal astrocytes ($p<0.05$) and increased SOD2 in both WT and HD striatal astrocytes ($p<0.05$). Oxidative stress indicators are evident in the striatum of HD mice where UCP2, UCP4, and SOD2 expressions are altered. In the cortex of HD mice, which experiences delayed disease features, alterations are evident at 8 months. BDNF notably reduces ROS in HD striatal astrocytes, potentially through increased SOD2 expression. BDNF's protective role against oxidative stress in HD could unveil novel therapeutic targets.

236. 152. **NEUROPROTECTIVE EFFECT OF POTATO PEEL POLYPHENOLS ON GLUTAMATE-INJURED NEURONAL CELLS**

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Oxidative stress is associated with many pathologies, such as neurodegenerative diseases, and polyphenols are antioxidants that could prevent this stress. Potato peel waste (PPW) is an abundant leftover from the potato processing industry. We have previously identified antioxidant polyphenols in PPW ethanolic extracts, such as chlorogenic acid, cafeic acid, and ferulic acid. This study aimed to analyze the neuroprotective effect of PPW *in vitro* on a neurodegenerative cell model and evaluate the mechanisms involved. To test the neuroprotective activity, we assayed subtoxic concentrations of PPW extract *in vitro* on neuronal HT-22 cells injured by glutamate. First, we demonstrated that pretreatment with these PPW extracts increased cell viability and protected the cells from glutamate-induced apoptosis. Our results showed that PPW polyphenols restored the $\Delta\psi$mit and ROS levels modified by glutamate. We also observed that the PPW extract pretreatment reduced glutamate-induced lipid peroxidation. These findings suggest that PPW extracts have effective neuroprotective properties *in vitro*, demonstrating that they would be a source of neuroprotective compounds to develop a dietary supplement with beneficial effects on human brain health.

237. 182. **STEVIA VS. SUCROSE CONSUMPTION: INFLUENCE ON THE BEHAVIOR OF JUVENILE RATS**

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In recent years, childhood obesity has grown rapidly in Argentina, following the global trend. This phenomenon is associated with an increase in type 2 diabetes, hypertension, and psychosocial problems. The main source of excess calories comes from sugary drinks that are widely available at low costs. For this reason, artificially sweetened beverages have emerged as an alternative to provide sweet taste with few or no calories. Despite its increasing use, little is known about the impact of sweetener consumption on children. Here we studied the effect of unlimited consumption of a *Stevia Rebaudiana* Bertoni (Stv) decoction in drink (0.5% V/V) in Sprague Dawley rats. Animals were exposed to Stv, sucrose (10% W/V, SAC) or only water (control) during youth. Then, they were tested for elevated plus maze (EPM) and novel object location (NOL) behavioral tests. In addition, TBARS levels and RAGE expression were measured in the prefrontal cortex (PFC) and in the hippocampus (HIP). Unlike the SAC group, the animals of the Stv group did not present differences with respect to the control group in the EPM and NOL tests, indicating that Stv consumption does not produce anxiety or memory alterations. However, in animals with high basal glycemias, only those that drank Stv recognized the novelty in the NOL (ANOVA, $p<0.05$). TBARS levels were not found to be modified by any of the beverages. The expression of RAGE was decreased in the PFC and the HIP of the Stv-group animals, and this decrease was associated with a better performance in the NOL memory test (ANOVA, $p<0.05$). Taken together, these results demonstrate that the consumption of Stv in early stages of development does not produce anxiety or memory alterations as occurs with SAC and, under hyperglycemic conditions, it could be beneficial for cognitive function.

238. 198. **SIGNALLING CASCADES INVOLVED IN THE FK506-DEPENDENT NEURODIFFERENTIATION**

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In previous works, we reported that the immunophilin ligand FK506 (tacrolimus) is a neurodifferentiating agent in vitro and in vivo. In this study, we aimed to evidence the signalling cascades involved in the early steps of neurodifferentiation in a model of mouse neuroblastoma Neuro-2a cells. Because neurons are dependent on insulin stimulation, whose mechanism of action involves the PI3K/AKT and MAPK pathways, the potential influence of this hormone on neuronal differentiation was also evaluated. Cells were incubated in a medium without other trophic factors (including serum) with either 1 μM FK506 or 1 μM retinoic acid (RA), a known differentiating agent. The neurite length was measured at different times. FK506 was more efficient than the standard agent RA, showing ~35% longer neurites after 24 h incubation. Western blots of cell lysates showed that immunophilin-induced profile of phospho-FKBP51 and phospho-FKBP52 isoforms was different for both agents, suggesting dissimilar mechanisms of activation. FK506 was used in the subsequent assays. The presence of 10 nM insulin (simultaneous or preincubated for 24 h) did not affect neurodifferentiation. The cell pretreatment during 5 h with 10 μM UO126, a MEK1-2 inhibitor, shortened the neurite length, implying a key role of the MAPK/ERK pathway. Then, the following inhibitors were assayed: 50 μM PD98059 (PD) (for ERK), 1LY2940022 (LY) (for PI3K), and SP600125 (SP)(for JNK). As it was expected, PD confirmed the previous observation made with UO126

for the involvement of the MAPK/ERK pathway, whereas the PI3K and JNK inhibitors exhibited only a mild effect on neurite outgrowth. Nonetheless, the number of neurites per cell was significantly greater in the presence of the JNK inhibitor. It is concluded that FK506 is a more efficient neurodifferentiating agent than RA, and it is proposed that the FK506 mechanism of action requires the MAPK/ERK pathway, whereas the JNK inhibition appears to favour neuronal arborization.

239. 204. **PRESERVED EXPRESSION OF THE NEURONAL CHLORIDE TRANSPORTER KCC2 AND ITS PHOSPHORYLATION AT SERINE 940 AFTER PROGESTERONE ADMINISTRATION IN AN EXPERIMENTAL MODEL OF SPINAL CORD-INJURY-INDUCED SPASTICITY**

Sol Ferreyra[1], Mariana Rey[2], Florencia Labombarda[3,4], Alberto Yorio[5], Héctor Coirini[2], Susana Gonzalez [1,3]
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Spinal cord injury (SCI) decreases the expression/activity of KCC2, a neuronal transporter involved in chloride homeostasis, promoting the development of spasticity. Our previous work demonstrated that progesterone (PG), a neuroactive steroid, improves functional outcomes and prevents injury-induced neuropathic pain. Here, we study whether PG can preserve the expression of KCC2 in the membrane of spinal motoneurons and the phosphorylation of serine 940 (pKCC2), a critical event for its functional activity. Male rats (SD) underwent spinal transection at T13 level and received daily PG (16 mg/kg sc, during 3 days post-injury, n=18) or vehicule (SCI, sc n=18). Uninjured rats were used as control (C, n=18). Transverse sections (20 µm) of the lumbar spinal cord were used for KCC2 immunohistochemistry and images of were acquired with a confocal microscope. Fluorescence intensity was measured at six points on randomly drawn lines at the motoneuron membrane (A1, B1, A2, B2, A3, and B3) and three linear areas in the neuropil near the motor neurons (C1, C2, and C3). KCC2 intensity of each neuron was defined as follows: KCC2 intensity = (A1 + B1 + A2 + B2 + A3 + B3) /6 - (C1 + C2 + C3) / 3. Total KCC2 and pKCC2 were evaluated in membrane fractions by Western blot. Immunofluorescence analysis showed that PG administration preserved the expression of KCC2 at the plasma membrane of spinal neurons ($p<0.01$ vs SCI; ns vs CTL). SCI animals showed a drastic reduction of pKCC2/KCC2 ratio ($p<0.001$ vs CTL), which was significantly increased in PG-treated animals ($p<.0.05$ vs SCI). After 3 days after PG administration, animals did not develop long-term alterations in the frequency-dependent depression of Hoffman (H) reflex a tool to assess spasticity (8 weeks after injury, at 4 and 8Hz, $p<0.001$ vs. SCI), which was impaired in SCI animals ($p<0.001$ vs C). Our findings add strong evidence to support the use of progesterone-based therapies for preventing SCI-induced spasticity.

240. 219. **GABAERGIC SYSTEM AND GNRH EXPRESSION DURING THE REPRODUCTIVE CYCLE IN THE PLAINS VIZCACHA, *Lagostomus maximus***

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The plains vizcacha, a rodent that inhabits Argentina, shows peculiar reproductive features such as the reactivation of the hypothalamic-pituitary-ovarian axis during pregnancy with follicular recruitment and pseudo-ovulation at mid-gestation. We showed that the hypothalamic gonadotropin-releasing hormone (GnRH) neurons are modulated by glutamate with different results according to the glutamate receptor subtype. Here we studied the relation of the GABAergic system with the hypothalamic GnRH expression during the reproductive cycle. Non-pregnant ovulating (NPO) and non-ovulating (NPNO) and pregnant (early-, mid- and term-pregnant) vizcachas were used (n=3-5/group). The expression and distribution of GAD65/67 and GABAB in the preoptic area (POA) and the median eminence/arquate nucleus (ME/ARC) was studied by immunohistochemistry, and their relation with GnRH expression by immunofluorescence and confocal analysis. GAD immunoexpression was detected in the neuropil of both POA and ME/ARC regions. The POA showed significant increased expression of GAD in the NPO, early-, and mid-pregnant animals, while the ME/ARC of NPNO and early-pregnant vizcachas showed increased expression than the other groups. In addition, close contacts of GAD immunoreactive terminals were observed surrounding GnRH neurons of the POA. Strikingly, redistribution of GAD was observed in NPO and mid-pregnant animals with conspicuously more expression around the vessels of the primary capillary plexus of the ME where GnRH axonic varicosities arrive. GABAB was determined in neurons of the ME/ARC located among GnRH axonal varicosities with radial distribution related to the third ventricle. However, GABAB expression was almost undetectable in the POA. In the ME/ARC, NPNO, early-, and mid-pregnant animals showed increased GABA-B expression. These results indicate variations of the GABAergic system throughout the reproductive cycle of the female vizcacha with a possible functional impact on GnRH neurons.

241. 235. **MITOCHONDRIAL OXIDATIVE METABOLISM IN MOUSE BRAIN CELL PRIMARY CULTURES**

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Glucose is the main source of energy for mammalian cells. Once glucose is transported to the cell, it is oxidized through glycolysis and Krebs cycle. Regarding brain energy metabolism, it is known that this organ is one of the most energetically expensive, showing a great neuronal dependence on obtaining energy through mitochondrial oxidative metabolism. Culture conditions play an important role in primary cultures from animal experimental models. However, chemical (composition of the medium, buffering system, pH) or physical (O_2 tension, temperature or extracellular matrix) variables are rarely studied, and they may not closely mimic the *in vivo* environment. Hence, the aim of this work is to study the adaptative mitochondrial oxidative metabolism in brain cell primary cultures. Using a fluorescent sensor genetically encoded for NADH and NAD^+, we determined the mitochondrial oxidative metabolism in neurons and astrocytes from cortical brain, as well as in astrocyte-enriched primary cultures. We observed an “anaerobic” glycolytic metabolism in the early stages of neuronal primary cultures, and an adaptive response tending to increase mitochondrial oxidative metabolism in later stages (19 independent experiments; n=4; *p<0,0121, **p<0,0032, ****p<0,0001; ns>0,9999). On the other hand, both astrocyte-enriched cultures and astrocytes co-cultured with neurons showed an “anaerobic” glycolytic metabolism (11 independent experiments; n=5, *p<0,0119; ns>0,9999), as the *in vivo* evidence shows. Future studies should be considered to analyze whether this metabolic adaptation is consistent with *in vitro* synaptic maturation, among other issues. All data in this study were normalized and treated with one-way ANOVA followed by a Bonferroni post-test.

242. 301. **CHRONIC DEPOLARIZATION OF OHC IMPAIRS THE MATURATION PROCESS OF THE MOC SYSTEM**

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The efferent pathway mediated by the medial olivocochlear (MOC) system regulates the excitability of outer hair cells (OHC). In response to sound overstimulation, the MOC system activates nicotinic acetylcholine receptor α9α10, which in turn, activates BK and SK2 channels, helping KCNQ4 to remove K^+, and to restore resting

membrane potential (RMP). Several conditions lead to chronic depolarization by K^+ accumulation (i.e. KCNQ4 impairment), damaging OHC and causing hearing loss. We hypothesized that the KCNQ4 absence, by altering RMP impacts the organization and function of the MOC system affecting the setting of the hearing process.
Using confocal imaging, we evaluated the location of MOC terminals on OHC in *Kcnq4*$^{+/+}$ and *Kcnq4*$^{-/-}$ animals at different stages: immature (2 postnatal weeks (W)), and fully developed (3, 4, and 10W). At mature ages, MOC terminals are exclusively located in the OHC basal domain in WT animals. At 2W, both genotypes possess 32% of synaptic contacts in the lateral domain. Subsequently, all terminals relocated to the basal domain in WT animals. However, in KO ones, 9.5%, 15% and 1.5% of the terminals remained in the lateral domain at 3, 4 and 10W, respectively. Moreover, we detected a decrease in both, the number of synaptic contacts per OHC and their volume, in 4 and 10W KO animals remaining unaltered in WT ones. On the other hand, we analyzed by qPCR the expression of the postsynaptic efferent components located in the MOC synapse. In 4W *Kcnq4*$^{-/-}$ animals, the mRNA expression of α10 subunit decreased 3.5-fold with no changes in α9 subunit; and BK and SK2 decreased 8-fold. However, at 10W, α10 expression returned to WT levels while BK increases 6-fold. These findings show that chronic depolarization affect the efferent innervation development and the expression of its components in OHC, impacting the MOC system function. This contributes to hearing impairment by compromising the precise tuning role exerted by the MOC system on OHC transduction.

243. 354. **THERAPEUTIC EFFECT OF METFORMIN IN EXPERIMENTAL OPTIC NEURITIS**

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In a previous work we have developed an experimental model of primary optic neuritis (NEO) in rats through the microinjection of lipopolysaccharide (LPS) directly into the optic nerve (ON), which reproduces the central hallmarks of primary human NEO. Currently, there are no effective therapies for the treatment of NEO. Beneficial effects of metformin have been demonstrated in several inflammatory diseases of the central nervous system. The objective of this work was to evaluate the effect of the treatment with metformin on the axoglial alterations of the ON and the retina induced by experimental NEO. To do this, adult male Wistar rats were injected with 1 µl of LPS (4.5 µg/µl) in one NO, whereas the contralateral ON was injected with vehicle (sterile saline). A group of animals was treated with metformin (i.p.) (100 mg/kg) at 24 h before and at 2, 4 and 6 days after the injection of LPS or vehicle (preventive treatment). Another group of animals received metformin (100 mg/kg) at days 4 and 6 post-LPS/vehicle (delayed treatment). At 21 days post-LPS/vehicle, the following parameters were analysed: i) visual pathway function (visual evoked potentials (VEPs)), ii) consensual pupillary reflex (CPR), iii) microglia/macrophage reactivity, iv) astrocytic reactivity, v) number of axons, vi) demyelination, and vi) number of retinal ganglion cells (RGCs). LPS induced a significant and persistent decrease in VEPs and RPC amplitude, increased Iba-1 immunoreactivity and ON astrocytosis, demyelination and loss of ON axons, as well as loss of RGCs ($P<0.01$ vs. vehicle). Pre-treatment with metformin significantly prevented the alterations these parameters ($P<0.01$ vs. LPS). Delayed treatment with metformin significantly reversed the decrease in VEPs and RPC amplitudes caused by LPS injection ($P<0.01$ vs. vehicle). In summary, these results suggest that metformin could be considered a new treatment for experimental NEO and a potential therapeutic strategy to treat NEO in humans.

244. 377. **SPHINGOSINE-1-PHOSPHATE SIGNALING IS ESSENTIAL FOR PRESERVING MORPHOLOGY AND FOCAL ADHESIONS OF RETINA PIGMENT EPITHELIAL CELLS**

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Cell-cell interactions between retinal pigment epithelium (RPE) cells provide the retina with a physical and metabolic barrier, the disruption of which characterizes many inflammatory and proliferative retinopathies. However, the underlying causes of this disruption are still ill-defined. We showed that the bioactive sphingolipids sphingosine-1-phosphate (S1P) and ceramide-1-phosphate (C1P) promote migration and inflammation in RPE cells. Using the human RPE cell line ARPE-19, we now analyzed whether S1P regulates cell morphology and RPE monolayer integrity. Inhibiting S1P synthesis with PF543, a sphingosine kinase 1 (SphK1) inhibitor, markedly decreased ARPE-19 cell migration in confluent cultures, without affecting cell survival. Using 50% confluent cultures, to better observe morphological changes, we determined that PF543 treatment promoted a remarkable cell retraction; highly elongated cells, absent in controls, augmented to 34±2% ($p>0.01$), their cell length/width ratio increasing to 5.3, from 1.6 in controls. S1P addition, 1 h after PF543 treatment, restored cell morphology, reducing elongated cells to 8±1.4% ($p>0.01$), suggesting that S1P inside-out signaling is required for preserving cell morphology. In contrast, C1P addition did not restore cell morphology in PF543-treated cells. When we preincubated cells with PF543 and JTE-013, a S1P2 receptor (S1P2) antagonist, before S1P addition, JTE-013 partially blocked S1P restoration of cell morphology. To analyze the mechanisms involved in cell adhesion, we determined distribution of paxillin, a scaffold protein in focal adhesions. While controls showed spot-like paxillin clusters in the cell periphery, these clusters disappeared in PF543-treated cells and were restored after S1P addition. These results suggest that inhibiting S1P synthesis leads to morphological changes and focal adhesion remodeling, and activation of the S1P/S1P2 axis is required for preserving cell morphology and establishing focal adhesions.

245. 409. **INFLUENCE OF ISOLATION ON MOTOR PERFORMANCE IN FEMALE (NFR/wr) MICE, A CONDITION WITH GENETIC SUSCEPTIBILITY TO MOTONEURON DEGENERATION**

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3. Unidad Académica 1, Departamento de Ciencias Fisiológicas, Facultad de Medicina, UBA, Bs. As., Argentina

Amyotrophic lateral sclerosis (ALS) is a fatal motoneuron disease characterized by progressive motor impairment leading to severe disability. ALS shows higher incidence in men and women are older at onset. The Wobbler (wr/wr) mouse is a recognized model of ALS. The autosomal mutation in the wr gene encodes for the vesicular protein sorting (Vps) 54 transport protein and causes motoneuron disease in homozygous condition. Heterozygous mice (NFR/wr) show a healthy phenotype. We postulate that genetic susceptibility to motoneuron degeneration is influenced by stressful situations. We studied the progression of motor performance on the accelerating rotarod in female NFR/NFR or NFR/wr mice at 2 ages (4- and 12-month-old) during 8 weeks. After training, animals were evaluated in the rotarod weekly during 2 weeks. Then, mice were separate in 2 groups during 6 weeks: 1) family or 2) socially isolated. Before sacrifice, isolated mice were subjected to acute stress during 2 hs. All animals were sacrificed during diestrus. We found that family-4-month-old NFR/wr mice showed a better performance than family-NFR/NFR ($p<0.01$) while family 12-month-old, NFR/NFR and NFR/wr, showed a similar performance. With regards to isolation, 2-way ANOVA followed by Tukey post-hoc test showed that NFR/wr ran shorter distance under isolation than family NFR/wr ($p<0.05$) at both ages. Body weight of 4-month-old NFR/NFR mice increased after 8 weeks of evaluation while both ages of NFR/wr showed sim-

ilar values. Adrenal gland mass significantly increased in socially isolated-4-month-old mice. Morphological analysis of neurons located in the ventral horn of the cervical spinal cord indicated that mice remaining in isolation showed smaller neuronal area ($p<0.05$) and a reduction of the number of MnSOD immunoreactive cells ($p<0.05$) than family-raised mice. Our data support a role of epigenetic factors in motor performance, in addition to genetic influence on this behaviour.

O2-NEUROSCIENCES

THURSDAY 16TH NOVEMBER 9:00 - 10:30

CHAIRS: ALEJANDRO VILLARREAL
SANDRA ZÁRATE

246. 21. **IMPACT OF CERAMIDE SYNTHESIS INHIBITION ON GLIAL ACTIVATION AND EXTRACELLULAR VESICLES LIBERATION IN AN EXPERIMENTAL ALZHEIMER'S DISEASE MODEL**

Melina Bellotto1,2, Ángeles Vinuesa1,2, Melisa Bentivegna1,2, Amal Gregosa1,2, Carlos Pomilio1,2, Nicolás González Pérez1,2, Jessica Presa1,2, Flavia Saravia1,2, Juan Beauquis1,2.

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Alzheimer's disease (AD) is the most prevalent neurodegenerative disease and the leading cause of dementia. One of its hallmarks is the deposition of amyloid β (Aβ) in the brain. Also, high levels of ceramides in plasma and CSF of AD patients could have a pro-inflammatory role. Recently, there has been growing interest in the role of small extracellular vesicles (SEVs) in AD. Previously, we reported that SEVs propagate inflammatory stimuli from microglia to neurons. Ceramides are important components of SEVs and can regulate their production. Here, we aimed to study 1) the effects of inhibiting ceramide synthesis in an AD murine model; 2) the impact of Aβ and palmitate (PA), a ceramide precursor, on glial activation and communication *via* SEVs and the regulation by ceramide synthesis. *In vivo*, we administered Myriocin (1 mg/Kg/dose, 9 doses, 3 weeks), an SPT inhibitor, to 8-m-old PDAPPJ20 transgenic mice. We found that transgenic mice performed worse than non-transgenic controls in short-term memory tests (ANOVA, $P<0.05$). *In vitro*, we treated BV2 microglia with Aβ1-42 peptides (0.5 μM) and studied the expression of IL1β and TNFα *via* RT-qPCR. Aβ increased cytokine expression (ANOVA, $p<0.005$). In parallel, we analyzed SEVs-enriched fractions derived from the conditioned media (CM) of Aβ- or PA-treated BV2 microglia by flow cytometry. Results suggest that PA induces SEVs secretion. Treatment of C6 astrocytes with PA failed to induce IL1β expression. However, CM from PA-exposed microglia did ($p<0.001$), and this effect was absent with a Cambinol pre-treatment, an inhibitor of nSMase2. Our results suggest a role for ceramide synthesis on 1) the cognitive status of PDAPPJ20 mice, model of AD, and 2) the induction and propagation of inflammation between glial cells, possibly through SEVs. In future experiments, we aim to determine the role of ceramides in Aβ-associated neurodegeneration.

247. 28. **PIAS4, A NOVEL REGULATOR OF TAU HOMEOSTASIS**

María Clara Sokn[1, #], Angel Ramón Torres Mc Cook[1, #], Vanina Giselle Velardo[1], Romina Paula Gobbini[1], Alejandra Attorresi[2], Sergio Senin[2], Eduardo Arzt[2], Tomás Falzone[2, 3], Ana Clara Liberman[1]

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Tauopathies are neurodegenerative diseases characterized by dysregulation of tau homeostasis. Accumulating evidence points to an important role of the PIAS SUMO ligases as key regulators of several proteins involved in neurodegeneration. The aim of this work was to evaluate the role of the PIAS family in the regulation of tau homeostasis. Using a Western blot (WB)-based screen in HT22 cells overexpressing human 2N4R WT tau (hTau) together with the PIAS family members, we observed a robust increase in total tau induced exclusively by PIAS4 (+0.75, $p<0.001$). In addition, we monitored tau dimerization using the bimolecular fluorescence complementation sensor Tau-BifC. Overexpression of PIAS4 increased the mean percentage of $BifC^+$ cells (+2.2, $p<0.0001$) as well as their mean fluorescence intensity (+0.28, $p>0.0001$), indicating that the enzyme promotes tau dimerization, a process implicated in pathological tau deregulation. N2a cell lines stably expressing endogenous levels of hTau (N2a_WT hTau) or SUMO mutant tau (N2a_K340R hTau) were used to complement the above methods. PIAS4 promoted not only WT hTau (+0.63, $p<0.0001$) but also hTauK340R accumulation (+0.46, $p<0.001$), suggesting that the enzyme activity on tau does not involve direct tau SUMOylation. Consistently, our niquel purification assays of his-tagged SUMO proteins confirmed that PIAS4 was unable to induce SUMO conjugation to tau. Furthermore, silencing PIAS4 with two different sh-RNA vectors significantly decreased total tau protein levels (-0.53, $p<0.01$ and -0.46, $p<0.05$, respectively) suggesting that PIAS4 may regulate tau degradation. Finally, we analyzed the effect of PIAS4 overexpression and silencing on autophagic flux, a cellular mechanism involved in the clearance of tau, using both WB and LC3/p62 immunofluorescence. PIAS4 overexpression inhibited autophagic activity, whereas PIAS4 silencing promoted it. We propose that PIAS4 is an autophagy modulator that regulates tau clearance.

248. 106. **REACTIVE ASTROCYTES DISPLAY DEFICITS IN ENERGY METABOLISM AND INSULIN SIGNALING IN AN EXPERIMENTAL MODEL OF ALZHEIMER'S DISEASE**

Melisa Bentivegna[1,2], Amal Gregosa[1,2], Daiana Vota[2], Carlos Pomilio[1,2], Ángeles Vinuesa[1,2], Nicolás González Pérez[1,2], Jessica Presa[1,2], Melina Bellotto[1,2], Flavia Saravia[1,2], Juan Beauquis[1,2]

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2. Dpto. de Química Biológica, Facultad de Ciencias Exactas y Naturales, UBA.

Alzheimer's disease (AD) is the most prevalent neurodegenerative disease and the leading cause of dementia. Besides amyloid beta (Aβ) and tau aggregation, inflammation and insulin resistance are common findings in AD brains. Astrocytes regulate these processes, maintaining homeostasis and coordinating inflammatory responses. Here, our objectives were 1) to study the metabolic and inflammatory status of PDAPP-J20 transgenic (TG) mice, model of AD, and 2) to evaluate inflammatory activation, energy metabolism and insulin signaling in astrocytes *in vitro* upon Aβ exposure. We hypothesized that Aβ-treated astrocytes adopt a proinflammatory phenotype and lose homeostatic functions. Insulin signaling (pAkt/Akt) was impaired in the hippocampus ($p<0.05$) but not in the hypothalamus or the liver, suggesting a region-specific deregulation. Also, hippocampal insulin receptor (IR) levels (immunoblot) were decreased ($p<0.05$). Immunolabelling for IR showed a tendency to decrease in GFAP+ astrocytes, that also displayed increased GFAP and S100b labelling ($p<0.05$), suggesting proinflammatory reactivity. Then, we evaluated the effect of Aβ on primary astrocytes *in vitro* and found increased NFkB nuclear translocation and S100b expression ($p<0.0001$). Immunofluorescence of IR showed higher overall optical density, though a lower membrane localization ($p<0.05$). AKT phosphorylation was found increased after Aβ exposure. Also, the number of mitochondria was decreased with increased superoxide production ($p<0.001$). The lipid droplets density was decreased but colocalized more with mitochondria ($p<0.01$), suggesting a metabolic switch to β-oxidation. Our results suggest that hippocampal insulin resistance and glial reactivity are concurrent events in experimental AD. Accordingly, Aβ-exposed astrocytes adopt a pro-inflammatory phenotype associated with enhanced insulin signaling and

mitochondrial defects. The study of these interrelated phenomena could help to understand AD pathophysiology.

249. 215. **INVOLVEMENT OF MICROGLIA ON THE LONG -TERM CONSEQUENCES OF EARLY LIFE STRESS IN THE VISUAL SYSTEM**

Juan Salvador Calanni[1], Laura Andrea Pasquini[2], Hernán Hugo Dieguez[1], Nathaly Bernal Aguirre, Damian Dorfman[1], Ruth Estela Rosenstein[1]

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Early life stress (ELS) is defined as a period of severe and/or chronic trauma, as well as environmental/social deprivation or neglect in pre/postnatal stage. Presently, the impact of ELS on the visual system in the adult stage is unknown. Using an animal model of maternal separation with early weaning (MSEW), which mimics early life neglect, we analyzed the long-term ELS consequences in the visual system. Mice were separated from the dams for 2 h at postnatal days (PNDs) 2-5, for 3 h at PNDs 7-9, for 4 h at PNDs 10-13, for 6 h at PNDs 14-16, and weaned at PND17. At the end of each separation period, mothers were subjected to movement restriction for 10 min. Control pups were left undisturbed from PND0, and weaned at PND21. At PND 60-75, MSEW did not affect the electroretinogram a- and b-wave amplitude, but decreased retinal ganglion cell (RGC) function and number, and increased retinal Iba-1(+) area, and cell soma size (by immunohistochemistry, $P<0.01$), consistently with an increased number of amoeboid microglial cells. At PND35, MSEW did not affect microglial and RGC number and function, whereas at PND45 microgliosis preceded RGC loss, supporting a key role of microglia in visual function alterations induced by ELS. To investigate this hypothesis, microglial depletion was induced by a treatment with 200 mg/kg of Sotuletinib (BLZ945), a Colony Stimulating Factor Receptor (CSF-1R) inhibitor was orally and daily administered from PND35 to PND60. BLZ945 alone did not affect the number or function of RGCs, but it significantly mitigated RGC function and number loss in MSEW mice at PND 60 ($P<0.01$). In summary, our results suggest that microglial cells could play a key role in long term consequences of early life stress on the visual system of adult mice.

250. 604. **NEURODEVELOPMENTAL ALTERATIONS IN FEMALE WISTAR RATS: LONG TERM-EFFECTS OF MATERNAL HYPERTHYROIDISM AND PRENATAL STRESS**

María Cecilia Michel Lara[1,2], María Belén Sánchez[1,3], Flavia Judith Neira[1,2], Luciana Belén Viruel[1,3], Pitton Josefina[1,3], Elisa Olivia Pietrobon[1,4], Marta Soaje[1,5], Graciela Alma Jahn[1], Juan Pablo Mackern-Oberti[1,5] and Susana Ruth Valdez[1,6]

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Thyroid hormones (THs) [thyroxine (T4) and triiodothyronine (T3)] play vital roles in physiological functions and are crucial for normal fetal brain development. THs can pass from mother to fetus during gestation. Hyperthyroidism (HyperT), resulting from excessive TH production, can lead to maternal, fetal, and lactational complications during pregnancy. Prenatal stress (PS) has been linked to changes in the structure and function of offspring brains, potentially contributing to neurodevelopmental and mental disorders. PS can also affect thyroid hormone and corticosterone (C, stress hormone in rats) levels, impacting reproductive health. We studied the long-term effects of the interaction between HyperT and PS on female offspring development. Maternal Wistar rats were divided into four groups: control (normal thyroid, Co), HyperT (administration of T4, 0.1 mg/kg/day doses, subcutaneous, throughout the protocol), Chronic Unpredictable Moderate Stress (CUMS), and HyperT+CUMS. CUMS was applied from gestational days 6 to 17. Female offspring were raised in standard conditions, and developmental parameters were assessed from birth to postnatal day (PD) 42. At adulthood (PD85-100), behavioral tests (OFT, EPM, FST) were conducted to evaluate the locomotor activity and anxious-depressive states. Truncal blood and adrenal gland (AG) histology were collected on PD110. One-way ANOVA statistical analysis was performed. Interaction between HyperT and PS led to reduced birth weight ($p<0.01$) and length ($p<0.001$) in offspring. HyperT+CUMS pups exhibited delayed eye-opening ($p<0.05$), incisor eruption ($p<0.001$), auditory startle reflex appearance ($p<0.001$), and aversion to heights ($p<0.001$). In adulthood, HyperT+CUMS rats displayed increased exploratory behavior in the OFT: greater distance traveled ($p<0.001$), entries to interest areas ($p<0.001$), entries ($p<0.01$) and time in the center ($p<0.001$). These rats also exhibited disinhibited behavior in risky situations in the EPM: increased entries ($p<0.05$) and time in open arms ($p<0.01$). Conversely, HyperT led to behavioral despair in the FST, evidenced by increased immobility time ($p<0.05$). C levels were lower in HyperT rats on PD110 ($p<0.01$), and both HyperT and HyperT+CUMS groups showed reduced AG fascicular zone area (C synthesis) ($p<0.001$). This suggests that the interaction between HyperT and PS could impact female offspring's physical and neurological development, potentially leading to behavioral disorders in adulthood.

P2-NEUROSCIENCES

THURSDAY 16TH NOVEMBER 14:00-15:30

CHAIRS: RUTH ROSENSTEIN
ALICIA ROSSI
LEONARDO DIONISIO

251. 85. **GENISTEIN (GEN) AMELIORATES NEUROPATHOLOGY, GLIAL ACTIVATION AND DYSFUNCTION IN A RAT MODEL FOR METABOLIC SYNDROME (MS)**

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Metabolic Syndrome (MS) is the medical term for the combination of at least three of the following factors: obesity, hyperlipidemia, hyperglycemia, insulin resistance and hypertension. The spontaneously hypertensive rat (SHR) is an accepted animal model for the study of human MS that reveals all the features of the syndrome when fed high-fat, high-carbohydrate diets. We have previously shown that this model for MS shows increased reactivity of both microglia and astrocytes in several brain regions, decreased neurogenesis in the hippocampus and reduced recognition memory. Genistein (GEN) is a phytoestrogen which is known to have neuroprotective actions. To elucidate if it exerts neuroprotective effects in MS we treated 25-week-old MS rats for two weeks with 10mg/kg daily s.c. injections of GEN. We found that MS rats showed a decreased number of DCX+ neural progenitors in the dentate gyrus and treatment with GEN increased this parameter. Expression of GFAP was increased in different brain regions (HC and PFC) and treatment decreased astrogliosis in all of them. We measured the expression of IBA1+ microglia in the same regions and classified microglia according to their morphology: we found that MS rats presented an increased proportion of the hypertrophied phenotype and GEN produced a shift in microglial phenotypes towards a ramified type. Furthermore, we evaluated the cognitive abilities of the MS rats by using the novel object recognition test (NOR), the Y-maze and the Object Location Task (OLT). MS rats showed cognitive impairments in all of these

tests and treatment with GEN showed significant improvements in the NOR, OLT and continuous Y-maze. These results indicate that GEN was able to exert neuroprotective actions increasing neurogenesis and improving cognitive impairments while decreasing astrogliosis and microgliosis in MS rats. Together, these results open an interesting possibility for proposing this phytoestrogen as a neuroprotective therapy for MS.

252. 87. **EARLY SIGNS OF NEUROINFLAMMATION IN THE POSTNATAL WOBBLER MOUSE MODEL OF AMYOTROPHIC LATERAL SCLEROSIS**

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1 Laboratory of Neuroendocrine Biochemistry, Instituto de Biología y Medicina Experimental-CONICET, Obligado 2490, 1428 Buenos Aires, Argentina
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3 Department of Human Biochemistry, Faculty of Medicine, University of Buenos Aires, Paraguay 2155, 1425 Buenos Aires, Argentina

The Wobbler mouse is an accepted model of sporadic amyotrophic lateral sclerosis. The spinal cord of clinically symptomatic animals (3–5 months old) shows vacuolar motoneuron degeneration, inflammation, and gliosis accompanied by motor impairment. However, data are not conclusive concerning pathological changes appearing early after birth. To answer this question, we used postnatal day (PND) 6 genotyped Wobbler pups to determine abnormalities of glia and neurons at this early age period in the spinal cord. We found astrogliosis, microgliosis with morphophenotypic changes pointing to active ameboid microglia, enhanced expression of the proinflammatory markers TLR4, NFkB, TNFalfa, and inducible nitric oxide synthase. The astrocytic enzyme glutamine synthase and the glutamate-aspartate transporter GLAST were also reduced in PND 6 Wobbler pups, suggesting excitotoxicity due to impaired glutamate homeostasis. At the neuronal level, PND 6 Wobblers showed swollen soma, increased choline acetyltransferase immunofluorescence staining, and low expression of the neuronal nuclear antigen NeuN. However, vacuolated motoneurons, a typical signature of older clinically symptomatic Wobbler mice, were absent in the spinal cord of PND 6 Wobblers. The results suggest predominance of neuroinflammation and abnormalities of microglia and astrocytes at this early period of Wobbler life, accompanied by some neuronal changes. Data support the non-cell autonomous hypothesis of the Wobbler disorder, and bring useful information with regard to intervening molecular inflammatory mechanisms at the beginning stage of human motoneuron degenerative diseases.

110. **BSY12: A PROMISING MOLECULE FOR NEUROLOGICAL DISORDERS**

Milagros Bürgi[1,2], Camila Scorticati[3,4], Matías Depetris[2,5] , Ricardo Kratje[1,2], Marcos Oggero[1,2]
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Neurological disorders (ND) include a wide range of pathologies affecting people´s life quality. In 2019, 1.5 billion people worldwide were diagnosed with some ND, which are expected to increase about 12% by 2030. Nowadays there are no effective treatments to slow their progression. Erythropoietin (EPO) represents a promising candidate considering its neurobiological action (NA). Nevertheless, its erythropoietic activity (EA) triggers undesirable effects. Consequently, we developed an EPO-derivative (BSY12) through glycoengineering to block the EA while preserving the NA. BSY12 was produced by CHO.K1 cells, immunoaffinity chromatography-purified and widely characterized. The protein structure and stability were evaluated by circular dichroism and thermal shift analysis. The EA, analyzed as the hematocrit increment, was evaluated in normocytic BALB/c mice. The BSY12 antiapoptotic capacity was studied in primary cultures of hippocampal neurons. Additionally, a Sholl analysis in CA1 pyramidal neurons of the hippocampus from BSY12-treated CF1 mice were carried out. The ability to cross the bloodbrain barrier (BBB) was studied in CF1, C57Bl/6N and SOD1G93A mice. The in vivo neurobiology performance was evaluated through the Complex Running Wheels test in healthy C57Bl/6N mice and in a model of amyotrophic lateral sclerosis using SOD1G93A mice. BSY12 presented a partially-folded structure. The EA was blocked but its NA was preserved taking into account that in hippocampal neurons, BSY12 reduces their in vitro staurosporine-induced apoptosis and increases their total dendrite extension and dendritic brunch intersections in vivo ($p<0,05$). BSY12 showed an improvement in motor-cognitive performance in healthy animals ($p<0,0001$) as well as a benefit in motor ability observed in SOD1G93A mice until an advanced stage of the pathology ($p<0,1$). In conclusion, these preliminary assays pave the way to validate BSY12 as a promising molecule for different models of ND.

253. 210. **HMGB1, TLR2, AND TLR4 EXPRESSION IN CELLULAR MODELS OF HUNTINGTON'S DISEASE**

Mateo Palmieri, Federico López Couselo, Julieta Saba, Diego Rivas, María Friser Frederiksen, Lila Carniglia, Daniela Durand, Mercedes Lasaga, Carla Caruso.
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Huntington's Disease (HD) is a neurodegenerative genetic disorder caused by a CAG repeat expansion in the huntingtin gene that generates motor, cognitive, and psychiatric symptoms in humans. 3-nitropropionic acid (3NP) is a toxin that generates mitochondrial dysfunction like HD. In glial cells, oxidative stress can cause an inflammatory response. HMGB1 is a nuclear protein that regulates gene expression and participates in DNA repair. HMGB1 can bind to toll-like receptors (TLR) and trigger an inflammatory response. We investigated the expression of HMGB1 and its receptors in zQ175 knock-in mouse model of HD (HD mice). The expression of HMGB1 and its receptors was determined in cultures of astrocytes and microglia from WT and HD mice striatum and cortex. By immunocytochemistry, we demonstrated that the expression of HMGB1 and TLR4 increases in cortical HD microglia ($p<0.05$) but not in untreated HD astrocytes, while the expression of TLR2 in both cell types remains unchanged. We evaluated the expression of C1qA only in microglia and WT and HD cortex microglia showed no differences in its expression. Our findings show that serum deprivation increases the expression of HMGB1 in astrocytes and HD microglia ($p<0.05$), while exposure to 3NP only increases HMGB1 levels in HD microglia ($p<0.05$) protein levels were determined in cortical astrocytes by western blot. In striatal cultures, we demonstrated that the expression of HMGB1 increased both in HD microglia and astrocytes ($p<0.05$), while the expression of TLR2 and TLR4 in astrocytes and microglia remains unchanged. Therefore, these results indicate that HMGB1 and its receptor TLR4 increase in HD glia and that this effect could be related to inflammation through the regulation of HMGB1 in microglia, while TLR2 and C1qA would not play a significant role in HD-related inflammation. These findings could enhance our understanding of HD and identify new therapeutic targets for this neurodegenerative disease.

254. 220. **EFFECT OF THE NOVEL AUTOSOMAL DOMINANT ALZHEIMER DISEASE VARIANT PSEN1 T119I ON Aβ SECRETION**

Luciana Isaja[1], Diego Cifarelli[1], Giulia Clas[2], Sofía Mucci[1], María Soledad Rodríguez Varela[1], Mariela Marazita[1], Patricio Chrem-Méndez[3], Ricardo Francisco Allegri[3], Gustavo Emilio Sevlever[1], María Elida Scassa[1], Ezequiel Ignacio Surace[2], Leonardo Romorini[1]
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2. Laboratorio de Enfermedades Neurodegenerativas, Instituto de Neurociencias (LEN-INEU-Fleni- CONICET), Buenos Aires, Argentina.
3. Centro de Memoria y Envejecimiento, Fleni

Mutations in the Presenilin-1 (*PSEN1*) gene are the most frequent cause of familial Alzheimer's disease (AD). We previously described the *PSEN1* T119I variant and generated a human induced pluripotent stem cell (hiPSC) line (FFAD1) from a carrier. To understand its implications on AD, we aimed to validate *PSEN1* T119I's impact on Aβ isoform secretion. Firstly, we generated a *knock-out* (KO) of *PSEN1* in HEK293T cells using CRISPR/Cas9 gene edition. We confirmed the absence of endogenous *PSEN1* protein expression in the selected clone by western blot. Next, we co-transfected HEK293T *PSEN1* KO cells with plasmids containing *wild-type* (WT) or mutant *PSEN1* variants (T119I and A246E), APP, and GFP. After 2 days, we measured transfection efficiency by flow cytometry of GFP+ cells and performed ELISA assays to quantify the Aβ 42 and 40 production of the cells. Secondly, we differentiated UOWi002-A (*PSEN1* WT), FFAD1 (*PSEN1* T119I) and UOWi003-A (*PSEN1* A246E) hiPSCs into cortical neurons for 70 days using a 2-dimensions based specific differentiation protocol. We verified the cortical phenotype of the neurons obtained by immunocytochemistry, flow cytometry and RT-qPCR of neural and cortical lineage markers (*TUJ-1*, *MAP2, TBR1*, *TBR2* and *CTIP2*). On day 70, we collected the pellets and supernatants and performed ELISA assays to quantify Aβ 42 and 40 isoforms. In both approaches,*PSEN1* A246E and T119I increased the Aβ42/40 ratio compared to wild-type *PSEN1*, although*PSEN1* T119I exhibited an intermediate phenotype. Overall, our results support the pathogenic role of *PSEN1* T119I variant on Aβ isoform secretion.

255. 225. **ASTROGLIAL RESPONSE IN A CONTEXT OF BRAIN EDEMA USING AN EXPERIMENTAL MODEL OF "COLD-INJURY"**

Emilia Frischknecht[1], Camila Vidos[1], Alberto Javier Ramos[1], Alejandro Villarreal[1,2]
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Astrocytes respond to Brain injury through a process of reactive astrogliosis increasing in size and morphological complexity, losing homeostatic functions and showing transcriptional changes. Reactive astrogliosis is a progressive phenomenon and can achieve time-stable phenotypic changes. Brain edema, which is an increase in interstitial water content, is an early event after many different brain injuries and pathologies. We hypothesize that transcriptomic changes underlying astrocyte reactive phenotypes in a context of brain edema are regulated by epigenetic mechanisms of histone posttranslational modifications. We here aimed to characterize astrocyte response in a model of cold injury. Such a paradigm of brain edema is promoted by localized cold exposure of only one brain hemisphere without direct physical disruption of neither skull, meninges nor nerve tissue. We conducted our studies in adult female and male mice (C57/4-5 months). The "non-exposed" (contralateral) hemisphere was used as an immunoreactivity base-line for comparisons. Using immunofluorescent co-labeling followed by epifluorescence and confocal microscopy we addressed immunoreactivity for astrocyte marker GFAP (glial fibrillary acidic protein), AQP4 (aquaporin 4) and C3 (complement 3). Our results showed an increase in GFAP immunoreactivity at 1DPL (days post lesion) which remained for 3 and 7 DPL. AQP4 immunoreactivity showed only to be increased at 1DPL. We further observed an increase in C3 immunoreactivity but, interestingly, with no colocalization with GFAP.
We conclude from this preliminary work that the cold-injury model of brain edema promotes a progressive astroglial response and results suitable for addressing epigenetic changes in reactive astrocytes at different time points of reactive astrogliosis. It is of note that this model will allow addressing cellular response in a context of brain edema, a pathological condition of high clinical relevance.

256. 329. **EXPLORING CDK5's ROLE IN THE REGULATION OF GENE EXPRESSION IN ROTENONE-STRESSED HUMAN PLURIPOTENT STEM CELLS-DERIVED NEURONS**

Mucci, Sofía; Rodríguez-Varela, Soledad; Allio, Camila Paola; Clas, Giulia; Isaja, Luciana; Sevlever, Gustavo; Scassa, María; Romorini Leonardo.
Laboratorio de Investigaciones Aplicadas a Neurociencias, Instituto de Neurociencias (FLENI-CONICET).

CDK5 was described as one of the molecular keys involved in neuronal development and homeostasis. Although several studies provide support for the involvement of CDK5 in neuronal homeostasis, the exact function of CDK5 remains elusive. Thus, aiming to understand the participation of CDK5 in rotenone-induced neuronal stress, we isolated RNA from control and rotenone-treated (1 μM, 24h) H9-human pluripotent stem cells (hPSCs)-derived neurons (n=3) and performed an RNA-Seq analysis. We ran the DESeq2 package in R Studio to identify the differentially expressed genes (DEGs) (alpha = 0.01). From the 43 DEGs detected, *CYGB*, *DUSP10*, *EDNRA*, *GDF15*, *JDP2*, *NHLH2*, *POU4F2* and *UNC5C* were selected according to their biological function and the *in-silico* reading levels, and validated by RT-qPCR in control and rotenone-treated H9 and FN2.1 hPSCs-derived neurons. Then, using FN2.1 *CDK5*$^{+/-}$ and *CDK5*$^{-/-}$ hPSCs lines generated by CRISPR/Cas9, we evaluated the mRNA levels of validated DEGs by RT-qPCR in neurons exposed or not to rotenone. Our results demonstrate that in untreated-hPSCs the basal expression levels of *GDF15*, *EDNRA*, *NHLH2*, and *UNC5C* are impacted by the absence of one allele of *CDK5*, and *CYGB*, *DUSP10*, *JDP2,* and *POU4F2* need complete CDK5 absence to change their basal expression relative to FN2.1 WT neurons. Regarding rotenone treatment, *CYGB*, *DUSP10*, *JDP2* and *POU2F4* transcripts lost their regulation in FN2.1 CDK5$^{-/-}$ neurons. *EDNRA*, *GDF15* and *UNC5C* mRNA levels regulation was affected in FN2.1 *CDK5*$^{+/-}$ and *CDK5*$^{-/-}$ derived-neurons. Finally, *NHLH2* expression levels did not vary between treated and untreated neurons in all tested neuronal lines. In conclusion, CDK5 directly or indirectly regulates the expression levels of some genes in rotenone-mediated stress in hPSCs-derived neurons.

257. 476. **ANALYSIS OF PERIPHERAL LEVELS OF mGlu3R IN HUMANS WITH MILD COGNITIVE IMPAIRMENT: AN APPROACH TOWARDS NOVEL ALZHEIMER'S BIOMARKERS**

Albany Sáez[1], María Micaela Castro[2], Eugenia Olivera[1], Pilar Garaventa[1], Romina Pavón[3], Alejandro David Moroni[2], Natalia Menite[2], Gastón Villafañe[2], Ana Clara Romero[1], Julieta Saba[1], Lila Carniglia[1], Mercedes Lasaga[1], Carla Caruso[1], María Laura Palumbo[2], Daniela Durand[1]
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Subtype 3 metabotropic glutamate receptor (mGlu3R) has a broad range of neuroprotective effects. Previously, we demonstrated that astroglial activation of mGlu3R protects hippocampal neurons from Aβ toxicity through both neurotrophin stimulation and Aβ clearance. Using the PDAPP-J20 Alzheimer's transgenic model, we showed that mGlu3R levels significantly decrease in hippocampus, predisposing to a state of neurotoxicity. Given this, we aim to determine if mGlu3R can be detected in human serum to be used as a new biomarker for cognitive impairment. Mild cognitive impairment (MCI) is an early stage of memory loss or other cognitive decline, which may progress to dementia, and constitutes a strategic time window for accurate diagnosis and therapeutic intervention. Individuals from Junín, Buenos Aires, aged 70.46±7.82 years were classified through various neurocognitive tests into Control and MCI groups (n=5-8). Blood samples were taken after informed consent, serum was isolated and Western blots for mGlu3R and Aβ were performed. Interestingly, mGlu3R could be detected in the serum of both groups.

We found a 34.5% decrease in the levels of the dimeric form of the receptor in the MCI group (p<0.05). The monomeric form of mGlu3R did not differ between groups (p=0.44). For Aβ, we detected different molecular weight bands. Levels of the 40kDa band were 26.9% lower in the MCI group (p<0.05). Bands around 25 kDa not significantly decreased in the MCI group (22.6%, p=0.22). Furthermore, correlation between mGlu3R and Aβ levels showed significant positive correlation only in the MCI group (Pearson r=0.9422, p<0.05 in MCI; Pearson r= -0.015, p=0.97 in control). In brief, based on the neuroprotective functions of mGlu3R and its dysregulation in AD mice, detecting mGlu3R in human serum opens a door for its study as a biomarker of neurodegeneration. The present results encourage the expansion of the cohort of individuals, in order to evaluate whether this pattern is repeated.

258. 480. **CRITICAL WINDOWS OF EARLY-LIFE FLUOXETINE-INDUCED RAT JUVENILE SOCIAL INTERACTION DEFICIT**

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Social interaction is a salient feature of humans for which our brain is highly specialized. Although some neurodevelopmental disorders exhibit social interaction deficits, little is known about their neurobiology. Interestingly, hyperserotonaemia has been hypothesised to underly social deficits in autism spectrum disorders. Preclinical studies have shown that early-life exposure to fluoxetine, a serotonin selective uptake inhibitor, produced abnormal emotional behaviours in rodents. However, these studies have not determined whether early-life exposure to fluoxetine disrupts juvenile social behaviour or if there is a specific time window for such disruption. Here, we addressed this gap by administering fluoxetine (sc.; 10 mg/kg) or saline to male Wistar rats during one of three putative critical windows: the postnatal (PN; P4-11), pre-weaning (PreWean; P12-19), or weaning (Wean; P20-27) developmental periods and assessed in the juvenile period (P30-40) the effects on locomotor activity and social and anxiety-related behaviours. Regardless of time of exposure, distance travelled or time spent in the centre or periphery of the open field remained unaltered. Wean treatment decreased the time spent in the open arms of the elevated plus-maze. Social behaviour was measured as pinning in an unfamiliar group-matched pair paradigm. PN group had longer pinning bouts, but similar latency to the first pinning and number of events. PreWean showed a tendency to increased latency to the first pinning but similar duration and number. Weaning group showed fewer pinning bouts, and a tendency to increased latency and duration. Our results demonstrate that Fluoxetine exposure during early-life has enduring effects into juvenile social behaviour, especially when exposure takes place around weaning time. These findings indicate a critical role of serotonin in the maturation of brain systems that modulate social behaviour and suggest novel targets for the treatment of social deficits.

259. 515. **ADMINISTRATION OF ANASTROZOLE, AN AROMATASE INHIBITOR, REDUCES MYELIN PROTECTIVE EFFECTS OF TESTOSTERONE IN THE SPINAL CORD OF A MURINE MODEL OF MOTONEURON DISEASE**

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Patients with amyotrophic lateral sclerosis (ALS) suffer muscle limb atrophy linked to degeneration of motoneurons. The Wobbler (WR) mouse, a model of ALS, exhibits motoneuron degeneration, astrogliosis and microgliosis in the cervical spinal cord (CSC). Previously, we demonstrated protective effects of testosterone (T) in male WRs. T exerts its effects directly or after metabolizing into dihydrotestosterone or aromatization into estrogens. Here, we explored the effects of the coadministration of T + anastrozole (A), an aromatase inhibitor, to WRs focusing on: 1) myelin status, assessed through semithin sections and electron microscopy, 2) mRNA expression of inflammatory factors, and 3) glutamate metabolism through the analysis of glutamine synthetase (GS) and GLT1, the major glutamate transporter, expression. T was administered via 10mm silastic tubes during 2 months. Anastrozole was dissolved in 10% DMSO and administered through Alzet osmotic micropumps (1mg/kg/day) s.c, starting 1 week before T. Four groups were constituted: a) WRs or (b) controls + empty silastic tubes + vehicle-filled pumps, c) WR+T (silastic tubes containing T) + vehicle-pumps, and d) WR+T+A. Regarding myelin status, control and WR+T showed compact myelin sheaths and lower g-ratios (inner axon diameter/total diameter of axon + myelin sheath) than WR and WR+T+A (p<0.001). WR and A+T treated-WRs showed detachment and rupture of myelin lamellae. Inflammatory factors were significantly increased in WRs (p<0.05 vs. control). T reduced their mRNA expression, persisting low in WR+T+A (p<0.0001 for IL18, p<0.01 for TLR4, TNF alpha receptor 1, and P2Y12R vs WR). However, GLT-1 mRNA and immunoreactivity as well as GS cell density were reduced in WR and WR+T+A (p<0.01 vs. controls), but elevated in WR+T (p<0.01 vs WR). Thus, T aromatization may play a role in myelin protective effects of T. In conclusion, T may be considered as a promyelinating therapeutic strategy for neurodegenerative diseases.

260. 524. **HISTONE ACETYLATION CHANGES AND ASTROCYTIC RESPONSE IN A CONTEXT OF BRAIN EDEMA**

Matías Monteverde Busso[1], Camila Vidos[1], Azul Millán[1], Lucas Manavela[1,2], Lourdes Florencia Gonzales Branchi[1,2], Alberto Javier Ramos[1], Alejandro Villarreal[1,2]

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Astrocytes respond to brain injury through a process of reactive astrogliosis involving transcriptional, phenotypic, and functional changes. Astrocyte functional changes have a high impact on brain injury outcome; however, the epigenetic mechanisms regulating gene expression, such as histone modifications, remain obscure. We have shown that astrocytes exposed to pro-inflammatory signals increase the level of histone acetylation. However, to date, there is no available description on early epigenetic changes in injury-affected astrocytes. We hypothesize that hypo-osmolar stress promoted by early edema has an impact on global histone modifications and astrocyte function. In a model of brain cortical injury by pial disruption in adult male Wistar rats, we addressed the levels of H3K9ac in astrocyte nuclei at 1.5- and 3.5-hours post injury. We observed, using immunofluorescence, a higher proportion of astrocytes with lower levels of H3K9ac at 3.5 hours when compared to non-injured hemisphere. Also, the injury promoted an increase in GFAP and AQP4 immunoreactivity near the injury core. In vitro, we exposed primary culture of astrocytes to hypotonic (20, 30 and 40% osm) culture medium to promote hypo-osmolar stress. We observed a decrease in the levels of H3K9ac and H3K27ac after 1 and 3 hours which were restored to control values 24h- after recovery in complete isotonic medium. Reduction in histone acetylation was prevented by treatment with histone deacetylase inhibitor Trichostatin-A. Interestingly, astrocytes exposed to lipopolysaccharide showed impaired NFkB (p65 subunit) nuclear translocation. Our results show that an edema-like microenvironment promotes a global histone acetylation reduction in astrocytes. During the recovery in histone acetylation levels, chromatin might be re-decorated but in a "reactive epigenome". However, reduced NFkB activation might be indicative of impaired astrocyte response during early stages of injury in a context of hypoosmolar stress.

261. 638. **INCREASE OF CD44 PROTEIN LEVELS IN HIPPOCAMPUS OF FEMALE BALB/C MICE EXPOSED TO**

CHRONIC MILD STRESS. REVERSION WITH GLATIRAMER ACETATE

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In previous studies, we observed cognitive deficit in female Balb/c mice exposed to chronic mild stress (CMS). Also, we found alterations in the hippocampus, a brain area involved in learning and memory and other functions, in CMS mice. Glatiramer acetate (GA), a synthetic amino acid polymer that can safely simulate the protective and reparative effects of autoreactive T cells, reversed the behavior and neuroimmune alterations induced by CMS. On the other hand, CD44 is the main receptor of the hyaluronic acid (HA), and the levels of CD44 are altered in Alzheimer's diseases or depression. In addition, studies have reported the role of CD44 in neurological processes like neural plasticity and neurodevelopment. Furthermore, we found that the enzymes responsible for the synthesis and catabolism of HA, HAS and Hyals, respectively were altered by CMS in female Balb/c mice. The aim of this work was to study the mRNA and protein levels of CD44, in the hippocampus of female Balb/c mice exposed to CMS model and injected with GA by RT-qPCR and western blot. Consistent with our previous studies, we found a poor performance in the Y-maze and different parameter in the open field test in CMS mice compared to control. We did not find a significant difference in the mRNA levels of CD44 in CMS mice with respect to control. However, we found an increase in the levels of the CD44 receptor protein in CMS mice compared to control mice (control PBS: 0.89 ± 0.28; CMS PBS: 1.63 ± 0.38; $p<0.05$). These alterations were reverted with the administration of GA. Although further analyses are required to determine this process. These results suggest that cells in the hippocampus with higher levels of CD44 receptor may have more interaction with the extracellular matrix, leading to less neural plasticity. Thus, this could explain the impairment in learning and memory in mice exposed to chronic stress.

262. 652. **A STEP FORWARD IN UNDERSTANDING SEROTONIN-OXYTOCIN INTERPLAY IN MICROGLIAL CELLS**

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High serotonin concentrations during early stages of development have shown to produce detrimental effects on social behavior. Besides, oxytocin is an important regulator of social interaction. In neurons, these two neurotransmitters are known to interact bidirectionally and when receptors are co-expressed, serotonin promotes oxytocin receptor (OXYr) desensitization. Considering the critical role microglia play in brain development and social behavior, the aim of this study was to shed light on serotonin-oxytocin crosstalk on these glial cells. We evaluated microglia reactivity and OXYr expression and distribution in hippocampal and cortical microglia exposed to serotonin. Microglial cultures were obtained at postnatal days 3-5 and immunostained for iba1 and OXYr. Cells were morphologically classified into type 1 or reactive (spherical, nucleus and cytoplasm are not differentiable), type 2 or in transition (rounded shape with defined nucleus and cytoplasm) and type 3 or non-reactive (ramified). Hippocampal microglia immunostained for iba1 showed increased proportion of type 2 and 3 cells and decreased proportion of type 1 cells after 1μM serotonin exposure. In this condition, total OXYr expression remained unaltered and located at the cell soma. No significant differences were seen with 10μM serotonin. Cortical microglia immunostained for iba1 responded to 20μM serotonin by increasing the proportion of type 3 cells and decreasing the proportion of type 1 cells. Total OXYr expression was conserved, and immunostaining located at cell soma. No significant differences were found with 1μM serotonin. Results indicate serotonin promotes a concentration-dependent effect on hippocampal and cortical microglia reactivity. In the assessed concentration range, serotonin-induced morphological changes are not paralleled to OXYr distribution on microglial cells. Our study opens the discussion regarding the serotonin role in serotonin-oxytocin interplay in microglia.

263. 672. **ELECTROMYOGRAPHIC MEASUREMENTS USING CUTANEOUS SURFACE ELECTRODES IN MALE HEMIPARKINSONIAN RATS**

Natalia González[1], Paloma Ramírez[1-2], Agustín Herrera[1-2], Francisco Jurado[1-2], Laura Medina[3], Ricardo Cabrera[1-3].
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Parkinson's disease (PD) is a neurodegenerative disorder. It is characterized by the irreversible loss of dopaminergic neurons in the substantia nigra. The neurotoxic 6-hydroxydopamine (6-OHDA) injected into the nigrostriatal pathway of rats destroys dopaminergic neurons by reducing dopamine (DA) levels. Electromyographs are devices used to measure the electrical activity of muscles, providing an overview of muscle activity. Contact with the muscle can be made through surface electrodes or needle electrodes. This work aimed to demonstrate that cutaneous surface electrodes coupled to an electromyograph are useful elements to capture muscle activity signals in freely moving animals without the need to use needle electrodes that require anesthesia and electrical stimulation. Adult male Sprague-Dawley rats were used, and neurodegeneration was induced by injecting 6-OHDA in the left striatum. Control (C) and Hemiparkinsonian (HP) rats were the experimental groups. Electromyographic measurements were performed through surface electrodes. The data were expressed as mean+SEM and analyzed by means of ANOVA 1. After performing the electromyographic measurements for the two experimental groups, a significant difference ($p<0.0001$) could be observed in group C since it presented a greater muscular electrical activity with respect to the HP group. The electromyograph developed in our laboratory, as well as the non-invasive cutaneous surface electrodes, allowed us to obtain a clear signal of the muscular activity in animals in free movement, obtaining an average amplitude of 0.1 mV in group C and an average of less than 0.05 mV in group HP. We conclude that this development is a great advance to determine in an early way the neurodegenerative phenomenon and the effect of the application of neuroactive steroids as a potential therapeutic for treating this disease.

264. 673. **NEUROMODULATION INDUCED BY NEUROACTIVE STEROIDS ESTROGEN AND PROGESTERONE ON THE ACTION OF KETAMINE IN MALE AND FEMALE RATS**

Natalia González[1], Paloma Ramírez[1-2], Sofía Fernández[1], Claudia Bregonzio[3], Ricardo Cabrera[1-2].
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3- Instituto de Farmacología Experimental (IFEC)- CONICET. Universidad Nacional de Córdoba. Córdoba.5000

Ketamine is a dissociative anesthetic that produces psychotomimetic effects in humans. It is an active ingredient widely used as a drug of abuse worldwide. In rodents, chronic administration of ketamine induces neuroadaptive responses that manifest with an increase in locomotor activity. This phenomenon is known as sensitization and involves dopaminergic, glutamatergic, and GABAergic neurotransmission alterations in the mesocorticolimbic system. Sex steroids play a key role in the differential response to drugs of abuse. The aim of this work was to study ketamine-induced neuroadaptations in an experimental model of psychosis in male and female rats asso-

ciated with s.c. administration of Estrogen (E) and Progesterone (P) combined with this drug. Castrated male rats and ovariectomized females of the adult Sprague-Dawley strain were used. The experimental groups were: 1. Females: saline + estradiol + progesterone (SEP), ketamine + estradiol + progesterone (KEP), ketamine + estradiol (KE), and ketamine + progesterone (KP). Males, under the same treatments (SEP), (KEP), (KE) and (KP). Using Ethowatcher to quantify meters run in 10 minutes, animals were evaluated in the open field. Data were expressed as mean+SEM and analyzed by ANOVA 1. In the open field, a significant increase in locomotor activity was observed in both females and males receiving ketamine together with combined E and P (P<0.0001). Independent E or P administration in ketamine-treated animals induced a significant decrease with respect to combined PE administration (P<0.0001). We conclude that E and P treatment produce differential neuroadaptive responses, whether they are applied alone or combined to both males and females in response to ketamine-induced hyperlocomotion and psycho-stimulation, suggesting non-genomic steroid-induced neuromodulatory effects.

P3-NEUROSCIENCES

FRIDAY 17TH NOVEMBER 14:00-15:30

CHAIRS: GABRIELA SALVADOR
SUSANA VALDEZ
MARCELA VILLAVERDE

265. 38. **NEUREGULIN-1 SELLECTIVELY MODULATES LPS-INDUCED IL-6 PRODUCTION IN BV2 MICROGLIAL CELLS**

Ana Julia Ticchi, Andrea De Laurentiis, Fernando Correa
CONICET, Laboratorio de Neuroinmunología, CEFyBO, Facultad de Medicina, UBA

Microglial cells play an important role in the central nervous system (CNS) innate immune response. Excessive or prolonged activation of these cells leads to CNS damage due to increased production of pro-inflammatory mediators. Several studies have revealed that neuregulins (Nrgs) are involved in normal brain function and psychiatric disorders. It has also been demonstrated that microglia express NRGs, and their levels are markedly increased in activated microglia and it has recently been shown that Nrg could modulate neuroinflammatory processes. Furthermore, Rac1, a downstream mediator of the Nrg1 signaling pathway, plays a central role in the inflammatory response and microglial neurotoxicity in the CNS. Objectives: To study the possible effects of Nrg1 on BV2 microglial cells in a model of inflammation induced by bacterial lipopolysaccharide (LPS) and to characterize the signaling pathways involved in the modulation of the microglial response. Material and Methods: Murine microglial cell line BV2 cultures were treated with LPS, Nrg1 and/or 1A116 (Rac1 inhibitor). Total proteins were collected for signaling pathway analysis and evaluation of the expression of three inducible mediators (COX-2, iNOS, HO-1) involved in neuroinflammation. Supernatants were also collected to study cytokine production. Results: We found that Nrg1 had no effect on the LPS-induced activation of ERK1/2 and JNK MAPK signaling pathway but changed the pattern of activation of p38 MAPK. Additionally, we found that Nrg1 increased the LPS-induced activation of NF-κB after 2h of stimulation. Next, we observed that Nrg1 prevented the increase of IL-6 production caused by LPS treatment, without affecting the expression of iNOS, COX-2, or HO-1. Conclusion: In the BV2 microglial cell line, Nrg1 modulates LPS-induced activation of the p38 MAPK and NF-κB signaling pathways, resulting in a diminished IL-6 production.

266. 45. **PARTICIPATION OF NMDA RECEPTORS IN FLUOXETINE EFFECTS ON MEMORY RECOGNITION AND THE MAPK GENES EXPRESSION IN MICE**

Juan Robledo Almonacid[1], María Belén Poretti[1], Mauro Gasparini Gatica[1], Mariela Fernanda Perez[2], Ana Carolina Martini[1] and Valeria Paola Carlini[1].
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Chronic fluoxetine (FLU) treatment impairs recognition memory, suggesting that this effect could probably be explained by FLU interference in the MAPK/ERK pathway. In order to elucidate the participation of the N-methyl-D aspartate glutamate receptor (NMDA) and the mitogen-activated protein kinases (Ca^{++}-MAMPK) pathway in FLU effects on behavioral memory expression, in the present project adult male Albino's Swiss mice were divided in groups (n=10/group) and treated for 28 days with saline/saline; NMDA/saline (NMDA: 75mg/Kg/day, i.p), saline/FLU (FLU: 10mg/Kg/day, p.o.) or NMDA/FLU. The last day of treatment mice were tested in the object recognition paradigm (TRO), and then sacrificed and their hipocampus were collected, to study gene expression of MAPK1, MAPK2 and MAPK3, by real time PCR. Data were analyzed by a two-way ANOVA, followed by Bonferroni test. FLU treatment decreases the percentage of exploration of the novel object in the TRO ($F=12.59$, $df=36$, $p\leq0.05$), while NMDA treatment significantly increases this parametrer ($p\leq0.05$). Animals treated with FLU and NMDA showed a behavior similar to controls. Regarding genes expression, animals treated with FLU showed a significant decrease in the relative expression of MAPK1 (F 6.65, $df=36$, $p=0.005$) and MARK2 ($F=9.31$, $df=36$ $p\leq0.05$). Conversely, NMDA administration significantly increased the relative expression of MAPK1 and MARK2 ($p\leq0.05$) in comparison to saline/saline, and the animals administered with MDMA and FLU showed genes expression similar to control. No significant effects were detected on the relative gene expression of MARK3 ($F=0.35$, $df=36$, $p>0.05$).
Our results show that the NMDA receptor activation during FLU treatment avoids FLU-induced memory impairment as well as down-regulation in MAPK/ERK pathway. Whether FLU has direct actions on NMDA receptors to induce both effects or they are mediated by other effectors that converge in the MAPK/ERK pathway remains to be elucidated.

267. 55. **ADVERSE CHILDHOOD EXPERIENCES AND ITS INCIDENCE IN EMOTIONAL HEALTH IN ADOLESCENTS**

Gabriela C. Ciavatta_SAFE2023[a], Diego Cohen[b], Gabriela B. Acosta[a].
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There is considerable evidence that adverse experiences in childhood cause chemical, functional, and behavioral changes in the neuronal development of the human being, which would negatively impact the emotional health of adolescents, exposing them to different vulnerable episodes. The objective of this work was to analyze whether the impact of adverse experiences in early stages of life affects the emotional health of adolescents and their psychological vulnerability. The methodology used was an observational, non-randomized, consecutive, analytical, open, prospective cohort study. Three scales were applied, two of which evaluate depression (Hamilton and Beck test) and one evaluates anxiety (Hamilton test). A sample of 186 adolescents divided into 3 groups: Control, 1st year students and 4th year students. Our results showed that the three scales used both in the 1st school year (Chi-square df; 19.40.1; ****P<0.001) and in the 4th year were significant with respect to the control group (Chi-square, df;43.65,1; **** P<0.001). We found that exposure to adverse childhood experiences, including physical or psychological abuse, significantly decreases anxiety and increases depression during adolescence. We conclude that the results of this exploratory study reveal that child maltreatment modulates emotional processing during adolescence.

268. 101. **EFFECTS OF THE MINERALOCORTICOID RECEP-**

TOR (MR) ANTAGONIST EPLERENONE IN EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS (EAE)

Guido Álvarez Quintero[1], Analía Lima[1], Paulina Roig[1], María Meyer[1], Alejandro F. De Nicola[1,2] and Laura I. Garay[1,2]
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There is growing evidence indicating that MR expression influences a wide variety of functions. In the context of the immune response, MR stimulation promotes proinflammatory responses and fibrosis. The present study explored if antagonism of the mineralocorticoid receptor reduces neuroinflammation in the spinal cord of mice with experimental autoimmune encephalomyelitis (EAE). Eplerenone (EPLE) (100mg/kg dissolved in 30% 2-hidroxypropyl-β-cyclodextrin) was administered i.p. daily from EAE induction (day 0) until sacrificed on day 17 post-induction. The mineralocorticoid receptor blocker (a) significantly decreased the inflammatory parameters TLR4, MYD88, IL-1β and iNOS mRNAs ;(b) attenuated HMGB1, NLRP3, TGF-B mRNAs, microglia and acquaporin4 immunoreaction reaction without modifying GFAP. Serum IL-1β was also decreased in the EAE+EPLE group. Moreover, EPLE treatment prevented demyelination and improved clinical signs. Interestingly, MR was decreased and GR remain unchanged in EAE mice while EPLE treatment restored MR expression, suggesting that a dysbalanced MR/GR associated with neuroinflammation. Thus, MR blockage with EPLE downregulated inflammation-related spinal cord pathology in the EAE mouse model of multiple sclerosis.

269. 147. **LONG-LASTING AMPHETAMINE EFFECTS OVER CENTRAL ANGIOTENSIN II RESPONSES INVOLVE AT_1-R**

María Josefina Piermarini[1], Osvaldo Martín Basmadjian[1], Victoria Belén Occhieppo[1], Gustavo Baiardi[2], Claudia Bregonzio[1].
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Angiotensin II (Ang II), a pleiotropic neuropeptide, plays a critical role in regulating the sympathetic and neuroendocrine systems through the activation of AT_1 receptors (AT_1-R). Studies on mammals have shown that administering Ang II through the intracerebroventricular (i.c.v) route increases water and sodium intake, as well as renal sodium excretion. Our group's previous findings have shown that AT_1-R are involved in behavioural and neurochemical sensitization induced by amphetamine (Amph) administration. Our current aim is to assess the functional and neurochemical response to Ang II, via the AT_1-R activation, in animals that have been previously exposed to Amph. Male Wistar rats (250-320g) were injected intraperitoneally with Amph (2.5mg/kg/day) or saline for 5 days, and implanted with i.c.v. cannulae. Twenty-one days after the last Amph administration, the animals received Ang II (400pmol) i.c.v. First group: the animals were tested in a free choice paradigm for sodium (2% NaCl) and water intake, and sacrificed for Fos immunoreactivity determinations. Second group: urine and plasma samples were collected for electrolytes and plasma renin activity determination. Third group, the animals were tested in the plus maze or the holeboard for anxiety and memory work evaluation, respectively. Previous Amph exposure altered the physiological and behavioral responses described for central Ang II administration. Remarkably, the AT_1-R blockade prevented most of these alterations but anxiety. Our results highlight that repeated Amph exposure attenuates the AT_1-R functionality in a long-lasting manner, modifying the brain Ang II-induced responses.

270. 183. **ACTION OF *Tessaria absinthioides* ON SPATIAL MEMORY AND OXIDATIVE STRESS IN RATS**

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Tessaria absinthioides Gillies (Hook. & Arn.) DC. (Asteraceae; TA) is popularly used to improve health. We previously described the effects of TA on the glucose and lipid metabolism of rats subjected to a sucrose overconsumption during the juvenile stage. The aim of this work was to evaluate whether TA improves spatial memory and produces central antioxidant effects in the same animal model. Male rats (SD) received water (CON), 10% W/V sucrose (SAC) or a TA decoction10% W/V with sucrose (TESSAC) from PND21-PND61. The novel object location (NOL) test was performed in the PND 68. In the test, the animal explores two identical objects placed in a quadrant for 5 minutes. After 2 hours, the animal explores for 5 minutes the device where one of the objects was relocated. The exploration rate (ER) is determined as the time spent exploring the relocated object over the time spent exploring both objects. Malondialdehyde (MDA) concentration was quantified by TBARs in hole homogenates of hippocampus (HC), hypothalamus (HT) and cerebral cortex (CC) obtained at PND69. SAC showed lower ER than CON (38.15%; $p<0.01$), whereas TESSAC had higher ER than SAC (31.98%; $p<0.01$) and similar to CON. In HT, the levels of MDA were higher in SAC than CON (72.37%; $p<0.05$). However, TESSAC had lower levels of MDA than SAC (34.07%; $p<0.05$) and similar to CON. In HC, no differences were found between SAC and CON, but TESSAC had lower levels of MDA than SAC and CON (86.02% and 88.72% respectively; $p<0.01$). In CC, no differences were observed between the groups. These results with those previously reported indicate that TA co-administration is capable of preventing alterations produced by sucrose overconsumption during critical stages of development. The properties of TA could be attributable to its high content of flavonoids, diterpenes and polyphenols. More complementary studies are necessary to consider TA for new therapeutic opportunities.

271. 353. **MITOCHONDRIAL COMPLEX I: STABILIZATION AND ACTIVITY IN A RAT MODEL OF CEREBRAL AMYLOIDOSIS**

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Bioenergetic dysfunction has been suggested as an early event and a cause of synaptic and cognitive deficiency in Alzheimer's Disease (AD). Previous reports showed impairments in mitochondrial Complex I (CI) activity associated to brain amyloidosis, however the molecular basis underlying this disturbance was not deeply established. Here we evaluated in McGill-R-Thy1-APP transgenic (Tg) rat, a model of AD-like cerebral amyloidosis, if there is a time-course disorganization of CI (a multimeric protein) in Supercomplexes (SCs) and if this process affects CI activity. Mitochondria were isolated from hippocampus of young (3 month) and old (9-12 month) animals (n=3/group) and the organization and abundance of SCs analyzed by electrophoretic runs on native gels. CI functionality was assessed by in-gel activity. T-test was applied for statistical analysis. We found by Western-blot (WB) similar amounts of the individual mitochondrial complexes (CI, CII, CIII, CIV and CV) and no differences in the assembly of SC1 ($I+III_2$), SC2 ($I+III_2+IV$) and SC3 (I_2+III_2) between genotypes. However, a lower CI activity was detected in the aged Tg as compared to young animals. To address if aged-associated impaired CI activity was linked to decrements of its relevant sub-units we assessed the expression of NDUFB8 (hydrophobic arm), NDUFA9 (hinge) and NDUFS2 (catalytic core). Similar levels of NDUFB8 and NDUFS2, and reduced amounts of NDUFA9 were detected in aged Tg vs. WT rats. NDUFA9 is encoded by a nuclear gene and translocates from cytoplasm to mitochondria, therefore we assessed its cytoplasmic levels in both genotypes. We detected higher cytoplasmic amounts of preprotein-NDUFA9 in aged Tg. NDUFA9 is imported to mitochondria after conversion of preprotein to mature one. We speculate that in a setting of high amyloid β levels, the processing of NDUFA9 may be impaired precluding its translocation and

stabilization of CI, a late step critical for CI biogenesis and activity.

272. 448. **METFORMIN REDUCES INFLAMMATORY RESPONSE AND RESTORES MICROGLIAL PROTEOSTASIS AND MITOCHONDRIAL STATUS IN EXPERIMENTAL MODELS OF TYPE 2 DIABETES MELLITUS**

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Type 2 diabetes is a metabolic disorder associated with cognitive dysfunction and a higher risk of developing Alzheimer's disease, being two highly prevalent disorders worldwide. Neuroinflammation mediated by microglial activation in the brain can lead to neuronal damage and cognitive impairment. Metformin is the primary drug for T2D treatment, but its effects on the brain and microglial cells are unknow. Our aim was to evaluate the therapeutic potential of metformin employing *in vivo* and *in vitro* experimental models that parallel some of the metabolic changes observed in T2D. In mice exposed to a high-fat diet (HFD) during 4 months since weaning -inducing insulin resistance along with neuroinflammation- systemic metformin administration (240 mg/kg i.p, 9 injections during 3 weeks) was found to diminish peripheral insulin resistance assessed by WB against AKT in liver. In the brain, metformin administration induced a reduction on microglial reactivity evaluated as soma size ($p<0.05$) in the hippocampus and led to a trend towards a reduced anxiety-like behavior measured in the open field test ($p=0.07$). Additionally, HFD administration disrupted hippocampal microglial proteostasis, evidenced by the accumulation of the autophagic substrate p62, and it was reverted by metformin ($p<0.001$). BV2 microglia-derived cells exposed to a metabolic insult as palmitate for 24 hours showed increased IL-1 expression and autophagosome accumulation, but metformin treatment during the last 2 hours reduced these parameters. Metformin also enhanced mitochondrial turnover, alleviating their accumulation within autophagosomes. Furthermore, the exposure of BV2 cells to palmitate decreased mitochondrial size and increased ROS production ($p<0.001$), both of which were restored by metformin treatment. These findings suggest that metformin has the capacity to restore proteostasis and prevent microglial cell activation, potentially contributing to the positive effects of metformin in a T2D context.

273. 449. **SELECTIVE DOPAMINE D2 RECEPTOR DELETION FROM NKX6.2 EXPRESSING CELLS CAUSES IMPAIRED COGNITIVE, MOTIVATION AND ANXIETY PHENOTYPES IN MICE**

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Abnormal dopamine neurotransmission is a common trait of some psychiatric diseases, like schizophrenia or bipolar disorder. Excessive dopaminergic tone in subcortical brain regions is associated with psychotic episodes, while reduced prefrontal dopaminergic activity is associated with impaired cognitive performance and reduced motivation, among other symptoms. Inhibitory interneurons expressing the calcium binding protein parvalbumin are particularly affected in both schizophrenia and bipolar disorder, as they set a fine-tuned physiological inhibitory/excitatory balance. Parvalbumin and somatostatin interneuron subtypes, are born from the medial ganglionic eminence and require the sequential expression of specific transcription factors for their specification, such as Nkx6.2. Here, we aimed at characterizing in detail interneuron subtypes derived from Nkx6.2 expressing progenitors by the generation of an Nkx6.2 Cre transgenic mouse line. We show that Nkx6.2 specifies over a third part of the total population of cortical somatostatin interneurons, preferentially at early developmental time points, whereas at late developmental stages, Nkx6.2 expressing progenitors shift to parvalbumin interneuron specification. Dopamine D2 receptor deletion from Nkx6.2 expressing progenitors causes abnormal phenotypes restricted to cognitive, motivation and anxiety domains but not to increased locomotor activity. Our results show that Nkx6.2 have the potential to specify both somatostatin and parvalbumin interneurons in an opposite timed program and that DRD2 expression is required in Nkx6.2 expressing progenitors to avoid impaired phenotypes commonly associated to the pathophysiology of psychiatric diseases.

274. 479. **STUDY OF mGlu3R FUNCTION IN PRIMARY GLIAL CULTURES FROM TRANSGENIC ALZHEIMER'S MICE**

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**These authors contributed equally*

Our group has demonstrated that the subtype 3 metabotropic glutamate receptor (mGlu3R) expressed in astrocytes exerts neuroprotective functions and promotes the non-amyloidogenic cleavage of APP. In turn, we described the presence of the splicing variant mGlu3Δ4 in the hippocampus of PDAPP-J20 murine AD model, where mGlu3R levels progressively decreased with age, while Δ4 levels increased prematurely. In vitro results suggested that mGlu3Δ4 could act as a negative regulator of mGlu3R. Here, we used glial cell cultures from PDAPP-J20 mice to test if the results observed in hippocampi were reflected on the glia. These in vitro assays would make it possible to test the effect of mGlu3R agonists as well. To achieve this, cultures of 1-month-old transgenic (Tg) and non-transgenic (Ntg) mice were performed using a Percoll gradient. We obtained glia-enriched cultures with a high proportion of ALDH1L1+ astrocytes (64.5 ± 7.03%), microglia CX3CR1+, and a low proportion of neurons (7.6 ± 2.8%). Surprisingly, only 2.16 ± 0.08% of the astrocytes were found to be GFAP+. We analyzed the expression of glial proteins implicated in AD. Western blot analysis showed a significant decrease in mGlu3R levels in Tg cultures ($p<0.05$). This result was corroborated by immunofluorescence ($p<0.01$). On the other hand, preliminary Western blot results indicated that the glutamate transporter GLT-1 and the scavenger receptor SR-A decreased in the Tg glia, while Aβ increased, and all these effects were reversed by the mGlu3R agonist LY379268. LY also reduced mGlu3Δ4 levels in the glia of Tg mice. Immunocytochemistry revealed higher membrane expression of C1q in Tg cells expressing mGlu3R, while CX3CR1 decreased in Tg glia. In summary, PDAPP-J20 glial cells show variations in mGlu3R and mGlu3Δ4 levels, consistent with previously found differences in the hippocampus. Activation of mGlu3R in glia might also play a neuroprotective and anti-amyloidogenic role in AD models.

275. 507. **MODELLING AMYOTROPHIC LATERAL SCLEROSIS/FRONTOTEMPORAL DEMENTIA USING PATIENT-DERIVED INDUCED PLURIPOTENT STEM CELLS**

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Amyotrophic lateral sclerosis (ALS) and Frontotemporal dementia (FTD) neurodegenerative diseases are part of a clinical-pathological continuum. A hexanucleotide repeat expansion, constitute the most frequent genetic cause of ALS/FTD, however, the precise molecular mechanisms involved in C9ORF72-mediated ALS/FTD pathogen-

esis remains elusive. Also, not much is known about repeat contraction in this gene and potential pathogenic effects. We aimed to establish an *in vitro* model of ALS/FTD through reprogramming patient somatic cells, and subsequent differentiation towards neural lineage. Peripheral blood mononuclear cells were obtained from a female ALS/FTD patient harbouring a heterozygous deletion within the *C9ORF72* hexanucleotide repeat region resulting in ~1.16 repeats, in contrast to the most frequent 2-repeat allele in the general population. The EF1a-hSTEMCCA-loxP lentiviral vector expressing OCT4, SOX2, c-MYC and KLF4 pluripotency genes was used to generate the iPSC line INEUi001-A using a feeder- and xeno-free reprogramming protocol. The presence of the deletion within the hexanucleotide was confirmed by sequencing. Transgenes silencing was evaluated by RT-qPCR using specific primers for exogenous expression. INEUi001-A iPSCs exhibited normal karyotype (46, XX; studied by G-banding) and showed high alkaline phosphatase activity. mRNA levels of SOX2, POU5F1, and NANOG genes confirmed stemness. Immunofluorescence staining (IFI) showed robust expression of stemness-associated markers OCT4, NANOG, SSEA4 and TRA1-60. *In vitro* spontaneous differentiation through an embryoid bodies-based method proved the pluripotent potential as judged by IFI analysis of smooth muscle actin, alpha-fetoprotein and b-III- tubulin expression. INEUi001-A iPSCs were further differentiated to neural stem cells (NSC). IFI to Pax6, Nestin and Sox2 validated NSC identity. The generated iPSC line may be useful to uncover physiopathological mechanisms that lead to neurodegeneration in ALS-FTD.

276. 537. **EVALUATION OF HIPPOCAMPAL AND PERIPHERAL LEVELS OF GSK-3β IN BALB/C MICE EXPOSED TO CHRONIC MILD STRESS. REVERSION WITH GLATIRAMER ACETATE**

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In previous reports, we have demonstrated that chronic stress induced cognitive deficit in BALB/c mice affecting the hippocampus and the immune system. These deleterious changes induced by chronic stress were reverted by glatiramer acetate (GA) administration. The glycogen synthase kinase 3 beta (GSK-3β) is an enzyme that has been related to neuronal plasticity, neurodevelopment and neurological diseases. GSK3-β is considered a key player in Alzheimer's disease pathophysiology and it was found in the lymphocytes of schizophrenic patients. The regulation of GSK-3β activity depends on the phosphorylation/dephosphorylation status of ser9 and/or tyr216 sites. The aim of the present work was to study the levels of GSK-3β in hippocampus, spleen and lymph nodes of female BALB/c mice exposed to chronic mild stress (CMS) model. Moreover, we evaluated the effect of GA treatment in CMS BALB/c mice. As we previously reported, we observed a poor performance in the open field test in CMS mice with respect to control mice. We found a decrease of GSK-3β mRNA levels in the hippocampus of CMS mice (p<0.05) compared to control, analysed by qRT-PCR. However, no differences were observed in spleen and lymph nodes in CMS mice with respect to control mice. Furthermore, we found an increase in the protein levels of pGSK-3β tyr216 (p<0.05) and a decrease in the levels of total GSK-3β (p<0.01) in the spleen of CMS mice detected by western blot. No differences were observed in hippocampus (pGSK-3β undetected) and lymph nodes in CMS mice regarding control mice. These changes induced by chronic stress could be reversed by GA. Our findings indicate that deregulated levels of GSK-3β phosphoisotypes could play a key role in the cognitive deficit observed in chronically stressed mice in both the hippocampus and the periphery. The knowledge of these mechanisms could be useful to find new peripheral therapeutic targets to revert the deleterious cognitive effects induced by chronic stress.

277. 600. **HORMONE DEPRIVATION INDUCES AN INCREASE IN OXIDATIVE DAMAGE TO MITOCHONDRIAL DNA IN THE HIPPOCAMPUS**

Micaela Avaca[1], Ricardo Gredilla[2], Ivana Villa[1], Analía Reinés[3], Tinna Stevnsner[4], Sandra Zárate[1]
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Ovarian hormone loss is related to brain mitochondrial dysfunction and oxidative stress. Base excision repair (BER) is the main mechanism to repair mitochondrial DNA (mtDNA) and is critical to avoid accumulation of mutations in this organelle. It comprises several steps involving lesion-specific glycosylases and endonucleases. We previously showed that the activity of glycosylases that repair oxidative lesions and APEendonuclease was decreased in mitochondria from the hippocampus of hormone-deprived rats. Conversely, the activity of those enzymes in cortical mitochondria was increased, suggesting a putative compensatory mechanism to avoid accumulation of oxidative lesions in this brain area. The aim of this work was to determine the level of mtDNA damage in the hippocampus (Hp) and cerebral cortex (Cc) of animals subjected to chronic deprivation of ovarian hormones induced by ovariectomy. For this purpose, adult rats were ovariectomized (OVX) or sham operated (SHAM). After 12 weeks, total DNA from the Hp and Cc was purified and subjected to long-amplicon PCR for a 13.4kb-fragment of mtDNA, which allows estimation of DNA polymerase blocking lesions. To estimate oxidized base lesions, DNA was pre-incubated with Fpg enzyme. The ratio of amplification of a short mtDNA fragment/nuclear DNA amplicon was used as an estimation of mtDNA copy number. In the Hp, Fpg-treated mtDNA from OVX rats showed a lower relative amplification than SHAM (p<0,05; Student´s t test), while no differences were found in the amplification of Fpg-untreated mtDNA or mtDNA copy number between groups (p=0,1; Student´s t test). Our results show that hormone deprivation increases mtDNA oxidative damage in the Hp, which correlates with previously observed lower mRNA levels and activity of BER enzymes in this brain region. Ongoing studies in Cc will determine mtDNA damage levels in this brain region and will help elucidate regional differential action of hormonal status on brain mitochondrial BER pathway.

278. 660. **EFFECT OF STRESS ON RELAPSE OF OPIOID ADDICTIVE BEHAVIOR IN MALE AND FEMALE MICE**

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Previous studies from our laboratory have shown sex differences in the behavioural, molecular and neurochemical responses to morphine (MOR) withdrawal syndrome. In addition, stress is an important factor to trigger relapse in substance use disorders. The aim of our study was to evaluate patterns of extinction and relapse of opioid addictive behaviour under stress conditions and to correlate these patterns with neurochemical changes in brain nuclei related to brain reward circuitry in prepubertal CD1 male and female mice. The paradigm used was conditioned place preference (CPP) which consists of 3 phases: preconditioning, conditioning and postconditioning (test). On the test day, the reinforcing effects of MOR (10 mg/kg, s.c.) were tested and 24 h later, the extinction phase was

carried out by 10 sessions of CPP administrating only saline (SAL) (0.10 ml/g, s.c.) in each session. The test was performed 24 h after the last extinction session, and CPP to MOR was considered extincted if the preference score was less than 15% of that obtained during the first test. After the extinction, animals were induced to relapse CPP by stress (immobilized for 15 min). Immediately after, brains were rapidly removed and homogenates were obtained. The supernatants were used for electrochemical detection coupled to HPLC of DA, 5-HT and their metabolites. Our results showed that MOR was able to induce reinforcing effects in mice of both sexes ($p<0.05$), but stress induced relapse of CPP to MOR only in female mice. ($p<0.05$). In addition, stress restored the levels (ng/ml) of DA (243 + 42,39) and its metabolite, DOPAC (32,33 + 2,028) in nucleus accumbens of mice that had extincted the CPP compared with their control groups (273,4 + 75,72 and 53 + 27,53, respectively). In conclusion, our results indicate that although the lack of sex differences in the reinforcing effects of MOR, stress was able to restore this effect only in female mice.

279. 674. **NEUROACTIVE STEROIDS ESTROGEN AND PROGESTERONE INDUCE NEUROPROTECTION BY DELAYING SIGNS OF DEPRESSION AND MOTOR IMPAIRMENT IN ORCHIECTOMIZED MALE HEMIPARKINSONIAN RATS**

Natalia González[1], Trinidad Parra[2], Ignacio Moreno[1], Esther Vargas[2], Emiliana Mashad[2], Ricardo Cabrera[1-2].
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Parkinson's disease (PD) is a neurodegenerative pathology. This disease is characterized by an irreversible loss of dopaminergic neurons in the substantia nigra. PD is more prevalent in men. Nigrostriatal degeneration is studied with experimental models in rodents by unilateral intrastriatal injection of the neurotoxic 6-hydroxydopamine (6-OHDA), which induces dopaminergic neurodegeneration and hemiparkinsonism in rats. The objective was to evaluate the neuroprotection induced by progesterone (P) and estrogen (E) on the motor alterations associated with neurodegeneration in orchidectomized male hemiparkinsonian rats. Adult male Sprague-Dawley rats were used, and neurodegeneration was induced by microinjection of 6-OHDA into the striatum. The experimental groups were: uncastrated control (C), hemiparkinsonian (HP), E-treated hemiparkinsonian (HPE), and P-treated hemiparkinsonian (HPP). Castrated control (CO), castrated hemiparkinsonian (HPO), castrated hemiparkinsonian treated with E (HPEO), and castrated hemiparkinsonian treated with P (HPPO). The animals were evaluated in two motor variables: forced swimming (PNF) and Raturn. Data were expressed as mean+SEM and analyzed by ANOVA 2 and Bonferroni post hoc. We observed a significant decrease in PNF in the swimming time of the C's with respect to CO ($p<0.0001$). No significant differences were observed between Cs with respect to HPE and HPP. HP animals showed a significant decrease in swimming time with respect to C ($p<0.0001$). A significant increase in this variable was observed in the HPPO and HPEO groups with respect to HP ($p<0.0001$). In the raturn test, the HP group presented a significant increase in rotational activity with respect to the C group ($p<.0001$). The HPP and HPE groups presented a significant decrease with respect to HP, bringing them to values of C. In the orchidectomized animals we observed a significant decrease in contralateral gyrations in HPPO and HPEO with respect to HP ($p<0.0001$). We conclude that both E and P have a neuroprotective effect enhanced by the decrease in testosterone induced by orchidectomy.

O1-ONCOLOGY

WEDNESDAY 15TH NOVEMBER 9:00 - 10:30
CHAIRS: ADRIANA CASAS
GEORGINA COLÓ

280. 37. **CROSSTALK BETWEEN ANDROGEN RECEPTOR AND WNT PATHWAYS IN ENDOCRINE RESISTANT BREAST CANCER MODELS**

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Endocrine therapy is the standard treatment for patients with luminal breast cancer. However, after treatment most patients develop hormone resistance, by mechanisms that may include dysregulation of growth factor signaling pathways. Fibroblast growth factor 2 (FGF2) consists of a secreted low molecular weight form and several nuclear high molecular weight forms (HMW-FGF2). We previously demonstrated that HMW-FGF2-overexpression in endocrine responsive T47D cell lines, induced hormone resistance, dysregulation of the WNT signaling pathway and an increase in androgen receptor (AR) expression. We hypothesize that FGF2 induces WNT/β-catenin pathway activation which, in turn, induces AR expression. The aim of this study was to evaluate if a crosstalk exists between the AR and WNT pathway and to identify the mechanisms underlying these connections in endocrine resistant breast cancer models. We used the endocrine resistant T47D-HMW-FGF2 and T47D-YB cell lines, the latter naturally expressing higher levels of HMW-FGF2 than the responsive T47D cells. Dihydrotestosterone (DHT; AR agonist) increased while ICG-001 (Wnt inhibitor) decreased cell proliferation. ICG-001 (0.5 μM) significantly reduced DHT-induced cell proliferation ($p<0.001$). In T47D-HMW-FGF2 cells, Wnt inhibitors reduced AR at the transcript and protein level, the latter both *in vitro* and *in vivo*. To assess if there was a direct effect of WNT pathway activation on AR expression we performed ChIP assays on TCF/LEF sites on the AR promoter. Preliminary data indicates that ICG-001 regulates the recruitment of β-catenin and LEF in the AR promoter which might modulate gene transcription. Our results suggest that in endocrine resistant cell lines with increased HMW-FGF2, an upregulated Wnt pathway may modulate AR expression which, in turn may guide tumor growth. In conclusion, we provide a mechanistic interpretation that may explain FGF2-induced endocrine resistance.

281. 194. **CLINICAL VALIDATION OF A URINARY TEST FOR THE EARLY DETECTION OF RECURRENCE IN NON-MUSCLE INVASIVE BLADDER CANCER**

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Aim Validation of the sensitivity, specificity and predictive values of the Uromonitor kit to be performed on urine samples, in a cohort of patients with a previous diagnosis of non-muscle invasive bladder cancer (NMIBC). Materials and methods: In patients with a history of non-muscle-invasive bladder cancer, routine cystoscopy was performed and urine samples from 50 patients were previously obtained by spontaneous urination. They were filtered through a pre-treated 0.80 μm nitrocellulose syringe filter (Whatman® Filter—Z612545, Merck, Germany) containing a home storage preservative buffer (10 mM glutathione, 1 M lithium chloride –urea 6 M–Biuret 30 mM, EDTA 2 M (E7889-100ML) (information on this process is available at https://www.youtube.com/watch?v=UgnfL3-hH6Y) Samples were kept in sealed and labeled bags in a refrigerator at 4°C for a maximum of 5 days (Monday to Friday) After obtaining all the weekly filters, they were sent together in a sealed container with a cold chain (a convenient procedure but not required by the manufacturer) to the molecular study laboratory (Argenomics SA – Pilar, Bs As), where the filters were stored at 2–8°C for a maximum of 72 hours until the DNA extraction procedure. Detection of TERT, FGFR3 and KRAS alterations was achieved via a tailor-made, robust and highly sensitive allele-specific competitive multiplex discrimination qPCR, allowing clear interpretation of the results. TERT, FGFR3, and KRAS tests were performed on approximately 25 ng of DNA extracted from cells. in each filtered urine. Extracted DNA was amplified and detected by real-time qPCR, using the proprietary chemistry for amplification and detection provided in the Uromonitor® Test Kit for targeted

nucleotide changes in the TERTp and FGFR3 gene. To screen for TERTp c.1-124 C>T and c.1-146 C>T alterations, an improved real-time allelic discrimination assay was developed using locked nucleic acid (LNA probes). The use of LNA probes allowed modulating the melting temperature on a probe-specific basis, improving the possibility of achieving preferential melting temperature in short probe sequences. LNA probes improve allelic discrimination, allowing greater stability in binding to a specific target. For the detection of selected FGFR3 mutations, we designed for each mutation, a mutation allele primer, a wild-type allele blocker, a locus reverse primer, and a fluorescent probe for real-time detection of the generated amplicon. The use of a molecular blocker completely abolishes the amplification of the wild-type allele so as not to interfere with the amplification of the mutant allele. Through this technique, we improve the current detection thresholds, improving the ability to detect a minimal amount of altered cells in a large pool of unaltered cells. To test the precision in urine samples, 6 samples negative for all mutations under study (confirmed by Sanger sequencing) were amplified "blank" (no DNA) for each alteration (false positive tests). In addition, 24 blind tests were analyzed from urine samples (7 tests for TERTp –124 assay, 7 tests for TERTp –146 assay, 5 tests for FGFR3 248 assay, and 5 tests for FGFR3 249 assay). Uromonitor® includes the detection of TERTp alterations by real-time PCR using LNA allelic discrimination probes. High GC content and thorough optimization of amplified TERTp abnormalities characterize this innovative test. Since the detection of TERTp mutations by current methods has a low sensitivity, the need arose to evaluate the detection limit of TERTp alterations included in the technology in the Uromonitor® test. To achieve this, we performed two-fold serial dilutions of genomic DNA containing the alteration studied (100% mutated DNA) into wild-type genomic DNA. Serial dilutions were then amplified for the corresponding detection assay. This procedure was repeated for the two TERTp alterations that make up the Uromonitor® test until the detection limit of the studied alterations was determined. For the statistical analysis, the collection of data and clinical information, demographic and histopathological data, results of the Uromonitor test and cystoscopies were collected in an Excel spreadsheet v.2205, 2019 - Microsoft Office Professional Plus 2019 package. For the statistical analysis, the program SPSS Statistical 21.0 - Package (SPSS, Inc., 220, 2003) was used. Descriptive statistics were performed and the results are expressed as median, percentages and mean ± Standard Deviation. The comparison between the diagnostic methods was made by analyzing the ROC (Receiver Operating Characteristic) curve and the area under the ROC curve (AUC). Results: Of the 50 urine samples, 44 were analyzed, consisting of 28 men (63%) and 16 women (37%), with a mean and median age of 71 years (range, 45–88). Molecular Characterization of Urine Samples: The urine samples were completely characterized by the alterations targeted by the Uromonitor® test and 7 detected one or more alterations. Of these, TERTp mutations were detected in 42.85% of the cases (100% presented with TERTp c.1–124C > T and 0% with TERTp c.1–146C > T) and FGFR3 mutations were detected in the 57.15% of the cases (25% in codon 248 and 75% in codon 249 of the FGFR3 protein). KRAS mutations were not detected in any sample. On the urine samples and comparing the results with Sanger sequencing, the TERTp–124 assay achieved a test accuracy of 100%, the TERTp–146 assay: 98.2%, the FGFR3-248 assay: 89.3 % and the FGFR3-249 assay: 94.1%. Overall, the Uromonitor® test had a pooled accuracy of 95.4%. Genetic Alterations, Distribution in Recurrences and Positive Cases: Of the total urine samples from the cohort of patients undergoing follow-up or diagnosis of tumor recurrence, they were positive for the Uromonitor assay as follows: TERTp mutations were detected in 6.8% of cases (6. 8% presented the –124C >T mutation and 0% the –146C > T mutation) and FGFR3 mutations were detected in 9.1% of the cases (2.3% in codon 248 and 6.8% in codon 249 of the FGFR3 protein). There were no concomitant alterations in TERTp and FGFR3 in any case. In no case did KRAS mutations appear. A single case in the follow-up cohort did not present any of the screened TERTp or FGFR3 abnormalities. Clinical Validation: Tumor recurrence follow-up cohort In the follow-up cohort (n=44), 18% (n=8) of the patients observed recurrence (confirmed by histology) after TUR, while the remaining 82% (n=36) were negative for recurrence (confirmed by cystoscopy). Uromonitor® performance comparison with cystoscopy: We analyzed and compared the detection of tumor recurrence in NMIBC by means of Uromonitor® in comparison with the study of routine use (gold standard) for the detection of bladder tumors such as cystoscopy. The sensitivity of Uromonitor® was 87.5% (1 false negative) in the detection of recurrence, with a specificity of 97% (1 false positive), both confirmed by TUR. The positive predictive power (PPP) of the test was 85% and the negative predictive power (PPN) 97%. The values were similar to the performance of cystoscopy, which in the follow-up series presented values of 75% (with 2 false negatives) and 97% (1 false positive) for sensitivity and specificity, respectively, with a PPP of 85% and a PPN of 94% Comparing the AUC-ROC values, Uromonitor has a higher AUC-ROC (0.846875) than Cystoscopy (0.7275). It is logical to infer that the combination of cystoscopy with urinary cytology obtains a greater benefit in the diagnosis of NMIBC than each method separately. When the Uromonitor® test was combined with cystoscopy in our study, 88.8% sensitivity and 97% specificity were obtained. The combined AUC-ROC = (0.888 * 0.97) / 2 ≈ 0.86064 demonstrating an improvement in sensitivity and maintenance of specificity. In our study, no KRAS mutations were detected in the samples analyzed, therefore, the sensitivity of TERT/FGFR3/KRAS was similar to that obtained in the detection of TERT/FGFR3. Conclusions: This work allowed us to validate the Uromonitor test for the detection of recurrence of bladder cancer. A 100% concordance was achieved in the reproducibility of the test. Our results show that this test is highly sensitive (87.5%) and specific (97%) and with great predictive value in the detection of tumor recurrence in patients with a history of NMIBC under surveillance. The pooled precision less than 100% is justified due to the detection of positivity by real-time PCR in samples for which Sanger sequencing does not detect the alteration due to lack of sensitivity. In all assays, the analytical detection limit was 6.25% mutant sequences against a wild-type DNA background. The presence of altered DNA in less than 6.25% of the total DNA in the sample may not be detected.
We can conclude that the Uromonitor®, with and without detection of KRAS mutations (Uromonitor® Tertp/FGFR3) could easily be used in association with cystoscopy in a specific context, since combined they increase sensitivity while maintaining a very high specificity rate, or be used even as a single method in patients unable to perform a cystoscopy. Our results prompt us to validate these findings in a robust and expanded independent series with an ongoing study that includes a group of benign conditions (renal lithiasis, urinary tract infections, prostatic hyperplasia, and others), as well as the utility of Uromonitor® in the initial diagnosis of all bladder cancers. We intend to test it and externally validate it to assess its cost-effectiveness and determine its value in both initial diagnosis and follow-up of urothelial cancer patients.

282. 273. **CRITICAL ROLE OF MIR-877-5P IN PROLIFERATION AND ADHESION OF TRIPLE NEGATIVE BREAST CANCER**

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Breast cancer (BC) is the leading cause of cancer death in women. Triple negative breast cancer (TNBC) is the molecular subtype that is known for having a poor prognosis and limited therapeutic options compared to other subtypes of BC. MiRNAs are small non-coding RNAs that regulate gene expression. Aberrant expression of miRNAs in body tissues and fluids are linked to pathologies such as BC. Previously, we identified several miRNAs whose expression is altered in BC tissue and correlates with patient survival using bio-

informatic approaches. In particular, we found that miR-877-5p was diminished in BC tissue compared to normal adjacent tissue (NAT) and its expression correlates with worse overall survival. The aim of this work was to investigate the effect of miR-877-5p in TNBC experimental models. Our hypothesis is that miR-877-5p has oncogenic functions in TNBC increasing tumor growth and progression. We first determined miR-877-5p expression levels in PAM50 basal-like BC tumors and NAT of patients from the TCGA BRCA data set. The miR-877-5p was significantly increased in PAM50 basal-like BC tissue compared to normal adjacent tissue. Analysis using UCSC Xena tool showed that its expression is increased in basal-like BC compared to the other PAM50 BC molecular subtypes. To investigate the effect of miR-877-5p in TNBC, we generated 4T1 stable-transfected cells with this miRNA overexpressed or control cells after clonning miR-877-5p into plasmid vector. Additionally, transient transfections with miRNA inhibitors were performed. Then, we evaluated the effect of miR-877-5p in proliferation, adhesion and migration of 4T1 cells through in vitro assays. We found that miR-877-5p inhibitor decreased cell viability and adhesion in this BC cell line. Our results suggest that targeting miR-877-5p could have potential therapeutic implications by impairing the survival and adhesive properties of 4T1 tumor cells, thereby potentially limiting their ability to spread and proliferate.

283. 472. **CHLORPYRIFOS PROMOTES CANCER STEM CELL SELECTION AND REGULATES THE EXPRESSION OF GENES LINKED TO ANTIESTROGEN THERAPY RESISTANCE IN BREAST CANCER**

Marianela Lasagna,[1-2] Daniel Zappia,[3] Mariana Mardirosian,[1-2] Gabriela Martin,[2] Noelia Miret, [2-4] Andrea Randi, [4] Mariel Nuñez, [2] Claudia Cocca [1-2]

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Loss of Estrogen Receptor α (ERα) expression is critical for breast cancer progression and considered to be one of the mechanisms of resistance to endocrine therapy. It is postulated that histone deacetylase 1 (HDAC1) interacts with ERα and suppresses ERα transcriptional activity. The organophosphorus chlorpyrifos (CPF) is currently classified as an Endocrine Disruptor (ED), as it can simulate hormone actions both *in vitro* and *in vivo.* EDs have been associated with endocrine therapy resistance. In this study, we investigated whether CPF can induce mechanisms associated with resistance to antiestrogen therapy such as the proliferation of cancer stem cells (CSC) and/or HDAC1 and ERα modulation. Our experiments were performed using MCF-7 cell line (ERα+) or MDA-MB231 and MCF10A (ERα-) cell lines. We analyzed if CPF (0.05 and 50 μM) induces CSC proliferation by mammosphere (MS1) assay and/or ERα, HDAC1 and the co-repressor SMRT expression in monolayer cells and MS1 by RT-qPCR. To elucidate the participation of ERα in the modulation of HDAC1 expression, we evaluated comparatively, the expression of this enzyme in MDA-MB-231 and MCF-10A. CPF at 0.05 μM increases the subpopulation of CSC derived from MCF-7 cells ($p<0.05$), decreases ERα and HDAC1 mRNA expression in monolayer-grown cells and MS1 ($p<0.05$; $p<0.001$, respectively), while CPF 50 μM decreases expression in MS1 ($p<0.001$), only. CPF 0.05 μM increases SMRT mRNA expression ($p<0.05$), but CPF 50 μM downregulates this marker ($p<0.05$) in monolayer cultures. In MS1, CPF 0.05 μM decreases SMRT mRNA ($p<0.01$). However, in MDA-MB-231, CPF 0.05 μM increased HDAC1 mRNA levels ($p<0.05$; $p<0.001$) in both monolayer and MS1, and CPF 50 μM increases the expression of this marker in MDA-MB-231 and MCF-10A monolayer-grown cells ($p<0.01$). The presence of the ERα determines the effect of CPF on HDAC expression and it may be a mechanism of resistance to antiestrogen therapy activated by the pesticide.

284. 509. **ANTI-TUMORAL ROLE OF PDX1 IN PANCREATIC DUCTAL ADENOCARCINOMA**

M. Jimena Mosna[1], Marcelo G. Stinson[1], Abel L. Carcagno[1]

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Pancreatic ductal adenocarcinoma (PDAC) represents a very aggressive type of pancreatic cancer worldwide. PDX1 is an important transcription factor both for embryonic development of the pancreas and for differentiation of progenitor cells into Langerhans islet beta cells. Although PDX1 overexpression has been reported in pancreatic cancer, its role in tumorigenesis is unclear. Therefore, our aim was to analyze the role of PDX1 on tumor aggressiveness of PDAC cells. To induce PDX1, PANC-1 cells were treated with BRD7552 for 9 days. Overexpression of PDX1 was confirmed by Western Blot analysis and no cytotoxic effect was observed by MTT or Trypan Blue assays on treated cells. Wound healing assay showed a significant reduction in migration rate compared to control. Preliminary results obtained by propidium iodide staining and flow cytometry assay revealed a higher proportion of cells in G1 phase in treated cells compared to control. Cell confluence assay showed a significant reduction in the area occupied by treated cells compared to control. However, immunostaining against Ki67 and Phospho-H3 revealed no significant difference in cell proliferation rate. Treated PANC-1 cells were implanted onto the chorioallantoic membrane of chick embryos and tumor growth was measured at different stages observing an increased reduction in tumor size between post-implantation days 3 and 7-8 compared to control. A hematoxylin-eosin staining showed a lower invasion of treated cells in the chorioallantoic membrane compared to control. Moreover, among tumors, different characteristics were observed according to morphology, coloration, and the presence of an angiogenic process. In conclusion, overexpression of PDX1 affects cell cycle, reduces the occupied area and inhibits the migratory potential *in vitro* as well as tumor growth *in ovo* in PANC-1 cells suggesting an anti-tumoral role for PDX1 in PDAC.

285. 611. **CHANGES IN CSCs PHENOTYPE PROMOTED BY GLYCOSAMINGLYCANS MODULATION IMPROVES PACLITAXEL-RESPONSE IN LUNG CANCER**

Flavia Piccioni[1], Marco Aurelio Díaz Gutierrez[1], Mariel Fusco[1], Lucía Victoria[1], Ludmila García[2], Paula Rosello[1], Manglio Rizzo[1,2], Mariana Malvicini[1].

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The success of conventional chemotherapy with taxanes employed in patients with non-small cell lung cancer (NSCLC) is restricted at least in part by cancer stem cell (CSC)-niches within the tumor microenvironment (TME). These cells express CD133, CD44, CD47, and SOX2, among other markers and factors. Hyaluronan (HA), a glycosaminoglycan of the TME can regulate CSC's function. We previously demonstrated that coumarin 4-Methylumbelliferone (4Mu) reduced HA expression in murine Lewis Lung Carcinoma (LLC) cells and increased their sensibility to paclitaxel (Pa) *in vitro*. Also, CD133+ cells differentially expressed HA compared to CD133- cells. In a mice model generated by subcutaneous injection of LLC cells, the Pa-4Mu combination significantly reduced the tumor volume compared to the Control group (C). In this work, we aimed to validate the effect of 4Mu combined or not with Pa on HA-metabolism and CSC-phenotype gene expression, previously analyzed *in vitro* in whole (w)LLC cells. We found that Pa increased the expression of CD133 compared to C ($p<0.01$), but when Pa was combined with 4Mu, its levels decreased drastically, below the C ($p<0.001$). Additionally, 4Mu decreased HAS-3 expression ($p<0.01$). To confirm that these effects were due to the differential expression of HA by CSC cells, we separated the CD133+ and CD133- fractions from

wLLC cells using magnetic beads. We observed that CD133+ cells expressed higher levels of HAS-3, ABCC5 HA-transporter, SOX2, and CD47 compared to CD133- cells ($p<0.01$). In addition, 4Mu decreased the expression of CD44 and HAS3 in the CD133+ fraction ($p<0.001$). We also found that Pa increased ABCC5 expression in both populations ($p<0.05$). Then, CSCs from NSCLC produce HA differentially and 4Mu modified their metabolism in isolated CD133+ cells. Notably, the CSC phenotype induced by Pa is restored to basal levels when combined with 4Mu in vivo. HA-modulation is a promising strategy to overcome CSC resistance to chemotherapy in NSCLC.

O2-ONCOLOGY

WEDNESDAY 15TH NOVEMBER 14:00-15:30
CHAIRS: MARIANO GABRI
VICTORIA FABRIS

286. 170. **DISSECTING THE THERAPEUTIC ROLE OF CARBON MONOXIDE (CO) IN PROSTATE CANCER**

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Several studies have placed carbon monoxide (CO) as an anti-inflammatory, anti-apoptotic, and anti-proliferative gasotransmitter. Over the past few years, CO releasing molecules (CO-RMs) have been developed to deliver controlled amounts of CO to cells and tissues. They are also being tested as potential therapeutic agents. We hypothesized that CO-RMs might modulate processes associated with prostate cancer (PCa) development. Therefore, in this work we aimed at identifying the impact of CO-RMs on PCa cells. First, we assessed the release of CO from three water-soluble CO-RMs (CORM-3, CORM-A1 and CORM-401) using a well-established myoglobin assay (spectrophotometric analysis of the conversion of deoxy-myoglobin to carbonmonoxy-myoglobin). Then, we treated PC3 cells (PCa cell line) with the 3 CO-RMs (25-200 μM, 6 h): 1) CORM-3 (ruthenium-based); 2) CORM-A1 (boronocarbonate-based); and 3) CORM-401 (manganese-based); and assessed cell viability (MTS assay), cell adhesion (cristal violet staining), cell migration (wound healing assay), oxidative stress (DCFH staining followed by confocal microscopy) and cell metabolism (ATP production assay). We did not observe changes in cell viability for the range of concentrations tested. However, we found a significant increase in the number of adherent cells ($p<0.05$) and a significant decrease in ROS levels ($p<0.05$) with the three CO-RMs evaluated. We also observed a significant decrease in cell migration after CORM-3 treatment ($p<0.05$). Further, we found that CORM-3 and CORM-401 modulated PCa metabolism impairing ATP production in PC3 cells ($p<0.05$). These findings evidence that CO-RMs might have multifaceted effects on PCa cells in the absence of the traditional toxicity reported for CO, highlighting the potential therapeutic implications of CO-RMs in PCa, opening up new avenues for intervention.

287. 205. **ESTABLISHMENT OF CELL LINES AND CULTURE OF CANCER STEM CELLS (CSCS) FROM VETERINARY PATIENT-DERIVED TUMOR SAMPLES ON LAB-ON-A-CHIP (LOC) MICRODEVICES**

Silvia Gómez [1 1], Ana Peña Herrera [2 2 2], Imanol Agüero [11], Matías Tellado [33], Betiana Lerner [22], Maximiliano Pérez [22], Denise Belgorosky[1 11] & Ana MarÍa Eiján[111 1]
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Cancer Stem Cells (CSCs) are a minority tumor cell population, associated with treatment resistance, as well as post-surgical recurrences. The identification, quantification and establishment of cell lines derived from primary tumors, can be a useful methodology to predict the evolution of patients and their response to treatment. The use of Lab-On-a-Chip (LOC) microdevices (MD), as a miniaturized support, enables the isolation and monitoring of CSCs. Objective: To develop an assay to study CSCs therapeutic response in LOC, in order to serve as a predictive platform for response to treatment in patients. Methods: Twenty-nine (29) veterinary tumor samples (Sarcomas (SA): 41%, Carcinomas (CA): 31%, Mastocytomas (MA): 17%, Melanomas (ME): 7%, Lymphomas (LY): 3%) were obtained, cultured in 2D and CSCSs were analyzed through sphere culture within LOC MD, using concentrations from $1x10^5$-$6x10^5$ cells/ml in low adhesion conditions, DMEM F12 , B27, FGF and EGF in absence of serum. Results: Among of the 29 tumors: 6 were established as 2D cell lines (solid thyroid CA, tubulopapillary CA, bladder CA, low and high grade MA); 5 samples were grown as spheres (% Sphere formation efficiency (SFE±SD) : SA: 0.37±0.2, Bladder CA:0.28±0.05, Thyroid CA: 0.37±0.3, Squamous cell CA: 0.1±0.02 and LY: 0.19±0.1). In a thyroid CA tumor sample, cell viability (MTS) was measured under carboplatin treatment, determining an inhibitory dose of 57 μM ($p<0.0001$). Furthermore, in one of the SA samples, treatment with Bleomycin (1.82 μM) decreased SFE and sphere size ($p<0.05$). Based on these results, the tumor types that we have been able to grow as spheres within LOCs have been high-grade CA and SA, and MA and CA were able to be cultured in 2D. The growth of CSCs within LOC microdevices and the possibility of culturing tumor cells derived from veterinary patients, encourages us to pursue the development of a predictive test prior to human clinical studies.

288. 242. **EXCESSIVE DOSE OF THE ANTI-APOPTOTIC FAMILY MEMBER SURVIVIN/BIRC5 INDUCES THE DEATH OF PANCREATIC CANCER CELLS**

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Pancreatic ductal adenocarcinoma (PDAC) is a highly aggressive disease with a survival rate to 5 years of less than 5%. In this context, Survivin/BIRC5 is a protein belonging to the IAPs family of apoptotic inhibitors. In adult tissues, Survivin expression is almost exclusively found in tumors. Having in mind those data, efforts have been made to find the ways to decrease the levels of Survivin that goes in detriment of cancer cells viability, but no study was made about the overexpression of this protein. In this study, we analyze the effects of an incremented dose of Survivin in pancreatic cancer cells. We constructed an expression plasmid of Survivin (Sur^{WT}) and a mutant version where lysines 90 and 91 were replaced by arginine to avoid the degradative ubiquitination of Survivn (Sur^{K90}). Interestingly, a scratch assay revealed that stabilization of Survivin, by mean of stable transfection of Sur^{K90} in the pancreatic cancer cell line Panc-1, significantly reduces migratory capacity of the cells. Furthermore, employing the "AlamarBlue" reagent, we observed a decrease in the viability of transiently transfected Panc-1 cells with Sur^{K90} and after of 24h of Sur^{WT}. Additionally, the decreased viability

becomes more pronounced with SurK90 in function of time ($p<0.05$). Moreover, by propidium iodide incorporation, though Panc-1 cells transfected with Sur^{WT} show an increase in apoptosis this effect is exacerbated with Sur^{K90} ($p<0.05$). In summary, our results demonstrate a relationship between the "dosage" of Survivin/BIRC5 and the decrease in viability in PDAC cells, along with a reduction in migratory capacity. These findings contradict the expected outcomes considering the anti-apoptotic and cell survival roles of this protein, leading to further investigation into the underlying mechanisms behind these observations.

289. 259. **EFFECT OF CANNABIDIOL (CBD) ON HLA-G EXPRESSION IN HUMAN CHORIOCARCINOMA (JEG-3) TUMOR CELLS**

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Human leukocyte antigen G (HLA-G) is a protein known for its key role in fetal-maternal immune tolerance. It is expressed preferentially in fetal tissues and a restricted manner in certain healthy adult tissues. Different tumors express this protein, which gives them the ability to evade or decrease the immune response.
There is extensive evidence on the palliative effects of cannabinoids (antiemetic, analgesic, anti-inflammatory, appetite stimulation, etc.) in patients with different tumors undergoing usual cancer treatments such as radiotherapy and chemotherapy. However, its therapeutic potential in oncology is not restricted to these effects. Several studies have demonstrated its antiproliferative, proapoptotic, antiangiogenic, and antimetastatic effects in various tumors, both *in vivo* and *in vitro*. Objectives. To evaluate the effects of different concentrations of cannabidiol (CBD) on the expression of HLA-G in the JEG-3 tumor line using molecular techniques. Materials and Methods. JEG-3 cells were cultured for 0, 12, 24 and 36 hours in the presence of 1 and 5 μM CBD, in DMEM + 0.4% DMSO medium, with their respective controls. After each incubation, HLA-G expression levels were analyzed by RT-PCR. Results. Through the t-test analysis ($p<0.05$) it was possible to observe that after 12 hours of culture, in cells incubated with 1 μM and 5 μM of CBD, the expression of HLA-G decreased significantly from 0.15 ± 0.03 (control value) to 0.0520 ± 0.0001 (value at 1μM 12 h) and 0.031 ± 0.003 (value at 5μM 12 h). This effect was maintained during 24 and 36 hours of incubation, without showing significant changes between them ($p<0.05$). Conclusion: our results show a marked decrease in the expression of HLA-G in JEG-3 tumor cells in presence of different concentrations of CBD. This could represent a new antitumor effector pathway of cannabinoids.

290. 389. **EFFECT OF INHIBITION OF NOTCH SIGNALING IN NON-SMALL-CELL LUNG CANCER**

Marcelo G. Stinson[1], M. Jimena Mosna[1], Abel L. Carcagno[1]

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Lung cancer is the second most frequent cancer worldwide, and the third one in Argentina. It is also the first cause of cancer-related deaths both globally and in Argentina. Non-small-cell lung cancer (NSCLC) represents approximately 85% of all lung cancer cases. 55% of NSCLC patients receive a diagnosis at advanced stages of the disease, with presence of distant metastatic foci, and have a 5-year relative survival rate of only 9%. There is accumulating evidence of the relevance of developmental pathways in carcinogenic processes. In particular, Notch signaling is a type of intercellular communication pathway with several roles in developmental processes. During development of the lung, Notch signaling plays crucial roles in processes such as proximo-distal patterning, cell fate choice, cell proliferation, and apoptosis. The aim of this work was to evaluate the role of Notch signaling in the tumorigenicity of NSCLC cells. We used DAPT, a pharmacological inhibitor of the γ-secretase, to downregulate the activity of the Notch pathway in A549 human adenocarcinoma cells as a model for NSCLC. A549 cells were treated for 7 to 9 days with 40 μM DAPT. Treated cells do not differ in viability from control cells (Trypan Blue exclusion). DAPT-treated cells show reduced adhesion time to the culture plate and decreased proliferative and clonogenic (low-density culture) capacities. Migration of treated cells is also impaired (wound healing). Furthermore, we also evaluated the effects of treatment with DAPT on spheroids of A549 cells. Treated spheroids are significantly bigger, and seem to be less densely packed than control spheroids. Treated spheroids were also grafted onto the chorioallantoic membrane of chick embryos, where their original differences in dimensions were lost. In conclusion, these results suggest that Notch signaling is relevant to the tumorigenicity of NSCLC cells, indicating its potential as a target for lung cancer treatment.

291. 517. **EFFECT OF METRONOMIC TREATMENT WITH CYCLOPHOSPHAMIDE (CY) AND LOSARTAN (LOS) ON THE TUMOR MICROENVIRONMENT (TME) HYPOXIA IN M-234p TRIPLE NEGATIVE MURINE MAMMARY ADENOCARCINOMA**

Fusini, Matías E[1].; Iriarte, Camila[1]; Martínez, Itati[1]; Pinzón Rivas, Johan[1]; Viviana R. Rozados[1,2]; O. Graciela Scharovsky[1,2,3]; Leandro E. Mainetti[1,2]; María José Rico[1,2]

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In breast cancer, TME hypoxia determines a state of immunosuppression and promotes tumor development, angiogenesis, vascular mimicry, metastasis and resistance to treatment. During the early stages of tumor cell growth, increased mitotic figures (MF) and $Ki67^+$ cells were observed with a significant reduction in the acute inflammatory infiltrate of eosinophils granulocytes (Eo) and concomitant increased extracellular deposition of collagen fibers, cancer associated fibroblasts (α-SMA^+) and tissue hypoxia ($HIF\text{-}1\alpha^+$). Our aim was to evaluate the efficacy of metronomic treatment with CY and LOS, with respect to the TME variables related to tissue hypoxia conditions. With this purpose, BALB/c mice were inoculated s.c. with M-234p tumor (n=36). When the tumor was palpable, the experimental groups were assembled: Control (no treatment), CY (25mg/kg/day), LOS (150mg/kg/day) and CY+LOS. Treatments were administered in the drinking water. Tissue samples (n=4/day) were obtained pretreatment (day 0), with treatment (days 7 and 14) and were fixed in 4% formalin and paraffin embedded. The tumor volume doubling time was higher in CY ($P<0.001$) and CY+LOS groups ($P<0.0001$) respect to Control, and on day 14 showed when comparing CY+LOS vs. Control, an increase in Eo ($P<0.05$), a decrease in FM ($P<0.05$) and $Ki67^+$ cells ($P<0.05$). Also, a decrease in the collagen area ($P<0.05$) and $HIF\text{-}1\alpha^+$ cells ($P<0.05$) were observed; αSMA^+ cells decreased in CY ($P<0.05$) and CY+LOS ($P<0.01$) respect to Control. The intra-tumoral vascular mimicry area was evidenced by the PAS^+ reaction. The CY+LOS group showed a lower density compared to the Control group ($P<0.05$). In conclusion, the combined treatment with CY and LOS: 1) increases of the concentration of eosinophils in the TME with a putative antitumor activity; 2) improves a TME with less hypoxia and consequent less desmoplasia; 3) decreases the vascular mimicry, and 4) CY+LOS synergy improves TME conditions favoring the decrease in tumor growth.

292. 545. **EFFECTS OF PHARMACOLOGICAL INHIBITION OF THE ATP DOMAIN OF HSP90 IN PROSTATE AND BREAST CANCER CELLS**

Nicolás Bruno[1], Iara Santa Cruz[2], Agostina Aramburu[3], Valentina Hansen[1], Camila Rubino[1], Alejandra Erlejman[1,4], Mario Galigniana[1,2], Gisela Mazaira[1,4].

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In recent decades, heat-shock protein 90 has become an attractive target for antitumor therapies since Hsp90 stabilizes many oncoproteins in their active conformation, it is overexpressed in tumor cells, and its inhibition simultaneously affects all hallmarks of cancer. It is accepted that the intrinsic ATPase activity of Hsp90 is directly responsible for its biological activity and therefore is the main target of pharmacological inhibition. The first generation of chaperone inhibitors was derived from geldanamycin (GA) and radicicol, two natural compounds that bind to the ATP-binding pocket of Hsp90. Although preclinical studies were promising, the clinical trials evidenced severe toxic effects, mainly nephro- and hepato-toxicity. The aim of this work was to analyze the biological actions of synthetic compounds predesigned by computational modelling according to their putative inhibitory effect on the ATPase activity of Hsp90. Such effect was inferred from their elemental molecular structures, which were chosen based on their proven low toxic effects. GA was used as a control in all assays. Results show that both the pyrazoline-derived compounds (C3 and C6) and the imine-derived compound (4f) reduce cell viability and cell migration for the two cell lines used as experimental models, i.e., PC3 prostate cancer cells and MDA-MB-231 breast cancer cells. It is shown that cell migration is affected by the reorganization of the actin cytoskeleton, whereas the effect on cell viability was due to an induced generation of reactive oxygen species. As predicted by in silico assays, all compounds significantly inhibited Hsp90 ATPase activity in vitro. Nonetheless, none of the compounds affected the migration to the nucleus of the glucocorticoid receptor, a known Hsp90-dependent process. Therefore, these results lead to propose that the intrinsic ATPase activity of Hsp90 is not directly responsible for all its biological functions.

P1-ONCOLOGY

WEDNESDAY 15TH NOVEMBER 14:00-15:30

CHAIRS: CECILIA PÉREZ PIÑERO
YANINA BENZO
ULISES ORLANDO

293. 56. **ANGIOTENSIN-(1-7) TREATMENT COUNTERACTS METASTASIS INDUCED BY RESISTANCE TO VEGFR INHIBITORS IN BREAST CANCER**

Agustina F. Carnevale[1], Pedro J. Salaberry[1], Ignacio E. Schor[1], Edith C. Kordon[1], Thomas Walther[2], Albana Gattelli[1] and Carolina P. Schere-Levy[1].
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Breast cancer metastasis promote by resistance to current therapies is a crucial factor precluding improvements in mortality. Triple negative breast cancer (TNBC) is the most aggressive and highly metastatic tumor subtype with absence of specific therapeutic options. Several inhibitors of tyrosine kinase activity (TKi) have been evaluated clinically to improve the survival of patients, including those that inhibit VEGFR which acts as the master regulator of angiogenesis promoting both tumor growth and metastatic spread. Different anti-VEGFR agents have been developed either TKis as Axitinib (Ax) or antibodies to block the binding of ligand (VEGF) as Bevacizumab (Bev). However, its effectiveness is under discussion since prolonged treatment develops resistance and patient relapse with metastasis in secondary organs. We studied the role of the renin-angiotensin system (RAS) in breast cancer. We reported that the pro-metastatic action of Angiotensin II (AngII) can be reverted by Angiotensin 1-7 [Ang-(1-7)]. Ang-(1-7) is generated by ACE2 enzyme. In renal carcinoma the treatment with Ax generates resistance by decreasing the expression of ACE2, being able to reverse this effect by adding Ang-(1-7). Herein, we found that treatments with Ax or Bev reduce the expression of ACE2 ($p<0,05$) in both TNBC and luminal cell lines, suggesting that a similar mechanism could be operating in breast cancer. In mouse allograft using two different metastatic TNBC tumor models (4T1/BALB/c, EO771/C57BL/6), independently of tumor size or time elapsed, we found that the sustained treatment either Ax or Bev induced lung metastases. Importantly, the addition of Ang-(1-7) to the regimen of treatment significantly reduced the development of lung metastatic foci ($p<0,02$) and decreased VEGF expression in tumor tissue. Our results indicate that combined treatment with Ang-(1-7) could reverses resistance to VEGFR inhibitors by reducing metastasis improving breast cancer therapy.

294. 62. **A PROSPECTIVE STUDY OF PREDICTIVE BIOMARKERS OF RESPONSE TO TREATMENT WITH BACILLUS CALMETTE-GUERIN (BCG) IN PATIENTS WITH NON MUSCLE INVASICE BLADDER CANCER. PRELIMINAR ANALYSIS OF URINARY LYMPHOCYTES**

José León Mellado[1], Joaquín Chemi[2], María Teresa Pombo[3], Mariana Aris[1], Roberto Villalba Bachur[2], Juan Camean[2], Jorge Jaunarena[2], Ximena Garcia[4], Gisela Coliva[4], Mora Amat[4], José Mordoh[1], Gustavo Villoldo[2], María Marcela Barrio[1]
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Intravesical BCG is the main therapy for non-muscle invasive bladder cancer (NMIBC) pts with a ~60% response rate. BCG induces a srong local inflammatory response that ultimately eliminates the tumor. In a previous study we defined a Th2-score evaluated in TILs of pre-BCG biopsies associated to the BCG response. We launched a prospective study to validate the score and to evaluate immune parameters in urine and blood samples before and on BCG treatment. In pre-BCG biopsies, the Th2-score was calculated combining T-bet+(Th1) and GATA-3+(Th2) ratio and the density/activation of EPX+ eosinophils by IHC. Urinary lymphocytes (UL) were analyzed by FACS. Single cell data from all the pts (74 UL samples, 205,067 $CD3^+FVS510^-$ cells) was modeled using unsupervised clustering algorithms (PhenoGraphv3.0) to significantly discriminate high and low score pts. The main results were: **1**-7pts had high (favorable) and 9 pts had low (unfavorable) score. **2**-PMN and monocytes were mainly present in the urine, and CD3+T cells could be recovered after 3-4 BCG doses; mostly CD4+>CD8+, and <10% NK/NKT cells **3**-$CD103^+$ T cells ($CD4^+$ and CD8+) tended to increase in high-score, but not in low-score pts. **4**-After induction (6- BCG) high-score pts had a higher proportion of $CD8+PD\text{-}1^+CD39^+TIM\text{-}3^+$ ($p= 0,03$) and $CD4^+CD39^+PD\text{-}1^+$ cells ($p= 0.048$) in UL than low-score pts, and were significantly reduced only in high-score pts with the advance of treatment (Post-M2) ($p= 0.02$. **5**-Clustering analysis revealed that $CD4^+CD103^+$ UL expressing distinct levels of activation/exhaustion molecules were significantly different between both groups: *Clusters 5* ($CD39^{low}PD1^{low}TIM\text{-}3^{low}$), *19*: ($CD39^{high}PD1^+TIM\text{-}3^+$ and *25* ($CD39^{low}$ $PD\text{-}1^-TIM\text{-}3^-$). Our results suggest that the initial response to BCG, with a greater activation of TILs (reflected in UL), occurs differentially between both groups, and could be relevant for the elimination of residual tumor cells. These results must be confirmed with the inclusion of more pts to the study.

295. 247. **CDU/5FC AND hIFNb GENE THERAPY IN COMBINATION WITH METFORMIN FOR THE TREATMENT OF GLIOBLASTOMA**

Daniela A. Pérez Visñuk, Gerardo Glikin, Liliana María Elena Finocchiaro, Marcela Solange Villaverde
Unidad de Transferencia Genética, Área Investigación, Instituto de Oncología Ángel H. Roffo, Facultad de Medicina. Universidad de Buenos Aires, Argentina.

Glioblastoma (GBM) is the most common and lethal primary tumor of the central nervous system (CNS). Although effective strategies have recently emerged for other tumors, overall survival of GBM patients remains dramatically poor (<18 months). Thus, denoting the need of research in this field. The aim of the present work was to determine the potential therapeutic effect of two different non-viral gene therapy approaches: 1) a yeast cytosine deaminase::uracil phosphoribosyl transferase/5-fluorocytosine (CDU/5-FC) suicide system and, 2) the lipofection of the human beta interferon gene, hIFNβ on U251, a human GBM cell line, alone or in combination of the metabolic modulator, metformin, (MET, a mitochondrial complex

I inhibitor). We found that both gene therapy approaches decreased GBM cells viability (crystal violet and APH assay; p<0.05). In addition, 3-5 mM MET increased this cytotoxic effect (hIFNβ vs hIFNβ / MET, p<0.05). Mechanistically, hIFNβ lipofection altered mitochondrial potential and produced intracellular oxidants (TMRM and DCF respectively, flow cytometry) particularly in combination with MET. Similar results were found during the combination of CDU/5FC and MET (DCF, p<0.05). In addition, we found that the combination of MET with the 5FU chemotherapy (the active product of CDU/5FC) reproduced these findings. These promising results encourage us to continue with the study of CDU/5FC and hIFNβ gene therapies alone or in combination with MET for the treatment of this extremely malignant disease.

296. 251. **AN ANTITUMOR PENICILLIN DERIVATIVE INHIBITS β-CATENIN LEVELS AND CELL MIGRATION IN MELANOMA CELLS SENSITIVE AND RESISTANT TO THE BRAF INHIBITOR DABRAFENIB**

Sofia V. Bajicoff[1], Florencia Cayrol[2], Mercedes Debernardi[2], Camila Chocan[1], Maria Florencia Arbe[3], Marcela Villaverde[3], Carina M.L. Delpiccolo[4], Nadia L. Martiren[4], Ernesto G. Mata[4], Viviana C. Blank[1], Leonor P. Roguin[1]

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Melanoma is a very aggressive form of skin cancer, with increased metastatic potential and high resistance to cytotoxic agents. In the search for effective treatments for resistant cells, we decided to study the mechanism of action of a previously characterized antitumor penicillin derivative (TAP7f) in different melanoma cells. After evaluating TAP7f cytotoxic potency (IC_{50} values) and the sensitivity to BRAF and MEK inhibitors, we selected two melanoma cell lines for further studies: A375S and A375R, sensitive and resistant to the BRAF inhibitor dabrafenib. By Western blot assays (WB), higher expression levels of β-catenin and p-ERK were found in A375R cells with respect to A375S cells (p<0.0001). When both melanoma cell lines were treated with TAP7f (20 μM) for 24 h, WB assays showed that the penicillin derivative reduced β-catenin expression levels in A375S (44,5 ± 8,2 %, p<0.001) and A375R cells (35,7 ± 7,4 %, p<0.01). Under the same experimental conditions, qPCR assays revealed that β-catenin mRNA levels were not modified, suggesting that β-catenin decreased expression is probably caused by proteasome degradation. Accordingly, after incubating each cell line with TAP7f in the presence of MG132, a proteasome inhibitor, a significant reversion of TAP7f inhibitory effect on β-catenin levels was observed (p<0.01). When the effect of the derivative on cell migration was studied by wound healing assays, results showed that although A375R cells exhibited a greater migratory ability than A375S cells, TAP7f decreased the percentage of wound closure in both cell lines in a concentration-dependent manner. Taken together, our results suggested that TAP7f exhibits antimetastatic activity both in sensitive and resistant melanoma cell lines through downregulation of β-catenin levels and inhibition of cell migration. These findings encourage us to continue studying this penicillin derivative as a potential candidate for the treatment of BRAFi-resistant melanoma cells.

297. 304. **ADAPTATION TO NUTRIENT STARVATION IN CANCER CELLS**

Sabrina E. Campisano[1], Caroline Gélabert[2], Irene C. Golán[2], T. N. Beyene[3], Carl-Henrik Heldin[2], Luciana Barbini[1,4], Patricia Sancho[3], Aristidis Moustakas[2], Laia Caja[2], Andrea Chisari[1,4]

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The aim of this work was to study how cancer stem cells from HLF and SNU499 HCC lines adapted to survive when the availability of glutamine or glucose was scarce. Cells were cultured under control concentration of nutrients and under different starvation conditions (with galactose instead of glucose, without glutamine or with low concentrations of glucose). It was determined the effect of nutrient deprivation on cell proliferation (MTS), ROS production (DCFH, MitoSOX and Cell Rox green probes), mitochondrial membrane potential, mitochondrial mass, glucose uptake capacity (CMXROS, Mitotracker Deep red and 2NBDG probes, respectively), stemness and epithelial mesenchymal transition by Extreme Limiting Dilution Analysis and N-cadherin, E-cadherin, Vimentin, Sox-2 and Fibronectin expression. HLF and SNU499 cells cultured during 72 hours with galactose instead of glucose or without glutamine proliferated less than control cells (p<0.001). After 7 days, both cell lines cultured under the different starvation conditions showed a lower proliferation compared to the control (p<0.001). Less overall ROS production in HLF cells cultured under the different nutrient starvation conditions was found compared to the control (p<0.001). HLF cells cultured without glutamine also produced lower levels of mitochondrial superoxide (p<0.05) which was correlated with a lower mitochondrial membrane potential and mitochondrial mass (p<0.001). SNU499 hepatocytes cultured without glutamine also produced lower levels of mitochondrial superoxide (p<0.001) and was correlated with a lower glucose uptake capacity (p<0.01). Both cell lines cultured without glutamine showed a lower stem cell frequency compared to the control (p<0.001). HLF cells cultured without glutamine increased E-cadherin expression (p<0.001) and decreased N-cadherin and fibronectin expression compared to the control (p<0.01). Inhibiting glutamine uptake in highly replicative cells could be an interesting therapeutic target.

298. 305. **ANTAGONISTIC ROLE OF RUNX1 IN THE REGULATION OF KLF4 GENE EXPRESSION IN TNBC CELL LINES AND THEIR CSC SUBPOPULATION**

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Triple negative breast cancer (TNBC) is an aggressive breast cancer subtype for which no effective targeted therapies are available. Growing evidence suggests that chemotherapy-resistant cancer cells with stem-like properties (CSC) may repopulate the tumor. Therefore, therapies that target CSC in combination with chemotherapy might prevent tumor recurrence. In TNBC patients, the expression of transcription factor RUNX1 correlates with poor prognosis. We identified that RUNX1 is relevant in tumor aggressiveness in TNBC cell models, for the regulation of oncogenes, cell migration and drug resistance. We recently reported that RUNX1 inhibition enhances drug sensitivity in TNBC-CSC. However, the mechanisms involved are still undefined. On the other hand, the transcription factor KLF4 is required to generate CSCs in TNBC and it has been described as a RUNX1 target gene in other tumors. Our goal was to investigate the regulation of *KLF4* by RUNX1 in TNBC cell lines. To consider intratumor heterogeneity we used two cell culture models: attached and forced suspension (mimicking CSC). To inhibit RUNX1 transcriptional activity we used the inhibitor AI-10-104. We found that, when RUNX1 activity is inhibited in attached MDA-MB-231 and -468 cell lines KLF4 mRNA and protein are increased, in a dose- and time-dependent manner (p<0.05). Growing MDA-MB-468 in forced-suspension significantly increased *KLF4*, *RUNX1*

and other stem genes levels. Interestingly, under forced suspension conditions, *KLF4* mRNA levels are reduced when RUNX1 was inhibited. Finally, we observed that *KLF4* mRNA is upregulated in MDA-MB-231-doxorubicin treated (0.01uM $p<0.05$) and -468-paclitaxel treated (5nM $p<0.05$). Taken together, these results suggest that RUNX1 may act antagonistically in tumor heterogeneity. The characterization of this dual gene regulation within the intratumoral cell variation is crucial for the understanding of drug resistance mechanisms and the development of future therapeutic strategies.

299. 371. **BORON BIODISTRIBUTION PILOT STUDY FOR BORON NEUTRON CAPTURE THERAPY (BNCT) MEDIATED BY BORIC ACID + GB-10 IN AN EXPERIMENTAL ORAL CANCER MODEL**

P.S. Ramos [1], M.A. Palmieri[2], A. Monti Hughes[1,3], V.A. Trivillin[1,3], M. Viale[3], E.C.C. Pozzi[4], I.E. Czornenki[1], D.N. Frydryk Benitez[1], G. Agüero[1], A.E. Schwint[1,3], M.A. Garabalino[1]

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[4]Departamento de Reactores de Investigación y Producción, Comisión Nacional de Energía Atómica (CNEA).

BNCT is based on the selective incorporation of ^{10}B carriers in tumors followed by neutron irradiation. A critical aspect of the therapeutic efficacy of BNCT is the biodistribution of ^{10}B in the tumor and in the dose limiting normal and precancerous tissues in the target volume. We previously performed biodistribution and BNCT studies with sodium decahydrodecaborate (GB-10) or Boric Acid (BA) in the hamster cheek pouch oral cancer model (HCPOCM), both at 50 mg^{10}B/kg. Although BA exhibited significantly higher boron accumulation in the tumor than GB-10, BA/BNCT induced a significantly higher radiotoxicity than GB-10/BNCT in the precancerous tissue surrounding tumors. Based on these results, the combination of BA+GB-10 would increase BNCT therapeutic effect while reducing radiotoxicity in precancerous tissue. For this aim, we performed boron biodistribution studies employing two administration protocols of BA+GB-10: (P1) BA+GB-10 (35+15) mg ^{10}B/kg, iv; (P2) BA+GB-10 (25+25) mg ^{10}B/kg, iv. Three hours after the administration of BA+GB-10, blood, tumor, precancerous tissue and normal pouch tissue samples were processed for absolute boron concentration measurements by ICP-OES. Mean boron concentration (ppm) in the tumor was significantly higher for P1 vs P2 (52.0 ± 6.2 vs 29.3 ± 13.2, respectively, $p=0.0401$). For both protocols, these values are therapeutically useful in BNCT (higher than 20 ppm). However, boron concentration in precancerous tissue and normal pouch tissue was higher in P1 vs P2 (62.1 vs 28.5 and 56.6 vs 28.6, respectively), potentially leading to higher BA+GB-10/BNCT induced radiotoxicity with P1. Finally, we observed similar blood boron concentration values between P1 and P2 protocols (30.3 ppm and 23.6 ppm, respectively) when we reduced the dose of BA. These results suggest that P2 could be a potentially useful protocol to be tested in BNCT *in vivo* studies. Ongoing boron biodistribution studies will complete these results.

300. 401. **CHANGES IN IMMUNE CELL PHENOTYPE AND CYTOTOXIC CAPACITY IN HER2+ BREAST CANCER PATIENTS RECEIVING HER2-TARGETED NEOADJUVANT THERAPY**

Ayelen Ivana Pesce Viglietti[1], María Belén Bordignon[1], Alexis Ostinelli[2], Manglio Miguel Rizzo[3], Gerardo Cueto[4], María Belén Sánchez[1], Florencia Perazzo[5], Mora Amat[2], Federico Coló[2], María Victoria Costanzo[2], Adrián Nervo[2], Jorge Nadal[2], Gabriel Crimi[5], Ignacio Mc Lean[3], Eunice Amancay[3], José Mordoh[1], Pablo Mandó[5] and Estrella Mariel Levy[1].

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The INMUNOHER study evaluated neoadjuvant chemotherapy (NACh) in combination with trastuzumab (TRZ) and pertuzumab (PER) x 6 cycles, in HER2+ breast cancer patients. Circulating immune cells were examined before (PRE samples; n=62) and after therapy (POST samples; n=49) for changes in phenotype and functionality by FACS. Eighteen markers were evaluated to characterize T lymphocytes and NK cells. Functionality was assessed in NK cells as IFNγ production and degranulation against the HER2+ cell line SKBR3 w/wo the addition of TRZ. In most patients GCSF (Granulocyte colony-stimulating factor), was indicated as primary prophylaxis of neutropenia while receiving NACh. After surgery, pathological complete response (pCR) was assessed. Samples from patients who had completed NACh regimens showed alterations in immunophenotype. The proportion of T-lymphocytes increased significantly at the end of treatment ($p<0.01$), in both, CD4 and CD8 subsets. Simultaneously, there was a reduction in the proportion of NK cells ($p<0.001$), and within this population, the phenotype shifted towards more immature cells (higher proportion of CD56brightNKG2A+ and lower proportion of CD57+). On the contrary, the adaptive population CD56dimCD57+NKG2C+ increased after treatment ($p<0.01$). Therapy also had an impact on NK cell function, resulting in a significant reduction in IFNγ production against SKBR3 cells alone ($p<0.01$), or with the addition of TRZ ($p<0.01$). In the context of pCR (pCR=51 vs no pCR=11), most changes were observed in the pCR cohort, however CD4 T cells decreased HLA-DR and CTLA-4 expression only in the non-pCR group ($p<0.05$). Our data suggest that the overall treatment-associated changes in immune cell proportions are predominantly in the pCR cohort. In contrast to other studies with TRZ alone, the addition of PER substantially increased the proportion of patients with pCR, but intervention is still necessary in those patients who do not respond.

301. 413. **AN IN VITRO MODEL TO STUDY IMMUNE CHECKPOINT INHIBITION (ICI) OF ANTI-TUMOR ASSOCIATED ANTIGEN (TAA) SPECIFIC T CELL CLONES**

Erika Schwab[1], María Belén Bordignon[1], José Mordoh[1,2], María Marcela Barrio[1]

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Chronic exposure to the Ags as it happens to TILs exposed to tumor cells, results in lymphocyte exhaustion and expression of immune checkpoints as PD-1. Periodic antigen presentation by dendritic cells (DC), acts as a boost necessary to maintain antitumor immune responses, as it happens after vaccination. Inhibition of immune checkpoints (ICI) aim to release physiological restraints that control immune function by revitalizing exhausted lymphocytes. Our objective is to study PD-1 inhibition of anti-TAA clones with nivolumab *in vitro,* at two different scenarios: i) chronic exposure to tumor cells and ii) presentation with peptide-loaded dendritic cells (DC-pep), as a boost with each vaccination. Anti-TAA CD8+ T cell clones under HLA-A0201-restricted anti gp100 (G154) and anti MART-1 (M26) CD8$^+$ clones were incubated with HLA-A0201$^+$ MEL-XY9 cells (1-6 days) or with DC-pep (48 hs) and analyzed. Phenotype (exhaustion/activation) was assessed by FACS, lytic capacity by Calcein assay, intracellular CD107a/IFNg by FACS and IFNg production by ELISA. Nivolumab or hu-IgG4 were assayed at 10 μg/ml. Clones chronically exposed for 6 days to tumor cells increased CD137 and PD-1 expression > 6 and >1.5-fold, respectively, while their lytic capacity decreased by~50% (5:1 E:T). Nivolumab restored the clones functionality, increasing tumor cell lysis > 6.5 fold and CD107a degranulation by 30%. MEL-XY9 PD-L1 expression increased 3-fold during coculture, probably induced by IFNg release. Clones boosted with DC-pep showed a 26% increase in PD-1 expression and nivolumab enhanced their degranulation capacity against tumor cells by 20%. We developed VACCIMEL, a therapeutic vaccine recently approved for adjuvant treatment of stages IIB, IIC and IIIA melanoma pts. VACCIMEL induced multiple circulating T cell clones reactive to TAA such as gp100 and MART-1, and to neoAgs. Given these results a potential impact of ICI on combination immunotherapy with VACCI-

MEL will be further investigated.

302. 430. **ASCL1 MODULATION INFLUENCES NEUROBLASTOMA CELL DIFFERENTIATION AND AGGRESSIVENESS**

Federico J. Garde[1], Candela D. Pastore[1], Daniela J. Di Bella[2], Abel L. Carcagno[1]

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Neuroblastoma (NB) is the most common extracranial pediatric cancer, originating from cells of the sympathoadrenal lineage. ASCL1 is a critical transcription factor in this lineage's development whose expression should be embryonic and transient, however, it is overexpressed in NB and has been linked to a worse clinical prognosis. The aim was to investigate the consequences of its knockdown (KD) in NB cells. We conducted ASCL1 KD in SK-N-SH cells. Additionally, we analyzed publicly available single-cell RNA sequencing (scRNA-seq) data obtained from tumors of NB patients, to gain insights into the heterogeneity of NB. ASCL1 loss of function results in the induction of differentiation of these cells. NB cells lacking ASCL1 acquire neuronal morphology and decrease their proliferative and migratory capacities, without affecting their viability. For our scRNA-seq analysis, we integrated the data into a single dataset, and clustered by dimensional reduction. We characterized the derived clusters by performing functional enrichment analysis and evaluating the expression patterns of ASCL1 with non-negative matrix factorization. Our findings revealed that NB cells share a common origin, specifically deriving from sympathoblasts of the adrenal medulla and that the observed heterogeneity can be explained by the grade of cellular differentiation and the predicted phase of the cell cycle. We discovered that ASCL1 is expressed across various clusters, and co-expresses with genes linked to neurogenesis and cell cycle. Finally, for future testing of ASCL1 KD, we optimized the implantation of SK-N-SH spheroids onto the chorioallantoic membrane of chicken. The results obtained led to two significant points: a) ASCL1 plays a role in blocking terminal neuronal differentiation in NB, thereby promoting tumor progression; b) the observed effects of reducing ASCL1 function *in vitro* that correlate with the role assessed *in silico* suggest that ASCL1 is a potential target for NB therapy.

303. 461. **CELLULAR REPROGRAMMING VIA ID4 MANIPULATION: A NOVEL APPROACH TO TACKLE TRIPLE NEGATIVE BREAST CANCER**

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Background: This study focuses on exploring the potential capacity of ID4 to reprogram triple negative breast tumors, a novel approach given the limited treatment options for triple negative breast cancer (TNBC). To address this, we focus on cell plasticity as a strategic possibility. This involves the reprogramming of TN tumors into luminal subtypes, thereby sensitizing them to endocrine therapies. Notably, ID4, an inhibitor of differentiation protein, assumes a central role in the reprogramming of mammary cells by controlling the transcription of lineage-specific genes. We hypothesize that the manipulation of ID4 expression could revert aggressive features of TN tumors by triggering luminal differentiation. Methods: In-silico analyses involved the evaluation of ID4 expression on human TN breast tumors of public datasets. In vitro experiments involved cell culture of MDA-MB231 estrogen receptor (ER-) breast cancer cell lines, ID4 expression was silenced by CRISPR-Cas9 technology. Gene and protein expression were analyzed by RT-qPCR and western blot. Results: In silico analysis stratifying ID4 expression in TN breast cancer tumors into "high" and "low" groups reveals distinct luminal and basal gene expression patterns. Notably, the low ID4 group exhibits a significant upregulation in luminal signature expression ($p<0.05$), concomitant with a downregulation in basal signature expression ($p<0.01$). Gene set enrichment analysis shows enrichment of "estrogen response late" and "estrogen response early" hallmarks, implying an interplay between ID4 expression and estrogen-responsive pathways in the breast cancer context under study. In vitro experiments corroborate these findings, demonstrating a substantial increase in ER and GATA3 expression (characteristic luminal genes) and a notable decrease in EGFR expression (a characteristic basal gene) upon silencing ID4 expression ($p<0.05$). Conclusions: This study introduces potential for TNBC reprogramming via ID4 modulation.

304. 467. **ANTITUMOR ACTIVITY OF COPPER-BASED METALLODRUGS AGAINST MURINE TRIPLE NEGATIVE BREAST CANCER**

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A range of metallodrugs is used to treat cancer, with cisplatin, carboplatin, and oxaliplatin being the most commonly administered. However, platinum toxicity and resistance to treatment have caused researchers to explore new compounds that may be effective in treating tumor growth. Copper-based metallodrugs have been found to possess promising anti-tumor and anti-metastatic properties for a variety of types of solid tumors. Our aim was to investigate the anti-tumor effect of two metallodrugs (1 and 2) on the mouse triple-negative breast cancer model 4T1. MTS assays were carried out to determine the IC_{50} values and assess cytotoxicity. The cells showed an IC_{50} value of $1.2 \pm 0.2\ \mu M$ for complex 1 and $1.1 \pm 0.2\ \mu M$ for complex 2. We used ethidium bromide and acridine orange staining to determine that both metallodrugs induced cytotoxicity through apoptosis. Following a 24-hour treatment of the cells with either drug, we conducted a clonogenic assay and noted a reduction in colony-forming capacity. Both compounds significantly reduced colony formation at even their lowest concentration ($IC_{50}/4$) with respect to control cells (70 ± 9 % for **1** and 69 ± 11 % for **2**, $p<0,05$). We also evaluated copper-based metallodrugs modulation on cell migration in a wound-healing assay. Following a 24-hour treatment period, we observed that both compounds effectively decreased cell migration capacity in comparison to the control cells when administered at a concentration equal to their $IC_{50}/2$ (54 ± 6 % for **1** and 48 ± 15 % for **2**, $p<0,05$). Overall, our findings demonstrate that both complexes possess anti-tumor effects on 4T1 cells. Further research into the mechanism of action of copper-based metallodrugs may yield potential alternatives for triple negative breast cancer treatments.

305. 533. **ANTITUMOR POTENTIAL OF *PLEUROTUS OSTREATUS* I-FRACTION IN CARCINOMAS WITH FEW THERAPEUTIC OPTIONS**

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Pleurotus ostreatus is an edible mushroom recognized by the Argentine Food Code. The antitumor activity of its polysaccharides, free or bound to proteins, has been demonstrated on different types of cancer. However, this potential in Triple-Negative Breast Cancer (TNBC) and Head and Neck Squamous Cell Carcinoma (HNSCC) is unknown. Therefore, our objective is to determine the direct antitumor activity of I-Fraction: a polysaccharide-enriched extract obtained from *P. ostreatus* in TNBC and HNSCC. Previously, we reported that I-Fraction decreases the viability of 4T1 cells (TNBC-murine) starting at 2.5 mg/mL and 24 h of treatment. In the present work, by crystal violet assay, we demonstrated that this effect is also displayed on MDA-MB-231 cells (TNBC-human) starting at 2 mg/mL and 48 h of treatment ($p<0.01$). HN13 cells (HNSCC-human) were less sensitive respect to TNBC cells, decreasing its viability after 5 mg/mL and 48 h of I-Fraction treatment ($p<0.01$). Through cell cycle analysis with PI staining, we demonstrated that treatment with I-Fraction (2.5 mg/mL, 48 h) increases the number of MDA-MB-231 cells in S phase ($p<0.001$). This increase was accompanied by a decrease of the cell population in G0/G1 ($p<0.001$) and G2/M ($p<0.05$) phase. The staining with Annexin V/PI of 4T1 cells treated with I-Fraction (2.5 mg/mL, 48 h) or vehicle, showed that the extract induces cell death by apoptosis ($p<0.001$) added to a lower induction of death by necrosis ($p<0.05$). Through wound healing assay, we demonstrated that I-Fraction (2 mg/mL) decreases the migratory capability of MDA-MB-231 cells ($p<0.01$). Furthermore, started with the characterization of I-Fraction, by phenol-sulfuric acid and Bradford method we determined that 68.72 % +/- 6.60 % of the extract are glucans and that 9.02 % +/- 3.32 % are proteins. In conclusion, these results demonstrate a direct antitumor activity *in vitro* of *P. ostreatus* I-Fraction and endorse the continuation of *in vivo* assays in TNBC murine models.

306. 565. **ANTIPARASITIC DRUG IVERMECTIN EXHIBITS ANTITUMOR ACTIVITY IN PANCREATIC DUCTAL ADENOCARCINOMA MODELS**

Florencia Gonzalez Moran[1,2], Luisina Solernó[1,2,3], Candela LLavona[1,2], Hernan G. Farina[1,3], Nelson Dusetti[2,4], Juan Iovanna[2,4], Valeria I. Segatori[1,2,3], Juan Garona[1,2,3], Daniel F. Alonso[1,2,3], Florencia Gottardo[1,2,3]

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Pancreatic ductal adenocarcinoma (PDAC) is the fourth leading cause of cancer-related deaths worldwide. In addition, its 5-year survival rate is less than 5% due to late diagnosis and limited treatment options. Ivermectin (IVM) is a widely used antiparasitic drug that has been repositioned as an antitumor agent given its capacity to reverse multidrug resistance, inhibit proliferation and decrease mitochondrial biogenesis. The aim of the present preclinical study was to investigate the antitumor effects of IVM in PDAC. Using TCGA GTEX databases and the UCSC Xena and GEPIA2 platforms (PAAD/n=179), we explored putative molecular targets associated with IVM mechanisms (i.e. CARL; PAK1; ERK1), confirming their overexpression in cancer tissue, and its direct correlation with worse prognosis. Employing the Tumor Immune Estimation Resource (TIMER2.0) platform we found a strong positive association between the expression of several IVM targets and the infiltration levels of cancer-associated fibroblasts as well as myeloid-derived suppressor cells in PDAC clinical samples. *In vitro*, PANC-1 and PANC02 cell lines showed a high sensitivity to IVM, obtaining an IC_{50} of ≈10 μM on cell viability. Treatment with low concentrations of IVM also inhibited colony-formation, clonogenic growth, tumor cell metabolism and chemotaxis. In addition, IVM treatment reduced mitochondrial transmembrane potential, a well-known immunogenic cell death molecular marker ($p<0.05$, ANOVA). *In vivo*, syngeneic mice were injected s.c with PANC02 cells, and after 15 days of cell inoculation, mice were treated daily with vehicle (control) or IVM (5 mg/kg i.p.). IVM treatment during three weeks impaired PDAC progression, reducing tumor growth by 60% ($p<0.05$, T test Graphpad Prism). Our results identified ivermectin as a promising co-adjuvant agent for the therapeutic management of PDAC, highlighting interesting correlations between its associated targets and the tumor immune landscape.

307. 574. **ANTITUMOR PROPERTIES OF YERBA MATE IN LUNG CANCER CELL LINES**

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Yerba mate (YM, *Ilex paraguariensis*) is a native tree from the subtropics of South America. In the last ten years, our phytomedicine research group has studied the antitumor properties of a YM extract. The physicochemical study revealed the high antioxidant capacity of the extract, associated with a substantial content of total polyphenols (particularly, chlorogenic acid). Recently, we demonstrated the *in vitro* inhibitory effect of YM extract on processes involved in tumorigenesis in breast and colon cancer cell lines. We reported a negative modulation of cell viability, as well as extracellular matrix degradation, cell adhesion and migration, which are key phenomena in tumor invasion. Based on these results, the aim of this study was to expand the *in vitro* evaluation of YM extract, using both human (NCI-H125) and murine (LL/2) lung cancer cell lines. High-density cell proliferation assays were performed at 24 and 72 hours of incubation to study the cytotoxic effect and the anti-proliferative properties of YM extract. *In vitro* results revealed that YM extract inhibits cell proliferation of NCI-H125 and LL/2 cell lines, with IC50 values of 0.079 ± 0.027 mg/mL and 0.062 ± 0.038 mg/mL, respectively. Cell adhesion experiments were carried out to analyze the effect of YM extract on binding capability of tumor cells to an artificial matrix. Wound-healing scratch assays were also conducted to study cell-cell and cell-matrix interactions, processes related to cell migration. We demonstrated the modulatory effect of the extract on cell proliferation, adhesion and migration, which are central cellular events linked to tumor progression and metastasis. In conclusion, our results showed the antitumor activity of YM extract in lung cancer cell lines. Further studies in different *in vitro* and *in vivo* lung cancer models are currently undergoing.

308. 576. **ANTITUMORAL ACTIVITY OF FUNGAL EXTRACT AGAINST MCF-7 CELLS**

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Cancer is one of the leading causes of death in every country in the world. In Argentina, the distribution of cases according to the type of tumor shows that breast cancer is the one with the highest incidence in women. Treatments are not always effective and it is essential to develop new therapies for its improvement. In this sense, interest in natural compounds has been renewed as possible therapies. Fungi represent a source of interest to obtain bioactive compounds such as proteins with antitumor activity. Therefore, the aim of this work was to evaluate the cytotoxic capacity on breast tumor cells MCF-7 of fungal protein extracts. To achieve this goal, fruiting from Pleurotus ostreatus (Po) and Agaricus bisporus (Ab) were lyophilized and

homogenized with Tris-Glycine buffer pH 8.4. Homogenates were centrifuged and the supernatants were treated with ice cold ethanol to precipitate soluble proteins. The precipitates were resuspended in phosphate buffer 50 mM, pH 7. Protein concentration was measured by Bradford method. An MTT assay was carried out to assess cellular metabolic activity. MCF-7 cells (100.000 cells/well) were seeded in 24-well culture plates and allowed to attach for 24 h. Culture medium was replaced with DMEM containing different protein concentration of Ab or Po extracts, and cells were incubated at 37 °C for an additional 24 or 48 h. At the end of incubation, cells were washed with PBS and incubated at 37 ºC for 30 min in the presence of MTT. Purple formazan crystals were solubilized with etanol and absorbance was measure. The results showed that Ab extract (0,1 mg/ml protein concentration) had a cytotoxic activity against MCF-7 cells after 48 h. On the other hand, Po extract at the same concentration decreased cell viability after 24h. And at 48 h, lower concentrations of Po extract also showed cytotoxic activity. Therefore, these extracts represent a possible option in the search for new treatments against breast cancer.

O3-ONCOLOGY

THURSDAY 16TH NOVEMBER 9:00 - 10:30

CHAIRS: DANIEL GRASSO
IGNACIO ESTEBAN LEON

309. 12. **BCG AS A SELECTION PRESSURE MECHANISM IN BLADDER CANCER THROUGH PD-L1 OVEREXPRESSION**

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Objective: Estimation of PD-L1 expression in relapsed tumors of patients with non-muscle invasive bladder cancer and determination of the expression rate in successive tumor relapses in response to BCG treatment. Materials and Methods: Tumor tissue fixed in paraffin from 86 patients with “de novo” and relapsed bladder cancer (with at least two recurrences) was analyzed by immunohistochemistry (automated immunohistochemistry with the Ventana PD-L1 (SP263) test). Benchmark System ULTRA Mouse Monoclonal Kit 22C3 - pharmDx (SK 006) Peroxidase reagent (Ventana Medical Systems, Inc / Roche).It was considered positive for ligand overexpression when PD-L1 ≥5%.The percentage of PD-L1 expression was quantified after the first transurethral resection (TUR) without having received BCG and after each new TUR after BCG instillation, using Lamb’s scheme, as an adjuvant treatment to surgery As a control, a tumor sample from the same patient was sent to both immunohistochemistry departments, and the results were compared. The prevalence estimate was summarized using percentages (95% confidence interval). For discrete data, percentage frequencies were used. Continuous data were summarized descriptively using the mean (standard deviation) or median (range). For the association between the categorical variables, odds ratio and Chi square were used with statistical significance ($p < 0.05$). Results: Of the 86 patients, 53 (72%) received at least one complete BCG cycle (15 total doses: 6 weekly induction cycles, followed by 3 weekly maintenance cycles every 3 months). 15 (18%) patients received 12 doses, 10 patients (11%) received 9 doses, and 8 (10%) patients only received the 6 induction doses before suffering a new relapse and requiring new surgery. 56 patients (66%) had two recurrences, 18 patients (21%) had three recurrences, 10 patients (11%) four recurrences, and only 1 patient (2%) had five recurrences. All patients received at least six weekly induction doses before re-operating and quantify PD-L1 expression. Follow-up of all patients was a maximum of five years. In all cases the tumors were of high or low grade of malignancy, but always not invasive muscles. In the literature, PD-L1 expression is observed in 7% of pTa tumors, 16% of pT1, 23% of pT2, 30% of pT3/4, and 45% of carcinoma tumors in situ (CIS). In our study, PD-L1 pre-BCG had a similar degree of expression, but post BCG (at least 6 weekly cycles) it was 11%(pTa), 21%(pT1), 25%(pT2a) and 31%(pT2b). Patients with 3 or 4 post-BCG tumor recurrences presented higher levels of PD-L1 expression. On average 19% (pTa), 23% (pT1), 27% (pT2a) and 33% (pT2b). Cis and patients who did not receive BCG after surgery were not analyzed due to their low representation in this sample. The 12 control cases presented similar results in both Centers. PD-L1 expression was associated with high-grade tumors (odds ratio) [OR] = 2.4, ($p = 0.009$) and tumor infiltration by mononuclear cells [OR] = 5.5, ($p = 0.004$) . The key determinants of stage progression were high-grade tumor pathology, according to the World Health Organization / International Society for Urological Pathology (WHO / ISUP) classification [OR] = 4.77; 95% confidence interval [CI]: 2.73-8.34; $p < 0.001$) and PD-L1 expression [OR] = 2.20, (95% CI: 1.18-2.74: $p = 0.012$). Furthermore, PD-L1 expression was found to be extremely abundant in BCG-induced bladder granulomas with an expression rate of 30% to 45% especially in patients who failed BCG treatment, being correlated with a higher rate of tumor recurrence, worse response to BCG adjuvant therapy and lower survival rate linked to the disease. Conclusions: In this cohort of patients, PD-L1 expression was found to be significantly increased in tumor recurrences compared to the primary tumor, after receiving intravesical BCG as adjuvant treatment. It is likely that this over-expression of PD-L1 in tumor cells arises due to an increased mitotic process and as an adaptive response to the BCG-induced inflammatory environment as another tumor escape mechanism from immunosurveillance. High grade and low grade bladder urothelial tumors of muscle invasive malignancy (although also non-invasive ones), are carriers of a greater overexpression of PD-L1 in relation to non-tumor cells, which suggests that this increased expression It would be related to greater tumor aggressiveness, given by the increased infiltrating capacity of the disease, the higher rate of recurrence, and greater metastatic power. We hypothesize that these patients with relapsed tumors that show increasing PD-L1 expression will respond less to BCG treatment and could benefit from Anti-PD-L1 drugs (Nivolumab, Pembrolizumab, Atezolizumab, etc.) either alone or in combination with BCG.

310. 20. **KANSL2 AS A MODULATOR OF GBM AGGRESSIVENESS BY REGULATING YAP/TAZ PATHWAY**

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Glioblastoma Multiforme (GBM) is the most aggressive and has the worst outcome of central nervous system tumors. These tumors characterize by their high cellular heterogeneity and therapy resistance. First, we analyzed the Glioblastoma multiforme (GBM) and low-grade gliomas (LGG) mRNA expression of The Cancer Genome Atlas (TCGA) database to investigate a correlation between KANSL2, a component of the non-specific lethal complex (NSL) involved in histone acetylations (H4K5, H4K8, and H4K16), with increased tumor aggressiveness ($p<0.05$) and molecular subtypes. We found that KANSL2 expression was associated with a reduction in the mesenchymal subtype ($p<0.05$). Additionally, high KANSL2 expression correlated with an enhanced cellular plasticity index

(p<0.01), indicating a more plastic phenotype. We also performed an RNA-seq analysis on tumor-derived stem cell-enriched spheroids obtained from independent patient samples. We found that KANSL2-knock down cells led to the upregulation of genes associated with epithelial-mesenchymal-like transition (p<0.001) and Hippo signaling pathway transcriptional targets (p<0.001), both processes linked to increased tumor aggressiveness. Importantly, these data were further validated using the "subtype me" GBM subtype classification tool, confirming KANSL2 decrease induces a transition to a mesenchymal subtype. Functional experiments in U251 and U87 GBM cell lines showed increased migratory capacity, elevated VEGF protein expression, and enhanced neovascularization in vivo of KANSL2 knock-down cells. Based on these findings, we propose KANSL2 has a role as a modulator of plasticity and aggressiveness in GBM, which is intricately affected by cellular subtype and migratory capacity. Additionally, KANSL2-RFP tagged overexpression in U87 cells led to a decrease in protein levels of intracellular YAP1, suggesting an inverse regulation of KANSL2 and the Hippo signaling pathway as regulators of different identities of plastic tumors.

311. 120. **METABOLIC EFFECTS OF T47D CELLS IN MAMMARY ADIPOSE TISSUE**

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In previous work we have observed that TNF is able to induce lipolysis in adipose tissue (AT) explants from mammary glands of C57-BL/6J mice and that the breast cancer cell line T47D secretes high levels of TNF. The aim of this work was to analyze the metabolic changes in AT produced by factors secreted for T47D cells. Wild type (wt) or TNF receptor 1 knock out (KO) AT explants were stimulated for 5 days with T47D cell line conditioned media (CM). The number of adipocytes per unit area of paraffin-embedded histological sections was measured to assess adipocyte size. The T47D CM induced a decreased in adipocyte size (1.8 ± 0.06 respect to wt basal, p<0.05). In addition, tissue total lipid content was studied by gravimetry, after Bligh & Dyer extraction and AT glycerol secretion by colorimetric assay. Stimulation with T47D CM decreased total lipid content (0.55 ± 0.04 respect to wt basal, p<0.05) and increased glycerol secretion (2.69 mg/l ± 0.92 wt T47D vs 0.42 mg/l ± 0.16 wt basal, p<0.05). To assess whether these lipolytic effects of T47D CM could be mediated by TNF, we performed these experiments in the KO AT model. T47D CM had no significant differences in total tissue lipid content respect to KO basal (1.05 ± 0.15 KO T47D; 0.98 ± 0.04 KO basal respect to wt basal; p<0.05). Although, T47D CM increased adipocyte size respect to KO basal (1.4 ± 0.1 KO T47D; 2.8 ± 0.1 KO basal respect to wt basal; p<0.05). Interesting, adipocytes in the KO AT were smaller than in the wt model (2.8 ± 0.1 KO basal respect to wt basal; p<0.05). Also, treatment with T47D CM increased glycerol secretion respect to KO basal (4.46 mg/l ± 0.42 KO T47D vs 0.44 ± 0.09 KO basal; p<0.05). In conclusion, we have observed that T47D stimulation induces a lipolytic profile in adipose tissue and, although TNF may be a lipolytic signal in the T47D CM, our results indicate that there are other factors that could also mediate these changes in AT lipid metabolism.

312. 145. **INCREASED EXPRESSION OF GEF-H1 IN THYORID CANCER PROMOTES CELL PROLIFERATION, MIGRATION AND INVASION**

Lucía Fernández Chávez[1], Vicente Bermúdez[1], Valentina Arenal[1], Exequiel Gonzalo Alonso[1], Karen Schweitzer[1], Pamela Pichel[3], Sergio Recio[3], María Julia Ferronato[1], María Eugenia Fermento[1], Eliana Noelia Alonso[1], Mateo Nicolás Campos Haedo[2], Cinthia Rosemblit[2], María Marta Facchinetti[1], Alejandro Carlos Curino[1] y Georgina Pamela Coló[1].

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RhoGTPases family is involved in several biological process including gene transcription, cell polarity, migration, and invasion. RhoGTPases switch between on and off states and are regulated by several GEFs (activators) and GAPs/RhoGDIs (inactivators). We identified a GEF-H1 as one of the main activators of RhoA. In addition, we have observed that GEF-H1 is involved in cell proliferation, cytoskeleton remodelling, cell migration and invasion, as well as in breast tumor development and metastasis. Rho GTPases contribute to tumor initiation and progression, however, the role of RhoA activators has not yet been study in thyroid cancer (TC). Hence, the aim of this work is to study the role of GEF-H1 in TC development. TC is the most prevalent endocrine neoplasia and the main cause of death is the metastasis. By bioinformatics analysis we found GEF-H1 mRNA and protein overexpressed in tumoral thyroid tissue (TT) compared with non-tumoral tissue (NT). In addition, the expression of GEF-H1 was significantly higher in papillary and anaplastic thyroid carcinomas than in NT (p<0.05) and increased in papillary carcinomas with lymph node invasion and/or metastasis (p<0.0001). We observed by immunostaining a significant increase in GEF-H1 protein expression in TC human biopsies compared with NT (n=89, p<0.0001). In addition, we observed by immunofluorescence staining high GEF-H1 protein expression in thyroid primary carcinoma cell culture compared with normal thyroid cells. To further study the role of GEF-H1 in tumor development, we generated GEF-H1-knock out (KO) cells using CRISPR/Cas9 technology from a thyroid papillary carcinoma cell line (TPC-1). A decrease in proliferation, migration and invasion rates was observed in GEF-H1-KO cells compared to wild type cells. Our results suggest that GEF-H1 could be used as a potential tumor biomarker and/or therapeutic target in TC, since it could be involved in controlling cell proliferation, migration, and invasion of TC cells.

313. 239. **THE DEVELOPMENT OF LUNG METASTASIS IN A MOUSE LUMINAL BREAST CANCER MODEL DEPENDS ON ITS PREVAILING PROGESTERONE RECEPTOR ISOFORM**

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We propose that luminal breast carcinomas with higher levels of the Progesterone Receptor isoform A (PRA) than the isoform B (PRB), named PRA-high (PRA-H), are those that are inhibited to grow by antiprogestins, whereas those with the opposite ratio (PRB-H) may be even stimulated with antiprogestin treatment. In previous studies, we suggested that PRB-H tumors are more proliferative and less metastatic than PRA-H tumors. To confirm our results, we decided to compare the growth and the number of lung metastatic foci using the C7-2-HI (PRA-H) murine mammary carcinoma and its antiprogestin resistant variant C7-2-HIR (PRB-H). Tumors were inoculated subcutaneously in BALB/c mice and then euthanized 42 and 90 days (n=3-5/group) later. Tumors and lungs were fixed, paraffin-embedded and stained with hematoxylin-eosin. A higher amount of foci were generated by the PRA-H tumor compared to the PRB-H counterpart (Mann-Whitney, 42 days: p=0.086; 90 days p=0.037), regardless of the fact that the growth rate of the former was almost 2 times lower compared with the latter (linear regression, p<0.01). At day 42, the metastatic foci generated by the PRA-H tumor tended to be larger than those generated by the PRB-H. However, after 90 days we observed that the few foci generated by the PRB-H tumors were larger than those generated by the PRA-H tumors. A higher degree of differentiation was observed in the metastatic foci that was independent of the tumors' PR isoform ratio. We also performed an RNA-Seq analysis of both tumors, which showed upregulation in motility and migration genes and a downregulation in the G2 phase genes (e.g.) in the C7-2-HI tumor (log fold change (LFC) > 1 and FDR < 0.05) compared to the PRB-H variant, in agreement with our

in vivo observations. These results confirm that tumors with a high PRA/PRB ratio are less proliferative but more metastatic than those with the opposite ratio.

314. 261. **HEME OXYGENASE-1 REGULATION OF STEMNESS AND METASTASIS ASSOCIATED GENES IN PROSTATE CANCER-BONE CROSSTALK**

Inés Achinelli[1,2], Agustina Sabater[1,2,3], Pablo Sanchis[1,2], Nicolás Anselmino[4], Sofía Lage-Vickers[1,2], Juan Bizzotto[1,2,3], Gastón Pascual[1,2], Rocío Seniuk[1,2], Maria Laura Lacreu[1,2], Javier Cotignola[1,2], Elba Vazquez[1,2], María Sol Ruiz[1,2], Geraldine Gueron[1,2], Ayelén Toro[1,2].

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Mortality in prostate cancer (PCa) is associated with bone metastasis and aggressive phenotypes promoted by prostate cancer stem cells (PCSCs). We have previously described that heme oxygenase-1 (HO-1) plays an antitumoral role in PCa. Here, we hypothesize that HO-1 is a key modulator of genes associated with stemness and metastasis during the crosstalk of PCa cells with bone progenitors.We used an indirect coculture system of PC3 cells (PCa) grown with MC3T3 cells (bone progenitors) to model PCa-bone crosstalk. PC3 cells were pretreated or not with hemin, a specific pharmacological inducer of HO-1. We performed RNAseq analyses and assessed the transcriptome of PC3 cells focusing on genes associated with stemness (*CD44, CD133* and pluripotency markers), metastasis and a stemlike signature previously identified by our group (*ADAM15*, *BCL2L1*, *LTBR*, *MBNL2*, *SPINT1*). PC3 cocultured with MC3T3 displayed upregulation of PCSC and pluripotency markers, as well as *BCL2L1* and *LTBR*, when compared with PC3 cells cultured alone. Interestingly, when pre-treating PC3 cells with hemin prior to the coculture, cells displayed an impaired upregulation of these genes, including PCSC marker *CD44*, pointing out to the protective effect of HO-1 induction towards the pro-stemness effect triggered by the coculture. We extended our analysis using PCa patients' survival and transcriptomic publicly available data (TCGA-PRAD (n=565), SU2C-PRAD (n=444), FHCRC-PRAD (n=176)). Results showcased that expression of *CD44* not only was higher in bone metastases vs. primary PCa tumors, but was also among the subgroup of genes associated with significant changes in patients' survival. Altogether, HO-1 expression modulates stemness and metastasis related genes in PCa cells, promoting a more differentiated state, counteracting the pro-stemness effect linked to the communication with bone progenitors. This supports the antitumoral role of HO-1 in PCa and highlights *CD44* as a relevant candidate for further studies.

315. 491. **DISTINCTIVE O-GLYCANS EXPRESSION IN CD44 AND CD15 POSITIVE GLIOBLASTOMA CANCER STEM-LIKE CELLS**

Jeremias Omar Castillo[1], Gretel Magalí Ferreira[1,2], Selene Rojo[1,2], Cynthia Antonella Gulino[1], Valeria Inés Segatori[1,2] and Mariano Rolando Gabri[1,2]

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Glioblastoma (GBM) is the most aggressive human brain tumor and is recognized for having a subset of cells known as Cancer Stem-like Cells (CSC), being the protagonists of recurrence and therapeutic resistance. In order to study CSC, Neurospheres cultures (NS) is an optimal in-vitro approach for GBM. Aberrant glycosylation plays an important role in malignant transformation and recent studies have elucidated that O-glycans and glycosyltransferases involved in their synthesis may be associated with CSC malignancy. Previously, we have established a glycosylation profile in multiple GBM cell lines cultured as monolayers, primarily characterized by N-glycans, with minimal expression of O-glycans. Ex post, we optimized NS growth conditions of U87-MG and confirmed a significant upregulation of CSC markers, including CD133, CD44 and CD15. In this work, O-glycan expression in NS and its association with CSC markers was evaluated. No NS formation was observed in the low-grade SW1008 glioma cell line, while U87-MG cells form an average of 27 ± 13 NS per field. By flow cytometry analysis, NS growing cells showed a significant O-glycan expression profile detected by lectins, and the presence of T and sT antigens (TA and sTA) were found in 7.8 ± 7.7% and 73 ± 12% of analyzed cells, respectively. Notably, subsequent analysis revealed significant CD44 and CD15 expression within these populations. Specifically, 76 ± 5% and 64 ± 17% of TA-positive cells expressed CD44 and CD15 respectively, while 54 ± 5% and 43 ± 6% of sTA-positive cells expressed these markers. Complementarily, 72 ± 13% of CD44-positive and 83 ± 8% of CD15-expressing cells were shown to express sTA. Immunocytochemical analysis on NS revealed a robust sTA expression with a weak TA expression. Our findings reveal a distinct O-glycan expression profile associated with the CSC population in U87-MG GBM cells, suggesting a characteristic glycosylation profile for this population.

P2-ONCOLOGY

THURSDAY 16TH NOVEMBER 9:00 - 10:30

CHAIRS: PATRICIA PENNISI
DENISE BELGOROSKY
ALEJANDRO URTREGER

316. 75. **EPITHELIAL-TO-MESENCHYMAL TRANSITION AND AKT PATHWAY ARE TARGETS OF SOLUBLE GUANYLYL CYCLASE BETA1 SUBUNIT IN HUMAN ENDOMETRIAL AND CERVICAL CANCER CELLS**

Lucas Acosta[1,2], María Victoria Rocca[1], María Teresa Pino[1,2] and Jimena Cabilla[1,2]

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Soluble guanylyl cyclase (sGC) is an enzyme constituted by two subunits, α1 and β1. Previously we showed that sGCα1 subunit independently promotes cell survival, proliferation, and migration in different cancer models; however the role of sGCβ1 subunit has yet to be elucidated. Since endometrial and cervical cancer are positioned among the most frequently diagnosed malignancies globally we aim to study the impact of sGCβ1 protein level restoration, using an adenoviral vector (sGCβ1-GFP incubated for 48 h) in endometrial (ECC-1) and cervical (HeLa) tumor cells. We analyzed the sGCβ1 overexpression effects in proliferation, survival (MTT and flow cytometry), migration (wound healing and transwell migration assay), cell signaling and epithelial-to-mesenchymal transition (Western blot) and cellular localization (epifluorescence microscopy). We observed that sGCβ1 transcript levels are reduced in endometrial and cervical tumors vs normal tissues from RNA-seq data ($p<0.05$). We found that sGCβ1, besides its well known cytoplasmic localization, can be also found in the nucleus, unlike sGCα1. Overexpression of sGCβ1 reduced cell viability and augmented apoptotic index ($p<0.0001$). Cell migration and invasion were also negatively affected ($p<0.05$ and $p<0.001$). All these effects were independent of sGC enzymatic activity since 1 μM ODQ, an enzymatic inhibitor, did not avoid sGCb1-driven actions. sGCβ1 reduced the expression of epithelial-to-mesenchymal transition factors such as N-cadherin and β-catenin ($p<0.001$ and $p<0.05$) and increased the expression of E-cadherin ($p<0.001$). sGCβ1 impacted cell signaling through significant downregulation of Akt pathway affecting GSK-3β ($p<0.01$) and c-Raf ($p<0.05$). Our results show for the first time the inhibitory effect of sGCβ1 in some of the key hallmarks of cancer in endometrial and cervical tumoral cells.

317. 90. **EFFECT OF OLIGO-FUCOIDAN PREBIOTIC AGENT AND BORON NEUTRON CAPTURE THERAPY (BNCT) ON MICROBIOTA IN AN EXPERIMENTAL ORAL CANCER MODEL**

M.A. Palmieri[1], S.I. Nemirovsky[2,3], I.E. Czornenki[4], J.A. Goldfinger[4], P.S. Ramos[4], E.C.C. Pozzi[4], S. Thorp[4], P. Curotto[4], G.G. Agüero[4], V.A. Trivillin[2,4], M.A. Garabalino[4], V.A. Medina[2,5], C. Costa[4], A.E. Schwint[2,4], M. Pezzoni[2,4], A. Monti Hughes[2,4]

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Boron Neutron Capture Therapy (BNCT) is particle radiotherapy, based on the administration of boron carriers incorporated preferentially by tumor cells, followed by neutron irradiation. BNCT clinical results for Head and Neck cancer have shown significant therapeutic efficacy. Oral microbiota is a heterogeneous group of microbial species colonizing the surfaces of the oral cavity. Microbiota could affect cancer therapy outcomes. Oligo-Fucoidan, isolated from *Laminaria japonica* brown seaweed, is considered a prebiotic agent as it stimulates beneficial bacteria in the gut. Our group showed, in the hamster cheek pouch oral cancer model, an enhancement in tumor control from 67% for BNCT to 94% for BNCT combined with Oligo-Fucoidan. Studies performed by our group described microbiota composition and proportion in a normal and cancerized hamster. In this study, we evaluated if Oligo-Fucoidan and/or BNCT are capable of changing microbiota composition and proportion in tumor-bearing hamsters. Samples of microbiota from the tumor, precancerous surrounding tissue and normal tissue were taken. DNA was extracted and bacterial diversity and taxonomic abundances were characterized by sequencing the 16S rRNA gene. During the cancerization process, we observed that Proteobacteria, Firmicutes and Actinobacteria decreased, while Bacteroidea and Fusobacteria increased (both previously reported in oral cancer patients). Preliminary studies showed that Oligo-fucoidan treatment increased Proteobacteria 4 times ($p<0.01$) and reduced Bacteroidea 1.4 times ($p<0.05$). Besides, Oligo-fucoidan tended to reduce Fusobacteria in cancerized pouches. The effect of BNCT on oral microbiota is currently under evaluation. These preliminary results showed that Oligo-fucoidan modulates cancerized hamster cheek pouch microbiota. Acknowledgments: Hi-Q Marine Biotech (Taiwan).

318. 103. **EXPLORING THE ANTIPROLIFERATIVE POTENTIAL OF THE ETHYL ACETATE EXTRACT FROM COLEUS NEOCHILUS**

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Coleus species have diverse ethnobotanical uses, most of them related to their medicinal properties. Previously, we described the antiproliferative effect of the ethanolic extract of Coleus neochilus (also known as boldo rastrero). This study focused on the effect of one of the subfractions of the ethanolic extract, the ethyl acetate fraction (BRAE), on human breast cancer cells. Four different fractions were obtained from the original ethanolic extract by using different solvents: hexane, chloroform, ethyl acetate and methanol-water. Incubation with BRAE 25 µg/ml reduced cell viability in MCF-7, T47-D, and MDA-MB-231 tumor cells (35%, 21% and 8% reduction, respectively; $p<0.05$, MTT assays), but not in MCF-10A non-tumor cells. The effect on the reduction of cell viability was also observed when cells were exposed to a 6 or 12 h pulse of BRAE, followed by a 24h-recovery period (14% and 55% reduction respectively, $p<0.05$), while a 3 h pulse of BRAE did not cause any effect. A concomitant reduction in p-ERK levels measured by western blotting was observed after 6 and 12 h pulses of BRAE. For MCF-7 cells, using a concentration of tamoxifen (Tx) that fails to significantly decrease viability (25 µM) we observed that Tx treatment after 8 h BRAE pulse followed by a 24 h-recovery period was more effective to reduced viability than BRAE alone (BR: 21%, BR+Tx: 66% reduction). For T47-D cells, the treatment of tamoxifen 25 µM (Tx) after a 8 h BRAE pulse followed by a 24 h-recovery period was more effective to reduced viability than Tx alone (BR: 22% reduction, Tx: 21% reduction, BR+Tx: 42% reduction). In addition, BRAE 25 ug/mL decreased 81% the cloning efficiency of MCF-7 cells ($p<0.05$). The antitumor effect of this ethanolic subfraction was stronger than that described previously for the total ethanolic extract. Therefore, this subfraction is a promising starting point for the purification and characterization of new compounds with potential applications in cancer treatment.

319. 111. **COMPLEXITY-INCREASING ALGORITHM FOR THE DETECTION OF MOLECULAR ALTERATIONS IN DIFFERENTIATED PEDIATRIC THYROID CARCINOMA**

Marisa Esther Boycho[1], Sandra Lorena Colli[2], Patricia Papendiek[3], Martín Medín[2], Ana Chiesa[3], Mercedes García Lombardi[4], Elena Noemí De Matteo[1,2], María Victoria Preciado[1], Mario Alejandro Lorenzetti[1]

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Differentiated thyroid carcinoma (DTC) is the most common tumor of the endocrine system in children and its incidence is rising. Molecular markers, either chromosome fusions or single nucleotide polymorphisms (SNPs) serve as prognostic and/or specific treatment-selection tools. Our aim was to characterize molecular alterations in a series of pediatric cases with DTC from Argentina and test a future laboratory algorithm for molecular diagnosis and stratification. FISH, IHC and Sanger sequencing were performed on 57 pediatric DTC biopsies enrolled between 2018 and 2022 at our hospital. The classic variant was predominant 25/57 (47%), followed by the Follicular variant 14/57 (25%). Initially, 4/57 (7%) cases were positive for pan-TRK by IHC and subsequently positive for NTRK3 by FISH. In independent FISH reactions, 2 cases were positive for *ETV6* as the fusion partner. The 2 uncertain cases were solved by NGS and also found to be *NTRK3-ETV6* fused. All negative cases were assessed for other fused genes by FISH. Considering all studied gene fusions, 17/57 (30%) harbored fusions in known oncogenes: 6 in *RET* (35%), 5 in *ALK (29%)*, 4 *NTRK3 (24%)*, 1 *BRAF (6%)* and 1 *MET (6%)*. BRAF c.1799A>T (p.V600E) SNP was detected in 7/57 (12%) cases by Sanger sequencing. When relating clinic-pathological features with molecular markers, tumor size (T1/T2 vs T3/T4) was significantly larger in those DTC with genetic alterations (P=0.027) and, initial risk assessment (high/intermediate vs low risk) was also statistically higher for cases with genetic alterations (P=0.018). The most commonly fused gene was *RET*, followed by *ALK* and *NTRK3*. Gene fusions were more prevalent than V600E SNP. Designing a laboratory algorithm, following an increasing order of complexity, will provide a reliable molecular testing platform to reduce the requirement on NGS service, which is not available in all laboratories. These results also broaden data on DTC alterations in children from our own geographic region.

320. 162. **FECAL MICROORGANISMS FROM CRC PATIENTS INDUCE THE ACTIVATION OF ONCOGENIC TRANSDUCTION SIGNAL PATHWAYS**

Juliana Lourdes Bernacchia[1], Alejandra Graciela Palma[1], Francisco Damián Rosa[1], María Cecilia Lira[1], Adriana Noemí De Paulis[2], Natalia Mangieri[2], Eugenia Bertona[2], Melisa Fernández[2], Adrián Sambresqui[3], Oscar Laudanno[3], Mónica Vázquez-Levin[4], María Fernanda Rubio[1], Mónica Alejandra Costas[1].

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Although numerous evidence demonstrates the importance of the microbiome in the development of multiple diseases, such as colorectal cancer (CRC), mainly due to its immunological action, we have previously shown that there are signals and pathways that could be induced directly by microorganisms in epithelial colorectal cells, independent of the immune system, that could probably contribute to CRC development. The relative abundance of some bacteria shows differences, according to healthy or disease conditions. To investigate whether such differences could correlate with different biological effects by direct action over CRC cells, in this work we performed experiments of infection of the human colorectal cancer HCT116 cell line with microorganisms from fecal samples from healthy (control) or CRC patients from the IDIM. Firstly, we performed experiments of cell survival and growth by means of cell staining with crystal violet and measure of absorbance at 570 nm, after infection by 4 h and incubated during 24 or 48 h with samples from CRC or control patients at multiplicity of infection (MOI) 5, 10 or 15 (aerobic plus anaerobic). We found that all the samples induce cell death at 24 h, not more than 50 +/- 10 % for MOI 15. However, the surviving cells continued to proliferate. Through immunofluorescence (IF) assays at MOI 5, we found that all the samples induced nuclear translocation of NF-κB, β-catenin and the nuclear receptor coactivator RAC3, which is highly expressed in cancer stem cells as we previously reported. However, the nuclear localization of β-catenin was significantly increased in cells stimulated with samples from CRC compared to control (40 +/- 15%), where an increased expression of Vimentin was also observed ($p<0.05$). Concerning E-cadherin, a significantly decreased expression was observed by IF ($p<0.05$), compatible with an increase in the epithelial-mesenchymal transition. We conclude that oncogenic and EMT signals could be directly induced by bacteria in colorectal epithelial cells, contributing to CRC development.

321. 228. **ALGORITHM TO QUANTIFY SPHERES DERIVED FROM CANCER CELL LINES**

Ana Belén Peñaherrera Pazmiño[1], Ramiro Isa-Jara[1], Silvia Gómez[2], Elsa Hincapié[2], Denise Belgorosky[2], Eduardo Imanol Agüero[2], Ana María Eiján[2], Maximiliano Pérez[1], Betiana Lerner[1].

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Introduction: Sphere formation assay is accepted as a method for selecting and enriching cancer stem cells (CSCs). They play a crucial role in chemoresistance and cancer recurrence. Thus, there is intense research that aims at a better understanding of CSCs behavior. In our laboratory we study the growth of CSCs in microdevices to develop predicting chemotherapy assays in cancer. Counting spheres cultured in different devices is laborious and operator dependent. Objective: the aim of this work is to develop a computational program that identifies, counts and measures spheres automatically. Methods: The software named Spheres Interface has been developed using Phyton. It is a graphical user interface (GUI) mainly based on PySimpleGUI and SKIMAGE libraries to detect regions with spheres. Using an inverted microscope, 10X images of U251 human glioblastoma cell line were acquired. Then, the algorithm was trained to calculate the area in square micrometers, and it quantifies the number of spheres in each image. This algorithm has been evaluated with images that present different environments such as uniform and non-uniform background. Results: It has been observed that the performance of the algorithm is better when images present uniform background. When images have non-uniform background, artifacts are interpreted as spheres. A number of 100 images acquired from U251 cells has been processed with the algorithm and the sphere number reported has been compared with the manual count. The mean difference between the two methods and the standard deviation of the difference were estimated (mean± SD: 2±4, ns by t-student test). Also, the algorithm allows to number the spheres, which enables the monitoring of individual spheres in the time course. Conclusion: This algorithm has allowed to count spheres derived from cell lines. Although it needs to be improved, it can be a useful tool for automated CSC quantification from cancer cell lines and primary culture cells.

322. 382. **DIFFERENTIAL MODULATORY EFFECT OF NICOTINE ON THE RESPONSE OF TRIPLE NEGATIVE BREAST CANCER CELLS TO CONVENTIONAL OR METRONOMIC THERAPY**

Abigail Vasquez[1], Yamila Sanchez[1], Maia Jalom[2], Ariel Kaminker[2], Francisco Pérez Kasten[2], Alejandro Español[1]

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Triple negative (TN) breast cancer is the subtype with the worst prognosis. In the conventional therapy (CT) the maximum tolerated dose of paclitaxel (PX) is used, which generates adverse effects requiring long drug-free intervals for patients´ recovery. An alternative strategy is metronomic therapy (MT) based on the administration of lower doses with short drug-free intervals. Previously we demonstrated that muscarinic receptors (MR) are expressed in breast tumor tissue but not in normal tissue, and that a metronomic combination of carbachol (Carb) (muscarinic agonist) with low doses of PX exerts an antitumoral effect. Breast tumor tissue also expresses nicotinic receptors (NR), and nicotine (NIC), the main active component of tobacco smoke, has been associated with the resistance to conventional oncologic treatment in lung cancer. The aim of this work is to evaluate if NIC differentially modulates the efficacy of the PX CT and the PX+Carb MT in TN breast cancer MDA-MB231 cells. By MTT assays we determined that NIC in a similar concentration to that of smoking patients' plasma (10^{-7} M) increased the cell viability (basal:100+/-8.2%; NIC:147.8+/-13.6%). Although both CT (PX 10^{-7}M) and MT (PX 10^{-8} M + Carb 10^{-11} M) decreased the cell viability (CT:67.2+/-3.2%; MT:73.6+/-3.0%), the presence of NIC only reduced the effectivity of CT (CT+NIC: 125.8+/-12.0%; MT+NIC: 63.6+/-6.1%, $p<0,001$) by a mechanism dependent on PKC, ERK1/2 and NF-κB, since their selective inhibitors reduced the effect (101,3+/-12,7%; 89,6+/-11,8% and 97.6+/-14.7% respectively). Furthermore, the cells´ sensitivity to PX was reduced by CT but not by MT (EC_{50}basal: $2.1x10^{-7}$ M; EC_{50}CT: $5.8x10^{-6}$ M; EC_{50}MT: $9.3x10^{-9}$ M). NIC+MT treatment the turns cells´ sensitivity similar to that of control cells (EC_{50}NIC+MT: $3.1x10^{-7}$ M) whereas NIC+CT treatment reduced it significantly (EC_{50}NIC+CT: $4.1x10^{-5}$ M). These results indicate that in NIC presence, the MT is more effective than CT in TN breast cancer cells.

323. 391. **EFFECT OF QUERCETIN IN A MODEL OF HEPATIC CARCINOGENESIS**

Daniela Romina Montagna[1,2], María Florencia Todero[1], Emilia Panarace[2], Gabriela Postma[2], Alejandra Duarte[1], Raúl Ruggiero[1]

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Cancer is often a disease of elderly. Previously, we suggested that senescence could play a promoting role in a model of liver carcino-

genesis induced by diethylnitrosamine (DEN) in C3H mice. To investigate the underlying mechanisms, we administered a senolytic drug – quercetin (Q) – in DEN-treated mice starting when livers show: a) numerous and large-sized hepatic nodules (HN), b) few and small-sized HN, c) absence of HN. All mice were sacrificed at 7 months post-DEN. DEN induces multifocal dysplastic foci that progress into adenomas and later into trabecular carcinomas. At state a) Q-treated mice exhibited a higher number of HN (Median [range] = 76 [60-86]) and a larger size (mm^2) per HN (8.1 [6.5-13.4] than controls: number of HN = 13 [7-17], $p<0.05$ and size per HN =1.3 [0.9-3.0], $p<0.05$. At state b) Q-treated mice displayed a lower number of HN (3 [2-33]) than controls: 13 [7-49], $p<0.05$, but a larger size per HN: Q-treated = 3.0 [1.1-4.0] vs. controls = 1.0 [0.5-1.7], $p<0.02$. Finally, at state c), Q-treated mice displayed both a lower number of HN (0 [0-1]) than controls (14 [0-49]), p<0-05 and a smaller size per HN: Q-treated = 0 [0-1] versus controls = 5.6 [0-11.3], $p<0.05$. Senescence would not promote tumor growth by a direct effect on tumor cells because, in such case, inhibition of HN should have been observed at all stages of carcinogenesis. Since DEN also produces liver injury, the tumor-promoting effect of senescence could be the result of an indirect mechanism associated with a progressively increasing ratio of tumor cells/normal cells upon the regenerative signals produced in the injured liver in which all tumor cells can proliferate while only the non-senescent fraction of normal cells can do it. This possibility could explain these results, only assuming that tumor cells can grow upon regenerative signals in a similar fashion than normal cells.

324. 416. **ACSL4 AND COX-2 EFFECTS ON CELL AGGRESSIVENESS AND RESISTANCE TO DRUGS IN COLON CANCER CELLS**

Paloma Martínez Ponce 1; Jesica Prada 1; Luciano Martin Quevedo 1; María Mercedes Bigi 1; Ana Fernanda Castillo 1, 2; Paula Mariana Maloberti 1, 2; Ulises Daniel Orlando 1.
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Tumor aggressiveness and drug resistance are major challenges for research in advanced colorectal cancer (CRC). Arachidonic acid (AA) metabolism plays an important role in colon carcinogenesis. Acyl-CoA synthetase 4 (ACSL4), an enzyme involved in AA metabolism, is upregulated in the transition from colon adenoma to adenocarcinoma. In other tumors, ACSL4 has been associated with resistance to treatment. AA is a substrate of cyclooxygenase-2 (COX-2), which is the isoform mainly involved in the pathogenesis of malignancy in CRC. Given that bioinformatic analyses have shown a significant increase in ACSL4 and COX-2 in patient samples of primary tumors, our aim was to relate ACSL4 and COX-2 expression to cell aggressiveness and drug resistance in different colon cancer cell lines. To this aim, we used SW48, SW480, Caco2, and HT29 cells, which differ in phenotype aggressiveness and drug resistance. We evaluated ACSL4 and COX-2 expression by western blot, and cell functionality through cell viability, proliferation, and clonogenicity assays. HT29 and SW480 cells had higher ACSL4 expression than SW48 and Caco2 ($p<0.001$), and neither SW48 nor SW480 cells expressed COX-2. However, HT29 cells had higher COX-2 expression than Caco2 ($p<0.001$). In pharmacological assays, HT29 cells treated with ACSL4 inhibitor rosiglitazone (75 μM) and COX-2 inhibitor ibuprofen (300 μM) showed the synergistic effect of combined treatment on the inhibition of cell proliferation relative to single-drug treatment ($p<0.01$), with comparable results in HT29 clonogenicity. Rosiglitazone -but not ibuprofen- affected SW480 cell growth. Also, HT29 cells were more resistant to high concentrations of oxaliplatin (20 μM), docetaxel (7.5 nM), and 5-fluorouracil (75 μM) than SW48 cells ($p<0.01$). Based on these initial results, we speculate that tumor cells with higher expression of ACSL4 and COX-2 have greater aggressiveness and resistance to treatment and that both enzymes may act in concert.

325. 426. **ENHANCING PACLITAXEL EFFECTS THROUGH COMBINATION WITH THE TELOMERASE INHIBITOR R1D2-10 IN HUMAN BREAST CANCER MODELS**

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Cellular immortality is one of the main features of cancer. Tumor cells have an unlimited replicative potential, principally due to telomerase activity. For this activity, it is necessary the assembly of many components, where the most relevant are the catalytic retrotranscriptase (hTERT), the RNA template (hTR), and dyskerin (DKC1). In our previous work, we developed and evaluated a novel telomerase assembly inhibitor, selecting the interaction between hTR and DKC1 as a target. Briefly, we performed a Docking-Based Virtual Screening against the PUA domain of DKC1 and selected R1D2-10 compound. It showed an *in vitro* inhibitory effect on telomerase activity and cell proliferation on the breast cancer cell lines MDA MB 231, MDA MB 468, and MCF-7. Furthermore, we demonstrated that chronic treatment with R1D2-10 caused telomere shortening and induced senescence and apoptosis. Then, we decided to explore the effect of R1D2-10 in combination with Paclitaxel on the triple-negative breast cancer (TNBC) cell lines MDA MB 231 and MDA MB 468. Our results demonstrate a synergistic effect of these drugs on the inhibition of cell proliferation in both cell lines (CI<0,8) in a broad range of concentrations (0,5-10 μM for R1D2-10 and 0,5-10 nM for Paclitaxel). In order to study the drug's combination effect in more detail, we decided to explore cell cycle and apoptosis processes by flow cytometry and RT-qPCR analysis. Our results showed a significant appearance of the Sub-G1 population after 24h of combined treatment in both cell lines. Furthermore, we found an increment in the expression of pro-apoptotic and cell cycle inhibitors genes compared to monotherapies. These results are promising, considering that our goal is to develop a combined therapy based on R1D2-10 that allows us to reduce paclitaxel dose and, subsequently, its side effects. On this basis, we are working on further studies to demonstrate the potential clinical use of R1D2-10 for breast cancer treatment.

326. 443. **EXPANDING PRECISION MEDICINE: DIGITAL-PCR IN LIQUID BIOPSIES TO IMPROVE NSCLC PATIENTS' FOLLOW-UP**

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Although precision medicine has significantly improved the life expectancy of cancer patients, a major challenge lies in its heavy reliance on next-generation sequencing (NGS), which is expensive and scarcely accessible in Argentina. Another obstacle is the low sensitivity of the techniques used for treatment follow-up, therefore therapeutic failure detection arrives late, when metastases is already present. Our aim is to improve the monitoring of NSCLC patients by determining biomarkers through liquid biopsies and digital PCR (ddPCR), a highly sensitive and cost-effective detection method. Here we present the validation and set up of the tool. Objectives: 1-Develop a methodology for treatment response monitoring to EGFR inhibitors (EGFRi) using liquid biopsy-based tumor burden analysis. 2-Generate detection panels for resistance mutations to EGFRi. Description & validation: 1-For tracking response to EGFRi, we analyzed in blood samples, the quantitative changes over time in the two main EGFR biomarkers (BM), L858R and 18 different deletions of exon-19. Quantitative changes in the BM reflected whether the tumor is responding well (decrease), partially (stable), or if it is resistant to the therapy (increase). This will enable early anticipation

of disease progression and provide a rapid parameters for treatment management. We used an *in vivo* xenograft model to validate the correlation between tumor burden and BM' concentration. 2-We generated three ddPCR panels to detect the major mutations that lead to EGFRi resistance: a.On-target EGFR mutations T790M and S797X; b.BRAF V600E and PI3K E545K; c.CNV of EGFR, HER2, and MET. We have confirmed the proper detection of each BM using liquid biopsy. This approach will offer an affordable tool to personalized therapies based on identified mutations, enabling broader, earlier, and proactive management of available therapeutic options. With these tools we expect to contribute to the improvement of patient treatment outcomes.

327. 455. **COMBINATION THERAPY OF PACLITAXEL AND UVB1 IN HNSCC AND TNBC CELLS**

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Head and Neck Squamous Cell Carcinoma (HNSCC) and Triple Negative Breast Cancer (TNBC) are heterogeneous and aggressive tumors with high mortality and difficult clinical management. Conventional chemotherapy remains as the main clinical management with the concomitant undesirable side effects for patients and time-limited positive responses. Further research is needed to find novel therapeutic strategies that prolong the patient survival and ensure a better quality of life. The aim of the present study was to evaluate the antitumor potential of the combination of the conventional chemotherapeutic agent Paclitaxel (PTX) with the non-hypercalcemic Calcitriol analog UVB1 in HNSCC and TNBC cells. Cell viability was evaluated by crystal violet assays in human HN13 and HN12 HNSCC and murine 4T1 TNBC cell lines treated with vehicle, UVB1, PTX or combination of drugs. The results show that UVB1 (1μM) with low concentrations of PTX displayed a greater reduction in viability with respect to control and monotherapies in all cell lines tested (120h of treatment, $p<0.001$). Apoptosis analyses were performed in 4T1 cells by flow cytometry with Propidium Iodide and Annexin V-FITC staining. The percentage of cells in early apoptosis was higher in cells treated with UVB1 or UVB1 (1μM) + PTX (1nM) compared to PTX alone or vehicle ($p<0.001$). In order to evaluate the Vitamin D Receptor (VDR) role in these antitumor effects, VDR was overexpressed in 4T1 cells with a pcDNA3-VDR plasmid. Transfected cells were selected and the overexpressed of VDR was checked by RT-qPCR, WB and IFI. The viability studies carried out with these cells showed that PTX displayed a higher antitumor effect in 4T1-pcDNA3-VDR cells compared to 4T1-pcDNA3-CTL cells ($p<0.001$). These results encourage us to continue evaluating this combination therapy in HNSCC and TNBC cells, as well as VDR role in the antineoplastic effects of these chemotherapeutic drugs.

328. 457. **EFFECT OF PHARMACOLOGICAL INHIBITION OF P300 ON THE EXPRESSION AND LOCALIZATION OF P53 IN TRIPLE NEGATIVE BREAST CANCER**

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Triple-negative breast cancer (TNBC) is a heterogeneous group of tumors that lack specific molecular targets. Therefore, it is necessary to investigate potential tumor markers for this subtype of BC. A relationship between p300 and cancer has been demonstrated; but its role remains unclear, as it has been documented both as a tumor suppressor and an oncoprotein. p300 functions as a transcriptional coactivator, histone acetyltransferase, and acetylates lysines of proteins involved in functions beyond transcription. This protein acts as a transcriptional coactivator of p53, regulating its activity through acetylation mechanisms in various tumor types. Hence, we decided to study whether the regulation of p53 mediated by p300 can influence its cellular localization and the alteration of its functions in TNBC. The aim of this work was to evaluate the effect of pharmacological inhibition of p300 on the expression and localization of p53 in TNBC cell lines (MDA-MB-231 and 4T1). The cell lines were treated with VV59 (an inhibitor of p300 acetylase function) or DMSO (vehicle) for 24 hours. In western blot assays, no significant differences were observed in p53 expression levels in either cell lines. However, a decrease in acetylated p53 levels was noted in the MDA-MB-231 cells treated with VV59 compared to control cells ($p=0.0370$). Through immunofluorescence, we observed that p300 inhibition reduced nuclear expression and localization of p53 ($p<0.0001$), accompanied by an increase in cytoplasmic p53 localization, compared to the vehicle, in both cell lines ($p<0.0001$). In silico assays revealed interaction pathways and high affinity between p300 and p53. These findings suggest that the acetylase function of p300 impacts the expression and localization of p53, providing potential insights into p53 regulation in TNBC cells.

329. 544. **CHARACTERIZATION OF R-SPONDIN3 GENE EXPRESSION REGULATION IN HUMAN BREAST CANCER CELLS**

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** Ana Laura Ortiz and Carla Maria Felcher contributed equally to this study.*

We have determined that R-spondin3 (RSPO3), a secreted protein that potentiates Wnt signaling pathway, is a key modulator of tumor progression and stem cell behavior in basal-like breast cancer (BL-BC) cells. Although the highest RSPO3 expression has been detected in cells of this cancer subtype, immunohistochemical analysis showed that a high proportion of human breast luminal tumors are positive for RSPO3. We have also found that blocking RUNX-CBFβ activity inhibited RSPO3 expression in the BL-BC cell line MDA-MB-231. In these cells it has been determined that RUNX1 binds to its DNA motif at the end of RSPO3 first intron, which constitutes a relevant putative regulatory region of the human RSPO3 gene, as indicated by combined bioinformatic studies of publicly available data from chromatin immune-precipitation and assay for transposase-accessible chromatin with sequencing (ChIP-seq and ATAC-seq) as well as transcription factor (TF) binding motives in the human genome. The goal of our present project is to analyze RSPO3 expression in a set of human BC cell lines, which includes both luminal (T47D and MCF-7) and basal (MDA-MB231 and BT-549) phenotypes, to determine relationships with information provided by publicly available data-sets about transcription modulators differentially recruited by the promoter and the intronic regulatory region in the human RSPO3 locus. Our results suggest that not only RUNX1, but also the estrogen receptor (ER), STAT3, co-repressor Groucho as well as the SWI/SNF chromatin remodeling complex may exert relevant differential roles in regulating RSPO3 expression in various breast cancer molecular subtypes.

330. 586. **EVALUATION OF A NOVEL BODIPY-BASED BORONATED COMPOUND FOR BORON NEUTRON CAPTURE THERAPY IN MELANOMA CELLS**

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Boron neutron capture therapy (BNCT) is a binary treatment that combines a selective accumulation of ^{10}B carriers in tumor cells and neutron irradiation to produce the ^{10}B neutron capture that generates the high linear energy transfer (LET) particles, ^{7}Li and α. The aim of this study was to evaluate a novel boronated compound for BNCT in human melanoma cells (A375). This new compound was synthesized by conjugating a boron cluster, sodium borocaptate (BSH) to a borondipyrromethenedifluoride (BODIPY), which presents an intrinsic fluorescence/BNCT duality. Cytotoxicity was determined by MTT assay after incubation of A375 cells with different concentrations of this compound (BODIPY-BSH) for 24 hours. No cytotoxicity was found up to 100 μM. For higher concentrations (1-100mM), significant cytotoxicity was detected. Cells were incubated with 100 μM BODIPY-BSH for different times (0-20 h) and the incorporation of this compound was detected by epifluorescence microscopy and intracellular boron concentration quantified by atomic emission spectrometry. Maximum take up was (3.01±1.23)exp+9 ^{10}B atoms/cell at 2 hours of incubation. BNCT was performed in A375 cells after incubation with 100 μM BODIPY-BSH for 2 h and neutron irradiation (thermal flux (2.9±0.3)exp+11 n/cm^2.min) with the RA-3 reactor facility from CNEA. Neutron irradiation of cells without BODIPY-BSH was also performed. Time of irradiation was calculated to obtain a total dose of 2 Gy, both for neutrons alone and BODIPY-BSH + neutrons. Control cells without treatment or incubated with the compound were sham irradiated. Response to treatments was determined by MTT assay. Cell viability reduction of (31.52 ± 9.74)% and (50.57 ± 5.06)% was found after neutrons alone and BNCT, respectively.
We conclude that this new compound is a promising agent for BNCT.

331. 594. **EFFECT OF ATORVASTATIN TOGETHER WITH RIFAXIMIN IN THE PREVENTION OF HEPATOCARCINOGENESIS GENERATED BY DIOXIN-TYPE TOXIC**

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Hepatocellular carcinoma (HCC) is the most common liver tumor. One of the main causes is exposure to dioxin-like compounds such as the endocrine disruptor hexachlorobenzene (HCB). A potential role of thyroid hormones (TH) in its development has been postulated, and statins prevent it. Rifaximin (RIF) has an anti-inflammatory capacity and reduces the expression of miRNAs involved in HCC. We show that HCB stimulates hepatic proliferation through TGF-β1 and TH, and that atorvastatin (AT) prevents it. Objective: To study the effects of HCB treatment on cell proliferation in Huh-7 cells, to analyze the ability of AT together with RIF to prevent such effects. MyM: Huh-7 line treated with HCB. Pretreatment with AT (30uM) and/or RIF (10uM) and/or exogenous TGF-B1 or T3. Specific objectives: A) Analyze: 1- proliferation (PCNA, cD1); 2-apoptosis (caspase-3 and Cytochrome-c); 3- cell regulation (TGF-β1, p27); 4- inflammation (TGF-β1 and Cox-2); 5- Metabolism of TH (DI) and avb3 receptors; 6- Cell migration. B) To study the prevention of AT with RIF, on the effect of HCB on the parameters listed in A. C) To analyze the role of TGF-β1 and T3 in the mechanism of preventive action of AT together with RIF, on HCB. HCB 5uM increased PCNA(39%,p<0.01), TGF-β1(45%,p<0.01), cox-2(25%, p<0.05), cytochrome-c(35%,p<0.01), caspase -3(25%,p<0.05) and promotes cell migration (30%,p<0.01) compared to controls, western blot and wound test. The same effect was observed in the avb3 receptor (28%,p<0.05); on the contrary, DI decreased (26%p<0.01), RT-PCR. The pretreatment with AT and RIF prevented the increase of the mentioned parameters and decreased the cell migration generated by HCB (60%,p<0.01). The dependence of HT on the preventive mechanism of AT has been demonstrated; however, the role of TH in the co-administration of AT and RIF remains unclear. Co-administration of AT and RIF shows non-additive antiproliferative, antimigratory and proapoptotic effects in preventing the development of HCC.

332. 610. **DEVELOPMENT OF MURINE MODELS OF ORAL CANCER ASSOCIATED WITH TOBACCO EXPOSURE TO STUDY THE CD44 RECEPTOR RELEVANCE IN TUMOR GROWTH, PROGRESSION AND RESPONSE TO IMMUNOTHERAPY**

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Head and neck squamous cell carcinoma (HNSCC) is the sixth most common cancer worldwide, causing around 3,000 deaths annually in Argentina. The curative option is surgical resection but most patients can access it because the diagnosis is usually in advanced disease. The elucidation of the genomic alterations of HNSCC that can promote recognition by the immune system (IS) and the approaches based on the activation of the antitumor immune response (immunotherapy) became an opportunity for HNSCC, and immune checkpoint inhibitors are approved for HNSCC. Primary and acquired resistance to therapies, including immunotherapy, could be mediated by tumor-initiating cells (CSCs), which are part of the tumor microenvironment (TME) and are characterized by markers such as CD133 and CD44, a different proliferation and self-renewal, and the ability to evade IS. Lately, an increase in CD44 expression levels was described in HNSCC tumors. We induced carcinogenesis that mimics the mutational complexity of tobacco-associated HNSCC by administering the drug 1-oxide 4-nitroquinoline (4NQO) in wild-type (WT) and deficient expression of the CD44 gene (CD-44KO) mice. After 22 weeks, we observed tumor lesions in both strains. While WT showed tumor growth in all mice exposed to 4NQO, CD44KO showed lesions in 50% of mice, suggesting a possible role of CD44 in tumor development. With the aim of obtaining a shorter and easier-to-follow-up model, we isolated, cultured, and establish WT or CD44KO cell lines. We phenotypically characterized lines and challenged WT or CD44KO C57 mice in the tongue or s.c with CD44+/+cells. We observed tumor growth in both models; however, tumor-bearing WTC57 mice showed a lower rate compared to CD44KO mice (p<0.05). Thus far, we have hypothesized about the role of non-tumor CD44 on tumor establishment. These models would allow us to study CD44 in the appearance and phenotype of HNSCC, describe the TME, and establish an *in vivo* platform for testing therapies.

O4-ONCOLOGY

THURSDAY 16TH NOVEMBER 14:00-15:30
CHAIRS: CATALINA LODILLINSKY
MARIANA MALVICINI

333. 74. **PRIMARY CULTURES OF HUMAN GLIOMAS FOR THE STUDY OF CANCER STEM CELLS INHIBITION**

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Introduction: Gliomas (GL) are a group of various glial origins, from low (LG) to high grade (HG) (1-4, WHO). Despite chemotherapy (temozolomide, TMZ), HGGL have poor prognosis, hence the importance of developing primary GL cultures for better therapeutic investigation. Inducible nitric oxide synthase (iNOS), a nitric oxide (NO) producer enzyme, could be a potential therapeutic target as NO is related to cancer stem cells (CSC) maintenance, responsible for therapy resistance and relapse. Objective: To analyze TMZ alone or in combination with iNOS inhibitor, S-methylisothiourea (SMT) on primary GL cultures. Methodology: Endorsements were obtained from 3 hospitals (Ángel Roffo, Clínicas, Ramos Mejía). Between 2018-23, a molecular panel (IDH, p53, ki67, 1p/19q, EGFR) was performed on samples from patients undergoing surgery. Samples were processed under sterile conditions by mechanical disintegration. Cultures with at least 1 passage were considered successful. In those, pluripotency (Nestin, qPCR) and iNOS (immunofluorescence) expression were assessed. TMZ (20μM), SMT (50μM) and TMZ+SMT effect were evaluated in 2D (crystal violet CV, MTS), 3D (sphere forming efficiency SFE in low adhesion conditions, diameter), and epithelial (E-cadherin) to mesenchymal (vimentin) transition (TEM) genes. Results: From 11 GL samples: 9% IDH+, 18% IDH-, 27% p53+, 27% 1p-/19q-, 18% EGFR+, 9% Nestin+, 36% iNOS+, 6 cultures were successful. In 1 HGGL, cell viability was reduced by TMZ, SMT showed no effect and TMZ+SMT was similar to TMZ (CV). In 2 HGGL, SMT inhibited SFE (>30%, p<0,05) and diameters (>50%, p<0,05), TMZ had no effect and TMZ+SMT was similar to SMT. In other LGGL, TMZ+SMT reversed the effect of single drugs. In other HGGL, it was observed that neither single drugs nor the combination had effects in 2D (MTS), 3D, nor TEM related genes. Conclusion: It was possible to collect GL samples, establish primary cell cultures and investigate iNOS as a potential target in GL CSC.

334. 83. **MIRNAS EXPRESSION AND MICROBIOTA IS ALTERED BY HIGH FAT DIET IN MICE STOOL**

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Prostate cancer (PCa) is the most common cancer type and the third cause of cancer death in Argentinean men. Metabolic syndrome (MS) is a risk factor for PCa and previous studies revealed that high-fat diet (HFD) is associated with PCa development and progression. The microbiome is made up of the environment of the microbiota that live in an organ of an individual. It impacts cancer development and varies according to different individuals metabolic states. MiRNAs are small non-coding RNA molecules that regulate gene expression. The interaction between the microbiome and the individual requires a regulatory network in which miRNAs could play a crucial role. Our aim was to characterize the bacteria and miRNA composition of the microbiome from mice with MS and PCa. Male C57BL/6J mice chronically fed with HFD or control diet (CD) were inoculated with the murine TRAMP-C1 cell line. After sacrifice, plasma, tumor, intestine, and stool samples were collected. We found that Bacteroidetes (B) and Verrucomicrobia (V) decreased significantly in HFD, while Firmicutes (F) and Proteobacteria (P) increased significantly. In addition, we observed that the obesity index, established by the F/B ratio, was significantly increased in the HFD. We examined a panel of 11 miRNAs selected from the literature (miR1a, miR16, miR20a, miR21a, miR27b, miR30c, miR34a, miR194, miR200a, miR200b, and let7c) for expression levels in stool, intestine, tumor, and mouse plasma by RT-qPCR. We found that miR34a and let7c were significantly altered in all tissues analyzed between diets, while the rest of the miRNAs analyzed were found to be significantly increased in different ways in each tissue. These results suggest that the altered expression of miRNAs obtained from stool and from the rest of the HFD mice tissues analyzed may be related to changes in the microbiota.

335. 275. **PKA ORCHESTRATES THE INTERACTION BETWEEN METASTATIC PROSTATE CANCER CELLS AND THE BONE MICROENVIRONMENT**

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Metastatic prostate cancer (PCa) cells soiling in the bone require a metabolic adaptation. Here, we identified the metabolic genes fueling the seeding of PCa in the bone niche. Using a transwell co-culture system of PCa (PC3) and bone progenitor cells (MC3T3 or Raw264.7), we assessed the transcriptome of PC3 cells modulated by soluble factors released from bone precursors. In a Principal Component Analysis using transcriptomic data from human PCa samples (GSE74685), the altered metabolic genes found *in vitro* were able to stratify PCa patients in two defined groups: primary PCa and bone metastasis, confirmed by an unsupervised clustering analysis. Thus, the transcriptional metabolic profile *in vitro* has a clinical correlate in human metastatic samples. Further, the expression levels of five metabolic genes (*VDR, PPARA, SLC16A1, GPX1* and *PAPSS2*) were independent risk-predictors of death (SU2C-PCF dataset) and a risk score model including this lipid-associated signature was able to discriminate a subgroup of bone metastatic PCa patients with a 23-fold higher risk of death. This signature was validated in a patient-derived xenograft pre-clinical model when comparing the PDX MDA-PCa-183 growing intrafemorally *vs.* subcutaneously and appears to be under the regulatory control of the Protein Kinase A (PKA), that emerges as a critical factor regulating the PCa-bone crosstalk. Following secretome analyses of conditioned media, we found fibronectin (Fn1) and type-1 collagen (Col1a1) as critical bone-secreted factors that regulate tumoral PKA. Additionally, we found that this kinase is critical for the regulation of key factors governing the metastatic process. We observed that the axis Col1a1-Fn1/PKA drives the expression of osteopontin, and of pro-inflammatory and pro-angiogenic genes. Overall, we identified a novel lipid gene signature regulated by PKA, a central hub of PCa metabolism and bone progression, outlining potential therapeutic targets to halt disease progression.

336. 402. **PROMISING DUAL ANTICANCER AND ANTIMETA-**

STATIC ACTION BY A NOVEL CU(II) COMPLEX DERIVED FROM ACYLHYDRAZONE ON HUMAN OSTEOSARCOMA MODELS

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Osteosarcoma (OS) is a frequent bone cancer, affecting largely children and young adults. Cisplatin (CDDP) has been efficacious in the treatment of different cancer such as OS but the development of chemoresistance and important side effects leading to therapeutic failure. Novel therapies including copper compounds have shown to be potentially effective as anticancer drugs and one alternative to usually employed platinum compounds. The aim of this work is to evaluate the antitumoral activity of a novel copper(II) compound with a acylhidrazone in 2D (monolayer) and 3D (multicellular spheroids) OS models. Using MTT assay we demonstrated that the complex significantly reduced the cell viability in MG-63 IC_{50}: 1,09±0,06μM and in non-tumoral mouse fibroblast L929 IC_{50}: 2,52±0,02μM ($p<0.0001$), showing that Cu complex has selectivity index value of 2.3 compared to CDDP(SI = 0.3). In addition, we observed that interact with calf thymus DNA (CT-DNA) suggesting that the complex binds to DNA in an intercalative manner. Reactive oxygen species (ROS) generation was determined by oxidation of fluorescence dye DHR-123, evidenced that the complex caused an increment in ROS production after 3 h in a dose-manner response between 10μM and 25μM ($p<0.01$). Flow cytometry studies demonstrated that the compound inhibits cell proliferation and conveys cells to early apoptosis at 0.5μM (26.6%) and late apoptosis 1.25μM (15.1%) ($p<0.0001$). On the other hand, MG-63 spheroids were cultured by the hanging drop technique and the effect of the compound on cell viability was evaluated by resazurin reduction assay (IC_{50}:16.3±3.1μM) showing that IC_{50} value was 4 times lower than CDDP (65±6μM) ($p<0.0001$). Finally, the compound reduced the spheroid cell migration in a dose-dependent manner from 7,5 to 20 μM suggesting a dual anticancer and antimetastatic actions. In summary, this copper complex displays a promising dual anticancer and antimetastatic action on OS 2D and 3D.

337. 406. **PALBOCICLIB RESPONSIVENESS OF THE MPA-INDUCED MURINE BREAST CANCER MODEL WITH DIFFERENT SENSITIVITY TO ENDOCRINE TREATMENTS**

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Luminal breast carcinomas represent more than 70% of all breast cancer (BC) patients. Palbociclib (PALBO), an oral CDK 4/6 inhibitor, is currently used in combination with endocrine therapy to treat advanced hormone receptor-positive, HER2-negative BC. However, about 25-35% of patients do not respond, and almost all patients, eventually, become resistant to this treatment. We have demonstrated that two tumor families from the MPA-induced BC murine model have a differential response to PALBO, regardless their response to an antiprogestin treatment (mifepristone, MFP), being the 59 family sensitive and the C4 family resistant to PALBO. The aim of the study was to evaluate the basal pRB expression, its regulation upon PALBO treatment and the transcriptome difference in both families that may shed light to understand their differential response to PALBO. RB phosphorylation levels were evaluated by IHC in 59-2-HI, 59-HI, C4-HI and C4-2-HI tumors after approximately 15 days of vehicle or PALBO treatment. RNA-Seq studies were carried out using RNA from untreated tumors. The differential expression and enrichment analysis were conducted with R/Bioconducter packages. As expected, pRB expression in the 59 tumors decreased after PALBO treatment ($p<0.001$). Contrarily, pRB levels increased after treatment in the PALBO-resistant tumors ($p<0.05$). In the latter, the basal pRB levels were lower than in the PALBO sensitive tumors. MFP was able to diminish pRB in C4-HI tumor, showing that the pRB axis is feasible of modulation. Preliminary analysis of RNA-Seq data highlights a down regulation of p18 (Cdkn2c; $p<0.05$) and an increase in Notch1 ($p<0.01$), in the PALBO resistant variants. Sensitive tumors show increases in pathways related with cell proliferation, such as S phase. We conclude that this model provides an excellent tool to dissect mechanisms related to PALBO resistance and to further investigate the link between p18, Notch 1 and RB phosphorylation mediating this effect.

338. 431. **HO-1 GENETIC VARIANTS AND ITS EFFECTS IN THYROID CANCER BIOLOGY**

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In previous studies on human thyroid cancer (TC), we observed elevated levels of hemeoxygenase-1 (HO-1) protein in both cytoplasmic and nuclear compartments. Additionally, increased HO-1 mRNA expression correlated with tumor progression. Activation of HO-1 through hemin treatment in the TPC-1 cell line promoted cell viability, proliferation, migration, and cell cycle progression, while inhibition via ZnPP had opposite effects. This study aimed to investigate the impact of genetically overexpressed HO-1 variants (full-length - FL, enzymatically inactive - H25A, and nuclear truncated - t-HO1) on cancer-related processes. Using stable transfections of these variants into TPC-1 cells, we observed that FL and H25A forms were predominantly overexpressed in the cytoplasm, while t-HO-1 accumulated in the nucleus. Overexpression of FL and t-HO-1 significantly enhanced cell viability ($p<0.0001$) and migration ($p<0.0001$) compared to controls. In contrast, H25A overexpression hindered these processes ($p<0.0001$) compared to FL. In primary cultures of human thyroid tumors and normal tissues, we identified HO-1 expression in the nuclei of normal cells and in nuclei/cytoplasm in tumor cells. Hemin treatment increased viability ($p<0.0001$) in tumor cells but decreased it in normal cells ($p<0.0001$). These findings correlate with prior evidence, demonstrating that hemin activation of HO-1 in tumor cells, through its enzymatic activity, exerts a protumor role. The current study revealed that FL and t-HO-1 variants independently promoted tumor-related processes, irrespective of subcellular localization. Notably, FL- impact on viability and migration seems tied to its enzymatic activity, as the H25A mutation impairs these effects. Intriguingly, nuclear HO-1 expression might differentially affect normal and tumor thyroid cells. Subsequent experiments will shed light on the relationship between HO-1's subcellular localization, enzymatic activity, and thyroid cancer progression.

339. 573. **NUCLEAR HO-1 INTERACTORS MIGHT DEFINE A NEUROENDOCRINE SIGNATURE IN PROSTATE CANCER**

Rocio Seniuk[1,2], Pablo Sanchis[1,2], Juan Bizzotto[1,2], Estefania Labanca[5], Nicolas Anslemino[5], Sofia Lage-Vickers[1,2], Gastón Pascual[1,2], Agustina Sabater[1,2,3], María Laura Lacreu[1,2], Julia Lechuga[1], Nora Navone[5], Elba Vazquez[1 2], Javier Cotignola[1,2], Pia Valacco[1,2,4], Ayelén Toro[1,2], Geraldine Gueron[1,2]

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Previous reports have shown that heme-oxygenase 1 (HO-1), an enzyme involved in free heme degradation, has a non-canonical anti-tumor effect in prostate cancer (PCa). It is believed that nuclear HO-1 influences the function of various transcription factors, although unraveling its non-canonical role is an unmet need. Thus, in this work we aimed at identifying HO-1's nuclear interactors and their association with aggressive PCa. PC3 cells were treated with hemin (80 μM, 24 h), a specific pharmacological HO-1 inducer. Next, nuclear HO-1 immunoprecipitation was performed and proteins were subjected to LC-ESI MS/MS analysis, identifying 11 differential nuclear proteins between control and hemin treated PCa cells (ILF3, ILF2, BCLAF1, SAFB, DDX17, SLC25A5, CASP14, PRDX1, BRIX1, CCDC175 and GPATCH1). To decipher how these factors are associated with the aggressive phenotype of PCa, we interrogated RNA-seq expression data of the MDA-PCa-PDXs series (Prostate Cancer Patient Derived Xenografts Program; MD Anderson Cancer Center), which captures PCa disease heterogeneity. Interestingly, an unsupervised clustering analysis considering the expression of HO-1's interactors showcased that samples with high expression of these genes derived from neuroendocrine tumors, negative for AR staining. Moreover, a Principal Component Analysis revealed ILF3 as the most relevant HO-1 interactor driving PDXs' samples variance, which was also associated with a significant decrease in relapse-free survival of PCa patients (GSE70770). Additionally, ChIP-Atlas data analysis evidenced that at least 36% of HO-1 nuclear interactors were reported as potential transcription regulators. Strikingly, KEGG pathways analysis revealed that their regulomes are significantly associated with Huntington disease, highlighting their relevance in neural processes. Overall, these findings suggest that HO-1 and its nuclear interactors may play a relevant role in neuroendocrine PCa.

P3-ONCOLOGY

THURSDAY 16TH NOVEMBER 14:00-15:30
CHAIRS: PAOLA DE LUCA
FLAVIA PICCIONI
CLAUDIA GENTILI

340. 57. **NEW RESULTS ON K-CARRABIOSE AS POTENTIAL ANTITUMOR AGENT**

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Carrageenans are sulfated galactans found in some red seaweeds with biological activities. In previous work, we have purified native and degraded carrageenans, including the disaccharides (carrabioses) and disaccharide-alditols as potential antitumor compounds and identified the active principle of the potential antitumor properties. κ-carrabiose was the most effective, showing high cytotoxic properties (IC50 0.043 ± 0.009 mg/ml), killing the murine mammary adenocarcinoma LM2 cells through an apoptotic pathway, showing a decreased motility and a decreased cell-cell and cell-matrix interactions. This work aimed to further explore the antitumor potential of κ-carrabiose. New results include the evaluation of the compound in a panel of tumour cell lines of various origins. The obtained IC50 values ranged from 0.051 to 0.099 mg/ml, suggesting that κ-carrabiose could be cytotoxic in several tissues. We also evaluated its cytotoxicity in the presence of different serum concentrations, which was not statistically different as compared to the non-serum control, showing that there are no serum enzymes degrading the compound into monosaccharides. We also evaluated κ-carrabiose with K^+ as a counterion of the free sulfate of the compound, compared to Na^+, the cation employed in the previous studies. κ-carrabiose did not change its cytotoxic activity, suggesting the action depends on the structure of the disaccharide and not on the counterion. Looking for a better approximation to the tumour environment, we evaluated its cytotoxicity in LM2 spheroids and, although the IC50 obtained (0.069 ± 0.02 mg/ml) was slightly higher than in the monolayer, the spheroids showed a response against the compound. These new results reinforce the idea of proposing κ-carrabiose as a novel antitumor agent, including using disaccharide units such as carrabioses coupled to antineoplastics to improve its cytotoxicity and antimetastatic properties.

341. 67. **MUC4 EXPRESSION AS A PROGNOSTIC BIOMARKER FOR TRIPLE NEGATIVE BREAST CANCER AND TNF BLOCKADE AS A NEW THERAPEUTIC APPROACH**

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We have demonstrated that TNF induces trastuzumab resistance through mucin4 (MUC4) expression, which also is an independent biomarker of poor response to adjuvant trastuzumab in HER2+ breast cancer. Here, we evaluated the role of TNF and MUC4 in the invasive capacity of triple negative breast cancer (TNBC) cell lines and its clinical impact. TNF blockade was achieved using etanercept (E), which blocks both the soluble (sTNF) and transmembrane isoforms, or dominant negative protein INB03 (DN) that only neutralizes sTNF. BT-549 and MDA-MB-231 TNBC cell lines treated with E or DN exhibited a decrease in the expression of MUC4 and mesenchymal markers by Western blot. Conditioned media (CM) of MDA-MB-231 and BT-549 cells treated with E (CM-E) or DN (CM-DN) impaired the invasion of both cell lines ($p<0.01$). Female *BALB/c* mice bearing the TNBC LMM3 tumors were treated with DN, anti-PD-1 or the combination treatment, and no impact on tumor growth was observed. However, DN+anti-PD-1 treatment prevented the appearance of lung metastasis ($p<0.05$). To evaluate the impact of MUC4 expression on the tumor microenvironment and aggressiveness, we studied, the tumor infiltrating lymphocytes (TILs) scored by H&E, and the expression of MUC4, Ki67, PD-L1, androgen receptor (AR) and cytokeratin 5, determined by immunohistochemistry in a cohort of 55 TNBC patients. MUC4 expression inversely correlated with TILs ($p=0.00013$), Ki67 ($p=0.016$), PD-L1 ($p=0.001$) and AR ($p=0.047$). To better understand how MUC4 modulates TILs infiltration, we evaluated the effect of BT-549-CM and MDA-MB-231-CM on T cell migration. CM-DN from both cell lines and CM-E of MDA-MB-231 increased T cell migration ($p<0.01$ and $p<0.05$, respectively). Moreover, MUC4 is associated with a higher metastasis risk ($p<0.005$) and proved to be an independent predictor of poor overall survival ($p<0.02$). We propose MUC4 as a predictive marker to guide a combined treatment of TNF blockers with chemotherapy or immunotherapy in TNBC.

342. 142. **NEXT-GENERATION OF CELLULOSE-DERIVED MATERIALS AS A NOVEL ALTERNATIVE FOR BREAST CANCER TREATMENT**

Aldana M. Schey, Lizeth Ariza Bareño, Luciana Cañonero, Andrés Bechis, Diego Britez Neira, Florencia Cámpora, Beatriz E. Pava Gómez, Ariel M. Sarotti. Alejandra Suarez, Rolando Spanevello, Marcela Villaverde, Laura B. Todaro, Alejandro J. Urtreger.

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Breast cancer is a health condition that affects millions of women worldwide. In this study, a second generation of compounds derived from the pyrolytic treatment of soybean husks has been investigated as an alternative for treating this disease. Fifteen new derivatives have been explored and compared with levoglucosenone (the leading compound) in their biological activity. Only three compounds (58A, 59A, and 37D) demonstrated the capability to induce cell death, exhibiting Inhibitory Concentration 50 (IC50) values of 5.9±2, 5.4±3, and 7.2±4 uM, for LM3 cells respectively. These values were notably lower than levoglucosenone's, making them promising candidates. Next, we investigated signaling pathways related to cell proliferation and survival. Western blot assays, revealed that 59A led to an inhibition of the ERK/MAPK pathway and an increase in apoptotic markers expression. In order to propose an improved therapy, we evaluated the combined effect with the chemotherapeutic agent doxorubicin. Doxorubicin showed an IC50 of 0.06 uM, but its combination did not generate a synergistic or additive effect. In a second stage, we combined the compounds with metabolism modulators, since all compounds induce an increase in glucose consumption and lactate production ($p<0.05$). We employed 2-deoxyglucose (2-DG: a glucose analog that inhibits glycolysis), 6-aminonicotinamide (6-AM: an antimetabolite that inhibits the pentose phosphate pathway) and metformin (a glucose levels modulator). Interestingly, only 2-DG significantly increased levoglucosenone-derivatives' effect ($p<0.05$). Loewe curves, from Combenefit software, described this interaction as a synergistic ($p<0.01$). Based on our findings, cellulose-derived materials demonstrate a promising biological profile, positioning them as an alternative for breast cancer management. These materials have the potential to be used either as standalone therapies or to enhance the effect of pre-existing therapies.

343. 195. **IMMUNOPHILIN FKBP51 SHOWS ONCOGENIC PROPERTIES IN A GLIOBLASTOMA CELL LINE**

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Immunophilins belong to the molecular chaperone family, constituting a subfamily of proteins that exhibit two specific properties: they show peptidylprolyl-(cis/trans)-isomerase activity and the capacity to bind immunosuppressive drugs. FKBP51 (*FK506-Binding Protein of 51-kDa*) is an Hsp90-binding immunophilin that was first discovered associated with steroid receptor heterocomplexes via its interaction with Hsp90. Recently, our laboratory discovered that FKBP51 is also a mitochondrial factor that shows antiapoptotic properties in normal fibroblasts. In this study, it is shown that FKBP51 is transported from mitochondria to the nucleus upon the onset of any type of stress (temperature, oxidative stress, toxins, etc.). This led us to think that in highly stressed cells such as cancer cells, FKBP51 should also show a predominantly nuclear localization. This was evidenced by confocal microscopy in several cancer cell lines, including N2a murine neuroblastoma cells and U87 human glioma cells. Also, Western blot analysis revealed that FKBP51 is overexpressed in all cancer cell lines compared to normal cell lines. To confirm this, NIH-3T3 fibroblasts were transformed into a tumorigenic cell line by transfection of the v-Ha-Ras oncogene. The transformed cells acquired a malignant phenotype, FKBP51 being mostly nuclear rather than mitochondrial and highly expressed. Inasmuch as TERT, the catalytic subunit of telomerase, is abundant in cancer cells and a known client factor of Hsp90, the recruitment of FKBP51 was investigated in U87 human glioma cells. Co-immuno-precipitation assays demonstrated the presence of FKBP51 in TERT•Hsp90 complexes, an oligomer that was disrupted by the Hsp90 inhibitor geldanamycin. Importantly, the overexpression of FKBP51 enhanced telomerase enzymatic activity. Based on these findings, we propose that a U87 glioma cell model could be useful to elucidate the role of FKBP51 role in glioblastoma development and progression.

344. 248. **FOXP3 EXERTS PROTUMORAL INTRINSIC EFFECTS IN GLIOBLASTOMA CELLS**

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Glioblastomas (GBM) are the most frequent and aggressive primary malignant brain tumors in adults. Although the survival of these patients is 12-18 months, their treatment remained unchanged for the last 20 years. Foxp3, a transcription factor required for the development and immunosuppressive activity of regulatory T cells has also been detected in several types of tumoral cells. However, the tumor intrinsic role of Foxp3 is poorly understood. We have previously shown that Foxp3 exerts intrinsic pro-tumoral effects in breast cancer cells and that administration of a cell-penetrating Foxp3 inhibitory peptide (P60) improves the efficacy of antitumor vaccines and exerts direct antitumor effects, inhibiting the progression of experimental breast cancer. Here we aimed to assess the intrinsic effects of Foxp3 in GBM cells. Our meta-analysis of transcriptomic data from GBM biopsies indicated that Foxp3 is expressed in these tumors and it is significantly associated with worse prognosis in GBM patients. We found expression of Foxp3 in murine and human GBM cell lines and patient-derived cultures, which was upregulated by chemotherapy ($p<0.05$). Inhibition of Foxp3 using P60 reduced cell survival in murine and human cell lines and enhanced their chemosensitivity to cisplatin and temozolomide. We developed an Adenoviral vector (Ad.P60) that encodes P60 and the reporter gene dTomato under the control of the CMV promoter in order to improve the availability of P60. Ad.P60 efficiently transduced GBM cells *in vitro* and *in vivo*. Transduction of GBM cells with Ad.P60 enhanced apoptosis and reduced cell viability, proliferation, migration and chemoresistance ($p<0.05$). Our results suggest that Foxp3 exerts pro-tumoral intrinsic effects in GBM. Thus, Foxp3 could constitute a valuable target to improve the treatment and diagnosis of these tumors.

345. 277. **EXPLORING THE LINKS: CANCER STEM CELLS, TGF-β PATHWAY, AND GPC3**

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We have demonstrated that GPC3 expression reverses the epithelial-to-mesenchymal transition (EMT) undergone by breast cancer cells. Given the role of the TGF-β pathway in this process, and reports indicating that the EMT confers stem-like attributes, we decided to analyze the putative relation among GPC3, TGF-β, and stem cells. To analyze the impact of GPC3 on the stem cell population, we evaluated the ability to form mammospheres of breast cancer cells with genetically modified GPC3 expression. GPC3 overexpression increased MDA-MB231 sphere formation by about 15%, and its silencing in MCF-7 cells reduced it by 45%. While control MDA-MB231 spheres were like "clusters", GPC3-overexpressing ones were large with cells concentrically arranged. qPCR assays showed that NANOG and OCT4 were poorly expressed in cell monolayers, but *in silico* studies exhibited upregulation of stem markers such as SOX, ALDH1A1, and KLF4, in human mammary tumors defined as "high GPC3" (FDR<0,05; Log2 fold change >0,5). Using the TCGA database, the gene enrichment analysis proved that several signatures involved in the stem regulation are upregulated in patients with "high GPC3" (as GOBP_STEM_CELL_PROLIFERATION, FDR<0.05, NES=1.39). Moreover, numerous related pathways appeared modulated, such signatures linked to the TGF-β signaling (FDR<0.05, NES=1.72). Our *in vitro* assays indicated that the expression of some members of this pathway are altered in GPC3-modified cell lines (like TGF-β1, TGFBRI, and TGFBRII). In addition, MDA-MB231-GPC3 cells exhibited lower Smad2/3 phosphorylation than controls, while MCF7-shGPC3 showed higher, reinforcing that GPC3 inhibits the TGF-β pathway. We decided to revert this inhibition by a recombinant TGF-β treatment. We found that MDA-MB231-GPC3 +TGF-β cells were viable under starving conditions, in contrast to cells treated with DMSO. In sum, our results suggest a role of GPC3 in stem population maintenance and the possible implication of the TGF-β pathway.

346. 316. **INHIBITION OF THE ONCOGENIC FUNCTIONS OF ROR1 USING A PHARMACOLOGICAL INHIBITOR**

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Melanoma is the deadliest type of skin cancer and has a poor prognosis when not diagnosed at early stages. Wnt5a is a secretory glycoprotein involved in the non-canonical Wnt signaling pathway that plays an important role in melanoma by increasing motility, invasion, proliferation and resistance to apoptosis. The downstream signaling is mediated by the tyrosine kinase receptors ROR1 and ROR2. In the present work we will study the role of ROR1 in melanoma progression and its inhibition with the inhibitor 1,2,3,4,6-Penta-O-galloyl-β-D-glucopyranose (PGG), a specific inhibitor of ROR1. To study the role of ROR1 in melanoma cells we transduced M2 melanoma cell line with ROR1 ADNc. Upon generation of ROR1-overexpressing cells, we observed that ROR1 expression induced marked morphological changes. ROR1 expression leads to reduced cell–cell contacts and increased cell scattering ($p<0.05$) suggesting a role for this receptor in Epithelial-Mesenchymal Transition (EMT) which is required for both tumor invasion and metastatic spreading. Moreover, ROR1 significantly promotes filopodia formation ($p<0.05$). Measurement of morphometric parameters also revealed a significant decrease in elongation and an increase in roundness in M2-ROR1 cells ($p<0.05$). These alterations were partially or completely reverted by incubation with PGG. We also found that ROR1 overexpression significantly increased the proliferation of M2 cells ($p<0.05$). To identify possible underlying mechanisms, we performed Western blots. We found that ROR1 overexpression significantly increased both Akt phosphorylation (T308 and S473) and STAT3 expression ($p<0.05$). Incubation with PGG significantly inhibited both cell proliferation and decreased Akt phosphorylation and STAT3 levels ($p<0.05$). These results show that ROR1 promotes proliferation and EMT in melanoma and that these effects can be reverted with a pharmacological inhibitor. Further studies are underway to confirm the usefulness of ROR1 as therapeutic target and of PGG as a novel therapy for melanoma.

347. 381. **HEXACHLOROBENZENE DIFFERENTIALLY MODULATES CONVENTIONAL AND METRONOMIC THERAPY IN TRIPLE NEGATIVE BREAST CANCER CELLS**

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The conventional therapy (CT) for triple negative breast cancer (TNBC) consists in the administration of the maximum tolerable dose of chemotherapeutic drugs, such as paclitaxel (PX) which produces several adverse effects requiring long intervals between treatment cycles to patient recovery. An alternative strategy to avoid that is metronomic therapy (MT) based on the administration of lower drug doses with short drug-free intervals. In this sense, our group has demonstrated the antitumor efficacy of a metronomic combination of PX and the muscarinic agonist carbachol (Carb) in breast cancer cells. Besides, the efficacy of the antitumor treatments may be modulated by environmental pollutants such as hexachlorobenzene (HCB) which has been described to reduce doxorubicin treatment efficacy in colon cancer. In this work we evaluated if HCB modulates the efficacy of PX CT and PX+Carb MT in TNBC MDA-MB231 cells. By MTT assays we determined that an environmental concentration of HCB (10^{-8}M) did not modify the cell viability (basal:100+/-8.2%; HCB:99.8+/-5.1%) but it significantly reduced the CT effect (PX 10^{-7}M)(CT:67.2+/-3.2%; HCB+CT:93.7+/-4.9%; $p<0.001$) whereas it did not modify the MT effect (PX 10^{-8} M+Carb 10^{-11} M) (MT:73.6+/-3.0%; HCB+MT:65.4+/-2.7%). Furthermore, the sensitivity to PX is reduced in the surviving cells after CT but not after MT (EC_{50}: basal:213.1 nM; CT:5.8 μM; MT:9.3 nM). HCB also reduces the sensitivity in CT surviving cells but not in MT surviving cells (EC_{50}: HCB+CT:76.5 μM; HCB+MT:10.5 nM). Then, by Western blot assays we determined that HCB and CT increased ABCG2 drug extrusion pump expression, related with chemoresistance, whereas MT reduced its expression even in HCB presence. In conclusion, in TNBC MDA-MB231 cells HCB reduces the efficacy of PX CT by the increment of ABCG2 expression, but do not modify the efficacy of PX+Carb MT. It indicates that in HCB presence, the replacement of the conventional therapy by a metronomic one would be beneficial

348. 387. **CHARACTERIZATION OF A NEW CISPLATIN-RESISTANT CELL LINE DERIVED FROM NON-SMALL LUNG CANCER (NSCLC) CELL LINE NCI-H125**

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Cisplatin (CDDP) is a non-small cell lung cancer (NSCLC) standard therapy. The frequent resistance acquisition to this treatment would be associated with the presence of cancer stem cells (CSC). The generation of CDDP-resistant cell lines is an important tool to study the mechanisms involved in this characteristic and the parallelism with what happened with the patients. In order to generate the H125 CDDP-resistant cell line (H125cpr), we treated the parental H125 cell line with CDDP 1μM in 72h weekly pulses during 12 weeks. After that, we escalate the treatment dose to 2μM in 72h weekly pulses for 20 more weeks. After 32 treatment weeks, H125cpr showed a IC50 of 6μM, while H125ct (control cell line without treatment) presented a IC50 of 1.5μM. To corroborate CDDP resistance, we evaluate the distribution of the cells in the different phases of their cell cycle by flow cytometry and propidium iodide dye. We observed that CDDP treatment produced an arrest in G2-M phase in H125ct, while this treatment had no effect in this parameter in H125cpr. Besides, we observed that H125cpr has a lower migratory capability than H125ct.

In the other hand, we observed that H125cpr has a bigger nucleus surface than H125ct. Besides, H125cpr presents more cells with cytoplasmic chromatin fragments (CCF) and a lower duplication rate than H125ct. These results indicated that H125cpr would have a more senescent/stem like phenotype than H125ct. So, we performed an oncosphere formation assay and we corroborated that H125cpr oncospheres showed higher growth rate than H125ct ones. Taking all together, we hypothesize that H125cpr has higher proportion cells with a phenotype more stem-like than H125ct. More experiments are needed to elucidate the mechanisms implied in the CDDP-resistance.

349. 437. **MACHINE LEARNING TOOLS IDENTIFY A PROGNOSTIC SIGNATURE IN PROSTATE CANCER THAT OUTPERFORMS CURRENT RISK PREDICTORS**

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In Argentina, prostate cancer (PCa) is the most common cancer in men. Based on histopathological characteristics, some PCa patients are grouped as intermediate risk, but they can vary significantly in the outcome of the disease. Our aim was to identify molecular biomarkers that could stratify the risk of progression in PCa patients, independent of histological grading and other clinicopathological variables. In this study, we performed protein extraction from formalin-fixed paraffin-embedded PCa and benign prostatic hyperplasia (BPH) tissue samples. Subsequently, tandem mass spectrometry (LC ESI-MS/MS) analysis enabled the identification of 109 proteins enriched in PCa compared to BPH samples. We then subjected these proteins to integrated bioinformatics analysis using publicly available transcriptomic databases (16 datasets, n = 2.954). For this purpose, we developed a sequential workflow including differential expression analysis, survival analysis, and identification of key predictors of PCa progression using machine learning techniques. Our results identified a gene expression signature capable of identifying patients at higher risk of progression of the disease, independent of the evaluated clinicopathological parameters, outperforming histological Gleason grading (GG) in the intermediate-risk patient subgroup. Further, we validated these findings in new PCa datasets that were not used during the training phase, underscoring the robustness of our methodology. In summary, leveraging experimental data, we established a workflow for transcriptomic data analysis and developed a gene signature outperforming GG risk prediction, even among patients previously classified as intermediate risk. This signature will enable more precise early diagnosis, facilitating personalized treatment, improving clinical outcomes, and reducing unnecessary interventions.

350. 466. **INHIBITION OF EPITHELIAL-MESENCHYMAL TRANSITION AND WNT CANONICAL PATHWAYS AS POSSIBLE MECHANISMS OF T2 ANTITUMOR ACTION ON TRIPLE NEGATIVE BREAST CANCER**

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In our previous investigations, we observed that T2 anti-tumor and anti-metastatic action was mediated by the induction of a more differentiated epithelial phenotype. In addition, T2 increased E-cadherin expression while simultaneously decreasing expression of α-SMA, suggesting that T2 treatment may encourage a reversal of the epithelial-mesenchymal transition (EMT) in 4T1 cells. To gain further insight into the regulation of genes during this process, Real-Time PCR was utilized to analyze the mRNA levels of three genes associated with EMT. Following a 24-hour T2 treatment, expression of Snail2 and Twist1 transcription factors was significantly decreased compared to the control group, at 57% ± 3% and 74% ± 5%, respectively ($p<0.05$). Vimentin mRNA, a classical mesenchymal marker, was also found to be markedly lower in treated 4T1 cells than the control group (20.0% ± 0.3%, $p<0.05$). Considering that Wnt canonical pathway is involved in the EMT of breast cancer cells, we also measured the mRNA levels of the Wnt transcriptional targets Axin2, CD44, and c-Myc, as well as the Wnt downstream effector TCF7. The expression of these genes was significantly downregulated in comparison to control cells after a 24-hour T2 treatment, according to our findings (13% ± 2%, 0.4% ± 1.6%, 25% ± 4%, and 18.0% ± 1.5%, respectively; $p<0.05$). Finally, to ascertain Wnt pathway involvement in the effects of T2, we utilized a specific reporter assay to evaluate the transcriptional activity of TCF/β-catenin. Preliminary results showed that a 24-hour T2 treatment decreased β-catenin transactivation induced by TGF-β compared to control in 4T1 cells (48% ± 14%). Based on our current and previous data, we arrive at the conclusion that T2 can reverse EMT in murine mammary tumor cells and consequently affect various phenotypic changes including growth, death, migration, along with metastatic and invasive capacities.

351. 503. **IN SEARCH OF A MOLECULE REPONSIBLE OF THE DIFFERENTIAL BIOELETRICITY OF LEFT/RIGHT-SIDED BREAST TUMORS**

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In a serendipitous discovery, our group found epigenetic differences between left and right-sided breast carcinomas (L-R). This unexpected data opened further research that allowed to deepen into the hypothesis that L-R tumors are not identical. Through diverse experimental approaches, we have been able to demonstrate that the epigenetic L-R differences are linked to differences in bioelectrical properties, proliferation rates, composition of stem cells, and patient survival rates. In this study, we aimed to explore an in-vitro approach by conditioning cultured cells with L-R mammary extracts, to evaluate the bioelectrical effect. For this, we used MDA-MB231 cultured cells conditioned with L-R bovine mammary extracts and measured bioelectric states with fluorescent probes (Mitotracker and Dibac) by flow cytometry. We were able to establish that the extracts from bovine mammary gland yielded similar effects as human glandular tissue: cells treated with L extracts exhibit a depolarized membrane potential as compared to the R-treated ones (One sample T-test, p=0,04). Expanding our insights, we separated the extracts in fractions obtained through sequential centrifugations and tested their bioelectrical effects on cells. We saw that the supernatant fraction derived from 50,000rpm centrifugations sustained the distinctive L-R effect of the whole extracts (Unpaired T-test, p=0,004), suggesting the involvement of a small, soluble, low-weight molecule, such as ions or neurotransmitters/hormones. Using a statistical bioinformatic tool R script-based, we conducted Differential Expression Analysis (DEA) on 722 TCGA L-R tumors and found a candidate signature of 7 GABA-related genes (Welch's t-test, p=0,002). Our findings suggest a potential window for differential treatment between L-R breast tumors, based on the repositioning of druggable ion channels.

352. 554. **FIRST EVIDENCE OF ANTITUMOR ACTIVITY OF DESMOPRESSIN ON INVASIVE SMALL INTESTINAL**

NEUROENDOCRINE MODEL

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Neuroendocrine tumors (NET) are a heterogenous neoplasms with a wide range of morphological and functional characteristics that can arise from any organ. In particular, small bowel tumors represent 45% of gastrointestinal neuroendocrine tumors (GI-NETs). Although they are rare tumors, their incidence has been increasing in recent years. For several years, we have studied the antitumor properties of desmopressin (dDAVP), a V2 receptor (AVPR2) agonist. dDAVP displays antiproliferative, antimetastatic, and antiangiogenic effects in numerous models such as colorectal cancer, breast cancer, and NE tumors such as small cell lung, among others. Considering that treatment and therapeutic resources are limited for the most aggressive GI-NETs and added to the lack of clinically relevant models for the study of NETs, this work aims to evaluate the effect of AVP analogs on key processes related to cancer progression on the intestinal neuroendocrine cell line, STC-1, as a model of GI-NET. In this study, AVPR2 expression in the STC-1 line was detected by immunofluorescence and confirmed by flow cytometry. Incubation of exponentially growing STC-1 cells with dDAVP (100 nM to 1,5 µM) resulted in a significant dose-dependent inhibition of proliferation with an IC50 of 0.95 µM (p< 0,0001). On the other hand, incubation with dDAVP for 7 days significantly inhibited the clonogenic growth of STC-1 showing an IC50 for dDAVP of 0.1µM. Sensitivity to chemotherapeutic agents as oxaliplatin and doxorubicin was measured by MTS assays. Also, we confirmed by flow cytometry that STC-1 express low levels of PDL-1. These results show the first evidence of the activity of the antitumor activity of dDAVP in a murine model of neuroendocrine-type invasive small intestine carcinoma. This research lays the groundwork for future explorations and approaches in the therapy of highly aggressive GI-NETs.

353. 613. **HEME OXYGENASE-1 IMPAIRS HORMONE-DEPENDENT BREAST CANCER CELL SURVIVAL THROUGH ITS ENZYME ACTIVITY**

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We have previously reported that induction of Heme Oxygenase-1 (HO-1), an enzyme that catalyzes heme degradation and releases iron, impairs breast cancer (BC) cell survival in both murine hormone-independent (LM3) and human triple-negative (MDA-MB-231) BC cell lines, most likely through ferroptosis induction. In this study, we aimed to evaluate the HO-1 modulation on hormone-dependent BC cell survival and to assess the involvement of HO-1 enzyme activity. To this end, we modulated HO-1 in T47D cell line by pharmacological induction (hemin, 36h) and by stable overexpression of wild-type HO1 (WT-HO1) or enzymatically inactive-H25AHO-1 (H25A). We studied cell viability (crystal violet), iron storage (Prussian blue), ROS levels (DFCA), lipid peroxidation (MDA accumulation) and the expression of ZIP14 iron importer (immunocytochemistry). We also carried out correlation studies between HO-1 mRNA levels and L-ferritin in BC subtypes (bioinformatics analysis). We found that hemin treatment and WT-HO1 overexpression decreased T47D cell viability ($p<0.01$ and $p<0.05$ respectively) and increased iron storage ($p<0.05$ in both), ROS levels ($p<0.01$ and $p<0.05$ respectively), MDA accumulation ($p<0.01$ in both) and ZIP14 expression. The treatment with an antioxidant (N-Acetylcysteine) and an iron chelator (deferoxamine) reversed the reduction of BC cell viability induced by hemin and by WT-HO-1 overexpression ($p<0.001$ and $p<0.05$ respectively). On the contrary, H25A cell viability was higher and the ROS levels and MDA accumulation were lower than in WT-HO1 cells ($p<0.05$). Bioinformatics studies confirmed a positive correlation between HO-1 mRNA and L-ferritin in BC subtypes (ER+, ER-, Basal-like, Normal-like, Luminal and HER2 enriched). In conclusion, HO-1 induction impairs cell viability in a hormone-dependent BC subtype through an increase in free iron accumulation, ROS production and lipid peroxidation, being the enzymatic activity of HO-1 necessary for its effect on cell viability.

354. 655. **IRRADIATED MDA-MB-231 BREAST TUMORS IN NUDE MICE. CAN HISTAMINE TREATMENT CONTROL GROWTH AND METASTASIS?**

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Ionizing radiation can promote epithelial-mesenchymal transition (EMT) activation and acquisition of cancer stem cell-like (CSC-like) properties in tumor cells that survive radiotherapy and thus facilitate metastasis. Previously we demonstrated in breast tumor cells the dual role of histamine on radio-induced EMT (favoring it at ≤ 1µM and hindering it at ≥10µM). Herein, we evaluated the *in vitro* histamine (Ha) effect on CSC-like enrichment and the link between CSC-like and EMT in MDA-MB-231 (MDA) breast cancer cells. Cells were treated with 1 or 20μM Ha and then γ-irradiated with a 2Gy dose (2Gy). After 5 days we assessed clonogenicity, mammosphere formation, and co-expression of CD44 (CSC-like marker) and Slug (EMT-associated transcription factor). In both non-irradiated and 2Gy cells, 1μM Ha did not modify clonogenicity vs controls, while 20μM Ha decreased it ($p<0.05$). 2Gy, 1μM Ha or their combination increased mammosphere formation, while 20μM Ha blocked the radio-induced rise ($p<0.001$). By indirect immunofluorescence we observed that 2Gy increased nuclear Slug and its co-localization with membrane CD44+ cells ($p<0.05$); 20μM Ha prevented radio-induced increments ($p<0.01$). *In vivo*, female nude mice were xenotransplanted with irradiated or non-irradiated MDA tumors and received or not histamine 5 mg/kg/day s.c. (HA) for 20 days. Tumor growth rate was similar in control and 2Gy tumors but lower in HA and 2Gy+HA ($p<0.05$), which also hindered vascularization. PCNA expression and mitosis paralleled these results. Intracellular TGFβ-1 (EMT promoter) was raised in HA, 2Gy and 2Gy+HA, but high vimentin expression (EMT marker) didn´t vary. Lung cellularity and metastatic foci were higher in 2Gy and 2Gy+HA mice ($p<0.05$). PCNA and vimentin-positive tumor cells in lungs were more numerous in HA, 2Gy and 2Gy+HA ($p<0.05$). Altogether, results show that even if HA may hinder control and 2Gy tumor growth it cannot prevent lung metastasis possibly due to a furthering effect on EMT.

355. 662. **HISTAMINE H_3 RECEPTOR ANTAGONISM, A POTENTIAL OPTION FOR LUNG CANCER TREATMENT?**

Paolo Laureta[1], Ignacio Ospital[1], Mónica A. Táquez Delgado[1], Melisa B. Nicoud[1], Michelle F. Corrêa[2], Gustavo A. Borges Fernandes[2], João P. S. Fernandes[2], Vanina A. Medina[1].
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Lung cancer is the leading cause of cancer-related deaths worldwide, accounting for the highest mortality rates among both men and women. The most common type of lung cancer is non-small cell car-

cinoma (NSCLC) and comprises squamous cell carcinoma and adenocarcinoma. The latter is the most common subtype and despite significant advances in therapeutics, the survival of most patients remains abysmal. It was previously reported that the expression levels of H_3R were significantly increased in NSCLC samples, and high levels of H_3R were associated with poor overall survival in NSCLC patients. The H_3R antagonist ciproxifan inhibited cell proliferation and epithelial to mesenchymal progression in lung adenocarcinoma cells. The aims of this work were to evaluate the expression of H_3R in lung cancer cell lines and to investigate the antitumoral properties of novel H_3R antagonists, 1-(2,3-dihydro-1-benzofuran-2-yl) methylpiperazines (LINS01 compounds). Cell viability, clonogenic proliferation, cell apoptosis (Annexin-V and TUNEL) and migration (wound-healing assay and transwell system) were assessed in human A549 adenocarcinona and H596 adenosquamous carcinoma cells. Results indicate that A549 and H596 cells show H_3R protein expression. Treatment of lung cancer cells with LINS01009, LINS01010, LINS01016, LINS01022 and LINS01023 compounds (0.001-50 µM) produced a significant concentration-dependent inhibition on cell growth, increasing cell apoptosis ($P<0.01$). The most potent responses were observed with LINS01016, LINS01022, LINS01023, showing a half-maximal inhibitory concentration (IC_{50}) of 7.9±1.2, 2.7±1.2 and 0.9±0.8 µM for A549 cells in the clonogenic assay. These compounds also produced cytotoxic effects on H596 cells, although to a lesser extent. These most potent compounds also exhibited the highest affinity constant at the H_3R. We conclude that H_3R could be a novel target for lung cancer treatment, offering therapeutic potentials for selective H_3R antagonists.

356. 668. **HISTAMINE H3 RECEPTOR ANTAGONISTS: THERAPEUTIC POTENTIAL AS ANTINEOPLASTIC AGENTS WITH THE ABILITY TO OVERCOME CHEMORESISTANCE IN TRIPLE NEGATIVE BREAST CANCER**

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Triple-negative breast cancer (TNBC) accounts for 10-15% of newly diagnosed cases of BC. It is recognized as the most aggressive subtype, carrying a particularly grim prognosis. Paclitaxel (PTX) is used as the standard of care. However, due to its limited solubility, secondary effects and the acquisition of chemoresistance, its application in the clinic has been challenged. Our earlier findings revealed the presence of the histamine H_3 receptor (H_3R) in human benign and malignant lesions, as well as in breast tissue derived cell lines. The aim of this work was to evaluate the expression of H_3R particularly in TNBC samples. In addition, we aimed to discover whether novel H_3R antagonists, LINS01022 and LINS01023, show antitumoral effects in murine 4T1 TNBC cells and if they could also potentiate PTX therapy, even in resistant cells (4T1R) *in vitro* and *in vivo* models. The H_3R expression was assessed in 50 TNBC samples by immunohistochemistry evidencing a higher H_3R expression in tumor samples when compared with peritumoral tissue. A high level of H_3R was associated with poor overall survival in TNBC patients. Results indicate that both LINS01022 and LINS01023 produced significant inhibition on cell proliferation and viability, inducing cell apoptosis in 4T1 and 4T1R cells, potentiating PTX-induced effects ($P<0.01$). Both compounds have potential for druggability estimated on ADMET calculation and toxicological profiles. The selectivity index (SI), the ratio of the IC_{50} against non-tumorigenic NMuMG and the TNBC cell lines, was more beneficial for LINS01022 with values of 2.7 in MDA-MB-231 and 2.8 in 4T1 cells. Therefore, LINS01022 was further evaluated in vivo. LINS01022 (20 mg/kg) reduced the tumor size and volume in 4T1 tumor-bearing mice, exhibiting a safe toxicological profile.

We conclude that the H_3R antagonists LINS01022 and LINS01023 are potent antineoplastic agents with the ability to overcome PTX resistance, showing promising application for TNBC treatment.

O5-ONCOLOGY

FRIDAY 17TH NOVEMBER 9:00 - 10:30

CHAIRS: MAURICIO MENACHO MÁRQUEZ
VIVIANA ROZADOS

357. 115. **PTHrP PROMOTES AN AGGRESSIVE BEHAVIOR OF CRC CELLS THROUGH ITS ACTION ON TUMOR AND STROMAL CELLS**

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The interaction of several cells and molecules in the tumor microenvironment (TME) promotes tumor-aggressive features. Previously we found that parathyroid hormone-related peptide (PTHrP), a cytokine from TME, promotes an aggressive phenotype in HCT116 colorectal cancer (CRC) cells and that conditioned medium from PTHrP-treated HMEC-1 endothelial stromal cells (CM) induces epithelial to mesenchymal transition (EMT) in CRC cells. The aim of this work is to further investigate the PTHrP effects on CRC and its TME. HCT116 cells were treated with the CM. Western blot (WB) analysis showed that CM modulates the expression and activation of Met, a receptor related to CRC ($p<0,01$), as well as cancer stem cell (CSC) features ($p<0.01$). As we previously found that in CRC cells PTHrP induces chemoresistance to CPT11 through Met signaling, we decided to analyze the potential role of the CM-Met axis in CRC cells drug resistance. Cells were pre-incubated with a Met inhibitor and subsequently treated with CM. Viability assays revealed that the increment in cell number due to CM exposure ($p<0.05$), is reversed by Met inhibition. Furthermore, CM induced CPT-11 resistance through Met pathway ($p<0.01$). On the other hand, WB analysis revealed increased expression of transforming growth factor beta 1 (TGFβ1) in PTHrP-treated HMEC1 cells. Given this result, we then analyzed TGFβ1 role from CM on tumor cells using an anti-TGFβ1 antibody. Viability assay shows that the neutralization of TGFβ1 protein levels reverses CM effect on the proliferation but not on CPT11 resistance. *In silico* analysis revealed differentially expressed genes among CRC and healthy stroma tissues associated with TGFβ signaling and Met activation ($p<0.05$). On CRC human samples, we observed a positive correlation ($p<0,001$) among the gene expression of markers studied herein. These findings suggest that PTHrP promotes CRC cells aggressive behavior through its action not only on tumor cells but also on stromal cells.

358. 125. **RSUME ACTS ON PRIMARY CILIA IN RENAL CARCINOMA DEVELOPMENT**

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RSUME, the product of the RWD domain containing 3 (RWDD3) gene, is involved in tumor angiogenesis, and its expression has been reported in organs prone to develop von Hippel-Lindau (VHL) syndrome tumors. RSUME acts as a negative regulator of VHL protein function in normoxia promoting Hypoxia-inducible factor alpha (HIF-α) stabilization. VHL ubiquitinates Aurora kinase A (AURKA) to control primary cilia formation, and VHL loss produces primary cilia disassembly and, in turn, renal cancer development. To deepen the mechanisms of RSUME action in clear cell Renal Cell Carcinoma (ccRCC), we first performed a Gene set enrichment analysis (GSEA) to compare RSUME high and low expression samples in five independent datasets with ccRCC tumor transcriptomic data. ccRCC tumor samples were obtained from The Cancer Genome Atlas (TCGA) and Gene Expression Omnibus (GEO) databases. We observed that cilium related pathways are enriched in tumors with a higher expression of RSUME (NES > 2; $p < 0.001$; FDR < 0.02), indicating that primary cilium could be a target of RSUME action on ccRCC. To validate RSUME molecular action in cilium pathway we used 10 genetically modified RCC cell lines generated in our laboratory, lacking endogenous VHL (RCC-786-O VHL-/-) expressing wild type VHL or different VHL mutants (VHL-Tyr112His, -Arg167Gln, -Leu188Val), combined with shRSUME or its scrambled control. By WB, we tested in these cell lines whether RSUME modulates AURKA levels. We demonstrate that in VHL absence or VHL *wild type* or VHL mutants' presence, RSUME silencing diminishes AURKA protein levels. To evaluate if RSUME and AURKA interact and participates in the same protein complex we performed immunoprecipitation assays, which show that RSUME and AURKA interact in a protein complex. RSUME regulation and interaction with AURKA may contribute to its action on ccRCC kidney disease development.

359. 192. **RUNX2 INHIBITION REDUCES THE PROLIFERATION AND THE MIGRATION OF HUMAN BREAST CANCER MODELS**

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We have previously shown that RUNX2 overexpression leads to an increased FGFR2 expression. Inhibitors against FGFR are used in breast cancer patients with endocrine resistance, but several patients are refractory to these inhibitors as well. Also, we have shown that RUNX2 overexpression generates endocrine and FGFR-inhibitor resistance in vivo. This work aimed to evaluate the effect of a RUNX2 inhibitor (CADD522: CAD) in the proliferation and migration of T47D or MCF7 control (C), RUNX2 overexpressing (RUNX2), and FGFR2 constitutively activated (R2CA) stably transfected cells. The results showed that CAD (10-50uM) diminished the proliferation of all cell lines in complete-supplemented media (C, RUNX2, R2CA vs CAD $p<0.0001$). Then, we studied if CAD (10uM) could revert the FGF2-induced proliferation in a hormone-depleted culture setup. RUNX2 and R2CA cells showed a higher proliferation rate in response to FGF2 (50ug/ml) as compared to control cells (C vs FGF2 $p<0.05$, RUNX2 and R2CA vs FGF2 $p<0.0001$). Only in T47D-C cells, CAD reversed FGF2-stimulation (C-FGF2 vs FGF2+CAD $p<0.05$), No reversion was detected in T47D-RUNX2 and -R2CA, maybe because CAD´s concentration was not enough to counteract the effect of endogenous RUNX2. Then, we analyzed if CAD could affect cell migration using wound healing assays with an automatized live cell analysis system. The results showed that T47D-RUNX2 and -R2CA cells migrate more than control cells (C vs -RUNX2, C vs -R2CA $p<0.05$). Also, CAD diminished T47D-R2CA and MCF7-R2CA migration as compared to their respective controls (R2CA-C vs R2CA-CAD 10uM $p<0.05$). In all RUNX2 cells, migration was not affected by the inhibitor. To conclude, our results suggest that CADD522 inhibitor affects cell proliferation and migration and could be used combined with standard hormone therapy in luminal breast cancer models. High RUNX2 expression levels could predict therapy outcomes and could correlate to a more aggressive phenotype.

360. 240. **STARVATION INDUCES SMALL EXTRACELLULAR VESICLES RELEASE IN PANCREATIC CANCER CELLS WITH THE PROPRIETY OF AUTOPHAGY INDUCTION EFFECT**

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Pancreatic ductal adenocarcinoma (PDAC) is a highly aggressive disease with a survival rate to 5 years of less than 5%. Autophagy, a cellular degradative and recycling process, plays an important role in the hypoxic and low nutrient tumor microenvironment of PDAC cells. Extracellular vesicles (EVs) are tiny vesicular structures released by all cell types with implications in intercellular communication. The aim of this work was to describe the tumor derived EVs response to starvation. Firstly, we set up the quantitative immunodetection of EVs by means of image cytometry. Additionally, we developed the quantitative total EVs detection using a red fluorescent dye. Then, the PDAC cell line Panc-1 was submitted to starvation for 30 and 60 min. In the conditioned medium, we detected a significant increase of released EVs for both times ($p<0,05$ and $p<0,001$ respectively). To verify the specificity of the response, we repeated the 1h starvation followed by 1h and 2h of cell recovery where we detected a normalization in the amount of released EVs. In the same conditions, we isolated EVs by ultracentrifugation and found that the fraction of small EVs is responsible for the starvation response ($p<0,001$). Moreover, by immunofluorescence, the small EVs increase is a consequence of CD81+ vesicles ($p<0,001$) with a stable proportional amount of CD63/CD81 double positive vesicles. Interestingly, conditioned medium from Panc-1 starved cells possess the capability of autophagy induction in the same cell line transfected with LC3-RFP. Finally, data suggest that PDAC cells respond to starvation with specific CD81+ small EVs with the effector capability of autophagy pathway induction.

361. 318. **S6 AND RB PROTEINS AND ASSOCIATED miRNAs AS BIOMARKERS OF BREAST CANCER PROGRESSION**

Micaela Vivanco[1], Karen Graña[1], María Cecilia Perrone[1], Andrea Werbach[1], Florencia Laura Cascardo[1], Natalí Salgueiro[1], Berenice Freile[2], Federico Waisberg[2], Alexis Ostinelli[2], Sergio Rivero[2], Adriana De Siervi[1] and Virginia Novaro[1].
Affiliations: 1. Instituto de Biología y Medicina Experimental (IBYME-CONICET) 2. Instituto Alexander Fleming (IAF) Buenos Aires, Argentina

Our group has focused on the study of alterations associated with the PI3K/AKT/mTOR and cyclin D1/CD4/6/Rb pathways, which are related to breast tumor growth and progression. They are also the target of selective kinase inhibitors. CDK4/6 inhibitors (palbociclib, abemaciclib and ribociclib) as well as PI3K (alpelisib) or mTOR (everolimus) inhibitors, have recently entered clinical practice. We have focused on the search for biomarkers that can be used to select the patients who will benefit from selective therapies. The determination of PIK3CAmut is not always feasible to perform in clinical practice, or even indicative of pathway activation. We first analyzed by immunohistochemistry 36 paired tumor-node samples from the same patient and 142 invasive carcinomas with a 5-year follow-up, 39 of which suffered disease relapse in that period. We observed an increase ($p<0.05$) in the expression of the ribosomal protein pS6 and the retinoblastoma protein pRb both in the tumor and lymph nodes of the patients who presented a relapse of the disease prior to 2 years. We next determined in plasma the presence of specific miRNAs related to the regulation of the PI3K/AKT/mTOR and cyclin D1/

CD4/6/Rb pathways. We evaluated miRNAs (i.e. miR-126, miR16, miR34a) by stem loop RT-qPCR, in tissue and plasma from patients starting selective treatment with kinase inhibitors. The paired samples were obtained at the diagnosis of advanced breast carcinoma and after starting the selective treatment, until the second relapse (average response time to CDK4/6 and PI3K inhibitors ~2 years).
In summary, we identified in an early and minimally invasive way, the patients who could respond better to selective CDK4/6 inhibitors or even to PI3K or mTOR inhibitors, according to the pS6 and pRb tumor profile, as well as associated circulating microRNAs. Through this predictive study, we hope to improve follow-up and optimize treatment strategies for patients with advanced luminal-type mammary carcinomas.

P4-ONCOLOGY

FRIDAY 17TH NOVEMBER 9:00 - 10:30
CHAIRS: FLORENCIA GOTTARDO
GABRIEL FISZMAN
LUCIANO VELLÓN

362. 34. **SIMVASTATIN-LOADED BIOACTIVE LIPID NANOPARTICLES AS A DRUG REPURPOSING STRATEGY AGAINST LUNG CANCER**

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In recent years, researchers have explored new uses for affordable medications approved for other diseases, a strategy called "drug repurposing". Statins (lipid-lowering drugs) exhibited anticancer effects in several cancer cell types; however, their therapeutic use has faced limitations related to solubility and toxic side effects. Nanotechnology has emerged as a powerful tool to overcome these drawbacks. Here, we developed Simvastatin (SV)-loaded lipid nanoparticles (LNP) to deliver and enhance SV activity against lung cancer cells. Two types of LNPs, in terms of composition, were produced by the ultrasonication method: LNP containing (NLC) or not (SLN) liquid lipids (LL) in the core. The morphology, size, z-potential (z-pot), and polydispersity (PI) were measured by DLS and TEM. Standard triglyceride (Crodamol®) or bioactive terpenes (linalool -LN-, geraniol -GN-, cineole -CN-) were used as LL. All LNPs exhibited a spherical shape with sizes from 115 to 165 nm in a narrow distribution (PI<0.3), along with a negative z-pot (-6 to -15 mV). The encapsulation of SV at 10 μM reduced A549 lung cell viability from 91.7% (free SV) to 54.0% (SLN/SV), 83.1% (NLC/SV), 56.5% (NLC/CN/SV), 37.1% (NLC/LM/SV), and 24.2% (NLC/LN/SV), respectively ($p<0.001$). NLC/LN/SV was selected for further research. The encapsulation efficiency (EE) and release profile of SV from NLC/LN/SV was measured by HPLC. The EE of SV exceeded 95%, and it was released in a controlled manner over 24 h. In contrast to SLN/SV, NLC/LN/SV remained highly stable for up to 4 months (4ºC, darkness). A549 cell death (CD) and cell migration (CM) were evaluated. CD increased from 9.0% (free SV) and 2.5% (free LN) to 34.9% for NLC/LN/SV ($p<0.001$). Also, SV encapsulation increased CM inhibition ($p<0.05$). Moreover, NLC/LN/SV were non-toxic in normal L929 fibroblasts and not hemotoxic against red blood cells. Our results support NLC/LN/SV as a promising and enhancer carrier system for SV repurposing against lung cancer.

363. 44. **NITRIC OXIDE INHIBITION CONTROLS LOCAL AND DISTANT INVASIVE BLADDER TUMOR DISEASE, REDUCING CHRONIC INFLAMMATION AND INCREASING THE INFILTRATION OF CYTOTOXIC IMMUNE CELLS**

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Bladder cancer (BC) is classified as non-invasive (NMI) and muscle invasive (MI) tumors. Inducible nitric oxide synthase (iNOS) is expressed in more than 50% of patients with BC and produces high levels of nitric oxide (NO). iNOS is a poor prognosis marker in BC, associated with invasion and early recurrence. Previously, using a murine BC model that express iNOS we demonstrated that NO inhibition with 0.5 g/L of L-NAME reduced subcutaneous tumor growth. Objective: to evaluate bladder tumor growth, immune cell profile and development of spontaneous metastasis in a MI murine BC model that express iNOS. Methods: Bladder tumor weight and lung incidence of metastasis were measured in tumor bearing mice (TBM) orthotopically inoculated with MB49-I (MI BC cells) and treated with L-NAME (0.5 and 1 g/L in drinking water). Bladder $CD8^+$, NK and Treg were evaluated by flow cytometry and iNOS and TGF-β expression by qPCR. Results: Treatment with L-NAME at doses of 0.5 and 1 g/L similarly reduced the orthotopic tumor growth ($p<0.01$), while only 1 g/L reduced spontaneous lung metastasis incidence ($p<0.05$). To analyze the chronic inflammation microenvironment, iNOS and TGF-β expression were measured in the bladder. MB49-I tumors constitutively expressed iNOS and TGF-β, whereas 1 g/L L-NAME reduced both expressions. Related to immune tumor microenvironment, only L-NAME 1g/L increased $CD8^+$ ($p<0.05$), NK cells ($p<0.01$) and $CD8^+$/Treg ratio ($p<0.01$) in the bladder. Our results show that 1 g/L of L-NAME not only controls local tumor growth in a bladder invasive stage, but also reduces distant disease. This effect is probably due to a reduction of chronic intratumoral inflammation mediated by iNOS and TGF- β that favors infiltration of cytotoxic cells into the tumor. These results, in iNOS+ bladder tumors are encouraging and should be future tested in combination with immunotherapy currently administered to patients.

364. 77. **PROGNOSTIC AND PREDICTIVE BIOMARKER VALIDATION FOR PERSONALIZED CARE IN A LATIN AMERICAN BREAST CANCER COHORT**

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Purpose: Several guidelines have been published for the appropriate use of different biomarkers and molecular signatures to determine the risk of recurrence and treatment decisions in patients with HR+HER2- breast cancer. However, data is still lacking for their usefulness in Latin American (LA) patients. Our aim was to evaluate the prognostic and predictive performance of different risk classifiers found in guidelines in a LA cohort. Patients and Methods: The Molecular Profile of Breast Cancer Study (MPBCS) is a LA breast cancer cohort study with 5-year follow-up. HR+HER2- stage I-IIIA patients (n = 633) stratified by node status (N0 and N+), that received adjuvant hormone-therapy, chemotherapy and their combination were considered for the analyses. Time-dependent ROC-AUC, univariate and multivariate Cox proportional hazards regression (CPHR) models were used to evaluate parameters associated with prognostic performance. Results: Most transcriptomic-based tests presented better prognostic performances (C-index 0.68-0.74 and tdROC-AUC 0.61-0.70 for N0 patients) than standard-risk classifiers (KI67 and NPI; C-index 0.55-0.60 and tdROC-AUC 0.56-0.57 for N0 patients). Between 42%-58% of N0 low-risk patients and 39%-61% of N+ low-risk patients were treated with adjuvant chemotherapy in addition to hormone-therapy. However, recurrence (RS), Endopredict (EP), Endopredict Clinical (EPClin), ROR-S and ROR-PC scores did not show benefit from chemotherapy for any risk group (interaction p-values range: 0.532-0.954) when adjusted CPHR models were applied. Conclusion: Transcriptomic-based signatures other than GENE70 discriminate better low- from high-risk of recurrence than methods based on Ki67% or clinical-based algorithms commonly used in LA. Tests based on these algorithms represent a valid tool for clinical decision making in LA patients with N0 breast cancer. Caution must be used when extrapolating absolute chemotherapy benefit from risk analysis performed in non-LA populations.

365. 93. **REGULATION OF PROLIFERATION, CELL CYCLE AND APOPTOSIS IN THE DEVELOPMENT OF RENAL TUMORS. ROLE OF PERIRENAL ADIPOSE TISSUE**

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The development of a tumor implies a deregulation of the cell cycle and apoptotic processes, and requires the interaction of tumor cells with the stromal environment. Adipose tissue is one of the most abundant stromal types. We recently demonstrated that the conditioned media (CMs) of human renal adipose tissue from patients with renal tumors (hRAT) regulates the behavior of tumor and non-tumor renal epithelial cells, differently from normal adipose tissue (hRAN). In this work, we evaluated changes in the expression of proliferation, cell cycle and apoptosis regulatory factors in: a) tumor (786-O, ACHN) and non-tumor (HK-2) human renal epithelial cell lines incubated with hRAT- or hRAN-CMs; and in b) fragments of human kidney tumors in different stages of development (different Fuhrman grade), by Western blot and immunohistochemistry. We observed a significant increase in the expression of cyclin D1 and pRB/RB in the three cell lines incubated with hRAT-CMs *vs.* hRAN- or control-CMs ($p<0.05$), without significant changes in p21 or PCNA expression. In addition, 786-O cells incubated with hRAT-CMs showed increased survivin expression relative to the same cells incubated with hRAN- or control-CMs ($p<0.05$). However, no clear regulation of hRAT-CMs on cell apoptosis was observed. In human kidney tumor fragments, we observed a significant decrease in the Bax/Bcl2 ratio and caspase 3 expression as the Fuhrman grade increased ($p<0.05$). In addition, we observed a higher expression of Ki67 and survivin in Fuhrman grade 2 and 3 tumors compared to grade 1 and 4 tumors ($p<0.05$). In conclusion, human renal peritumoral adipose tissue secretes factors that could regulate the cell cycle of tumor epithelial cells, favoring renal tumor development. On the other hand, renal tumors present a decreased apoptotic activity the more developed the tumor is; however, the surrounding adipose tissue does not seem to be important in this apoptotic regulation.

366. 141. **SHIGA TOXIN FROM ENTEROHEMORRHAGIC *E. COLI* AS A NOVEL AGENT AGAINST TRIPLE NEGATIVE BREAST CANCER**

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Shiga toxin (Stx) is responsible for producing the hemolytic-uremic syndrome. There are two types of Stxs, Stx1 and Stx2 which are about 60% homologous. Classically, the Stx cytotoxic effect is mediated by its receptor globotriaosylceramide (Gb3). Gb3 has a restricted profile of tissue expression in normal human cells and it is overexpressed in many neoplastic cells, including breast tumors. Breast cancer is the most common malignancy and the leading cause of cancer-related death in women worldwide. Triple negative breast cancer (TNBC) is the most aggressive and difficult to treat from all breast tumors. Thus, the aim of this study was to determine the potential of Stx as a novel cytotoxic agent in the TNBC human cell line MDA-MB-231. For this purpose, cells were treated with Stx1, Stx2 or the antibody anti-Gb3. Moreover, the non-tumorigenic mammary epithelial cell line NMuMG and VERO cells (highly sensitive to Stx kidney epithelial cells extracted from the African green monkey) were used as a negative and a positive control of Gb3 expression. Gb3 content and Stx uptake were observed by immunofluorescence in MDA-MB-231 and VERO cells. MTT results showed that 10 ng/ml of Stx1 and Stx2 reduced respectively 50% and 40% the cell viability after 48h ($p<0.001$), and anti-Gb3 reduced 10% the cell viability after 48h ($p<0.05$). Moreover, 10 ng/ml of these toxins significantly increased the number of cells with karyorrhexis ($p<0.0001$) and autophagy ($p<0.001$), reduced the mitosis rate ($p<0.001$), the incorporation of BrdU ($p<0.0004$) and the migration rate ($p<0.001$). Treatment with PPMP (an inhibitor of Gb3 synthesis) significantly reverted all the observed effects produced by Stx1, Stx2 and anti-Gb3. MDA-MB-231 cells are susceptible to Stx1, Stx2 and anti-Gb3, suggesting that Stx could be used as an antineoplastic agent in TNBC. However, further studies in different cell lines and in vivo models are needed to confirm these findings.

367. 146. **POTENTIAL OF SUPERPARAMAGNETIC IRON OXIDE NANOPARTICLES (SPIONs) AS RADIOSENSITIZER ON CANCER THYROID CELLS**

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Thyroid carcinomas are generally well-behaved malignancies that respond to standard treatments. However, a subset of thyroid carcinomas of follicular epithelial origin (well-differentiated, WD; poorly differentiated, PD; or anaplastic, A) is highly aggressive. External beam radiotherapy could be a treatment option for high risk patients. Superparamagnetic iron oxide nanoparticles (SPIONs) have been used in cancer diagnosis and therapy. SPIONs can increase reactive oxygen species (ROS). This characteristic could be combined with radiotherapy to optimize the clinical outcome. The aim was to study the radiosensitizing properties of SPIONs in thyroid cells. Methods: SPIONs were synthesized and stabilized by methyl- poly(ethylene glycol) (mPEG). Thyroid cancer (WD: TPC-1, PD: WRO and A: 8505c) cells were incubated with different concentrations of mPEG-coated SPIONs. Cell viability was measured by MTT method. Intracellular SPIONs content by measuring the Fe concentration *per* cell was performed by ICP-AES at 2, 4 and 24 hours. Intracellular ROS levels using the fluorescent dye 2', 7'- dichlorofluorescein-diacetate (DCFH-DA) and survival fraction at 2 Gy (SF2) were measured. Results: 24 hours incubation with SPIONs did not affect cell viability (0- 100 μg/ml). Intracellular iron content significantly increased at 2, 4 and 24 hours in cells incubated with SPIONs ($p<0.01$ for 50 and 100 *vs.* 25 μg/ml at 24 hours). Intracellular ROS levels were higher in cells incubated with SPIONs (50 and 100 μg/ml vs. control for TPC-1 and 8505c cells). Plating efficiency diminished in all cells incubated with 100 μg/ml SPIONs ($p<0.05$). SF2 values decreased in cells treated with 50 μg/ml SPIONs and irradiated ($p<0.05$ for TPC-1, WRO and 8505c). Conclusions: SPIONs treatment increased intracellular iron concentration, ROS levels, and decreased SF2 values combined with radiation, showing promising radiosensitizing properties.

368. 211. **OVEREXPRESSION OF HPV-16 E6/E7 PRODUCES DYSPLASTIC EPITHELIAL CHANGES IN THE ORAL TONGUE IN A CONDITIONAL TRANSGENIC MOUSE MODEL**

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Oral squamous-cell carcinomas (OSCC) is a heterogeneous group of tumors involving distinct anatomical sites with varying etiological factors including smoking as well as infection with high risk human papilloma viruses (HPV-16 and 18). Incidences of HPV-associated anal SCC and OSCC have raised, particularly oropharyngeal SCC. Previously, we have established and validated specific HPV-16 E6/E7 genetically engineered mouse model to study HPV related carcinogenesis by crossing the driver line K14CreER^{TAM} with a Rosa26-rtTA-IRES-EGFP-rtTA^{flox}/Tet-E6/E7 bi-transgenic line (E6/E7 mice). In this model expression is achieved, in a Cre-dependent manner, after doxycycline (DOX) administration. Here, we study the oral phenotype in order to characterize the impact of E6/E7 overexpression in the oral mucosa. Up to 2 months after induction of the system, E6/E7 mice (N=8) and their control littermates (N=8) did not present any overt phenotype and had no significant differences in survival time. However, histological evaluation revealed hyperplasia, acanthosis and increased proliferative activity in the basal layers of the tongue epithelium (80%, 4/5) and one case of mild dysplasia (20%, 1/5). DOX withdrawal reverts the above mentioned phenotype (100%, 3/3). After 4 months of E6/E7 overexpression the transgenic tongue developed dysplastic changes (100%, 5/5) which ranged from mild to moderate. The dysplastic areas showed increased Keratin 14 expression. Since mTOR activation is a widespread feature of OSCC we studied pS6 expression, downstream target of the PI3K/Akt/mTOR pathway. E6/E7 tongues (2 months) did not present differences versus control however dysplastic areas of tongues from 4 months showed increased expression of pS6. We conclude that lingual epithelium was susceptible to a prolonged overexpression of E6/E7. These results warrant further analysis in order to understand the possible role of PI3K/Akt/mTOR pathway in the development of these E6/E7 premalignant lesions.

369. 229. **ROLE OF ENDOTHELIAL CELLS AND BMP7 IN CISPLATIN CHEMORESISTANCE OF CERVICAL CANCER CELLS**

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Cisplatin continues to be the main cytostatic used in the treatment of cervical cancer (CC), despite its use, a significant population develops chemoresistance both at the beginning and during the disease. The interaction between tumor cells and their tumor microenvironment, through soluble factors, may be related to said resistance. We previously observed that endothelial cells with characteristics related to those found in the tumor microenvironment and bone morphogenetic protein 7 (BMP7) acting on these cells are involved in processes associated with cell plasticity in CC. This work aimed to evaluate the effect of these endothelial cells and BMP7 on chemoresistance to cisplatin in CC. First, the endothelial HMEC-1 cells were treated for 24 hours with conditioned media from CC-derived HeLa cells (TCM) to acquire the characteristics of endothelial cells found in the niche of the tumor microenvironment. Then, the medium was renewed to obtain a new conditioned medium (ECM-T) and study the release of soluble factors. The number of viable HeLa cells was counted by trypan blue dye exclusion test to evaluate the effects of cisplatin with or without ECM-T. We observed that these ECM-T attenuated the cytotoxic effect of cisplatin in HeLa cells. The same result was obtained using the neutral red technique. Then, we studied if BMP7 released by tumor cells promotes characteristics similar to those of the tumor niche in endothelial cells. Using qRT-PCR, we observed that exposure of HMEC-1 cells with TCM from HeLa cells increases the mRNA levels of endoglin, a protein overexpressed in tumor endothelial cells. This increase was partially reversed by pre-incubating TCM with an antibody against BMP7. The neutralizing of BMP7 with an antibody also attenuated the response of HeLa cells to EMC-T in the presence of cisplatin. These results suggest an indirect role of BMP7 in the chemoresistance to cisplatin in CC by acting on endothelial cells.

370. 246. **ROLE OF MIRNAS IN DOXORUBICIN RESISTANT TRIPLE NEGATIVE BREAST CANCER**

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Breast Cancer (BCa) is the most prevalent global malignancy and one of the leading causes of cancer deaths. Despite the novel therapies, resistance to chemotherapeutic drugs is a major challenge for effective therapy. Doxorubicin (DOXO) is a drug vastly used for BCa treatment which makes it an interesting target for the study of drug resistance. MiRNAs are short non-coding RNAs that act as post-transcriptional regulators of gene expression. Our aim was to identify a panel of miRNAs as possible biomarkers for DOXO resistance in triple negative breast cancer (TNBC) cells. We have selected the murine 4T1 cell line (IC50= 0.33+ 0.08) to generate a DOXO-resistant variant cell line (4T1ADR *IC50=1.37+-0.11)* by chronic exposition to sublethal increasing doses of DOXO for 8 months. We characterized the resistant cell lines by clonogenic and cell cycle analysis. Additionally, Balb-c mice were randomly divided into two groups (n=16 per group): mice injected with 4T1 WT or 4T1ADR. When the tumors were palpable, half of each group received weekly DOXO and the rest received a vehicle. Tumors were measured during the experiment to assess response to DOXO and miRNAs expression was measured in plasma and tumor. Tumor size was augmented in 4T1ADR compared to control. Also, tumor size was diminished by DOXO treatment only in 4T1 WT. miR-191-5p expression was diminished in 4T1ADR tumors compared to 4T1WT

and DOXO treatment decreased miR-191-5p only in 4T1WT tumors. These results suggest an important role for miR-191-5p in DOXO resistance mechanisms in mice. Furthermore, we measured miRNAs expression at tumor samples from 12 BCa patients from pre- and post-neoadjuvant treatment. We found that 16-5p was significantly increased in post- compared to pre-neoadjuvant treatment. Further research is needed to understand the mechanisms and the role of miRNAs in drug resistance in BCa in order to elucidate effective biomarkers for predicting response and potential therapeutic targets.

371. 300. **RUNX1 TRANSCRIPTIONAL ACTIVITY FAVORS A FINGERPRINT OF DRUG RESISTANCE IN TNBC CELL LINES**

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Triple negative breast cancer (TNBC) is associated with epithelial-mesenchymal transition (EMT) and an enrichment in cancer stem cells (CSC) which are both involved in tumor chemoresistance. Our group has shown that RUNX1 is implicated in the aggressiveness of this breast cancer subtype by promoting cell migration and regulating tumor gene expression, such as the oncogene RSPO3, the tumor suppressor gene GJA1 and the EMT regulator gene SOX4. Moreover, RUNX1 protein expression in TNBC correlates with poor patient prognosis. Our aim was to evaluate RUNX1 relevance during drug treatment in human TNBC. Here we show that RUNX1 mRNA is significantly upregulated in doxorubicin (Doxo)- and paclitaxel (Px)-treated TNBC MDA-MB-231 and -468 cell lines (*p* values<0,02). Using a RUNX1 transcriptional activity commercial inhibitor (AI-10-104) in both cell lines we can see a decrease in cell viability and an increase in apoptosis. Interestingly, we observe that this loss of RUNX1 transcriptional activity significantly enhances Doxo and Px toxicity in TNBC cell lines (*p* values <0,0001) measuring both cell viability and apoptosis. In addition, we found that mRNA expression of *ABCG2* and *ABCC1* transporters are reduced in TNBC cell lines treated with AI-10-104, accompanied by an increase in the intracellular accumulation of Doxo. Furthermore, in a forced suspension cell model (which promotes a CSC phenotype), RUNX1 expression is increased compared to the adhered cell population, and the inhibition of RUNX1 transcriptional activity decreases *OCT4*, *ABCC1* and *ALDH1* expression in the suspended cell population. Finally, RUNX1 inhibition in the MDA-MB-231 cell line prevents mammosphere formation capacity. Also, mammospheres already established and then treated simultaneously with Doxo and AI-10-104 show a significant reduction in their number compared with Doxo alone. Therefore, our data strongly suggests that RUNX1 may be involved in the generation of TNBC chemoresistance.

372. 307. **STUDIES OF CELLULAR MECHANISMS INDUCED BY BNCT THAT AFFECT ITS RADIOSENSITIVITY**

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Introduction: BNCT is a radiotherapeutic modality for the treatment of tumors based on the nuclear reaction ^{10}B (n, ^{4}He)^{7}Li. In our laboratory we have previously described different molecular mechanisms induced by BNCT such as the DNA damage response (DDR) and the TGF beta/Smad independent pathway that are activated to maintain genomic hemostasis. Nuclear sirtuins (Sirt) 1, 6, and 7 have been described to be directly involved in the maintenance of genome integrity. The objective of this study was to evaluate the radiosensitivity to BNCT using a TGF beta receptor inhibitor**.** Materials and methods**:** Cells from the human colon cancer line (HT29) were seeded in 60 cm^3 or 96-well plates and divided into the following 5 groups: Control; BNCT; BNCT(Ly); NCT; NCT (Ly). LY2109761 was added at a concentration of 10 μmol/L for 2 h. The plates were irradiated with the thermal neutron beam from the nuclear reactor (RA3) (neutron flux=1.10^{10} n/cm^2 sec-1) at different times to obtain total physical doses between 0 and 5 Gy. Results: The study of cell viability by MTT showed a decrease in survival at 10 days as a function of the total absorbed physical dose for all treatments, being significantly higher for the groups treated with the inhibitor (Ly) ($p<0.01$). On the other hand, qPCR studies showed an increase in the expression of rTGF-B1 and Smad 7 for the BNCT group and a decrease in their expression for the BNCT (Ly) group ($p<0.05$). This same expression pattern was found for Sirts 6 and 7. At 24 h, less cell activation (Ki67+) was found for the groups with the inhibitor. **Conclusions:** The addition of the Ly compound could inhibit the TGFbeta/Smad pathway and Sirts 6 and 7, causing decreased survival and cell proliferation, pointing out its potential use as a BNCT radiosensitizer.

373. 373. **NORCANTHARIDINE TREATMENT INHIBITS TRIPLE NEGATIVE BREAST CANCER PROGRESSION**

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Triple negative breast cancer (TNBC) is an aggressive subtype characterized by the absence of estrogen and progesterone receptors, as well as HER2 overexpression. Due the lack of specific targeted therapies, there is a clear imperative to explore new therapeutic strategies. Norcantharidin (NCTD), a promising natural compound, has previously shown antitumor effects against lung and liver malignancies. However, its impact on TNBC remains unknown. Therefore, our work aimed to investigated the potential therapeutic implications of NCTD in TNBC. Using human (HS578T) and murine (4T1) TNBC cell lines, we observed a significant antiproliferative effect of NCTD with IC50 values of 56 µM and 35 µM respectively, as determined by the MTS assay. Moreover, fluorescence microscopy (acridine orange/BrEt staining), flow cytometry (Annexin V/IP staining) and Western blot analysis (modulation of cleaved caspase 3 and Parp levels) revealed apoptosis induction. Additionally, NCTD reduced adhesive and migratory capacities in both cell lines, along with a notable decrease in MMP-9 secreted activity ($p<0.05$, Anova test). The study of the molecular mechanisms underlying these biological effects revealed that NCTD treatment inhibits ERK/MAPK and AKT signaling pathways associated with cell proliferation and survival respectively (Western blot). In vivo assays using BALB/c mice further supported our findings. Systemic administration of NCTD (2.5 and 3.75 mg/kg) significantly reduced both tumor size and local recurrence ($p<0.001$and $p<0.01$ respectively). However, pretreatment of 4T1 cells with NCTD resulted in an increased number of experimental lung metastatic nodes. In conclusion, our study highlights the significant antitumor activity of NCTD in TNBC, offering promising prospects for its application as a therapeutic option. However, further research is necessary to optimize NCTD's efficacy, explore combination therapies, and fully elucidate the molecular mechanisms involved in its action.

374. 563. **OVERCOMING COLORECTAL CANCER RESISTANCE TO 5-FLUOROURACIL BY TARGETING RAC1**

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Colorectal cancer (CRC) is the third most commonly diagnosed type of cancer worldwide. 5-fluorouracil (5-FU) is a chemotherapy

drug used in CRC treatment; however, half of CRCs are resistant to 5-FU-based therapies. Rac1 is a key member of the Rho GTPases family. Rac1 modulates cell adhesion and movement, and is highly expressed in tumors. Increasingly studies are reporting the role of Rac1 as a potential target for tumor therapy. The aim of this work was to evaluate the impact of Rac1 in CRC resistance to therapy. In previous work, we identified genes and pathways associated with recurrence after 5-FU-based therapies suggesting that Rac1 inhibition could be of benefit to overcome resistance. As approximately 30–40% of CRCs carry a KRAS mutation, we began evaluating the relevance of RAC1 expression in KRAS wild-type/mutated CRCs. For this, we downloaded the TCGA-COAD dataset and separated patients according to RAC1 expression levels, determined using the "Survminer" package. In turn, we added an additional filter separating patients according to KRAS gene status. We found that high expression of RAC1 was associated with poor prognosis when KRAS is mutated ($p<0.05$). We extended this evaluation for Rac1 GEFs.
To continue characterizing the role of Rac1 in CRC resistance, we evaluated parameters associated to Rac1 activity like cell and nuclear sizes and actin arrangement in 5-FU-resistant CRC cells. Control and resistant cells (generated by overexposure to 5-FU) were plated, treated with Rac1 inhibitors or vehicle, fixed and stained with phalloidin-rhodamine and DAPI to visualize actin cytoskeleton and nuclei. Measurements were performed by Image J. We noted that Rac1 inhibition was enough to overt morphological changes associated to resistance, and re-sensitize cells to 5-FU ($p<0.01$). All our data allowed us to postulate that targeting Rac1 represents a promising avenue for the development of new therapies for patients with CRC resistant to 5-FU-based therapies.

375. 579. **OPTIMIZED CURCUMIN NANOMEDICINES SYNTHESIS FOR CANCER TREATMENT**

Irina Muntaabski[1], Rodrigo Lloyd[1], Sofia Hernández Krasuk[2], Alma Katz[2], Greta Bolzi[1], Julia Gallino[1], Luciano Fiore[1], Noelia Anahí Soria[1], Diego Chiappetta[3], Marcela Morettón[3] and Lucia Policastro[1]

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Curcumin (Cur) is a polyphenol isolated from *Curcuma longa* that has beneficial properties demonstrated in multiple chronic diseases. In this context, Cur emerges as a promising therapeutic alternative in oncology, due to its proved anticancer effectiveness. Nevertheless, Cur is highly insoluble in aqueous media, which decreases its bioavailability *in-vivo*, creating the need to optimize delivery alternatives of Cur. In this scenario, the administration of novel anti-tumor drugs in 100 nm-size nanovehicles (NV) optimizes the localization of drugs in tumor tissue. This occurs manly due to the enhancement permeably retention effect that reduce peripheral toxicity and increase the tumor local therapeutic effectiveness. NV conventional synthesis technologies have limitations and are inefficient with variations in the batch-to-batch procedures. However, microfluidic technology-assisted synthesis significantly improves the processes and allows reproducibility between different batches. The aim of this work is the synthesis and characterization of NV such as liposomes (LP) and polymeric micelles (MP) for the encapsulation of Cur through conventional synthesis and microfluidic-assisted synthesis, in order to obtain an optimized synthesis method for *in-vitro* evaluation of Cur-NV therapeutic efficacy. We encapsulated of Cur in LIP (Cur-LIP) and MP (Cur-MP) and evaluated the size, polydisperse indices (PDI) by DLS, and we observed the Cur-NV structure by TEM. Finally, the concentration and % of encapsulation of Cur were determined by spectrophotometry and the cellular uptake and therapeutic effect was evaluated *in-vitro* by MTT and fluorescent microscopy techniques. As results and conclusions, the microfluidic assisted-synthesis optimized Cur-NV significantly in size, PDI, and the amount of Cur encapsulated respected traditional methods. Cur-NV was found to be effective for the treatment of *in-vitro* cancer model.

376. 597. **PRECLINICAL VALIDATION OF RAC1 INHIBITOR 1A-116 ANTITUMOR ACTIVITY IN A MURINE COLORECTAL CANCER MODEL**

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Colorectal Cancer (CRC) is the second cause of death by malignant neoplasms in Argentina. Standard CRC treatments include a surgical resection in combination with radiotherapy, chemotherapy and/or immunotherapy. Despite these therapeutic approaches, nearly half of patients develop recurring CRC. The emergence of several types of targeted drugs has revealed new prospects for CRC treatment. RAC1 is a small GTPase that has a critical role in cancer progression, regulating different processes related to tumor growth, chemoresistance and metastasis. Importantly, RAC1 proved to be overexpressed in CRC and significantly associated with tumor stage. 1A-116 is a small molecule previously developed by our group designed to target RAC1 activation and has proven to be effective in preclinical settings of different cancer types. The aim of the present work was to explore the antitumoral effects of 1A-116 in the murine aggressive CRC model CT-26. First, 3D spheroids were established by orbital shaking centrifugation. Spheroids were treated twice/week with different concentrations of 1A-116 for 15 days. 1A-116 showed a significant cytostatic activity on 3D spheroid growth in the range of 25-100 uM ($p<0.05$). In addition, we determined cell viability on these 3D spheroids by confocal microscopy using Calcein-AM as a vital stain. Further, we established a syngeneic mouse model in Balb/c mice. CT-26 tumor-bearing mice were daily treated with 1A-116 10 mg/kg/day or vehicle via i.p. 1A-116 showed antitumor activity, significantly reducing tumor growth ($p<0.05$). These preclinical results lay the ground of 1A-116 as an interesting and promising therapeutic tool in CRC. Further studies are warranted to establish its therapeutic value in combinational schemes with conventional chemotherapy and immunotherapy.

377. 616. **PRELIMINARY EVALUATION THROUGH THE GRADIENT BOOSTING ALGORITHM (GBM) OF CD146 OVEREXPRESSION IN SPORADIC COLORECTAL CANCER PATIENS**

Florencia Adriana Lohmann[1], Pamela Rosalez[2], Mónica Alejandra Loresi[1], Maximiliano Hernán Dádamo[1], Laura Raquel Soto[1], Julieta Natalia Soarez[1], Walter Hernán Pavicic[1], Andrea Romina Cajal[1], Juan Pablo Santino[3], Marcelo Raúl Risk[1], Carlos Alberto Vaccaro[1,4], Tamara Alejandra Piñero[1]

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Colorectal cancer (CRC) ranks as the third most prevalent malignant neoplasm globally and stands as the third leading oncological cause of death in Argentina. The projected global incidence of CRC for the year 2030 is expected to reach 2.2 million new cases, accompanied by an anticipated 1.1 million associated deaths. This underscores the critical need to discover novel biomarkers capable of enhancing therapeutic strategies. CD146 has emerged as a biomarker of

angiogenesis within the tumor microenvironment across various cancer types. Accumulated evidence strongly suggests that CD146 overexpression correlates with the early development of the primary lesion or progression to metastasis. The aim of this study was to evaluate the relationship between CD146 overexpression and anatomoclinicopathological, as well as epidemiological variables in CRC. The degree of CD146 overexpression was assessed within formalin-fixed, paraffin-embedded (FFPE) tissue samples obtained from 41 surgically treated sporadic CRC patients at the Hospital Italiano de Buenos Aires, Argentina. CD146 overexpression was detected in 56% (23/41) of cases and exhibited a correlation with histological grade and Duke stage (χ^2=16.64; P=0.05). By employing the Gradient Boosting Machine (GBM) machine learning algorithm, commonly utilized for statistical classification and regression tasks, a predictive model was constructed, consisting of an ensemble of weak prediction models, typically represented as decision trees. Through this analysis, variables such as tumor site (38.5%), body mass index (BMI (21.7%), cancer age (13.7%), sex (8.1%), and the presence of colorectal polyps during the first 6 and 12 months of follow-up were identified as factors exerting more influence on CD146 expression. CD146 shows promise as a consequential prognostic biomarker in CRC, emphasizing the necessity of jointly assessing with patient progression as a prospective tool of precision medicine.

378. 632. **SMYD2 AS A NEW THERAPEUTIC TARGET FOR HEPATOCELLULAR CARCINOMA**

Barbara Bueloni[1], Maria Jose Cantero[1], Lucia Lameroli[1], Catalina Atorrasagasti[1], Mariana Garcia[1], Esteban Fiore[1], Juan Bayo[1], Guillermo Mazzolini[1]

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Introduction: Current hepatocellular carcinoma (HCC) therapies have limited survival impact, driving the need for novel treatments. The methyltransferase SMYD2 is a potential therapeutic target due to its role in transcriptional regulation and its non-epigenetic substrates, such as the tumor suppressor p53 and the oncogenic transcription factor β-catenin. In this line, Wnt/β-catenin pathway is frequently overactivated in immunologically unresponsive tumors. Our aim was to explore whether the pharmacological inhibition of SMYD2 in HCC could trigger an antitumoral effect while also reducing the immunosuppressive nature of “cold” tumors. Methods: SMYD2 expression levels and correlated relevant pathways were explored using public HCC datasets. SMYD2 inhibitors (AZ505 and LLY507) impact on HCC cells survival, cell cycle and apoptosis was assessed by MTT assay and flow cytometry. RNA-seq analysis was performed on LLY507—treated HuH7 cells. The PM299L cell line bearing hyperactive β-catenin was used to study the effect of SMYD2 inhibition on Wnt pathway activation. ***In vivo*** effect of SMYD2 inhibitors was evaluated on an orthotopic HCC murine model. Genes expression was assessed by qPCR. Results: SMYD2 is upregulated in HCC tumors and negatively correlates with immune-related genes and apoptotic processes that are downregulated in HCC. LLY507 induces cell cycle arrest and apoptosis on HCC cells, and downregulates aggressive and cell cycle-related genes as revealed by RNA-seq. On J774 macrophages, SMYD2 inhibition induces the expression of IL-1β but reduces TGFβ production. Next, we confirmed the upregulation of β-catenin targets in PM299L cells, as well as its downregulation after AZ505 treatment. Notably, LL507 and AZ505 strongly inhibit tumor growth ***in vivo.*** Tumors treated with AZ505 exhibit an upregulation of inflammatory genes, as well as a decrease in the expression of immunosuppressive cytokines.Principio del formulario. Conclusions: Our results indicate that SMYD2 inhibition is a potential therapeutic strategy for HCC that reverts oncogenic and immunosuppressive transcriptional programs.

O6-ONCOLOGY
FRIDAY 17TH NOVEMBER 14:00-15:30
CHAIRS: PAOLA ROJAS
NATALIA RUBINSTEIN

379. 212. **TARGETING PIN1 TO OVERCOME TMZ RESISTANCE IN GLIOBLASTOMA MODELS**

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Glioblastoma (GBM) stands as the most prevalent and lethal primary tumor of the central nervous system in adults. Its elevated mortality arises from cellular resistance to temozolomide (TMZ), the front-line therapy. The main mechanisms that contribute to TMZ resistance encompass heightened expression of the DNA repair enzyme, MGMT, and the acquisition of a stem cell-like phenotype. It has been demonstrated that the peptidyl-prolyl isomerase PIN1 is implicated in the regulation of these mechanisms through the activation of the NF-KB signaling pathway. PIN1 is an enzyme that plays a key role in regulating critical cellular processes in tumor progression in different types of cancer, including GBM. Consequently, we have developed a PIN1-specific inhibitor, PI-7, that exhibits antitumoral activity in GBM cell lines. Based on this, the objective of this study is to establish a combined therapy to enhance TMZ’s effect by inhibiting PIN1 in resistant GBM models. Therefore, an acquired-resistance model, U251-TR, was generated by chronic exposure to TMZ. In addition, LN18 cells served as an intrinsic-resistance model. Initially, the impact of PI-7 on proliferation was assessed in U251-TR and LN18 cell lines, resulting in IC50 values of approximately 60μM after 3 and 6 days. In parallel, the antiproliferative effect of TMZ was evaluated after 6 days of treatment, with IC50 values of 500μM and 700μM for LN18 and U251-TR, respectively. Moreover, PIN1 was found to regulate active levels of NF-KB, and PI-7 reduced the transcription of genes modulated by this pathway. Consequently, PI-7 could potentially restore TMZ sensitivity in TMZ-resistant models. Finally, we performed proliferation assays with PI-7 and TMZ treatment for 6 days in both cell lines, revealing an enhanced inhibitory effect. In conclusion, this study highlights the role of PIN1 in TMZ-resistance mechanisms in resistant GBM models. Indeed, PI-7 emerges as a promising therapeutic coadjuvant strategy for GBM therapy..

380. 312. **TUMOR BANK OF DR. ARTURO OÑATIVIA HOSPITAL: A DIVERSE SOURCE OF SAMPLES FOR ONCOLOGICAL RESEARCH.**

Sequeira Gonzalo R., Bazzoni Paola L., Martinez Marisa M., Díaz Macarena, Monteros-Alvi Marcelo N.

OBJECTIVES: 1) Establish the Tumor Bank for Biomedical Research at Dr. Arturo Oñativia Hospital in the province of Salta. 2) Establish intramural workflow and operational protocols. MATERIALS AND METHODS: Samples: obtained from oncology patients at Dr. Arturo Oñativia Hospital. Collected: 1) Pre-surgical peripheral blood, from which plasma and white blood cells are separated and stored. 2) Tumor specimen: Three areas are distinguished: surrounding normal tissue, transitional tissue, and the tumor itself. Stored: 1) In a blister pack with freezing medium (OCT) and immediately taken to -20°C. 2) Three fragments of 1 mm x 3 mm from each of the mentioned zones, stored for molecular studies. 3) The remaining tumor tissue is distributed into 3 cryovials with 10% DMSO in fetal bovine serum and placed at -80°C in a cool cell, then finally stored at -196°C.RESULTS: Since its creation in May 2022, samples from patients have been collected. The stored tumor types are: thyroid (40%), breast (36%), kidney (8%), and parotid gland (5%), with the remaining 11% being other tumors. The Tumor Bank holds: 129 blisters (for immunofluorescence), 244 cryovials (for PDXs), 494 of tumor tissue, 217 of transitional tissue, and 309 of normal tissue tubes for molecular biology studies, 710 plasma tubes (liquid biopsy), and 293 tubes of white blood cell pellets (germline mutations). CONCLUSIONS: The Tumor Bank in the city of Salta operates in compliance with legal, administrative, and workflow requirements.

The range of activities involved in the Tumor Bank is appropriately integrated into our multidisciplinary team, allowing us to make the most of this tool. Additionally, the main objective of the Tumor Bank since its inception has been to contribute to the development of translational research projects, and we can conclude that it is now ready to actively participate in such initiatives."

381. 366. **SYNERGISM OF SMALL MOLECULES TARGETING VDAC WITH SORAFENIB, REGORAFENIB OR LENVATINIB ON HEPATOCARCINOMA CELL PROLIFERATION AND SURVIVAL**

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Voltage dependent anion channels (VDAC) in the outer mitochondrial membrane regulate the influx of metabolites that sustain mitochondrial metabolism. The small molecules X1 and SC18 induce mitochondrial dysfunction and cell death. X1 antagonizes the inhibitory effect of tubulin on VDAC. SC18 occupies an NADH-binding pocket in the inner wall of all VDAC isoforms. Here, we hypothesized that X1 and SC18 have a synergistic antiproliferative effect with sorafenib (Sor), regorafenib (Reg) or lenvatinib (Len), that are currently FDA-approved drugs to treat hepatocarcinoma (HCC). We used the well differentiated Huh7 and the poorly differentiated SNU-449 HCC cells to determine cell proliferation (colony formation assay), and cell survival (calcein/propidium iodide confirmed with trypan blue exclusion). Synergism was calculated by the Chou-Talalay method. A Coefficient of Interaction (CI)<1 indicated synergism. The inhibitory effect of X1, SC18, Sor, Reg and Len as standalone treatments, was concentration and time dependent ($p<0.05$ vs. Control). IC_{50s} to inhibit clonogenic capacity in SNU-449 cells were 0.6μM, 23.2μM, 5.3μM, 11.2μM, and 4.1μM for X1, SC18, Sor, Len and Reg, respectively. In Huh7 cells the IC_{50s} were 0.3μM,10.8μM and 1.7μM for X1, SC18 and Sor. IC_{50s} for inhibiting colony formation were lower compared to concentrations required to induce cell death in both cell lines. IC_{50s} for cell death in SNU-449 cells were 10.8μM, 52.1μM, and 4.6μM for X1, SC18, and Sor; and 2.5μM, 36.6μM and 5.9μM for X1, SC18 and Sor in Huh7 cells. At IC_{50s} to inhibit cell proliferation, SC18 arrested cells in G0/G1 ($p<0.05$). SC18 at 0.25-2 IC_{50s} inhibited the clonogenic capacity synergistically with Sor, Reg or Len (CI<1) in SNU-449, and with Sor in Huh7 cells. X1 or SC18 also induced cell death synergistically with Sor at 0.5-2 IC_{50s} in SNU-449 cells (CI<1). These results suggest that small molecules targeting VDAC may represent a potential new class of drugs to treat liver cancer.

382. 376. **ACRIFLAVINE MODULATES ANGIOGENESIS AND TUMOR PROGRESSION IN OVARIAN CANCER**

España De Marco María José[1], Marinoni Rocio[1], Tesone Marta[1], Pérez Piñero Cecilia[2].

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Ovarian cancer is one of the most lethal gynaecological malignancies. The development of new therapies is needed, as the five-year survival rate is less than 50%. Hypoxia is a feature of solid tumors associated with an aggressive phenotype. The main transcriptional factor involved in this process is Hypoxia Inducible Factor 1 alpha (HIF1α). The aim of this work was to study HIF1α inhibition by Acriflavine (ACR) in SKOV3 and IGROV1, both human ovarian cancer models, *in vitro and in vivo*. We have previously shown that ACR diminished cell migration. Then, we found lower levels of HIF1α in SKOV3 cells after 24 h of incubation with ACR by Western Blot. Proliferation assays showed that both cell lines diminished proliferation after 72 h of ACR 0.5 μM. In SKOV3 cells, we found lower levels of CCND1, pERK/ERK ratio, VEGFR2, and TIE2 after 24h of ACR treatment by Western Blot. For the *in vivo* experiments, $5x10^6$ cells (IGROV1 or SKOV3) were s.c. injected into the flank of NSG mice. Mice received daily ACR i.p. injections (8 or 12 mg/kg, 15 days). All ACR-treated tumors were significantly smaller than control tumors (8mg/kg: from day 8 SKOV3 $p<0.001$, from day 11 IGROV1 $p<0.0001$; 12mg/kg: from day 8 SKOV3 $p<0.01$ and IGROV1 $p<0.05$). We found a lower Ki67 index (SKOV3) and an increase in active caspase 3 (IGROV1 and SKOV3) in ACR-treated tumors. We studied HIF1α downstream targets, VEGF and GLUT1, and angiogenesis-related proteins in tumor samples. We found lower levels of GLUT1, VEGF and PDGFB, and a reduced endothelial vascular area (CD31) in SKOV3 ACR-treated tumors vs control. Also, IGROV1 ACR-treated tumors showed lower levels of VEGFR2. Our results suggest that ACR prevents new vessel formation and the recruitment of pericytes *in vivo*. In summary, HIF1α plays an important role in the proliferation, migration, and angiogenesis of ovarian cancer models. ACR could be a potential drug for treating ovarian cancer in combination with other therapies.

383. 463. **THE INFLUENCE OF ECO-EVOLUTIONARY DYNAMICS ON THE FINE-TUNING OF CANCER INCIDENCE ACROSS MAMMALIAN SPECIES**

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Cancer is associated with the accumulation of mutations throughout the life of a multicellular organism. Therefore, the probability of developing cancer increases as an individual gets older. Since cancer negatively impacts survival of individuals, classic evolutionary explanations have had to account for the prevalence of cancer across the tree of life. Cancer-promoting gene variants have been traditionally associated with either negative or neutral fitness value. In the first case, these variants are proposed to be related to other phenotypic traits on which positive selection operates (antagonistic pleiotropy). Alternatively, they are conceived as neutral traits since cancer tends to occur after reproduction. However, some mammals present molecular mechanisms that confer strong or even full resistance to developing tumors, confirming that multicellularity can evolve without cancer as an inevitable by-product. On the other hand, the existence of other mammalian species with high incidence of malignant tumors suggests that cancer may also be acting as a phenoptosis mechanism on less reproductive adults. Here, we present the hypothesis that cancer mortality rates across the tree of life might have been fine-tuned by evolution. A high incidence rate could have a neutral, negative or positive adaptive value depending on species' evolutionary history and context. Using public cancer-related mortality databases, we show that species with higher intraspecific competence exhibit high cancer mortality rates; while those with cooperative and

caring habits display lower rates. Mathematical modeling reveals that higher mortality rates in older less reproductive individuals can lead to an increase in the population size in a context of intraspecific competition (hydra effect) whereas in a cooperation context, population size increases as mortality rates of older and less reproductive individuals decrease. Our results approach the phenomenon of cancer from a multidimensional perspective and are compatible with a co-evolution of cancer incidence and other physiological, ecological, and behavioral aspects across the tree of life.

384. 502. **THE LATERALITY OF BREAST CANCER: ION CHANNELS AS DETERMINANTS OF LEFT–RIGHT FUNCTIONAL DIFFERENCES**

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Breast cancer is a heterogeneous disease that displays diverse molecular subtypes and clinical outcomes. Although it is known that the location of tumors can affect biological behavior, the underlying mechanisms are not fully understood. In our previous study, we found a differential methylation profile and membrane potential state between left (L) and right (R) sided breast tumors. In this current study, we aimed to identify the ion channels responsible for this phenomenon and determine any associated phenotypic feature. To achieve this, experiments were conducted in mammary tumors in mice, human patient samples, and with data from public datasets. Results from the mouse model revealed that L-sided tumors have a more depolarized state than R-sided (unpaired t-test, p=0.01). We identified in-silico a 6-ion-channel-gene signature (CACNA1C, CACNA2D2, CACNB2, KCNJ11, SCN3A, and SCN3B) associated with the side: L-tumors exhibit lower expression levels than R-tumors (Mann–Whitney test, p=0.005). Additionally, the signature correlates inversely with DNA methylation writers (r= -0.42, Spearman correlation test, p < 0.0001) and with key biological processes involved in cancer progression, such as proliferation and stemness scores (r=-0.47 and r =-0.62 respectively, Spearman correlation test, p< 0.0001) (ROC curve analyses for proliferation and stemness respectively: AUC=0.74, SE=0.02, 95% CI=0.698 to 0.782, p<0.0001; AUC=0.79, SE=0.01, 95% CI=0.761 to 0.837, p< 0.0001). The signature also correlates inversely with patient survival rates (Kaplan Meier, p< 0.0001). In vivo, we confirmed that KI67 and CD44 markers were increased in L-sided mice tumors (paired t-test, p<0.05) and a similar tendency for KI67 was found in patient L-tumors (unpaired t-test, p=0.2). Overall, this study provides new insights into the potential impact of anatomical location on breast cancer biology and highlights the need for further investigation into possible differential treatment options.

385. 578. **THE INTERACTION OF INTEGRIN ALPHA V (IAV) WITH THE UROKINASE-TYPE PLASMINOGEN ACTIVATOR RECEPTOR (UPAR), AND THE SUBSEQUENT AKT SIGNALING IN GLIOBLASTOMA, IS DEPENDENT ON THE PRESENCE OF COMPLEX AND HYBRID N-GLYCOSYLATION**

Gretel Magalí Ferreira[1,2], Cynthia Antonella Gulino[1], Selene Rojo[1,2], Jeremías Omar Castillo[1], Valeria Segatori[1,2], Mariano Gabri[1,2]
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Integrin alpha V (IαV) and urokinase-type plasminogen activator receptor (uPAR) are described as tumor-associated proteins in glioblastoma (GBM), being both associated with poor survival as well as resistance to treatment. uPAR participates in the modulation of cell behavior by binding to integrins and receptor tyrosine kinases on the plasma membrane to form complexes that actively participate in cell signaling. While their interaction has been documented in other malignancies, it remains uncharacterized in GBM, the most common and aggressive primary brain tumor. Both proteins have N-glycosylation sites that can potentially be involved in the interaction with other proteins. The aim of this work was to analyze the role of N-glycosylation in the interaction between IαV and uPAR, and its impact on AKT signaling pathways. By employing IαV immunoprecipitation (IP) and MS/MS analysis, an oligomannose N-glycan profile was shown in low-grade glioma cells, while high-grade cells primarily exhibited complex and hybrid N-glycan structures and abundant sialic acids. uPAR expression was only detected by western blot in the high-grade cell line A172, and co-IP confirmed its interaction with IαV. Same result was observed by confocal microscopy (CM), quantified by Pearson's coefficient analysis. Interestingly, this interaction was abrogated by treatment with the N-glycosylation inhibitor Swainsonine (SWN), and PHA-L lectin preincubation, which recognizes complex and hybrid N-glycans by both co-IP and CM (***p< 0.001). In contrast, this interaction was not interfered when cells were preincubated with ConA, a lectin that recognizes oligomannose N-glycans. SWN treatment resulted in a down-modulation of the AKT signaling pathway, as evidenced by a reduction in the pAKT/AKT ratio. In conclusion, our results suggest that complex and hybrid N-glycans are involved in the interaction between IαV and uPAR in GBM modulating its downstream AKT signaling.

P5-ONCOLOGY

FRIDAY 17TH NOVEMBER 14:00-15:30
CHAIRS: MARIEL NUÑEZ
MARIA GISELLE PETERS
ELIANA ALONSO

386. 48. **STUDY OF THE IMPACT OF GEN MYCN ON RETINOBLASTOMA TUMOR PHENOTYPE**

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The MYCN protein is a transcriptional regulator that controls various cellular processes and is essential for embryonic development, especially as part of the nervous system. While its role as a driver of aggressive tumor behavior has been demonstrated in some pediatric tumors like neuroblastoma, its significance in retinoblastoma (Rb), the most frequent pediatric intraocular neoplasm, remains unclear. Despite most Rb are initiated by biallelic inactivation of RB1, some retain *RB1* with *MYCN* amplification probably as the tumor driver. These are highly aggressive tumors resistant to clinically used chemotherapy agents. Also, *MYCN* deregulation is important in *RB1*null tumors as we reported that 60% of patients with metastatic disease exhibit gains or amplification in this gene. The objective of this study is to determine the contribution of MYCN alteration in tumor aggressiveness and pharmacological sensitivity in Rb. The primary cell line HPG-RBG1 (MYCNampl RB1$^{+/+}$) and commercial cell line Y79 (MYCNampl RB1$^{-/-}$) were infected twice with lentiviralpLKO vector shMYCN3 (HPG-RBG-1*MYCN3*; Y79*MYCN3*) or a control shRNA (HPG-RBG-1*MYCN0*; Y79*MYCN0*). The expression of MYCN protein was assessed by Western blot. Cell viability was determined by trypan blue dye exclusion assay and pharmacological sensitivity to melphalan was assessed by means of MTT assay. MYCN was highly reduced at the protein level in HPG-RBG-1*MYCN3* and Y79-*MYCN3* compared to HPG-RBG-1*MYCN0* and

Y79*MYCN0*, respectively. Transfection with shMYCN3 hindered HPG-RBG-1 cell growth. A reduction of more than 50% in cell viability was observed in Y79*MYCN*3 cells compared to Y79*MYCN0*. Y79*MYCN3* cells were two fold more sensitivite to melphalan than control. Preliminarily, we observed that MYCN plays a role in aggressiveness and pharmacological sensitivity in retinoblastoma. More studies on its implication in the sensitivity to commonly used drugs for retinoblastoma will be conducted.

387. 89. **SYNERGISTIC *IN VIVO* ANTITUMOR EFFECT OF 2'-NITROFLAVONE AND SAFINGOL COMBINATION**

Juan Manuel Anselmi Relats, Leonor Roguin, Magalí Cercato, Mariel Marder, Julieta Marino, Viviana Blank
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The sphingosine kinase 1 (SphK1)/sphingosine-1-phosphate (S1P) axis has been widely studied in cancer research due to its role in modulating sphingolipid metabolism, which determines cell survival or death. In this sense, the development of SphK1 inhibitors has emerged as a promising strategy due to their ability to increase pro-apoptotic ceramide and decrease oncogenic S1P levels. In addition, it has been reported that certain flavonoids exert antitumor activity through an increase in ceramide levels. Previously, we demonstrated that the synthetic flavonoid, 2'-nitroflavone (2NF), and the SphK1 inhibitor, safingol, synergistically inhibited cell proliferation and induced apoptosis *in vitro* in breast tumor cells. In this work, we studied the effects of the 2NF and safingol combination in an *in vivo* syngeneic LM3 breast cancer murine model. Animals were treated with either vehicle, 2NF (0.7 mg/kg), safingol (0.5 mg/kg) or the combination of these drugs three times per week for two weeks. Results showed that the administration of 2NF reduced tumor volume by 38% ($p<0.05$), whereas safingol had no significant effect compared to control mice. However, the co-administration of both drugs diminished tumor volume by ~80% ($p<0.0001$). We also studied the mechanism underlying these effects by performing western blot assays in tumor lysates. Pro-apoptotic Bax and cleaved PARP proteins were increased ($p<0.01$) and anti-apoptotic Bcl-xL and Bcl-2 were diminished ($p<0.05$) in samples from mice treated with both drugs, compared to those treated with each compound alone or vehicle. The combination also reduced PCNA immunofluorescence staining in tumor sections. Drug doses did not have any toxic effect, since neither mice weight nor hematoxilin-eosin-stained tissues showed differences between treatments. In summary, results suggested the potential benefit of combining an antitumor flavonoid with a regulator of sphingolipid metabolism to promote an enhanced cell death response.

388. 131. **THE TUMOR STROMA MODULATES CHEMORESISTANCE IN OSTEOSARCOMA CELLS**

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Osteosarcoma (OS) is the most frequent malignant bone tumor, affecting 2% of the world pediatric population with cancer, with lung metastases as a clinical challenge. Patients without metastases diagnosis have a 5-year survival rate of 70%, while this rate drops to 30-50% with metastases. Doxorubicin (Dox) is an essential chemodrug in OS treatment. Survival statistics have been stagnant since 1970 mainly due to chemoresistance developed in response to therapy. The tumor microenvironment (TME) is a key OS progression modulator, with stromal cells such as fibroblasts and mesenchymal stem cells (MSC) establishing a bidirectional communication with OS cells. Using an OS tumor model with lung metastatic and non-metastatic components, we assessed the effects of extrinsic and intrinsic TME factors on OS cells' drug resistance ability. For this we evaluated the functional effects of mesenchymal lineage cells' secretomes and of the chemosensitizing drug CBD on the drug resistance response of non-metastatic (SAOS2) and metastatic (LM7) OS cells, by determining the IC50 of Dox under the different modulations. Fibroblasts were effective in significantly lowering the IC50 of Dox in LM7 (3.93 times less $p<0.01$) and SAOS2 cells (5.01 times less $p<0.0001$) while MSC were able to lower the IC50 only in SAOS2 cells (7.66 times less $p<0.01$). CBD was not able to significantly decrease Dox IC50 values in SAOS2 cells coinciding with the intranuclear localization of Dox. These results indicate that MSC' secretome and its differentiated progeny increased drug sensitivity in OS cells and further, the stemness state of the stromal cells' secretome would differentially affect drug sensitivity on target tumor cells with diverging metastatic behavior. The identification of factors that increase drug sensitivity would contribute to the design of treatment strategies that require lower chemodrugs concentrations and therefore less probabilities of developing chemoresistance and adverse effects.

389. 132. **A CD105+ SUBPOPULATION OF STROMAL CELLS FROM PRIMARY TUMORS OF BREAST CANCER PATIENTS PROMOTES MESENCHYMAL-LIKE STEM CELL STATES**

Tiago Martín Osinalde[1], María Belén Giorello[1], Francisco Raúl Borzone[1], Geismar Alex[1], Juan Carlos Calvo[1], María del Rosario Padin[2], Alejandra Wernicke[2], Alejandra Chasseing[1], Luciano Vellón[1].
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Within the tumor microenvironment, certain subpopulations of stromal cells are able to trigger aberrant tissue reparative processes that include the acquisition/loss of cancer stem cell (CSC) states. We aimed to study whether conditioned media (CM) from CD105+ CD34- and CD105- CD34- subpopulations of spindle stromal cells (CD105+/CM and CD105-/CM, respectively) from primary tumor of breast cancer (BC, invasive ductal carcinoma, stage I-II) patients were able to affect CSC states in BC-derived MCF-7 and MDA-MB-231 cells. Previously, we observed that CD105+/CM increased more drastically mammosphere formation ability in MDA-MB-231 when compared to MCF-7 cells. Here, we tested CM from more patients, individually as well as pooled, and found that this trend was conserved, since CD105+/CM induced a 7,9-10,7 fold increase in mammosphere frequency in MDA-MB-231 cells, and a 2,2-3,15 fold increase in MCF-7 cells, whereas CD105-/CM did not generally induce significant changes in mammosphere formation in both cell lines when compared to control CM, as quantified by extreme limiting dilution assay (ELDA) and further statistical analysis with a specialized software (http://bioinf.wehi.edu.au/software/elda). Interestingly, when MCF-7 mammospheres, generated in the presence or the absence of CD105+/CM and CD105-/CM, were allowed to attach and spread onto gelatin under differentiating conditions (complete DMEM/F12 culture medium), we observed that mammospheres generated in CD105+/CM remained more frequently as such and spread less than those generated in control or CD105-/CM. These results support our previous findings that CD105+/CM promote the generation of mesenchymal-like CSC states (MDA-MB-231) rather than epithelial-like CSC states (MCF-7), suggesting that CM from different subpopulations of stromal cells from the breast of BC patients not only differentially affect stem states, but may also affect differentiation and migration ability of BC-derived epithelial cells.

390. 160. **UNRAVELING THE ROLE OF CLCA2 DURING THE *IN SITU* TO INVASIVE TRANSITION IN BREAST CANCER**

Naiara Rodriguez Padilla[1], Marianela Sciacca[1,5], María del Pilar Carballo[2], Ezequiel Lacunza[3,6], Martin Abba[3,6], Lina Marino[2], Érica Rojas Bilbao[2], Marcela Villaverde[1,5], Pablo Saez[4], Ana María Eiján[1,5] and Catalina Lodillinsky[1,5].
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Ductal carcinoma in situ (DCIS) in breast cancer (BC) is defined as a proliferation of epithelial neoplastic cells contained within the lumen of mammary ducts. DCIS are the precursors of infiltrating carcinomas (IDC) but it is not yet possible to predict which or when they will progress to more advanced stages. Intraductal inoculation of human MCF10DCIS.com cells into the murine mammary gland generates DCIS that spontaneously progress to IDC. We have shown that MT1-MMP, a membrane metalloprotease with collagenase activity, is essential for the DCIS to IDC transition. In the invasive state, MT1-MMP is over-expressed at the invasion front, defining two cell populations: MT1-MMP^{high} and MT1-MMP^{low}. The transcriptome analyses of these populations was compared against a set of human high-grade DCIS. *Chloride Channel Accessory 2* (CLCA2) is overexpressed, in both, worse prognosis-human DCIS and MT1-MMP^{high} population, which suggests that this gene is involved in the early transition of BC. CLCA2 expression was assayed by immunofluorescence analyses on paraffin sections of invasive tumor obtained after intraductal injection of tumor cells. We observed an increase in expression in the peripheral areas as well as in contact with the stroma, similarly to MT1-MMP. CLCA2 protein levels in BC were assayed by IHC in a cohort of patients (n=58). The H-score (% of labeled cells multiplied the intensity of the label) for CLCA2 was higher in DCIS vs to normal tissues (p<0,0001). Particularly number of positive cases was higher in nuclear grade III-DCIS (X^2 p=0.048). Furthermore, no statistical difference between DCIS vs IDC was observed, however the number of CLCA2 positive cases was lower in IDC vs DCIS (x^2 p=0,0427). Thus, after validation of CLCA2 expression in our experimental model, we report that CLCA2 is up-regulated in DCIS tumors while its levels drop in IDC. Analysis at the functional level awaits us ahead to understand the role of CLCA2 in the early progression of BC.

391. 291. **STUDY OF PROGENITOR CELLS NICHE ALONG THE ORAL TONGUE IN A MURINE REPORTER SYSTEM**

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The squamous epithelium in the oral mucosa relies on epithelial stem cells for self-renewal and tissue regeneration. Lingual keratinized epithelial cells have a rapid tissue turnover rate within mammals and are thought to be the source of SCC of the tongue. Our main goal is to map the anatomical subregions of the tongue while classifying niches of stem cells based on their oncogenic potential. Using a tamoxifen-inducible reporter line, K14-CreER^{TAM}/TdTomato, we characterize the target cells along with their cellular products by analyzing diverse anatomical sub-sites of the tongue. We did a time-course analysis to study the appearance and loss of TdTomato (TOM) + cells. Tongue sub-sites: dorsal: D, ventral: V. Quantification by Image J. We confirmed that TOM expression occurs exclusively after tamoxifen induction. We quantitatively analyzed TOM+ cell density and fraction at 1, 5, and 16 weeks, and 8 months after induction (N=4-5 each time). Higher density and fraction of TOM+ cells were observed on V after 1 week, reaching values of 10.1±1.2 TOM+ cells/um and 0.64±0.08 compared to the D (8.0±0.8 TOM+ cells/um and 0.36±0.03 p<0.05). This difference was similar in both layers. In all the regions, a gradual decrease in the number of labeled cells was observed over the course of time. This decline was similar during the first 4 months; however, D differs from V due to the small number of remaining cells after 8 months of induction (D vs V: 0.38±2 vs 5.7±1.4 TOM+ cells/um). We studied the expression of Krt14 as a differentiation marker. Krt14 was found at the highest level in the basal layer. At 1, 5, and 16 weeks Krt14 positive cells include TOM+ cells in the basal layer. TOM+ cells represent small fraction of the Krt14 pool of cells in basal layer**.** Conclusion: putative quiescent stem cell density differs between D and V tongue region in our reporter system. These results warrant further analysis to define the role of these quiescent stem cells in oral carcinogenesis.

392. 309. **THE EFFECT OF T4 ON MAMMARY TUMOR CELLS DEPENDS ON THE INTERACTION WITH OVARIAN STEROIDS**

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Hypothyroidism seems to be a protective factor against breast cancer (BCa) but long-term exposure or overdoses of thyroid replacement therapy with thyroxine (T4) may increase BCa risk. We previously observed that hypothyroidism prolonged the latency of appearance, reduced incidence, and retarded growth of tumors in rats, and that T4 regulated mammary carcinogenesis by interacting with other hormone pathways. In the present study, we analyzed the biological activity of T4, alone or combined with steroid hormones, on proliferation, viability, adhesion, and migration of human BCa cell lines. MCF-7 (REα+, REβ+, PgR+, TRβ1+) were treated with 10^{-9} M of T4; 17β estradiol (E2) and/or progesterone (P4), or DMEM/F12 with 1% FBSc as control. We evaluated proliferation and adhesion by MTT, viability by trypan blue and migration by wound healing. To verify if the biological effects were mediated by the genomic and/or non-genomic action of T4, the experiments were repeated using the TR inhibitor (1-850, Cayman Chemical, MI, USA). We evaluated the expression of proteins related to proliferation and apoptosis by western blot. Statistical analysis was performed using Student T test, ANOVA I and Bonferroni as post-test (p<0.05). T4 induced cell proliferation (p=0.012), viability (p=0.025) and PCNA expression (p<0.05) on MCF7 cells after 24h treatment compared to control. These effects were reversed with the administration of 1-850. Furthermore, the combination with E2 also increased the proliferation (p= 0.013) and viability of MCF-7 after 48 h of incubation, as well as co-administration with P4 (p= 0.003). Finally, administration of the three hormones together further augmented proliferation of MCF-7 (p<0.001). However, none of the treatments modified cell adhesion, or migration. In conclusion, T4 promotes the proliferation and viability of hormone-sensitive breast tumor cells through binding to TR and its effects are affected by the interaction with ovarian steroids.

393. 411. **THE COMBINED TREATMENT OF ATRA AND LAPATINIB INDUCES CYTOTOXIC EFFECTS AND MODULATES RETINOID GENES EXPRESSION ON CANCER STEM CELL COMPONENT OF TRIPLE-NEGATIVE BREAST CANCER CELL LINE**

Diego Javier Britez Neira[1], Andrés Bechis[1], Lizeth Aixa Ariza Bareño[1], Luciana Cañonero[1], Aldana Schey[1], Alejandro Jorge Urtreger[1], Laura Beatriz Todaro[1].
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Cancer stem cells (CSCs) are resistant to both chemotherapy and radiotherapy and are considered the seed of metastasis. In order to validate a new therapeutic strategy against this component, throughout the current study, we examined the effect of the combined treatment of All-Trans Retinoic Acid (ATRA) and Lapatinib on several parameters related to CSCs and metastatic dissemination in human triple-negative mammary tumor cell lines (HS578T and 4T1). The Lapatinib treatment dose for both cell lines was previously determined as 5μM for HS578T and 1μM for 4T1 which correspond to their respective IC50 values. A decreased clonogenic capacity was observed in the combined treatment for both cell lines. However, in HS578T cells, a significant decrease was observed upon Lapatinib treatment alone. Furthermore, a decrease in cell adhesion and an increase in EGFR expression were evident in HS578T cells under the combined treatment. Regarding CSCs, on monolayers treated over 48hs less compact spheres with a clustered appearance could have been observed for both Lapatinib and the combined treatment,

accompanied by a significant decrease in diameter and an increase in the number of spheres (P<0.05). The qPCR analysis of retinoid system genes revealed a significant increase in RARγ (nuclear retinoic receptor involved in stemness) in both ATRA and combination treatment. The cytotoxic effects on cell monolayers observed in previous studies, with the effects evidenced on CSCs of Lapatinib, either alone or in combination with ATRA in both triple-negative breast cancer models, provide further in vitro evidence of the potential repositioning of these drugs for the treatment of HER2-negative breast cancer.

394. 428. **THE PROTUMORAL ROLE OF P300 IN THYROID CANCER**

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Thyroid Carcinoma (TC) is the most prevalent endocrine tumor worldwide. p300 is a protein that functions as a transcriptional cofactor, histone acetyltransferase, and lysine acetyltransferase for proteins involved in functions other than transcription. A relationship of p300 with cancer has been demonstrated, however, its role is still unclear since it has been documented as a tumor suppressor and/or as an oncoprotein. In our laboratory, we have established an association between p300 and breast cancer, observing a protumoral role. Due to the limited studies on p300 and TC, it is interesting to investigate the expression and the cellular and molecular mechanisms through which this protein could be involved in the oncogenesis and tumor progression of TC. The objective of this work was to study the expression of p300 and the effect of inhibiting the acetylase function on the processes of apoptosis and metastasis in human TC cells. We observed through In silico assays that p300 is expressed (both RNA and protein) in these tumors. The treatment of human papillary TC cell line, TPC-1, with VV59 (inhibitor of p300 acetylase function) or its vehicle (DMSO) produced a decrease in the number of cells compared to the vehicle (crystal violet assay and manual counting, p<0.0001). When we analyzed the cell cycle by flow cytometry, we detected an increase in the sub G0/G1 phase and a decrease in the G0/G1 phase in the cells treated with VV59 compared to those treated with the vehicle (p<0.001). On the other hand, we detected that pharmacological inhibition of p300 decreases migration (wound healing assay, p<0.0001), invasion (matrigel assay, p<0.0001), and cell adhesion (crystal violet assay, p<0.0001). Taken together, these results demonstrate an antitumoral role for pharmacological inhibition of p300 acetylase function in the human TC cell line.

395. 519. **TARGETING ANGIOGENESIS IN OSTEOSARCOMA: ADDITION OF REPURPOSED HEMOSTATIC DRUG DESMOPRESSIN TO BEVACIZUMAB AS A THERAPEUTIC STRATEGY**

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Angiogenesis plays a crucial role in osteosarcoma (OSA) progression, the most common primary malignant bone tumor. In these highly metastatic and vascularized tumors overexpression of VEGF correlates with poorer outcomes. Although promising, adding anti-VEGF bevacizumab to chemotherapy didn't provide significant clinical benefits in OSA. Desmopressin (dDAVP) is a repurposed hemostatic drug in oncology that acts as a selective agonist for the AVPR2 receptor present in blood microvessels and some cancer cells. dDAVP has shown potent angiostatic and antimetastatic activity in other aggressive tumors but its antiangiogenic effect in OSA has never been studied. The objective of this work was to evaluate dDAVP effects on OSA-associated angiogenesis, alone or in combination with bevacizumab. After exploring interactive gene expression web servers GEPIA2 and TIMER2.0 (SARC-TCGA/n=257), AVPR2 showed a positive prognostic impact on overall and disease-free survival in sarcoma patients, and negatively correlated with proangiogenic genes (VEGFA, MEK, MTOR), as well as protumoral immune infiltrates (MDSCs and M0 macrophages). Its expression also correlated with different antitumoral immune cells such as NK cells, M1 macrophages, mast cells and CD4+ T cells. Moreover, AVPR2 was detected in human MG-63 OSA cells by qPCR and IHC. In an *in vivo* modified matrigel plug assay, dDAVP treatment (12 μg/kg i.v., 3 doses/week) notably reduced early angiogenic response in MG-63 incipient lesions. In nude mice bearing growing MG-63 xenografts treatment with dDAVP (12 μg/kg i.v., 3 doses/week) in combination with bevacizumab (5 mg/kg i.p., 2 doses/week) significantly inhibited tumor progression, enhancing the anti-OSA effects of both monotherapies. Results were significant at p<0.05 (t test or ANOVA, GraphPad Prism). dDAVP plus bevacizumab exhibits cooperative antiangiogenic activity in OSA, revealing an interesting correlation between AVPR2, angiogenic markers and the tumor immune landscape.

396. 525. **SYNERGISTIC ANTIPROLIFERATIVE EFFECT OF THE COMBINATION OF 2'NITROFLAVONE AND GEFITINIB IN TRIPLE NEGATIVE BREAST CANCER CELLS**

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Gefitinib is an epidermal growth factor receptor (EGFR) inhibitor used for the treatment of cancer which has recently been proposed for the treatment of certain types of breast cancer. 2'nitroflavone (2'NF) is a synthetic flavone that was obtained in our institute which has previously demonstrated to affect the EGFR pathway activation as well as the expression of receptors related to EGFR activity in breast cancer cells. As EGFR is associated with tumorigenesis and is believed to be involved in the mechanism of action of flavonoids, a combinatory therapy between 2'NF and gefitinib is proposed. The aim of our investigation is to analyze if there is a synergistic effect that justifies the combination of these two molecules for the treatment of breast cancer. To assess our objective, MDA-MB-231 breast cancer cells were treated with 2'NF, gefitinib or a combination of both for 48 h. The effect on cell proliferation was determined by hexosaminidase assay using concentrations in a range between 0.1 μM and 150 μM. Afterwards, the synergistic effect was determined using Compusyn software that calculates a combinatory index (CI) which value must be under 1 to have a synergistic effect. Besides, the results were confirmed by flow cytometry using concentrations of 5 μM and 10 μM. Results were analyzed by ANOVA, p<0.05 was considered statistically significant. Results showed an CI of 0.62 which according to the software refers to the category of synergism (n=6). The software also indicates that due to this synergism the IC_{50} can be reduced 2.95 times for 2'NF and 3.49 times for gefitinib. These results were reconfirmed by flow cytometry showing a significative increase in cell death when the molecules are combined.
In conclusion, a combinatory treatment using 2'NF and gefitinib demonstrated to have a synergistic effect on breast cancer cells which justifies the use of them together as a potential new therapy.

397. 526. **TUMORAL PD-L1 MODULATES CD206+ MACROPHAGE IMMUNOSUPPRESION DURING BREAST CANCER PROGRESSION**

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One of the main immunosuppressive mechanisms during tumor progression is the expression of PD-L1, the ligand for T-cell inhibitory receptor PD-1. Despite PD-1 is also expressed in the myeloid lineage, it is not clear which macrophage-specific immune evasion mechanisms are modulated by tumor cell PD-L1. To interrogate this, we generated a PD-L1 KO TNBC-like tumor model in the murine EO771 cell line using CRISPR/Cas9 editing, allowing us to profile the immune infiltrates of the tumoral microenvironment (TME) *in vivo*. Profiling tumor growth in WT vs. PD-L1 KO tumors, we found that tumoral PD-L1 is partially required for EO771 tumor growth. Using flow cytometry (FC) to characterize the immune infiltrates of early vs late-stage WT tumors, we found a decrease in F480h CD206+ TAMs in late-stage tumors, suggesting that inhibition of CD206+ polarization is involved in immune evasion. Interestingly, analyzing late-stage PD-L1 KO vs WT tumors, we found that tumoral PD-L1 inhibits CD206+ macrophage polarization. Furthermore, WT tumor progression triggered PD-1+ and MHCII+ expression in CD206+ TAMs. Comparing PD-L1 KO vs WT tumors, we found that tumoral PD-L1 promotes MHCII expression in CD206+ TAMs, suggesting that PD-L1 fosters antigen presentation by MHCII *in vivo*. On the contrary, FC analysis of *in vitro* experiments showed that direct contact of tumoral PD-L1 inhibits MHCII expression in CD206+ macrophages, suggesting that indirect TME mechanisms compensate the immunosuppressive inhibition of MHCII mediated by tumoral PD-L1. Interestingly, using FC to analyze GFP+ tumor cell phagocytosis in CD11b+ F480+ TAMs, we found that tumoral PD-L1 directly suppresses phagocytosis both *in vivo* and *in vitro*. Altogether, these data suggest that tumor-intrinsic PD-L1 plays a key role in TNBC progression by triggering immune suppression mechanisms in CD206+ TAMs. By interrogating these non-canonical mechanisms, we could gain insights into novel mechanisms of resistance to PD-L1/PD-1 therapies.

398. 605. **EFFECT OF GLYCODRUGS IN THE CHEMOTHERAPEUTIC RESPONSE IN PANCREATIC CANCER**

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Drug glycosylation has emerged an alternative approach to improve pharmacokinetic properties, bioavailability and reduce the toxicity. Using as model pancreatic ductal adenocarcinoma (PDAC), the most common histological subtype of pancreatic cancer, we aimed at evaluating the anti-tumoral properties of glycosylated version of two polyphenolic chemotherapeutic agents (4-methylumbelliferone (4MU) and resorcinol (R) alone or in combination with standard cytotoxic therapy for PDAC. The enzymatic addition of the disaccharide rutinose using a diglycosidase was performed to obtain the respective glycodrugs named 4-methylumbelliferilrutinose (4MUR) and resorcinol-rutinoside (RR). Our experiments showed that the monotreatment with the glycodrugs did not affect significantly cell viability, however, the combination with gemcitabine, a commonly used chemotherapeutic agent, show a synergistic effect in PDAC cell models. In Panc-1 cells, 4MUR showed an antineoplastic effect decreasing the cell viability 44% (100 nM of Gemcitabine/ 50nM of 4-MUR, 48 h, $p<0.05$) and in MiaPaCa-2 cells, the cell viability was 70% after the co-treatment with RR (100 nM of Gemcitabine/100 nM of RR, 48 h, $p<0.05$). Next, in search of the mechanism underlying this combination, we evaluate the expression of genes related to hyaluronic acid metabolism (CD44, HYAL2, HAS2 and HAS3) and the ECM degraded compounds gene MMP-2 in PDAC cells by qPCR after 4MUR and RR treatment. Genes related to hyaluronic receptors and synthesis were downregulated and, simultaneously, Hyal2 gene was upregulated. Remarkably, MMP-2 expression was downregulated after glycodrugs treatments. In summary, our findings demonstrate that glycodrugs improve gemcitabine therapeutic effectiveness in PDAC, suggesting glycosylation as a novel and effective approach for PDAC treatment.

399. 612. **THE IRON EFFECT ON THE CELL SURVIVAL OF BREAST CANCER DEPENDS ON ITS OVERLOAD LEVEL**

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Cancer cells develop metabolic alterations to sustain an increased proliferation. The iron is an essential element required for many biological processes and its metabolism is disrupted in breast cancer (BC) cells. It has been reported that high cellular iron concentration accelerates the proliferation of BC cells. However, recent works described that the iron overload induce cell death by ferroptosis, a form of cell death caused by iron-catalyzed excessive peroxidation of polyunsaturated fatty acids; being a promising therapeutic target for therapy-resistant cancers. In this study we aimed to analyze the behavior of BC cells exposed to an increasing iron overload. To that end, the murine BC cell line, LM3, was treated with increasing ferric ammonium citrate (FAC) concentrations (0- 400 μM) for 48 h and cell viability (by crystal violet), intracellular iron (by Prussian Blue), reactive oxygen species (ROS) (by DFCA), lipid peroxidation (TBARS) (by MDA accumulation) and the expression of divalent metal transporter 1 (DMT1) by immunocytochemistry, were analyzed. LM3 cell viability increased after FAC treatment with 25 μM and 50 μM ($p< 0.05$) but decreased with 200 μM and 400 μM FAC ($p< 0.05$ and $p< 0.01$, respectively), respect to vehicle. The ROS levels increased after FAC treatment with 50 μM ($p< 0.05$), 200 μM ($p< 0.001$) and 400 μM ($p< 0.001$) compared to vehicle. In addition, we detected an increase in lipid peroxidation in LM3 cells treated with 200 μM and 400 μM of FAC compared to vehicle ($p< 0.01$, in both). Also, we found iron accumulation as hemosiderin form and high DMT1 importer expression in LM3 cells treated with 200 μM and 400 μM of FAC, compared to vehicle. Altogether these results suggest that the effect of iron on cell viability depends on its overload level and that a high iron overload promotes the iron entry through DMT1 and its accumulation as hemosiderin inducing lower cell viability through lipid peroxidation-dependent mechanisms.

400. 633. **DIFFERENTIAL REGULATION OF MULTIDRUG RESISTANCE ASSOCIATED PROTEIN 4 (MRP4/ABCC4) EXPRESSION IN RESPONSE TO EPIDERMAL GROWTH FACTOR (EGF) IN HUMAN PANCREATIC DUCTAL ADENOCARCINOMA AND HEPATOCELLULAR CELL LINES**

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Goméz[1], Ismael Barosso[2], Carlos Davio[1], Carolina Ghanem[1,3].
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Multidrug resistance associated protein 4 (MRP4/ABCC4) and receptor (EGFR) are associated with poor overall survival in Hepatocellular carcinoma (HCC) and pancreatic ductal carcinoma (PDAC). Also, overexpression of both proteins is associated with high cell proliferation in several types of cancer. Our previous results show that EGF induces MRP4 expression in the pancreatic cell line BxPC3. The aim of this work is to evaluate the effect of EGF on MRP4 expression using human cancer cell lines, BxPC3 and HEPG2, which differ in their origin and basal expression of EGFR. The expression of EGFR was detected only in BXPC3 but not in the hepatocellular derived cell line, HEPG2, by western blot (WB). To confirm this result, we stimulated both cell lines with EGF, the canonical agonist of the receptor, and evaluated the response measuring P-JNK/JNK relation by WB. After 5 minutes of exposure, the pathway was activated in both cell lines. However, the magnitude of the response was significantly higher in BxPC3 compared to HEPG2 (2 fold). Additionally, we demonstrated that EGF (0.3 ng/μl) induces the transcriptional expression of MRP4 only in BxPC3, increasing its mRNA and protein levels at 24 and 48 hours respectively, with no modification of its expression in HEPG2. Finally, the induction of MRP4 expression was abolished in the presence of a selective EGFR inhibitor (CL 387-785; 1μM). Furthermore, the basal expression of MRP4 is also associated with the level of EGFR expression, being higher in the PDAC cell line and lower in HEPG2. In summary, these data suggest that the expression and activation levels of EGFR may be associated with the induction of MRP4 expression in vitro. Additionally, it is known that PGE2, a typical substrate of MRP4 can transactivate EGFR, probably suggesting that both suggested PDAC and HCC poor prognosis markers could be co-regulated, enhancing their effect upon each other.

401. 636. **THE COMBINATION OF DAPT AND ENZALUTAMIDE IMPROVES *IN VIVO* PC3 PROSTATE TUMORS**

Agustina Chimento[1,2], Sofía Herrera[2], Daiana Vitale[2,3], Licina Tessone[4], Laura Alaniz[2,3], Carolina Cristina[2,3].
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Prostate cancer (PCa) is one of the most frequent cancers among males. New AR inhibitor agents such as Enzalutamide (Enz) have been released; but, their efficacy is insufficient. There is strong evidence that involves Notch pathway in prostate development but its role in PCa generation and progression is poorly understood. We previously demonstrated AR and Notch receptor expression in prostate cancer PC3 cells *in vitro* and we observed significantly reduced viability of PC3 cells treated both with Enz (p=0.0018;n=3), an inhibitor of Notch signaling DAPT (p=0.0027;n=3) and also with the combined Enz-DAPT treatment (p=0.0043;n=3). In this work, we aimed to study the effect of Notch system inhibition together with Enz treatment in prostate tumor development *in vivo*.–We injected PC3 cells subcutaneously in Nude mice and we treated them three times/week during 3 weeks with DAPT (i.p. 12 mg/kg,n=4), Enz (i.p. 10 mg/kg,n=3) or the combination of Enz-DAPT (n=3). Control animals received vehicle (DMSO) (n=4). No differences were observed in the single treatments, but tumor growth reached a reduction along the second week in the Enz-DAPT animals compared to controls (p<0,05). For proliferation index calculation, Ki67+ immunostaining was performed and nuclei in the hotspot areas were manually quantified and normalized to total nuclei in these areas. We found a trend of reduction of Ki-67 index in the group of Enz-DAPT treated animals compared to controls. We observed a trend of reduction of a target gene of Notch *Hes1* and *Notch1* receptor by RT-qPCR in the combined treatment as well. Our study shows *in vitro* and *in vivo* activation of the Notch pathway in tumors PC3 cells, which would be involved in tumor cell proliferation. Based on our results arised from *in vivo* experiments, a combined approach with inhibitors of both pathways could be more effective than any isolated one. Further studies are needed to potentiate this combined treatment thinking of patients with aggressive PCa.

402. 647. **TARGETING FOXP3 TUMOR INTRINSIC EFFECTS USING ADENOVIRAL VECTORS IN EXPERIMENTAL BREAST CANCER**

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Regulatory T cell master transcription factor, Forkhead box P3 (Foxp3) has been detected in cancer cells, but its role in breast tumor pathogenesis remains controversial. Here we assessed Foxp3 tumor intrinsic effects in experimental humen breast cancer cells using a Foxp3 binder peptide (P60) that impairs Foxp3 nuclear translocation. Foxp3 expression varies among triple negative breast cancer (TNBC) cells, being higher in MDA-MB-468 (60%), than in MDA- MB-231 (25%). Nevertheless, Cisplatin upregulated the Foxp3 expression in MDA-MB-231 (43%; *p<0.05, Student´s t test*). Foxp3 inhibition with P60 enhanced chemosensitivity and reduced cell survival and migration in human breast tumor cells. We also developed an adenoviral vector encoding P60 (Ad.P60) that efficiently transduced breast tumor cells, reduced cell viability and migration, and improved the cytotoxic response to cisplatin (*p<0.05, ANOVA*). Intratumor administration of Ad.P60 in breast tumor-bearing mice significantly delayed tumor growth and inhibited the development of spontaneous lung metastases (*p<0.05*, *Multiple regression analysis* and *Student´s t test*). Our results suggest that Foxp3 exerts protumoral intrinsic effects in breast cancer cells, and that gene therapy-mediated blockade of Foxp3 could constitute a therapeutic strategy to improve the response of these tumors to standard treatment.

O1-PHARMACOLOGY

WEDNESDAY 15TH NOVEMBER 9:00 - 10:30
CHAIRS: VENTURA SIMONOVICH
SUSANA GORZALCZANY
DANIELA QUINTEROS

403. 70. **THE BISPHOSPHONATE ALENDRONATE EXERTS A DIRECT ACTION ON ADIPOSE TISSUE IN A MODEL OF HYPOESTROGENISM AND OBESITY**

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The expansion of adipose tissue plays a crucial role in the progression of obesity, a chronic disease that generates oxidative stress and an imbalance in the production of proinflammatory adipokines promoting a deleterious impact in many tissues. Bisphosphonates are drugs employed as first line therapy for bone-related diseases, such as postmenopausal osteoporosis. However, several extraosseous effects have been demonstrated in the last decades. Postmenopausal women exhibit a higher prevalence of obesity and overweight, so that, in this work we investigated the impact of the bisphosphonate alendronate (ALN) on adipose tissue in a model of hypoestrogenism and obesity. For this purpose, retroperitoneal adipose tissue was isolated from bilaterally ovariectomized Wistar rats fed with a high-fat diet (27%). For in vitro treatments, slices of adipose tissue (40 mg) were incubated with 5 µM ALN for 18 h. Control group received vehicle only (phosphate buffered saline). Hydrogen peroxide (H_2O_2), TBARS and leptin levels were measured. Firstly, we detected that serum H_2O_2, TBARS and leptin levels in obese rats were higher than those detected in normal weight rats (6, 65 and 122% above control, respectively, $P<0.05$), compatible alterations with oxidative stress and inflammatory conditions. When adipose tissue was incubated with ALN, a marked reduction in H_2O_2 levels with respect to the control group was detected (2731 ± 419 vs 1642 ± 89 nmol H_2O_2/g of tissue, control vs ALN respectively, $P<0.002$, fluorometric assay). We also observed that treatment with ALN significantly inhibited the production of TBARS (12% vs control, $P<0.0001$, colorimetric assay). The bisphosphonate significantly reduced leptin levels released to the culture medium compared to the control group (31% vs control, $P<0.0025$, ELISA kit). In conclusion, the results presented suggest a novel extraosseous effect of ALN through a direct action on adipose tissue, reducing oxidative stress and leptin production.

404. 258. **REGULATORY MODULATION OF NF-κB ACTIVITY BY HSP90-BINDING IMMUNOPHILINS AND β-CATENIN**

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β-Catenin is a ubiquitous client protein of the chaperone Hsp90 that activates the Wnt-dependent transcriptional pathway responsible of cell adhesion, cell development, and a variety of diseases, including cancer. Its aberrant activation leads to the nuclear accumulation of β-catenin promoting the induction of many oncogenes. NF-κB is a transcription factor that plays key roles in inflammation, stress response, tumour growth, and apoptosis. Previously, we reported that two highly homologous Hsp90-binding immunophilins regulate transcriptional activity of NF-κB, where FKBP52 is an activator and FKBP51 is an inhibitor. Therefore, we hypothesised that these Hsp90-binding immunophilins, β-catenin, and NF-κB may be integrated in a common functional pathway. To evaluate whether a signalling crosstalk exists between β-catenin and NF-κB pathways, HEK cells expressing an NF-κB-Luc reporter gene, NF-κB, and increasing concentrations of β-catenin were stimulated (or not) with 0.1 ug/ml phorbol-12-myristate-13-acetate for 7 h. Under both conditions, β-catenin showed a strong inhibitory effect on NF-κB activity. As expected, the overexpression of FKBP52 enhanced NF-κB biological activity, whereas β-catenin impaired the immunophilin effect. On the other hand, the overexpression of FKBP51 showed inhibitory action on the NF-κB activity, and the expression of β-catenin greatly improved that effect in a concentration-dependent manner. Confocal microscopy studies demonstrated that the mere overexpression of the p65 subunit of NF-κB showed β-catenin translocated into the nucleus in unstimulated cells. Both factors, p65 and β-catenin, exhibit nuclear colocalization. Moreover, β-catenin coimmunoprecipitated with p65, indicating the existence of complexes. Interestingly, increased levels of p65 stimulated β-catenin expression. In summary, this study evidences a novel crosstalk between β-catenin and NF-κB pathways that is regulated by the Hsp90-binding immunophilins FKBP51 and FKBP52.

405. 425. **QUINUCLIDINE ETHER DERIVATIVES AS NOVEL LIGANDS OF THE ALPHA7 NICOTINIC RECEPTOR**

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The α7 nicotinic acetylcholine receptor is a ligand-gated cation channel expressed in the brain, mainly in cortex and hippocampus, where it contributes to cognition, attention, and working memory. Its reduced activity has been associated to schizophrenia and Alzheimer's disease. α7 is also expressed in non-neuronal cells, such as astrocytes, microglia and lymphocytes, where it plays a role in inflammation and immunity. Therefore, potentiation of α7 has emerged as a therapeutic strategy for neurological, neurodegenerative and inflammatory disorders. The quinuclidine scaffold was used for the development of nicotinic agonists, with the hydrophobic substituents at position 3 providing selectivity for α7. Here, six new ligands (4–9) containing a 3-(pyridin-3-yloxy)quinuclidine moiety were synthesized, and its pharmacological activity upon α7 was evaluated by two-electrode voltage-clamp and single-channel recordings. Only ligand 4 activated α7. Ligands 5 and 7 had no effects on α7, but ligands 6, 8, and 9 potentiated the ACh-currents. Ligand 6 was the most potent and efficacious of the potentiating ligands, with a EC_{50} of 12.6 ± 3.32 µM and a maximal potentiation of EC_{20} ACh responses of 850 ± 120%. The concentration–response curve of ACh was shifted to the left by 10 µM ligand 6 (control EC_{50} = 125 ± 25 µM; ACh + ligand 6 EC_{50} = 96 ± 30 µM; $p<0.05$). At the single-channel level, the potentiation exerted by 10 µM ligand 6 was evidenced by the appearance of prolonged bursts of channel openings (1.08 ± 0.32 ms) compared to the control (0.38 ± 0.08 ms, $p<0.001$). The burst duration is the most sensitive parameter to determine potentiation and relates to the efficacy of the modulator. Computational studies revealed the preference of ligand 6 for an intersubunit site in the transmembrane domain and highlighted some putative key interactions that explain the different profiles of the synthesized ligands. We conclude that ligand 6 is a novel positive allosteric modulator of α7.

406. 593. **THERAPEUTIC DRUG MONITORING IN EPILEPSY: LEVETIRACETAM**

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Introduction and Objective: Therapeutic Drug Monitoring (TDM) applicability in newer anticonvulsants (AED), such as Levetiracetam (LEV) is controversial. However, it is known that inter and intra individual variability exists, often related to brand changes or variable renal function. Hence, TDM in these patients would be of clinical importance. We aim to present the validation of a quantitative method using High-Performance Liquid Chromatography (HPLC) for AED in serum samples, and its potential application in pharmacokinetic analysis. Materials and Methods: A HPLC-UV reverse phase system was used, at 40°C. The isocratic mobile phase consisted of a mix-

ture of 7.5 mM phosphate buffer/methanol. Theophylline 100 μg/ml as Internal Standard (IS) was added to each 500 μl samples, and then the supernatant was injected. Furthermore, levels of patients undergoing AED treatment from an epidemiological study were measured. Anthropometric data and treatment condition were also collected. Results: The retention times of LEV and IS were 9.8 and 12.5 minutes, respectively. As for specificity, no interferences were detected. Linearity was assessed across levels of 6 to 60 μg/ml, with an R^2 of 0.9984 and a CV% < 15%. Recovery was 101.8% ± 3.8% RSD. Precision was confirmed with intra- and inter-day coefficients of variation below 10%. Stored samples remained stable for 72 hours at 4°C, one week at -20°C, and two years at -80°C. All these parameters were validated. Using this method, 22 samples were quantified. The 6 patients (one of whom was resistant to LEV) exhibited at least one level below the reference range (12-45 μg/mL). During the pharmacokinetic analysis, the resistant patient was differentiated from the responders. Conclusion: The method proved to be simple, fast, robust, cost-effective, and meets the specifications outlined in FDA and local guidelines. Based on the results, this technology would enable the TDM of the local population, with the potential for individualization and therapeutic optimization.

407. 598. **EVALUATION OF THE CARDIOVASCULAR EFFECTS AND PHARMACOKINETICS OF THE COENCAPSULATION OF CARVEDILOL AND QUERCETIN IN NANOMICELLAR DISPERSIONS IN AN EXPERIMENTAL HYPERTENSION MODEL**

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Introduction: Carvedilol is a β-blocker used for the management of arterial hypertension, that is conditioned by its rapid systemic elimination. In previous studies, we showed that encapsulation of carvedilol in nanomicellar dispersions improved oral bioavailability and extended half-life of elimination. This technique also allows the coencapsulation with lipophilic agents. Quercetin is a flavonoid with a potential antihypertensive effect since reduces inflammation and oxidative stress. Based on this evidence, the combination of carvedilol and quercetin would represent a potential strategy to improve target organ damage protection. Aim: To evaluate the pharmacokinetic and hemodynamic response, as well as the impact on target organ damage, of the oral administration of carvedilol, carvedilol/quercetin and quercetin in spontaneously hypertensive rats (SHR). Methodology: Soluplus nanomicellar dispersions were prepared with 3mg/ml carvedilol, 2mg/ml quercetin and 3/2mg/ml carvedilol/quercetin. The pharmacokinetic profile, hemodynamic effects and cardiac/vascular damage markers were evaluated in SHR after acute (single dose) and chronic treatment (8 weeks). Results: Single-dose carvedilol/quercetin showed a statistically greater area under the curve of carvedilol plasma levels compared to carvedilol (1461±210ng/ml.h vs 916±142ng/ml.h), and a greater reduction in heart rate (-23.2±2.8 vs -10.7±1.1%). After 8 weeks, carvedilol/quercetin induced a similar antihypertensive response compared with carvedilol (-12.7±0.7% vs -13.2±1.1%). However, the combination showed more protection against target organ damage than carvedilol, with a statistically significant decrease in cardiomyocyte size (902±51 vs. 610±30μm2) and the left ventricular fibrosis (5.98±0.5 vs. 3.72±0.3μm2). Conclusion: The coencapsulation of carvedilol/quercetin in nanomicellar with a single dose increases the bioavailability of carvedilol and potentiates its bradycardic effect. While, in chronic treatment, the combination offers greater protection against target organ damage in SHR.

P1-PHARMACOLOGY

WEDNESDAY 15TH NOVEMBER 9:00 - 10:30

CHAIRS: NATALIA ALZA
SANTIAGO ZUGBI
CLAUDIA BREGONZIO

408. 164. **EXPLORING THE ANTINOCICEPTIVE AND ANTIDEPRESSANT POTENTIAL OF 3,3-DIBROMOFLAVANONE: A NOVEL THERAPEUTIC CANDIDATE FOR PAIN MANAGEMENT WITH IMPROVED SAFETY PROFILE OVER MORPHINE**

Natalia Colettis#, Josefina Higgs#, Cristina Wasowski, Valentina Pastore* and Mariel Marder*

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In the search for lead compounds with reduced side effects compared to opioids, the synthetic phytochemical 3,3-dibromoflavanone (3,3-DBF) emerged as a promising pain management candidate. 3,3-DBF displayed dose-dependent antinociceptive activity mediated by the μ-opioid receptor in central and peripheral pathways. Unlike morphine (MOR), chronic 3,3-DBF administration demonstrated antinociceptive effects without inducing tolerance, impacting locomotor activity, motor coordination, or causing constipation. This study aimed to explore 3,3-DBF effects in mice, focusing on dependence and depression, a typical pain comorbidity. The dependence effect was examined in mice divided into three group treatments, receiving 3,3-DBF, MOR, or vehicle twice daily for three consecutive days, with doses doubling daily to reach 60 mg/kg (MOR) and 100 mg/kg (3,3-DBF). On day four, half of each group treatment received either naltrexone (1 mg/kg), to induce withdrawal syndrome, or saline as a control. Withdrawal signs induced by 3,3-DBF were assessed using the Gellert-Holtzman scale. Two-way ANOVA followed by Dunnet indicated 3,3-DBF didn't elicit withdrawal signs ($P>0.05$) comparable to MOR ($P<0.0001$). Additionally, acute 3,3-DBF injection (10-30 mg/kg) showed antidepressant-like activity comparable to imipramine (25 mg/kg, $P<0.001$, one-way ANOVA followed by Dunnet) in Forced Swimming and Tail Suspension Tests, reducing immobility time ($P<0.05$, one-way ANOVA followed by Dunnet). In conclusion, 3,3-DBF displayed antinociceptive effects without inducing tolerance or dependence, while exhibiting antidepressant properties. These findings underscore 3,3-DBF's potential as a promising therapeutic agent for pain management, with advantages over MOR in minimizing side effects. The results contribute to developing flavonoid-derived drugs targeting the central nervous system, offering effective, low-side-effect medications for pain treatment and its comorbidities.

409. 187. **ACUTE INFLAMMATORY PAIN MECHANISMS IN LPS PAW-INJECTED MICE**

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Animal models of inflammatory pain have used a number of different irritants injected into skin, paws, muscles, joints and visceral organs. These irritants include those that produce acute inflammatory pain. Lipopolysaccharide (LPS) is one of the most studied pathogen-associated activator of inflammation. Subcutaneous administration of LPS into the subplantar region of rodent hind paws elicits an acute localized inflammatory reaction leading to swelling. Similar inflam-

matory reactions are elicited by other agents as Carrageenan, Zymosan and formalin. Acid-sensing ion channels (ASICs) play important roles in pain conditions. MitTx-1 (a toxin that activates ASIC1) injected in the mouse paw has also been documented to trigger pain behavior in mice. So far however, ASIC1 has only been barely described in the skin. We have previously analyzed the hindpaw injection of formalin to assess acute pain showing ASIC1 levels increased at the spinal cord, anterior cingulate cortex, and dorsal root ganglia after formalin injections. In this work, we analyzed LPS-paw injected mice to focus on localized acute pain and ASIC1 involvement in pain perception. We employed a range of techniques: western blot, immunohistochemistry, von Frey and the hot plate tests. Our initial analysis demonstrated a heightened expression of ASIC1 in the hind paw following LPS-induced inflammation (4-fold increase in LPS-injected versus PBS-injected, p=0.0031, Student`s t-test). The inflammatory process was marked by observable signs such as swelling (20% increase within 4 hours), neutrophil infiltration, and increased sensitivity to mechanical stimuli (more than 5-fold increase in 50% von Frey threshold) and thermal stimuli (45% increase in latency times in the hot plate test).Collectively, our results suggest a significant presence of ASIC1 channels in the skin, contributing to mechanisms underlying pain perception. This insight introduces potential therapeutic interventions for pain relief treatments.

410. 216. **EXPLORING THE POTENTIAL OF 4',5'-DIMETHYL-3-CHLORO-2'-HYDROXYCHALCONE ON BIOLOGICAL TARGETS ASSOCIATED WITH NEURODEGENERATIVE DISEASES**

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The complexity of neurodegenerative diseases (NDDs), such as Alzheimer's disease (AD) and Parkinson's disease (PD), requires multidirectional treatment approaches. The combination of cholinesterases (ChE) and monoamine oxidases (MAO) inhibition, along with strategies to counteract amyloid β (Aβ) aggregation, has the potential to restore neurotransmitter levels and offer a multitarget approach for NDD treatment. Chalcones, a subgroup of flavonoids, have gained attention in the field of NDDs due to their potential therapeutic properties. Extensive research has shown that chalcones possess neuroprotective effects by mitigating oxidative stress, reducing neuroinflammation, and modulating key pathways involved in neurodegeneration. In this study, the synthetic chalcone 4',5'-Dimetyl-3-chloro-2'-hydroxychalcone (**1**) was evaluated on biological targets related to NDDs. *In vitro* experiments were conducted to assess its capacity to inhibit human recombinant monoamine oxidases A and B (hMAO-A and hMAO-B) (Amplex Red method), murine acetylcholinesterase/butyrylcholinesterase (mAChE/mBChE) (Ellman's method), and β-amyloid peptide aggregation (thioflavin T method). *In vivo* studies on male Swiss mice involved cognitive evaluations with the Y-maze test and behavioral assessments using a chemical model of PD (administering 1.5 mg/kg/day of rotenone for 7 days). Chalcone **1** exhibited selective and reversible inhibition of hMAO-B (IC_{50}= 0,354 ± 0,084 μM), selective inhibition of mAChE (IC_{50}= 4,37 ± 0,83 μM), and inhibition of Aβ aggregation (51,6 ± 11,3% at 10 μM). Moreover, it demonstrated positive effects on memory *in vivo* and was able to reverse motor damage induced by rotenone in the PD model. These findings contribute to the existing knowledge on chalcones and highlight the potential of chalcone **1** as a therapeutic tool for NDDs.

411. 293. **GLUCOCORTICOID RECEPTOR TRANSCRIPTIONAL ACTIVITY MODULATES HISTAMINE H1 RECEPTOR SIGNALING**

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Antihistamines and glucocorticoids are commonly used to treat various inflammatory conditions, such as allergic rhinitis and asthma, and they are often administered together. In our previous work, we demonstrated in vitro that histamine H1 receptor (H1R) ligands enhance the anti-inflammatory effects of glucocorticoids (GCs), showing therapeutic benefits in a murine asthma model. Now, our focus has shifted to understanding the reverse scenario: how glucocorticoids affect the signaling of the H1R. To investigate this, we transfected HEK293T cells with plasmids coding to H1R and glucocorticoid receptor (GR). Following a 48-hour treatment with 100 nM of the GC Dexamethasone (Dex), we observed a significant 40% reduction in H1R-induced intracellular calcium mobilization ($p<0.05$), with a t1/2 of 2.4 hours, indicating the desensitization of the H1R in response to Dex treatment. To further explore the underlying mechanism, we conducted binding experiments using the specific H1R ligand [3H]-mepyramine and found a 2-fold increase in specific membrane radiolabel after 48 hours of the GC treatment ($p<0.01$). We then quantified the levels of β-Arrestin gene expression, known to be involved in H1R desensitization, using qPCR. After 6 hours of Dex exposure, we observed a 2-fold increase in β-Arrestin 1 expression ($p<0.05$), which further increased to 3-fold after 24 hours of treatment ($p<0.01$). However, no changes were observed in β-Arrestin 2 levels. Collectively, our results suggest that the GC induces desensitization of H1R calcium signaling through the upregulation of β-Arrestin 1 expression. Moreover, this reduction in H1R signaling may trigger a compensatory mechanism involving receptor overexpression. Considering the co-expression of H1R and GR in several physiological systems and their widespread joint use, the interactions we describe could have significant implications for antihistamine-based therapy, warranting further research.

412. 340. **PHARMACOLOGICAL INHIBITION OF THE RH DOMAIN OF GRK2 INCREASES ENDOTHELIN RECEPTOR RESPONSE IN CARDIOMYOCYTES**

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Endothelin-1 is a potent vasoconstrictor that exerts its effects through two Gq protein-coupled receptors, ETA and ETB. We previously described that ETA desensitization is mediated by GRK2 through two mechanisms: one involving phosphorylation and the other mediated by its RGS-homologous (RH) domain. Considering that ETA desensitization can lead to heterologous desensitization of the insulin receptor and taking into account that arterial hypertension and insulin resistance are comorbidities where GRK2 is up-regulated, we aimed to study the molecular mechanisms linking these three components in isolated rat cardiomyocytes. In isolated hearts of spontaneously hypertensive rats, a model that besides of hypertensive is insulin resistant, we observed by qPCR a 2.7-fold increase ($p<0.01$) in ET1 levels compared to normotensive WKY rats. We assessed whether ET1 pre-treatment could desensitize the cardiomyocyte response to insulin in primary cultured neonatal rat cardiomyocytes. We found that exposure to ET1, for prolonged durations (24 hours), reduced AKT phosphorylation in response to insulin stimulation. We next evaluated the role of GRK2 in the regulation of ET1 response. Incubation of cells with inhibitors of GRK2 developed in our lab targeting the RH domain, led to an enhanced Ca2+ response to ET1, resulting in a leftward shift in the EC50 or an increased maximal response in concentration-response curves. These findings suggest that in cardiomyocytes, the ET1 response is regulated by the RH domain of GRK2, and that desensitization of the ET1 response leads to a diminished insulin response suggesting that targeting the RH domain of GRK2 could hold promise as a potential approach to overcoming insulin resistance.

413. 346. **GRK2 AND HISTAMINE H2 RECEPTOR AS MOLECU-**

LAR TARGETS FOR ACUTE MYELOID LEUKEMIA

Gisela Eliane Gómez[1], Emiliana Echeverría[2], Ana Sahores[1,2], Maximiliano De Sousa[2], Carlos Davio[2], Natalia Fernández[2], and Carina Shayo[1].
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Previously we demonstrated that increases in intracellular cAMP play an important role in acute myeloid leukemia (AML) cell proliferation/differentiation. In this regard, histamine (Hist), through H2 receptor (H2R), rises cAMP levels but fails to promote cell differentiation due to the rapid H2R desensitization (DES) mediated by GRK2. Structurally, GRKs possess an RGS-homology domain (RH) responsible for G-protein activity regulation, a kinase domain engaged in receptor phosphorylation, and a region responsible for membrane localization. Through a docking-based strategy, we identified inhibitors of the RH domain of GRK2 with the ability to interfere with RH actions. The aim of this study was to evaluate the effect of combining Hist treatment with GRK2 inhibitors targeting different domains, on the proliferation of AML cell models. We selected compounds that inhibit RH activity towards Gαs-GPCRs (C3 and C5) or Gαq (C5 and C13) in the screening model, and CMPD as a commercially available GRK2-kinase inhibitor. First, we assessed U937 and HL60 cell proliferation after 72h treatment with 100μM Hist and different concentrations of GRK2 inhibitors, either by cell count or by using Incucyte® cell imaging. We detected a concentration-dependent anti-proliferative effect of C3, C5, and CMPD, with Hist enhancing C3 and CMPD effect ($p<0.05$). Next, we evaluated in U937 cells their capacity to inhibit the DES of the cAMP response induced by 30min Hist pre-exposure, by a radio-binding protein assay. We found that C3 and CMPD increase the Hist-remaining response ($p<0.05$). We also explored public dataset of AML revealing co-expression of H2R and GRK2 in all AML subtypes. No correlation was detected between GRK2 levels and patient survival. In summary, our results contribute to the rational basis of a polypharmacological approach in AML using Hist and GRK2 inhibitors, which deserves further investigation.

414. 394. **DEVELOPMENT OF A TOOL FOR THE DIAGNOSIS AND PLANNING OF THE DEGREE OF MATURITY AND PERCEPTION OF PHARMACOVIGILANCE SYSTEMS**

Reinaldo Adolfo Sanchez de Leon[1], Guillermo Alberto Keller[1], Jose Arturo Berardo[1].
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Background: Pharmacovigilance is an essential task in the public health system of a country. Its development has been implemented irregularly, leading to the existence of heterogeneity in its operation. Aim: to develop a diagnostic instrument useful to provide detailed information on the maturity of a pharmacovigilance system. Methods: A survey of information was carried out (regulations, perceptions of specialized professionals, and experiences of professionals). Data was classified and evaluated by a group of experts and made it compatible with international standards, defining numerical maturity scales for each of the conceptual axes detected. Results: more than 60 interviews with professionals from different fields related to pharmacovigilance (regulatory authorities, professionals, institutions, drug providers, associations, prescribers, and patients). 59 variables were identified, processed and grouped into 6 major groups: System, Documentation, Decentralization, Notifications, Communication and prevention, and Risk evaluation. A numerical assessment instrument was developed for each of the groups of variables with a rating from 1 to 5, which in turn can be grouped into a global rating of the system from 1 (immaturity) to 5 (maturity). The evaluation of different organizations taken in an initial pilot test allowed good discrimination of maturity and the confrontational evaluation of members of the same system showed that the tool allows to assess patients and prescribers perceptions. Likewise, the existence of specific criteria for each score allows forecasting the degree of future maturity based on the completion of action plans. Conclusions: A standardized tool for evaluating the degree of maturity of the pharmacovigilance system can be implemented for the diagnosis, planning of improvements, and evaluation of the perception of pharmacovigilance system.

415. 477. **CANNABIDIOL INHIBITS BK CHANNELS**

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Cannabidiol (CBD), one of the main *Cannabis sativa* bioactive substances, is receiving attention with the speculation that it can be useful in a wide-range of conditions. In particular, CBD is approved for the treatment of major epileptic syndromes. In the central nervous system, several CBD targets have been proposed, including endocannabinoid receptors, and different types of ion channels such as TRPV1, VDAC1, voltage-operated Na^+, and T-type Ca^{2+} channels, among others. The large conductance voltage- and calcium-activated K^+ (BK) channel is involved in fast action potential repolarization, and regulates its shape and duration. BK channel inhibition has been proposed as an anticonvulsant mechanism. Here, we explore the effect of CBD on the BK channel heterologously expressed in HEK cells. Using the patch clamp technique in the inside-out configuration we observed that CBD inhibits BK channel currents in a concentration-dependent manner showing an IC50 of 282,9 nM (pIC50: 6.55 ± 0.06; Hill slope: 0.98 ± 0.12, n=5-9). The direct inhibition of the BK channel by 1 μM CBD results in a half-decrease in the maximal conductance (G_{max}: 0.52 ± 0.06, n=8) and a shift in activation curves to more depolarized voltages ($\Delta V_{1/2}$: 22.9 ± 4.4, n=8). Moreover, CBD significantly delays BK activation kinetic, suggesting a closed channel stabilization (for 1 μM CBD, at +100 mV: τ_{CBD}= 15.5 ± 2.1 ms vs. τcontrol= 5.5 ± 1.3 ms n= 5, $p<0.05$). In addition, the inhibition of the BK channel is also observed when 1 μM CBD is applied from the extracellular face of the cellular membrane in the whole-cell configuration (% of inhibition: 48.3 ± 5.3, n=8). Our results indicate that CBD directly inhibits the BK channel, reducing its voltage dependence and delaying channel activation. These effects could be involved in the probed anticonvulsant activity of this cannabinoid adding a new target to CBD effects.

416. 571. **NEUROTRANSMISSION, A STORY BEHIND THE HISTORY**

Héctor Alejandro Serra, Sebastián Alejandro Alvano, Marcelo Adrián Estrín, Daniel Oscar Fadel, Laura Ruth Guelman, Rubén Iannantuono, Fabiana Ibelli.
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Introduction: The receptor idea radically changed the modern pharmacology and therapeutics. A part of this idea was due to the development of autonomic neurotransmission (NT). In Argentina, the group led by Professor De Robertis developed a fundamental tool for understanding NT functioning, the synaptosome. For more than 40 years the authors of this communication have recognized one of the members of De Robertis group, as their mentor and teacher, the late Professor Luis María Zieher. Objective: Highlight the NT concept and the Professor Zieher role in this history, as a tribute to his work. Methodology: Literature and web search of relevant articles on NT and those written in this regard by Professor Zieher. Results: NT is an intracellular communication by substances inside the nervous system and towards its effectors. In the past, two theories about this were formulated: the electrical one, coming from observations on neuromuscular excitability, and the humoral one, coming from chemical substances effects on peripheral nervous system. The Otto Loewi experiments, around 1920, using a heart preparation in tandem, definitively demonstrated the existence of chemical neu-

rotransmitters released by stimulated nerves. Then, synaptic vesicles were described with the electron microscope help and, in this field, a young Zieher began his career. In his first works, around 1963, he demonstrated that axonal vesicles from rat hypothalamus were the source of the observed norepinephrine high concentration. And then, together with Jaim Etcheverry, he proposed thecotransmission concept. This was contrary to the prevailing idea at that time, proposed by Henry Dale, "a neuron, a neurotransmitter". Conclusion: Seen today NT is a complex, robust and versatile concept, but it should not be forgotten that the human species development and its culture has depended, depends and will depend on the brain cells communication. We owe a small part of this knowledge to Luis María Zieher.

417. 625. **EXPLORING SEROTONIN-GATED ION CHANNELS THROUGH REPURPOSING STRATEGIES**

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A drug repurposing strategy that considers the chemical structure and molecular action on serotonergic Cys-loop receptors offers valuable guidance for the rational reuse of existing drugs, thereby reducing the time and costs associated with developing new medications. We focused on nematode and vertebrate 5-HT-gated ion channels and tested several drugs in clinical use using electrophysiological techniques. In nematodes, a unique serotonin-activated chloride channel, MOD-1, is emerging as a new target for antiparasitic drugs. In humans, 5-HT3A is involved in emesis and is an important player in the enteric nervous system. We previously demonstrated that tryptamine and its derivatives could be good candidates for anthelmintic therapy, acting on the serotonin MOD-1 receptor. We found that sumatriptan and eletriptan, from the triptan family, inhibit 5-HT-induced currents of MOD-1 receptor in a concentration-dependent manner. By using the nematode *Caenorhabditis elegans*, we revealed the anthelmintic actions of sumatriptan and eletriptan at the behavioral level. Our locomotor activity assays showed that both drugs produced a decrease in worms' activity, with eletriptan being more potent than sumatriptan. Mutants lacking MOD-1 were partially resistant to both drugs. Also, we revealed novel aspects of MOD-1 function from the molecular level to the organism level, which may contribute to provide new directions for anthelmintic drug discovery and drug repurposing. By electrophysiology techniques we revealed that the anthelmintic piperazine (PZE), which acts at nematode GABA and MOD-1 receptors, decreased human 5-HT3A macroscopic currents elicited by 5-HT. The analysis showed that PZE acts as a negative allosteric modulator; thus PZE or its derivatives may be explored as promising therapeutic tools that may replace classical orthosteric antagonists. Our drug repurposing strategy contributes to identify new targets and potential uses of drugs on a rational basis.

418. 642. **OBTAINING AND MOLECULAR CHARACTERIZATION OF IONIC COMPLEXES WITH SODIUM PHENYLBUTYRATE AND PHENYLBUTYRIC ACID AS A STRATEGY FOR ALLEVIATING AVERSION IN THE TREATMENT OF UREA CYCLE DISORDERS**

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Sodium phenylbutyrate (SPB), employed for the treatment of urea cycle disorders, exhibits an aversive flavor that undermines treatment adherence. Eudragit EPO (EuE), a cationic polyelectrolyte, can interact with acidic drugs, typically necessitating the incorporation of inorganic counterions to enhance compatibility. The formation of EuE-SPB or EuE-PBA (phenylbutyric acid) complexes emerges as a viable strategy to ameliorate the disagreeable taste, while conferring minimal risk of perturbing the oral absorption profile due to its aptitude to limit release under salivary conditions, which dissipate at pH<5. The objective of this study involves the identification of complexation conditions and molecular characterization thereof. SPB or PBA were interposed with EuE dissolved in ethanol (1 ml/g) at EuE:SPB or PBA ratios of 1:0.5, 1:0.75, 1:1, and 1:1.25, both with and without pre-neutralization using 0.25 HCl. Subsequent to desiccation, materials were subjected to FTIR spectroscopy and thermal analyses (DSC/TGA and hot stage microscopy), utilizing precursors and physical mixtures as references. EuE-PBA spontaneously forms a semisolid material of intricate manipulability at all assessed ratios. Ionic interactions between the dimethylamino groups of EuE and the carboxylic acids of PBA, alongside hydrophobic interactions within lipophilic domains of both substances, were established. Thermal evaluation elucidated a reduction in glass transition temperature with absence of PBA fusion, coupled with no discernible weight loss, implying a loading capacity exceeding 100%. The products were anhydrous, and HCl pre-neutralization can be avoided since it did not induce differences in the evaluated properties. Characterization of EuE-SPB yielded akin outcomes, albeit yielding a manageable solid product with a loading capacity ≤ 50%. EuE-SPB or PBA complexes emerge as prospective candidates for the development of solid oral formulations possessing enhanced organoleptic attributes.

419. 669. **HEPG-2 SPHEROIDS AS A MODEL OF RESISTANT HEPATOCELLULAR CARCINOMA TO STUDY ABC TRANSPORTERS INHIBITORS FOR REVERSION OF MDR**

Andreina Quevedo[1], Natalia Poznanski[1,2], Daniel Zappia[1,3], Roxana Peroni[1,2].

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Hepatocellular carcinoma (HCC) is the most common form of liver cancer, that in turn is the third leading cause of cancer-related mortality. Chemotherapy is the first-choice treatment, but ABC transporter over-expression (MDR) in tumoral cells frequently leads to treatment failure. Since 2D culture drug screening assays had shown poor extrapolation to the clinic, it is crucial to generate MDR models with high prognostic value. Objectives: To evaluate 3D spheroids as models of MDR in hepatocellular carcinoma. Methods: HepG-2 cells were cultured in DMEM with 10% FBS, 100U/mL penicillin/streptomycin at 5%CO_2-95%O_2, 37°C. To form spheroids, cells were seeded onto agar-coated plates (3000 cells/well) for 5 days, monolayers were tested at 70/80% confluence. The induction of ABC transporters was generated by chemically induced hypoxia (100 uM $CoCl_2$ for 24/48 h) or by the presence of the cytostatic doxorubicin (0,1-10 uM; DOX). Viability was tested by the acid phosphatase assay and ABC transporters (BCRP and P-gp) expression was assayed by real-time PCR. Results: Treatment with $CoCl_2$ for 24 h reduced the viability of spheroids by 10% and the monolayer by 15%, but elevated levels of the hypoxia-inducible factor (HIF-1) messenger were selectively obtained in spheroids. DOX significantly reduced cell viability at 0.1uM in monolayer ($p<0.01$) and at 1uM in spheroids ($p<0.001$). Treatment with DOX for 24 hours induced the expression of BCRP and P-gp messengers in both conditions but it was significantly higher in spheroids ($p<0.05$). Conclusions: The spheroids showed a better ABC-related multidrug resistance phenotype compared to the monolayer culture. The present results allow us to suggest that HepG-2 spheroids are a cost-effective and simple, optimized model for the screening of ABC transporter inhibitors that could be used to reverse MDR in hepatocellular carcinoma.

P2-PHARMACOLOGY
WEDNESDAY 15TH NOVEMBER 14:00-15:30
CHAIRS: GUILLERMINA HERNANDO
DANIELA QUINTEROS
SILVINA ALVAREZ

420. 313. **THYROID HORMONE RECEPTOR ALPHA IS EXPRESSED IN A MODULE HIGHLY CORRELATED WITH PROSTATE CANCER TRAITS WITHOUT ANDROGEN RECEPTOR GENE INVOLVEMENT**

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Thyroid hormones' (THs) role in prostate cancer (PCa) development is still unclear. Since genes interact in complex biological networks and an androgen-TH crosstalk has been described, gene network analysis that extracts modules (clusters) of highly correlated genes could be useful to better understand TH action in PCa. To investigate the role of THs through their receptors THRA and THRB and their interaction with androgen receptor (AR) in complex biology networks associated with PCa, we performed weighted gene co-expression network analysis in the RNA-seq TGCA-PRAD dataset by WGCNA package in R. The correlation between modules and PCa clinical traits (PSA value, Gleason score o GS, biochemical recurrence, and clinical T grades) was calculated and the modules with higher correlations were selected. Module membership (MM>0.8) and clinical trait correlation (cor. gene Trait significance > 0.2) were set to identify hub genes, and the NDEx database was used to assess its relationship with existing networks. To study the relationship between selected modules with THs and androgens, MM of THRA, THRB and AR was reported, and enriched gene ontology (GO) terms related to THs action were searched for in the Enrichr database. From the 12 modules obtained, we selected two modules highly correlated to GS, tan (r = 0.46, p = 9e-24) and cyan modules (r= 0.29, p= 7e-10). Tan's hub genes were mainly present in cell cycle networks while cyan's were involved in invasion and metastasis pathways. Tan module only co-express AR gene (MM= 0.21, p=1e-05) and cyan module THRA (MM= 0.28, p= 1e-09). In tan module there were not significantly enriched GO terms related to TH action, whereas, in cyan module we found two terms, "Cellular Response to Thyroid Hormone Stimulus (GO:0097067)" and "Response to Thyroid Hormone (GO:0097066)". Conclusion: TH action through THRA, which co-express in a module highly associated to GS and invasion, could act alone because AR is not involved in this module.

421. 19. **SCREENING OF FDA-APPROVED DRUGS AGAINST *GIARDIA LAMBLIA* TROPHOZOITES VIABILITY**

Jerónimo Laiolo[1,2], Gabriel Luna Pizarro[1], Constanza Feliziani[1], María Laura Guantay[3], Gastón Soria[4], Andrea Silvana Rópolo[1] and María Carolina Touz[1]

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Giardia lamblia is a parasitic protozoan that resides in the small intestine of humans and other vertebrates, causing a disease called giardiasis. Globally, *G. lamblia* is the third most common cause of diarrheal disease in children under 5 years old, with an estimated 280 million clinical cases worldwide. Due to the increasing resistance of the parasite and the side effects of the standard drugs used in giardiasis treatment, there is a need to search for new therapeutic agents. Thus, the objective of this study was to evaluate The Spectrum Collection library, consisting of drugs approved by the United States Food and Drug Administration (FDA), to identify fast-acting and effective compounds against *G. lamblia.* The drugs were initially tested at a concentration of 50 μM, and the viability of *G. lamblia* WB/1267 trophozoites was evaluated using an inverted light microscope and the MTT method. As a positive control, the reference drug metronidazole (MTZ) was also used at a concentration of 50 μM. Out of the 1250 drugs examined, 43 exhibited encouraging results in effectively killing the parasite. Among these, gambogic acid, a naturally occurring prenylated xanthone moiety secreted by the *Garcinia hanburyi* tree, caught our attention. This compound demonstrated superior activity compared to MTZ against *G. lamblia* strains WB/1267 and GS/H5, as well as their MTZ-resistant counterparts developed in our laboratory, with a half maximal inhibitory concentration (IC_{50}) of 3.25±0.33, 3.27±0.46, 3.77±0.01, and 7.32±0.45, respectively. Finally, the effect of gambogic acid on the parasite's ultrastructure was demonstrated through fluorescence confocal microscopy using commercial and monoclonal antibodies. These findings emphasize the importance of optimizing and utilizing existing drugs, including those already in clinical use, to improve giardiasis therapy. Further research and development efforts in this direction hold great potential for overcoming the challenges posed by drug resistance and providing more efficient treatments for giardiasis patients.

422. 32. **UTILIZATION STUDY OF FOSFOMYCIN IN THE BACTERIAL MULTIRESISTANCE CONTEXT IN A POLYVALENT ICU**

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Introduction: The problem of bacterial resistance, and in particular the appearance of multiresistant strains, has led to the revival of old antibiotics, such as colistin and fosfomycin. Objectives: The objectives of this study have been the following: Quantify the degree of exposure to fosfomycin in relation to the most commonly used antibiotics in the ICU in the term of 6 months. Describe which antibiotics and how many cycles thereof were indicated prior to the indication of fosfomycin as an antibiotic plan. Describe the eventual adverse events observed with fosfomycin. Materials and methods: Observational, longitudinal, prospective, and descriptive study, of six months of duration, where the degree of exposure to fosfomycin was quantified according to the indicator DDD-100 bed-day, also we describe the type of antibiotic and the number of cycles of them prescribed prior to the indication of fosfomycin, as well as the eventuals adverse effects attributable to the fosfomycin have been evaluated. Results: During the study period, 6 patients received Fosfomycin, with a consumption of 4.42 DDD-100 bed-day. On average, these patients had an antibiotic cycle prior to the indication of Fosfomycin, except for one patient who received two cycles. Conclusions: In the present study 6 patients received fosfomycin as an antibiotic plan, ranking sixth in the percentage of antibiotic prescriptions in the ICU during the study period.Regarding the consumption of fosfomycin, evaluated by the indicator DDD-100 bed-day, it is 4,42. One patient received two antibiotic cycles, the rest received an antibiotic cycle prior to the indication of fosfomycin.No adverse events attributable to fosfomycin have been observed.

423. 43. **A LECTIN FROM *HELIANTHUS ANNUUS* RECOGNIZES GP120 FROM HIV-1 AND PROMOTES AN INFLAMMATORY PHENOTYPE IN DENDRITIC CELLS**

Maia Chop[12], Melisa Radicioni[13], Marianela Del Rio[13], Juan Sabatté[14], Mariana Regente[13], Christian Rodriguez Rodrigues[12].

1- CONICET 2- Departamento de Química, Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Mar del Plata. 3- Instituto de Investigaciones Biológicas, CONICET-UNMDP. 4- Instituto de Investigaciones Biomédicas en Retrovirus y Sida.

Background: Helja is a mannose-binding lectin that is isolated from sunflower seeds. HIV-1 envelope glycoprotein (gp120) contains high-mannose and complex N-glycans. The aim of this study was to analyze whether Helja recognizes gp120 and induces maturation and pro-inflammatory cytokine transcription in dendritic cells (BM-

DCs). Methods: Helja was purified from Helianthus annuus seeds using D-mannose-agarose affinity chromatography. Molecular interactions between Helja and recombinant gp120 were evaluated by dot and ligand blot approaches using anti-Helja antibodies. BMDCs were cultured in complete RPMI medium supplemented with FLT3-L. Cell phenotype was evaluated in Helja-treated BMDCs by measuring membrane proteins (CD86, CD40, MHCII, CD11c, CD80, Clec9a, Ly6G, SIPRα, CD103 and SiglecH) by FACS. BMDCs cytokine profiles were analyzed using RT-qPCR (il-6, tnf-α, il-12, inf-α tgf-β, and il-10). Results: Helja-gp120 interactions were measured by immunodetection and confirmed by ligand blot assays. A signal dot was observed at a MW of 95 kDa. Next, BMDCs were stimulated with Helja (10 μg/ml) to evaluate cell activation. The lectin induced a marked upregulation of MHCII, MHCI, CD80, CD40 and CD86 (n=3, **p<0.01 Helja-treated BMDCs vs control, *p<0.05 Helja+LPS-treated BMDCs vs LPS control). No changes in Clec9a, Ly6G, SIRPα, CD103 and SiglecH were detected. Finally, Helja also induced the transcriptional activity of pro-inflammatory and antiviral cytokines (il-6, tnf-α, il-12 and inf-α (n=3, *p<0,05, **p<0,01, ***p<0,001 vs control) resulting in an additive effect with LPS. No increase in anti-inflammatory cytokines (tgf-β, il-10) was observed. Conclusions: Helja recognizes specific glycosidic residues and arrangements of gp120 in HIV-1, which induces dendritic cell maturation. This promotes immune activation, leading to the release of proinflammatory cytokines by BMDCs. These results suggest that this lectin has potential biomedical applications as an antiviral agent.

424. 88. **FUCOIDAN EXTRACT ISOLATED FROM *UNDARIA PINNATIFIDA* INHIBITS HSV-1 INDUCED NF-κB SIGNALING PATHWAYS ACTIVATION IN D407 CELLS AND PREVENTS THE BETA AMYLOID SYNTESIS**

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2. Departamento de Química, INQUISUR, Universidad Nacional del Sur (UNS)-CONICET, 8000 Bahía Blanca, Buenos Aires, Argentina.

3. Grupo de Investigación y Desarrollo en Acuicultura y Pesca (GIDTAP), Facultad Regional Chubut, Universidad tecnológica Nacional – CONICET.

Currently, the use of antiviral drugs has been limited due to the generation of drug-resistant viral strains. Additionally, in certain age groups, their implementation is not recommended due to their high toxicity and limited effectiveness. In the present time, the trend in scientific and clinical research is towards the use of natural antiviral compounds as a more attractive option, as they are biodegradable and have minimal or no side effects. Fucoidans are sulphated polysaccharides obtained from brown seaweed. Studies have shown that certain fucoidans could reduce neurotoxic and inflammatory effects and have antiviral action. However, the role of fucoidans from *Undaria pinnatifida* on inflammation and amyloid beta peptide formation induced by viral infections in retinal pigment epithelial cells has not been explored yet. Objectives: To employ fucoidans derived from *Undaria pinnatifida* algae as potential antivirals and determine their role in inflammation and amyloid beta peptide formation in retinal pigment epithelial cells (D407). For this purpose, we used the HSV-1 virus, which is closely related to neurodegenerative pathologies. Results: the treatment with these fucoidans reduced viral titers in D407 cells, with an IC50: 0.8 μg/ml and a CC50: 736.9 μg/ml, resulting in an SI: 921.1. Additionally, we observed that treatment with a dose of 100 μg/ml reduced amyloid beta peptide synthesis by almost 100% at 24 hours by immunocytochemistry techniques, significantly inhibited the p65NFkB´s phosphorylation by western blot techniques, and reduced cell death by MTT assay. Additionally, a significant reduction of IL-6 was observed through real-time PCR techniques. These data together suggest that the fucoidan extract Isolated from *Undaria pinnatifida* Inhibits HSV 1-Induced NF-κB Signaling Pathways activation in D407 cells and prevents the beta amyloid formation.

425. 137. **DNA DAMAGE AFTER IN VITRO IRRADIATION WITH β-EMITTER IODINE-131**

Lara Negrin[1], Laura Mazzitelli-Fuentes[1], Virginia Venier[1], Humerto Romano[1], Lucía Pereira[2], Jeronimo Leberle[3], María Soledad Ausas[1], Vanesa Biolatti[1].

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Objectives: The study of the radiobiological aspects related to metabolic therapy represents an essential tool for understanding the basics of radionuclide action and designing potential therapeutic optimization strategies. The aim of this work was to study the effect of *in vitro* irradiation with β-emitter ^{131}I in peripheral blood cells, exposed to different irradiation periods, in order to analyze DNA damage. Materials and Methods: Blood samples from healthy volunteers were incubated with 10, 50 or 100 μCi of ^{131}I, during 1, 4 and 24h. Estimated absorbed doses (D) ranged from 1-223 cGy. After each incubation time, DNA damage response was determined by γ-H2AX foci and dicentric assays. Results: An increase in the number of foci per cell was observed in irradiated samples for 1 and 4h, compared to control samples. Remarkably, the 24h sample did not show an increment in foci number, revealing a change in damage kinetics. Regarding the dicentric assay, an increase in chromosomal aberrations (CA) was detected in samples exceeding D of ~10 cGy, for all time periods. When compared with external X-ray acute irradiation data, a similar amount of CA was observed for D lower than 100 cGy. However, for higher doses, after 24h of continuous irradiation CA frequency was lower than its equivalent for X-rays. Conclusion: *In vitro* analysis showed the dynamics of damage induction is time-dependent: once the system was perturbed, a rapid increase in the number of foci was observed, which decreases over time, despite the persistence of irradiation by the radionuclide. Likewise, the number of CA found after 24h was lower than in acute X-ray exposures, suggesting continuous irradiation is less genotoxic than acute exposures. This work demonstrates that continuous exposure promotes a particular cell response. Its radiobiological characterization will help elucidate the dose-response relationship in clinical radionuclide treatments and provide the biological basis to optimize therapy planning.

426. 206. **IN SILICO STUDY OF ECHINOCOCCUS GRANULOSUS GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE ASSOCIATED WITH BISPHOSPHONATES**

Facundo Ariel Aguero[1,2], Andrea Maglioco[1,2], Margot Paulino[3], Alejandra J Juaréz Valdés[1], Emilio AJ Roldán[1], Alicia G Fuchs[1,4]

1-centro de altos estudios en ciencias humanas y de la salud-universidad abierta interamericana. 2-consejo nacional de ciencia y tecnología. 3-departamento de experimentación, teoría de la estructura de la materia y sus aplicaciones, facultad de química, universidad de la república, uruguay. 4-instituto nacional de parasitología "dr. mario fatala chaben"-administración nacional de laboratorios e institutos de salud "dr. carlos malbrán"

The *Echinococcus granulosus* (Eg) produces cystic echinococcosis, a zoonotic disease, in ungulate animals and in humans. Argentina reported 630 human cases in 2018-2019. In our laboratory we work using a cellular line from Eg G1 protoscolices, EGPE. The bisphosphonates (BF) on EGPE decreased cell growth and ATP, increased the total calcium but decreased the Ca2+. The ethidronate (EHDP) was effective and the alendronate (AL) had no effect. We identified the Eg's glyceraldehyde-3-phosphate-dehydrogenase (GAPDH), a tetramer, UniProt W6UJ19 by MS/MS. We investigated whether GAPDH, could be druggable with EHDP and AL. Materials and Methods: A validated GAPDH model bound to NAD+, substrate, Pi and BF was subjected to molecular dynamics simulation (MD) for 100 ns in NAMD2. Structures were analyzed and visualized with software VMD 1.9.3 and MOE2022. Potential energy, RMSD and RMSF from the system with or without BF (control) were calculated. Binding free energy with ligands was calculated by MMPBSA in the last 100 frames of the MD. T-test was used to compare control

and samples. Results: The EHDP was placed close to the Pi site and did not allow the Pi binding and the AL interacted with Thr154, Gln211, Thr 212, Gly213 and Arg235 impeding the substrate binding. Binding free energy for EHDP and GAPDH was favorable only in monomers A and B. The control showed favorable interaction with substrate only in monomer A (-10.7±5.1 Kcal/mol), while in presence of EHDP, the substrate had favorable interaction with 3 monomers (A, C, D). Significant differences were found between the binding free energy estimated for the substrate with each monomer with or without EHDP ($p<0.05$). Binding free energy for AL and GAPDH was favorable only in monomer B (-1.3±2.2 Kcal/mol) and binding free energy for NAD+ increased in the monomers C and D ($p<0.01$) in presence of AL. Conclusion: Both BF affected the enzyme in different manner but if the enzymatic activity is modified by BF must be study.

427. 380. **ANTIBACTERIAL PROPERTIES OF A FUNGAL EXTRACT AGAINST *SALMONELLA SPP***

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Salmonella spp is a gram-negative bacteria responsible for salmonellosis, a foodborne disease of significant public health concern. Infection in humans occurs through the ingestion of contaminated water or food. This type of disease is treated with antibiotics; however, its excessive use has generated strains resistant to treatment. Therefore, the search for new treatments is essential. Compounds obtained from fungi present great potential due to their diversity and unique bioactivity. Different fungal antibacterial compounds like secondary metabolites, polysaccharides and proteins have been isolated. Taking this into account, the objective of this work was to find new fungal extracts with antibacterial activity against *Salmonella spp*. In order to achieve this goal, fruiting bodies of *Agaricus bisporus var brunnescens* (portobello) were lyophilized and homogenized with 50 mM phosphate buffer (pH 7). Homogenates were centrifuged and the supernatant was size-fractionated into two molecular weight fractions: F- (< 10 kDa) and F+ (>10 kDa), using ultrafiltration. Protein concentration was measured by Bradford method. The antimicrobial activity of the different fractions against *Salmonella enterica* subsp. *enterica* serovar Typhimurium and *Salmonella enterica*, subsp. enterica serovar Enteritidis was evaluated through the microdilution method. Wells of a microcuvette were inoculated with the strains under study and different concentrations of fungal fractions. After 24 h of incubation at 37ºC, turbidity was measured by spectrophotometry. To confirm the data obtained, plate count method was performed. Our results showed that the F- fraction (0.5 mg/ml protein concentration) was able to completely inhibit the growth of both strains. Therefore, the fraction obtained represents a potential alternative for the treatment of diseases caused by *Salmonella spp*, as well as an alternative to reduce the presence of these microorganisms in food.

428. 414. **LIVE-CELL IMAGING ANALYSIS FOR DETECTION OF NEUTRALIZING ANTIBODIES AGAINST PATHOGENIC NEW WORLD MAMMARENAVIRUSES**

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Pathogenic clade B New World Mammarenaviruses (NWMs) are the etiological agents of Argentine, Venezuelan, Brazilian, and Bolivian hemorrhagic fevers. NWMs include Junín (JUNV), Guanarito (GTOV), Sabiá (SABV), Machupo (MACV), Chapare (CHAV), and a new Sabiá-like (SABV-L) viruses. Viral Pseudotyped Particles (VPPs) with NWM glycoproteins are widely used for Virus Internalization Assays (VIA) as BSL2-safe alternatives to live virus assays. VIA of VPPs can be quantified by flow cytometry through the detection of fluorescent cells that express a GFP reporter gene. However, this method is time-consuming, requires cell fixation in suspension, and is unsuitable for living cells. To address these limitations, we propose the adoption of Live-Cell Imaging (LCI) as an alternative approach for VIA quantification. In this study we compared VIA of NWMs in the presence of plasma samples from Argentine Hemorrhagic Fever convalescent patients (P-AHF) comparing LCI with flow cytometry. VPPs of NWM and Lassa virus (LASV) were generated transfecting HEK-293T cells with a three-vector system: a) MuLV gag/pol polyprotein; b) eGFP; and c) surface glycoproteins GP1/GP2 for each virus. VIA using VPPs was concurrently conducted through LCI and flow cytometry to quantify the neutralizing activity of two different P-AHF along with a negative control. With LCI we observed that high titer P-AHF (1:10240) completely blocked the internalization of JUNV VPPs and partially blocked MACV, while showing negligible effects on CHAV, SABV-L and LASV VPPs. A lower titer P-AHF (1:320) significantly blocked JUNV but did not affect the other VPPs. Importantly, equivalent results were observed in parallel using flow cytometry. In conclusion, LCI demonstrates potential as an alternative tool to flow cytometry for a reliable and fast VIA of NWMs. This methodology holds promise for the titration of neutralizing antibodies within blood samples and the evaluation of novel agents that aim to viral entry inhibition.

429. 417. **MANIFESTATION OF ACUTE INTERMITTENT PORPHYRIA: BIOINFORMATICS ANALYSIS OF THE *NR1L2* GENE**

Priscila Pagnotta[1,2], Johanna Zuccoli[3], Viviana Melito[1,3], Victoria Parera[3] y Ana María Buzaleh[1,3]
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Acute Intermittent Porphyria (AIP) is a metabolic disease in which the mutation in Porphobilinogen deaminase is not enough for the manifestation of the symptoms. We observed that *ABCB1* variants would contribute to its triggering either bioinformatics or experimentally. The aim was to evaluate *in silico* the influence of variants in *NR1I2*, a gene that encodes for PXR receptor, regulator of *ABCB1* expression, on AIP onset in relation to porphyrinogenic drugs. Four *NR1I2* SNVs (rs12721613, rs2472677, rs12721607 and rs12721608) and the databases gnomAD, PharmGKB, Gene Expression Omnibus, UniProt and GenBank were used. Allele frequencies varied among different geographic regions and ethnicities, reinforcing the relevance of local control group analysis. T allele of rs2472677 was associated with a phenotype of toxicity, a differential metabolism and efficacy for drugs contraindicated for AIP (Isoniazid, Rifampicin and Efavirenz). Considering models to infer liver toxicity, Rifampicin induced down expression of *ABCB1* and 8 *CYP*s genes, including *CYP3A4*, in primary culture of human hepatocytes (GSE139896); Isoniazid caused differential expression of 11 *ABC*s genes (27.3% down expressed) and 18 *CYP*s genes (61.1% down expressed) in a human liver cancer cell line (GSE168473). No data were reported for the other variants and the association with toxicity or differential metabolism in the analyzed databases. It is interesting to investigate the role of drugs on gene expression both in drug metabolizing and transport systems, in addition to the allelic and genotypic frequencies of the corresponding variants. Genetic variants of *NR1I2* and their levels of expression could contribute as a possible actor in the drug-mediated AIP triggering factors. It is therefore of interest to continue experimentally exploring variants in this gene in controls and AIP patients.

430. 676. **HEMODYNAMIC AND CARDIOPROTECTIVE EFFECTS OF SUSTAINED-RELEASE SUBCUTANEOUS AND ORAL FORMULATIONS OF CARVEDILOL IN SPONTANEOUSLY HYPERTENSE RATS**

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1 University of Buenos Aires, Faculty of Pharmacy and Biochemistry, Department of Pharmacology, Buenos Aires, Argentina. 2University of Buenos Aires, Faculty of Pharmacy and Biochemistry, Institute of Pharmaceutical Technology

and Biopharmacy (InTecFyB), Buenos Aires, Argentina. 3University of Buenos Aires, Faculty of Pharmacy and Biochemistry, Department of Pharmaceutical Technology, Buenos Aires, Argentina. 4University of Buenos Aires, Faculty of Medicine, Department of Pathology, Institute of Cardiovascular Physiopathology (INFICA), Buenos Aires, Argentina.

Objectives: To evaluate the in vivo release and cardioprotective efficacy of carvedilol subcutaneous implants developed with ε-polycaprolactone (PCL) and Soluplus (SP) (Imp-C), carvedilol-loaded polymeric micelles (Micelles) and PCL microparticulate system containing carvedilol (SM-C) in spontaneously hypertensive rats (SHR). Methodology: Male SHR were treated with Imp-C 100:100:50 mg (CAR:PCL:SP) (n=5), SM-C 1:6 mg (CAR:PCL) (n=5, Micelles (5mg/mL) and Control (n=5) for 2 months. Serum carvedilol levels, systolic blood pressure (SBP) and echocardiographic parameters were determined throughout treatment. Target organ damage was evaluated histologically and by means of the ventricular hypertrophy index, and molecular markers of fibrosis and inflammation were measured by Western blot. Results: All treated animals presented plasmatic concentrations of carvedilol up to 300 ng/mL. All treatments significantly reduced indirect SBP values compared to the control group. Echocardiographic parameters were significantly improved in the treated rats. Left ventricular fibrosis assessed by histology was significantly lower in the treated groups compared to the control group. (LV Fibrosis %, Control vs Imp vs Sm-C vs Micelles : 7.92 ±1.81 vs 2,11 ± 0.22 vs 2.03 ± 0.31 vs 5,63 ±1,17; p<0,05) LV weight was lower in SM-C and in Implants compared to control (LV weight/Animal weight : 3.0±0.1 mg/g vs 3.1±0.1mg/g vs 3.9±0.2mg/g; p< 0.05). The expression of TGFB, Il-6 and TNF-α was significantly lower in treated animals compared to controls. Conclusion: The formulations provided stable plasmatic levels of carvedilol during the course of treatment, providing a sustained decrease of peripheral and central SBP and the reduction of molecular markers of fibrosis and inflammation. The subcutaneous extended-release systems of carvedilol developed represent an effective treatment for the prevention of end-organ damage associated with arterial hypertension.

431. 78. **ANTIBIOTICS PRESCRIBED FOR THE TREATMENT OF LOWER RESPIRATORY TRACT INFECTION IN PATIENTS AFFILIATED OF THE SOCIAL SECURITY, CORRIENTES 2022**

Juliana Pujol, María Teresa Rocha, Sergio Daniel Morales, Rocío Paola Cardozo, María Eugenia Horna, Lorena dos Santos Antola, Isabel Hartman.
School of Medicine. National University of the Northeast

Objective: to characterize the prescriptions of antibiotics (ATB) for the lower respiratory tract infections (LRTI) treatment in outpatients of a social security institute of Corrientes. Drug utilization study, prescription-indication type. All prescriptions from affiliates of any age and sex, containing ATB for systemic use indicated for LRTI during four month of 2022 were included. Variables: sex, age, diagnosis of the prescription, pharmacological group, ATB prescribed; rationality of the prescription (according to clinical practice guidelines). A total of 810 prescriptions were registred, 484 (59.8%) corresponded to respiratory infections. Women 57%; average age 31 (± 22 years). In addition, 191 (40%) were for LRTI. Taking into account the relationship between diagnoses and ATB prescribed according to age, the most frequent were: acute bronchitis -AB- (66%): azithromycin, levofloxacin, amoxicillin+clavulanic acid, clarithromycin were prescribed in adults (>18 years) in order of frequency. In children and adolescents: amoxicillin, azithromycin, clarithromycin, amoxicillinca + clavulanic acid, levofloxacin. For pneumonia (16%): in adults: amoxicillin+ clavulanic acid, levofloxacin, clarithromycin, ampicillin+dipyrone+guayphenesin; in children and adolescents: amoxicillin, azithromycin, amoxicillin+clavulanic acid, clarithromycin, cephalexin. For Influenza-flu (12%): adults: levofloxacin, amoxicillin+clavulanic acid, azithromycin, amoxicillin; in children and adolescents: amoxicillin, amoxicillin+clavulanic acid, azithromycin, clarithromycin, amoxicillin+ambroxol, amoxicillin+acetylcysteine. Conclusions: the most frequent pathology found was BA and the treatment instituted was with ATB macrolides in adults and beta-lactams in children, according to the recommendations of scientific societies. However, the available evidence does not show efficacy in the ATB treatment of BA (the majority of viral origin); so we can suspect an unnecessary prescription.

P3-PHARMACOLOGY

THURSDAY 16TH NOVEMBER 9:00 - 10:30
CHAIRS: NATALIA ALZA
SANTIAGO ZUGBI
ANDREA CARINA CUMINO

432. 49. **PHARMACOKINETIC INTERACTION BETWEEN TACROLIMUS AND MEROPENEM IN PEDIATRIC KIDNEY TRANSPLANT PATIENTS**

Lucas Brstilo[1,2,3], Natalia Riva[4], Ignacio Bressan[5], Karen Larsen[2,6], Maria Agustina Astolfo[5], Manuel Molina[1], Santiago Zugbi[1], Guido Trezeguet Renatti[1,2], Marcos Paz[7], Andrea Bosaleh[8], Nieves Licciardone[9], Oscar Imvertaza[10], Marta Monteverde[7], Guillermo Virkel[2,6], Paula Schaiquevich[1,2]
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[4]Department of Pharmaceutical Technology and Chemistry, University of Navarra. [5]Laboratory of Mass Spectrometry, Hospital Italiano de Buenos Aires. [6]Pharmacology Laboratory, Centro de Investigación Veterinaria Tandil (CIVETAN). [7]Renal Transplant Unit, Hospital de Pediatría JP Garrahan. [8]Pathology, Hospital de Pediatría JP Garrahan, [9]Central Laboratory, Hospital de Pediatría JP Garrahan. [10]Liver Transplant Unit.

Introduction. Individualized therapy with tacrolimus is essential to ensure graft survival in patients with solid organ transplants. Tacrolimus is mainly metabolized by CYP3A4/5. The concomitant use of drugs possessing CYP3A4/5 modification activity may affect tacrolimus levels, impacting treatment effectiveness and safety. Objectives. To describe the incidence and characterize the interaction mechanism between tacrolimus and meropenem in pediatric kidney transplant patients. Methods. A longitudinal, observational, retrospective and prospective evaluation was developed in pediatric renal transplant patients treated with tacrolimus and meropenem. Dose-normalized tacrolimus trough concentrations (C0/D) were compared with and without meropenem. The Drug Interaction Probability Scale (DIPS) was performed to assess the probability of the drug interaction causation. Microsomes obtained from pediatric livers were genotyped for CYP3A5 polymorphisms and used for the *in vitro* drug-drug interaction study. Results. 26 patients aged 13.4 years (range: 3.1-18.0) were included of whom 10 (38.5%) presented the interaction which was classified as 'probable'. In this subgroup of patients, the median (range) C0/D increased by 76.6% (range: 40.8-463.7) in presence of meropenem (p<0.01). An uncompetitive inhibition mechanism was observed *in vitro*. The maximum metabolism rate (Vmax) and the Michaelis-Menten constant (Km) of tacrolimus decreased in presence of meropenem by 56.9 and 34.8%, respectively. The intrinsic clearance of tacrolimus decreased by 34% in presence of meropenem in microsomes with CYP3A5*3/*3 genotype while no change was observed in microsomes with CYP3A5*1/*3 genotype. Conclusions. We report a novel interaction between tacrolimus and meropenem in pediatric kidney transplant patients and confirm the mechanism using human liver microsomes. Close monitoring of tacrolimus when co-administered with meropenem, particularly in patients with CYP3A5*3/*3 genotype, is recommended.

433. 68. **RELEVANT CYTOTOXIC ACTIVITY OF A NOVEL PEGYLATED CHRYSIN ON HUMAN HEPATOMA CELL LINE (HEPG2)**

Fátima Belén Gasser[1], Julia Oggero[2], Ma. Florencia Beltramino[1], Carolina Engler[1], Candela Simonetto[1], Ma. Eugenia Baravalle[1], Hugo Ortega[1], Santiago Vaillard[2], Victoria Vaillard[2].
[1] Centro de Medicina Comparada, Instituto de Ciencias Ve-

terinarias del Litoral (ICIVET Litoral UNL-CONICET), Universidad Nacional del Litoral (UNL), Esperanza, Santa Fe, Argentina.
[2] Instituto de Desarrollo Tecnológico para la Industria Química (INTEC UNL-CONICET), Universidad Nacional del Litoral (UNL), Santa Fe, Argentina.

Chrysin is a natural flavonoid found in honey and plants which has several biological properties such as antioxidant, anti-inflammatory and antitumoral capacity. PEGylation of hydrophobic drugs improves their aqueous solubility, cellular uptake and overall therapeutic efficacy. In this study, we investigated the effect of a novel PEGylated chrysin (CP), achieved by substitution reaction, on in vitro cytotoxicity, reactive oxygen species (ROS) and Lactate dehydrogenase (LDH) production, genotoxic activity and cell migration in human hepatoma cells (HepG2). The cytotoxic activity of CP was evaluated after exposure with increasing concentrations (10-200ug/ml) and Paclitaxel (PTX) as positive control for 48h through the MTT assay. The leakage of LDH into the medium as an indicator of cell membrane damage was detected with an LDH kit (Wiener Lab) and triton X-100 was used as positive control. Intracellular production of ROS was evaluated using staining with dihydroethidium and analyzed by flow cytometry. The genotoxic activity of CP was evaluated using micronucleus assay and cell migration evaluation was carried out using the in vitro scratch wound-healing assay. A dose-dependent decreasing cell viability curve and increasing LDH production was obtained in HepG2 cells. The IC_{50} value obtained for CP was 55 μg/ml. Treatment with CP at concentration of IC_{50} and IC_{50}/2 values produces dose-dependent ROS compared to untreated cells ($p<0.05$). These results suggest cell membrane damage and oxidative stress as possible action mechanisms for CP on HepG2 cells. In addition, the percentage of MN in binucleated cells treated with CP were not significant compared to the untreated cells ($p<0.05$), suggesting a low mutagenic activity. In addition, treatment with IC50/2 concentration inhibiting cell migration compared to untreated cells ($p<0.05$). Therefore, the novel CP might be a potential anti-metastatic chemotherapeutic agent for both cancer prevention and treatment.

434. 99. **CONSUMPTION OF ANTIDIABETIC DRUGS IN A POPULATION WITH SOCIAL COVERAGE. CORRIENTES-ARGENTINA, 2022**

Isabel Hartman, Rocha María Teresa, María Eugenia Horna, Sergio Daniel Morales, Dos Santos Lorena.
Department of Pharmacology. School of Medicine. National University of the Northeast.

The objective of the study was to determine the pattern of consumption of antidiabetic drugs (ATD), insulins and non-insulins among affiliates with diabetes mellitus (DM) of a social security, in Corrientes-Argentina. Observational, descriptive and cross-sectional study on ATD consumption from a drug dispensing database from January to December 2022. As an indicator of consumption, the Defined Daily Dose per 1000 inhabitants per day (DHD) suggested by the WHO. The data were loaded into an Excel 2013 spreadsheet and analyzed using descriptive statistics, with the Epi Info 7 program. Estimating the mean and its standard deviation (SD) for the numerical variables and proportions for the categorical variables. A total of 5,231 ATD prescriptions were made for the 560 members with DM, 40% for women and 60% for men. Average age 59 years, SD±11. The total DHD was 37.13. Corresponding to insulins 7.02 DHD (analogous 6.32 DHD and human 0.7DHD) and non-insulin drugs 30.10 DHD (metformin 11.42 DHD, gliptins 7.39 DHD, sulfonylureas 5.59 DHD, gliflozins 4.60 DHD, incretins 0.80 DHD, glitazones 0.2 4DHD and glinids 0.05DHD). Monodrugs: 81.66% of prescriptions. Of the 9 fixed-dose drug combinations, 8 contained metformin. In conclusion, ATD consumption predominates in men. Most of the insulins were analogous, which are the most expensive and not necessarily the most effective. Among the non-insulin medication, the consumption of metformin predominates, the drug of first choice in the treatment of DM2, which is the most prevalent. Search about the consumption of ATD is of vital importance as a source of information to design coverage policies aimed at the rational use of drugs in this disease with high morbidity and consequences for the individual, the family and social security.

435. 181. **GENUINE ESSIAC (ESSIAC LATINOAMERICA): RADIOPROTECTIVE PROPERTIES OVER IONIZING RADIATION AND CHEMICAL SUBSTANCE PROMOTERS OF REACTIVE SPECIES PRODUCTION**

Veronica L. Martinez Marignac[1], Gloria S. Oertlin[1], Lucia Cervantes[1,2], Leonel Mondragon[1,3], Jose Luis Favant[1,2].
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We demonstrated previously that ESSIAC tea, a freely available 8 herbs tea in the United States and Canada, has an antioxidant and repairing action against radiation damage. ESSIAC tea has shown potent thus controverted antitumor activity, and its herbs ROS scavenger feature.Here we intend to evaluate its capacity to prevent or repair damage produced by chemical ROS production substances, in this case by very low dose of chloroform, a model developed to study hepatic fibrosis.On a validated murine model, we performed acute exposure to 40mSv XRay and chloroform injection, we divided randomly BALB/c male mice in 6 groups: controls, tea drinkers, irradiated, chloroform injected, tea plus irradiated and tea plus chloroform injected mice. XRay and chloroform were acute administered on week 11, while tea was administered ad libitum instead of water from week 6 and we perform sacrificed on week 11 (24 or 48h after chemical or physical injures). ROS production was confirmed by alkaline comet assay (ACA) on peripheral blood after 24 and 48h; a significant comet % reduction on animals receiving ESSIAC plus chloroform or IR administration (pValue<0.01) at 48 h after injures. Liver, small and large intestine sections were subjected to standard H&E staining and examined under light microscope.The necrosis and inflammation were accessed and scored by single blind procedure.Comparisons showed significant differences on tissue damage and on inflammation, in chemical and physical damage exposed animals (pValue<0,02) at 24 or 48h after administration of procedures. While in those animal receiving ad libitum tea showed features non different from control mice tissue. We conclude that ESSIAC Latinoamerica protected the tissues (blood, liver and intestine) from chemical and IR injuries, principally due to ROS production. We need to perform more analysis to evidence that ROS is the only or principal factor of harm produced to the tissues, in order to endorse scavenger feature of ESSIAC.

436. 202. **IDENTIFICATION OF CALCIOTROPIC ACTIVE PRINCIPLE IN *SOLANUM GLAUCOPHYLLUM* LEAVES IN THE RíO SALADO BASIN (PROV. OF BUENOS AIRES)**

M. Alejandra Sequeira[1,2], Marcos J. Lo Fiego[1], J. Daniel Coria[3], Ana J. Recofsky[4], Anabella M. Morales Del Mastro[1] y M. Belén Faraoni[1,2]
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Solanum glaucophyllum (Sg) is a species of the Solanaceae family, widely distributed in the flooded area of the Río Salado basin, Buenos Aires province of Argentina. It has been studied since 1960 for its production of calciotropic metabolites, derived from vitamin D_3, particularly 1α,25-$(OH)_2D_3$. Its assimilation from the plant by breeding cattle, gives rise to a specific calcium over absorption disease, known in Argentina as enzootic calcinosis or “enteque seco”, of relevant concern for leading to the detriment of the argentine livestock economy. Nowadays, “enteque seco” disease can be controlled through herbicide treatment or grazing restriction to the

disease-disseminated areas. Inspired on providing an ecological and sustainable solution, the objective of this work is to contribute with knowledge about the metabolomics related to the calciotropic active principle of vitamin D_3 in *Sg* (relevant to the toxicity index of the species), depending on environmental factors, such as different months of the year, and different locations along the Río Salado basin. To this purpose we present the development of a five-consecutive-steps methodology for 1α,25-$(OH)_2D_3$ identification, throughout: 1-selective collection and conditioning of *Sg* leaves; 2- obtaining of extract enriched in intermediary products of the primary metabolism of vitamin D_3; 3-acid hydrolysis of the extract; 4- hydrolysate purification from column chromatography; 5- analytical identification of metabolites. The results obtained evidenced the presence of the calciotropic active principle in the months of November, February and April (2021), and in fields situated in both towns of General Conesa and Dolores, Province of Buenos Aires. In addition, these results lay the groundwork to progress in the development of a quantitative technique, contributing to improve the management and control scheme of this species, in order to avoid the loss of livestock in the Río Salado basin.

437. 233. **PREDICTORS OF ALLOGRAFT INJURY IN LIVER TRANSPLANTATION: HLA EPLET MISMATCH, TACROLIMUS VARIABILITY AND DONOR-SPECIFIC ANTIBODIES**

Guido Trezeguet Renatti[1,2], Julia Minetto[1], Cintia Y Marcos[1], Agostina Arrigone[1], Gabriela Aboud[1], Florencia Degrave[1], Santiago Cervio[1], Hayellen Reijenstein[1], Leandro Lauferman[1], María F D'Arielli[1], Agustina Jacobo Dillon[1], Diego Aredes[1], Daniela Fernandez Souto[1], Cecilia Gamba[1], Marcelo Dip[1], Oscar Imventarza[1], Esteban Halac[1], Paula Schaiquevich[1,2]

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As a consequence of alloactivation, immunological injury to the liver allograft may contribute to the low long-term allograft survival as half of the recipients will require a new organ after 10 years post-transplant. Thus, it is of most importance to identify risk factors of allograft injury to preserve its function. Our aim was to evaluate the correlation between BPAR and demographic, immunological, pharmacological, and transplant-related factors and to characterize the development of *de-novo* DSA (dnDSA) in pediatric liver transplantation. Pediatric patients transplanted between 2018 and 2021 were included and prospectively followed. Donor/recipient pairs were HLA-typed by NGS and the HLA eplet mismatch (eMM) was quantified using the HLA Matchmaker algorithm. Tacrolimus variability was quantified as tortuosity and DSA was evaluated from available serum using LIFECODES Single Antigen Assays. Univariate and Cox multivariate models were used for risk assessment. Sixty-six of the 112 liver transplant patients recruited had full available data and were on tacrolimus as primary immunosuppression. BPAR-free survival at 1 and 2 years post-transplant was 68.1% (95%CI, 57.4-80.8) and 58.7% (95%CI, 47.3-72.8), respectively. Tacrolimus tortuosity (HR 11.50, 95%CI, 4.55-28.97; p<0.001) and HLA-DQ antibody verified (ab) eMM (HR 1.20, 95%CI, 1.02-1.40; p=0.026) were identified as independent risk factors for BPAR. Fourteen of 53 patients with available post-transplant data developed DSA: 11 anti-HLA class II, 2 anti-HLA class I, and 1 patient developed anti-HLA class I and II antibodies. The most frequent DSA observed was anti-HLA-DQ (n=8). No associations between dnDSA and the evaluated risk factors were found in this cohort. In this large cohort of patients, we emphasize the role of tacrolimus variability and immunological risk as factors associated with BPAR. The presence of dnDSA in liver transplantation should be further studied in association with allograft injury.

438. 255. **DEPRESCRIPTION OF DRUGS IN OUTPATIENTS BELONGING TO A SOCIAL SECURITY SYSTEM OF CORRIENTES, PERIOD 2022-2023**

Sergio Daniel Morales, María Teresa Rocha, Isabel Hartman, María Eugenia Horna, Ramon Martínez, Lorena Dos Santos Antola.

School of Medicine. National University of the Northeast.

The objective was the analysis of an educational intervention on outpatients who were chronically prescribed proton pump inhibitor (PPI) drugs (prescription not adjusted to the diagnosis or prolonged use longer than the time stipulated by the clinical practice guidelines) belonging to a Social Security Institute of Corrientes during the years 2022-2023. Chronic prescription is considered when medications are prescribed for 6 months or longer. A drug use study of prescription-indication type was carried out before and after an educational intervention aimed at consumers. Long-term treatment plan request forms (6-month duration plans) in a 1-year period that contained drugs belonging to the PPI group were analyzed. The variables analyzed were: sex, age, diagnoses, medications. Of a total of 850 forms, 43 (5%) corresponded to PPI prescriptions for patients diagnosed with gastritis (n=28), chronic gastritis (n=12) and without diagnosis (n=3). Average age: 59 years. According to sex: 25 corresponded to the male sex and 18 to the female sex. Prior to the educational intervention, the most prescribed drugs were: omeprazole (n=16), esomeprazole (n=12), pantoprazole (n=12), lanzoprazole (n=2), dexlanzoprazole (n=1). After the educational intervention of the 43 observed forms, PPI deprescription was achieved in 8 patients: 3 patients did not present justification or another diagnosis that justified its use, 3 diagnosed with gastritis and 2 with chronic gastritis. Through the educational intervention, it was possible to avoid the overprescription of PPI, in clinical situations in which their chronic use could be considered inappropriate.

439. 345. **TRANS RETINOIC ACID-ASSOCIATED DIFFERENTIATION SYNDROME AS A COMPLICATION IN THE TREATMENT OF ACUTE PROMYELOCYTIC LEUKEMIA. CASE REPORT**

Leonardo Uranga[1,2], Pablo Astrada[2], Silvia Zidarich[2], Nilda Brizuela[1]

1 Chair General Pharmacology. FCM. UNC

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Clinical Pharmacology: We present two clinical cases of patients with acute promyelocytic leukemia (APL) who developed ATRA (trans retinoic acid or tretinoin) syndrome. Case 1:78 years old male who starts with fever and generalized weakness, asthenia and bruising in limbs. Pancytopenia without renal failure. Start with piperacillin/tazobactam plus filgrastim and blood products. Sternal bone marrow puncture-aspiration (PAMO) with confirmation of APL. It begins with trans retinoic acid with increased leukocytes but associates acute respiratory distress syndrome (ARDS) with pleural effusion, grade 3 renal failure (AKIN). He required MRA (mechanical respiratory assistance) , dialysis and dexamethasone, dying with multiple organ failure. Case 2:52-year-old male with asthenia and generalized non-traumatic hematomas and febrile episodes. Pancytopenia with PAMO showing APL. Initiates piperacillin/tazobactam associated with filgrastim and blood products, idarubicin and trans retinoic acid. After 5 days of treatment adds respiratory failure, fever, with evidence of respiratory distress syndrome [ARDS] plus bilateral pleural effusion. Start with dexamethasone. It requires MRA and complicates with pneumonia. ATRA syndrome physiopathologically resembles ARDS with systemic inflammatory response, endothelial damage with capillary leak syndrome plus microcirculation obstruction and ARDS. Dexamethasone 30 mg per day is the treatment of choice and discontinuation of ATRA. Treatment with ATRA generates in up to 25% of cases the differentiation syndrome or ATRA in patients with acute promyelocytic leukemia (APL) manifesting with fever, ARDS, pleural effusion, generalized edema and hepatomegaly. It has high mortality and may be confused or overlapped with pneumonia or nosocomial infection, heart failure or pericarditis. Treatment includes stopping ATRA and using dexamethasone. Both cases presented renal failure and respiratory failure, requiring intensive therapy and mechanical ventilation.

440. 392. **COMPARATIVE ANALYSIS OF THE FREQUENCY OF DRUG INTOXICATION DURING THE COVID-19 PANDEMIC AND PRE-PANDEMIC IN A TOXICOLOGY UNIT**

María Laura Ferreiros-Gago[1], Guillermo Alberto Keller[2], Carlos María Falco[2].

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[2] Hospital General de Agudos Donación Francisco Santojanni, Departamento de Urgencias.

Background: drug intoxication is a frequent problem in clinical practice. During childhood, accidental intoxication predominates, while intentional intoxication predominates in adolescence. Aim: Study the frequency of drug intoxications in a pediatric hospital toxicology service in the period before and during the covid pandemic. Methods: This study combines an ecological observational study (telephone consultations) and a before-after comparison, where the exposure (intervention) would be the epidemiological event of COVID-19 pandemic. Results: 5,572 drug intoxication were registered during two years of pre-pandemic period (2018-2019) and 4,936 during a pandemic period (2020-2021). The pandemic was associated with a lower risk of drug intoxication (RR 0.90 95%CI 0.87-0.94 p < 0.01). Registered drug intoxications were more frequent in females (53%-RR1.03 95%CI 0.99-1.06 p 0.92) and in the preschool age group. During the pre-pandemic, a greater risk of drug was observed (RR 1.07 95% CI 1.03-1.11 p < 0.01) in preschoolers, while during the pandemic a greater risk was observed in adolescents (RR 1.38 95% CI 1.24-1.54 p < 0.01). The pandemic period was associated with a higher risk of intentional intoxication (RR 1.16 95%CI 1.07-1.26 p < 0.01). In 15% of cases two or more drugs were involved. In the pandemic period, there was a higher risk of acetaminophen intoxication (RR 1.61 95% CI 1.42-1.82 p < 0.01), meanwhile ibuprofen has greater number of intoxications during the prepandemic period (RR 1.30 IC95% 1.21-1.38 p < 0.01). The pandemic was associated with a higher risk of intoxication with ATC N03 (RR 1.13 95%CI 1.04-1.22 p 0.022) and N06 group drugs (RR 1.49 95%CI 1.21-1.82 p < 0.01). Intentional cases were associated with a higher risk for N03 (RR 3.03 95% CI 2.80-3.27 p 0.02); N05 (RR 3.21 95%CI 2.83-3.65 p < 0.01) and N06 group (RR 3.66 95%CI 2.99-4.47 p < 0.01). Conclusions: The pandemic was associated with a lower risk of drug intoxication. Drug intoxications predominate in pre-school during the pre-pandemic period, and in adolescents in the pandemic. Higher risk of paracetamol, ATC N03 group, and N06 intoxicaction were detected in pandemic period.

441. 393. **ADVERSE DRUG REACTIONS IN A EMERGENCY DEPARTMENT SETTING**

Guillermo Alberto Keller[1,2], Pablo Roberto Manjarin[1], Roberto Alejandro Diez[2], Carlos Maria Falco[1].
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2 Universidad de Buenos Aires, Facultad de Medicina, Centro de Vigilancia y Seguridad de Medicamentos.

Background: Adverse drug reactions increase morbidity and mortality, prolong hospital stay and increase healthcare costs. Aim: To determine the prevalence of emergency department visits for adverse drug reactions and to describe their characteristics. Methods: A pharmacovigilance committee receives all notifications of adverse reactions. They were classified according to their severity, seriousness, and seriousness criteria. The comparison with the available casuistry of the number of visits to the emergency department allowed to determine the frequency of reactions in each area and the frequency in which they generate hospitalization. Results: 247 reports of adverse reactions were registered. Most of them (225, 91.1%) were not serious. Among the serious ones, those that generated hospitalization (14, 5.7%), prolonged a pre-existing hospitalization (2, 0.8%), life at risk (4, 1.6%), or generated disability (2, 0.8%) predominated. Among non-serious events, the most frequent adverse reactions were gastrointestinal (85, 34.4%), hematologic (40, 16.2%), cardiac (35, 14.2%), and neurologic (31, 12.6%). Among the serious adverse events, hematological (11, 50.0%), dermatological (6, 27.3%) and hepatic (5, 22.7%) predominated. The number of total consultations of patients from which the reports generated by the professionals came is estimated in the same period at 12148, which allows establishing an incidence of adverse reactions in the emergency setting of not less than 2%, being at least 0.2 % cause of serious adverse reactions, often linked to hospitalization. Conclusion**:** Adverse drug reactions in the emergency setting are frequent and generate significant morbidity. The report is lower than previously reported in other centers, suggesting the existence of underreporting. Even so, it makes it possible to establish the existence of indicative levels and frequency of occurrence of adverse reactions for the planning of risk minimization measures.

442. 568. **NOVEL INSIGHTS INTO BASIC RESEARCH: EXPLORING MOLECULAR EFFECTS OF TRANS-CINNAMALDEHYDE AND EUGENOL ON α7 AND MUSCLE ACETYLCHOLINE RECEPTORS VIA ELECTROPHYSIOLOGICAL ASSESSMENT**

Guillermina Hernando, Juan Facundo Chrestia, Sol Leda and Cecilia Bouzat
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Naturally occurring bioactive compound have been used in traditional medicine for the treatment of various diseases for many centuries. In modern times, there has been a renewed interest in natural compounds and essential oils for their medicinal properties. In recent years, it has been demonstrated that many of these natural compounds act on neurotransmitter receptors, including Cys-loop receptors such as the nicotinic acetylcholine receptor (nAChR). nAChRs form a family of ACh-gated ion channels found in the central and peripheral nervous systems, involved in processes like muscle contraction, memory, and attention. In this project, we assessed the molecular-level effects of trans-cinnamaldehyde (TC) and eugenol (EGN) -the two naturally occurring phenylpropanoids present in *Cinnamomum* oil- on two types of mammalians nAChR, which are involved in several pathological disorders. As TC and EGN are multitarget drug, there is a need to establish the molecular mechanisms by which they may exert therapeutic as well as adverse effects. Through single-channel recordings, we observed that TC exerts a negative modulatory effect on both α7 and muscular nAChR. On the one hand, TC leads to a marked reduction of α7 activity by decreasing the frequency of activation episodes without causing changes in amplitude and in the open durations. On the other hand, recordings from muscular nAChR exhibit a concentration-dependent reduction in open channel duration within the micromolar range, induced by both TC and EGN. This change is accompanied by a shift towards shorter durations in the main closed component. The modulation of nAChRs is of pharmacological relevance and should be considered in the evaluation of the potential therapeutic uses of TC and EGN as. Our findings offer insights into how natural compounds influence Cys-loop receptors, which remain unexplored and are important targets in various therapeutic approaches.

443. 582. **THERAPEUTIC MONITORING OF ITRACONAZOLE IN PATIENTS WITH CHRONIC PULMONARY ASPERGILLOSIS: A POSSIBLE SOLUTION TO A COMMON PROBLEM**

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Chronic pulmonary aspergillosis (CPA) is an infectious disease that without treatment, causes a slow and progressive destruction of the lung parenchyma. The monitoring of serum concentrations of antifungals is a pillar of successful treatment, since a sublevel can generate treatment failure and a supralevel, toxicity. However, long-term treatment is far from giving an optimal response to all patients. One of the possible causes of this low efficacy, as well as relapses, could be a deficient plasmatic level of itraconazole. Aims: Implement the therapeutic monitoring (TM) of itraconazole, using a sensitive, precise and fast method and, in case of detection of suboptimal levels, evaluate the possible serological and clinical relationship. Methods: To perform the TM, blood samples were obtained from patients diagnosed with CPA, followed-up by Dr. Javier Muñiz Hospital (CABA), who gave their consent. Samples were analyzed by liquid chromatography (HPLC) after deproteinization with acetonitrile, and

plasma levels were compared with the trough therapeutic range of itraconazole (0.5 to 4.0 ug/ml). Results: Samples were taken from 24 patients diagnosed with CPA and culture, serology, and itraconazoleTM were performed. Those whose plasma level was found to be below the therapeutic range, were re-dosed. The values obtained were between 0.15 and 5.46 ug/ml with a mean level of 1.75 ug/ml. A total of 3 patients (12.5%) showed sublevels of the antifungal. One of them did not make their serology negative and did not show clinical improvement. On the other hand, 1 patient showed levels higher than those recommended. The developed chromatographic method was accurate, economic and fast. Conclusions: Itraconazole TM is a useful tool to improve the efficacy of CPA treatment and the prognosis of the pathology.

444. 644. **DETERIORATION OF ORAL HEALTH IN PEOPLE WITH ANKYLOSING SPONDYLITIS**

[1]Betina Orman, [2,6]María Lis Bianchi, [1]Castro Escalante Angélica, [3]Guillermo Corró, [3] Carlos David Bruque, [4]Sebastián I. Costa, [5]Débora A. González, [2,6]Teresita Ferrary.
Cátedras de [1]Farmacología, [2]Medicina interna, [4]Odontología Integral Adultos, [5]Biofísica y bioestadística. Facultad de Odontología. Universidad de Buenos Aires. [3]Unidad de Conocimiento Traslacional Hospitalaria Patagónica, Hospital de Alta Complejidad SAMIC, El Calafate, Santa Cruz, [6]Instituto de Rehabilitación Psicofísica Buenos Aires. Argentina.

OBJECTIVE: to evaluate the oral status of people with ankylosing spondylitis (AS) METHODS: an observational and cross-sectional study was performed, with 62 patients with AS according to modified New York criteria and 62 controls without rheumatic inflammatory diseases, matched by age (44 ± 12 years) and gender (74% male). Salivary flow at rest (RWS) and stimulated with citric acid (SWS) (ml/5 min) was measured. Decayed and missing teeth were counted (DMFT). To assess periodontal status, clinical attachment loss in mm (CAL) and probing depth (PPD) were measured, expressed as mean (mm) or as number of sites ≥ 4 mm. Periodontal disease (PD) was diagnosed according to the American Academy of Periodontology (1999). Results were expressed as median [min-max], as mean ± SD, or as percentage, and compared using Anova and chi-square, *p<0.01. RESULTS: patients with AS presented lower salivary flow: RWS 1.9 ± 0.7 vs 2.7 ± 0.8 * and SWS 4.2 ± 1.5 vs 5.2 ± 0.9 *. They also showed higher DMFT (9 [0-30] vs 7 [0-16] *), more sites with PPD ≥ 4 mm (7 [0-57] vs 0 [0-9]) *, higher average PPD (3.2 ± 1.6 vs 1.5 ± 1.0) *, higher average CAL (3.4 ± 1.8 vs 1.9 ± 1.6) *. Periodontal disease was diagnosed in 84% of AS patients and 39% of controls *. Among the patients with PD, 52% in the EA group and 25% in the control group had a moderate or severe condition *. CONCLUSIONS: AS was associated with reduced resting and stimulated salivation, and worse dental and periodontal status.

O2-PHARMACOLOGY

THURSDAY 16TH NOVEMBER 14:00-15:30
CHAIRS: GUILLERMINA HERNANDO
HUGO HECTOR ORTEGA
PAULA SCIBONA

445. 26. **IN VIVO AND EX VIVO PHARMACOLOGICAL EVALUATION OF FLUAZURON UPTAKE BY THE CATTLE TICK *RHIPICEPHALUS (BOOPHILUS) MICROPLUS***

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The control of *Rhipicephalus (Boophilus) microplus* is crucial due to its economic impact on cattle production. Fluazuron (FZN), a potent acaricide, has been widely used to combat tick infestations. An understanding of its uptake mechanism by ticks is essential to develop control strategies. This work evaluated *in vivo* and *ex vivo* pharmacological aspects of FZN uptake in *R. (B.) microplus*. In Phase 1, 10 Braford heifers, naturally infested with *R. (B.) microplus* and exposed to a continuous natural challenge with ticks until the end of the trial, were treated with a pour-on formulation of FZN 2.5% (2.5 mg/kg). Plasma and semi-engorged female tick samples were serially taken after treatment. In Phase II, a heifer was experimentally infested with *R.* (*B.*) *microplus* and then, 20 ticks were placed into a tick feeding unit (TFU). The heifer was topically treated with FZN and tick samples from TFU and free ticks (FT) were collected at 12 and 24 h post-treatment. The artificial feeding system was carried out with ticks fed with blood obtained from the heifer before (AFc) and 12 h (AF_{12}) and 24 h (AF_{24}) post-treatment with FZN. FZN levels in both phases were characterized by HPLC. Whereas no correlation was found between the AUC_{0-14} of FZN in plasma and in ticks (p=0.3813), a significant positive correlation was identified between the partial exposure within the 3 to 14-day period (r= 0.8142, p=0.0041). Mean FZN levels in FT at 12 h post-treatment (185.3 ng/tick) were higher than mean FZN levels in FT at 24 h post-treatment (5.70 ng/tick) and in TFU-fed ticks for 12 and 24 h (11.2 and 5.24 ng/tick, respectively) ($p<0.05$). FZN detected in AF_{12} and AF_{24} were similar to those of TFU-fed ticks. Therefore, it appears that within the initial 12-48 h, the uptake of FZN by ticks is primarily through the parasite cuticle. Subsequently, tick concentrations reflect those found in the plasma of treated cattle. The therapeutical implications of these findings should be carefully evaluated.

446. 379. **CROSS-TALK BETWEEN HISTAMINE H2 RECEPTOR AND GLUCOCORTICOID RECEPTOR INFLUENCES CELL PROLIFERATION, DIFFERENTIATION AND APOPTOSIS IN ACUTE MYELOID LEUKEMIA CELLS**

Valeria Torralba-Agu[1], C Daniel Zappia[1], Carina Shayo[2], Luciana Rocha-Viegas[3], Federico Monczor[1].
1. Instituto de Investigaciones Farmacológicas, ININFA UBA CONICET. Argentina.
2. Instituto de Biología y Medicina Experimental, IBYME CONICET. Argentina.
3. Instituto de Fisiología, Biología Molecular y Neurociencias. IFIBYNE UBA CONICET. Argentina.

Numerous studies investigated the individual administration of histamine (HA) and glucocorticoids (GCs) for treating acute myeloid leukemia. However, the joint application of these drugs remains unexplored. Our previous findings revealed that HA and amthamine (Amtha), a histamine H2 receptor agonist, increases the transcriptional activity of the dexamethasone (Dex)-induced glucocorticoid receptor. In this study, we aimed to assess whether this cross-talk had any impact on cell proliferation, differentiation and apoptosis using the U937 leukemic cell line. 96-hour exposure of U937 cells to low doses of Dex (1-10 nM) led to an approximately 50% increase in cell proliferation, whereas higher doses (>1 µM) caused a 50% reduction in cell proliferation ($p<0.01$). Consistently, the assessment of membrane expression of the differentiation marker CD11b, as well as annexin V and propidium iodine staining for cell apoptosis, showed that 1-10 nM Dex reduced spontaneous cell differentiation and apoptosis, while 1 µM Dex increased both cell populations. For its part, though 100 µM HA or 10 µM Amtha exerts no noticeable effect, its co-administration with Dex inhibits the pro-proliferative, anti-differentiation, and anti-apoptotic effects of low Dex doses, while not altering the effects of higher doses. Notably, the MEK inhibitor PD98059 also prevents the increase in cell proliferation of low Dex doses. Examination of Dex's influence on ERK phosphorylation reveals that the GC prompts an increase in pERK levels, an effect counteracted by Amtha. Taken together, our results suggest that ERK phosphorylation underpins the effect of low Dex doses on cell proliferation. Given the potential clinical utility of GCs in restraining leukemic cell proliferation, the fact that HA and Amtha inhibit undesired proliferative Dex effects bears therapeutic significance.

447. 569. **DOES COMPLEXATION WITH COPPER(II) IMPROVE**

PHARMACOLOGICAL PROPERTIES OF SULFADIAZINE?

Juan José Martínez Medina[1], Cristian Villa Pérez[2], Juan Fernando Cadavid Vargas[3], Ana Laura Di Virgilio[2] and Delia Beatriz Soria[2]

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Objectives. The main purpose of this study was to synthesize a ternary Cu(II) complex of sulfadiazine (SDZ) - an antibacterial and antimalarial drug - which includes the 2,2′-bipyridine (bipy) as co-ligand, named Cu(SDZ)bipyCl. The assessment of this complex's toxicological along with the antimicrobial and antitumor features is reported here. Methods. The safety profile was assessed in terms of mutagenicity by the Ames Test using two *Salmonella* strains and acute toxicity by the *Artemia salina* assay. The antimicrobial activity was studied against bacteria and fungi by the agar dilution method. The minimum inhibitory concentration (MIC) was determined against 10 ATCC strains and 10 clinical isolates strains. Moreover, the cytotoxic activity was determined using the MTT method in two tumoral cell lines. Results. Our findings showed that this complex induces neither mutagenicity (frameshift mutations on *S. typhimurium* TA98 or base-pair substitution mutations on *S. typhimurium* TA100) nor acute toxicity on *A. salina* nauplii (until 600 μg mL^{-1}). Besides, the ligand SDZ showed MIC values with clinical relevance (≤ 1000 µg mL^{-1}) against five strains (*P. aeruginosa* ATCC 27853, *E. coli* ATCC 25922, *S. aureus* ATCC 25923, *S. aureus* ATCC 29213, and *C. krusei*). The complex showed relevant antimicrobial activity against these strains and other additional ATCC strains (*S. epidermidis, E. faecalis, C. albicans*, and *C. parapsilosis*) and clinical isolates (*Escherichia, Staphylococcus,* and *Candida* genus). Moreover, the complex showed a harmful effect in human osteosarcoma (MG-63, IC_{50} = 41.8 ± 6.5 µM) and human lung carcinoma (A549, IC_{50} = 37.5 ± 6.7 µM) cell lines with a concentration-dependent behaviour from 10 to 100 µM. Conclusions. Overall, the complexation with Cu(II) and bipy improved the pharmacological properties of SDZ, which resulted in a promising strategy for developing novel antitumoral or antimicrobial agents with an acceptable safety profile.

448. 615. **IN SEARCH OF THE MECHANISM BEHIND CANNABIDIOL'S (CBD) CONTROL OF EPILEPTIC SEIZURES: MODIFICATIONS TO CBD MOLECULAR TARGETS INDUCED BY CONVULSIVE STRESS**

Claudia Taborda[1,2], Florencia Fernandez[1,3], Franco Moscovicz[1,2], Natalia Borda[1], Alberto Lazarowski[1], Jerónimo Auzmendi[1,2]

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Cannabidiol (CBD) is used as an adjuvant in the treatment for the control of drug-resistant epileptic seizures, increasing the plasmatic concentration of antiepileptic drugs (AEDs) presumably by blocking their metabolization. However, DAEs without hepatic metabolization are also increased. In previous work we demonstrated that CBD blocks ABCt. On the other hand, hypoxia and inflammation modify hepatic CYPs and ABCt levels. Since seizures produce a systemic hypoxic/inflammatory state, we hypothesized that the targets on which CBD presumably acts are modified as a consequence of seizures. Using databases (UniProt, PDB, Ensembl, CDD and RGD) we analyzed the correspondence between human-rat cytochromes by sequence homology and conservation of functional domains. Then, we employed the pilocarpine (30mg/kg) induced Status Epilepticus (SE) model and extracted liver RNA to evaluate the expression of CYPs (2C9, 2C19, 2D6 and 3A4) and ABCt (B1a and G2) by RT-PCR. In addition, we evaluated phenytoin consumption using hepatocytes in primary cultures obtained from the same animals. The human-rat sequence identities were between 78.89-84.51% with over 50% coverage. Despite this, the functional domains were conserved. SE induction had an efficiency of 81.1%, mean duration of 127±48 min and lethality of 9.51%. Evaluation of CYP expression showed a decreasing trend that was significant for CYP 2C9 (p=0.0029) and 3A4 (p=0.0212), while ABCt expression was significantly increased (p=0.0110) as a consequence of SE. Consistently phenytoin consumption was decreased 3-5% after SE. Our results show that SE induces a variation of CYPs and ABCt expression similar to those described for inflammatory/hypoxic conditions, suggesting that CBD could increase the plasma concentration of DAEs, in particular those without hepatic metabolism, by blocking ABCt.

449. 623. **ANTI-ECHINOCOCCAL ACTIVITY OF CANNABIDIOL AGAINST *ECHINOCOCCUS GRANULOSUS*: *IN VITRO* AND *IN VIVO* STUDY.**

Albani Clara María[1,2], Fuentes Giselle[1,5], Ramírez Cristina[3,4], Pensel Patricia Eugenia[1,2], Gatti Florencia[1,2], Albanese Adriana[1,2], Elissondo María Celina[1,2]

1- Instituto de Investigaciones en Producción Sanidad y Ambiente (IIPROSAM CONICET-UNMdP); Facultad de Ciencias Exactas y Naturales – UNMdP; Centro Científico Tecnológico Mar del Plata – CONICET; Centro de Asociación Simple CIC PBA, Mar del Plata, Argentina.

2- Laboratorio de Zoonosis Parasitarias, Facultad de Ciencias Exactas y Naturales (FCEyN), Universidad Nacional de Mar del Plata (UNMdP), Mar del Plata, Buenos Aires, Argentina.

3- Departamento de Química y Bioquímica, Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Mar del Plata, Funes 3350, 7600, Mar del Plata, Buenos Aires, Argentina.

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5- Centro de Investigaciones en Abejas Sociales, Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Mar del Plata, Mar del Plata, Argentina.

Cystic echinococcosis (CE) is a global parasitic zoonosis caused by infection with the larval stage of *Echinococcus granulosus sensu lato*. CE affects more than 1 million people worldwide, causing important economic costs in terms of management and livestock associated losses. Albendazole is the drug of choice for the treatment of human CE. However, its low aqueous solubility, poor absorption, and consequently erratic bioavailability are the cause of its chemotherapeutic failures. Based on the problematic described, new treatment alternatives are urgently needed. In the present study, the *in vitro* and *in vivo* efficacy of cannabidiol (CBD), the second most abundant component of the *Cannabis sativa* plant, was demonstrated against *E. granulosus sensu stricto*. CBD (50 μg/mL) caused a decrease in protoscoleces viability of 80 % after 24 h of treatment which was consistent with the observed tegumental alterations. Collapse of the germinal layer was observed in 40 % of cysts treated with 50 μg/mL of CBD during 24 h. In the clinical efficacy study, all treatments reduced the weight of cysts recovered from mice compared with control group. However, this reduction was only significant with ABZ suspension and the CBD + ABZ combination. The co-administration of CBD with ABZ suspension enhance the *in vivo* efficacy of drugs alone, although the differences were not significant. Moreover, the ultrastructural alterations observed in cysts recovered from mice treated with the combination were greater than that provoked with the monotherapy. Further *in vivo* studies will be performed by adjusting the dosage and frequency of CBD and CBD + ABZ treatments.

P4-PHARMACOLOGY
THURSDAY 16TH NOVEMBER 14:00-15:30
CHAIRS: JERÓNIMO LAIOLO
VENTURA SIMONOVICH

NATALIA FERNANDEZ

450. 112. **PROTECTIVE ACTIVITY ELICITED BY *CANNABIS SATIVA* AND *TILIA X VIRIDIS* EXTRACTS AGAINST GLUTAMATE INDUCED OXIDATIVE STRESS IN HT-22 NEURONS**

Elina Malén Saint Martin[1], María Laura Barreiro Arcos[2], Ignacio Peralta[1], Carla Marrassini[1], Laura Cogoi[1], María Rosario Alonso[1], Claudia Anesini[1].

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Oxidative stress (OS) affects the central nervous system in epilepsy. The prevention of neuronal cell death induced by OS might be an interesting therapeutic approach in the treatment of this and other neural disorders. *Cannabis sativa* L. is used in the treatment of epilepsy, being cannabidiol (CBD) its main anticonvulsant compound. *Tilia x viridis* is widely distributed in Argentina and has antioxidant and sedative activities. The aim of this work was to evaluate the effect of an ethanolic extract of *C. sativa* (CSRD), and an aqueous extract of *T. x viridis* (TE) and their association on the OS induced by glutamate (Glu) in the HT-22 cell line. The main compounds in CSRD and TE were identified and quantified by HPLC-MS/MS and HPLC-UV. Cells were pre-incubated with the extracts for 2 hs and challenged with Glu 5 mM for 12 or 24 hs to assess: cell viability with MTT spectroscopically, and reactive oxygen species (ROS) with 2',7'-dichlorodihydrofluorescein diacetate (DCF-DA) in a fluorescence reader and microscope. Results were expressed as media ± SEM. *p< 0,05, ****p<0.0001 Student's t test vs. basal; ## p<0,01, #### p< 0,0001; $ p< 0,05, $$ p<0,01 ANOVA + Dunnett's test vs. Glu and vs. CSRD 1 μg/ml + Glu respectively. Results: CBD in CSRD: 62.36 ± 1.57%w/w. Epicatechin (E) in TE: 0.16 ± 0.003%w/w. Viability: Basal: 100.00 ± 0.81%; Glu: 25.47 ± 0.44%****; CSRD 1 μg/ml + Glu: 47.88 ± 3.51####; TE 500 μg/ml + Glu: 31.96 ± 0.84%####; TE 500 μg/ml + CSRD 1 μg/ml + Glu: 77.79 ± 8.27%$$. ROS: Basal: 100.00 ± 5.15%; Glu: 130.26 ± 10.82%*; CSRD 1 μg/ml+ Glu: 124.40 ± 31.54%; TE 500 μg/ml + Glu: 50.86 ± 2.75%##; TE 500 μg/ml + CSRD 1 μg/ml + Glu: 62.42 ± 6.31%$.

Conclusions: Glutamate reduced cell viability and increased ROS. CSRD 1 μg/ml improved cell viability and reduced ROS production (no statistically significant). TE enhanced CSRD effects. Results suggest the association of *C. sativa* with *T.* x *viridis* could be interesting to assess in animal models of epilepsy.

451. 151. **DEVELOPMENT OF NANOPARTICLES WITH A GALLIC ACID**

[1]Francisco Gualdieri, [1,2]Exequiel Giorgi, [3]Martin Desimone, [1,2]Mauricio De Marzi, [1,2]Liliana N. Guerra

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Nanotechnology is a useful approach to deliver antioxidants into cells. Our aim is to produce silica nanoparticles (SiNPs) with an antioxidant polyphenol molecule, gallic acid (GA), one of the principal bioactives included in carqueja (*Baccharis articulata).* SiNPs were prepared by Stöber method. Carqueja extract (CE) was obtained with a sample of 30 mg dried leaves/mL heated at 70°C for 14 min. We determined its antioxidant capacity (AC) by the DPPH radical method and polyphenol concentration (Pph) by Folin technique (which renders gallic acid content). We evaluated CE effect on neutral lipid content in Hep-G2 cells, used as a model for non-alcoholic fatty liver disease (NAFLD cells), by Oil-Red-O staining. NAFLD was assessed by treating cells with 0.05mM oleic acid for 48h. CE showed AC of 51.5 ± 1.3 % and Pph of 427 ± 50 μg/mL; CE decreased lipid content in NAFLD cells, which is set to 100 arbitrary units (AU) (100 ± 14 AU [NAFLD cells] *vs* 51 ± 19 AU [CE + NAFLD cells], p<0.05). We prepared SiNPs with TEOS as a precursor. We obtained spherical SiNPs, size of 110 ± 21 nm (ANP) and 376 ± 67 nm (BNP). SiNPs were homogeneous population (dynamic light scattering analysis) and had negative potential Z. A portion of the SiNPs were positivized with APTES. Different concentrations of gallic acid (0.2 to 8 mg/mL) were adsorbed on 8 mg/mL SiNPs by constant agitation at 25°C for 24h. Between 0.2-4 mg/mL GA, adsorption on SiNPs(-) is directly proportional with its concentration but logarithmic for the SiNPs(+). With a maximum efficiency of 100% for both ANP (- and +), 85% for BNP(-) and 94% for BNP(+). When 8 mg/mL of GA was studied, only 30% was adsorbed for both SiNPs(-) while the SiNPs(+) presented 40% adsorption. We concluded that high GA concentration could saturate the active surface of these SiNPs. CE decreases intracellular lipid content. Therefore, this tool could help in delivering gallic acid into the cells to evaluate this antioxidant effects on lipid content.

452. 252. **SYNERGISTIC EFFECTS OF THYMUS VULGARIS ESSENTIAL OIL IN COMBINATION WITH ANTIFUNGAL AGENTS AND INHIBITION OF VIRULENCE FACTORS OF CANDIDA ALBICANS**

Alan Blanc[1], Maximiliano Sortino[1,2], Estefanía Butassi[1], Laura Svetaz[1]

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The aim of this study was to evaluate the antifungal activity of a mixture of thyme essential oil (ThyEO) and posaconazole (PSZ) and its capacity to inhibit virulence factors of *Candida albicans*. Materials and Methods: Minimum inhibitory concentration and minimum fungicidal concentration were evaluated using the microbroth dilution assay. Microdilution checkerboard assay was used to assess interactions. Ergosterol and methylene blue assays were used to detect effects on fungal membrane, while the sorbitol assay was used to detect effects on fungal cell wall. Inhibition of yeast virulence factors (adherence to epithelial cells, germ tube and pseudomycelium formation, secretion of hydrolytic enzymes, and biofilm formation) was assessed using previously reported methods. Results: the combination ThyEO/PSZ (31.25/0.0039 μg/ml) showed partial synergism against *C. albicans* strains. Furthermore, this mixture was fungicidal. ThyEO/PSZ, its components alone, and thymol have been shown to disrupt the fungal cytoplasmic membrane, increasing its permeability. At sub-inhibitory concentrations, ThyEO/PSZ significantly decreased the ability of *C. albicans* to adhere to buccal epithelial cells. ThyEO/PSZ, ThyEO and PSZ were able to reduce the pseudomycelium production of *C. albicans* while thymol completely inhibited its formation. ThyEO/PSZ, its components, and thymol inhibited biofilm formation and preformed biofilms of *C. albicans*. For the assays, means and standard deviations were determined. Differences between treatment groups were analyzed using ANOVA, Kruskall-Wallis, and Dunn tests; *p*-values<0.05 were considered significant. Notably, ThyEO/PSZ showed synergistic and fungicidal activity against a resistant strain of *C. albicans*, reducing the PSZ dose 4-fold. Conclusions: These findings make ThyEO and ThyEO/PSZ mixture valuable candidates for the development of alternative antifungals with a lower incidence of adverse effects.

453. 302. **USE OF BIOTIC ELICITORS IN *Tagetes erecta* TO IMPROVE THE PRODUCTION OF PHOTODYNAMIC SECONDARY METABOLITES WITH ANTIFUNGAL ACTIVITY**

Luz Amira Rivero[1], Laura Svetaz[1], Ma Victoria Rodríguez[2], Ma Sol Strebot[2], Candela Araujo[2], Maximiliano Sortino[1,3]

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To generate changes in the production of photodynamic antifungal secondary metabolites in the ornamental and medicinal plant Tagetes erecta, it was grown on a substrate inoculated with different treatments: A) sterile water (control) and in the presence of live microorganisms: B) Gluconacetobacter diazotrophicus (Gd), C) Trichoderma harzianum (Th), and D) Fusarium solani (Fs), either alone or in combination: E) Gd+Th, F) Gd+Fs, G) Fs+Th, and H) Gd+Fs+Th. Exomorphological parameters (stem length, number of leaves, midrib length, and leaf area) were measured, and chlorophyll content (total, a, and b) was quantified using spectrophotometry. Antifungal activity of leaf, stem, and root hexane extracts against Candida albicans ATCC10231 was evaluated by spot and developed bioautography, and the amount and intensity of the bands obtained were compared by thin layer chromatography (using CAMAG® Automatic TLC Sampler 4-ATS 4) at 254 and 366 nm wavelengths (λ). Exomorphological parameters were evaluated using the Kruskal-Wallis test, finding significant differences from control treatments D-H with fewer leaves and B-H in the rate of change in stem length. A higher growth rate in leaf area and midrib length was observed for treatments with Gd alone or combined with a fungus. Treatments E, F, and G resulted in a greater decrease in the percentage of chlorophyll. Through spot bioautography, the antifungal activity of stem extracts was observed for treatments C, D, and H, and in all root treatments, but not in leaf extracts. In developed bioautography, two active bands were detected for all stem treatments; for the root, three bands were detected for D, E, F, G, and H; four bands for A and C; and five bands for treatment B. Differences in intensity and number of bands for both λ were observed. The microorganisms affected the exomorphology and production of secondary metabolites in T. erecta. Extracts from plants treated with Gd showed higher inhibition and number of active bands.

454. 352. **SYNERGISTIC POTENTIAL OF *PHYTOLACCA TETRAMERA* EXTRACTS AND PHYTOLACCAGENIN WITH POSACONAZOLE AGAINST *CANDIDA ALBICANS*: INSIGHTS INTO THE MODE OF ACTION AND INHIBITORY EFFECT ON BIOFILM FORMATION**

Estefanía Butassi[1], Juan Carlos Cortés[2], Juan Carlos Ribas[2], Laura Andrea Svetaz[1]
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Combination therapies can improve efficacy, decrease toxicity, and reduce the development of drug resistance, making them the standard treatment for several diseases. In a previous study, four synergistic mixtures were found between methanol, dichloromethane, and butanolic extracts obtained from *P. tetramera* berries and their active compound fitolacagenin with posaconazole against *C. albicans.* The aim of the present study was to evaluate the mechanisms of action of these mixtures and their ability to inhibit biofilm formation against the clinical isolate *C. albicans* CCC125-2000. Antifungal mechanisms were studied by cellular [Minimum Inhibitory Concentration (MIC) determination in presence of ergosterol and sorbitol] and enzymatic assays [enzymatic activity of β1,3-D-glucan synthase (GS) and chitin synthase (ChS)] targeting the fungal membrane or cell wall. Biofilm formation inhibition assay was performed in 96-well flat-bottomed microtiter plates and metabolic activity was determined using the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay. The MIC of the mixtures increased in the presence of different concentrations of exogenous ergosterol, similar to that of amphotericin B, which acts by binding to fungal membrane ergosterol. Nevertheless, MIC values in presence of the osmotic stabilizer sorbitol did not shift to higher values compared to those without sorbitol, as is the case with caspofungin, which is a GS inhibitor. Also, these mixtures did not inhibit the enzymatic activities of GS and ChS. These results suggest that the mechanism of antifungal action of the mixtures could be binding to ergosterol in the membrane and would not act directly through the inhibition of cell wall synthesis or assembly. These mixtures inhibited biofilm formation in a dose-dependent manner. These results justify the studies toward the development of these combinations as new antifungal herbal medicinal products containing an Argentinean plant.

455. 355. **EFFECTS OF POLEO (*Lippia Turbinata Griseb*) ON ISOLATED WISTAR RAT UTERINE MUSCLE**

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The practice of traditional and complementary medicine is increasing. However, most of the plants used lack scientific support. Poleo (*Lippia Turbinata Griseb*) has been employed as an antispasmodic and also as an uterine stimulant (abortifacient). In this study we evaluated the effects on tone, amplitude and frequency of contractions induced by extract of Poleo in isolated uterus. Virgin female rats of the Wistar line, aged 3-4 months, were used. Poleo extract in alcoholic solution in 30% dilution was used to know the effects of the active principles on the tissue to be studied. An isolated experimental model organ was used to simulate the physiological conditions in which the tissue is found within the organism. The strips of uterus was extracted from the sacrificed animal and placed in the organ bath until its contractile activity was stabilized. Then, Poleo extract was added in volumes of 300 microliters. Muscle contractility variations were recorded through a voltage transducer connected to a Beckman Type RB electrophysiograph. From these recordings, statistical comparison of the results was performed using the Student's T method. Exposure of the uterus to Poleo extract produced a significant increase in the frequency and amplitude, and a decrease in the duration of uterine contractions ($p<0.01$). Preliminary results would explain the "popular" use of Poleo as an abortifacient agent. However, future studies are needed to elucidate its pharmacodynamics.

456. 359. **SKIN ULCERS AT INJECTION SITES UNDER INTERFERON-BETA 1A IN A MULTIPLE SCLEROSIS PATIENT: CASE REPORT**

Tovar Grimoldi L, Diez RA, Amato MF.
Biosidus

Interferon β-1a (IFN-β 1a) represent first-line therapy for multiple sclerosis (MS) with immunomodulatory effects. It´s been in the market for 25 years and has proven to be an efficacious drug capable of reducing rate and severity of relapses, besides improving disease parameters, measured by MR imaging techniques. We present the case of a 69-year-old male patient, with a late diagnosis of MS, who developed skin ulcers at injection sites after 7 months of treatment with this agent (44 μg, thrice weekly, subcutaneous injection). The cutaneous lesions started as bruises at application sites on the legs that 20 days later evolved into ulcers. He continued under treatment rotating application sites into arms but after visualizing the same reaction the treating physician decided to discontinue IFN-β 1a. Meanwhile a biopsy of the lesions was performed and evidenced in deep skin tissue a dense inflammatory infiltrate (predominantly polymorphous nuclear) and necrosis affecting medium caliber blood vessel. Despite the fact that it was not possible to test neutralizing antibodies against IFN-β 1a (NABs) a possible Arthus reaction or a type III hypersensitivity reaction may have occurred, in which the role of NABs in the development of the skin lesions has to be considered. We performed a pharmacovigilance analysis by literature review and by consulting a group of specialists. A search of PubMed and International Pharmaceutical Abstracts was conducted using the MeSH terms Interferon β-1a with skin ulcers, skin lesions, hypersensitivity reaction. We found several case reports, one systematic review which indicate that skin lesions at injection sites under IFN-β 1a rarely occur, but they must be considered. Even though it was not possible to test neutralizing antibodies against IFN-β 1a (NABs) a possible Arthus reaction or a type III hypersensitivity reaction may

have occurred, in which the role of NABs in the development of the skin lesions has to be consider.

457. 362. **COMPARISON OF POLYPHENOL CONTENT IN ARBEQUINA AND BARNEA VARIETALS OF EXTRA VIRGIN OLIVE OILS FROM LA RIOJA**

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Extra virgin olive oil (EVOO) is characterized by a maximum free acidity expressed in oleic acid of 0.8 g/100 g of oil. It mainly contains triglycerides, triterpenoids and polyphenols (PT). The PT content is related to oxidative stability, and the difference between the varietals is more than 45% of the PT. The PT found in the highest proportion are secoiridoids, oleuropein and ligustroside aglycones. During the olive oil extraction process, changes occur in the chemical structure of the secoiridoids of the fruit, giving rise to simpler forms such as hydroxytyrosol and tyrosol. From the perspective of human health, the importance of these compounds is due to their antioxidant capacity and the role they play in preventing degenerative diseases. The aim of this work was to determine the PT content in the EVOOs obtained from Barnea and Arbequina varietals produced in La Rioja. The extracts were analyzed by liquid chromatography with a UV detector, using syringic acid as an internal standard and tyrosol as an external standard. Conventional extraction method and calibration curves were performed. T- Student test was applied to determine the difference among the means ($P<0.05$). Significant differences were recorded between both EVOOs. Barnea varietal presented 248 mg/kg and the Arbequina varietal 268 mg/kg of PT. Regarding hydroxytyrosol, 1.8 mg/kg and 3.0 mg/kg respectively were detected. Taking these factors into account and considering that the same extraction and analysis method were applied, it can be seen that the EVOO extract of var. Arbequina from La Rioja presents a total phenol content, which is higher than that of the Barnea variety and, in turn, is within the range of values recorded for Arbequina oils of European origin. This shows that 20 grams of this oil provide the amount of PT recommended in the daily diet, and indicates that it can be an interesting raw material for the development of food supplements and/or natural cosmetics.

458. 375. **POTENTIAL OF COLOMBIAN AMAZON PLANT EXTRACTS BASED ON THE *IN VITRO* INHIBITION OF *GIARDIA LAMBLIA* TROPHOZOITES**

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Giardiasis is a parasitic disease caused by the protozoan *Giardia lamblia* and is one of the main causes of diarrheal diseases worldwide resulting from the consumption of food and water contaminated with this parasite. Currently, exist eight genotypes (A-H) but only two of them—genotype A (including different strains like strain WB) and genotype B—have the capability to infect humans. The primary drugs used globally for treatment belong to the 5-nitroimidazole family, including metronidazole (MTZ) and tinidazole. However, a therapeutic failure rate of up to 20% has been observed, along with cross-resistance between different therapeutic agents. This study aimed to evaluate extracts of native plants from the Amazon region in Colombia with the primary goal of identifying new sources of active compounds capable of inhibiting the in vitro growth of *G. lamblia* trophozoites. In an initial stage, the giardicidal action of 17 extracts (at a concentration of 500 μg/mL) was analyzed on the WB/1267 strain. Those extracts demonstrating favorable biological activity underwent calculations to determine their median inhibitory concentration (IC_{50}). Among these, the extracts from *Astrocaryum chambira*, *Attalea butyracea*, and *Bactris gassipae* stood out, presenting IC_{50} values of 133.4±43.5 μg/mL, 93.1±31.6 μg/mL, and 332.1±62.9 μg/mL, respectively. A synergism test was also conducted using MTZ in combination with the extracts of interest. The IC_{50} of these extracts was computed for MTZ-resistant WB/1267 strains and GS/H7 (genotype B), encompassing both the original strains and MTZ-resistant strains. This study contributes to the potential expansion of treatment options for giardiasis by identifying novel plant extracts with promising inhibitory effects on *G. lamblia*. The findings hint at the possibility of developing alternative therapies to address the challenges posed by therapeutic resistance in combating this parasitic disease.

459. 408. **ANTI-INFLAMATORY PROPERTIES OF DEACYLCYNAROPICRIN FROM Cyclolepis genistoides**

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Chronic inflammation is considered a common pathological mechanism in many diseases including cancer, heart disease, diabetes, arthritis, and neurodegenerative disorders. Combating inflammation with plants and natural compounds is thought to be a strategy for replacing current therapy that causes severe side effects. The aim of our work was to study the anti- inflammatory properties of bioactive constituents from the aqueous extract of Cyclolepis genistoides D. Don (Asteraceae). This plant has been used in folk medicine in northern and central Argentina for bone pain (analgesic properties) and as a diuretic in kidney diseases. The metabolite analysis of the aqueous extract by LC-HRMS revealed the presence of coumarins (isofraxidin, fraxetin), phenolic compounds (caffeoylquinic acids and their sulfate derivatives, luteolin and its glucuronide, luteolin-7-sulfate) and two sesquiterpene lactones, deacylcynaropicrin (DACP) and its 11,13-dihydro derivative (DH-DACP). In our lab, we previously demonstrated the ability of C. genistoides and DACP to modulate the transcription factor NFμB by blocking its nuclear translocation. To gain more insight in the anti-inflammatory potential, the pharmacological activity was also evaluated in other inflammation-related cellular in vitro models. We found that DACP (20 μM) inhibited not only NFμB1 but also COX-2 gene expression in PMA-differentiated and LPS-stimulated THP-1 cells. In addition, nitric oxide production was inhibited by DACP in microglial BV-2 cells exposed to LPS and IFN-γ (IC50 = 10.4 +/- 0.7 μM). However, DH-DACP (20 μM) had no effect on the studied pro-inflammatory pathways. The difference in pharmacological properties of both sesquiterpene lactones could be explained by the Michael acceptor moiety present in the DACP structure. Taken together, we hypothesize that DACP could be a lead compound for the development of anti-inflammatory agents due to its ability to inhibit NFκB pathway.

460. 453. **ISOLATION AND CHARACTERIZATION OF AN ABIETANE DITERPENE FROM COLEUS NEOCHILUS WITH POTENT ANTIPROLIFERATIVE ACTIVITY ON A HUMAN BREAST CANCER CELL LINE**

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Coleus spp. have diverse ethnobotanical applications, with their most prevalent use being attributed to their medicinal properties. We have previously described the antiproliferative effect of the ethanolic extract from *C. neochilus* (also known as "boldo rastrero") on different human breast cancer cell lines. The present study is focused on the extraction and isolation of compounds responsible for this effect. The ethanol extract (BRET) was subjected to solvent-solvent extraction, yielding four different sub-extracts: hexane (BRHX), chloroform (BRCL), ethyl acetate (BRAE), and methanol-water (BRAM). Remarkably, incubation with BRAE (50 µg/ml) was more effective in reducing the viability of human breast cancer cell line MCF-7 than the other fractions (BRET: 23%, BRHX: 29%, BRCL: 52%, BRAE: 68%, and BRAM: 10% reduction; $p<0.05$) as determined by MTT assays. Given these results, BRAE was subjected to further fractionation. A column chromatography using silica gel and mixtures of dichloromethane and methanol as mobile phase rendered eleven fractions, among which seven demonstrated a significant reduction in cell viability (50 µg/ml, $p<0.05$). Notably, fractions 6 (F6) and 7 (F7) exhibited an even greater reduction in cell viability compared to BRAE (BRAE: 74%, F6: 80%, F7: 98%, reduction; $p<0.05$). Subsequently, a second separation was conducted on F7 using the same chromatographic conditions. This led to the isolation of an abietane diterpene identified by 1D and 2D NMR experiments as 7α,12β,17-triacetoxy-6β,19-dihydroxy-13β,16-spirocicloabiet-8-ene-11,14-dione. This diterpenoid showed a significant reduction in MCF-7 cell viability (25 µg/mL: 96%, 10 µg/mL: 26% reduction; $p<0.05$). Therefore, this metabolite could be responsible, at least in part, for the antiproliferative activity of *C. neochilus*. To our knowledge, this compound has been isolated for the first time from *C. neochilus* and this is the first report of its antiproliferative effect on breast cancer cells.

461. 548. **QUALITY BY DESIGN IN THE DEVELOPMENT OF AN ORAL SELF-NANOEMULSIFYING SYSTEM FOR PAIN TREATMENT**

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A novel oral pharmaceutical composition of morphine (MOR) and omega-3 fatty acids (O3) generated a synergistic analgesic effect and reduced adverse events associated with MOR in a murine model (patent P-20120100854). Considering that a self-nanoemulsifying drug delivery system (SNEDDS) could enhance the oral absorption of O3, this study aimed to optimize a MOR-O3-loaded SNEDDS composition, maximizing the loading capacity of the oil phase. First, the limits of each component in the blank SNEDDS were explored using pseudo-ternary phase diagrams to then optimize them by means of a D-Optimal design, using ethanol, propylene glycol (Pg), kolliphor (K), and the oily phase (krill oil, AK) as independent variables. The key responses measured were polydispersity index (PDI) and mean droplet size (nm). Mathematical models were used to establish correlations between variables and responses, and a desirability function was applied to determine the optimal formulation (PDI ≤ 0.2 and smaller size). After optimization, component limits were adjusted for MOR-O3 loaded SNEDDS, which were evaluated similarly to blank SNEDDS, using Pg, K, and the oily phase: AK + MOR-O3 as independent variables. The predictive capability of the design space was confirmed with two independent optimized formulations, comparing obtained and predicted responses. Both the PDI and droplet size of blank SNEDDS and MOR-O3-loaded SNEDDS fitted significant mathematical models, defining design spaces. The most desirable blank SNEDDS formulation contained 28% Pg, 50% K, and 22% AK while the one loaded with MOR-O3 contained 33% Pg, 45% K, and 22% of oily phase, being transparent and of low flow with a droplet size of (15 ± 8) nm and PDI of 0.17 ± 0.05. In addition, it allowed the loading of MOR in the oily phase used in previous preclinical studies. In conclusion, this study offers a robust and predictive approach for developing MOR-O3 loaded SNEDDS suitable for future pain treatment applications.

P5-PHARMACOLOGY

FRIDAY 17TH NOVEMBER 9:00 - 10:30
CHAIRS: NATALIA ALZA
HUGO HECTOR ORTEGA
SILVINA ALVAREZ

462. 95. **NEUROPROTECTIVE ACTIVITY OF *N*-SUBTITUTED TRITERPENIC AZINES SYNTHETYZED FROM LUPEOL**

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Currently, Parkinson's disease (PD) is the second most common neurodegenerative disorder after Alzheimer's disease, and its prevalence has doubled in the past 25 years. The main hallmark of PD is the progressive loss of dopaminergic neurons in the *substantia nigra*, which led to the typical motor symptoms. Despite decades of intensive efforts in finding a cure for PD, treatments alleviate symptoms through restoring dopamine deficiency or surgery. Our aim was to test the potential neuroprotection of a series of triterpenic azines in a cellular model of PD using the neurotoxic 6-hydroxydopamine (6-OHDA). Firstly, a semisynthetic approach was used to obtain a series of azines (C=N-N=C), interesting molecules for their biological properties. From the natural triterpene lupeol isolated from the plant *Chuquiraga erinacea*, we prepared 30-oxolupeol by allylic oxidation, being this latter the template for azine synthesis. Combining 30-oxolupeol different aromatic hydrazones led to 16 azines through a microwave-assisted method, with good yield. Secondly, the neuroprotective activity of these compounds was evaluated *in vitro*. Neuroblastoma cells IMR-32 were exposed to non-cytotoxic concentrations of azines in the presence of 6-OHDA (25 μM), and cell viability was determined by the MTT assay. Variable efficiency in neuroprotection was observed between azine derivatives. Whereby all of them showed some degree of restoration of cell viability at 50 μM, only three compounds displayed strong defense against 6-OHDA neurotoxicity at 10 μM, reestablishing control levels. The more active azines have the structural features of being *N*-substituted with a *para*-metoxy or *meta*-metoxy benzene at position 31, or with a furane ring. To conclude, these azines obtained from 30-oxolupeol are potential neuroprotective agents against 6-OHDA neurotoxicity and could be an inspiration for the development of new drugs for PD treatment.

463. 107. **EFFECT OF *Ligaria cuneifolia* INFUSION ("Argentine mistletoe") ON THE LIPID PROFILE AND ERYTHROCYTE AGGREGATION KINETICS IN DYSLIPIDEMIC PATIENTS**

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Ligaria cuneifolia (Lc), popularly known as "*Creole mistletoe*" is a hemiparasitic plant whose infusion is used in folk medicine to improve blood fluidity and decrease plasma cholesterol levels. Objectives: to analyze the effect of *Lc* infusion on plasma cholesterol levels (Cho), triglycerides and erythrocyte aggregation in patients with Cho>200mg/dl. Methods: blood samples were collected for basal determinations (C) from eight patients aged 50±15 years old (male)

were studied, prior to signing an informed consent. All received envelopes with dried extract of leaves and stems of *Lc* (2.6 gr each) and a printed instruction to prepare the infusion to be ingested three times a week, for a month (T*Lc*). Determinations made in plasma: Total Cho by the esterase-oxidase method, HDLCho and LDLCho by colorimetric methods, triglycerides (TG) and fibrinogen (FB) by enzymatic methods; all concentrations were expressed in mg/dl. In whole blood: erythrocyte aggregation (AE), by optical densitometry, getting the average size (s) of the aggregates and the initial velocity (v) of the process. Statistical analysis was performed using Wilcoxon test. Results: Median and confidence interval (CI 95%). Cho: C:223.5 (197-257) vs. T*Lc*: 222.5 (206-261) ns; HDLCho: C: 49 (42-62) vs T*Lc*: 48.5 (48-68) ns; LDLCho: C: 157.5 (155-168) vs. T*Lc*: 146 (138-166) *; TG: C:182 (112-184) vs. T*Lc*: 136 (109-149) *; FB C:396.5 (380-597) vs. T*Lc*: 334(293-481)*; V: C: 0.44 (0.21-0.69) vs T*Lc*: 0.52 (0.32-0.58) ns; S:C:1.83 (1.78-1.86) vs. T*Lc*: 1.79 (1.7-1.86) ns. (*p<0.01 vs C; ns: non significative vs. C). Conclusion: in the patients studied, treatment with *Lc* generated a significant decrease in LDLCho, TG and FB blood levels, without causing changes in the evaluated parameters of erythrocyte aggregation. Besides, considering that plasma LDLCho and TG levels are related to atherosclerosis developing, these results would support the *Lc* treatment as a feasible strategy for the prevention of cardiovascular diseases.

464. 173. **ARGENTINE SPECIES WITH POTENTIAL EFFECT ON THE CENTRAL NERVOUS SYSTEM: PHYTOCHEMICAL ANALYSIS AND ANTIOXIDANT ACTIVITY**

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We investigate potential CNS effects of medicinal plants for neurodegenerative diseases (ND) and comorbidities. Our study focused on infusions and tinctures from native plants with historical medicinal use. Selected species were *Achyrocline satureioides* ('Marcela', flowering aerial parts), *Erythrina crista-galli* ('Ceibo', leaves, *Minthostachys verticillata* ('Peperina', sterile aerial parts), *Heteropterys glabra* ('Tilo del campo', fruit), *Aloysia citriodora* ('Cedrón', sterile aerial parts), *Stigmaphyllon bonariense* ('Papa de río', secondary stem/fruit). Authenticated samples were dried, grounded and extracts prepared per Argentine Pharmacopeia 7th edition (infusions 5% w/v, tinctures 10% w/v). Phytochemical analysis included: Total phenol content estimated by Folin-Ciocalteu method (infusions: 77.07-183.33; tinctures: 97.35-162.23 mg gallic acid eq/g dry extract); flavonoid content by modified Maksimovic technique (infusions: 1.92-104.24; tinctures: 6.59-121.53 mg rutin eq/g extract); hydroxycinnamic acids at Absorbance λ = 328 nm in ethanol (infusions: 14.59-168.97; tinctures: 17.93-148.74 mg chlorogenic acid eq/g extract); presence of condensed tannins assessed using proanthocyanidin method. Oxidative stress and CNS iron accumulation are linked to ND. Extracts' ability to scavenge DPPH radicals (EC_{50} (μg/mL): infusions: 28.1-365.9; tinctures: 27.4-191.7) and ABTS (EC_{50} (μg/mL): infusions: 11.0-65.5; tinctures: 9.6-38.9) was determined, Trolox as reference (EC_{50} (μg/mL): DPPH: 16 ± 6, ABTS: 13 ± 4). Ferrozine method evaluated iron-chelating capacity (EC_{50} (mg/mL): infusions: 1.59-6.31; tinctures: 2.66-5.56), EDTA as reference (EC_{50} (mg/mL): 0.0049). TBARS was determined in mouse brain homogenate (% inhibition of TBARS formation [1 mg/mL]: infusion: 80.4-89.0; tincture: 70.8-94.7). These findings guide the selection of extracts for the development of novel powerful natural therapeutic agents for ND and CNS pathologies.

465. 191. **L-CARNITINE, A NEW AND PROMISING PROTECTOR AGAINST CYCLOPHOSPHAMIDE-INDUCED GONADO TOXICITY**

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Objective: Evaluate the effect of L-carnitine (L-CAR) on folicular development and ovarian reserve in a rodent model of premature ovarian failure (POF) induced by cyclophosphamide (CTX). Materials and methods: F1 mice (Balb/c x C57) (8-10 weeks, n=6 per group) were injected intraperitoneally (IP) with CTX (75 mg/kg; CTX Group) to generate the POF group. The control group (without CTX) was injected IP with the CTX vehicle (saline solution), in the same way as the animals with POF. The CTX+L-carnitine groups received IP injection of CTX on day 1 of treatment. In addition, these groups received five doses IP injection of L-carnitine (100 and 200 mg/kg in saline solution) on days: 1, 3, 5, and 10 of treatment and were sacrificed on day 15. The fifth dose was administered two days before the first day of treatment. Ovaries were isolated for histology and immunohistochemistry (IHC) for DDX4 (oocytemarker). Statistical analysis was performed using ANOVA followed by Tukey's test. Results:The results showed that CTX reduced the % of primary, early antral, and antral follicles compared to the control group ($p<0.05$). CTX+L-CAR (200 mg/kg) increased the % of these structures. Moreover, CTX augmented the % of atretic follicles compared to the control ($p<0.05$) while CTX+L-CAR decreased it ($p<0.05$). IHC for DDX4 revealed that CTX diminished the number of primordial follicles compared to the control ($p<0.05$), where as L-CAR increased it ($p<0.05$). Conclusion: These results suggest that L-CAR preserves the ovarian reserve and improves folicular development. L-CAR might represent a low-cost and non-invasive treatment to preserve female fertility in patients undergoing chemotherapy.

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466. 196. **EVALUATION OF THE ANTICANCER ACTIVITY OF A NOVEL NARINGIN-V(IV)O COORDINATION COMPLEX IN HUMAN LUNG CANCER A549 CELLS**

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Coordination complexes of bioactive compounds often display an enhanced pharmacological profile compared to the original compound. Their pharmacological effects are influenced by factors such as the identity and quantity of ligands, the metal atom, its oxidation state, coordination mode, and geometry. Variations in these factors can impact properties such as interaction with specific biomolecules, complex stability, solubility, and bioavailability. In a previous study, we prepared two coordination complexes derived from the glycosylated flavonoid naringin and the oxidovanadium(IV) cation, $[VO(Narg)_2]\cdot 8H_2O$ (VONarg) and $[VO(Narg)(Phen)Cl]\cdot 3H_2O$ (VONargPh). These complexes exhibited enhanced biological activity when compared to naringin. The aim of this study was to modify the coordination site of the oxidovanadium(IV) cation with the flavonoid and assess its anticancer potential against the A549 lung cancer cell line. Physicochemical analysis revealed that the new complex, synthesized at pH = 12, $K_2[VO(Narg)(H_2O)_2]\cdot 3H_2O$, coordinates with naringin through the glycosidic region, in contrast to the initial two complexes, which coordinate through the 5-C-O^- and 4-C=O groups. It demonstrated higher solubility in aqueous media and exhibited a notable reduction in cell viability (42 %) at 100 μM after 24 h. These results surpassed the effectiveness of VONarg (20%). Additionally, the treatment led to a 112% increase in the production of reactive oxygen species after 4 h of incubation. Furthermore, the levels of the endogenous antioxidant glutathione decreased by 46%, and the mitochondrial membrane potential reduced 12 % in comparison to

the control. Moreover, alterations in cellular morphology indicated a loss of cytoplasm and the formation of apoptotic bodies. These findings collectively point to the potential of this complex as an effective agent against cancer cells by inducing oxidative stress (to which cancer cells are more susceptible) and triggering apoptosis.

467. 203. **ANTIOXIDANT ACTIVITY OF *HALAMPHORA COFFEAEFORMIS*: A FUCOXANTHIN-PRODUCING MICROALGA WITH POTENTIAL HEALTH BENEFITS**

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Fucoxanthin (Fx) is a major photosynthetic light-harvesting carotenoid found in diatoms and brown seaweeds, presenting numerous beneficial properties for health, such as antioxidant, hypoglycemic, antimicrobial, anti-obesity, anti-aging anti-metastatic, among others. We carried out previous studies with cultures of the marine diatom *Halamphora coffeaeformis*, isolated from the Bahía Blanca Estuary (Argentina), evidencing a significant Fx production. The aim of this work was to evaluate the antioxidant capacity of *H. coffeaeformis* extracts by implementing an optimized protocol for 2,2'-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) diammonium salt radical ($ABTS^{\cdot+}$) bleaching test. Cultures were performed in duplicate in Erlenmeyers with f/2 medium at 33‰, and with a light:dark photoperiod of 12 h:12 h, adapted to a photosynthetically active radiation intensity of 100 $\mu E\ m^{-2}\ s^{-1}$. Antioxidant activity was determined by a Jasco V-630 UV-Vis spectrophotometer by $ABTS^{\cdot+}$ scavenging activity measurement after the addition of Fx enriched culture extracts. The obtained results showed that $ABTS^{\cdot+}$ discoloration assay is an efficient antioxidant activity indicator of *H. coffeaeformis´* extracts, demonstrating for concentrations less than 0,01 mg mL^{-1}, similar $ABTS^{\cdot+}$ bleaching results than ascorbic acid, used as a reference positive control. Fx and associated pigment production during the stationary growth phase in cultures were evidenced by HPLC chromatography. *H. coffeaeformis* cultures under studied conditions showed strong antioxidant properties, with the effective concentration for 50% scavenging (EC_{50}) of $ABTS^{\cdot+}$ being 0,02 mg mL^{-1}. In conclusion, the obtained results suggested that *H. coffeaeformis* is a sustainable Fx source, encouraging future studies of its bioactive properties in health.

468. 323. **ANTIDIABETIC POTENTIAL OF EXTRACTS FROM DIFFERENT VARIETIES OF *SORGHUM***

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Type 2 diabetes mellitus is a chronic condition that covers approximately 90-95% of cases. In the current market, there is a wide variety of drugs for its treatment. In recent years there has been a growing interest in alternative approaches such as the use of natural products. There are references about the usefulness of sorghum as a hypoglycemic agent referred to the species cultivated in other regions of the world, but the study of the species adapted to our region (NEA) is of the utmost importance since it is known that they can vary their chemical composition due to edaphoclimatological influences. The objective of this work was to evaluate the hypoglycemic potential of sorghum by measuring the inhibitory capacity of its extracts on the enzyme alpha glucosidase (APG) responsible for glucose absorption at the intestinal level. Samples of red and white variety sorghum were used with 3 different granulometries for each case, 500um sieve (ST500), 500um flour (H500) and 177um fine flour (HF177). The material was macerated for 48h in a previously optimized mixture of solvents, filtered and dried in a rotatory evaporator. The in vitro APG inhibition assay was performed in a microplate reader using extracts (0.02mg/ml) and acarbose (1mg/ml) as positive control. Final results were expressed as the ratio of inhibition relative to acarbose. It was observed that all the extracts were more active than the positive control (Ir>1). However, the red variety presented greater inhibitory capacity than the white variety. For red sorghum, the most active extract was ST500 (ir=75.68) followed by H500 (Ir= 56.31). In the case of white sorghum, the extract that was most active was ST500 (Ir=46.96) but lower than all the cases of red sorghum. Our findings show that there are significant differences in the hypoglycemic potential of sorghum extracts according to the variety and size of the material used.

469. 324. **INTERACTION OF A SUNFLOWER MANNOSE-BINDING LECTIN WITH INFLUENZA VIRUS**

Radicioni M[1,4], Del Rio M[1,4], Cagnoni A[2,4], Lerman A[3], Cimmino C[3], Silva A[3], Uez O[3], Mariño K[2,4], Regente M[1,4].
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Influenza virus circulates in the world causing disease in humans. To establish an infection, the viral genome must replicate in the epithelial cells of the upper respiratory tract. In our laboratory, a mannose-binding jacalin-like lectin of sunflower seeds, Helja, was isolated and identified. The ability of Helja to bind glycoconjugates could be of biomedical interest as an antipathogenic agent. Previous evidence obtained by hemagglutination inhibition, ligand-blot, and competition assays on mannose-agarose affinity matrices, suggests the binding of Helja to Influenza virus particles. The aim of this work was to analyze the interaction of Helja with different types of Influenza viruses through biophysical assays and to evaluate its ability to inhibit viral binding to buccal epithelial host cells (BECs). Through solid phase assays, biotinylated Helja showed the ability to bind to all the immobilized viral particles analyzed, displaying greater affinity for Influenza B Yamagata. Viral particles labeled with FITC and following by fluorescence confocal microscopy were used to evaluate the effect of the lectin on the virus binding to BECs. We observed that the preincubation with Helja decreases the viral interaction to the host cells for all the tested strains, showing greater inhibition for the Influenza B Yamagata particles. Our results indicate that Helja interacts differentially with the envelope glycoproteins of different Influenza A and B strains, suggesting its capacity as an effective tool to prevent virus entry and replication in host cells. Future studies could contribute to the design of a new antiviral agent based on the use of Helja as a bioactive compound.

470. 347. **SUNFLOWER HULLS EXTRACTS EXHIBITS ANTIFUNGAL PROPERTIES AGAINST *C. albicans***

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Candida albicans is an opportunistic fungus causing superficial and systemic infections. The search for safe and efficient antifungal compounds is a challenge for researchers. Sunflower husk (SH) is an agroindustrial by-product rich in phenolic compounds with bioac-

tive properties as natural therapeutic agents. The objective of this work was to explore the antifungal activity of extracts from SH on *C. albicans*. Phenolic extracts (PE) were prepared by maceration of SH in 80%, 50%, 30% and 0% ethanol. Total polyphenols were quantified with the Folin-Ciocalteu method, and the characterization of their composition was analyzed by high performance liquid chromatograph technique (HPLC) to tentatively identified major phenolic compound. Fungal growth inhibition assays were performed in liquid medium in the presence and absence of different doses of each extract and OD reading at 630nm was determined. Also, viability assays were performed by (3-(4,5-dimethylthiazol-2-yl)-2 ,5-diphenyltetrazolium bromide) (MTT) protocol. According to HPLC results, chlorogenic acid (CGA) and caffeic acid (CA) were identified as the major phenolic acids in PE 80, 50 and 30; while in the aqueous extract only caffeic acid was detected. Regarding their biological activity, PE 80 was the extract with the best antifungal behavior displaying a MIC50 0.6 mg/ml phenols acids, while PE 50 and PE 30 showed less potent activity and the aqueous extract did not exhibit antifungal action in the evaluated doses. The yeasts treated with PE 80 were vacuolized, indicating loss of permeability of the plasma membrane, loss of typical yeast shape and presence of cell agglomerates. Our results support the study of sunflower husk ethanolic extracts as a promising tool for the treatment of human fungal diseases.

471. 447. **EFFECTS OF ORAL TREATMENT WITH SOY ISOFLAVONES OR MACA FLOUR (*Lepidium meyenii*) IN RATS ON CARDIAC DYSFUNCTION AFTER ISCHEMIA/ REPERFUSION AND SEX-DEPENDENCE**

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Soy isoflavones (SI) are used to treat menopausal symptoms, while rooth maca flour (*Lepidium meyenii* W.) is used as food supplement. Both products contain the phytoestrogens genistein and daidzein, which individually had demonstrated cardioprotection against ischemia and reperfusion (I/R) associated to mKATP channels activation. Here we have evaluated whether the effects of oral subacute SI or maca was dependent on the sex, and if they induce antioxidant mechanisms. Methods: Wistar rats non-treated controls (C) or administered orally with SI 100mg/kg/day or with maca 1g/kg/day were done. After treatment, isolated hearts were arterially perfused (11 ml/min) and introduced inside a flow calorimeter for simultaneously measuring left intraventricular pressure (LVP), changes in diastolic P (ΔLVEDP) and total heat flow (Ht, $mW.g^{-1}$). Maximal LVP of contraction (P) and muscle economy (Eco= P/Ht) were calculated. Hearts stimulated at 5 Hz were stabilized and exposed to 30 min I/60 min R. At the end, hearts were frozen up to determination of thiobarbituric acid reactive substances (TBARS) and reduced glutathione (GSH). Results: in female rat hearts, SI improved the post-ischemic contractile recovery (PICR) from 21±4% (C, 14) to 45±8% (8) of pre-I P, but maca did not change it. In male rat hearts, maca improved PICR from 27±16% (C, 11) to 76±12% (5) at start of R, while SI increased it to 51± 14% (6). However, maca increased appearance of fibrillations. ΔLVEDP during I/R was not modified by anyone. Moreover, female rat hearts treated with SI or Maca showed lower TBARS than C (52±5%, 34±3% of C, respectively). Males did not change TBARS, as neither was GSH in any sex. Conclusions: a) SI were more cardioprotective in female than in male rats, b) Maca prevented cardiac stunning only in male rats at start of R, but afterwards fibrillated, c) the SI effect in females could be related to inhibition of lipid peroxidation, while maca was not enough cardioprotective.

472. 460. **SYNTHESIS AND EVALUATION OF A XANTHONE ANALOGUE AS A NEW AKT PROTEIN INHIBITOR IN SKELETAL MUSCLE CELLS**

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Akt is a crucial regulator of cell survival, growth and proliferation and in consequence, its inhibition is an attractive strategy for the treatment of diverse types of cancer. The aim of this study was the synthesis of a xanthone analogue and the investigation of its potential as an inhibitor of Akt activation (Akt phosphorylation) in normal and cancerous skeletal muscle cells. The xanthone analogue was synthesized in three steps: (1) 1,3-dihydroxyxanthone was obtained through the intermolecular condensation between salicylic acid and phloroglucinol using $ZnCl_2$ and $POCl_3$. (2) Selective Williamson etherification of the phenolic hydroxyl group at the C3 position was carried out by nucleophilic displacement with 1,5-dibromopentane in the presence of K_2CO_3 and dry acetone. (3) Amination of the bromopentyl-substituted intermediate with diethylamine in dry acetone at room temperature, resulted in the xanthone analogue, later used in biological and molecular assays. Cell viability assays were developed with Trypan blue stained technique on C2C12 and RD cell lines exposed to 1.0 μM of the xanthone analogue for 24 and 48 hours. The results did not show any significant effect on the viability of the C2C12 cells. However, the number of viable RD cells decreased after 24 hours of treatment, with a percentage reduction of 48.4% ± 24.4 (SD), $p<0.05$ (*). Western Blot assays revealed that the xanthone analogue acts as an effective inhibitor of Akt phosphorylation in both cell lines. Inhibition is highly significant at 0.5 μM for 24 hours in C2C12 cells, with a percentage of inhibition of 64.3 % ± 4.9 (SD), $p<0.001$ (***); and at 1.0 μM for 24 hours in RD cells with a percentage of inhibition of 46.3 % ± 3.4 (SD), $p<0.001$ (***). Altogether these results show that the xanthone analogue reduces cell viability in the tumor cell line and inhibits Akt activation in both cell lines studied, which could be a potential therapeutic option in cancer treatment.

473. 675. **DEVELOPMENT AND VALIDATION OF A HPLC-UV ANALYTICAL METHOD FOR DETERMINATION OF MELOXICAM IN BANDURRIA (*THERISTICUS MELANOPIS*) PLASMA**

Passini Sabrina[1], Albarellos Gabriela[1], Lois María Fernanda[2], Demergassi Natalia[2], Falzone Martin[2], Montoya, Laura[1]

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Meloxicam (MLX) is a non-steroidal anti-inflammatory widely used in veterinary medicine to treat inflammatory and painful pathologies. Pharmacokinetic studies are necessary to recommend and protocolize dosing therapeutics schemes. The southern bandurria (*Theristicus melanopis*) is a native bird that, although it is wild, it may need to be treated for inflammatory pathologies in rescue and rehabilitation centers. The objective of this work was to validate an analytical method by High Performance Liquid Chromatography (HPLC) for the determination of MLX in bandurrias plasma. The research presented in this work was carried out following the pertinent Argentine regulations (Ley 22.421, Wildlife Conservation). Detection of MLX was adapted from previously HPLC/ UV methodologies described using a Thermo Fisher Scientific UltiMate3000®, Gilson UV/Vis151.The mobile phase was a mixture of diammonium hydrogen orthophosphate buffer, methanol, and acetonitrile (50% buffer, 40% MeOH, and 10% ACN) with a reverse phase C18 column with precolumn as the stationary phase. A flow rate of 1 ml/min and UV detection at 364 nm were used. Standard curves were prepared in mobile phase and bandurria plasma in a concentration range of 10 ug/ml to 0.03 ug/ml of MLX. Plasma extraction was carried out after protein precipitation with acetonitrile, N2 evaporation and resuspension with mobile phase. The validation criteria of the chromatographic method were taken as recommended by Guidance for Industry, bioanalytical Method Validation. The developed method was linear (0.03ug/ml-10ug/ml) $R2>0.9995$. Precision and accuracy were less than 15% included the quantification limit (0.03 ug/ml). The extraction percentage was 86% (CV3,5%). The method for the determination of MLX in bandurria plasma was precise and accurate. A small volume of

organic solvent is used, which produces less environment waste. This method is sensitive enough for its application in pharmacokinetic studies of meloxicam in bandurrias.

P6-PHARMACOLOGY

FRIDAY 17TH NOVEMBER 14:00-15:30
CHAIRS: JERÓNIMO LAIOLO
DANIELA QUINTEROS
MARIA LAURA RUIZ

474. 73. **5-HT$_{2A}$ RECEPTOR DEFICIENCY ALTERS LOCOMOTION AND PREFRONTAL CORTEX GENE EXPRESSION INDUCED BY A SINGLE ADMINISTRATION OF THE ATYPICAL PSYCHEDELIC NORIBOGAINE IN MICE: GENDER DIFFERENCES**

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Ibogaine is the main indole alkaloid isolated from the root bark of the African shrub *Tabernanthe iboga*. It is an atypical psychedelic drug capable of inducing oneirogenic effects (waking dream-like states) and vivid memory recall. Although the subjective and behavioral effects they induce are quite dramatic, they possess little addictive potential when compared to other drugs. Ibogaine is metabolized mainly by CYP2D6 to the primary metabolite Noribogaine (Noribo). The main objective of this study was to analyze the contribution of 5-HT2A receptor (5HT2AR) on the molecular and behavioral responses of Noribo since it has been suggested that at least some of the effects of are linked to 5-HT$_{2A}$ activation. We used the 5-HT$_{2A}$ receptor knockout (KO) (5-HT$_{2A}$ $^{-/-}$) or wild type (WT), male and female mice. Mice were injected with a single Noribo dose (10 and 40 mg/kg), and qPCR was performed on several immediate early genes (IEG), glutamate receptors and 5-HT2AR in the medial prefrontal cortex (mPFC). Noribo decreased locomotion 30 mins. after injection in KO mice for both sexes but only WT males showed decreased locomotion. For male mice, we found that Noribo increased IEG expression (Npas4, Egr1, cFos) for KO mice ($p<0,05$), but no differences were found for WT. Noribo increased GRIA1 mRNA in both genotypes ($p<0,05$), GRIN2A only showed increased expression in WT ($p<0,05$). For female mice, Egr1 and cFos expression were increased only for WT ($p<0,05$), Npas4 expression increased only in KO ($p<0,05$). Noribo increased GRIN2A expression in KO ($p<0,05$). For both sexes 5HT2AR expression increased in WT mice following Noribo injection ($p<0,05$), but males only showed changes for 10 mg/Kg and females for 40 mg/Kg. As expected, KO mice showed decreased 5HT2AR mRNA ($p<0,05$); ANOVA. Our results showed that Noribo altered the expression of 5HT2AR and further induced changes in locomotion and mRNA expression within mPFC a in a sexually dimorphic, genotype and dose-dependent manner.

475. 130. **DEWORMING WITH FENBENDAZOLE ADMINISTERED IN DAIRY SUPPLEMENT IN BALB/C AND C57BL/6JROFFO BREEDING MICE**

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Syphacia obvelata is one of the most commonly detected parasites in mice, with a high prevalence in animal facilities. Its presence was diagnosed at Angel H. Roffo Oncology Institute (IOAHR) animal facility in 2019. The aim of this study was to eradicate this parasite in BALB/c and C57BL/6JRoffo breeding mice and their offspring (aged 12 days or older), in order to obtain more reliable and reproducible experimental results and to improve sanitary quality. Based on this, treatment with Fenbendazole and water and bedding sterilization were chosen. Since there is no commercially available food formulated with this anthelmintic in our country, we have developed a novel administration option by adding it to the dairy supplement (250 mg of Fenbendazole per 100 g of supplement) provided to the mice in our animal facility. Portions of 1 gram were administered per 50 grams of body weight (adjusted weekly) daily for 21 days. To monitor the anthelmintic action, the Graham Test and necropsy with intestinal recovery of adult parasites (RIDA) diagnostic techniques were chosen. The Graham Test was conducted on one identified adult animal per cage as a baseline sampling, showing a 7% positivity in BALB/c strain samples and 35% in C57BL/6JRoffo strain samples. A positive result was considered if at least 1 egg compatible with *Syphacia obvelata* was present. Subsequent controls during and post-treatment were negative. At the end and 30 days post-deworming, 10 animals randomly selected for RIDA testing were also negative. Although more control instances are pending, the obtained results are encouraging. The Piperazine-Ivermectin therapy, used in mouse animal facilities to effectively eliminate pinworm infections, presents adverse effects such as toxicity in young animals and poorly tolerated by breeding females. Administering Fenbendazole in the dairy supplement is a feasible alternative utilizing the available resources, and is practical for large-scale dosing and well-accepted by the animals.

476. 169. **BIOLOGICAL IMPACT OF MAGNESIUM(II)-LUTEOLIN COMPLEX: ANTICANCER AND ANTIMETASTATIC ACTIVITY**

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Introduction Cervical cancer is the fourth most common malignancy in women worldwide and the second-most prevalent gynecological cancer. Although standard treatments are beneficial for early-stage cervical cancer, their efficacy for locally advanced is restricted due to significant side effects, drug resistance, and metastases. In this context, natural products, such as flavonoids, can be considered as a potentially effective replacement for chemotherapy drugs. Objective The main goal was to evaluate the anticancer and antimetastatic capacity of Mglut, a newly synthesized and characterized Magnesium(II)-luteolin complex. The basis of our hypothesis is that flavonoids structurally modified by coordination show improved biological properties. Methods The MTT test was performed to assess the impact of the compounds on HeLa cell viability. Intracellular reactive oxygen species ROS levels (CM-H$_2$DCFDA probe), mitochondrial membrane potential (PMM) (DIOC$_6$ probe) were studied to determine the probable mechanism of action. The induction of apoptosis and morphological changes were measured with flow cytometry (Annexin V-IP) and staining with crystal violet, respectively. The inhibition of cell invasion, migration, and adhesion was also investigated. Results Lut and Mglut inhibited the growth of HeLa cells in a dose-dependent manner being its IC$_{50}$ values >100 and 70.8 μM, respectively, and both reduced slightly the HaCat cells viability. The HeLa cells incubated with 100 μM Mglut displayed morphological changes typical with apoptosis, such as cellular shrinkage and disruption. The free ligand and complex increased ROS levels while decreasing PMM levels in dose-dependent ways. Likewise, lut an Mglut induced apoptosis and necrosis. At non-cytotoxic concentrations, both compounds decreased cell migration (82.8 and 62.1%), invasion (84.3 and 60.7%) and collagen type I adhesion (6.8 and

25.9 %). Conclusion Antitumor and antimetastatic activity of luteolin enhance upon coordination.

477. 178. **EXPLORING NEUROPROTECTIVE EFFECTS OF STEVIOSIDE, A GLYCOSIDE DERIVED FROM *STEVIA REBAUDIANA*: *IN VITRO* AND *IN VIVO* STUDIES**

Pastore Valentina, Colettis Natalia, De Tezanos Pinto Felicitas, Marcos Alejandra, Marcucci Carolina, Rademacher Marina, Marder Mariel.
Instituto de Química y Fisicoquímica Biológicas Prof. Dr. Alejandro C. Paladini (IQUIFIB, UBA-CONICET). Facultad de Farmacia y Bioquímica, UBA. Junín 956, (1113) CABA, Argentina

Epilepsy is a chronic neurological disorder characterized by recurring seizures, with neuronal hyperexcitability and oxidative damage from free radicals being key factors in its development. Current antiepileptic drugs control seizures in 70% of cases, while 30% are treatment-resistant. In Latin America and the Caribbean, 68.2% of countries use natural resources for seizures. Medicinal natural products and structural modification of active compounds are sought for high-impact public health disorders. Several preclinical and clinical studies suggest the use of stevia (*Stevia rebaudiana* B.) and its derivatives with therapeutic and pharmacological applications, as they exhibit a variety of biological activities. Here, we evaluate the neuroprotective activity of a stevia derivative, stevioside (STV). We worked *in vitro* with human neuroblastoma cells (SH-SY5Y), which were treated under 4 conditions: A) STV (1-100 μM, 24 h); B) PTZ, a cytotoxic convulsant compound (20 mM, 24 h); C) H_2O_2 (1 mM, 48 h); D) STV (30 and 100 μM, 24 h) plus B or C. It was observed that STV is not cytotoxic up to 100 μM. Furthermore, pretreatment of cells with STV prior to PTZ and H_2O_2 treatments reversed both the damage caused by PTZ and oxidative damage from H_2O_2, suggesting that STV is a promising agent capable of preventing oxidative stress inherent to seizure episodes. On the other hand, in *in vivo* assays in male Swiss mice, following National Institute of Health (NIH) protocols, STV at 100 mg/kg, i.p., provided 75% protection of mice treated with PTZ (85 mg/kg, s.c.) at 4 hours after administration. Additionally, mice brains were homogenated and antioxidant assays were performed. STV showed a decrease in TBARs formation (P<0,0001) and an increase in endogenous antioxidant agents, GSH (P<0,01), compared to the PTZ group. Therefore, STV appears to be a potential anticonvulsant agent whose mechanism of action could be associated with the inhibition of reactive oxygen species production.

478. 281. **GERANIOL PROTECTS AGAINST OXIDATIVE STRESS AND PROTEOTOXICITY IN C. ELEGANS PARKINSON'S DISEASE MODELS**

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Due to the escalating life expectancy, the prevalence of age-related disorders like neurodegenerative diseases (ND) has surged. Oxidative stress (OS) has emerged as a key accelerator in ND progression. In Parkinson's disease (PD), for instance, compromised free radical scavenging capacity has been linked to worsened α-synuclein aggregation and proteotoxic damage. Geraniol (GER), a plant-derived essential oil, has recognized antioxidant properties. Considering that OS contributes to ND progression, compounds with antioxidant activity have been postulated as potential therapeutic agents. Leveraging the suitability of *Caenorhabditis elegans* as a model organism in biomedical research due to its genetic homology with mammals, including cytoprotective proteins, we aim to assess GER's biological efficacy in a *C. elegans* PD model and delve into the underlying molecular mechanisms. Our results first confirmed the in vivo antioxidant activity of GER. We cultured wild-type animals with GER and then exposed them to Juglone (oxidative agent). Locomotion was employed as a survival proxy using the Worm MicroTracker device. Strikingly, GER exhibited a significant increase in animal survival under OS conditions (P=<0.001). To unravel the precise mechanism driving GER's protective effects, we analyzed null mutants within key molecular pathways associated with OS response. Intriguingly, our preliminary findings showed that either DAF16/FOXO, HSF1 or SKN1/NRF2 are involved in mediating GER's protective effect. Given the link between OS and PD, we also evaluated the GER´s impact within a *C. elegans* PD model. We found that GER improves the impaired-locomotion phenotype of this model (P = 0,011). So far, these results indicate a potential antiproteotoxic effect of GER in C. elegans PD models. To comprehensively dissect GER's effects, we propose an integrative approach involving genetic analyses, advanced microscopy techniques, and behavioral assessments.

479. 315. **PROPRANOLOL ATTENUATED A CONTEXTUAL FEAR MEMORY AFTER FEAR GENERALIZATION IN MALE AND FEMALE MICE**

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Destabilization-reconsolidation, a retrieval-dependent process, allows for the modification of established memories, which is why this phenomenon has been considered an opportunity for attenuating the negative features of pathological memories. Memory attenuated in this way suggests an effective therapeutic strategy to provide long-term relief. However, very aversive memories are often resistant to this process. Here, after the induction of a resistant fear memory in mice using robust fear conditioning, we examined whether it is possible to render it susceptible to pharmacological disruption according to the degree of generalized fear. For this, based on the perceptual similarity between the associated context (CA) and non-associated contexts (CB, CC, and CD) to the aversive event, we established an ordered gradient of generalized fear (freezing and risk assessment). We observed that as the exposure context became less similar to CA, the conditioned response decreased (CA-CB vs CC-CD, p<0.05). Next, in conditioned mice, we injected propranolol, a known reconsolidation interferent, after exposure to the different contexts. In males, propranolol treatment resulted in a decreased fear response following exposure to CA or CB, but not CC or CD, compared to the control group (p<0.05). In females, the decrease in fear response due to propranolol was observed after exposure to CC, but not to the other contexts, compared to the control group (p<0.05). Taken together, these results indicate the possibility of indirectly manipulating a robust contextual fear memory by controlling the level of generalization during recall, highlighting that it is not necessary to expose animals to context conditioning as is commonly done. From a clinical standpoint, this would be of considerable relevance since, following this strategy, the treatment of psychiatric disorders associated with traumatic memory formation would be more effective and less stressful.

480. 319. **EXOGENOUS KETONE BODIES MODULATION OF GABAERGIC SIGNALING IN *C. elegans***

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Mutations in PTEN (a negative regulator of the PI3K pathway) are associated with neurodevelopmental disorders, epilepsy, and schizophrenia. Several reports suggest that an increase in the excitation/inhibition (E/I) ratio in the brain is a hallmark of these disorders. The *C. elegans* Neuro-Muscular system, where both excitatory (ACh) and inhibitory (GABA) neurons innervate muscles, provides a suitable model for studying E/I balance. Combining pharmacological and behavior assays, we found that *daf-18* (*C. elegans* ortholog for PTEN) mutants are hypersensitive to cholinergic drugs, suggesting a deficit in GABAergic signaling. *daf-18* mutants are deficient in eliciting complex movements such as the "omega turn", a sharp turn in which the GABAergic inhibition on dorsal muscles plays a critical role. Moreover, using microscopy techniques, we observed that

daf-18 mutants exhibit morphological defects in GABAergic neurons (abnormal branching, and incomplete commissures). In contrast, we did not find significant differences in the morphology of cholinergic neurons. DAF-18 specific rescue in GABAergic neurons partially rescued the defective phenotypes, suggesting an autonomic role of the PI3K pathway in GABAergic function. In addition, we found that the GABAergic deficit in *daf-18* mutants is entirely dependent on the inactivation of the transcription factor DAF-16/FOXO. Ketogenic Diets have been used for refractory epilepsy. The mechanisms underlying this therapeutic effect remain elusive. We found that exposure to the ketone body hydroxybutyrate (βHB) during early development ameliorated GABA defects in *daf-18* mutants. Interestingly, this ketone body does not alleviate the defects observed in daf-16/FOXO mutants, suggesting an essential role of this transcription factor in the βHB effect. Since fundamental processes are highly conserved throughout the animal kingdom, this study may contribute to the understanding of disorders associated with E/I imbalances in mammals.

481. 325. **INTRAVAGINAL ADMINISTRATION OF PHARMABIOTIC/PHYTOBIOTIC CAPSULES WITH AUTOCHTHONOUS LACTIC BACTERIA FOR THE PREVENTION OF INFECTIONS OF THE BOVINE REPRODUCTIVE TRACT**

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The postpartum bovine reproductive tract is susceptible to infections that are treated with antibiotics and hormones, or a combination of them. The growing problem of the transmission of resistance to antimicrobials requires to reduce their use and look for alternative therapies, such as probiotics and phytobiotics. The objective of this work is to advance in the design of pharmabiotics/phytobiotics formulas with bovine beneficial autochthonous lactic acid bacteria (BBALB) for the prevention of reproductive tract infections. Hard gelatin capsules containing BBALB ($3x10^{10}$ CFU), individually or combined with phytoderivatives (Malva and Lapacho) were administered intravaginally to bovine females (n=30). Two doses were applied with an interval of 15 days, before the probable date of delivery, and two postpartum doses. The modification of the autochthonous microbiota from vaginal washings and the permanence/colonization of the inoculated BBALBs were evaluated, and the safety of the designed formulas was determined through nutritional-clinical and hematological-biochemical parameters in blood and serum. All the females of the different experimental groups (EG) remained healthy before parturition (5±0.0 RS). During the postpartum period, two cows from the BBALB MG group and one from the Control group showed signs of reproductive infections. The females of the different GE maintained or slightly decreased their postpartum weight (304.5 ± 4.95 KgLW). All the animals showed normal hematocrit (36.44±1.08%), and leukocyte formula within bibliographic reference values (RV) in the BBALB Vg, BBALB Vg+VE and BBALB MG+VE groups, without significant differences between the animals EG and with Control. Glycemia was lower at RV=40-88.2 mg/dl, with no significant differences between EG and Control at the same sampling time. Metabolic parameters (glycemia, proteinemia, albuminemia and C-reactive protein) were normal in all animals throughout the trial. The normal culturable microbiota of the bovine vagina was slightly modified after the administration of the capsules. Total aerobic mesophiles increased slightly in all EGs after parturition. Culturable Enterobacteriaceae remained at 2.50±0.05 logUFC/ml during the assay in all EGs, except in BBALB Vg+VE. Cultivable lactic bacteria increased in all postpartum EGs (BBALB Vg:2.15±0.07, BBALB Vg+VE:2.00±0.02 and BBALB MG:1.91±0.05 logUFC/ml). The results indicate that the intravaginal administration of pharmabiotic/phytobiotic capsules is safe and does not produce adverse effects when administered to pre- and postpartum cows.

482. 326. **BENEFICIAL AND SAFETY CHARACTERISTICS OF AUTOCHTONOUS LACTIC BACTERIA (LB) WITH PROBIOTIC POTENTIAL FOR CANINE PUPPIES**

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Probiotics are defined as "live microorganisms that, when administered in adequate amounts, produce a health benefit in the host". For the selection process of new autochtonous probiotic microorganisms, functionality, safety, and technological aspects must be taken into account. This work is aimed at evaluate different beneficial properties and safety of LB isolated from mother's milk and fecal matter of different dog breed, in order to advance in the design of an homologous probiotic formula with LB to reconstitute the intestinal microbiota and protect from infections in dogs. 100 different strains isolated from dogs: 79 LB (17 from mother's milk, 62 from puppy fecal material) together with 21 other LB (adult dogs) available in the group were evaluated in the a) production of beneficial enzymes (protease, lipase, amylase, cellulase and feruloyl esterase), b) H_2O_2 production and c) innocuity (gelatinase, hemolysin and lecithinase). Protease was determined in agarized skim milk, lipase in MRS-milk cream, showing an inhibition halo. Amylase production was evaluated in MRS-starch agar medium (revealed with Indole), feruloyl esterase in 1% in methanol (w/v) ethylferulate 1g/L added to MRS agar without glucose, cellulase in MRS-carboxymethyl cellulose agar (revealed with lugol). H_2O_2 production was detected using the tetramethyl benzidine-MRS agar (TMB-MRS) plate method. Hemolysin activity was determined on blood agar (BHI agar+5% human blood), lecithinase on egg yolk agar and gelatinase on BHI agar+3% gelatin. The LB in study were 13 cocos and 87 bacilli. The survey of beneficial enzymes showed 24% strains with protease activity, 20% with cellulase activity and 19% with feruloyl esterase activity. 39% of the strains evidenced H_2O_2 production (12% weak, 9% strong and 18% very strong). Hemolysis was produced in 68% as partial (alpha), 2% total (beta) and 30% gamma. No isolates expressed lecithinase and gelatinase activity. This work allowed us to select the LB strains with the best characteristics, and to correlate them in a way to define the optimal combinations, supported also with the origin and breed of the isolate. The strains sharying properties are being subjected to genotypic identification, in order to advance in the design of probiotic formulas with homologous strains for the health of canine puppies.

483. 350. **α7 NICOTINIC EXPRESSION AND FUNCTION IN HUMAN RETINAL PIGMENT EPITHELIUM CELLS**

Julieta Ailen Mader, María Florencia Fernández Delias, Juan Facundo Chrestia, Florencia Anahí Sotelo, María del Carmen Esandi, Cecilia Bouzat
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The α7 nicotinic receptor (nAChR) is one of the most abundant nAChRs in the nervous system and is also present in non-neuronal cells, including immune and epithelial cells. It is involved in cognition, memory, pain, neuroprotection and inflammation and its potentiation has emerged as a therapeutic strategy for neurological, neurodegenerative, and inflammatory disorders. Given that the increase in oxidative stress in retinal pigment epithelial cells contributes to the development of age-related macular degeneration and that α7 activation exerts cell protective effects, we explored the presence and functional relevance of α7 in D407 retinal pigment epithelium cells. By confocal microscopy using the α7 specific antagonist α-bungarotoxin labeled with Alexa 488 and real time-PCR we demonstrated the presence of α7 in these epithelial cells. To simulate the events occurring in age-related macular degeneration, we treated cells with 500 μM ferric ammonium citrate (FAC) for 48 h to induce stress damage and measured reactive oxygen species (ROS) with the fluorescent probe DCFH-DA and cell viability by the MTT assay. FAC treatment resulted in a significant 56 ± 23% increase in ROS levels with respect to the control. To determine if α7 protects against oxidative damage, we exposed cells for 4 h to a specific α7 agonist, PNU-282987, before the FAC treatment. This exposure reduced 29

± 3% basal levels of ROS. Notably, PNU-282987 exhibited protective effects against the FAC treatment, leading to a reduction of 46 ± 15% in ROS levels when compared to the treated cells. In line with these observations, exposure to the α7 agonist restored the 20% reduction in cell viability induced by FAC. Overall, we demonstrated for the first time the presence of α7 in the D407 cell line, which is a model system for studying various retinal diseases, and its protective role against oxidative damage, a key factor linked to the onset of macular degeneration.

484. 360. **DIFFERENTIATION OF N2A CELLS: COMPARISON BETWEEN HDAC INHIBITORS AND DIFFERENTIATING AGENTS**

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Differentiation is a crucial process upon cell development. Epigenetic mechanisms play an important role in it and HDACs, a family of enzymes whose major function is to acetylate histones and, therefore, regulate gene expression is one the main character in cell differentiation. The aim of this work was to compare differentiating agents in the N2a cells (mouse neural crest-derived cell line) where its neuronal stem cell could differentiate into neurons. Cells were treated with differentiating drugs such as: dbcAMP (cAMP analogue), resveratrol (polyphenol) and two selective HDACi (HDAC inhibitors) MS-275 (class I) and MC-1568 (class IIa). N2a were incubated with DMEM high glucose, 1% Glutamax and 0,5% SFB at 37°C and 5% CO_2. For 4 days cells were observed and counted if necessary. Finally, cells were characterized by phase-contrast or fluorescence microscopy. We first determined cell viability at day *in vitro* 4 (DIV4) and noticed that MS275 500 nM significantly reduced the total number of viable cells (p= 0,021) while other conditions did not affect viability. After that, we analyzed the number of differentiated cells in each condition: 24% Resveratrol 6.2 μM vs 9% ethanol, 27% dbcAMP 0.5 mM vs 3% water and 20% MS-275 50 nM vs 14% DMSO. Finally, we observed morphological changes in N2a cells comparing presence or absence of typical neuron structures such as dendrites, axons and filopodia. Here we found that HDACi induced differential transformation in cell morphology. We saw that MS-275 increased the number of axons (p=0.037) and dendrites (p=0.013) while MC-1568 showed an increase of filopodia (p=0.08) and dendrites (p=0.02). These results show differentiating agents which can convert neuronal stem cells into mature neurons as we observed. Although further work is still needed, we can assume that class I HDACs are necessary to maintain a tumor-like conformation and, evenmore, that HDACi stop tumoral growth and lead to N2a neuron-like differentiation.

485. 670. **INVOLVEMENT OF $GABA_B$ RECEPTORS IN THE CONTROL OF THE ANALGESIC RESPONSE OF MORPHINE USING A MODEL OF NEUROPATHIC PAIN IN MALE AND FEMALE BALB/C MICE**

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Neuropathic pain (NP) is a type of chronic pain that affects between 7-10% of the world population and continues without effective treatments. It has been suggested that the efficacy of combining pharmacological treatments lies in using analgesics that have different mechanisms of action. In previous studies of our laboratory, a GABAergic-opioid interaction was evidenced in a pain model. In this line, baclofen (BAC, $GABA_B$ agonist) was able to increase the antinociceptive effect of morphine (MOR) in the hot plate. On the other hand, it has been reported that the nerve injury that causes NP induces changes in the expression of brain derived neurotrophic factor (BDNF) in different brain areas related to pain. BDNF is a potential biomarker for NP because it promotes neuronal growth, maintenance, survival, and neurogenesis. The aims of the present study was to evaluate the participation of $GABA_B$ receptors in a model of neuropathic pain in mice of both sexes from a pharmacological approach. In addition, we analysed the expression of BDNF in areas related with pain. The behavioral results showed that MOR was able to reduce the NP in males and females ($p < 0.05,0.001$, respectively), while BAC increased this effect only in males ($p < 0.05$). In contrast, 2-OH-saclofen ($GABA_B$ antagonist) blocked the antinociceptive effect of MOR in males ($p<0.001$) and this effect was only attenuated in females ($p<0.05$). These results confirm the involvement of $GABA_B$ receptors in the analgesic effect of MOR in a NP model. $GABA_B$ receptors could be considered as a potential therapeutic target for the NP pain treatment.

P1-REGENERATIVE MEDICINE AND NANOMEDICINE

WEDNESDAY 15TH NOVEMBER 9:00 - 10:30
CHAIRS: JULIETA MAYMÓ
ESTEBAN FIORE
ANA TORBIDONI

486. 166. **CHEMICAL MATURATION OF HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED CARDIOMYOCYTES**

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Cardiovascular diseases are the main cause of death wordwide. Efforts to model these pathologies are continously made in order to improve therapy and drug treatment. In the last years, human induced pluripotent stem cells (hIPSCs) models have yield a variety of tools in order to design new clinical approaches. hIPSCs derived cardiomyocytes (CMs) are an advanced model that had brought the opportunity to study cardiac cells. Nevertheless, hIPSC-CMs lack crucial characteristics present in the adult human heart since they are differentated in short term protocols and thus resemble embryonic CMs. In this work we differentiated hIPSCs into pure immature CMs (day 21) and then applied a maturation cocktail consisting of the hormone T3, dexamethasone, a PPARa small molecule agonist and palmitic acid in low-glucose DMEM medium until day 38. As a control, we cultured immature CMs in RPMI B27- base medium. We evaluated the expression of cardiac maturation markers by RT-qPCR. These included structural components, metabolic genes and specific ion channels. We found that the maturation medium significantly upregulated the expression of metabolism (COX6A2, CPT1B) and ion transport (RYR2, ATP2A2, CX43), and enhanced the structural proteins isoform switch from immature to mature (MYL7 to MYL2 and TNNI1 to TNNI3). Using quantitative bioinformatic analysis, we evaluated a key property of mature CMs, which is polynucleation/poliploidy. By DAPI staining of CMs in maturation vs control media, we confirmed that CMs in the mature media have a greater population of 4n nuclei. Finally, we evaluated the proliferative capacity of both conditions, with the control population yielding a near 1.5 fold in the amount of cells at the end of the protocol. In conclusion, we show that this protocol robustly generated pure mature like hIPSCs-CMs in 40 days with well-defined sarcomere structures and key maturative traits, generating a unique model to target key questions about cardiac regeneration.

487. 179. **DIFFERENTIAL EXPRESSION OF HIPPO PATHWAY MEADIATORS IN FETAL AND ADULT OVINE HEARTS.**

PRELIMINARY RESULTS

Agustina Scharn[1], María del Rosario Bauzá[1], Ayelén Emilce López[1], Mariano Nicolás Belaich[2], Alberto José Crottogini[1], Fernanda Daniela Olea[1], Paola Locatelli[1].
1. IMETTYB, Instituto de medicina Traslacional, Trasplante y Bioingeniería, Universidad Favaloro-Conicet.
2. Universidad Nacional de Quilmes.

Over the past years several cardiac regenerative therapies have been investigated. One possible approach would be to target the Hippo pathway, which plays a pivotal role in controlling organ size and tissue growth. Objectives: To assess the expression levels of several Hippo pathway mediators (*Fgf6*, *Amotl1*, *Ctgf*, *Cyr61*, *Tead1*, *Birc5*, *Ankrd1, Yap* and *Taz*) in fetal and adult sheep hearts. Methods: On account that ovine cardiomyocytes are mitotic until approximately day 100 of gestation, cardiac tissue samples from 70-day gestation time ovine fetuses (F-70, n=4), 120-day gestation time (F-120, n=4) and adult sheep (AS, n=4) were harvested. RT-qPCR was performed to evaluate the expression of *Fgf6*, *Amotl1*, *Ctgf*, *Cyr61*, *Tead1*, *Birc5*, *Ankrd1*, *Yap* and *Taz*. Results: *Amotl1*, *Taz*, *Birc5*, *Tead1*, *Fgf6* and *Ctgf* expression were increased in F-70 vs AS (*Amotl1* fold increase: 2.06±0.8, p<0.01; *Taz*:1.88±0.5, p<0.01; *Birc5*: 103.7±15.9, p<0.0001; *Tead1*: 2.93±0.3, p<0.001; *Fgf6*: 12.8±7.5, p<0.01; *Ctgf*: 5.15±3.1, p<0.01) while *Birc5* expression was increased in F-120 vs AS (68.13±39.1, p<0.001). Contrarily, expression of *Ankrd1* was significantly higher in AS vs F-70 (0.14±0.05) and F-120 (0.11±0.05) both p<0.0001. Statistical analyses: X±DS, ANOVA-Tukey and non-parametric). No difference was detected in *Cyr61* and *Yap* expression levels. Conclusion: Fetal myocardium showed higher expression levels of *Amotl1*, *Taz*, *Birc5*, *Tead1*, *Fgf6* and *Ctgf*, while *Ankrd1* had lower expression levels when compared to AS. The overexpression of these genes may be a potentially useful strategy to promote cardiomyocyte proliferation.

488. 238. **LIVER OVEREXPRESSION OF THE NOVEL PAN-TGF-β INHIBITOR BRECEPT AMELIORATES LIPID METABOLISM DISORDERS IN A MAFLD RAT MODEL**

Carolina Anahí Cámara[1], Anabela La Colla[1], Tania Melina Rodriguez[2], Stella Maris Echarte[1], Carolina Natalia Sendón[1], Victoria González Scarvaglieri[3], Ricardo Alfredo Dewey[2,4,5], Andrea Nancy Chisari[1]
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Metabolic-associated fatty liver disease (MAFLD) is characterized by the pathological accumulation of triglycerides in hepatocytes, obesity, and metabolic dysfunction. Treatments for MAFLD patients are limited. The TGF-β pathway modulates lipid metabolism in hepatocytes. Brecept (Br), a pan TGF-β inhibitor, was developed by fusing TβRII-SE, a soluble TGF-β type II receptor (TβRII) peptide encoded by a novel splice variant, to the Fc portion of human IgG (TβRII-SE/Fc). Previous results evidence the potential effect of Br against metabolic syndrome. Thus, we aimed to study the effect of lentiviral-mediated liver overexpression of Br (Lv-Br) on lipid metabolism in a rat model of MAFLD induced by Western Diet (WD). We compared three groups: control, WD, and WD that received an intrahepatic injection of the lentiviral vector encoding Br (WD+Lv-Br) at week 10. In week 20, WD+Lv-Br group had a lower abdominal circumference than WD group(p<0.05). In week 21, animals were sacrificed. WD+Lv-Br group, compared with WD group, significantly decreased the percentage of gonadal and abdominal adipose tissue(p<0.05) and had lower triglycerides and cholesterol liver content(p<0.05). Furthermore, comparing WD+Lv-Br with WD group, we found a significantly decreased serum triglyceride concentration(p<0.05) but non-significant differences in serum total cholesterol. However, we observed an evident trend to decrease the concentration of lipoproteins that have the highest concentration of triglycerides in their structure (VLDL-ch+IDL-ch). Finally, three metabolic indexes were calculated with these data, and we observed a significantly improved LDL-ch quality (Tg/HDL-ch index, p<0.01) and a trend to diminish endothelial dysfunction (Kannel index) and decrease atherogenic lipoprotein concentration (non-HDL-ch), comparing WD+Lv-Br with WD group. Overall, these results suggest that Brecept liver overexpression ameliorates lipid metabolic disorders associated with MAFLD induced by WD in rats.

489. 361. **CONTRIBUTION OF GLAST+ WNT1+ BONE MARROW STROMAL PROGENITORS TO THE INFARCTED HEART**

Maximiliano Borda[1], Gisela Romina Bustos[2], Francisco Alvarado[1], Matías Martino-Garcet[1], Gianlucca Giardelli[1], Alejandro Montaner[3,4], Germán González[2,4] & Jorge B. Aquino[1,4]
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Objectives: To analyze the contribution of GLAST^{+} WNT1^{+} bone marrow stromal progenitors (BMSPs) to the heart after acute myocardial infarction (AMI). Materials and methods: Wnt1^{Cre};Rosa26^{tdTomato} and GLAST^{CreERT2};Rosa26^{tdTomato} (tamoxifen-injected at postnatal days 2 or 60) mice were used. AMI was induced by permanent or transient (30 minutes) ligation of a branch of the left coronary artery. After 7 or 30 days, heart samples were sectioned and immunostained. Results: A contribution of GLAST+ WNT1+ BMSPs with neovessels in remote areas of the left ventricle was likely found; however, such a contribution seems transitory. One month after the AMI, Tom+ cardiomyocytes were found in subepicardial areas, adjacent to the area of the lesion. In addition, very few cells morphologically resembling endothelial, sinus node and Purkinje cells were also found within the infarcted area. Conclusions: GLAST^{+} Wnt1^{+} BMPs would probably contribute endothelial cells and cells of cardiogenic lineage after AMI. Although the significance of these findings remains to be addressed, they may help the development of new applications for regenerative medicine.

490. 482. **BACULOVIRAL GENE THERAPY OVEREXPRESSING TBX20 PROMOTES ANGIOGENESIS AND CELL PROLIFERATION IN SKELETAL MYOBLASTS**

María del Rosario Bauzá[1], Pilar Ferrer[1], Agustina Scharn[1], Jorge Alejandro Simonin[2], Mariano Nicolás Belaich[2], Alberto José Crottogini[1], Fernanda Daniela Olea[1].
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Objectives**:** Gene therapy has been proposed as a possible treatment for peripheral arterial disease (PAD) in order to restore blood flow to the ischemic area. We have previously reported that overexpression of the transcription factor Tbx20 through baculoviral vector (Bv-Tbx20) induced an increase of cell proliferation and angiogenesis in ischemic myocardium. We hypothesized that Bv-Tbx20 administration would promote angiogenesis in PAD. Our aim was to evaluate the effects of overexpression of Tbx20 in rabbit skeletal myoblasts (Msk) culture on angiogenesis and cell proliferation at different days of culture. Methods: msk were transduced with Bv-Tbx20 or Bv-Null (as control) and at 2 and 5 days post transduction were measured cell proliferation by MTS assay and gene expression by RT-qPCR. In addition, supernatants from transduced cells were used to perform a tubulogenic assay in HMEC cells. Results: At 2 days post-transduction, BvTbx20 group showed higher percentage of cell proliferation than BvNull (140.2±22.2 vs. 100±35.8%, *p<0.05*), Tbx20 transgene expression (2310±613 vs. 1.1±0.2, *p<0.05*) and angiogenic regulatory gene expression (Prok2: 6±9.2 vs. 1.3±0.7, *p<0.05*; ProkR1: 5.2±6.6 vs. 1.1±0.8, *p<0.05*; and angiopoietin: 1.5±0.6 vs. 1.06±0.4, *p=0.07*). Also, at 5 days post-transduction, Bv-Tbx20 showed higher percentage of cell proliferation (121.7±16.2 vs. 100±19.7 %, *p<0.05*), Tbx20 transgene expression (60.5±42 vs 0.9±0.1, *p<0.05*) and gene expression of Prok2 (2.8±1.9 vs 0.9±0.6, *p<0.05*) and angiopoietin (2.2±0.8 vs. 1.2±0.8, *p<0.05*).In the tubulogenic assay a higher amount of rings was found in the BvTbx20 group vs. BvNull at 2 (2.2 ±0.4 vs 0.7± 0.4, *p<0.05*) and 5 days (2.3±0.3 vs 1.1±0.2, *p<0.05*).

Conclusion: Tbx20 overexpression in Msk increased cell proliferation, gene expression levels of angiogenic genes and promoted angiogenesis *in vitro*. These preliminary results suggest that Tbx20 could be a therapeutic alternative for tissue regeneration in PAD.

491. 549. **ADMINISTRATION OF DERMATAN SULFATE/CHITOSAN NANOPARTICLES LOADED WITH IRW TO INCREASE THE RESPONSE OF HUMAN COLORECTAL CANCER CELLS TO 5-FLUOROURACIL. IN VITRO STUDIES**

CURCIO Sofía[1], BLACHMAN Agustín[1], BIROCCO Ariadna[1], QUIROZ Lucía[1], BARAKIAN Benjamín[2] and CALABRESE Graciela C[1].

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The gold standard drug for colorectal cancer treatment, 5-Fluorouracil (5-FU), is known to induce pharmacological tolerance in long-term management. Recently, we described the capability of polyelectrolytes complex nanoparticles of dermatan sulfate (DS) and chitosan (CS), loaded with the tripeptide IRW (DS/CS-IRW), to sensitize colorectal cancer cells to 5-FU. The aim of the present work is to evaluate two different ways of administration of DS/CS-IRW for future *in vivo* studies. DS/CS-IRW were obtained by ionotropic gelification and characterized by Dynamic Light Scattering (DLS) and Transmission Electron Microscopy (TEM). Intravenous administration was evaluated on healthy serum. Samples were incubated in the presence DS/CS-IRW (10–50μg/mL equivalent DS) for 2h at 37°C to evaluate the adsorbed protein corona (PC) effect by polyacrylamide gel electrophoresis. Besides, erythrocyte hemolysis (EH) was determined spectrophotometrically at 570nm, after DS/CS-IRW addition. Oral administration was evaluated in a static *in vitro* digestion model. The digestion involves the exposure of DS/CS-IRW (10μg/mL equivalent DS) to gastric fluid (GF) (2h at 37°C, stirring) and intestinal fluid (IF) (4h at 37°C, stirring). After centrifugation (15min at 5000rpm) DS/CS-IRW characteristics were studied by DLS on the supernatant of GF, IF and GF+IF. DS/CS-IRW showed complexes with a single size population of 482(±75)nm (100%), a PDI value of 0.38(±0.09) and a Z potential +42(±0,54)mV. No changes in the electrophoretic profile for human albumin was detected, neither EH after DS/CS-IRW treatment. DLS studies carried on GF, IF and GF+IF supernatants showed a slight increase in the DS/CS-IRW size (505,9±79.4; 549,3±55,9 and 538,1±49.9; respectively). In conclusion, results suggest that both oral and intravenous administration could be employed in *in vivo* experiments. Further long-term studies are required to evaluate bioavailability and their effect on immune response.

492. 661. **CONTRACTILE FUNCTIONALITY IS COMPROMISED IN PLURIPOTENT STEM CELL-DERIVED CARDIOMYOCYTES WITH PARTIAL SILENCING OF HIWI2**

Nicolás Posteguillo, Guadalupe Amin, Julia Halek, Joaquín Smucler, Ariel Waisman, Lucía Moro, Gustavo Sevlever, Santiago Miriuka, Agustina Scarafía, Alejandro La Greca.

Laboratorio de Investigaciones Aplicadas a Neurociencias (LIAN), Instituto de Neurociencias (INEU) Fleni-CONICET.

PIWI proteins, from the Argonaute family of RNA-binding proteins, can associate with small non-coding RNAs called piRNAs to perform several well documented functions in germline cells. Recently, increasing amounts of evidence on uncharacterized functions in somatic cells and contexts were published, including modulating neuronal differentiation. Previous work done in our laboratory showed that *HIWI2* (*PIWIL4*) transcript is upregulated during embryonic stem cells (ESC) differentiation towards cardiomyocytes (CM). Considering all these results, we hypothesized that *HIWI2* participates in the process of CM differentiation. To study its role in the process, we generated a CRISPR/Cas9-based heterozygous *HIWI2* knockout hiPSC line (*HIWI2*$^{+/-}$) and differentiated it to CMs alongside wild type cells (WT). Decreased expression of *HIWI2* in *HIWI2*$^{+/-}$ was corroborated at transcript and protein level by qPCR and Western blot, respectively. Appreciable differences were not found neither in morphology or size of differentiating cells during bright field microscopic inspection, nor in the expression dynamics of relevant cell-stage gene markers (*NANOG* and *OCT4* in pluripotency, *MESP1* and *EOMES* in mesodermal progenitors, and *GATA4*, *TBX5* and *TNNT2* in cardiac progenitors) by qPCR or in percentage of TNNT2 positive cells assessed by flow cytometry. Despite all this, we observed that *HIWI2*$^{+/-}$ CMs lacked spontaneous contractile activity, and that contractility could not be restored by external electrostimulation. Further investigation revealed that expression levels of *NKX2.5*, *CX43*, and several genes involved in sarcomeric structures (*TNNI1*, *TNNI3*, *MYL7*, *MYL2*, *MYH6* and *MYH7*) and ionic transport (*RYR2* and *KCND3*) were altered in *HIWI2*$^{+/-}$, supporting our observations. In conclusion, our results suggest that *HIWI2* would not be critically involved in the differentiation process of CMs, but may be pivotal for assembling the structures and components required for contractility.

O1-REGENERATIVE MEDICINE & NANOMEDICINE

WEDNESDAY 15TH NOVEMBER 14:00-15:30

CHAIRS: HEBE DURAN
MARCELA BOLONTRADE

493. 22. **DEVELOPMENT AND EVALUATION OF CELL-FREE BONE SUBSTITUTE FOR THE TREATMENT OF CRITICAL BONE LESIONS**

María Eugenia Balañá[1,2], Sabrina Kokubu[3], Gabriel Kokubu[2,3], Patricia Mandalunis[4], Karin Hagelin[1], Alejandro Montaner[1], Gustavo José Leirós [1,2].

[1] Instituto de Ciencia y Tecnología Dr. César Milstein (FPC-CONICET). [2] Instituto de Investigación en Medicina y Ciencias de la Salud (IIMCS-Universidad del Salvador). [3]Consultorio Kokubu-Yamauchi. [4]Carrera de Especialización en Periodoncia-Escuela de Odontología (Facultad de Ciencias de la Salud, Universidad Maimónides).

Autologous or allogeneic bone grafts used in case of bone loss, provide osteogenic cells and factors but they have certain limitations. Metallic or ceramic implants provides mechanical support but show poor integration. Therefore, it is crucial to develop bone substitutes based on a biocompatible and osteogenic matrix. The aim of this study was to generate a cell-free bone substitute using collagen matrices (MCol) loaded with BMP-2, IMT-504 oligonucleotide, or a combination of both, to promote bone regeneration in critical lesions. Lesions of 6mm in diameter were created in Wistar rat calvaria and grafted with MCol combined with the following treatments: MCol+BMP-2, MCol+IMT-504, MCol+BMP-2+IMT-504. A matrix-only (Control) and an untreated lesion control (Control Abs) were also included. Previously we reported no differences among the groups in radiographic studies 10 days after graft while all groups exhibited a significant decrease in lesion size after 40 days. Only MCol-BMP2 and the Control group showed better lesion closure than the absolute Control, whereas MCol+IMT-504 got worse wound closure compared to the Control. This effect was partially reversed by BMP-2 in the MCol+BMP-2+IMT-504 group. In this work, histological studies conducted 40 days after graft demonstrated that BMP-2 improved osteoconductive and osteoinductive effects of collagen matrix. Notably, the presence of newly formed bone exhibited a continuous and more organized structure throughout the lesion compared to the Control Abs, which only showed new bone at the edges or compared to the Control group, which displayed “islands” of new bone. Conversely, IMT-504 exhibited diminished efficacy for bone regeneration than the Control Abs, potentially attributed to its anti-inflammatory effect upon topical application.

494. 66. **IMT504 AMELIORATES LIVER FIBROSIS IN MICE**

Maximiliano Borda[1], María J. Cantero[1], Esteban J. Fiore[1,3], Gianlucca Giardelli[1], Francisco Alvarado[1], Mercedes Díaz Pedraza[1], Alejandro Montaner[2,3], Guillermo D. Mazzolini[1,3] &

Jorge B. Aquino[1,3]
[1]IIMT CONICET-Universidad Austral. [2]ICT Milstein. [3]Consejo Nacional de Investigaciones Científicas y Técnicas

Objectives: To evaluate the effect of IMT504 during fibrogenesis or on a fibrotic liver in mice. Materials and methods: Liver fibrosis was established by applications of thioacetamide (TAA) or by bile duct ligation, and IMT504 was i.p. injected. A direct role of IMT504 and/or conditioned media (CM) of bone marrow stromal progenitors (BMSPs) subpopulations, pre-treated or not with IMT504, on relevant cellular types was analyzed. Histochemical, immunostaining, and qPCR assays were applied. Results: IMT504 inhibited liver fibrogenesis in both in vivo models, and accelerated the regression of a fibrotic liver in the TAA model, almost to normal. In mice treated with TAA for 2 or 8 weeks, and 1 day after the application of IMT504, it was found that the application of this ODN and the CM of GLAST+ Wnt1+ BMSPs pre-incubated with IMT504 reduced the mRNA expression levels of profibrogenic and pro-inflammatory factors, and increased the levels of anti-inflammatory markers, in samples of whole liver. Within the fraction enriched in parenchymal cells, markers involved in proliferation, hepatocyte functionality, and DNA repair and quality control of DNA synthesis were found to be overexpressed, while Gadd45α (a marker of cellular stress, DNA damage and cell cycle arrest) was down-regulated. Furthermore, these treatments: directly converted fibrotic macrophages towards a tolerogenic and pro-regenerative (upregulation of HGF) phenotype; significantly deactivated hepatic stellate cells and induced overexpression of HGF and IGF-I in primary cultured hepatocytes. Conclusions: IMT504 has a potent antifibrotic effect through different parallel mechanisms, and at the same time it also induces liver regeneration.

495. 76. **FHL1-LINKED MUSCULAR DYSTROPHY: PATIENT DERIVED IPSCS AND DIFFERENTIATION TO CARDIOMYOCYTES FOR IN VITRO DISEASE MODELING**

Federico Zabalegui[1], Sheila Lucia Castañeda[1], Guadalupe Amín[1], Carolina Bárbara Belli[2], Ariel Waisman[2], Gustavo Emilio Sevlever[1], Santiago Gabriel Miriuka[1], Lucia Natalia Moro[1].
1.Instituto de Neurociencias (INEU-CONICET), Laboratorio de Investigación Aplicada en Neurociencias (LIAN), Fundación para la Lucha contra las Enfermedades Neurológicas de la Infancia (FLENI), Escobar, Provincia de Buenos Aires, Argentina.
2.Instituto de Medicina Experimental (IMEX-CONICET), Academia Nacional de Medicina, Buenos Aires, Argentina.

The *FHL1* gene locates in the Xq26 region and encodes for four and half LIM domain protein 1, which is mainly expressed in skeletal and cardiac muscle tissues. This gene plays a crucial role in cytoskeletal remodeling, myoblasts differentiation, sarcomere assembly, and autophagy regulation. Unfortunately, mutations in FHL1 have been linked to muscular dystrophy (MD) and associated with limited life expectancy. The aim of this research was to generate and characterize induced pluripotent stem cells (iPSCs) derived from a patient with MD as consequence of a hemizygous missense mutation in FHL1 (c.377G>A, p.C126T) in order to facilitate *in vitro* disease modeling and personalized therapy development. To generate MD-iPSC, a blood sample was taken from the patient for erythroblasts expansion and reprogramming by transduction with STEMCCA lentiviral vector carrying the reprogramming factors Oct4-Sox2-Klf4-cMyc. After MD-iPSCs isolation and expansion we confirmed the presence of the c.377G>A mutation in FHL1 by Sanger sequencing, STEMCCA silencing, and normal karyotype. The pluripotency of the MD-iPSCs cell line was validated through assessments of alkaline phosphatase activity and the expression of pluripotency genes (*OCT4*, *NANOG*, *SOX2*) by RT-qPCR and immunohistochemistry. Additionally, MD-iPSCs were capable of differentiating into cells representing the three germ layers using embryoid body formation. Furthermore, MD-iPSCs were successfully differentiated into cardiomyocytes, observing high expression levels of cardiac markers (cTNT, NKX2.5) on day 21 of differentiation, using RT-qPCR and immunohistochemistry analyses. In conclusion, an iPSCs line derived from a patient with MD was successfully generated and differentiated into muscle cells (cardiomyocytes). It remains to determine the molecular and cellular consequences of the mutation, which will be elucidated through the differentiation and study of this cell line, as well as the development of a personalized therapy.

496. 226. **BLOCK OF CCL2 SECRETION INHIBITS PRO-INFLAMMATORY MACROPHAGE ACTIVATION IMPROVING AXONAL REGENERATION AFTER SEVERE SPINAL CORD INJURY**

Julieta Schmidt [1], Ana Uceda [1], Alejandra Sgariglia [1], Héctor Ramiro Quintá [1,2]
[1] Laboratorio de Medicina Experimental "Dr. J Toblli", Hospital Alemán – Argentina.
[2] Consejo Nacional de Investigaciones Científicas y Técnica.

Spinal cord injury (SCI) is a traumatic pathology that results in temporary or permanent damages in the spinal circuits, disrupting the flow of information between brain and SC. This study aimed to investigate the dynamic association between mechanical injury and inflammatory response in order to improve the axonal regeneration and motor recovery. The size of the spinal lesion caused by the mechanical injury increases after the secondary damage promoted by the inflammatory response. This process is triggered by recruitment of pro-inflammatory macrophages to the injury site. The protein that initiates it is the Chemokine C-C Ligand2 (CCL2), secreted by reactive astrocytes. Since our group has an extensive experience in the use of Netrin-1 (N1) as a therapeutically approach to promote axonal regeneration, we detected that N1 treatment, furthermore, inhibited significantly the increased lesion size after secondary damage ($p<0.05$), in a rat model of SCI. We observed an intense CCL2 fluorescent signal surrounding the lesion in vehicle-treated rats. In contrast, CCL2 signal was markedly reduced in N1 treated rats ($p=0.0423$). Notably, in the confocal images from vehicle treated-rats, we determined cells with pro-inflammatory macrophage-like phenotype displaying the CCL2 signal inlaid into astrocyte ramifications. However, N1 treated rats only presented a few CCL2 clusters inlaid in the astrocyte processes, and no diffuse fluorescent CCL2 signal was present in the spinal cord tissue. Besides, only in N1 treated rats, the regenerated axons were able to cross the epicenter of the lesion to relay with second order neurons ($p<0.05$). In summary, even though macrophage activation improves tissue cleanliness, this affects negatively the regenerative process. Thus, preventing the CCL2 secretion, the environment of axonal regeneration after SCI could be improved. These results provide a proof of-concept evidence of neuromodulation in neuronal repair after SCI.

497. 308. **OPTIMIZED IN VITRO DIFFERENTIATION PROTOCOL OF HUMAN PLURIPOTENT STEM CELLS TO SKELETAL MUSCLE CELLS FOR MYOPATHY MODELING**

Sheila Lucía Castañeda[1], Federico Zabalegui[1], Guadalupe Amín[1], Gustavo Emilio Sevlever[1], Santiago Gabriel Miriuka[1], Ariel Waisman[1], Lucía Natalia Moro[1].
1. Instituto de Neurociencias (INEU-CONICET), Laboratorio de Investigación Aplicado a Neurociencias (LIAN), Fundación para la Lucha contra las Enfermedades Neurológicas de la Infancia (FLENI).

Myopathies are skeletal muscle disorders mainly characterized by muscle weakness, determined by failure of cytoskeletal structure, mitochondrial physiology, glycogen or lipid metabolism, or ion channel function. Skeletal myocytes (SMs) differentiated from myopathy patient-derived induced pluripotent stem cells (PSCs) represent a source of study for disease modeling, drug discovery and personalized therapy development. The aim of this work was to establish an *in vitro* two dimensional (2D) differentiation protocol of human PSCs to SMs that will be used for myopathies *in vitro* modeling. In order to exit pluripotent state and induce presomitic mesoderm we tried CHIR99021 (CHIR) incubation with 2 or 7 days of LDN193189 (LDN) combined with and without SB431542 (SB), reaching the lowest expression of *NANOG* and *OCT4*, and the highest expression of *MSGN*, *MYF5*, *TBX6* and *TBXT*, at day 3 by qPCR with 7 days of LDN without SB and 2 days of LDN with SB conditions. Next step was to obtain premyogenic progenitors and both conditions were

followed by 1 week culture with fibroblast growth factor (GF), hepatocyte GF and insulin GF. We obtained the highest expression of *PAX3* at day 13 by qPCR with 7 days of LDN without SB. We terminally differentiated myoblasts to SMs following the culture with horse serum or a combination of CHIR, prednisolone and SB, obtaining with the latter one the highest expression of *MYOD*, *MYOG* and *PAX7* at day 30 by qPCR. As culture replating expands and purifies myoblasts, we tried this by replating at day 12 or 30 and sampling 2 weeks later. We got higher *DES*, *MYOG* and *TTN* expression at day 12 condition, which significantly reduces the protocol duration. Lastly, we detected NANOG and OCT4, TBX6 and TBXT, and DES expression at days 0, 3 and 26 respectively of the optimized protocol by immunofluorescence staining. In conclusion, we successfully differentiated NANOG/OCT4+ PSCs to DES+ SMs by an optimized 2D protocol by which myopathies will be studied *in vitro*.

P2-REGENERATIVE MEDICINE AND NANOMEDICINE

THURSDAY 16TH NOVEMBER 9:00 - 10:30
CHAIRS: LUIS IBARRA
MARIANO SCHUMAN

498. 91. **EVALUATION OF BIODEGRADABLE PRP-BASED SCAFFOLDING SYSTEMS SUITABLE FOR TISSUE ENGINEERING STRATEGIES AND REGENERATIVE MEDICINE**

Angelini Marquiani G. A.[1]; Rizzo M. E.[1]; Valenzuela Alvarez M.[1]; Bolontrade M.F.[1]

1 *Instituto de Medicina Traslacional e Ingeniería Biomédica (IMTIB) / CONICET-Hospital Italiano Buenos Aires (HIBA)-Instituto Universitario del Hospital Italiano (IUHI), Buenos Aires, Argentina*

Critical sized bone fractures are difficult to regenerate into functional structures through the body endogenous healing mechanism alone. External intervention with biomaterials is necessary for an effective recovery. Allogeneic and autologous bone grafts are widely used as bone substitutes with a high risk of immunological rejection and donor site-associated morbidity, respectively. Platelet-Rich Plasma (PRP) is an advantageous material and candidate for manufacturing scaffolds for bone regeneration, in combination with cells involved in tissue repair, such as fibroblast (FIB) and mesenchymal stem/stromal cells (MSC). We analyzed and characterized different properties of PRP as a scaffold suitable for cellularization, and established a protocol for PRP activation. Both calcium gluconate and calcium chloride ($CaCl_2$) generated a gel-like structure, with $CaCl_2$ being effective at lower concentration ranges (2-5 mg/ml vs. 5-10 mg/ml). Using two different cellularization strategies we assessed diverse niche-cell interactions, demonstrating that PRP scaffolds were not cytotoxic and that both FIB and MSC proliferated on it, degrading scaffolds at a 4,34±0,27 (% loss of weight/day) rate. We also showed that FIB and MSC could differentiate into osteoblastic lineage under standard culture conditions in PRP scaffolds. Using inactivated PRP as a culture medium additive replacing fetal bovine serum (SFB), we found that it generated a proliferative profile similar to SFB, however, when PRP replaced SFB in the osteoblastic differentiation medium, the cells surprisingly differentiated into an adipoblastic lineage. Finally, we evaluated cell adhesion on polylactic acid (PLA) scaffolds with and without a PRP coating. PRP was not critical in favoring cell adhesion on PLA. These results offer further information on the use of PRP for regenerative medicine strategies, pointing at the advantages and limitations for its use as a cellularizable scaffold or as a cell culture additive.

499. 119. **IMT504 ENHANCES WNT SIGNALING PATHWAY IN GLAST+ WNT1+ BONE MARROW STROMAL PROGENITORS**

Maximiliano Borda[1], María J. Cantero[1], Esteban J. Fiore[1,3], Gianlucca Giardelli[1], Francisco Alvarado[1], Abalo Agustina[1], Camila Becerra[1], Alejandro Montaner[2,3], Guillermo D. Mazzolini[1,3] & Jorge B. Aquino[1,3]

[1]IIMT CONICET-Universidad Austral. [2]ICT Milstein. [3]Consejo Nacional de Investigaciones Científicas y Técnicas

We previously found that the oligodeoxynucleotide IMT504 induces the proliferation and mobilization of GLAST+ Wnt1+ bone marrow stromal progenitors (BMPs), with no or little effects on other BMP subpopulations. Objectives: To evaluate the effect of IMT504 on the mRNA expression levels of ligands and markers of the Wnt signaling pathway in two subpopulations of BMSPs propagated in vitro. Materials and methods: Passaged 8 GLAST+ Tom+ and Tom- BMSP subfractions, obtained from $GLAST^{CreERT2}$;$Rosa26^{tdTomato}$ (tamoxifen-injected at postnatal day 2), were incubated separately with the oligodeoxynucleotide (ODN) IMT504 for 2 hours and left to recover for 4 or 22 hours. The mRNA expression levels of Wnt ligands, as well as Wnt signaling pathway/target genes, were analyzed by qPCR. Results: A highly significant increase in Wnt1, Wnt3a and Wnt5a as well as in beta-catenin and Axin2, Lef1 and Sp5 was observed in GLAST+ Wnt1+ BMPs, without affecting Tom- cells. Consistently, a downregulation in E-cadherin was also observed in Tom+ cells treated with IMT504. And longer incubation after IMT504 treatment of Tom+ cells resulted in further significant changes in the mRNA expression levels of the same genes. Longer incubation after IMT504 treatment in serum-deprived culture medium appears to induce this expression pattern, even in naïve Tom+ cells. Conclusions: GLAST+ Wnt1+ BMPs are a unique cellular subpopulation, specifically targeted by IMT504, inducing the expression profile of Wnt ligands and signaling molecules, likely acting in an autocrine/paracrine manner to improve their proliferative and mobilization capabilities.

500. 341. **EVALUATION OF E2Fs INHIBITION ON CELL CYCLE, VIABILITY, AND GENE EXPRESSION IN HUMAN PLURIPOTENT STEM CELLS**

Vautier M[1], Rodríguez Varela MS[1], Mucci S[1], Isaja L[1], Sevlever GE[1], Scassa ME[1], Romorini L[1]

1. Laboratorio de Investigación Aplicada a Neurociencias, Instituto de Neurociencias, Fundación para la Lucha contra las Enfermedades Neurológicas de la Infancia (LIAN-INEU-Fleni-CONICET), Buenos Aires, Argentina.

Human pluripotent stem cells (hPSCs), like embryonic (ESCs) and induced pluripotent stem cells (iPSCs), exhibit an unusual cell cycle with a shortened G1 phase. E2F transcription factors (E2Fs) regulate the G1/S transition where hPSCs initiate cell fate decisions. In these cells, the role of E2Fs and their regulation remains uncertain. Thus, this work aimed to identify the effect of E2Fs inhibition on cell cycle, viability, and gene expression in hPSCs. First, we determined higher mRNA levels of canonical E2Fs in hPSCs (H9 hESCs and FN2.1 hiPSCs) arrested in early G1 with PD0332991 compared to human fibroblasts (HF). Intriguingly, western blot assays revealed heightened E2F3a and diminished E2F1 levels in hPSCs compared to HF. Then, we treated both hPSC lines with the E2Fs general inhibitor, HLM006474. The effectiveness of the inhibitor was determined by studying the cell cycle profile of treated hPSCs using propidium iodide (PI) staining followed by flow cytometry analysis. Cell viability was assessed by Trypan blue and PI staining, and XTT colorimetric assay. We chose 20 μM HLM006474 for 24 hours for our experiments as these conditions increased G1 cell population and preserved cell viability in both hPSCs lines. Then, we analyzed the expression levels of stemness markers (*OCT-4, NANOG*, *SOX-2* and *LIN-28*) by RT-qPCR. Interestingly, *OCT-4* mRNA and protein levels decreased in both treated hPSC lines relative to controls as judged by RT-qPCR and flow cytometry analysis. We also analyzed by RT-qPCR the expression levels of *CYCLIN E1, CYCLIN A2, E2F1, SIRT1* and *RUNX3* due to their known E2F-mediated regulation. Notably, E2F inhibition led to reduced *CYCLIN A2* mRNA levels in H9 hESCs and elevated E2F1 mRNA levels in FN2.1 hiPSCs relative to controls. Prior research also brought out a decrease in *CYCLIN E1* mRNA after 7 days of treatment. Our results highlight the connection between the cell cycle machinery that regulates G1/S phase transition and the pluripotency network.

501. 403. ***IN VITRO* AND *IN VIVO* ANGIOGENIC EFFECT OF *MEIS1* OVEREXPRESSION**

Ayelén Emilce López[1], María del Rosario Bauzá[1], Jorge Alejandro Simonin[2], Agustina Scharn[1], Araceli Castro[1], Cintia Silvestro[1], Mariano Nicolás Belaich[2], Alberto Crottogini[1], Fernanda Daniela Olea[1], Paola Locatelli[1]
[1] Instituto de Medicina Traslacional, Trasplante y Bioingeniería (IMETTYB- Universidad Favaloro- CONICET), [2] Laboratorio de Ingeniería Genética y Biología Celular y Molecular Área Virosis de Insectos, Universidad Nacional de Quilmes.

Objectives: to assess the angiogenic effects of Meis1 overexpression in neonatal rat cardiomyocytes (NRCMs) and ovine hearts. Methods: NRCMs were transduced with a baculoviral vector encoding *Meis1*(Bv.Meis1) The effect was evaluated at 2 and 5 days post-transduction(PT). Angiogenic gene expression was assessed by RT-qPCR, and *in vitro* angiogenesis by HMEC cell proliferation (MTS assay) incubated with the supernatant of *Meis1* transduced NRCMs at the same time points. 12 sheep with acute myocardial infarction (AMI) received intramyocardial injections of Bv.Meis1 or Bv.Null. 7 days after AMI, sheep were euthanized and samples of the injected zone were harvested. Angiogenic gene expression (RT-qPCR) and microvascular densities (immunohistochemistry) were quantified. Statistics: T test or Mann Whitney´s test (significance: p <0.05). Results: Angiogenic gene expression was increased in NRCMs transduced with Bv.Meis1 compared to CMs-Bv.Null at 2 days PT (*Vegf* fold increase:1.91±0.62 vs. 0.89±0.06, p<0.01, *Angiogenin* fold increase:1.19±0.139 vs. 1±0.015, p<0.05 and *Hif1α* fold increase:1.30±0.20 vs. 0.90±0.01, p<0.05). MTS assay showed increased cell division in cells with NRCM-Bv.Meis1 supernatant compared to NRCM-Bv.Null at 2 (Cell proliferation:117.4±10.70% vs. 100±18.56%, p<0.01) and 5 (Cell proliferation:118.7±20.99% vs. 100±12.91%, p<0.05) days PT. Expression of genes involved in angiogenesis was increased in Bv.Meis1 sheep group vs Bv.Null (*Vegf* fold increase:1.17±0.194 vs. 0.68±0.18, p<0.01, *Angiogenin* fold increase:3.45±1.84 vs. 1.36±0.77, p<0.05). Microvascular densities were increased in Bv.Meis1 group in comparison to Bv.Null (capillary density:1545±372.1 vs. 1234±345.2 capillaries/mm^2, p<0.05; arteriolar density:13.68±3.98 vs. 9.36±2.44 arterioles/mm^2, p<0.05). Conclusion: Transduction of NRCMs with Bv.Meis1 induced angiogenesis. This effect was reproduced *in vivo* through the injection of Bv.Meis1 in infarcted sheep, resulting in significant angio-arteriogenesis.

502. 538. **IDENTIFICATION OF LINC881 LOCUS AS A KEY REGULATOR OF MASTER TRANSCRIPTION FACTOR NKX2-5 IN CARDIOMYOCYTES**

María Agustina Scarafía, Julia Halek, Nicolás Posteguillo, Joaquín Smucler, Sheila Castañeda, Guadalupe Amin, Carolina Colli, Lucía Moro, Ariel Waisman, Santiago Miriuka, Alejandro La Greca.
Laboratorio de Investigaciones Aplicadas a Neurociencias (LIAN), Instituto de Neurociencias (INEU) Fleni-CONICET.

The decisions leading cells to determine their fate occur very early during development. Among the elements that comprise the regulatory gene network of cardiac development, genes *TBX5*, *GATA4*, and *NKX2-5* stand out as central transcription factors in the heart. However, the mechanisms involved in their regulation and the decision-making process of cardiac lineage commitment are not fully understood. In previous results, we identified *LINC881*, a primate-specific long non-coding RNA (lncRNA) transcribed from a cardiac Super-Enhancer (SE49551), as a potentially relevant gene in cardiac differentiation of human pluripotent stem cells (hPSC). Loss-of-function assays employing CRISPR/Cas9 to knockout (KO) the region comprising the first exon of *LINC881* and part of SE49551, revealed that while cardiac differentiation of KO cells was not compromised, nor their contractile activity, expression of *NKX2-5* was severely reduced. Overexpression of *LINC881* transcript in KO cells and their differentiation into cardiomyocytes failed to restore *NKX2-5* expression to levels similar to those in the Wild Type (WT) cell line, indicating that *LINC881* transcript is not sufficient to rescue *NKX2-5*, and that the genomic locus would be necessary for its regulation. Transcriptomic analysis revealed 134 differentially expressed genes (DEGs, p-adj<0.1) affected by the deletion, none of which are reported regulators of *NKX2-5*. Examining DEGs in more detail, we found that the deleted locus does not regulate genes in close chromosomal proximity (distance>4Mb), suggesting that the regulatory mechanism might be exerted on distant regions, through direct or indirect interactions. Our findings suggest a key role of the studied locus during cardiac differentiation of hPSCs, a previously unknown aspect in *NKX2-5* regulation.

503. 580. **SILICA NPS@TGFβ COMPLEXES PRESENT IMMUNOMODULATORY ACTIVITY OVER MONOCYTES**

Exequiel Giorgi[1,2], Sofía Genoves[3], María Eugenia Diaz[1,2],-Mauricio De Marzi[1,2], Martin Desimone[3]
(1) Instituto de Ecología y Desarrollo Sustentable (Universidad Nacional de Lujan-CONICET), (2) Universidad Nacional de Luján, Departamento de Ciencias Básicas, (3) Universidad de Buenos Aires, IQUIMEFA-CONICET, Facultad de Farmacia y Bioquímica (FFYB)

Inorganic nanoparticles (NPs) can display a high capacity to adsorb proteins over their surface. In particular, they could transport proteins with immunomodulatory activity for biomedical purposes. Our objective was to obtain nanocomplexes of silica NPs with TGFβ that presented immunomodulatory activity over human monocytes. Silica nanoparticles (SiOH NPs) with a diameter of 111±11 nm and a negative potential of -23.6 mV were synthesized. A portion of these were chemically modified with APTES obtaining NPs with positive potential (+13.3 mV) named $SiNH_2$ NPs. NPs were characterised by TEM, FTIR and DLS. TGFβ was successfully immobilized over both NPs. SiOH NPs@TGFβ complexes had a adsorption capacity (AC) of 27.7 μg/mg and $SiNH_2$ NPs@TGFβ presented an AC of 25.2 μg/mg. THP-1 cells (human monocytes) were cultured in presence of NPs and nanocomplexes (300 μg NPs/mL) at 24, 48 and 72 h. Also, THP-1 cells were treated with TGFβ alone in the same concentration that TGFβ adsorbed over NPs. Controls of TGFβ alone generated a slight decrease in the cell metabolic activity. Metabolic activity of cells treated with NPs were compared with cells treated with nanocomplexes. We found that SiOH NPs@TGFβ diminished 30% the metabolic activity in comparison to SiOH NPs at 24 h. In the other hand, $SiNH_2$ NPs@TGFβ also generated a reduction of metabolic activity in comparison with $SiNH_2$NPs at 24 h (22%). In both cases, this tendency was even more evident at 48 and 72 h. Both nanocomplexes presented similar effects regardless of the original chemical nature of the NPs. We also found that while SiOH NPs provoked a higher nitric oxide (NO) expression by monocytes at 24 h, while the nanocomplexes did not generate changes in comparison with the NO levels in control cells. We demonstrated that the nanocomplexes presented a similar activity than TGFβ, but it was enhanced and maintained through the time. This provides a model of a possible nanoimmunomodulator to be applied in inflammatory diseases.

504. 591. **ISOLATION AND CHARACTERIZATION OF HUMAN AMNIOTIC MESENCHYMAL CELLS AS STEM CELL SOURCE FOR CORNEAL ENDOTHELIAL REGENERATION**

Rodrigo Riedel[1], Antonio Pérez-Pérez[2], Mariana Jaime[3], Pilar Guadix[4], Víctor Sánchez-Margalet[2], Cecilia Varone[1], Alejandro Berra[5], Julieta Maymó[1].
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The amniotic membrane from the human placenta at term is a valuable stem cell source, including human mesenchymal stromal cells (hAMSCs). They express embryonic stem cell markers and have the ability to differentiate towards different tissues. Moreover, hAMSCs possess immunomodulatory, anti-fibrotic, and anti-inflammatory properties, thus allowing their use in cell therapy. Corneal diseases

are the fourth principal cause of blindness worldwide, such as corneal endothelial dysfunction. However, the available treatments have several obstacles. Recently, mesenchymal stem cells (MSC) have been spotlighted as an alternative corneal endothelial cell source because of their origin in the walnut crest. This work aimed to isolate and characterize the human mesenchymal stromal cells from the amniotic membrane as stem cells. First, we have successfully performed an isolation protocol using trypsin-collagenase digestion. We have isolated approximately 1,5x10^7 cells with 70% viability by Trypan Blue staining and Neubauer chamber counting, respectively. Phenotypic characteristics of freshly cells were analyzed by flow cytometry and bright field microscopy. We have obtained 90% positive cells for MSC markers (CD73, CD90, CD105) and negative for hematopoietic markers (CD45, Gly A). In addition, isolated cells presented typical MSC morphology with adherent growth, spindle shape, and whirlpool patterns, in culture. We also observed that vimentin expression was retained until day 14, measured by Western blot. Moreover, we have determined by qRT-PCR that hAMSCs express significant levels of stem-cell markers such as OCT-4 and NANOG, during at least 7 days in culture. Finally, we found that cell viability significantly increased during 14 days in culture, evaluated by MTT assay. Isolation and characterization of hAMSCs will allow us to continue studying their application in the ophthalmological health area.

505. 649. **THE HUMAN HEPATOCARCINOMA HUH-7 CELLS UNDERGO APOPTOTIC PROCESS AFTER AMNIOTIC MEMBRANE CONDITIONED MEDIUM TREATMENT**

Luciano A. Pérez[1], Rodrigo Riedel[1], Nataly De Dios[1], Antonio Pérez-Pérez[2], Mariana Jaime[3], Víctor Sánchez-Margalet[2], Cecilia Varone[1] and Julieta Maymó[1].

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Recently, the placental stem cells have been positioned as a central tool for the regenerative and reparative medicine. Their therapeutic potential to treat different diseases, including cancer, has been highly reported. There is plenty evidence about the anti-tumoral effects of the human amniotic membrane given by their antiproliferative, antiangiogenic and propoptotic properties. Liver cancer is the fifth cause of cancer in the world, with a poor prognosis and survival. Alternative treatments to radio- or chemotherapy have been searched. We and other groups demonstrated the antitumoral effects of the amniotic membrane and their stem cells, but there is still a great lack of knowledge about the molecular and cellular mechanisms involved. We have previously showed that the amniotic membrane conditioned medium (AM-CM) inhibits hepatocarcinoma cells proliferation and survival, and promotes HepG2 cells apoptosis. In this work, we aimed to analyse the AM-CM propoptotic effect in a more aggressive hepatocarcinoma cellular model, Huh-7. First, we observed cell morphology changes by bright field microscopy. Hepatocarcinoma HepG2 and Huh-7 cells shrinked and cytoplasm density increased after AM-CM treatment. We have also analyzed the diversity of AM-CM effects on cell viability since they are obtained from different human placentas with intrinsic variability. MTT viability assay confirmed this variability. Considering these results, we have treated Huh-7 cells with the more effective AM-CM media. We determined a significant increase in Bax/Bcl-2 ratio expression measured by qRT-PCR. We also observed through immunofluorescence (IFI) that the expression of AIF, a mitochondrial proapoptotic protein, increased significantly after treatment with AM – CM. Cytochrome-c expression also increased, measured by WB. Finally, we have found by IFI that there is a significant increment in Caspase-3 cleavage. Our results position amnion derived cells as emerging candidate for alternative antitumoral treatments.

O2-REGENERATIVE MEDICINE & NANOMEDICINE

FRIDAY 17TH NOVEMBER 9:00 - 10:30

CHAIRS: LEONARDO ROMORINI
LUCÍA MORO

506. 109. **SOLUPLUS® NANOMICELLES ENHANCE IgG NEUTRALIZING PROPERTIES AGAINST SHIGA TOXIN TYPE 2**

Daniel Girón[1,2], Gabriela E. Gómez[3,4], Juan J. Casal[1,2], José M. Delfino[3,4], Fernando Gomez[1,2], Cristina Ibarra[1,2], Maria M. Amaral[1,2], Diego Chiappetta[5,6,7], Marcela Moretton[5,6,7], Flavia Sacerdoti[1,2].

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[7] Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina.

Shiga toxin type 2 (Stx2) is the main virulence factor of Shiga toxin producing *Escherichia coli* (STEC) and is responsible for the development of typical Hemolytic uremic syndrome (HUS). Nowadays, no specific treatment exists for this disease. The aim of this work was to develop Soluplus® nanomicelles (NM) associated to specific IgG (IgG-Stx2), characterize them, and evaluate their neutralizing capacity against Stx2 to propose them as a treatment for HUS. IgG-Stx2 or control (IgG-CT) were purified from bovine colostrum by affinity chromatography. NM-IgG association (NM-IgG-Stx2 or NM-IgG-CT) was achieved by the magnetic stirring method. Characterization of NM-IgG was performed by dynamic light scattering (DLS), UV-Vis, fluorescence, and circular dichroism (CD) spectroscopies, while morphology was analyzed by transmission electron microscopy (TEM). Neutralization capacity of NM, NM-IgG-CT, NM-IgG-Stx2, IgG-CT or IgG-Stx2 against Stx2 was evaluated on Vero cells and HGEC. Additionally, neutralization of supernatants (SN) from STEC strains containing Stx2 was also assayed on Vero cells. NM showed a monomodal size distribution with a hydrodynamic diameter (Dh) of 69.2 ± 0.3 nm, while IgG showed a major Dh peak of 19.3 ± 1.8 nm. NM-IgG showed a single peak with a Dh value of 75.1 ± 0.5 nm, significantly higher compared to NM alone (*$p<0.05$). TEM analysis revealed a spherical morphology for both NM and NM-IgG. The UV-Vis and fluorescence spectra point to a moderate increase in IgG absorbance in a range of Soluplus® concentration (up to 1 mg/ml). Consistently, CD spectra in the far UV region showed an increase in the magnitude of the absolute signal intensity (most evident at 216 nm), due to the solubilizing effect of Soluplus®. Finally, NM-IgG-Stx2 improved by 20% the neutralization capacity of purified Stx2 or Stx2 derived from different STEC strains SN compared to IgG-Stx2 alone (**$p<0.01$) *in vitro*. These results suggest that IgG associate with NM and this interaction enhances IgG neutralizing properties against Stx2 due to a better availability of IgG in the presence of NM.

507. 254. **BONE REGENERATION WITH TERIPARATIDE-IMPREGNATED BOVINE BONE GRAFT FOR THE REPAIR CRITICAL-SIZED BONE DEFECTS IN RATS**

Gretel G. Pellegrini[1,2], Marina Bonanno[3], Macarena Gonzales Chaves[4], Magali Zeni Coronel[1], Ricardo Orzuza[4], Susana N. Zeni[1], Panos N. Papapanou[2].

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College of Dental Medicine; [3] Department of General Biochemistry and Oral Biology, School of Dentistry, Buenos Aires University (FOUBA); [4] Department of Histology and Embryology, FOUBA.

Bone grafts are routinely used for bone defect repair in maxillofacial areas, and bovine bone (BB) is one of the most used bone grafting materials. Intermittently-administered parathyroid hormone 1-34 (PTH), or teriparatide (T), has anabolic effects on bone. We hypothesized that BB impregnated with T would enhance bone formation in the repairing of critical-sized bone defects (CSBD) in the tibiae of adult Wistar rats. To test this hypothesis, we evaluated the effect of T in impregnated BB particles on bone homeostasis; systemic bone remodeling; bone volume (BV) and microarchitecture; and new bone formation. The experimental protocol involved the following animal groups: Control CSBD without graft; BB: CSBD grafted with BB; BB & T: CSBD grafted with BB impregnated with T; BB & T + subcutaneous T (sc): CSBD grafted with BB impregnated T accompanied by injections of T for 30 days. Animals were sacrificed on day 45. Serum samples were obtained at multiple time-points to evaluate bone homeostasis and bone turnover markers. Bone microarchitecture by micro-CT and new bone formation by histomorphometry were evaluated at the treated defects. Results: % change (D= at 45 vs. 0 days) of serum PTHi, amino-terminal propeptide of type I procollagen (P1NP) and carboxy-terminal telopeptide of type I collagen (CTX) were substantially and statistically significantly increased, while osteocalcin was decreased when compared to control ($p<0.05$, assessed by one-way ANOVA after Bonferroni corrections). BB & T presented with higher P1NP and periostin than BB & T + T sc. Histologically, BV exhibited a non-significant increase in the T-treated groups. Micro-CT analysis showed higher BV/ total volume, trabecular number and lower trabecular spacing in BB & T. Conclusion: Impregnation of T in BB enhances the bone repair process in CSBD and may represent a novel therapeutic alternative for the repair of oral bony defects, without discernible systemic effects.

508. 412. **NEW HLA-G ISOFORMS IN UMBILICAL CORD MESENCHYMAL STEM CELLS**

Ailén Iribarne[1,2], Diana Tronik-Le Roux[2,3], Isabelle Poras[2,3], Marina Daouya[2,3], María Belén Palma[1,4], Laura Andrini[1], Pablo Pelinski[5], Edgardo D. Carosella[2,3], Santiago Miriuka[4] and Marcela N. Garcia[1]

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4. LIAN-INEU-CONICET, FLENI Escobar, Buenos Aires, Argentina

5. Hospital Español, La Plata, Buenos Aires, Argentina

Chronic venous ulcer (CVU) is the loss of substance that does not heal with conventional treatment after correcting the factor that produced it, leading to the appearance of infections and complications in the patient. Treatments based on the use of autologous mesenchymal stem cells (MSC) have been successful; however, their implementation entails complications. Umbilical cords (UC) offer an extensive source of MSC from Wharton's jelly tissue with the same features for clinical applicability and avoiding difficulties. The umbilical cord mesenchymal stem cells (ucMSC) express the Human Leukocyte Antigen G (HLA-G), an immuno-checkpoint (IC), which produces a local inhibition of the system that would allow allogeneic transplantation playing a significant role in regenerative medicine. The present work aims to identify which HLA-G isoforms are present in ucMSC. First, immunohistochemistry (IHC) was used to detect the HLA-G presence directly in UC and surface labeling for specific antigens using flow cytometry (FC) was carried out in ucMSC. To detect different HLA-G isoforms, RT-PCR was performed using specific primers. In the results by IHC and FC the HLA-G expression was low. Then, RT-PCR analysis showed that 77.8% of UC were HLA-G positive. Until now, all HLA-G isoforms described have the α1 domain. However, within the positive UC, only 12% of them amplified α1 domain by PCR. The other 88% of UC have isoforms that lack this domain, which would correspond to the detection of new HLA-G isoforms. These data explain the low detection with IHC and FC, since the employed antibodies target α1 domain. The presence of this IC would modulate the activity of the immune system and could play a key role in the activation of angiogenesis depending on the isoform present. These different functions of HLA-G allow allogeneic transplantation of ucMSCs and a favorable environment for tissue regeneration, so these ucMSCs could be a new therapeutic alternative for the treatment of UVC.

509. 496. **USE OF SMALL MOLECULE SB4 AS A POTENTIAL ANALOG OF BMP4 IN DIFFERENTIATION PROTOCOLS INVOLVING HUMAN INDUCED PLURIPOTENT STEM CELLS**

Carolina Romano Florit[1], Gonzalo Tomás Chirino Felker[1], Guadalupe Amin[1], Joaquín Smucler[1], Federico Sevlever[1], Ariel Waisman[1], Sheila Castañeda[1], Santiago Gabriel Miriuka[1], Lucía Natalia Moro[1], María Andrea Camilletti[1,2].

1. Laboratorio de Investigación Aplicada a Neurociencias (LIAN), Instituto de Neurociencias (INEU, FLENI-CONICET); 2. Instituto de Biociencias, Biotecnología y Biología translacional (iB3), Depto Fisiología y Biología Molecular y Celular, Facultad de Ciencias Exactas y Naturales (FBMC, FCEyN-UBA-CONICET).

Bone morphogenetic protein-4 (BMP4) mediates essential signalling pathways in the early steps of human development. Recombinant BMPs have been synthesised for *in vitro* studies and therapeutic purposes, but they are very costly and display low stability. In this context, a small molecule known as Sb4 was identified as a potential analog of BMP4 in human renal cells. Since this has not been studied in human induced Pluripotent Stem Cells (hiPSCs), we set out to evaluate the ability of Sb4 to activate the BMP signalling pathway in hiPSCs and induce differentiation toward the mesodermal lineage. First, the cytotoxicity of Sb4 in hiPSCs was determined by calculating the percentage of living cells after 24h of Sb4 treatment using Trypan Blue and Neubauer's chamber. For concentrations 0.01-50uM, cell viability was above 80%, whereas 100uM caused it to drop below 30%. Next, for mesoderm-differentiation assays, hiPSCs were incubated in RPMI supplemented with Activin(A)+rBMP4 or A+Sb4(10 uM) for 72hrs, and RNA samples were collected for RT-qPCR. Results revealed an upregulation of mesoderm-associated genes (*TBXT,EOMES,MIXL1*) and a downregulation of pluripotency genes (*OCT4*,*NANOG*) in both conditions. To explore if Sb4 effects are partly mediated by a SMADs-dependent BMP pathway, SMAD1 expression was evaluated by western blot in hiPSCs treated with Sb4 alone or in combination with rBMP4. Interestingly, SMAD1 increased after Sb4 treatment, and this effect was more significant for the combined group (vs.control hiPSCs). Additionally, preliminary results showed positive pSMAD1/5 immunostaining in hiPSCs after 30' treatment with Sb4, mirroring the response with rBMP4, suggesting an activation of the pathway. Finally, biological BMP4 targets *ID1* and *ID3* were upregulated after 24h of Sb4 treatment. To sum up, our findings indicate that Sb4 activates the BMP pathway in hiPSCs, and might be used as a cheaper alternative to rBMP4 for hiPSCs differentiation towards mesodermal lineages.

510. 564. **REPURPOSING FLUBENDAZOLE TO INHIBIT THE INVASION OF BREAST CANCER CELLS THROUGH ITS DELIVERY BY DERMATAN SULFATE/CHITOSAN POLYELECTROLYTE COMPLEXES**

BIROCCO Ariadna[1], BLACHMAN Agustín[1], CURCIO Sofía[1], QUIROZ, Lucía[1], DI GIANVINCENZO, Paolo[2], MOYA Sergio[2], CALABRESE, Graciela[1].

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Although conventional therapies have improved the survival rates in triple negative breast cancer (TNBC), recurrence and metastasis are still inevitable, emphasizing the need to develop new therapeutics. The antihelmintic Flubendazole (FLU) has recently been repurposed as a novel antitumoral agent; however, its bioavailability is low because of its poor water solubility. We have reported the effective targeting of dermatan sulfate/chitosan polyelectrolyte complexes (PECs) as an effective nanocarrier towards TNBC cells, because of the selective interaction between DS and the CD44 receptor. The aim of this work is to evaluate the capability of PECs to load and distribute FLU to CD44+ cells, using a TNBC cell model. FLU-PECs were obtained by ionotropic gelification and characterized by Dynamic Light Scattering (DLS) and Transmission Electron Microscopy (TEM). The MDA-MB-231 cell line, in 2D and 3D cultures, was treated with FLU (1-10 μM) or FLU-PECs (10μg/mL equivalent DS) for 4 and 24hs. Cytotoxicity studies were performed by the MTT assay; cell uptake was analyzed by flow cytometry and fluorescence studies, and cell invasion was addressed by wound healing assay and collagen adhesion assay. The nanoformulation displayed a single population of 556(±80)nm, with a Z-potential of +40(±4)mV. FLU and FLU-PECs show a similar decrease in cell viability (23±8% in 4h and 46±9% in 24h respectively). Flow cytometry and confocal microscopy studies confirmed that both unloaded and loaded FLU-PECs can be internalized by 2D and 3D cells, which correlates with a high expression of CD44. Regarding its effects, both FLU or FLU-PECs show a similar inhibition in 2D cell migration, with no statistical differences. In short, FLU-PECs deliver FLU to CD44+ cells and exert their expected effect, thus the encapsulation of this repurposed drug could prove a novel therapeutic alternative for TNBC.

P1-REPRODUCTION

WEDNESDAY 15TH NOVEMBER 9:00 - 10:30

CHAIRS: SOLEDAD ROSSI
JORGELINA BUSCHIAZZO

511. 53. **EFFECT OF RESVERATROL AND TROLOX SUPPLEMENTATION ON REACTIVE OXYGEN SPECIES PRODUCTION IN VITRIFIED AND WARMED PORCINE OOCYTES**

Stephania Madrid Gaviria[1,2], Sergio Adrián Morado[1,2], Mariana Córdoba[1,2], Pablo Cetica[1,2].
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The aim of this research was to evaluate the effect of the antioxidants trolox and/or resveratrol on reactive oxygen species (ROS) production in vitrified and warmed porcine oocytes. Immature cumulus-oocyte complexes (COCs) were matured *in vitro* in 199 medium added with porcine follicular fluid (FFP), cysteine, FSH and LH, at a 39°C, 5% CO_2 in humidified air for 44 h. Then, oocytes were denuded by incubation with hyaluronidase for 5 minutes at 37°C. Oocytes were vitrified and warmed by a minimum volume method. To evaluate the effect of the antioxidants, the vitrification and warming solutions were supplemented with resveratrol and/or trolox at a concentration of 2 μM and 50 μM, respectively. After warming, oocytes were cultured in 199 medium + FFP for 3 h to allow their recovery. After this time, oocytes were stained with 10 μM of DCH_2FDA for 30 minutes in dark. A small group of oocytes from each treatment was stained with 0.12 μM of FDA for 15 minutes in dark. Then, they were washed three times in PBS-PVA and placed on a slide for their observation under an epifluorescence microscope. The exhibited fluorescence by each oocyte was analyzed using the IMAGEJ software from the images obtained. As fluorescence levels detected by DCH_2FDA are dependent on esterase activity, the ratio between the fluorescence intensity for each oocyte measured by DCH_2FDA and the mean fluorescence detected by FDA for each treatment was considered a better indicator of ROS levels. Data were analyzed by ANOVA ($p<0.05$). It was found that the vitrification-warming process generates a significant increase in ROS production compared with fresh oocytes ($p<0.05$). Nevertheless, the addition of trolox, resveratrol or their combination at the studied concentrations, did not control the detected ROS increase unlike what we have found in previous studies where resveratrol, alone or in the presence of trolox, partially avoided the increase in active mitochondria caused by this cryopreservation protocol.

512. 94. **COMBINATION OF PRENATAL ANDROGENIZATION AND HIGH-FAT DIET ON SEXUAL DEVELOPMENT AND ADULT REPRODUCTIVE FUNCTION OF FEMALE MICE**

Pedro Javier Torres[1], Rocío Maldonado[1], Nicolás David Ramírez[1], Pablo Aníbal Pérez[2], Eugenia Mercedes Luque[1], Verónica Inés Cantarelli[1], Marina Flavia Ponzio[1], Silvina Gutierrez Oschman[2] y Ana Carolina Martini[1].
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2. Centro de Microscopía Electrónica, Facultad de Ciencias Médicas, Universidad Nacional de Córdoba e INICSA-CONICET/UNC, Córdoba, Argentina.

Polycystic ovary syndrome (PCOS) is frequently associated with female subfertility. Injecting dihydrotestosterone (DHT) into pregnant dams leads to a PCOS-like phenotype in the female offspring. This study aims to evaluate whether coadministration of a high-fat diet (HFD) to the pups exacerbates the effects of prenatal androgenization and modifies plasma and ovarian Ghrelin (Ghrl) levels/expression. Pups received either a control diet (CD) or a HFD (commercial pellet + 30% pork fat) from weaning. We evaluated the impact of four treatments (DHT-CD; C-CD; DHT-HFD; and C-HFD) on sexual development, adult reproductive function, and plasma/ovarian Ghrl levels/expression (measured by western blot). Statistics: ANOVA (two-way or repeated measures); n=2-8 litters/treatment. No significant differences were found in pups' weight gain. DHT delayed or even inhibited vaginal opening, with this effect being exacerbated by the HFD (% vaginal opening on postnatal day 39: DHT-HFD^{a}=25.0, DHT-CD^{bc}=64.3, C-HFD^{b}=100, C-CD^{bd}=100; $p<0.05$ a vs b and c vs d). In adulthood, DHT-exposed females (DHT-CD and DHT-HFD) exhibited significantly lower pregnancy rates than controls (C-CD and C-HFD); 67% vs 100%. The addition of the HFD decreased litter size, with the most pronounced effects seen in DHT-HFD females, who also displayed morphological abnormalities in their uteri. Although plasma Ghrl levels did not vary significantly (C=13.72±5.93pg/ml vs DHT=12.05±2.33pg/ml; n=9 pups/treatment), ovarian Ghrl expression tended to decrease with DHT (37.51±6.12 vs 47.49±10.10; n=6 pups/treatment) and increase with the HFD (49.25±6.72 vs 35.75±9.27; n=6 pups/treatment). Our findings highlight the adverse effects of prenatal androgenization on the sexual development and fertility of female offspring. Moreover, they shed light on the synergistic impact of a HFD, which exacerbates those effects, emphasizing the intricate interplay between hormonal factors and dietary influences in shaping reproductive outcomes.

513. 97. **PATERNAL PROGRAMMING OF ALTERATIONS IN THE PLACENTA OF FETUSES OF MALE DIABETIC RATS**

Irune Pirrone, María Laura Leonardi, Alicia Jawerbaum, Evangelina Capobianco.
Laboratory of Reproduction and Metabolism, CEFYBO-CONICET, School of Medicine, University of Buenos Aires, Argentina.

The Paternal Origin of Health and Disease paradigm studies how paternal condition influences offspring health. Paternal Type 2 Diabetes programs metabolic alterations in the offspring. The placenta is involved in fetal programming, but it is unknown if it is affected by paternal diabetes. Peroxisome proliferator activated receptors (PPARs) are transcription factors needed for the appropriate development of the placenta. Aim: to study the paternal programming of alterations in the expression of PPARs in the placenta of fetuses in a model of paternal diabetic rats. Methods: Male control (C) and diabetic (D, streptozotocin-induced, 90 mg/kg) rats were mated with

healthy females (n=8). Paternal sperm was collected from cauda epididymis to count spermatozoa and measure motility. On day 21 of gestation, pregnant rats were euthanized, and the placenta and the fetuses were sexed and weighed. In paternal and fetal plasma, glycemia, triglyceridemia (TG), and cholesterolemia (Ch) were measured by colorimetric assays. The placenta was stored at -80°C for the evaluation of PPARα, PPARγ and PGC1α (by Western blot). Results: Glycemia, TG and Ch was higher in D than in C males ($P<0.05$). A significant reduction in motility and sperm count was found in D males when compared to C (54% and 46%, respectively, $P<0.01$). Fetal glycemia and Ch, and placental and fetal weight were similar in paternal C and D groups, but TG were increased in male fetuses from paternal D group (12%, $P<0.05$). In fetuses, a decrease in PPARγ (females 39%; males 77%, $P<0.05$) and an increase in PGC1α (females 15%; males 84%, $P<0.05$) and PPARα (males 45%, $P<0.05$) were found in the placenta of paternal D group. Conclusion: In this model of diabetes, we showed alterations in male reproductive functions and the paternal programming of sex-dependent alterations in PPARs, master genes that regulate the development and function of the placenta, an organ highly related to the programming of the diseases of the offspring.

514. 135. **MITOCHONDRIAL DYNAMIC IS ALTERED IN THE OVARIES FROM RATS WITH POLYCYSTIC OVARY SYNDROME**

Mayra Bordaquievich[1], Melanie Neira[1], Candela Velazquez[1], Yamila Herrero[1], Rocío Marinoni[1], Fernanda Parborell[1] and Dalhia Abramovich[1]

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Introduction: Polycystic ovary syndrome (PCOS) is the most common endocrine disorder, affecting 5-10% of women in reproductive age. In spite of being described eighty years ago, the pathological mechanisms of PCOS are not completely understood and therefore, studying the molecular mechanisms of this syndrome is essential to improve diagnosis and treatment of these women. Objective: To evaluate the involvement of mitochondria in the ovarian alterations in a rat model of PCOS. Methods: 21 days old Sprague Dawley rats were treated with dehydroepiandrosterone (DHEA) for 15 days (PCOS group). Control group received vehicle. At day 16, rats were sacrificed and the ovaries recovered to perform histology and western blot. Histological slides were stained with picrosirius red to evaluate fibrosis or H&E. Results: Unlike the control group, we observed the presence of ovarian cysts and the ovarian anomalies previously described in the PCOS group. We found a significant decrease in TOMM-20 protein, in the mitochondria-shaping proteins MFN-2 and DRP-1 and in Sirtuin-1, with no changes in OPA-1. Ovarian fibrosis was increased in the PCOS group. Conclusions: Our results suggest a decreased in the number of mitochondria and a deregulation in mitochondrial dynamic that could be involved in ovarian dysfunction in this rat model of PCOS. Therapies that target mitochondria, such as metformin, are worth to study in this pathology to improve ovarian performance.

515. 201. **ELEVATED CHORIONIC GONADOTROPIC HORMONE IN TRANSGENIC MICE INDUCES PARTHENOGENETIC ACTIVATION AND OVARIAN TERATOMAS**

Susana B. Rulli[1,2], Laura D. Ratner[3], Matti Poutanen[2], Ilpo Huhtaniemi[2,4]

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Both male and female reproductive functions are impacted by altered gonadotrophin secretion and action, which may also influence the development of endocrine tumors. Female transgenic (TG) mice overexpressing both the α- and β- subunits of human chorionic gonadotropin (hCG), produced high levels of bioactive hCG and presented with precocious puberty, infertility, and enhanced gonadal steroidogenesis. The objective of this work is to ascertain if chronic hypersecretion of hCG contributes to the development of gonadal tumors. Ovaries from double TG female mice for hCG were analyzed at different ages. By the age of two months, ovarian tumors with characteristics of teratomas developed with 100% penetrance. Tissues such as keratinized epithelium, hair follicles, cartilage, sebaceous glands, neural tissue, and intestinal and respiratory-like epithelium were identified in the TG ovaries. Teratomas were also seen in wild-type ovaries orthotopically transplanted into TG mice, demonstrating an endocrine mechanism for the hCG-induced ovarian tumorigenesis. Both *in vitro* and *in vivo* experiments showed oocyte parthenogenetic activation in TG females, developing up to the blastocyst stage. In addition, ovaries showed reduced ovulatory gene expression, inhibited ERK1/2 phosphorylation, and impaired cumulus cell expansion ($p< 0.01$). In conclusion, persistently high endocrine hCG activity causes parthenogenetic activation and development of ovarian teratomas, along with altered follicle development and impaired ERK signaling, offering a novel mechanism associated with the molecular pathogenesis of ovarian teratomas.

516. 296. **EFFECT OF ESTRADIOL-17β INJECTIONS DURING THE LUTEAL PHASE ON THE PLASMA PROGESTERONE CONCENTRATION IN LLAMAS**

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The aim of this study was to evaluate the effect of estradiol-17β (E2) injections during the luteal phase on the plasma progesterone (P4) concentration in llamas. Twenty-one females were induced to ovulate by GnRH injection in presence of a follicle ≥ 8 mm and randomly assigned into one of three groups: control (n=7) without treatment; G7-8 (n=7) treated with 1.6 mg of E2 IM on Day 7 and 0.8 mg of E2 IM on Day 8; and G7-10 (n=7) treated with 1.6 mg of E2 IM on Day 7 and 0.8 mg of E2 IM from Day 8 to 10 post-GnRH. Blood samples were collected every other day from Day 0 to 7 and daily until Day 15 post-GnRH to determine plasma P4 concentration by RIA. The data were analyzed using an ANOVA test, and the effect of days within groups was compared by a Tukey Test with Bonferroni adjustment. Mean plasma P4 concentration increased from Day 0 to Day 8 post-GnRH in all females. In the control group, mean plasma P4 concentration decreased to below 1 ng/ml on Day 10, being statistically different from treated groups ($P<0.01$). In the G7-8, mean plasma P4 concentration decreased between Day 11 and 12 post-GnRH, being in those days significantly lower than in llamas from G7-10 ($P<0.01$). In conclusion, E2 injection from Day 7 post-induction of ovulation results in a luteotrophic effect in llamas. The E2 administration from Day 7 to Day 10 post-GnRH prolongs the corpus luteum function for three more days than in control animals, which gives further support to the hypothesis that E2 would be involved in the process of maternal recognition of pregnancy in this species.

517. 327. **DOES PRENATAL CANNABIS EXPOSURE INFLUENCE OFFSPRING DEVELOPMENT AND REPRODUCTIVE OUTCOMES?**

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Cannabis is the most commonly used illegal drug worldwide, especially among people of reproductive age. Its biological effects are mediated by the endocannabinoid system (eCS), which is a complex lipid signaling system composed of the endogenous ligand, their receptors (CB1 and CB2), and their biosynthesizing and catabolizing enzymes. The main psychoactive component of *Cannabis sativa* is Δ9-tetrahydrocannabinol (THC), a highly lipid soluble molecule that can cross the placenta and the blood-brain barrier with ease, accumulating in fetal tissues, particularly the brain. Several studies have shown that the eCS plays an important role in reproduction, from egg fertilization to parturition. Therefore, the aim of this study was to investigate whether prenatal cannabis exposure is associated with adverse effects throughout pregnancy and offspring behavioral and cognitive development. We administered THC oil (0,3 μg/μl) intragastrically since day 1 (vaginal plug observation) until the day before the onset of labor. We analyzed different gestation parameters and observed that THC mice presented lower reproductive efficiency (36.9%) than vehicle (57.2%) and control mice (71.4%). We also observed that pregnant mice treated with THC presented lower body weight on day 16 of gestation vs vehicle and control. This trend persisted during lactation. However, no differences were found on offspring physical development parameters (body weight, pinnae detachment, incisor eruption and eye opening). Additionally, we observed increased mortality of the pups in the first week of life exclusively within the THC group. We performed behavioral tests when the offspring reached adulthood and found differences in the THC-exposed group when compared to vehicle and control mice. In conclusion, our results suggest that chronic use of cannabis oil with high THC content during pregnancy could potentially lead to adverse pregnancy outcomes.

518. 343. **CLINICAL UTILITY OF ANTIMÜLLERIAN HORMONE REGARDING ANTRAL FOLLICLE COUNT IN THE EVALUATION OF THE OVARIAN RESPONSE**

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igual contribución*

Objectives: To analyze the total and mature number of oocytes recovered in women undergoing an assisted reproduction treatment (ART) who have discordant values of antimüllerian hormone concentrations (AMH) and antral follicle counts (AFC). Materials and Methods: 208 patients (35-39 years) who underwent ART at CIGOR between 2021 and 2022 were analyzed. Based on the cut-off values for AFC 5 and AMH 1.2 ng/ml, patients were divided according to the concordance/discordance of both values in 6 groups: AFC<5 and AMH<1.2 concordant or AMH≥1.2 discordant; AFC between 5-9 and AMH<1.2 discordant or AMH≥1.2 concordant; AFC≥10 and AMH<1.2 discordant or AMH ≥1.2 concordant. Mean±SD for the total and mature number of oocytes recovered were compared, and data analyzed by Mann-Whitney test, considering $p<0.05$ as significant. Results: For patients with a poor prognosis (AFC<5; N=51), 92% were concordant and only 8% discordant, with similar clinical results for both groups. In AFC≥10 (N=68), 87% of the patients presented concordant AMH and only 13% discordant, with significant differences in total oocytes (12.3 vs 6.4 concordantly/discordantly respectively; p=0.0015) and mature oocytes (9.8 vs 5.4 concordantly/discordantly respectively; p=0.02). In the group with the AFC 5-9 (N=89), 28% were concordant (AMH≥1.2) and 71% were discordant (AMH<1.2), with statistically different results between the number of total oocytes (7.6 vs 4.7 concordantly/discordantly respectively; p=0.036) and that of mature oocytes (6.2 vs 3.7 concordantly/discordantly respectively; p=0.013). Conclusion: Considering both data (AFC and AMH) allows a better prediction of ovarian response, especially in those patients with AFC between 5-9 and discordant AMH values.

519. 419. **ALTERATION OF CHROMATIN-MODIFYING ENZYMES EXPRESSION IN PRE-OVULATORY FOLLICLES IN ADULT COWS GESTATED UNDER HIGH TEMPERATURE-HUMIDITY INDEX**

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In cattle, gonadal development and gametogenesis occur during gestation. When dairy cows suffer high temperature and humidity conditions at pregnancy, the fertility of their offspring can be compromised. This could be consequence of variations in expression of proteins involved in follicular growth and development, which are regulated by different enzymes acting on chromatin structure, such methylating DNA enzymes. The aim of the study was to analyze the expression of DNA Methyltransferases (DNMTs) in preovulatory follicles of cows gestated under different environmental conditions. Adult Argentinean Holstein cows (n=24) gestated under different THI conditions during their *in utero* development were used. Ovarian samples were obtained by ovariectomy after estrous synchronization and gene and protein expression of DNMTs (DNMT1, DNMT3a and DNMT3b) was determined by RT-PCR and immunohistochemistry in pre-ovulatory follicle. Gestation was divided into two periods (P1: 0-150 days; P2: 151 days-birth); and three trimesters (T1: 0-90 days; T2: 91-180 days; T3: 181-birth days) in which the exposure to high Temperature-Humidity Index (THI) was considered. Negative associations between gene expression of DNMTs and THI during all gestation and particularly in the second trimester were observed. Also, negative associations between DNMT1, DNMT3a protein expression in granulosa cells of preovulatory follicles and THI in T3 and P2 were observed. Instead, positive associations between DNMT3a in granulosa cells and THI in T1 were detected. Besides, DNMT3b expression in theca interna were positively associated to high THI during all gestation. This data suggests that exposure to high THI during intrauterine development influences the expression of genes and proteins associated with epigenetic modifications and may affect the expression of molecules critical for the development and function of pre-ovulatory follicles in adult life.

520. 486. **ROLE OF HUMANIN IN THE MODULATION OF ANTRAL FOLLICLE DEVELOPMENT *IN VITRO***

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The regulation of apoptosis and steroidogenesis are crucial for correct antral follicle development. Recently, we demonstrated the expression of Humanin (HN, a small mitochondrial peptide) in rat antral follicles. Also, we reported that inhibition of endogenous HN *in vivo* increases the number of atretic follicles. However, the direct effect of HN on apoptosis and steroidogenesis in antral follicles has not yet been elucidated. The present study aimed to evaluate the direct participation of HN in the regulation of apoptotic genes expression and steroidogenesis in antral follicles *in vitro*. To evaluate this, ovaries from diethylstilbestrol-treated prepuberal rats were used. Isolated antral follicles were individually cultured for 6 h for gene expression (n=12) or 24 h for hormonal levels (n=16) in the presence or absence of HN (1μM). To assess the gene expression of apoptotic regulatory genes (*Bcl-2*, *Bax,* and *Bad*) and steroidogenic enzymes

(*3β-HSD* and *aromatase*), antral follicles were stored at -80°C for subsequent RNA extraction and quantitative RT-PCR (qRT-PCR). mRNA expression was normalized to the endogenous control, *β-Actin*. Culture media was stored at –80°C for further determination of progesterone (P4) and estradiol (E2) levels by electrochemiluminescence. Normalized mRNA results for the assessed apoptotic regulatory genes (*Bcl-2, Bax*, and *Bad*) showed no significant differences in the presence of HN ($p>0.05$). Concerning steroidogenesis, HN interestingly increased the P4 levels in the culture media ($p<0.05$) without modifying *3β-HSD* mRNA expression ($p>0.05$). Regarding E2, HN did not change either the E2 levels or *aromatase* mRNA expression ($p>0.05$). In conclusion, our results suggest that HN may probably modulate antral follicle development by increasing P4 levels.

521. 555. **DETRIMENTAL EFFECTS OF LINDANE ON THE BOVINE OVIDUCTAL EPITHELIAL CELLS**

Maximiliano De Boeck[2], Ignacio Abel Angel-Spiess[1], Mariela Roldán-Olarte[1,2], Milda Alejandra Vella[1,2], Sergio Antonio Cuozzo[3], Pablo Alberto Valdecantos[1,2]

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Lindane, the **γ-**isomer of the hexachlorocyclohexane, was a widely used organochlorine pesticide in agriculture and public health. Due to its high environmental persistence, bioaccumulation, and toxicity, in the present work we investigate its effects on primary cultures of the bovine oviductal epithelial cells (BOEC), focusing on cell viability, proliferation, migration, and genotoxicity. Different lindane concentrations (12.5 **μ**M, 25 **μ**M, 50 **μ**M, 100 **μ**M and 200 **μ**M) were tested on 80% confluent BOEC monolayers in DMEM 5% FSB at 38.5°C, 5% CO_2 and 100% humidity. Then cell viability was evaluated by the trypan blue exclusion test. Cell viability was above 86% at all the lindane concentrations assayed. However, concentrations starting from 25 μM exhibited a significant decrease in cell number/mL compared to the control ($p<0.05$). We selected 50 **μ**M to evaluate the lindane effect on the proliferative capacity of BOEC by a clonogenic assay. Cells at low density (500 cells/60 mm plates) were seeded and incubated overnight for cell attachment. The medium was then replaced with or without lindane (control) and cultured for 7-10 days. Colonies were then stained with Giemsa and scored; a substantial reduction in the colony formation was observed ($p<0.05$). Cell migration was evaluated by the wound healing assay; scratches were made in confluent BOEC cultures and incubated at 3 h, 6 h, and 12 h in DMEM 5% FBS with or without lindane (50 **μ**M). Cells in the presence of lindane displayed a tendency to delay cell migration. Lindane's genotoxicity was evaluated by a micronucleus assay after 48 h of exposure to 50 **μ**M of lindane. Cells fixed in 4% formaldehyde and stained with Hoëchst 33342 exhibited a 2,4-fold increase in micronucleus formation. Results suggest that lindane have detrimental effects on the oviductal epithelium altering essential cellular processes that potentially impair female fertility.

522. 589. **EVALUATION OF HISTOMORPHOMETRIC CHANGES IN THE UTERUS OF PRENATAL RESTRICTED LAMBS**

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The aim of the study was to describe the histomorphometric characteristics of the uterus of 45-days-old lambs belonging to mothers nutritionally restricted or controls from the second half of gestation. Merino pregnant ewes carrying single fetuses were randomly assigned to restricted (R) or control (C) groups and fed with 75% or 125% of metabolic energy requirements from ~80 days to ~140 days of gestation, respectively. Uterus samples from Merino female lambs of 45-days-old (n=7 for each group) were evaluated. For the histomorphometric evaluation, 3 cuts of 4 μm thickness of each uterine horns were obtained and stained with H-E in order to evaluate the following parameters: *height of the luminal epithelium (HLE) and the glandular epithelium (HGE)*, considering the distance between the basal membrane and the apical end of 30 cells of each of the epithelia with a magnification of 100x. *Glandular density (GDe)*, the number of glands in 10 fields at 40x magnification and *Glandular area* (GA) using the following formula: GA=π×(Axis 1)/2×(Axis 2)/2 were evaluated. The axes were the average of two right-angled diameters in a minimum of 30 glands per section at 100x magnification. Samples were analyzed by two observers. The results were analyzed by ANOVA. Data are expressed as mean ± SD. Results were considered significant with $P\leq0.05$. HLE and AG did not differ between groups (**R**: 11.73±0.18; 1347.43±36.74 and **C**: 11.81±0.18; 1277.54±36.74; respectively; $P\leq0.05$). The HGE and GDe were significantly higher for lambs from R than lambs from C group (**R:** 13.34±0.18; 25.19±0.59; **C:** 12.86±0.18; 22.32±0.64; respectively; $P\leq0.05$). The pre-natal maternal nutrition could affect the development of uterine glands in their lambs. In this study, a higher HE and GDe was observed in the uterus of lambs born from nutritional restricted mothers. Future studies are necessary to explain these results.

523. 602. **INNOVATIVE THERAPEUTIC STRATEGY FOR AUTOIMMUNE EPIDIDYMO-ORCHITIS IN RATS: LOCAL INJECTION OF MELATONIN WITHIN THERMOSENSITIVE PEO-PPO COPOLYMERS**

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Experimental autoimmune epididymo-orchitis (EAO) is a well-established rodent model of organ-specific autoimmunity associated with infertility. In EAO oxidative stress negatively impacts on spermatogenesis. Our study aims to explore the potential of intratesticular injection of melatonin (MLT), an antioxidant hormone with poor solubility in aqueous medium, to ameliorate the effects of inflammation on epididymal sperm parameters. For this objective, we used Pluronic® F127, a biocompatible polymeric micelle (PMs) nanoplatform with *in vivo* thermosensitization seeking to optimize MLT delivery and availability within the testes. Two groups of adult male Wistar rats were evaluated: non-immunized (normal, N) and experimental rats which were immunized with testis homogenate and adjuvants to induce EAO. Rats received a single intratesticular injection of F127 25% (w/v) with MLT (2.5mg) or F127 25% (w/v) in saline (as control) and were sacrificed 10 days after. In EAO rats MLT prevents reduction in cauda sperm viability (% media±SEM, N+F127: 84,8±0,6; EAO+F127: 47,4±8,1; EAO+F127-MLT: 71,4±9,9 $p<0.05$ vs EAO+F127) and motility (% media±SEM, N+F127:78,5±2,4; EAO+F127: 37,8±6,1; EAO+F127-MLT: 61,9±11,1 $p<0.05$ vs EAO+F127). Head and tail sperm abnormalities were also reduced by MLT [% media±SEM, Head: N+F127: 7,90±0,5, EAO+F127: 37,6±1,6, EAO+F127-MLT: 10,4±0,8 $p<0.01$ vs EAO+F127; Tail: N+F127: 14,75±0,07, EAO+F127: 48,4±1,9, EAO+F127-MLT: 17,1±2,6 $p<0.01$ vs EAO+F127. Sperm count significantly decreased in all EAO groups vs. N rats. F127 25% (w/v) increased the maximum solubility of MLT by 14 times vs saline, potentially amplifying its effect. MLT injected within the testis might act on stored epididymal sperm mitigating the detrimental action of the inflammatory microenvironment possible through the strong antioxi-

dant properties of MLT. Our study suggested that local MLT delivery through thermosensitive PMs, might be a good strategy to address orchitis-induced male infertility.

524. 666. **COCULTURE OF PIG EMBRYOS WITH SPHEROIDAL VESICLES OF OVIDUCTAL EPITHELIAL CELLS**

María Soledad Lorenzo[1,2], Carolina Griselda Luchetti[1,2], Analía Bertonazzi[3], Daniel Marcelo Lombardo[1,2]
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The co-culture of monolayer porcine oviductal epithelial cells (POEC) with in vitro-produced (IVP) pig embryos enhances embryonic development and reduces reactive oxygen species. When scraped from the oviductal mucosa, POEC spontaneously form ciliated spheroidal vesicles (SV), which remain suspended in culture. To replicate the oviductal environment, this study aimed to determine whether the co-culture of POEC-SV with IVP embryos promotes embryo development and quality. POEC-SV were obtained from diestrus slaughtered female oviducts, and viability, cilia presence and glycoprotein secretion were verified. IVF-produced embryos: oocytes from slaughterhouse ovaries were *in vitro* matured 44 h and coincubated with refrigerated semen for 4 h ($1x10^6$ zs/mL). Presumptive zygotes were cultured in 50 μl NCSU23 drops (20 embryos/drop, 38.5°C, 5% CO_2, 7% O_2) randomly distributed in 20 SV/culture drop (n= 298), 10 SV/culture drop (n=241) and control (without SV n=253). At 48 h, cleavage was assessed, and embryos were cultured in NCSU23+glucose without SV. Day 7 blastocysts, cells/blastocyst (Hoechst), and apoptosis (TUNEL) were assessed. Cleavage and blastocyst rates were analyzed by *chi*-square test, cells/blastocyst and TUNEL+/total cells by ANOVA (significant P≤0.05). 10 SV did not show differences with control in cleavage (38.5% vs 30.4%) or blastocysts (7.47% vs 4.74%). 20 SV decreased cleavage (14.7%) and blastocysts (3.02%). Cells/blastocyst was similar in all groups: control (64.33±7.34), 10 SV (53.45±7.00) and 20 SV (44.25±6.21). TUNEL+/total cells showed no differences between groups (7.02; 7.44; 11.76). Conclusion: POEC-SV did not show the embryotrophic effect observed with monolayer POEC coculture. The highest amount of SV harms embryonic development, possibly due to competition for nutrients between POEC-SV and embryos. It would be interesting to evaluate the POEC-SV coculture in a culture system that allows a higher availability of nutrients, such as a four-well plate.

O1-REPRODUCTION

WEDNESDAY 15TH NOVEMBER 14:00-15:30
CHAIRS: SUSANA RULLI
FERNANDA PARBORELL

525. 189. **CELL-FREE DNA LEVELS IN PREGNANT WOMEN WITH THROMBOPHILIA: A POTENTIAL TOOL FOR HIGH-RISK PREGNANCY**

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Cell-free DNA (cf-DNA) has been proposed as a valuable tool for pregnancy monitoring and predicting adverse obstetric outcomes. We investigated cf-DNA levels in plasma samples from pregnant women with thrombophilia, a condition associated with an increased risk of abnormal coagulation and potential pregnancy complications. First-trimester pregnant women (aged 28-41 years) were recruited from Laboratorios BLUT and Clínica de Ginecología y Maternidad Central in Santa Fe, Argentina. The patients were divided into two groups: those with thrombophilia (TBF group, n=6) and a control group without thrombophilia (C group, n=12). The TBF group included acquired (n=5) and hereditary (n=1) thrombophilia cases. Plasma cf-DNA was isolated using QIAmp DNA blood mini kit (QIAGEN). Actin-β (*ACTB*) gene was quantified by real-time quantitative PCR using an *ACTB* standard curve. Median cf-DNA levels were compared between the groups using the Mann-Whitney test. In addition, the groups were compared for age, thromboprophylaxis, and obstetric history using the Exact Fisher test. No statistically significant differences in age or gestational age were found between the TBF and C groups. In the TBF group, 83% (5/6) of patients used heparin and aspirin for thromboprophylaxis, which was significantly different (p=0.0007) from the control group (0%). Moreover, all women in the TBF group had a history of pregnancy loss, which differed from 33% (4/12) in the C group (p=0.0028), suggesting an association between thrombophilia and adverse pregnancy outcomes. Finally, plasma cf-DNA levels were significantly higher in the TBF group than in the C group (43156 copies/mL vs 14697 copies/mL; p=0.0009). Our findings emphasize the potential of cf-DNA as a non-invasive biomarker for identifying high-risk pregnancies in the first trimester, offering valuable insights for early intervention and more vigilant monitoring of individuals at risk.

526. 209. **EXPOSURE OF MINIMUM DOSES OF GLYPHOSATE DURING THE POSTNATAL PERIOD ALTERS THE OVARIAN FUNCTION AND ESTROUS CYCLE**

Ferman Delfina Sol, Dasso Marina Ercilia, Centola Cecilia Lucía, Cruz Mariana, Dascal Eduardo Raúl, Gamaleri Yamila, Pennisi Patricia Alejandra, Peluffo Marina Cinthia
Centro de Investigaciones Endocrinológicas "Dr. César Bergadá" (CEDIE) -CONICET–FEI–División de Endocrinología, Hospital de Niños "Ricardo Gutiérrez"

Glyphosate is one of the most widely used herbicides in agriculture worldwide. It has been demonstrated that at high doses it can act as an endocrine disruptor. Also, *in vitro* and *in vivo* studies imply that glyphosate changes ovarian function. However, there are some controversies and most of the studies focus on prenatal period exposure at high doses. Thus, the aim of this study was to assess the effect of glyphosate on ovarian function when chronically administrated at low doses during the postnatal period. To accomplish this, female Sprague Dawley rats (n=16, 1 month old) were daily weighted and treated for 8 weeks. Rats were randomly assigned to one of the following treatment groups: control group (n=8, receiving water) and treated group (n=8, receiving an oral dose of glyphosate 1mg/kg day). The length and stages of the estrous cycle were evaluated by daily vaginal cytology. Once vaginal smears were collected and dried on a slide, they were stained with Giemsa and observed under a microscope to determine the different cell types present in each smear to assign their stage of the cycle. On the day of the sacrifice, blood was obtained by intracardiac puncture to further assess serum estradiol (E2) and progesterone (P4) levels, and the ovaries were weighted. No significant differences were observed in the weights of the animals or the ovaries of the different groups. Interestingly, we observed that the exposure to chronic low doses of glyphosate significantly decreased the number of days the rats stayed at the diestrus stage in comparison to the controls (p<0.05). Also, treatment with glyphosate induced a significant decrease in the E2 levels compared to the control group (p<0.05), with no significant changes in the P4 levels. In summary, these results demonstrated that even at a low dose, chronic exposure to glyphosate during the postnatal period has an impact on ovarian function and the estrous cycle.

527. 214. **MOUSE SPERM LACKING CALCIUM CATSPER CHANNEL ARE ABLE TO UNDERGO ACROSOMAL EXO-**

CYTOSIS DURING CAPACITATION *IN VITRO*

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During capacitation, sperm acquire the ability to fertilize the egg. To this end, the gametes undergo acrosomal exocytosis (AE), a specialized event that requires an increase in intracellular calcium (Ca^{2+}) levels. The sperm-specific Ca^{2+} channel CatSper plays a key role in capacitation, but its participation in AE is still controversial. By using CatSper1 KO mice that express EGFP in the acrosome and DsRed2 in the flagellum (CatSper1 KO-EGFP), we investigated the role of CatSper in AE. First, by flow cytometry, we registered the number of acrosome reacted cells (without EGFP) and acrosome intact cells (with EGFP). No significant differences were observed between CatSper1 WT and KO mice in the percentage of acrosome reacted sperm after exposure to Ca^{2+} ionophore A23187 (10 μM), indicating that the fusion machinery and the downstream effectors are not affected. Similar results were observed after exposing the cells to thapsigargin (2.5-20 μM), which induces the release of Ca^{2+} from the intracellular reservoirs and triggers AE. Next, AE was induced *in vitro* with progesterone (30-100 μM). Both CatSper1 WT and KO sperm were able to undergo AE in a concentration-dependent manner and no significant differences were observed with the exception of progesterone 30 μM ($p < 0.05$). The effect of progesterone on mouse sperm seems to be independent of ABHD2, as previously reported, since incubation with inhibitors against this hydrolase did not prevent the occurrence of AE. Finally, to evaluate the *in vivo* occurrence of AE in the upper segments of the oviductal isthmus, CatSper1 KO-EGFP sperm were observed by fluorescence microscopy within the female reproductive tract after mating. However, CatSper KO sperm were unable to ascend in the oviduct due to the impeded hyperactivation. No sperm were found in the upper isthmus or ampulla four hours after ovulation. In conclusion, the lack of CatSper channels in mouse sperm do not significantly affect the occurrence of AE.

528. 263. **DYSLIPIDEMIA: NEGATIVE EFFECT ON OOCYTE MATURATION AND IMPACT ON OVARIAN INTRAFOLLICULAR LIPID PROFILE**

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Dyslipidemia, defined as alterations in plasma lipid levels, shows high and increasing global prevalence. Ovarian follicular fluid (FF) is produced by granulosa cell (GC) secretion and by diffusion from the theca capillaries. The association between dyslipidemia and intrafollicular lipids or oocyte maturation competence remains unclear. This work aimed at analyzing: (I) the association between neutral plasma lipids and the percentage of metaphase II oocytes (% MII) and (ii) the influence of dyslipidemia on the lipid composition of FF and the hydrophobicity of intracellular lipid droplets in GC. We collected FF and GC from 109 women with no reproductive pathologies with dyslipidemia (n=57, age 32.3 ± 6.4 years) and without dyslipidemia (n=52; age 31.5 ± 5.5 years). These women include male factor infertility, donors, and women with the desire to gestate genetically related children. Follicular fluid lipids were quantified using enzymatic methods. Hydrophobicity of intracellular lipids was evaluated by fluorescence spectroscopy using the lipophilic dye Nile Red. To compare women with the most contrasting plasma lipid profiles, we selected the extreme quartiles (Q1 and Q3, women with the lowest and highest plasma lipid levels, respectively) based on principal component analysis. In Q3, the % MII oocytes negatively correlated ($p<0.05$) with total cholesterol and LDL-cholesterol in plasma. Both quartiles exhibited positive correlations between plasma and follicular lipids. Mean values of total cholesterol, HDL-cholesterol, and triglycerides of FF were statistically higher in Q3 than in Q1. Hydrophobicity of intracellular lipid droplets did not show differences between quartiles. Findings from our work showed that dyslipidemia exerts a negative effect on oocyte maturation and that dyslipidemic women have a distinctive lipid profile in the FF compatible with an "intrafollicular dyslipidemia".

529. 280. **ALTERED GLUCOCORTICOID RECEPTOR ACTIVATION DURING PREOVULATORY PERIOD SHIFTS INFLAMMATORY SIGNALS IN THE OVARY OF DAIRY CATTLE UNDER STRESS CONDITION**

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Ovulation has been described as a localized inflammatory-like reaction. During this, glucocorticoids, through its receptor (i.e., the glucocorticoid receptor (GR)), increase in the follicular microenvironment to limit the inflammatory process. Here we aimed to evaluate GR expression in the preovulatory follicle and effector mechanisms related to its activation in inflammatory pathways both in the preovulatory follicle and in peripheral blood mononuclear cells (PBMC) of cows treated with adrenocorticotropin (ACTH). To this end, 100 IU of ACTH (AG; n=7) was administered to Holstein cows every 12 hours for 4 days before ovulation when ovariectomy was performed. Control group (CG; n=5) received saline solution. Previous to ovariectomy, blood samples were taken to isolate PBMC. In ovaries, GR, nuclear factor κB inhibitor-α (NFκBIα), interleukin (IL) 6, tumor necrosis factor α (TNFα) and IL1 system (IL1α, IL1β, IL1R2) expression was evaluated by immunohistochemistry along with GR/NFκB coexpression. Also, IL4 and TNFα production was analyzed by flow cytometry after exposing PBMC to autologous follicular fluid. Statistical analysis was performed using t-student's test. In the AG, GR nuclear expression resulted to be lower in theca cells ($p<0.05$). TNFα, IL1α and IL1R2 immunoexpression was lower in theca cells of the AG in comparison to CG ($p<0.05$). On the other hand, both in granulosa and in theca cells the nuclear coexpression of GR/NFκB resulted to be lower in the AG ($p<0.05$). However, NFκBIα immunoexpression was similar ($p>0.05$). After follicular fluid exposure, the percentage of IL4+ PBMC was higher in the AG group, while the percentage of TNFα+ was lower ($p<0.05$). Our results show that in the preovulatory follicle GR nuclear activation is impaired after ACTH exposure resulting in altered expression of inflammatory mediators related to ovulation. Also, this expression pattern could be attributed to an imbalance in the intricate cross-talk between the GR and the NFκB.

530. 368. **cBIMPs IS A cAMP ANALOG THAT POTENTLY ENHANCES HUMAN SPERM MOTILITY**

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Cyclic AMP (cAMP) orchestrates different aspects of sperm function that are necessary for the acquisition of fertilizing capacity. It has a role in the development of motility and hyperactivation (HA) and in the occurrence of acrosomal exocytosis (AE) by the activation of a protein kinase (PKA), leading to protein phosphorylation on tyrosine residues (pY). Such responses can be mimicked by cAMP analogs,

which have been used in the management of sperm *in vitro*. The objective of our study is to analyze the effect of a new cAMP analogue, Sp-5,6-DCl-cBIMPs (cBIMPs), on human sperm functional parameters. Motile sperm from normozoospermic men were incubated for up to 4 h with cBIMPs (0.01, 0.03 and 0.1 mM) or DMSO (0.2 %) as a control. Motility parameters were evaluated by a CASA system (SCA, Microptic), protein phosphorylations in PKA substrates (pPKA) and pY by Western blot, AE by *Pisum sativum* agglutining staining and DNA fragmentation by the TUNEL assay. The results showed a significant increase in % total and progressive motility, in all kinematic parameters and in % HA for all cBIMPs concentrations in comparison to the control (n=6; p<0.001). Sperm exposed to cBIMPs depicted an increase in pPKA and pY, whereas no significant changes in % AE (n=5) or % DNA fragmentation (n=4) were found. The effects of cBIMPs upon sperm motility were maintained for up to 4 h even after removing the compound, and they were higher than those obtained with other known cAMP analogs: dbcAMP and 8-Br-cAMP (n=5; p<0.01). Moreover, the addition of cBIMPs (0.03 mM) during the swim-up procedure led to a higher recovery of motile cells compared to the control (n=11, p<0.01). In conclusion, these results indicate that cBIMPs is a potent cAMP analog that can enhance human sperm motility and HA, without inducing premature AE or DNA fragmentation. This compound can also be used in the handling of human sperm, increasing the efficacy of sperm selection during assisted reproduction techniques.

531. 398. **EXPRESSION LEVELS OF THE POTENTIAL REGULATORS OF *FMR1*, MIR-92A-3P AND MIR-19B-3P, DURING FOLLICULOGENESIS IN THE RAT**

Marina Luz Ingravidi[1], Liliana Dain[1,2], Laura Kamenetzky[1], Ianina Ferder[1]

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The *FMR1* gene is located on the X chromosome and is implicated, among other genetic disorders, in the Fragile X-Associated Primary Ovarian Insufficiency. Several studies have shown dysregulated microRNA (miRNA) expression in patients with Fragile X-associated diseases and that some miRNAs can potentially regulate *FMR1* expression. In previous *in silico* studies from our lab, we found 2 miRNAs, miR-92a-3p and miR-19b-3p, that are highly expressed in the rat's ovary and are good candidates to bind to *Fmr1*. In the present work, we aimed to validate the expression of these 2 miRNAs during folliculogenesis, using a rat model of follicular development. We performed real time quantitative PCRs (RT-qPCR) using isolated RNA from rat's ovaries that were enriched in 3 different types of follicles: preantral follicles (prepubertal rats), early antral follicles (DES-treated rats) and preovulatory follicles (PMSG-treated rats). For each follicular stage, ovaries from 10-30 rats were randomly divided in at least 4 different pools. Expression of each miRNA was normalized to U6. Additionally, we measured *Fmr1* expression in the same follicular stages. Statistical analysis was performed using fgStatistics. We found that the expression of both miRNAs were 3-fold higher (p-value<0.05) in the early antral follicles compared to the preantral stage. The expression level of miR-92a-3p was also higher (3-fold, p<0.05) in early antral follicles in comparison to preovulatory follicles. In addition, levels of *Fmr1* are increased in the early antral follicles (1.92-fold, p-value=0.05) compared to the preovulatory stage. In conclusion, we found a differential expression of our candidate miRNAs during folliculogenesis. This differential expression could indicate a role for these miRNAs in the regulation of this process. Future studies will be conducted to increase the number of ovaries in the different stages and to analyze *in vitro* the binding of these miRNAs to *Fmr1*.

P2-REPRODUCTION

THURSDAY 16TH NOVEMBER 9:00 - 10:30

CHAIRS: DALHIA ABRAMOVICH

DANIELA PÉREZ SIRKIN

532. 13. **REGULATION OF SERTOLI CELL (SC) PROLIFERATION BY LACTATE**

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The final number of SCs, reached during the proliferative periods, defines the spermatogenic capacity in adulthood. It is recognized that FSH is the main mitogen targeting SCs and that it exerts its action, at least partly, through the activation of the PI3K/Akt/mTORC1 pathway. We have recently shown that FSH simultaneously upregulates proliferation and aerobic glycolysis in an mTORC1-dependent manner, and that aerobic glycolysis is required to achieve full mTORC1 activation. In the last decade, the study of metabolites as second messengers capable of modifying signalling pathways activity has gained interest. New evidence suggests that lactate, the end product of aerobic glycolysis, is responsible for the complete activation of mTORC1 in proliferating cells. Although the necessity of a high glycolytic flux in immature SC to maintain the proliferation rate has been evinced, the molecular mechanisms remain obscure. The aim of this study was to assess whether lactate (Lac) is able to regulate mTORC1 signalling pathway and consequently SC proliferation. SC obtained from 8-day old rats were maintained under basal conditions (B) or stimulated with 20 mM Lac in the absence or presence of 1nM rapamycin (Rap), an mTORC1 specific inhibitor. Phosphorylated (P)-mTORC1 and P-p70S6K levels by Western blot, and bromodeoxyuridine (BrdU) incorporation by immunocytochemistry were evaluated. It was observed that Lac increased P-mTORC1 and P-p70S6K levels, as well as BrdU incorporation. In addition, Rap was able to block Lac effect on SC proliferation (B: 8.5±0.7^{a}, Rap: 7.3±0.5^{a}, Lac: 11.7±1.3^{b}, Lac+Rap^{a}: 8.3±0.9% BrdU positive cells, mean±SD, n=3, different letters indicate statistically significant differences, P<0.05). These results suggest that Lac upregulates SC proliferation in an mTORC1-dependent manner, and we postulate Lac might be the mediator in the contribution of glycolysis in the regulation SC proliferation by FSH.

533. 24. **IMPACT OF CONTROLLED OVARIAN HYPERSTIMULATION ON MOUSE EARLY EMBRYO DEVELOPMENT: POSSIBLE MODULATORY EFFECT OF GHRELIN**

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Controlled ovarian hyperstimulation (COH) is commonly used in assisted reproduction in order to increase oocytes retrieval. However, the resulting supraphysiological levels of sex-steroids have been associated with negative reproductive outcomes. COH might also alter the levels of ghrelin (Ghrl), a hormone involved in embryo development and implantation. We aimed to analyze the effects of COH on ghrelinemia and early embryo development and to evaluated if the administration of a Ghrl antagonist (Ant=(D-Lys3) GHRP6) can ameliorate the negative effects of COH. Firstly, the COH protocol treatment was set by hyperstimulating adult female mice with different doses of pregnant mare´s serum gonadotropin (PMSG=5IU, 7.5IU, 10IU and 15IU) and human chorionic gonadotropin (10IU), and evaluating sex-steroid levels and oocytes quantity/quality. Natural cycling (NC) females were used as controls. After selecting the best protocol (PMSG=10IU), Ghrl concentrations were assessed. A second group of females [NC-females, COH-fe-

males and COH+Ant-females (treated with 6nmol/animal/day of Ant during hyperstimulation); n=13-20/group] were mated with untreated males and euthanized on day 3 of pregnancy, to evaluate embryo developmental status. Data were analyzed by Mann Whitney/Kruskall Wallis test; a $p<0.05$ was considered significant. COH significantly increased ovulation rate vs. NC-females (27.82±2.92 vs. 10.15±0.71; $p<0.05$), with no effects on oocytes quality. Also, COH significantly increased vs. NC-females, progesterone (6.16±1.05ng/ml vs. 0.78±0.29ng/ml) and Ghrl (16.45±6.01 vs. 4.12±0.79) levels, but not those of estradiol. Finally, COH significantly increased the proportion of morulas (77.5% vs. 25.0%) and decreased that of blastocysts (18.3% vs. 75.0%); being these effects reversed by Ant (% morulas=13.3% and % blastocysts=54.6%).
Our results show that COH delays early embryo development, being this effect associated, at least in part, to increased Ghrl levels consequent of COH.

534. 47. **CHANGES IN CERVICAL ENDOCANNABINOIDOME IN NON-PREGNANT AND PREGNANT MICE**

Carolina Marvaldi[1]; Fernando Correa[2]; Clare Johnson[3]; Ayelen Mirón Granese[1]; Julieta Aisemberg[1]; Ana Maria Franchi[1]; Heather Bradshaw[3]; Manuel Luis Wolfson[1].
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The endocannabinoidome (eCBome) is a complex lipid signaling system composed of the endocannabinoids (eCB), eCB-related molecules, their receptors, and their metabolic enzymes. Despite cumulative evidence showing the important role of the eCBome in different aspects of female reproduction, little is known regarding their function in the physiopathology of the cervix. The cervix is a critical organ for pregnancy maintenance. Dynamic alterations in the structure of the cervical extracellular matrix drive flexibility and mechanical strength of the cervix throughout pregnancy, labor, and postpartum. Therefore, the aim of this work was to evaluate the participation of the eCBome in the cervical remodeling during gestation. No significant differences were observed between these groups for eCB receptors (CB1 and CB2) and NAPE-PLD protein levels. However, we found that FAAH protein levels were decreased in cervix from pregnant mice on day 15 of gestation when compared to non-pregnant mice ($p<0.05$). Anandamide (*N*-arachidonoyl ethanolamine) is the main eCB involved in pregnancy events. We compared the cervical content of AEA and observed that the cervix from pregnant mice presented lower levels of AEA than the cervix from non-pregnant mice. Furthermore, we found that several eCB-related molecules belonging to the family of *N*-acyl ethanolamines (NAEs) were diminished in the cervix from pregnant mice when compared to the cervix from non-pregnant animals (e.g., OEA, PEA, SEA, and LEA). Nevertheless, the cervical content of *N*-docosahexaenoyl ethanolamine (DHEA) was increased in pregnant animals. Regarding the 2-acyl glycerols, 2-arachidoloyl glycerol (2-AG) levels were increased in the cervix from pregnant mice in comparison with non-pregnant. The same pattern was observed in 2-OG, 2-PG, and 2-LG levels. Free fatty acids and N-acyl glycines levels were also modulated during pregnancy. Our data suggests that a switch in eCBome profile is needed for cervical remodeling and maintaining a successful pregnancy.

535. 50. **PORCINE OOCYTES MEIOTIC PROGRESSION AND ITS RELATION WITH REACTIVE OXYGEN SPECIES PRODUCTION AND MITOCHONDRIAL ACTIVITY DURING IN VITRO MADURATION**

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During *in vitro* maduration (IVM) a series of modifications take place in the oocyte, which lead to its developmental competence and involve a variation in reactive oxygen species (ROS) production. The aim of our study was to evaluate meiotic progression, ROS production and mitochondrial activity in porcine oocytes during IVM. Cumulus-oocyte complexes (COCs) obtained by aspiration of antral follicles from ovaries of slaughtered gilts were incubated in 199 medium supplemented with 50 µg/ml gentamicin sulfate, 10% (v/v) porcine follicular fluid, 0.57 mM cysteine, 0.5 µg/ml FSH and 0.5 µg/ml of LH at 39ºC, 5% CO_2 in a humidified atmosphere for 44h. At 0, 12, 24, 36 and 44h, cohorts of COCs were recovered from the IVM medium and denuded with a glass Pasteur pipette. Using epifluorescence microscopy, meiotic progression was assessed by Hoechst 33342, ROS production by DCH_2FDA, active mitochondria by MitoTracker Green and internal mitochondrial membrane potential by JC-1 stain. Digital microphotographs were obtained and processed using IMAGE J software to calculate oocyte fluorescence intensity. Between 0 and 12h oocytes progressed from germinal vesicle stage to germinal vesicle breakdown, observing a concomitant decrease in ROS production and a significant increase in active mitochondria and mitochondrial membrane potential ($p<0.05$). Around 24 and 36h most oocytes reached metaphase I, while others progressed further to intermediate stages between metaphase I and II. In that time frame ROS production tended to increase, while active mitochondria and mitochondrial membrane potential tended to decrease. At 44h, when most of the oocytes were arrested at MII stage, metabolic parameters showed similar values to 0h. In conclusion, meiotic progression proved to be related with a decrease in ROS levels and an increase in mitochondrial activity. These metabolic variations could be associated with moments of active protein synthesis.

536. 51. **GLYCOLYSIS AND OXIDATIVE PHOSPHORYLATION ARE NECESSARY FOR VASOACTIVE INTESTINAL PEPTIDE-MEDIATED MIGRATION IN TROHPOBLAST CELLS**

Fátima Merech[1], Brenda Lara[1], Daiana Ríos[1], Vanesa Hauk[1], Daniel Paparini[1], Guillermina Calo[1], Mariela Videla[2], Mariela Garcia[2], Mathias Chemen[1], Rosanna Ramhorst[1], María Eugenia Monge[2], Claudia Pérez Leirós[1] and Daiana Vota[1]
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Trophoblast cells display different functional phenotypes to support placental development and fetal growth. Extravillous cytotrophoblast cells (EVT) acquire a migratory and invasive phenotype, regulate immune responses and vascular transformation maintaining placental homeostasis. A highly active metabolism of trophoblast cells is required to attend the dynamic demands of the placenta and fetus throughout pregnancy but how EVT integrate metabolic signals remains unclear. We have previously shown that vasoactive intestinal peptide (VIP) stimulates cytotrophoblast cell glucose and amino acid metabolism in vitro and in vivo, as well as it promotes cell migration in vitro. Our goal was to deepen into the metabolic pathways activated upon VIP stimulation of EVT, their role in cell migration and VIP effect on TCA and glycolysis metabolites accumulation. The human EVT-like cell line Swan-71 was cultured with VIP (10-100 nM). Cell migration was evaluated by wound healing assay using 2-DG or Rotenone as glycolysis and electron transport chain inhibitors. Lactate, pyruvate, succinate and other metabolites were assessed by Accutrend Plus System or semi-targeted metabolomic approaches. Long chain fatty acids (LCFAs) uptake and lipid droplets were analyzed by flow cytometry with BODIPY-FL C12 and 493/503 fluorescent probes. Expression of LCFAs transporters FATP1/2/4, metabolic regulator PPARg and lactate transporters MCT1/4 by RT-qPCR. VIP promoted EVT migration via glycolysis and oxidative phosphorylation (*$p<0.05$). VIP stimulation induced LCFAs uptake and lipid droplet accumulation (*$p<0.05$) along with the upregulation of FATP2 and the downregulation of PPARg expression (*$p<0.05$). Lactate production and MCTs/FATP1/4 expression remained unchanged.

Our findings shed light on the mechanisms involved in the effect of VIP as a metabolic and functional placental regulator.

537. 176. **INHIBITION OF NLRP3 *INFLAMMASOME-DEPENDENT INFLAMMATION IMPROVES TESTOSTERONE PRODUCTION IN TESTES OF AGED SYRIAN HAMSTERS***

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Male aging studies have shown that *testosterone levels decline with age. Our group previously described* a significant increase in testicular inflammation in aged Syrian hamsters (*Mesocricetus auratus*). Autophagy, a cellular recycling system, plays critical roles in inflammation. This study aimed to examine the relevance of inflammation and autophagy on testicular testosterone production during aging. Young and aged (5 and 22 months old) hamsters kept in a normal photoperiod (14 h light per day) were used. Testicular expression of inflammatory (NLRP3 inflammasome, caspase 1, IL1β) and autophagy (P62) markers was determined by western blot; testosterone levels were quantified by RIA. In aged hamsters, we found diminished circulating testosterone levels and testicular StAR expression but increased testicular NLRP3, caspase 1 and IL1β expression which was accompanied by a reduced autophagy characterized by significantly elevated protein levels of P62. When testicular fragments from aged hamsters were incubated in the presence or absence of a potent and specific inhibitor of NLRP3 (MCC950 10 μM) or autophagy inducers (rapamycin 200 nM and metformin 50 μM), protein expression levels of NLRP3, IL1β and P62 were markedly decreased. MCC950 significantly increased protein StAR expression and the levels of testosterone secreted to the incubation media, while metformin and rapamycin showed a tendency to improve them. In summary, these results suggest that testicular aging is associated to increased inflammation, reduced autophagy and a diminished testosterone production. Treatment with NLRP3 inhibitors would exert a protective effect improving testosterone production in the aged testis. Autophagy inducers might also have a beneficial effect on steroidogenesis in testicular aging. These studies represent an important advance promoting future interventions focused on preventing inflammatory processes during aging in the male gonad.

538. 224. **CHARATERIZATION OF OVIDUCTAL PROTEINS THAT PARTICIPATE IN THE SELECTION OF SPERM**

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In previous works, we identified to DMBT1 and ANXA2 as oviductal proteins that interact with sperm. Isolated DMBT1 reduce viability and motility *in vitro* and thus, involved in negative sperm selection, while ANXA2 may be involved in oviductal reservoir formation. The objective of this work was to evaluate the transcriptional and translational expression of DMBT1 and ANXA2, the presence of isoforms and subcellular locations of both proteins in oviductal epithelium through the porcine estrous cycle. Also, to analyze the effect of no isolated DMBT1 present in different subcellular fractions on sperm physiology. Oviducts were classified according its estrous cycle stage and processed to obtain oviductal fluid (OF), oviductosomes (OVS) and plasma membrane of epithelial cells (MEC). Transcriptional and translational expression were assayed by RT-qPCR and Western blot, respectively. Sperm were incubated in capacitating medium supplemented with OVS (mOVS), OF (mOF) or MEC (mMEC) corresponding to each estrous stage. The specificity of the effect of DMBT1 present in each fraction was evaluated by blocking its interaction with sperm using anti-DMBT1 antibody and pre-immune serum as control. The results show that expression DMBT1 and ANXA2 are regulated at transcriptional level through the estrous cycle ($p < 0.05$) but only DMBT1 is regulated at translational level ($p < 0.05$). Both proteins were detected in OVS, OF and MEC and the presence of alternative transcripts and putative protein isoforms for ANXA2 and DMBT1 were also demonstrated. Greater viability was found in mOF of luteal stage and in mFO and mOVS of follicular stage all supplemented with the anti-DMBT1 antibody ($p > 0.05$), indicating an effective blocking effect of the antibody in these fractions. Also, greater motility in mOF of luteal stage supplemented with antibody was found. These results are in line with previous hypothesis of the role of DMBT1 in negative selection by reducing viability and motility of sperm

539. 287. **STUDY OF MOLECULAR COMPENSATION MECHANISMS LEADING TO THE LACK OF MALE FERTILITY DEFECTS IN A METABOLIC SYNDROME MOUSE MODEL**

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The prevalence of metabolic syndrome (MS) has increased at alarming rates in recent years, coinciding with reproductive age, and becoming a risk factor for fertility disorders. However, there is still a controversy on the relationship between MS and male fertility. In this sense, our group has addressed the possible effect of MS on male fertility in a murine model without finding evidence of this association. In that study, we observed an important increase in gonadal fat of MS animals, and two regions were identified within it according to their location with respect to the epididymis: distant gonadal fat (DG) and nearby gonadal fat (NG). We found that NG is less susceptible to the metabolic injury of MS than DG, thus generating a possible protective microenvironment for epididymal sperm stored in the cauda region. To continue the study of possible compensation mechanisms behind the absence of fertility alterations in animals with MS, in the present study we evaluated the oxidative stress in the testis and cauda epididymis of MS mice. Even though in the testis there was no effect in lipid peroxidation ($p>0.05$), we found a significant increase in the activity of antioxidant enzymes (catalase -CAT- and superoxide dismutase -SOD-) ($p<0.01$; $p<0.05$, respectively). When we analyzed the epididymal cauda, we did not detect lipid peroxidation damage ($p>0.05$) nor any increase in CAT or SOD activity ($p>0.05$). In summary, these results show a redox balance generated by the antioxidant enzymes in the testis that, together with those results related to the potential protective effect given by the NG to the cauda, could explain the lack of alterations in male fertility and sperm parameters observed in our MS murine model.

540. 330. **EVALUATION OF OXIDATIVE AND INFLAMMATORY STATUS IN MEN OF COUPLES UNDERGOING IVF TREATMENT**

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Worldwide, approximately 15% to 20% of couples suffer from infertility, of which up to 30% can be attributed to the male factor. Although conventional semen analysis is fundamental in the evaluation of male infertility, it has notable limitations, as it does not assess any parameters related to the oxidative-inflammatory balance of the seminal microenvironment. The aim of this work was to study in semen the redox and inflammatory markers in men of couples undergoing fertility treatment. Fifty-nine semen samples aged between 24– 64 years, were categorized into two groups: (A) untested fertility (no offspring) and (B) tested fertility (with at least one offspring). Control group consisted of 25 healthy men with at least one offspring (C). We determined in seminal plasma a) oxidative stress markers: malondialdehyde (MDA) and nitrite (NO_2^-) concentrations; b) enzymatic antioxidant defenses: superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx); c) Interleukin 6 (IL-6) and Tumor Necrosis Factor Alpha (TNF-α). Statistical analyses were performed by InfoStat software and were considered significant at

p<0,05. Semen parameters were similar in all groups. However, Kruger morphology was significantly lower in group A respect to the other groups. MDA levels were similar in all groups, but patients in group B showed higher concentrations of NO_2^- and lower SOD and GPx activity compared with C [NO_2^- μM: A= 4,1 (2,8-7,2); B= 5,3 (2,8-11,1); C= 3,1 (1,8-5,1); SOD mUSOD/mg prot: A= 556,5 (360,0-958,1); B=679,7 (327,9-876,9); C= 945,0 (708,6-1078,0)]. TNF-α level was higher in A and B groups respect to C [TNF-α pg/mL: A= 12,2 (4,9 - 26,8); B=8,5 (3,8 - 28,5); C=5,9 (2,7 - 9,3)]. These findings show increased inflammatory status and variations in oxidant-antioxidant conditions in the seminal plasma of men undergoing IVF treatment. Study semen redox environment would contribute to highlight underlying imbalances that could condition the fertilizing capacity of sperm.

541. 390. **THE IMPACT OF OVERNUTRITION AND EXPOSURE TO ENVIRONMENTAL POLLUTANT 3-METHYLCHOLANTHRENE ON TESTICULAR INTEGRITY IN PREPUBERTAL RATS**

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Previously, we observed that male rats that were overweight and/or exposed to 3MC exhibited reduced sperm count and motility, without no significant changes in testicular weight. This study aims to assess whether exposure to the environmental pollutant 3-methylcholanthrene (3MC), known to be an obesogen, may enhance the adverse effects caused by overnutrition on testicular integrity and spermatogenic process. To this end, prepubertal male rats fed standard or cafeteria diet were exposed to a vehicle or a 0.1 mg/kg dose of 3MC three times per week for 40 days. Leading to four experimental groups: controls (SDV), overweight rats (CDV) and SD rats exposed to 3MC (SD3MC), CD rats exposed to 3MC (CD3MC). The body weight and gonadal fat weight were measured at 61 days of age, and the testes were collected. In each group, eight sections of the same testis from five different animals were analyzed. CDV rats had significantly higher body weight than SDV rats (10% p<0.05), and 3MC reduced this increase to SDV values (p<0.05). Moreover, CDV rats showed a significant increase in gonadal fat compared to the SDV rats (p<0.001). In addition, the CD3MC group exhibited a relative increase in gonadal fat compared to the SD3MC group (24% p<0.05). In contrast, the CDV group exhibited a significant increase in the diameter of seminiferous tubules (95% p<0.001), a decrease in epithelial thickness (11% p<0,05), and an increase in luminal diameter (138% p<0,01) compared to the control group. Furthermore, overweight rats presented a higher number of disruptions in the epithelium (disruptions/field) (344% p<0,001) compared to the SDV group. The results suggest that the combination of both overnutrition and exposure to 3MC, an obesogenic environmental pollutant, adversely affect the structure of microtubules, leading to alterations in morphology that could impair the process of spermatogenesis in a developing organism.

542. 418. **COMPARATIVE STUDY OF LEPTIN ANTIAPOPTOTIC EFFECT IN BEWO AND SWAN-71 CELLS UNDER HYPOXIC CONDITION**

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The placenta is a major source of leptin in the fetomaternal circulation, although its physiological role remains to be clarified. Leptin promotes proliferation and survival of trophoblastic cells and is proposed to be a marker of acute stress in the fetus. Moreover, leptin prevents cellular stress in trophoblastic cells and is incremented in different pregnancy pathologies such as preeclampsia, as a compensatory response to hypoxia or oxidative stress present in placental cells. In this work we compared some parameters associated with the antiapoptotic effect of leptin against hypoxic stress, generated by a hypoxia chamber with a defined amount of oxygen (2% O_2) or a model of chemical hypoxia using $CoCl_2$ treatment that stabilizes HIF-1α transcription factor. We used Swan-71 cells, a first trimester cytotrophoblast human cell line and BeWo cells, a human choriocarcinoma cell line. Cell models were grown in a hypoxia chamber, or treated with $CoCl_2$ (100μM) with or without leptin (100ng/ml). Treatment with 100μM PD98059 or 50nM Wortmannin, pharmacological inhibitors of MAPK and PI3K respectively, were also used. The expression of PARP-1, Caspase-3, P53 and Mdm-2 was determined by Western blot. Swan-71 cell migration was evaluated by Wound healing assay. Our results showed that leptin regulates p53 signaling pathway under hypoxic condition through MAPK and PI3K in both Swan-71 and Bewo cells. Leptin also prevents Caspase-3 cleavage through PI3K pathway in Bewo cell line after HIF-1α stabilization. Cells grown in a hypoxia chamber showed increased apoptosis determined by cleaved Caspase-3 and PARP-1. These effects were reversed by leptin treatment. On the other hand, we observed that leptin promotes Swan-71 cell migration and this effect is partially blocked after $CoCl_2$ treatment. These results suggest that leptin increased cell survival of trophoblastic cells, grown under hypoxic conditions or with HIF-1α stabilized by $CoCl_2$ treatment, involving MAPK and PI3K pathways.

543. 421. **ADENYLATE CYCLASE ISOENZYMES PARTICIPATE IN HYALURONIC ACID CAPACITATION OF BOVINE SPERMATOZOA**

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The aim of this study was to evaluate the effect of adenylate cyclase isoenzymes inhibition in bovine sperm functional parameters, such as capacitation, motility, viability/acrosomal integrity, mitochondrial activity and *in vitro* fertilization (IVF). Hyaluronic acid (HA) was used as a sperm capacitation inducer, LRE-1 as soluble adenylate cyclase inhibitor, and 2,5-dideoxyadenosine (2,5-D) as membrane adenylate cyclase inhibitor. Five treatments were performed with frozen-thawed semen in TALP medium at 38°C: control, HA, HA/LRE-1, HA/2,5-D, and HA/LRE-1/2,5-D. Sperm capacitation was evaluated by the chlorotetracycline epifluorescent technique and viability/acrosomal integrity by trypan blue vital staining with differential interferential contrast. Sperm motility was evaluated by microscopy and analyzed with the ISAS-Prosier software. Mitochondrial membrane potential of spermatozoa was measured using the fluorochrome JC-1. Cleavage rate was analyzed 48 hours after IVF. Data were analyzed by ANOVA and Tukey's test (P<0.05). Adenylate cyclase inhibitors produced a significantly decrease in percentage of capacitated spermatozoa (HA/LRE-1 4.67 ± 2.31 %, HA/2,5-D 6.00 ± 1.63 %, HA/LRE-1/2,5-D 3.33 ± 1.15 %)compare to HA samples (23.60 ± 5.90 %) and a mitochondrial membrane potential percentages diminish respect to HA samples (P<0.05). Total and progressive motilities, amplitude of lateral head displacement and beat-cross frequency also decreased when inhibitors were added in sperm samples, respect to HA treatment (P<0.05). Cleavage percentages in IVF showed a tendency to decrease in the presence of the inhibitors. Both adenylate cyclase isoenzymes participate in the intracellular signal mechanism induced by HA during capacitation.

544. 469. **EFFECT OF TROLOX AND RESVERATROL ON ACTIVE MITOCHONDRIA AND CYTOSOLIC OXIDATIVE STATUS DURING *IN VITRO* MATURATION OF BOVINE OOCYTES**

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During oocyte *in vitro* maturation (IVM), the oxidative status in the cytosol and the activity of mitochondria are indicators of the oocyte's ability to carry out the processes of nuclear and cytoplasmic maturation. The properties of the antioxidants trolox (T) and resveratrol (R) and the concentrations in which they could exert an effect are being analyzed in different cells. The aim was to study the effect of T and R during IVM on the active mitochondria and the cytosolic oxidative status of the oocytes. Ovarian follicles were puncture-aspirated and the oocyte-cumulus complexes (COCs) were recovered. COCs were matured in 199 medium for 22 h at 39°C with a humidified atmosphere of 5% CO_2 in air (control) or added with T 25 μM (T_1), 50 μM (T_2) or 100 μM (T_3) or R 0.1 μM (R_1), 1 μM (R_2) or 5 μM (R_3). To determine active mitochondria and the cytosolic oxidative status, denuded oocytes were incubated for 30 min with MitoTracker green FM and RedoxSensor red CC-1. The individual luminosity of each oocyte was assessed by IMAGE J. Oocyte nuclear maturation was evaluated by assessing the metaphase II with Hoechst. Data were analyzed by ANOVA ($p<0.05$). The active mitochondria in the matured oocytes in the presence of T_3 decreased significantly respect to the control ($p<0.05$), they also decreased in the presence of R_1, R_2 and R_3 respect to the control ($p<0.05$), not observing significant differences between them. Likewise, the cytosolic oxidative status decreased in the presence of T_3 compared to the control ($p<0.05$), while in the presence of R_2 and R_3 this parameter increased compared to the control ($p<0.05$). No significant differences were observed in oocyte nuclear maturation with the different treatments. We can conclude that supplementation of the IVM medium with T or R modifies both mitochondrial and cytosolic oxidative activity in the bovine oocyte without affecting nuclear maturation. However, their effects on oocyte cytoplasmic maturation remain to be elucidated.

545. 485. **POLYAMINES IN THE IMMATURE SYRIAN HAMSTER TESTIS: A PRELIMINARY STUDY OF THEIR CONTRIBUTIONS TO SERTOLI AND TESTICULAR PERITUBULAR CELL PHYSIOLOGY**

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Polyamines (PA) are ubiquitous polycationic compounds. They have many functions, contributing to immune response and redox balance, among others. The specific role of PA in testicular physiology, though, has not been fully elucidated in part, most likely, due to the complex cellular organization of the testis. The first aim of this study was to quantify PA levels in the immature Syrian hamster testis. Using thin layer chromatography (TLC) we detected differences ($p<0.0001$; n=8) in testicular concentrations of putrescine (Pu: 7.8±0.2mM), spermidine (Sd: 73.1±2.6mM) and spermine (Sp: 107.8±3.2mM). Next, we analyzed the possible contribution of different cell populations of the immature Syrian hamster testis to local PA synthesis. Ornithine decarboxylase mRNA expression was found in Leydig cells, testicular macrophages, Sertoli cells, testicular peritubular cells and germ cells. TLC was able to detect, at least, two of the main PA intracellularly in all of the above cell populations. Following Sertoli cell (SC) and testicular peritubular cell (TPC) isolation from immature Syrian hamsters' testes, potential effects of Pu, Sd and Sp on cellular physiology were addressed. In SC cultures, Ldha mRNA expression was stimulated by Pu (10 μM), Sd (10 μM) and Sp (1-10 μM) while Catalase expression was induced by Sp (10μM) ($p<0.05$; n=3; qPCR). Sod1 and Pxr1 mRNA expression levels remained unchanged ($p>0.05$; n=3; qPCR). In TPC, Sd (10 μM) and Sp (1-10 μM) reduced ($p<0.05$; n=3; qPCR) expression levels of contractility markers (α-Sma, Calponin, and Myh11) and some extracellular matrix components (Col4a1 and Col1a2) while others (biglycan and decorin) remained mostly unaffected by PA incubation. Pxr1 and Sod1 expression in TPC was up-regulated by Pu (0.1μM) and Sd (0.1-1-10μM) while Catalase expression was mainly up-regulated by Sd (1-10μM). These results suggest that PA may differentially influence the functionality of SC and TPC.

546. 492. **EFFECT OF THE LIPOPOLYSACCHARIDE FROM *E. COLI* IN THE OVARY IN A RAT MODEL OF SUBCLINICAL INFECTION**

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Subclinical infections cause dysregulation of immune homeostasis that could have serious consequences for pregnancy. It has been postulated that alterations in the inflammatory response might cause placental dysfunction impacting progeny development. Previous results from our laboratory show that the administration of lipopolysaccharide from *Escherichia coli* (LPS) as an infection stimulus to rats during early gestation is associated with a decrease in fertility as it affects the maternal-fetal interface and the neurodevelopment of the offspring. The aim of this study was to elucidate if the effect of LPS was caused by a direct action on the uterus, or indirectly by a systemic contribution via the ovary. For this, pregnant rats of the Wistar strain received vehicle (saline, control) or intraperitoneal LPS (20 kg/mg on day 6 + 50 kg/mg on days 7, 8 and 9 of gestation). The animals were euthanized on day 15 of gestation and the ovaries were surgically removed and stained with hematoxylin and eosin. The number and size of the corpora lutea, and the number and type of luteal cells were quantified. Data were analyzed using one-way ANOVA. Differences were considered significant when $p<0.05$. The general architecture and the size of the ovaries were similar in rats treated with LPS compared to the ovaries of control rats. Leukocyte infiltration was not observed. No differences were registered in the number or size of the corpora lutea between treatments. Moreover, the number of large and small luteal cells was similar between control and LPS ovaries. In conclusion, these results provide evidence that subclinical infections do not affect the structure of the ovary in our model, supporting the hypothesis that our previous results are caused by a local infection of the maternal-fetal interface. More experiments are being carried out to determine the serum levels of estrogen and progesterone to study ovarian functionality.

547. 543. **RELEVANCE OF THE CYSTEINE-RICH SECRETORY PROTEIN (CRISP) FAMILY FOR FEMALE FERTILITY**

Abril Rebagliati Cid, Visacovsky, Nicolás[1]; Mariana Weigel Muñoz, Valeria Sulzyk and Cuasnicú, Patricia S.
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Cysteine-Rich Secretory Proteins (CRISP1-4) play key roles in mammalian fertilization in both sexes. In females, CRISP1, 2 and 3 are expressed in reproductive organs and/or cumulus cells that surround the egg. Whereas single female knockout (KO) mice for CRISP1 and CRISP2 are fertile, double KO (DKO) females for CRISP1 and CRISP3 are subfertile, suggesting the existence of compensation among CRISP family members. To further investigate the relevance of CRISP proteins for female fertility, triple KO for CRISP1, 2 and 3 (TKO) and quadruple (QKO) were generated by CRISPr/Cas9 and their fertility examined by caging one mutant and one control female with a fertile male, followed by evaluation of born pups. Results showed that fertility of TKO and QKO mice was significantly impaired compared to controls ($p< 0.01$) and not different between mutant groups, consistent with the lack of CRISP4 in females. As CRISP2/CRISP4 DKO females generated in our lab are

fertile, fertility defects in TKO/QKO seem to be mainly due to the lack of CRISP1 and CRISP3. Based on this, *in vivo* fertilization studies were performed in superovulated instead of estrous single CRISP1 KO females to analyze the contribution of CRISP1 to female fertility in a more demanding condition. Results showed a significant decrease in the percentage of fertilized eggs recovered from the ampulla, supporting the relevance of female CRISP1 for fertility. As no single KO for CRISP3 was available at our lab, and with the aim of analyzing the contribution of CRISP3 to female fertility, female KO mice lacking this protein were generated by CRISPr-Cas9 and fertility analyzed. Differently from CRISP1 and CRISP2 single KO colonies, a significant decrease in fertility was observed for CRISP3 KO females. Together, these results support the relevance of female CRISP proteins, especially CRISP3, for fertility, contributing to a better understanding of the molecular mechanisms underlying mammalian female fertility.

O2-REPRODUCTION

THURSDAY 16TH NOVEMBER 14:00-15:30

CHAIRS: CAROLINA LUCHETTI
MARÍA PAULA DI YORIO

548. 30. **HYPOXIA INDUCIBLE FACTOR-1 ALPHA (HIF-1α) REGULATES LUTEINIZATION AND LUTEAL ANGIOGENESIS**

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Ovarian angiogenesis is key to follicle and corpora lutea development. Hypoxia inducible factor-1 alpha (HIF-1α) is the main angiogenesis inductor capable of sensing low oxygen concentration. Our aim was to elucidate the role of HIF-1α in the ovary during luteinization and its impact in corpus luteum function. Prepubertal female F1 mice (BALB/c x C57) received 5 IU of eCG i.p followed by 5 IU of hCG i.p 48h later. One group of animals received i.p 5 mg/kg of the HIF-1α inhibitor Acriflavine (ACR) the same day of hCG injection (low ACR) and another group received i.p 10 mg/kg of ACR both the same day of hCG injection and the next one (high ACR). The control group received saline. 48h after hCG, mice were sacrificed and the ovaries and serum recovered. WB, ELISA and histological techniques were performed. Statistical analyses were carried out by unpaired t-test. The % of structures was similar among groups but there was an increase in total cysts in the high-dose ACR group. Ovarian VEGF was decreased in both ACR groups and HIF-1α was decreased only in the high ACR group. The levels of ANGPT2, PDGF-B and N-Cadherin did not change. Endothelial and periendothelial cell areas were decreased within the corpora lutea of ACR groups. Serum progesterone did not change after ACR administration. Ovarian StAR levels were decreased only in high ACR group, while p450scc and 3ß-HSD levels did not change. p-Erk showed a tendency to decrease in high ACR ovaries and p-Akt increased significantly in low ACR ovaries. The % of corpora lutea suffering luteolysis increased in low ACR group. In summary, ACR decreased VEGF, endothelial and periendothelial cell areas in the recently formed corpora lutea leading to cyst formation. Therefore, HIF-1α inhibition modifies ovarian angiogenic factor levels leading to an alteration in blood supply to the newly formed corpora lutea. Although serum progesterone did not change, decreased ovarian StAR shows HIF-1α inhibition affects corpora lutea function.

549. 39. **EXTRACELLULAR VESICLES FROM RAM SEMINAL PLASMA TRANSPORT DECAPACITATION PROTEINS**

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Extracellular vesicles (EVs) are micro and nanovesicles present in body fluids. Seminal plasma (SP) contains EVs derived from the epididymis, seminal vesicle, prostate and bulbourethral glands. They contain lipids, proteins and small regulatory RNAs from the original tissues and play a key role in the exchange of information with the sperm. Although EVs of various species have been characterized, there are not many studies in ram SP. This species of productive interest, presents a low performance in artificial insemination and *in vitro* fertilization using cryopreserved sperm. We have studied the role of small SP proteins termed as “decapacitation factors (DFs)” as suitable treatments to reduce and revert sperm cryodamage. The aim of this work was to isolate and characterize EVs from ram SP and determine if they participate in the transport of DFs. Three methods were used: ultracentrifugation (UC), precipitation with different ratios of polyethylene glycol (PEG) PEG:PS, and molecular size exclusion column (SEC). Total protein content, protein profile and the presence of CD9 tetraspanin as a vesicle marker were analyzed. Anti fibronectin (Fn) was used to study the presence of DFs from the Binder of Sperm Proteins family. Morphology was characterized by transmission electron microscopy. The total protein content was higher in EVs obtained by UC and SEC. The protein profile analyzed by SDS-PAGE showed that the EVs obtained by UC have a wide range of molecular weight (Mw) proteins, while those obtained by the other methods showed predominance of high or low Mw, according to the method. The EVs obtained by UC, SEC and PEG:PS 1:3 and 1:5 revealed positive for anti CD9 and Fn antibodies. This allows us to select three methods for the isolation of SP EVs and demonstrate that EVs transport decapacitation proteins, being a potential tool for supplementation of seminal extenders and to deep study the interaction of EV and ram sperm.

550. 118. **METFORMIN AND FEMALE REPRODUCTION: EFFECTS ON PHYSIOLOGICAL CONDITIONS**

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Objectives: 1. To study the effects of metformin (Met) on female mice reproduction. 2. To study the effects of metabolic syndrome (MS) and Met on female mice reproductive performance. Materials and methods 1.14-weeks old C57BL/6 mice were divided into 2 groups. One group received Met orally. The other, water only. After 4 weeks, animals were split into 3 subsets. In one subset, blood, adipose tissue and the ovaries were collected and estrous cycle was determined. The other subset was mated and the time to pregnancy and offspring features were analyzed. The third subset underwent in vitro fertilization (IVF). 2. 21-days old C57BL/6 mice were fed for 15 weeks with regular chow or high fat diet (HFD) (45% Kcal fat). Body weight was determined once a week. At week 11, some HFD animals received Met orally for 4 weeks. Then, animals from the three groups were split and analyzed as explained in 1. Results: 1. Met reduced visceral adipose tissue, cycle duration, primary follicles, pup’s weight, ovarian angiogenesis, progesterone, 3βHSD and StAR. Estradiol, aromatase and preantral follicles were higher in the Met group. No differences were found after IVF. 2. HFD mice had higher body weight, adipose tissue, glycemia, area under glycemia curve, total cholesterol, HOMA IR index, ovarian angiogenesis, fibrosis and genomic damage, time to pregnancy, 3βHSD and progesterone, with no differences in estradiol. Met restored most of these alterations. We found a smaller number of pups per litter

with similar weight. Met restored these alterations. Cycle duration was also reduced. The % of newly-formed corpora lutea was lower in HFD. Met reversed this effect. After IVF, fewer oocytes were retrieved, with no effect of Met. Conclusions: Met has an effect on ovarian performance in physiological and pathological conditions, being able to restore most of reproductive alterations caused by MS. Altered ovarian angiogenesis may be one of the mechanisms that explains MS and Met effects on the ovary.

551. 184. **ROLE OF CCR2 CHEMOKINE RECEPTOR WITHIN THE FOLLICULAR ACTIVATION PROCESS**

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Follicle activation is an irreversible physiological process, consisting of the recruitment and awakening of the quiescent pool of ovarian primordial follicles, constituting the ovarian reserve. Hence, its activation is a key biological checkpoint that controls female reproductive potential. However, the molecular mechanisms responsible for initiating such a process are not fully understood. Preliminary results from our laboratory suggest that chemokines may be directly involved in this process. Thus, the present study aimed to evaluate the effect of CCR2 chemokine receptor activation in gene expression of key regulators of follicle activation. To evaluate this, ovaries from adult domestic cats (*Felis catus,* n:18) were used. Using a tissue slicer, the ovarian cortex was cut into 1x1x0.5 mm3 cortical pieces. Each piece was examined under a dissecting scope and only tissues (n=127 total, 3 experiments) that showed primordial follicles were used. Ovarian tissues (n=3-4 per well) were cultured in defined culture media for 4 hr at 38°C in 5% CO2 in atmospheric, and incubated under different treatments: control group (medium alone), CCR2 antagonist (1μM), and MCP1 (10 ng/ml and 100 ng/ml). At the end of the culture period, the fragments were stored at 80ºC for RNA extraction. Following cDNA synthesis, quantitative real-time PCR was performed to evaluate the mRNA expression of key genes involved in follicle activation (FOXO3, mTOR, AKT, KIT, KITL, and PIK3Ca). Normalized results (GAPDH plus 18S) showed that MCP1 treatment significantly increased the mRNA expression of KIT, FOXO3 (10 ng/ml), and AKT (100 ng/ml) compared to the control ($p<0,05$; ANOVA). Also, significant differences ($p<0,05$) were observed for FOXO3 mRNA expression in the presence of CCR2 antagonist. The mRNA expression of KITL, mTOR, and PIK3Ca did not significantly differ among groups. In summary, these results suggest that the CCR2-MCP1 pathway may be a key player in the regulation of follicle activation.

552. 208. **POLYCYSTIC OVARY SYNDROME ALTERS THE ENDOCRINE PROFILE AND INCREASES HISTOMORPHOLOGICAL ALTERATIONS IN THE UTERUS OF AGED RATS IN THE LONG-TERM**

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This study aimed to investigate the long-term impacts of polycystic ovary syndrome (PCOS) on rat uterine health. To achieve this, we evaluated: a) Levels of serum steroid hormones , b) determination of uterine lesions incidence and multiplicity, c) evaluation of uterine collagen remodeling, and d) infiltration of eosinophils. PCOS was induced in female Wistar rats through sc injection of dehydroepiandrosterone (6mg/100g bw) from 21 to 40 days of age. The CONTROL group received injections of sesame oil. Animals were euthanized at 18 months (CONTROL18 and PCOS18) and at 24 months (CONTROL24 and PCOS24). Blood samples were collected for the quantification of sex steroids, and uterine were dissected and processed for histomorphological analysis. At 18 months, levels of 17β-estradiol, progesterone and testosterone were comparable between the groups, revealing distinct alterations in luminal epithelium and glands. PCOS18 rats exhibited an increased in multiplicity of glands with squamous metaplasia and gland conglomerates. Additionally, elevated of collagen remodeling was observed in subepithelial stroma along with a reduction in the myometrium. Eosinophil infiltration remained unchanged. At 24 months, PCOS24 rats displayed lower serum progesterone compared to CONTROL24 rats (CONTROL24: 22.4 ± 6.2 vs. PCOS24: 5.9 ± 1.9 ng/mL, $p<0.05$). PCOS24 rats exhibited the same epithelial alterations identified at 18 months, along with an augmented incidence of intraepithelial lumens and glands displaying cytoplasmic vacuoles. In this group, collagen remodeling increased in subepithelial stroma while decreasing in the myometrium. Additionally, higher eosinophils counts were noted in both compartments. Our findings underscore that PCOS expedites the onset of uterine alterations observed in 24-month-old rats. The alterations found were linked to heightened estrogen stimulation that was inadequately balanced by progesterone, which could promote the emergence of neoplasia.

553. 320. **COVID-19 AND THE OVARIAN VASCULATURE: ACUTE AND LONG-TERM CONSEQUENCES**

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A correct ovarian function depends on a healthy vascular system, this allows a proper supply of nutrients, oxygen, and hormones to the developing follicles. The vascular system requires the coordinated action of angiogenic factors. We previously observed alterations in angiogenic parameters up to 9 months post-infection that include a reduction in VEGF levels in the follicular fluid (FF) of recovered COVID-19 patients, decreased endothelial cell migration, and increased genomic damage in an endothelial cell line (EA. hy926) stimulated with FF from recovered COVID-19 patients. So far, no study has evaluated the effect of SARS-CoV-2 infection on angiogenesis over longer periods in the female gonad. The objective was evaluate angiogenic parameters in patients who have suffered from COVID-19 up to 18 months post-infection. A total of 85 women (21–43 years old) under ART were recruited from 4 reproductive medicine institutions. The patients were classified into: the control group (n=30) (no positive test for COVID-19) and the post-COVID-19 group (n=55) (at least one positive test by PCR). EA.hy926 cells were incubated with FF from either control or post-COVID-19 patients. The endothelial migration, angiogenic markers (VEGF, ANGPT-1, ANGPT-2), and nuclear DNA damage were analyzed. Statistical analysis was performed using ANOVA followed by Tukey's test or ***t***-test. After 18 months of COVID-19 infection, VEGF levels in FF from recovered COVID-19 patients did not differ from those in the control group. Stimulation with FF from post-COVID-19 patients resulted in a significant decrease in endothelial cell migration compared with control FF ($\boldsymbol{p} < 0.0001$). In addition, ANGPT-1, ANGPT-2, VEGF, and γH2AX protein expression unchanged as well as in protein levels when cells were stimulated with either control or post-COVID-19 FF. In conclusion, our results show that ovarian an-

giogenesis is partially restored 18 months after COVID-19 infection.

554. 529. **SPERM ENERGY RESTRICTION AND RECOVERY (SER) TREATMENT IMPROVES SPERM FUNCTION IN THE BOVINE MODEL**

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The cattle industry relies on the successful application of assisted reproductive technologies to produce animals with higher economic value. In recent years, the use of *in vitro* produced embryos in the cattle industry has been on the rise on a global scale. Improvement of embryo production that increases the number and/or quality of embryos able to generate healthy offspring would have a considerable financial impact on the agricultural industry. We have recently shown that the use of sperm energy restriction and recovery (SER) treatment improved sperm function and increased fertilization and embryo development rates after *in vitro* fertilization (IVF) in mice.
Here, we optimized the application of SER treatment in bull sperm and used it prior to intracytoplasmic sperm injection (ICSI) without exogenous chemical activation. Our results show an improvement on sperm motility after SER. In addition, when used prior to ICSI, the percentage of 2-cell embryos obtained with SER-treated sperm increased over 3-fold when compared to controls (50% SER, 16% control; P≤ 0.05). Moreover, 17% of the SER-derived embryos reached blastocyst stage while none of the control-derived embryos did (P≤ 0.05). We then investigated if these differences could be due to SER treatment facilitating the egg activation mediated by sperm. We found higher percentages of eggs that displayed more than 3 Ca^{2+} peaks after ICSI in the SER treatment in comparison to controls (40 % to 10%, respectively; P≤ 0.05). As sperm PLCz correlates with the occurrence of Ca^{2+} oscillations, we evaluated the localization of this protein in bull sperm. We observed no changes in PLCz localization in the sperm head after SER treatment in comparison to controls. Overall, our results indicate that SER treatment improves sperm function and facilitates egg activation and suggest that manipulation of sperm incubation conditions can increase the efficiency of *in vitro* embryo production.

P3-REPRODUCTION

FRIDAY 17TH NOVEMBER 9:00 - 10:30
CHAIRS: JULIETA AISEMBERG
JUAN MANUEL TEIJEIRO

555. 23. **DO PHYSICAL EXERCISE AND/OR GHRELIN ADMINISTRATION REVERSE THE NEGATIVE EFFECTS OF OBESITY ON SPERM QUALITY?**

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**D Machuca and P Guantay contributed equally to this work*

 # AC Martini and EM Luque should be considered last joint authors.

Obesity (Ob) is a highly prevalent pathology all over the world, characterized by dyslipidemia, hypertension, heart disease, meta-inflammation and premature mortality. Our hypothesis was that "in rats, physical exercise (Exc), with or without the co-administration of Ghrelin (Ghrl), improves the metabolic profile and the sperm quality altered by obesity". In male Wistar rats we studied, the effects of an obesogenic diet (ObD=pelleted food +30% pork fat +water with 5% fructose) from weaning (day 21), on body weight evolution and, in adulthood, the metabolic profile, visceral fat and sperm quality. We evaluated also if Exc (standardized forced walk in wheels, 15min/day, 3 days/week from day 65 to 103), with or without Ghrl (6 nmol/animal/day s.c. from day 85 to 103) modified these parameters. Animals were divided in five groups: a) control diet (CD: pelletd food+ water) b) ObD, c) ObD+Ghrl, d) ObD+Exc and e) ObD+Ghrl+Exc. Results were analyzed by ANOVA with a p<0.05; n=7animals/group. ObD increased significantly (day 103) body weight evolution (g) (ObD=406.6±5.8 vs CD=334.3±10.4), visceral fat (g) (ObD=12.4±0.7 vs CD=6.6±0.6), total cholesterol (mg/dl) (ObD=79.1±4.9 vs CD=51.4±2.5), triglycerides (TG) (mg/dl) (ObD=130.7±9.7 vs CD=79.1±4.9) and LDL (mg/dl) (ObD=27.5±4.9 vs CD=11.9±2.2). Exc (ObD+Exc) significantly reversed these effects (body weight=356.7±15.4; visceral fat=7.71±0.85, total cholesterol=66.8±3.0; TG=56.1±4.3), but not Ghrl. Exc and Ghrl (together or separately) significantly increased the values of sperm concentration (10^6/ml) (CD=25.3±0.7; ObD+Exc=24.5±2.2; ObD+Ghrl=26.4±2.7; ObD+Ghrl+Exc=24.4±1.0 vs ObD=18.8±0.7) and sperm motility (%) (CD=57.9±3.14; ObD+Exc=53.3±3.6; ObD+Ghrl=58.1±3.2; ObD+Ghrl+Exc=59.2±3.8 vs ObD=44.2±3.4) altered by obesity. In conclusion, Exc was very effective in reversing all the negative effects of obesity; Ghrl was only for sperm quality. The combination of both treatments did not show more effectiveness than Exc alone.

556. 25. **EFFECT OF A DIET ENRICHED IN EXTRA VIRGIN OLIVE OIL ON PLACENTAL EXPRESSION OF MIR-22 AND GLUTS IN WOMEN WITH GESTATIONAL DIABETES MELLITUS**

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Introduction: Previous works have shown that a diet enriched in Extra Virgin Olive Oil (EVOO), rich in oleic acid (ω-9), regulates metabolic and inflammatory parameters in maternal plasma and in the placenta of women with Gestational Diabetes Mellitus (GDM). MiR-22 is a microRNA involved in the regulation of inflammatory and metabolic pathways. GLUTs are glucose transporters, being GLUT4 negatively regulated by miR-22 in different tissues. Aim: To evaluate in patients with GDM whether a diet enriched in EVOO regulates maternal plasma fatty acid percentual composition and placental expression of miR-22, GLUT4 and GLUT3. Methods: Women with GDM and healthy controls (C; n=15) were enrolled between 24-28 weeks of pregnancy. All of them received a standard diet for pregnancy whereas a group of women with GDM also received 36 mL/day of EVOO (GDM, n=15; GDM-EVOO, n=15). Fatty acid percentage composition was determined in term maternal plasma by GC-FID. miR-22, GLUT4 and GLUT3 expression was evaluated in term placentas by qPCR. Results: In maternal plasma, an increase in erucic and nervonic acid was found in the GDM-EVOO group (p<0.05 vs. C). In the placenta, miR-22 was decreased in the GDM group (p<0.05 vs. C), an alteration prevented by the EVOO-enriched diet (p<0.05 vs. GDM). GLUT4 mRNA levels showed no changes among the three evaluated groups. GLUT3 expression was decreased in the GDM group (p<0.05 vs. C), an alteration prevented by the EVOO-enriched diet (p<0.05 vs. GDM). Conclusion: The increase in maternal plasma levels of erucic and nervonic acid, ω-9 elongation products of oleic acid, is possibly the result of the good adherence to the EVOO supplementation in the GDM-EVOO group. In the GDM group, the reduced placental levels of miR-22, a microRNA involved in inflammatory and metabolic pathways, were regulated

by the EVOO-enriched diet, although not related to the expression of GLUT4. GLUT3 mRNA levels in the GDM-EVOO group possibly reflects changes of maternal metabolic parameters.

557. 104. **THE MIR-122-SIRT1-PPARALPHA PATHWAY IN THE DECIDUALIZED UTERI FROM PREPUBERAL OFFSPRING OF DIABETIC RATS: EFFECT OF A MATERNAL DIET ENRICHED IN OLIVE OIL**

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Introduction: Reduced markers of decidualization and an increased prooxidant environment have been previously found in the decidualized uteri of the offspring of diabetic rats, an alteration prevented by a maternal diet enriched in olive oil. In other systems, a pathway that involves the microRNA-122 (miR-122), the sirtuin SIRT1 and the nuclear receptor PPARα regulates the prooxidant/proinflammatory environment. Aim: To evaluate the miR-122-SIRT1-PPARα pathway in the decidualized uteri of prepuberal offspring of diabetic rats fed or not with an olive oil-enriched diet. Methods: A mild pregestational diabetic rat model was induced in F0 females by neonatal administration of streptozotocin (90 mg/kg sc). Control and diabetic females were mated with healthy males and received a 6% olive oil-enriched diet or a standard diet from day 1 of pregnancy until parturition. The offspring were fed a standard diet and the uteri of the female offspring (F1) were evaluated on postnatal day 30, after induction of decidualization with PMSG (50 UI) and hCG (50 UI). MiR-122, SIRT1 and PPARα was evaluated by RT-qPCR. Results: The offspring of diabetic rats (DG) showed a reduction in miR-122 levels in the decidualized uteri (-89%, $p<0.01$ vs. Controls), an alteration prevented by the maternal diet enriched in olive oil. *Sirt1* mRNA levels and *Ppara* mRNA levels were increased in the decidualized uteri of DG (+68% and +84% respectively, $p<0.05$ vs. Controls), an alteration prevented by the maternal diet enriched in olive oil. PPARα target genes *Cat* and *MnSod* also increased their expression in the decidualized uteri of DG (+61% and +52%, respectively $p<0.05$ vs. Controls), an alteration prevented by the maternal diet enriched in olive oil. Conclusion: A stimulated miR-122-SIRT1-PPARα pathway in the decidualized uteri suggest a compensatory response to regulate the increased prooxidant/proinflammatory environment. This activation is prevented when the mothers received the antioxidant/anti-inflammatory diet enriched in olive oil.

558. 108. **THE ENRICHED ENVIRONMENT IMPROVES THE PHYSIOLOGY OF THE OVARY IN PREGNANT MICE**

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Maternal lifestyle modulates reproductive physiology impacting reproductive performance. We have previously demonstrated that exposure preconceptionally and during gestation to an enriched environment (EE) regulates uterine physiology, promoting vascular remodeling during early gestation and pregnancy success. We hypothesize that these events are also influenced by the regulation of ovarian activity. Therefore, we decided to study the effect of EE exposure on ovarian physiology during early gestation. For this, six-week-old female mice were housed in EE or control cages for six weeks and then mated with control fertile males. Pregnant mice were sacrificed on day 7 of pregnancy. Ovaries were extracted and the number of corpora lutea and primordial, primary, preantral, antral, and atretic follicles was counted in hematoxylin and eosin-stained slices. Five slices per ovary were analyzed. Serum was collected to measure progesterone and estrogen levels. Data was analyzed with t-tests using the statistical software Infostat. Differences were considered statistically significant when the p-value was equal to or less than 0.05. Ovaries from enriched females presented more corpus luteum than control females, although their size did not differ between groups. No differences were detected between the groups for the number of primordial, primary, preantral, antral, and atretic follicles. Besides, enriched females presented higher progesterone levels, while estrogen levels did not change. Our results demonstrate that the maternal environment regulates ovarian physiology, and this could promote the benefits previously reported. Even considering the limitations of an animal model, the positive effects of EE support the idea that a maternal enrichment protocol could be recommended for women seeking pregnancy. This non-pharmacological intervention might be advantageous for women, and especially for those who have to overcome a fertility treatment.

559. 171. **EFFECT OF LIRAGLUTIDE ON BLOOD-TESTIS BARRIER FUNCTION IN JUVENILE RATS**

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Liraglutide (Lira), an analogue of glucagon like peptide 1, is widely used for treating adult patients with obesity and type 2 diabetes. The rates of obesity continue to grow in children and therefore it has been recently approved for use in children over 10 years old. However, little is known about Lira effects on testicular function. The blood-testis barrier (BTB), composed of junctions between Sertoli cells (SC), isolates germ cells, providing an adequate microenvironment for their development. Consequently, defects in the BTB lead to harmful effects on testicular function. At present, no studies have been conducted to assess if early-life administration of Lira represents any potential risk to BTB establishment. Thus, the aim of this work was to evaluate the effects of Lira on BTB integrity *in vivo*. To this end, Sprague Dawley male rats were randomly assigned to the following groups: Lira (receiving daily 0,2 mg/kg Lira s.c.) and control (C; sterile saline solution s.c.) from postnatal day (Pnd) 20 to Pnd33, period of life that is essential to complete a functional BTB. The BTB integrity was evaluated on Pnd34 using a biotinylated tracer. Histological analysis was also performed. The results showed that Lira group exhibited a significant increase in BTB permeability compared to controls (C: 5.5±1.5; Lira: 11.9±3; $n=9$; $p<0.001$). However, no differences in testicular histology were observed. To evaluate possible mechanisms that could explain the effects on BTB permeability, intratesticular testosterone levels and the expression of intercellular Sertoli cell junction proteins (claudin11 and occludin) were examined. Lira treatment did not modify intratesticular testosterone levels or claudin11 and occludin mRNA levels. Taken together, these results indicate that Lira has a deleterious effect on BTB integrity in juvenile rats. However, further studies are necessary to determine mechanisms involved and the impact of this effect on adult fertility.

560. 266. **IMPACT OF AGING ON OVARIAN FOLLICLE ASPIRATION EFFICIENCY AND OOCYTE MITOCHONDRIAL MEMBRANE POTENTIAL IN DONKEYS**

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** equal contribution*

Studying aging on donkey fertility holds particular importance in the context of their conservation as some wild asses are critically endangered. Moreover, domestic donkeys could become an interesting animal model for gaining insights into similar aging processes in humans. Hence, our study investigated the influence of age on transvaginal follicle aspiration (TVA) efficiency and the quality of the retrieved immature oocytes by evaluating mitochondrial membrane potential. Three young (5-10 years) and three old (18-25 years) female donkeys were used for this experiment. Three young domestic mares (5 to 10 years) served as an additional control group. Immature oocytes were obtained by TVA, held in holding media at 20ºC for 20-24 h, and subjected to analysis of mitochondrial mass (MMS) and mitochondrial membrane potential (MMP) by measuring intensity levels post-confocal analysis of Mitotracker Green and TMRE, respectively. To independently assess MMP relative to MMS, the MMP/MMS ratio was calculated. Results showed no significant differences in oocytes recovered per aspirated follicle (mares: 0.5 ± 0.1; young donkeys: 0.7 ± 0.2; old donkeys: 0.5 ± 0.3). Older donkey oocytes displayed higher MMS and MMP ($p<0.05$), but a lower MMP/MMS ratio compared to young donkeys ($p<0.05$). Young mare oocytes had higher MMS ($p<0.001$) with no MMP difference from young donkeys. Interestingly, MMP/MMS ratio was higher in young donkeys ($p<0.001$) than young horses. In summary, our findings reveal that aging compromises mitochondrial function in asses. Remarkably, old donkey oocytes might display a proactive mechanism, potentially increasing mitochondrial numbers in attempting to counteract their lower activity. Moreover, donkey oocytes could have more active mitochondria compared to horses. These discoveries contribute to our comprehension of aging and fertility.

561. 286. **EVALUATION OF METABOLIC PLASTICITY IN THE ACQUISITION OF MURINE SPERM FERTILIZING ABILITY**

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Mammalian sperm must suffer functional and structural changes in the female reproductive tract to gain the ability to fertilize the egg. This process, known as capacitation, requires an efficient management of energy. Our previous work showed the relevance of mitochondrial function for hyperactivation and the development of sperm fertilizing ability during capacitation. However, it remains unclear which substrates sustain this activity. Although most capacitation media contain glucose (G), pyruvate (P) and lactate (L), the nutrients sperm find in the female tract are poorly defined. Hence, in this work we evaluate the development of sperm fertilizing ability in different metabolic environments. We showed that exogenous non-glycolytic substrates maintained the mitochondrial membrane potential (MMP) increase, differently from the G-only condition ($p<0.05$). MMP was even lower ($p<0.05$) when the entrance to the mitochondria of P produced by addition of only G was inhibited, showing the utilization of endogenous P by mitochondria. However, in the G-only condition, sperm presented a similar hyperactivation ($p>0.05$) but a higher fertilization rate than control ($p<0.05$). The addition of P to the G-containing medium produced a decrease in fertilization compared to G-only ($p<0.01$) and control ($p<0.05$) media, suggesting a possible negative feedback towards the oxidation of G. Likewise, when glycolysis is inhibited either by the omission of G or by the addition of 2-deoxyglucose, fertilization decreases significantly ($p<0.05$), revealing the importance of G catabolism for development of sperm fertilizing ability. To sum up, the use of glycolytic and non-glycolytic substrates shows that glycolysis and oxidative phosphorylation can sustain different functional sperm parameters suggesting an inherent metabolic plasticity capacity. Moreover, the need of both pathways for the sperm fertilizing ability reveals a functional link between them, previously controversial in these cells.

562. 306. **MATERNAL DIABETES REDUCED MEGALIN AND CUBILIN LEVELS IN THE FETAL KIDNEY**

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Background: Maternal diabetes causes an altered development of different fetal organs, including the kidney, related to the programming of diseases in the offspring. The multiligand endocytic receptors Megalin and Cubilin are key receptors in the process of renal reabsorption, their expression can be modulated by the nuclear receptor PPARα. Megalin is a functional receptor for matrix metalloproteinase 9 (MMP9), mediating its endocytosis and catabolism. MMP9 is a proteolytic enzyme involved in morphogenesis, but also in pathological processes when overproduced. Aim: To evaluate PPARα mRNA levels and protein expression and localization of Megalin, Cubilin and MMP9 in the fetal kidney of control and diabetic rats on day 21 of gestation. Methods: Pregestational diabetic rats were obtained by neonatal administration of streptozotocin (90 mg/kg sc) and control rats were injected with vehicle. Both groups were mated with healthy males and fetal kidneys were obtained on day 21 of gestation to evaluate PPARα mRNA levels (RT-PCR) and megalin, cubilin and MMP9 (IHQ). Results: A decreased fetal kidney/fetal weight was found in the diabetic group (-30%,$p<0.01$). In the fetal kidney from diabetic rats a reduced *Ppara* mRNA levels was found together with a reduction in Megalin (-87%,$p<0.05$) and Cubilin (-36%,$p<0.05$ vs controls) in the proximal convoluted tubule, where reabsorption takes place. An increased MMP9 (+263%,$p<0.001$ vs controls) was observed not only in the proximal and distal convoluted tubule but also in collecting ducts of the fetal kidney from diabetic rats. Conclusion: Maternal diabetes induces a decrease in PPARα possibly related to the downregulation of Megalin and Cubilin expression. The reduction of Megalin in the proximal convoluted tubule suggests an altered clearance of MMP9, which was increased in the collecting ducts of fetal kidneys of diabetic rats. These alterations may be related to the programming of renal dysfunction in the offspring of diabetic mothers.

563. 351. **RESISTANCE-ASSOCIATED PROTEIN 4 (MRP4) REGULATES CAMP-DEPENDENT SIGNALING PATHWAY ASSOCIATED WITH HUMAN SPERM CAPACITATION**

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Cyclic AMP (cAMP) has been reported to be essential for events associated with sperm capacitation, including regulation of motility and changes in motility pattern known as hyperactivation. There is also evidence that many of the effects of cAMP in sperm are mediated by activation of the cAMP/PKA pathway, with an increase in PKA substrates (pPKA) and tyrosine residues (pY). Previously we determined that MRP4 plays an essential role in mouse and bovine sperm capacitation and characterized MRP4 in human sperm. This work aimed to evaluate the participation of MRP4 in the regulation of cAMP-dependent signaling pathway during human sperm capacitation. Sperm (from 42 normospermic samples) were incubated for 6 h in capacitating conditions (HTF medium; 25 mM HCO_3^-;5 mg/ml BSA) (CAP) with or without MK571 (MK) (50 μM), a MRP4 inhibitor. The presence of MK increased intracellular cAMP levels diminishing cAMP extrusion after 30 min of capacitation ($p<0.05$). We observed a decrease in pPKA substrates and pY levels in spermatozoa incubated in CAPMK compared to those incubated in CAP ($p<0.05$). Furthermore, MRP4 blockade increased or decreased the phosphorylation of specific PKA substrates. Lastly, we investigated the role of cAMP efflux by MRP4 in the regulation of sperm motility. The presence of MK in CAP decreased the hyperactivation index (~50%) and reduced several kinematic parameters, such as VCL (CAP:133,67±12,74; CAPMK:121,77±13,68) and

ALH (CAP:2,74±0,27; CAPMK:2,53±0,30) (p<0.05). The addition of non-permeable cAMP to CAP reversed the MK effect on sperm motility, but not on hyperactivation. These results suggest that MRP4 transporter is involved in the regulation of intracellular cAMP levels by modulating phosphorylation levels in nucleotide-dependent signaling pathways in human sperm. Moreover, our results support the importance of cAMP efflux through MRP4 in sperm capacitation and suggest a role in the regulation of some key motility kinematic parameters in this species.

564. 356. **ALTERATION OF THE SENESCENCE-ASSOCIATED SECRETORY PHENOTYPE AT THE MATERNAL-EMBRYO INTERFACE IN A RAT MODEL OF ADVANCED MATERNAL AGE**

María Laura Leonardi, Dalmiro Gomez Ribot, Lautaro Recchia, Alicia Jawerbaum, Romina Higa
Laboratory of Reproduction and Metabolism. CEFYBO-CONICET. School of Medicine, University of Buenos Aires, Argentina.

Background: Pregnancy at Advanced Maternal Age (~35 years, AMA) induces obstetric complications and neonatal adverse outcomes. During early pregnancy, an optimal decidual function is essential for a correct placenta and embryo development. MMP2, a protease of senescence-associated secretory phenotype (SASP), have an important role in tissue remodeling during trophoblast invasion and embryo morphogenesis. We have previously observed that decidua of a rat AMA model showed increased MMP2 and embryonic growth impairments. Here, our aim was to evaluate in the decidua from AMA rats, the localization of 4HNE (marker of lipoperoxidation) and mRNA levels of p21 (marker of senescence) during placenta formation. Also, to evaluate mRNA levels of p21 and MMP2 in the placenta and the embryo from AMA rats. Methods: 3 months old (Control) and 10 months old (AMA) Wistar rats were mated with young males. Decidua, placenta and embryos were obtained on day 12 of pregnancy. mRNA levels of p21 and Mmp2 were measured by RT-qPCR (n=8) and 4-HNE by immunohistochemistry (n=5). Results: The decidua of AMA showed increased mRNA levels of p21 (+54%, p<0.05) and increased 4HNE immunostaining in the mesometrial region (+71%, p<0.05). In contrast, p21 (-27%, p<0.01) and Mmp2 (-26%, p<0.05) were reduced in the placenta of AMA rats. Embryos from AMA group showed decreased mRNA levels of Mmp2 (-33%, p<0.05) but no changes in p21 between groups. Conclusion: In AMA rats, the higher senescence in decidua increases SASP-molecules as MMP2 that, together with it increased oxidative status may affect placenta formation. Indeed, in the placenta the decreased p21 and MMP2, needed for trophoblast invasion, suggest that placentation is altered. Importantly, embryos showed reduced MMP2 probably related to the embryonic developmental impairments previously observed.

565. 357. **ZIKA VIRUS INFECTION OF TROPHOBLAST CELLS MODULATES NEURAL PROGENITOR CELL SURVIVAL AND METABOLISM**

Mathias Chemen[1, 2], Soledad Rodriguez-Varela[3], Daiana Rios[1], Agostina Marquez[2], Daniel Paparini[1], Fátima Merech[1], Vanesa Hauk[1], Claudia Pérez Leirós[1], Leonardo Romorini[3], Cybele García[2], Daiana Vota[1]
[1]Laboratorio de Inmunofarmacología. [2]Laboratorio de Estrategias Antivirales. Instituto de Química Biológica de la Facultad de Ciencias Exactas y Naturales (IQUIBICEN), CONICET-Universidad de Buenos Aires, Buenos Aires, Argentina. [3]LIAN-CONICET, Fundación FLENI

Aim: Zika virus (ZIKV) infection during the first stages of pregnancy was pointed out as a risk factor related to a higher incidence of congenital neurological disorders. We have previously demonstrated that ZIKV infection of first trimester cytotrophoblast-derived cells (Tb) impaired cell migration and reduced brain-derived neurotrophic factor (BDNF). Interestingly, the vasoactive intestinal peptide (VIP) produced by the placenta decreased Tb susceptibility to ZIKV infection, restoring cell migration and metabolism. We aim to investigate the impact of Tb cell infection with ZIKV on human neural stem cell survival and the possible extended antiviral role of VIP during ZIKV infection of neural progenitors. Methodology: First trimester Tb-derived cell line Swan-71 (Tb) was infected with or without a local ZIKV isolate for 8h to obtain Tb- conditioned media (CMTbZ/CMTb). Neural stem cells (NSCs) derived from human embryonic stem cells were cultured with CMTb or CMTbZ or infected with ZIKV in the presence/absence of 10-50 nM VIP. Apoptosis, fatty acid and lipid droplet accumulation was measured by flow cytometry using a specific fluorescent Annexin V/IP kit or BODIPY FL C12 and BODIPY 493/503 fluorescent dyes, respectively. Infective viral particle production was measured by plaque assay. Results: CMTb protected NSCs from apoptosis (P<0.05) but the effect was lost in CMTbZ. Moreover, differential modulation of cell metabolism induced by CMTb or CMTbZ was observed. On the other hand, ZIKV infection of NSCs in the presence of VIP decreased viral particle production with respect to cells infected in the absence of VIP. These results suggest that ZIKV infection of first trimester Tb cells altered their soluble factors secretion pattern impacting indirectly in NSCs survival and metabolism modulation. Also, VIP not only modulates placental susceptibility to ZIKV infection but also ZIKV propagation in human neural progenitor cell cultures.

566. 378. **THE IMPACT OF BUTYRATE IN MATERNAL AND OFFSPRING METABOLISM IN A RAT MODEL OF MATERNAL OVERWEIGHT**

Heinecke, F; Labiano, M; Flores Quiroga, J P; Meneghini, M A; Faletti, A G and White, V
Centro de Estudios Farmacológicos y Botánicos (CEFYBO-CONICET-UBA), Facultad de Medicina, Buenos Aires, Argentina

In a rat model of maternal overweight, we previously observed that maternal oral administration of butyrate, a postbiotic from the gut microbiota, prevented fetal overgrowth and liver lipid overaccumulation, while in mothers it prevented hypertriglyceridemia, although liver lipid overaccumulation persisted. Our aim was to evaluate whether butyrate could also ameliorate the negative program induced by maternal overweight in the offspring and improve maternal metabolic parameters. Methods: Female Wistar rats were fed standard (CT rats) or high saturated fat (FD rats) chow for 8 weeks and mated with CT males. Butyrate (3%) or vehicle was orally administered daily during gestation and 3 days per week during lactation (FDB rats). Mothers were euthanized after weaning and the offspring were euthanized at 140 days of age. The offspring were fed a control diet. Maternal and adult offspring hepatic levels of triglyceride (TG) and cholesterol ester (CE) were assessed by TLC, mRNA levels by RT-qPCR, and circulating activity of hepatic enzymes (ALT and AST) by the IFCC method. Results: Maternal liver showed overaccumulation of TG, CE (191% p<0.001 and 85% p<0.05) and increased mRNA levels of Srbp-1c (95% p<0.05) in both FD and FDB groups, with a negative correlation with triglyceridemia (p<0.01). Butyrate prevented the increase in ALT activity observed in FD maternal serum (16% p<0.05), whereas AST activity decreased in the FDB group (34% p<0.05). The offspring showed an overaccumulation of hepatic TG (40% p<0.05) and an increase in circulating ALT and AST levels (35% p<0.05), all of which were prevented by butyrate (34% and 19% p<0.05). Conclusions: Butyrate was able to prevent liver tissue damage and lipid overaccumulation in the offspring, improving the metabolic program. In the mothers, it partially prevented liver damage, but lipid overaccumulation persisted, possibly due to the overnutrition and the increase in Srbp-1c mRNA levels.

567. 464. **EXPLORING GENISTEIN'S IMPACT ON CELL ADHESION IN BOVINE OVIDUCTAL EPITHELIAL CELLS**

Vella, MA[1]; De Boeck, M[1]; García, DC[3]; Valdecantos, PA[2]; Roldán-Olarte, M[1,2]
[1]*INSIBIO-CONICET-[2]FBQF - UNT. Chacabuco 461. 4000. Tucumán. Argentina. [3]Univ. de Santiago del Estero (UNSE).*

An optimal oviductal microenvironment is pivotal during the early phases of the reproductive process in mammals. The adhesion of cells that constitutes the oviductal epithelium is of importance to contribute to the maintenance of structural integrity and the efficien-

cy of gamete transport, sperm capacitation and ensures the proper exchange of nutrients between the interstitial cellular space and the oviductal lumen. Genistein (GNT), an isoflavone with multiple biological properties, is capable of diffusing through the oviductal epithelium. Given the significance of preserving oviductal integrity and the diverse properties of GNT, the aim of this work was to evaluate its impact on cell adhesion in bovine oviductal epithelial cells (BOECs). Cells were obtained for mechanical pressure from recently slaughtered heifer oviducts. Explant and monolayer cultures were stimulated with GNT at various concentrations (0.1 μM - 10 μM) for 24 hours. Subsequently, BOEC adhesion was assessed. To achieve this, monolayer cultures were dissociated with 0.25% trypsin, and 10^4 live cells were seeded in wells previously coated with type I collagen, derived from dissected bovine tendons. After 2 hours, cells were washed with PBS solution to remove debris and unattached cells; then were stained with 0.1% Crystal violet for adherent cell visualization and count them with ImageJ software. A decrease in the number of adherent BOECs per mm² was observed with all tested GNT concentrations, highlighting that with 10 μM the most pronounced effect was recorded. The *in vitro* expression of *PTK2* and *PXN*, genes involved in focal adhesion formation, was evaluated using RT-PCR. In GNT- treated cultures, a reduction in the expression of both genes was evident. These findings lead us to conclude that GNT-treated cultures showed reduced expression of both genes, suggesting that this isoflavone negatively impacts in BOEC adhesion in a dose-dependent manner, possibly by influencing focal adhesion-related genes.

568. 473. **EXPRESSION AND LOCALIZATION OF CRISP PROTEINS IN EPIDIDYMAL EPITHELIUM AND EPISIDIDYMOSOMES**

Rebagliati Cid A[1], Carvajal G[1], Valeria Sulzyk[1], Battistone A[2], Breton S[2], Weigel Muñoz M[1], Cuasnicú PS[1].
Ibyme Conicet 1. Mgh Harvard 2.

Mammalian sperm acquire their fertilizing ability during a maturation process that occurs in the epididymis, mainly as a consequence of the association of epididymal proteins with the sperm surface. Results from our laboratory showed that double knockout mice for two such proteins, epididymal CRISP1 and CRISP4, were subfertile and exhibit clear defects in epididymal epithelium differentiation and luminal acidification critical for sperm maturation. In view of this, in the present work, we studied cell expression and localization of CRISP1 and CRISP4 along the epididymis. Analysis of protein expression by Western Blot and indirect immunofluorescence showed that whereas CRISP1 was expressed in the three regions of the organ (i.e. caput, corpus and cauda), CRISP4 was mostly detected in the caput and proximal corpus, being almost absent in the cauda. The use of specific markers for each epididymal epithelial cell type revealed that whereas CRISP1 and CRISP4 were not detected in basal cells, both were expressed not only in principal cells, as previously reported, but also in clear cells known to be involved in proton secretion, supporting the involvement of these proteins in luminal pH regulation. Considering the important role that extracellular vesicles (i.e epididymosomes) play in protein association with sperm during maturation, the presence of CRISP1 and CRISP4 in epididymosomes from different regions of the organ was examined. After confirmation of successful isolation of the epididymosomes by electron microscopy, the presence of CRISP1 and CRISP4 in these vesicles was analyzed by Western blot. Results showed that whereas CRISP1 was present in those vesicles obtained from either the caput or the cauda, CRISP4 was only detected in caput epididymosomes. Together, these results support the relevance of epididymal CRISP proteins for sperm maturation, contributing to a better understanding of the mechanisms underlying epididymal physiology and male fertility.

569. 522. **EVALUATION OF ENZYMES AND METABOLITES ASSOCIATED TO LIPIDS AND KETONE BODY METABOLISM IN DAIRY COWS WITH FOLLICULAR PERSISTENCE**

Cattaneo Moreyra ML[1,2], Gareis NC[1,2], Angeli E[1,2], Hein GJ[1,3], Ortega HH[1,2], Salvetti NR[1,2], Rodríguez FM[1,2], Rey F [1,2].
[1]Laboratorio de Biología Celular y Molecular Aplicada, ICi-Vet-Litoral (UNL-CONICET), Esperanza, Santa Fe.[2]Facultad de Ciencias Veterinarias-UNL, Esperanza, Santa Fe. [3]Centro Universitario Gálvez (CUG-UNL), Gálvez, Santa Fe.

Reproduction can be negatively affected due to disruption in lipid and ketone body metabolism. Therefore, we evaluate, by immunohistochemistry, the protein expression of PPARγ receptor, acetyl-CoA carboxylase (ACC, fatty acid synthesis enzyme), and relevant enzymes of cholesterol metabolism and ketone bodies (3-hydroxy-3-methyl-glutaryl-coenzyme A reductase <HMGR and synthase <HMGS; succinyl-CoA:3-ketoacid CoA transferase <SCOT). In addition, we measured the concentrations of β-hydroxybutyrate (BHB), non-esterified fatty acids (NEFA), cholesterol (CHOL) and triglycerides (TG) by using commercial kits. Cows with healthy reproductive tract (n=25) were subjected to estrous cycle synchronization with a G6G-Ovsynch protocol. While cows from the control group (C; n= 5) were only synchronized, those from the persistence groups were treated with progesterone intravaginally (P4) to induce follicles with 0 (P0; n=5), 5 (P5; n=5), 10 (P10; n=5) and 15 days of persistence (P15; n=5). Ovaries were obtained by bilateral ovariectomy to finally extract follicular fluid (FF) and ovarian histological sections. A lower expression of ACC and PPARγ was observed in theca cells of P15 compared to C ($p<0.05$). While HMGS expression was lower in the granulosa cells of P10 compared to C, P0 and P5 ($p<0.05$), in theca cells, its expression was lower in the P10 compared to C ($p<0.05$). In theca cells, the HMGR expression was lower in all the persistence groups compared to C ($p<0.05$). Also, the SCOT expression was lower in the granulosa cells of P5, P10 and P15 compared to C ($p<0.05$). In FF, the concentrations of BHB, CHOL (total, HDL and LDL), TG and NEFA were similar in all the groups ($p>0.05$). These results suggest a local alteration of the enzymes related to the lipids and ketone body metabolism in the initial stages of persistence, without evidencing significant changes in their related metabolites in FF. Probably, another extraovarian origin contributes to the ovarian microenvironment.

570. 532. **THE EFFECT OF RECOMBINANT OF S100 A9 ON SPERM ACROSOME REACTION IS DEPENDENT ON THE STRUCTURE OF THE EXPRESSED PROTEIN**

Estefania Massa, Gastón Prez, Sergio Ghersevich
Area of Clinical Biochemistry, Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, Suipacha 531, (2000) Rosario, Argentina

S100 A9 was identified in the oviduct secretion and a commercial human recombinant S100 A9 (hrS100 A9) was able to affect parameters of sperm function *in vitro*. The aim of this study was to express and purify human recombinant S100 A9 and to assess the effect of the expressed protein on sperm induced acrosome reaction (AR). The S100 A9 cDNA was inserted into the PGEX-2T plasmid, alongside a built-in sequence of glutathione S-transferase (GST), and were cloned in *E. coli*. The expressed GST-S100 A9, was purified using glutathione-agarose beads. The fusion protein was treated with agarose-thrombin beads to remove GST moiety (pS100 A9). The recombinant proteins were analyzed by Western blot. Human sperm were obtained from normozoospermic donors (n=5). Motile sperm (swim up) were incubated with increasing concentrations (0.1, 1.0, and 10.0 μg/ml) of: commercial hrS100 A9, pS100 A9, or GST-S100 A9 in medium with 0.5% BSA for 6 h at 37°C - 5% pCO_2. Aliquots of each sperm suspension were incubated with 20 μM progesterone to induce the AR, which was detected using fluorescein isothiocyanate-*Pisum sativum* agglutinin and results were presented as % of inducible population (% IP). Neither of the assayed proteins affected sperm cell viability or progressive motility, which were always higher than 85% and 70%, respectively. The results were: IP-control: 8.1±3,2%; IP-GST-S100A9(0.1 μg/ml): 8.2±2.3%; IP-pS100A9(0.1 μg/ml): 27.0±17.4%; IP-hrS100A9(0.1 μg/ml): 24.2±6.7%. Both pS100 A9 and hrS100 A9 caused a significant increase ($p<0.001$) of the IP at the dose of 0.1 μg/ml, while the GST-S100 A9 did not affect the AR. The cloning expression procedure successfully led to the recovery of GST-S100 A9, which was further treated to obtain pS100 A9. The pS100 A9 (without the GST moiety) was able to affect the induced AR, while the fusion protein

did not. The presence of GST in the fusion protein may impede the activation by S100 A9 of the mechanism that modulates the sperm induced AR.

P-SIGNAL TRANSDUCTION

WEDNESDAY 15TH NOVEMBER 9:00 - 10:30
CHAIRS: GRACIELA PIWIEN PILIPUK
NATALIA CALVO
CLAUDIA GENTILI

571. 63. **LEPTIN EFFECTS ON THE EXPRESSION OF RAC3 COACTIVATOR**

Iara Castellanos, Francisco D. Rosa, María Cecilia Lira, Juliana L. Bernacchia, María Florencia Quintanilla, Alejandra G. Palma, Mónica A. Costas and María Fernanda Rubio
Laboratorio de Biología Molecular y Apoptosis, IDIM UBA-CONICET

Adipocytes account for the largest proportion among the cells that comprise breast tissue. Although they are considered to be a critical cell type in the tumor microenvironment of breast cancer, it is still unclear the molecular mechanisms that control their behavior in this context. We have demonstrated that the expression of the coactivator RAC3 decreases during adipogenesis and was increased in adipose tissue adjacent to breast tumors. Therefore, the aim of this work was to study if leptin was able to modulate RAC3 expression.
From the systematic review of 20 papers, we confirmed the association between serum leptin levels and the presence of breast cancer in postmenopausal women (test for overall effect Z=11.98, p<0.00001). Then we studied the effect of leptin over a reporter vector that contain the RAC3 promoter upstream of the luciferase gen. For this HEK293 cell line was transfected with this construction and stimulated with leptin (L1:100ng/ml and L2:200ng/ml) and we observed that both doses of leptin induced an increase in luciferase activity (L1 2.08±0.11 and L2 2.48±0.08 vs B 1.00±0.08 p<0.0001). The values were relativized to basal condition and results are showed as the media±SD. Previously we reported that NF-κB is a transcription factor involved in RAC3 expression, then we studied by immunofluorescence the presence of its phosphorylated subunit p65 in nucleus of 3T3-L1 derived-murine adipocytes. The cells were stimulated with L1 for 15, 30 and 45 minutes and we observed a greater fluorescence intensity in nucleus in a time dependent manner and in Chromatin Immunoprecipitation (ChIP) assays, we observed that p65 was recruited to κB sequences in the RAC3 promotor and this recruitment displaces the presence of histone deacetylase 1 in a dose-dependent manner. These results suggest that leptin could be an adipokine that may be involved in the increased expression of RAC3 that we have observed in patients with breast cancer.

572. 395. **REGULATION OF RAC 1 GENE EXPRESSION BY vGPCR. INFLUENCE OF POST-TRANSCRIPTIONAL PROCESSING VARIANTS**

Mercedes Montani[1], Victoria Napoli[1], Emilia Feuerstein[1], Pedro Salaberry[1], Ángela Lara Montero[1], Julián Naipauer[1], Omar Coso[1]
1 Instituto de Fisiología, Biología Molecular y Neurociencias – Universidad de Buenos Aires/ CONICET

Kaposi's sarcoma (KS), an AIDS-defining cancer caused by the KS herpesvirus (KSHV), is a vascular sarcoma characterized by angiogenesis and spindle-cell proliferation. KSHV-encoded G protein-coupled receptor (vGPCR) can initiate KS-like tumors in mice. Moreover, it has been found that endothelial cells expressing vGPCR, show RAC1 upregulation, and inhibition of RAC1 activation in these cells diminishes tumorigenesis *in vivo*. This oncogene is important for tumor formation, progression, and metastasis and it has been widely reported in different subcellular compartments, but mainly in the plasma membrane when is activated. In the present work, we hypothesized that vGPCR would impact RAC1 expression and/or activation levels by regulating the production of different mRNA isoforms generated by alternative polyadenylation (AP), changing the mRNA stability and protein localization of RAC1. Therefore, we evaluated the existence of different mRNAs of RAC1 generated by AP, its stability, and subcellular distribution in the murine endothelial cell lines SVEC and SVEC vGPCR. We performed 3'RACE assays and found two mRNA isoforms of RAC1 synthesized by AP differentially expressed in both cell lines. In addition, we analyzed the RAC–GTP levels and detected that vGPCR upregulates RAC activation (p<0.05). Immunofluorescence of RAC1 revealed that vGPCR not only regulates RAC1 activation but also regulates its subcellular distribution. To determine if these events are associated with 3'UTR regulation, we designed fluorescent expression vectors that allow us to overexpress the different mRNA isoforms of RAC1 as fusion proteins with GFP and study RAC1- GFP subcellular distribution and evaluate the stability of each RAC1 mRNA isoform by RT-qPCR. Our results revealed that different isoforms of RAC 1 mRNA are synthesized by AP; this mechanism can be regulated by vGPCR and could be associated with RAC1 mRNA stability and protein subcellular localization regulating RAC1 activity.

573. 559. **REGULATION OF P53 UNDER NUCLEOLAR STRESS BY THE MAGEB2 ONCOPROTEIN**

Micaela Escalada [1], Candela Vidal [1], Melisa Suberbordes [1], Emanuele Buratti [2], Franco Pascucci [1], Martín Monte [1]
1 Laboratorio de Oncología Molecular, Dpto. Química Biológica, FCEN-UBA. Instituto IQUIBICEN, UBA-CONICET, Buenos Aires, Argentina.
2 Molecular Pathology Lab, International Centre for Genetic Engineering and Biotechnology (ICGEB), Trieste, Italy.

MAGE-I proteins are specifically expressed in normal germ-line cells, however, they exhibit anomalous re-expression in cancer cells leading to altered regulation of cell cancer-signaling pathways and enhanced tumor phenotype. Our group has been involved in the characterization of these proteins as nuclear oncogenic drivers, primarily focusing on the regulation of the p53 tumor suppressor pathway. MageB2 displays a predominantly nucleolar localization that is uncommon for MAGE-I proteins. We reported that MageB2 is a pro-proliferative protein that confers resistance to nucleolar stress (NoS). To investigate the mechanism by which MageB2 maintains its proliferative potential under NoS, we carried out an immunoprecipitation/mass spectrometry (IP/MS) approach in cells treated with BMH-21 (RNA Pol I inhibitor that induces NoS). We detected that MageB2 could interact with MYBBP1A, and this interaction is enhanced during NoS. MYBBP1A is a protein that, similar to MageB2, accumulates in the nucleolus and relocalizes to the nucleoplasm in the presence of NoS. Notably, when this relocalization takes place, MYBBP1A plays a role in the activation of p53. We corroborated the MageB2/MYBBP1A protein interaction in HEK293T cells. Furthermore, our results indicate that p53 activation measured by the mRNA levels of its target genes (p21waf1, bax, and puma) throughout RT-qPCR, is lower in HCT116 WT cells when compared to HCT116 MageB2 KO cells under NoS. No alterations in p53 activity is observed in MageB2 KO cells in the absence of stress. Our results suggest that MageB2 could inhibit p53 specifically under NoS and that the mechanism could involve the interference with the MYBBP1A/p53 axis.

574. 601. **BUILDING A SUBCELLULAR ATLAS OF AKT FOR PREDICTING ITS PHYSIOLOGICAL AND PATHOLOGICAL FUNCTIONS**

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[1] Instituto de Biociencias, Biotecnología y Biología Traslacional (iB3), Universidad de Buenos Aires (UBA), Buenos Aires, Argentina.
[2] Departamento de Fisiología, Biología Molecular y Celular (DFBMC), Facultad de Ciencias Exactas y Naturales (FCEN), UBA, Buenos Aires, Argentina.
[3] Instituto de Fisiología, Biología Molecular y Neurociencias (IFIBYNE), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET)-UBA, Buenos Aires, Argentina.

Protein kinase AKT is associated with various processes, including

cell metabolism, proliferation, and survival, as well as pathological conditions like cancer. AKT is regulated by numerous posttranslational modifications (PTMs) and recruited to diverse subcellular compartments. However, the mechanisms through which a cell determines the substrates and functions that AKT should regulate remain largely unknown. We hypothesize that the profile of AKT PTMs determines its subcellular localization, and vice versa, thus establishing the subset of AKT substrates and defining the functions displayed by AKT in response to diverse stimuli and cellular contexts. This work aims to develop an atlas of AKT subcellular localizations as a predictive tool of AKT physiological and pathological functions using *in silico* and experimental approaches. Using a combination of bioinformatic resources, we performed a spatial analysis of the AKT interactome network, linking subcellular localizations with biological processes and diseases. This analysis revealed an important subset of AKT substrates and interactors (S&I) linked to nuclear speckles (NS), mRNA splicing, and congenital malformations diseases. However, to date, no reports show AKT recruitment to NS. Here, we demonstrate through fluorescence microscopy (FM) experiments that AKT and phosphorylated AKT substrates colocalize with NS. The *in silico* analysis also showed that a subset of S&I linked to the endomembrane system was associated with cell death, proliferation, response to stress, autophagy, and cancer. Quantitative analysis of FM images showed a tight coregulation of AKT recruitment to Golgi, endoplasmic reticulum, and lysosome membranes, in response to stressing signals like serum starvation and oxidative stress, aligned with predicted processes like autophagy and cell death. This study sheds light on the complexity of the AKT signaling pathway, enabling us to explain and even predict AKT's subcellular and functional code.

575. 619. **MAGEA9 ONCOPROTEIN AND THE DNA DAMAGE REPAIR AND WNT/ B-CATENIN PATHWAYS**

Suberbordes Melisa [1], Micaela Escalada[1], Candela Vidal[1], Franco Pascucci[1], Martin Monte[1]
1.Laboratorio de Oncología Molecular, Dpto. Química Biológica, FCEN-UBA e Instituto IQUIBICEN, UBA-CONICET.

MageA9 is a tumor-expressed protein. Like most MAGE type I proteins (Melanoma Antigen Genes) its gene expression is silenced in normal somatic tissues, abnormally re-expressed in tumors and associated with poor prognosis. However, little is known about MageA9 protein and its mechanisms of action. Particularly, MageA9 expression correlates with enhanced cell proliferation, resistance to radiation and metastasis occurance. To better understand the mechanisms of action of MageA9, we search for potential protein interactions by exploring BioPlex3.0, a curated proteomic database in both HEK293T and HCT116 cell lines. Data analysis of those MageA9 interactors detected in both cell lines indicated that it could be associated with DNA repair and damage response, Wnt/β-Catenin signaling, and the epithelial-mesenchymal transition(EMT) processes. Concerning the DNA damage response, we recently found that MageA9 inhibits the transcriptional activity of p53. To look for p53-independent mechanisms associated with the DNA damage response, we start studying the potential interaction between MageA9 and SMC1, a key protein of this process. By performing a co-immunoprecipitation assay we could corroborate the interaction. Then, to address whether MageA9 expression could impact on the β-Catenin pathway, we assessed the transcriptional activity of β-Catenin by means of reporter gene assay (TOP-Flash-Luc). Increased luciferase activity was observed in cells expressing MageA9 and the β-Catenin reporter gene. Furthermore, we also observed upregulation of endogenous β-Catenin target genes such as cMyc and N-Cadherin by RT-qPCR, in cells overexpressing MageA9. In addition, enhanced N-Cadherin levels could be associated with the EMT. This first approach to understanding the molecular function of MageA9 suggests further investigation on the regulation of DNA repair and damage response, Wnt/β-Catenin signaling, and the EMT processes as part of MageA9 association to poor prognosis.

P1-TOXICOLOGY

WEDNESDAY 15TH NOVEMBER 9:00 - 10:30
CHAIRS: CLAUDIA COCCA
SILVINA GUTIÉRREZ
NATACHA PILONI

576. 17. **SEARCHING FOR POTENTIAL TARGETS OF BENZOPHENONE-3 ACTION INVOLVED IN THE MAMMARY GLAND ALVEOLAR GROW AND FUNCTION**

Gonzalo Schierano-Marotti[1,2], Gabriela Anahí Altamirano[1,2], Ayelen Luciana Gomez[1,2], Sofia Oddi[1], Mónica Muñoz-de-Toro[1,2], Laura Kass[1,2].
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The UV-filter benzophenone-3 (BP3), commonly used in sunscreens, has been shown to alter the mammary gland development. Previously, we have shown that intrauterine exposure to BP3 alters the histoarquitecture of the lactating murine mammary gland by modifying the alveolar area, alveolar size ratio and the myoepithelial linear density, without changing the expression of milk proteins. Potential targets of BP3 action involved in alveolar growth and function could be signal transducer and activator of transcription 5 (STAT5a), Ets transcription factor 5 (ELF5) and glucocorticoid receptor (GR). Therefore, our aim was to evaluate the expression of these molecules in the lactating mammary gland of the F1 female offspring after the intrauterine exposure to BP3. Pregnant F0 C57BL/6 mice were dermally exposed to vehicle (sesame oil; control) or 50mg BP3/kg/day (50BP3) from gestation day 8.5 to 18.5. At 8 weeks old, F1 female offspring were bred, and mammary gland samples were obtained on lactation day 10. The mRNA expression of STAT5a, ELF5 and GR was evaluated by real-time RT-PCR. The 50BP3 group showed similar expression of STAT5a, ELF5 and GR to the control group ($p > 0.05$). In conclusion, STAT5a, ELF5 and GR do not seem to be involved in the alveolar modifications induced by intrauterine exposure to BP3 in the lactating murine mammary gland.

577. 81. **PESTICIDE IMIDACLOPRID ENHANCES MAMMARY EPITHELIAL CELL MOTILITY AND ALTERS MAMMARY BRANCHING MORPHOGENESIS**

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The neonicotinoid imidacloprid (IMI) is a widely used pesticide which binds to the nicotinic acetylcholine receptor (nAChR) and its endocrine disruptor actions is being studied. As mammary gland development is regulated by hormones, we hypothesize that IMI exposure exerts alterations in the breast that favor tumorigenesis. We have reported that IMI boosts NMuMG mammary epithelial cell migration, metalloproteases (MMP)2 and 9 activity, and G protein-coupled estrogen receptor (GPER) expression. Herein, we aimed to delve into the IMI mechanism of action to promote NMuMG cell motility and to investigate whether it induces alterations in the mammary gland. NMuMG cells were treated with IMI (0.01-10 μM) for 24 h and the α7-nAChR protein levels were examined, finding an increment at 10 μM (190% $p<0.001$, western blot, WB). The c-Src activation, a kinase downstream of GPER and α7-nAChR, was enhanced at 10 μM IMI after 1, 2 and 4 h (150%, 80% and 60% respectively, $p<0.01$, WB). Next, we tested the role of GPER on NMuMG cell migration (wound healing assay) and MMP2 and 9 activity (gel zymography) using the GPER specific inhibitor G15 (1 μM). Results showed that IMI (10 μM) stimulates both processes in a GPER-dependent manner ($p<0.001$). Finally, female pre-pubertal BALB-c mice were

exposed to IMI (0.01, 0.1 and 10 mg/kg/day) orally for 4 weeks. The whole mammary gland was mounted and tissue sections were stained with hematoxylin-eosin for morphological and histological studies. IMI (10 mg/kg/day) enhanced ductal hyperplasia (140% p<0.01) and the number of terminal end buds (TEBs, 140% p<0.05). IMI (0.1 mg/kg/day)-treatment induced ductal growth (46% p<0.05) but reduced branch density (33% p<0.05). In conclusion, IMI increases mammary epithelial cell motility trough GPER and alters mammary branching morphogenesis, likely leading to preneoplastic lesions and retaining TEBs. Our results support the hypothesis that IMI represent a risk factor for breast cancer.

578. 100. **ENDOMETRIOSIS ASSOCIATED ANGIOGENESIS INDUCED BY ENDOCRINE DISRUPTORS**

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Endometriosis is a common gynecological disease suffered for reproductive-age women, which is defined by the growth of endometrial tissues outside the uterine cavity. It often causes chronic pelvic pain, and infertility. Angiogenesis is critical in endometriosis progression, is a complex process involving endothelial cell migration, proliferation and tube formation. Vascular endothelial growth factor (VEGF) is a powerful angiogenic factor. Endocrine-disrupting environmental pollutants are thought to play a role in the development of this disease. Hexachlorobenzene (HCB) is an organochlorine pesticide that increases microvessel density and VEGF levels in a rat endometriosis model. Chlorpyrifos (CPF) is an organophosphate insecticide that acts as an Endocrine Disruptor. Both are weak Aryl Hydrocarbon Receptor (AhR) ligands. The present study examined the effect of HCB and CPF on endometriosis angiogenesis *in vitro*. T-HESCs cells (endometrial stromal cell line) were exposed to HCB (0.005, 0.05, 0.5 and 5 μM), CPF (0.05, 0.5, 5 and 50 μM) or vehicle (ETOH) for 48 h, and the conditioned media (CM) were then used to expose EA.hy926 endothelial cells. The results showed that HCB and CPF induced VEGF secretion in T-HESCs. Moreover, the CM of HCB treatment enhanced the endothelial cell proliferation (PCNA expression and MTT assay), migration (scratch motility assay) and tube formation (tube-like structure formation in Matrigel assay); increasing total tube length (0.005 μM; 0.5 μM) and branching points (0.5 μM). The assays with CPF CM showed an increase in endothelial cell proliferation, migration, and tube formation, enhancing total tube length (5 μM). In addition, we evidenced that the enhancement in angiogenesis induced by HCB and CPF exposure was mediated by AhR and VEGF-R2 signaling pathways. Our results demonstrated that HCB and CPF exposure induces VEGF secretion in human endometrial cells triggering angiogenesis, a critical event for the endometriosis progression.

579. 134. **ACTIVITY OF HUMAN PLACENTAL PARAOXONASE (PON) THROUGH THE USE OF DIFFERENT SUBSTRATES**

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It has been reported that women living close to agricultural areas at the Alto Valle of Río Negro and Neuquén region are at risk of organophosphate pesticides (OP) exposure. Paraoxonases (PONs) are enzymes linked to OP detoxification and oxidative stress defense. Three isoforms are known: PON1, PON2 and PON3 with dissimilar activities towards bioactivated OP, which also exhibit arylesterase and thiolactonase activity. The objective was to evaluate the PON expression and activity in human placenta. Expression was evaluated by qPCR and western blot. The hydrolase activity of PON was evaluated using different substrates, by 4-nitrophenyl acetate, and phenyl acetate the arylesterase activity and with dihydrocumarin the lactonase activity evaluate. Substrate-activity curves were performed, and concentrations selected were 2.5 mM (4-nitrophenyl acetate), 1 mM (phenyl acetate) and 4 mM (dihydrocumarin). Since PON activity is calcium dependent different concentrations of EDTA were used to evaluate the inhibition of the enzymatic activity. Six (6) placentas were analysed. The placenta showed the expression of PON2 and PON3 transcripts. PON2 protein expression was confirmed by Western blot. The placenta demonstrated arylesterase and lactonase activities. For arylesterase the results obtained indicate a mean activity (mean ± SD, umoles/min/ug prot) of 31.93 ± 12.48 for 4-nitrophenyl acetate and 33.93 ± 16.02 for phenyl acetate. The mean lactonase activity was 45.34 ± 17.61 umoles/min/ug prot for dihydrocumarin. The results obtained clearly indicate the presence of active PON isoforms in the placenta, with both arylesterase and lactonase activities. Since the PON family participates in the protection against oxidative damage and lipid peroxidation, the modulation of stress, the detoxification of reactive molecules, the implication of PON regulation by chemical contaminants in the placenta is worthy to be studied.

580. 136. **ANGIOGENESIS IN BREAST CANCER: PESTICIDES ACTIVATE ENDOTHELIAL CELLS VIA VEGF RECEPTOR-2 SIGNALING**

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Breast cancer is one of the leading cancers among women worldwide. Given the evidence that pesticides play an important role in breast cancer, interest has grown in pesticide impact on disease progression. Organochlorine hexachlorobenzene (HCB) and organophosphate chlorpyrifos (CPF) are pesticides agonists of aryl hydrocarbon receptor (AhR), a transcription factor involved in vascular development and tumor progression. Previously, we have reported that HCB and CPF exposure induced vascular endothelial growth factor (VEGF) secretion through AhR signaling in MDA-MB-231 breast cancer cells. VEGF binds to the VEGF–receptor 2 (VEGFR2) increasing endothelial proliferation, migration, and tubulogenesis. Our aim was to examine whether the conditioned media (CM) of MDA-MB- 231 tumor cells exposed to HCB or CPF are capable of activating EA.hy926 endothelial cells, promoting a) proliferation (PCNA levels by Western Blot), b) migration (wound healing assay), and c) tubulogenesis (tube-like structure formation in Matrigel assay). Besides, we analyzed whether the effects are dependent on VEGFR2, using the ABYO inhibitor. Endothelial cells were exposed to the CM of MDA-MB-231 cells treated to ethanol, HCB (0.05 μM) or CPF (0.05 μM), in the presence or absence of ABYO. Results showed that CM from tumor cells exposed to HCB or CPF increased PCNA expression levels (55% and 87%, respectively; p<0.05), indicating greater proliferation. Moreover, endothelial cells showed a significant increase in migration in the presence of CM of HCB or CPF (32% and 28%, respectively; p<0.01; p<0.05). Finally, both CM of HCB and CPF promoted the formation of tubules, increasing the total tube length (60%, p<0.01) and branching points (44%, p<0.05). The CM effects on the biological functions analyzed depended on the VEGFR2 pathway. In conclusion, the exposure to HCB and CPF pesticides stimulates angiogenesis processes, promoting breast cancer progression.

581. 144. **IN VITRO EFFECTS OF THE PYRETHROID PESTICIDE DELTAMETHRIN ON THE APOPTOSIS AND VIABIL-**

ITY OF HUMAN THYROCYTES

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Humans are exposed to pesticides present in their environment. Among these contaminants, the pyrethroid deltamethrin (DM) was detected in vegetables. In-vitro studies have suggested that deltamethrin induces oxidative damage mediated by reactive oxygen species formation. Particularly, the thyroid is a gland sensitive to the disruptive effects of pesticides. However, it is unknown what direct effects and mechanisms of action are exerted by DM in this gland. Thus, we aimed to study the effects of DM on thyrocyte apoptosis and viability. To achieve our goal we performed a series of experiments using the normal human thyrocyte cell line Nthy-ori 3-1. First, cells were incubated for 6 h with 0, 1, 0.1, and 0.01 μM deltamethrin in the presence or absence of 500 μM H_2O_2, a proapoptotic stimulus. Apoptosis was assessed by Caspase-3 activity (EnzChek Caspase-3 Assay Kit #2, Molecular Probes). DM showed no apoptotic effects, while the concomitant incubation with H_2O_2 proved no synergic or protective effects of this pyrethroid ($p<0.01$, ANOVA). Viability (MTS) was tested after cells were incubated for 6 and 24 h with DM and 0.01 nM estradiol (E2), a major proliferative factor in the thyroid. Both time points showed an expected increase in viability by E2 ($p<0.05$, ANOVA) and no cytotoxic actions of the pyrethroid *per se*. After 6 h, viability in cells incubated with both E2 and 0.1 or 0.01 μM DM augmented compared to their respective groups without estrogen ($p<0.05$, ANOVA). On the contrary, after 24 h, 1 and 0.1 μM DM prevented the increase in cell viability induced by E2 ($p<0.05$, ANOVA). The 0.01 μM concentration of DM was not able to reduce E2-increased viability at this time point ($p<0.05$, ANOVA). Our results suggest that DM is not a proapoptotic or cytotoxic agent in Nthy-ori 3-1 cells. In addition, it inhibits proliferation induced by E2 in a concentration and time-dependent manner.

582. 200. **METALLOESTROGENS CADMIUM (Cd) AND ARSENIC (As) AFFECTS PROLIFERATION AND MITOCHONDRIAL ACTIVITY OF HUMAN CERVICAL CARCINOMA CELLS THROUGH ESTROGEN RECEPTOR BETA (ERβ)**

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Endocrine disruptors (EDC) are known as a heterogeneous group of chemical compounds, whether natural or synthetic, capable of interfering with the endocrine system's homeostasis. Once inside the organism, EDCs mimic the effects of natural hormones by stimulating or inhibiting crucial cellular metabolic processes with severe consequences for health. Cd is considered a type I carcinogen while As is a highly prevalent EDC in Argentine soils and groundwater. The characterization of an EDC is focused on its ability to stimulate or inhibit mechanisms mediated by the estrogen receptor alpha (ERα) and androgen receptor. ERα-mediated estrogenic effects of Cd and As has been well characterized. ERβ has been largely considered to counteract ERα-driven effects although its expression is retained in several hormone-responsive tumors. However, the effects of Cd and As on ERβ has not been addressed yet. The aim of this study is to explore whether these metals or a mixture of them could trigger an estrogenic signal through ERβ. A human cervical carcinoma model (HeLa cells) was used since it retains functional ERβ and G-protein-coupled ER (GPR30) but lacks canonical ERα expression. Experiments were conducted after 72 h of exposure to 10 nM Cd, 10 nM As, or a mixture of both, with or without the natural ERs ligand (17β-estradiol, E2), an ER inhibitor (ICI 182,780), an ERβ agonist (DPN), and an ERβ antagonist (PHTPP). Results showed that Cd and As increased mitochondrial activity (MTT assay, $p<0.05$), G2/M cell cycle phase (flow cytometry, $p<0.05$), and cyclin A levels (western blot, $p<0.05$) and reduced G0/G1 phase ($p<0.05$), p-Rb/Rb ratio ($p<0.05$), and cyclins D1 and E ($p<0.05$) in a ERβ-dependent manner. These results suggest that both Cd and As can display xenoestrogenic actions through ERβ, and therefore, the effects mediated by this receptor should be studied in depth.

583. 294. **NEONICOTINOIDS EFFECTS ON DNA METHYLATION AND SIRTUIN EXPRESSION IN HUMAN PLACENTA EXPLANTS**

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Argentina's apple and pear production is centered in the Alto Valle of Rio Negro and Neuquén. This production relies on the intensive application of pesticides, such as Neonicotinoids (NEO). Pregnancy is proposed as the first exposure scenario in human life, as environmental chemicals can access the placenta and affect its function. Recently, the exposure to pesticides has been associated with epigenetic alterations, which can affect the expression of various genes. The study aimed to explore epigenetic alterations caused by NEO exposure in human placenta explant cultures, focusing on the expression of DNA methylation enzymes and sirtuins (Sirts), histone deacetylase proteins. Explants were obtained from normal-term pregnancies and exposed to the NEO Acetamiprid (Ace) and a commercial formulation (AceCF) at concentrations ranging from 0.1 to 10 μM for 24 h. To ensure the explants' functionality and viability, MTT (Thiazolyl Blue Tetrazolium Bromide), LDH (Lactate Dehydrogenase) and hCG (chorionic gonadotropin) assays were conducted. Ten-Eleven-Translocation enzymes TET2, TET3 and DNA methyltransferase DNMT3A, DNMT3B and Sirts 1, 2, 4, and 5 expressions were studied by qPCR. After 24h of NEO treatment, explants remained viable, as indicated by no significant changes in MTT, LDH, and hCG tests (One-way ANOVA $p>0.05$), suggesting the suitability of the explants for evaluating molecular NEO toxicity. The expression of DNA methylation enzymes TET2, TET3, and DNMT3A remained unchanged, while DNMT3B levels were increased in Ace treatments starting from 1 μM concentration and in AceCF starting from 0.1μM. Sirt 1, 4, and 5 showed a 60% decrease with Ace, and AceCF exhibited a 50% reduction at the lowest concentration before returning to baseline. Sirt 2 expression was undetectable for both pesticides. In summary, this preliminary study presents the first evidence of epigenetic changes associated with NEO exposure, suggesting potential disruption of several cellular processes.

584. 415. **IMPACT OF HERBICIDES ON HUMAN CELLS: STUDY OF THE MOLECULAR MECHANISMS IMPLIED ON THEIR TOXICITY**

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The massive use of herbicides in various parts of the world has established a discussion on their consequences on human health, with a large amount of research analyzing their toxicity. However, the molecular mechanisms through which these agents act are not completely described yet. In our work, we evaluated the effects of three commonly used herbicide formulations -RoundUp® (RU, active ingredient or a.i.: glyphosate), GesaPrim® (GP, a.i.: atrazine)

and Paraquat Insuagro® (PI)- on the regulation of the Unfolded Protein Response (UPR) pathway in human cells. This pathway has key regulatory roles that can determine the fate of a cell, controlling many biological functions, such as cell survival, proliferation, cell migration, and apoptosis. The deregulation of this pathway has been reported in many human diseases, including several cancers. For all exposure experiments, we evaluated herbicide concentrations far below agricultural recommendations: 3.5 µg/mL for RU, 4.7 µg/mL for GP, and 100 µM for PI. The HeLa Kyoto cell line was used as a model, evaluating three different exposure times: 15 minutes, 2 hours, and 24 hours. First, we analyzed if there were any significant differences in the levels of several UPR proteins such as ATF4, ATF6, and XBP, by means of a t-Student test. Second, we employed fluorescent reporters to evaluate their activation in single cells and in real time. Our results revealed an activation of the PERK branch of the UPR through the expression of ATF4, since we found significant differences in its levels for the highest exposure time in all three of the formulations. Moreover, we also observed the activation of the IRE1 branch by detecting increasing XBP1s (active form) levels for all herbicides on 24hs exposure time. In conclusion, activation of both branches revealed that all formulations induced a cellular stress response at long exposure times, triggering the UPR.

585. 590. **A REPRESENTATIVE RAT MODEL OF HUMAN GLYPHOSATE EXPOSURE AND ITS CONSEQUENCES ON BODY WEIGHT AND REPRODUCTIVE HEALTH**

María Emilia Racca[1,2], María Paula Gastiazoro[1,3], Julieta Cepeda[1], Milena Durando[1,3], María Mercedes Milesi[1,3], Jorgelina Varayoud[1,3]

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When evaluating the toxicity of glyphosate-based herbicides (GBHs), variations in the window and length of exposure and administration routes may influence the effects that are observed. In this sense, prior investigations have demonstrated the impact of glyphosate (Gly) and GBHs on health when exposed during critical developmental periods using different experimental approaches. Nonetheless an evaluation of long-term oral exposure that better mimics the human condition has yet to be conducted. This study aims to investigate whether long-term oral exposure to a safe dose of a GBH alters body weight and reproductive outcomes. Female Wistar rats were fed a control (CON) and GBH supplemented chow-based paste (GBH, 2 mg of Gly/kg bw/day) diets from weaning until adulthood (postnatal day (PND)100). Food and tap water were administered *ad libitum*. Data of body weight and food intake was registered. On PND100, a group was sacrificed and uteri were removed for morphometric analysis. The remaining animals were mated and euthanized on gestational day 19. To assess reproductive outcomes, the number of corpora lutea, implantation and resorption sites were determined. Feto-placental parameters were evaluated using data of fetal and placental weight and placental index. Long-term exposure to GBH increased body weight, with no changes in food intake. Endometrial hyperplasia was detected in 100-day-old females exposed to GBH, evidenced by an increased density of uterine glands and stromal nuclei. GBH females had an increase in the rate of pre-implantation embryo losses and a decrease in fetal weight and length. These results suggest long-term oral exposure to GBH induces uterine hyperplasia and affects rat weight, fertility and fetal development. We emphasize the need to deepen the mechanisms related to these findings, and to include long-term and oral exposure models for assessing the toxicity of pesticides for being more representative of human exposure.

586. 618. **EARLY SUBFERTILITY AND OOCYTE DEPLETION IN FEMALE OFFSPRING BORN TO DAMS PERINATALLY EXPOSED TO BENZOPHENONE-3**

Valentina Galliani [1], Julián Abud[1,2], Clarisa Santamaria[1,2], María Laura Zenclussen[1,2], Horacio Adolfo Rodríguez[1,2]

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Benzophenone-3 (BP3) is a UV filter widely used in cosmetics, to which we are significantly exposed. Previously, we have shown that female offspring born to dams dermally exposed to 50 mg of BP3/kg bw.day or olive oil (control) from gestational day 9 to postnatal day 21, showed an early decline of fertility in a Fertility Assessment by Continuous Breeding" (FABC). Based on these results, our current objective was to examine whether the reduced fertility is associated to changes in germ cells population of the offspring perinatally exposed to BP3. To achieve this, ovaries and testes were studied before and after FACB. Sperm analyses were carried out on male offspring, whereas counting and diameter measurements of primordial follicles, counting of antral and total follicles, along with estradiol, testosterone, and progesterone serum levels were performed in female offspring. Our results showed no alterations in the number of motile spermatozoa (motile/total) at the beginning of the FACB. However, we did observe a lower percentage of primordial follicles at the start and the end of the FACB, with the antral follicle population unchanged. The number of total oocytes remained unaltered at the beginning and the end of FACB. The diameters of primordial oocytes were smaller than controls both before and after FACB, suggesting a potential disruption in their activation process. Finally, decreased estradiol and elevated progesterone serum levels at the end of FACB were observed, with no variation in testosterone. We conclude that female offspring born to dams perinatally exposed to BP3 developed a severe reduction of their stockpile of primordial follicles. When subjected to the FACB protocol, these females became subfertile earlier than the control animals. Altogether, these results point to the BP3-induced reduction of the stock of primordial follicles as an underlying cause of early subfertility.

O-TOXICOLOGY

THURSDAY 16TH NOVEMBER 9:00 - 10:30

CHAIRS: MARISA REPETTO
GABRIELA SALVADOR

587. 16. **GLYPHOSATE MODIFIED THE EXPRESSION OF ANGIOGENIC GENES IN A TROPHOBLASTIC CELL LINE WITHOUT ALTERING THE ANGIOGENIC PROCESS**

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Glyphosate (G), the active component of herbicides, has been detected in biological samples from pregnant women and umbilical cord samples. Adverse pregnancy outcomes are associated with G exposure, including shortened gestational length in humans and vascular alterations at embryo implantation sites in rats. Considering that the trophectoderm defines successful decidualization through migration, invasion, and spiral arteries remodeling, we aimed to analyze the effects of G on angiogenesis using a trophoblastic cell line. HTR-8/SVneo cells were exposed to 0.625, 1.25, and 2.5 μM G. The mRNA expression of genes reported to be involved in cellular migration and angiogenesis, such as IL11, vascular endothelial growth factor (VEGF), C-C motif chemokine ligand 2 (CCL2), and coagulation factor II thrombin receptor (F2R), was assessed after 6 and 12 h of treatment with G by qRT-PCR. Tube–formation assay was performed to study the angiogenic response to G. At 6 h, an overexpression of CCL2 was observed at 1.25 μM G compared to the vehicle (P=0.024) and to 0.625 μM G (P=0.045). In addition, higher

VEGF levels were observed at 1.25 μM G compared to 2.5 μM G (P=0.025), and the same trend was seen when compared to the vehicle (P=0.053). However, at 12 h, exposure to G had no effect on the mRNAs evaluated. In the tube-formation assay, no differences in the number of branches, tube total length, and branching points were observed between G and the vehicle. *In vitro*, G did not appear to alter the angiogenesis process; however, an acute effect on the expression of several angiogenic genes was observed. Therefore, G exposure could contribute as a risk factor for developing pregnancy complications, mainly in case of pre-existing diseases.

588. 153. **ALTERED UTERINE MECHANISTIC PATHWAYS IN RATS ASSOCIATED WITH IMPLANTATION FAILURE AFTER PERINATAL EXPOSURE TO GLYPHOSATE OR A COMMERCIAL FORMULATION**

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Embryo implantation requires close communication between the blastocyst and a receptive uterus. The uterus is a target organ for sex steroid hormones and is consequently vulnerable to chemicals with endocrine-disrupting properties, such as glyphosate herbicide. In female rats, we showed that perinatal exposure to a glyphosate-based herbicide (GBH) or its active ingredient, glyphosate (Gly), causes subfertility by increasing the rate of preimplantation embryo losses. This study aims to explore the mechanisms of action of GBH and Gly, analyzing key endocrine pathways for endometrial receptivity. Pregnant Wistar rats (F0) were treated orally with GBH or Gly (2 mg of Gly/kg/day) from gestational day (GD) 9 until weaning. Sexually mature F1 females became pregnant and uterine samples were collected on GD5 (preimplantation period). Hematoxylin-eosin uterine sections were assessed for morphological parameters: luminal epithelial height, glandular density, and subepithelial stroma (SS) and myometrial thickness. Moreover, protein expression levels of Ki-67, a proliferation marker, the cell cycle regulators PTEN, cyclin G1, p27, and IGF1Rα, and estrogen receptors (ERα and ERβ) involved in controlling proliferation, were evaluated by immunohistochemistry. Glandular differentiation markers, such as FOXA2, β-catenin, and Wnt5a, were also assessed. GBH and Gly reduced uterine glandular density, associated with decreased expression of FOXA2, Wnt5a, and β-catenin in glands. Both GBH and Gly groups showed increased proliferation in the SS. In this compartment, GBH rats showed an increase in ERα and ERβ expression, while Gly rats exhibited an increase in PTEN and cyclin G1 expression. In conclusion, perinatal exposure to GBH and Gly disrupts SS proliferation and glandular differentiation, which are crucial processes for proper endometrial receptivity. These alterations might explain the implantation failures observed in GBH- and Gly-exposed rats.

589. 218. **ASSESMENT OF EPIGENETIC MODIFICATIONS AS A MECHANISM OF ACTION OF GLYPHOSATE**

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Previously, we detected that perinatal exposure to a glyphosate-based herbicide (GBH) or glyphosate (Gly), its active ingredient, induces implantation failure in rats. Hence, we investigated whether epigenetic alterations in the rat uterus during the receptive stage could be a possible mechanism of action of the herbicide implicated in the implantation failures. F0 dams were exposed to a GBH or Gly through food in a dose of 2 mg Gly/kg bw/day, from gestational day (GD) 9 until lactational day 21. F1 adult female rats were pregnant and uterine tissues were analyzed on GD5 (preimplantation period). The transcripts levels of *Hoxa10 gene,* a key molecule for endometrial receptivity, and major epigenetic enzymes were assessed by RT-qPCR. To analyze the methylation status of *Hoxa10*, enzyme-specific restriction sites were searched *in silico* in the regulatory regions of this gene and assessed using the methylation-sensitive restriction enzyme-PCR technique. To determine changes in histone post-translational modifications, histone 3 and 4 acetylation (H3Ac and H4Ac), and histone 3 methylation (H3K27me3) along the different regulatory regions of *Hoxa10* were evaluated by chromatin immunoprecipitation assays. GBH and Gly reduced *Hoxa10* mRNA expression in association with increased DNA methylation. Moreover, GBH and Gly exposure increased histone H3 and H4 acetylation and enriched H3K27me3 marker at three out of four sites analyzed. GBH and Gly increased mRNA transcripts of Enhancer of Zeste homolog 2 (EZH2) enzyme which specifically methylates H3K27. In conclusion, both GBH and Gly induce epigenetic changes that might explain the down-regulation of *Hoxa10* gene. We propose that Gly and GBH could act through similar disruption pathways, as comparable results were detected.

590. 333. **CHLORPYRIFOS IN HUMAN PLACENTA: EFFECTS IN CES AND PON ACTIVITY AND EXPRESSION**

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The organophosphates (OPs) are the most widely used pesticides worldwide. In Argentina, the OP chlorpyrifos (CP) was widely utilized until 11/2022. This study aimed to determine the CP level, and key enzymes for OP detoxification, carboxylesterase (CES) and paraoxonase (PON), in the placenta of women residing in the North Patagonia. The study included 104 healthy pregnant women from Neuquén city (CG, n=47) and rural areas (RG, n=57). CP levels were determined by GC-ECD and confirmed by GC-MS. CES activity was determined using α-naphthyl acetate (α-NA) and 4-methylumbelliferyl acetate (4-MUBA) substrates. PON activity was measured using phenylacetate (AE) and dihydrocoumarin (LA) substrates. Transcript and protein levels were assessed by western blot and qPCR. Quantitative and categorical variables underwent Multiple Factor Analysis. CP mean levels (ng/g lipid) in RG (195.9 ± 290.6) were 55% higher than UG (86.3 ± 156.7) (p=0.007, Mann-Whitney), as well as the CP detection frequency (RG: 73% vs UG: 49%). CES activity (α-NA) showed lower levels in RG vs UG (p=0.016, M.W.) while CES (4-MUBA) showed non significant changes (p=0.914, M.W.). Both CES1 and CES2 transcripts were significantly upregulated in RG vs. UG (p=0.010, M.W.), as well as CES 2 protein expression. PON AE and LA activities were higher in RG vs UG (p=0.048, p=0.030, M.W.). PON2 transcript and protein expression levels were increased in RG vs UG (p=0.008, p=0.030 M.W.). Altogether, results indicate that pregnant women are exposed to CP. Low CES activity (α-NA), and the presence of CP was significantly associated with rural areas, making it a valuable indicator for exposure assessment. A compensatory response leading to increased CES1, CES2, PON2 expression and, PON AE and LA activity was also observed in RG. However, augmented CES expressions might not sufficiently counteract the inhibition of CES activity (α-NA). PON induction might be dampening other toxicity mechanisms as oxidative stress.

591. 336. **GLYPHOSATE CONCENTRATION DETECTED IN HUMAN BREAST MILK ALTERS MILK PROTEINS LEVEL IN A MOUSE MAMMARY CELL LINE**

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Previously, we demonstrated that milk protein synthesis is affected by environmental estrogens using a 3D in vitro model. In recent years, the broad-spectrum herbicide glyphosate (GLY) has been classified as a potential estrogenic and androgenic compound. Here, our aim was to analyze whether direct exposure to glyphosate-based herbicide (GBH) and its active ingredient GLY affects milk proteins synthesis during mammary differentiation using the murine cell line HC11. Cells were grown in medium supplemented with epidermal growth factor, insulin and fetal bovine serum (FBS) until confluent. Cells were then cultured for 72 h with medium supplemented with 2% charcoal-stripped FBS, after which they were differentiated with lactogenic hormones (prolactin, insulin and dexamethasone) and exposed for 72 h to: a) Vehicle (phenol red-free medium), b) 0.01 and 0.5 µM GBH and c) 0.01 and 0.5 µM GLY. The concentrations of GBH and GLY were selected considering the detected values of GLY both in human milk and in serum and urine of pregnant women. After 72 h of differentiation and exposure to GBH and GLY, cell viability assay was carried out using the WST-1 kit. Also, the mRNA levels of milk proteins: beta-casein (CSN2) and whey acidic protein (WAP), considered markers of functional differentiation, were analyzed by real-time RT-PCR. No significant differences in cell viability were found between the treatments. On the other hand, the mRNA level of CSN2 was increased in HC11 cells treated with 0.01 μM GBH and GLY ($p<0.05$). In addition, the WAP mRNA level was only increased in cells treated with 0.01 μM GBH. These findings show that direct exposure to a concentration similar to that found in breast milk (0.01 µM) alters milk protein synthesis. In conclusion, exposure to the herbicide could interfere with the mammary functional differentiation and, therefore, with the growth and health of the offspring.

592. 344. **EFFECTS OF EXPOSURE TO BENZOPHENONE-3 ON EARLY MOLECULAR MARKERS OF MAMMARY INVOLUTION IN HC11 CELLS**

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Mammary involution is a period of intense tissue remodeling, and its alteration may result in inadequate lactation or predispose to tumor development. In this sense, the UV-filter benzophenone-3 (BP3) has been shown to have estrogenic activity and to alter apoptosis, which could affect this process. Here, we investigated whether exposure to BP3 modifies the expression of early molecular markers of involution in a murine mammary cell line. HC11 cells were grown to confluence and exposed to vehicle (0.1% ethanol) and 1 or 1000 nM of BP3 for 72 h. The cells were then differentiated with lactogenic hormones for another 72 h, after which the hormones were withdrawn. Cell viability was assessed immediately after BP3 exposure. Beta-casein (CSN2), Stat3, Bcl2 and Bax mRNA expression was analyzed at 0, 24, 48 and 72 h, whereas DNA methylation of Bcl2 and Bax promoters was assessed by bisulfite pyrosequencing at 24 h after removal of the lactogenic hormones. Cell viability and Stat3 mRNA expression were not affected by BP3 exposure. The exposure to BP3 1 nM increased the mRNA expression of CSN2 (11.9-fold), Bcl2 (5.3-fold) and Bax (1.5-fold) at 24 h, and reduced CSN2 expression (9.8-fold) at 72 h compared to vehicle. Bcl2 promoter showed reduced DNA methylation at 24 h in cells exposed to BP3 1 nM compared to vehicle, and no differences were observed in the Bax promoter. Our results showed that BP3 exposure could interfere with the normal involution of the mammary gland and modify the expression of key molecular markers of this process.

593. 508. **EVALUATION OF THE POTENTIAL EFFECT OF HERBICIDES ON KEY BIOLOGICAL PROCESSES**

Mercedes García Carrillo[1], Daiana Ailín Ameghino[1], Julien Gigan[2], Rafael Argüello[2] & Matías Blaustein[1,3]
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A growing body of evidence points to herbicides' negative impact on human cells. Herbicides have been shown to interact with particular proteins such as PI3K, AKT, PERK, eIF2α, and ATF4, affecting the regulation of critical biological processes for the maintenance of homeostasis such as protein synthesis and cell signaling, which in turn could trigger various diseases. The aim of this work was to perform a comparative analysis of the potential toxicological effects of three herbicide formulations on human cells: RoundUp (a.i.: glyphosate), Gesaprim (a.i.: atrazine), and Paraquat Insuagro (a.i.: paraquat). First, to identify the proteins that could be affected by herbicide exposure, we built herbicide-protein interaction networks from curated and experimental data sources. We then performed Gene Ontology and KEGG Pathways terms enrichment analysis to identify biological processes and diseases potentially associated with these networks. Results from bioinformatics revealed that herbicide-protein interaction networks were linked to the regulation of key signaling pathways involved in cell proliferation, cell death, stress response, cell survival, and in the regulation of protein synthesis, including the PI3K/Akt pathway and the Unfolded Protein Response (UPR). Besides, an enrichment in cancer-associated categories was detected for all the herbicides. Second, we compared the effect of these herbicides on protein translation in white blood cells by using the SUnSET technique. Consistent with our bioinformatic results showing that these herbicides could interact with pathways regulating proteostasis, such as PI3K/Akt and the UPR, SUnSET experiments revealed that translation was significantly inhibited in the B and dendritic cell populations for all herbicides. Overall, our results reveal that herbicides can deregulate the Akt and UPR pathways, affecting proteostasis in human blood cells and potentially altering crucial physiological and pathological processes.

P2-TOXICOLOGY

THURSDAY 16TH NOVEMBER 14:00-15:30
CHAIRS: NATALIA GUIÑAZÚ
LAURA ÁLVAREZ
ANA MARÍA BUZALEH

594. 213. **COMPARISON BETWEEN A COMMERCIALLY AVAILABLE DIET AND AN ORGANIC PESTICIDE-FREE DIET ON THE CIRCULATING LEVELS OF FREE THYROXINE (T4)**

Denise Echechurre[1], Santiago Jordi Orrillo[1], Mariela Nordera[2], Alejandra Sastre[2], Victoria Lux-Lantos[3], Marina Fernández[3], Rubén Cardozo[1]
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Endocrine disruptors (ED) can be defined as exogenous chemicals that interfere with the physiology of natural blood-borne hormones present in the body. Among the known sources of ED, food is one of the most relevant. An analysis performed by our group showed that commercially available lab rodent food pellets are contaminated with pesticides. These agents could interfere with the normal functioning of endocrine axes. In particular, the thyroid axis is sensitive to the disruptive effects of pesticides. Our aim was to study the effects of the chronic intake of an organic pesticide-free diet and a commercially available diet on T4 serum levels. Adult male and female Wistar rats (4 months age) were fed with organic pesticide-free pellets (PFP) and commercial pellets (CP) (Gepsa Feed) during 30 and 60 days. Animals ate both type of diets equally throughout the experiment (two-way ANOVA: NS). No increase in body weight (BW) was detected among male rats (two-way ANOVA: NS). On the contrary, females fed with PFP during 60 days showed a significant increase in their BW compared to rats fed with CP during 60 days and rats fed with PFP during 30 days (two-way ANOVA, $p<0.05$). Serum free T4 (FT4) was quantified by the electrochemical immunoassay Elecsys FT4 kit (Roche). Circulating FT4 decreased in male rats fed with PFP after 30 days compared to males fed with CP (two-ways ANOVA, $p<0.05$). No significant alterations were detected in serum FT4 levels in female rats (two-way ANOVA: NS). Our results suggest that the consumption of an organic pesticide-free diet induces time and sex-dependent effects on the circulating free T4 levels. Therefore, diet should be carefully chosen during experimental design.

595. 282. **IN-VITRO EFFECTS OF BISPHENOL A, BENZOPHENONE 2 OR BENZOPHENONE 3 ON AUTOPHAGY MARKERS PROTEIN EXPRESSION IN IMMORTALIZED GONADOTROPIN-RELEASING HORMONE (GNRH) NEURONS**

J.M. Riaño Gomez, V.A.R. Lux-Lantos, E.M. Sorianello and M.O. Fernandez

Benzophenones (BP, UV filters) and Bisphenol A (BPA, a monomer of polycarbonate plastics) are endocrine disrupting chemicals (EDC). In this study, we evaluated the effects of the exposure to BPA, BP2 or BP3 ($1x10^{-7}$ or $1x10^{-9}$ M) on autophagy factors protein expression in GnRH-expressing cell lines, GT1-7 or GN11 cells. Cells were plated in 6-well plates, in DMEM high glucose, with 10% FBS and antibiotics. Twenty-four hours later, media were replaced by phenol red-free DMEM, with 10% charcoal-stripped FBS and antibiotics. Cells were exposed to BPA, BP2 or BP3 ($1x10^{-7}$ or $1x10^{-9}$ M), alone or in combination with Chloroquine (CQ, an inhibitor of the degradation of the autophagosome), for 12 or 24 hs. Media were removed and plates frozen -70 C for western blot analysis. Cells were lysed in RIPA buffer with protease inhibitors and protein concentration measured by Bradford. Proteins were separated in polyacrylamide gels and transferred to PVDF membranes. LC3, p62 and tubulin were detected with specific antibodies. Results were expressed as Media±SE and analyzed by Repeated Measures Two-way ANOVA or by ANOVA (Statistica). In GT1-7 cells, CQ increased LC3-II ($p<0.001$) and p62 ($p<0.05$) after 24 h treatment. BP2 $1x10^{-9}$ M decreased LC3-II protein expression relative to control values [LC3-II (AU) C: 1.1±0.1. C-CQ: 1.9±0.3, BP2-7: 1.3±0.2, BP2-7-CQ: 1.8±0.3, BP2-9: 0.6±0.2, BP2-9-CQ: 0.8±0.2; Repeated Measures Two-way ANOVA: Main Effect Treatment with the EDC: BP2-9, BP2-9-CQ different from C, C-CQ, $p<0.05$; Main Effect treatment with CQ: C-CQ different from C, $p<0.001$; n=4]. Exposure to the EDCs for 24 h did not significantly modify p62 protein expression. In GN11 cells, CQ increased LC3-II ($p<0.01$) and p62 ($p<0.05$) after 24 h treatment; in this cell line none of the EDCs had a significant effect. Our results show that exposure to EDCs alter the autophagy process in mature immortalized GnRH neurons. More experiments are underway to further explore the effects observed.

596. 358. **STUDY OF PHASE I AND II ENZYMES IN HTR-8/SVNEO AND BEWO TROPHOBLASTS AFTER EXPOSURE TO PESTICIDES**

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Chemical contaminants can be detoxified by cellular mechanisms, mainly by phase I and phase II enzyme metabolism. Carboxylesterases (CES) are phase I enzymes that detoxify carboxylic esters and 3 isoforms exists, CES1, CES2 and CES3. Glutathione S-transferases (GST) are phase II enzymes that ligates reduced glutathione (GSH) to the toxic compound, to increase solubility and facilitate its excretion. Several isoforms of GST have been reported. Both CES and GST have demonstrated beneficial impacts during pesticide intoxication. The objective of this work was to evaluate phase I and II enzymes in HTR-8/SVneo and BeWo, first and third trimester trophoblasts, after exposure to the organophosphate pesticide chlorpyrifos (CP).HTR-8/SVneo and BeWo cells were incubated for 24 h at different concentrations (0.1-100 uM) of CP 99.9% pure. DMSO 0.04% was the control condition (C). Basal expression of the CES1, CES2, CES3 and GST-P and GST-M isoforms were evaluated by conventional RT-PCR. CES activity was determined by fluorometric technique with 4-MUBA substrate. The GST activity was determined by Habig's method. GSH content was determined by Ellman's method. Preliminary results indicate that BeWo cells mainly expressed CES1 while HTR-8/SVneo mainly CES2 isoform. Both GST-P and GST-M were expressed by BeWo and HTR-8/SVneo cells. CES and GST activities showed non-significant changes, in both cell lines. GSH content was significantly increased in CP 100 μM (44.3±14.2) vs C (17.15±8.9) (mean±SD) ηmol/mg prot ($p=0.0481$, Dunnett) only in HTR-8/SVneo cells. These results suggest that CES isoforms are differentially expressed in first-and third trimester cells. CES 1 and 2 have shown different sensibility to OP inhibition, however incubation with CP did not alter the activity of CES. Similarly, CP did not modify GST activity in both cell lines. The significant increase in GSH suggests the possible induction of other enzymes important for the GSH levels, such as glutathione reductase.

597. 370. **SEX-DEPENDENT HISTOMORPHOLOGICAL CHANGES IN THE THYROID GLAND OF JUVENILE CAIMAN LATIROSTRIS PRENATALLY EXPOSED TO ENDOSULFAN**

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Endolsulfan (END), a banned persistent organochloride pesticide with thyroid-disrupting properties, has been detected in *Caiman latirostris* eggs collected near crop fields in Argentina. Our aim was to identify potential long-lasting effects of in ovo exposure to END on the thyroid gland of *C. latirostris*. Eggs from areas with low human intervention were incubated at male or female producing temperature and exposed to 20 ppm of END or ethanol (VEH). At juvenile stage, thyroid glands were extracted, processed, and paraffin embedded. Microscopic assessment of thyroid sections stained with PAS was employed to quantify the percentage of follicles exhibiting varying degrees of hyperplasia (from 0 to 3), according to morphological criteria established by Galoppo et al. (2020). Our results, presented as mean ± SEM, demonstrate that in males, END exposure increased the percentage of follicles exhibiting grade 3 hyperplasia (8,44±4,76 *vs* 61.30±4,60; $p=0.0006$). This hyperplasia correlates with higher percentages of follicles displaying over three epithelial layers (8,19±3,80 *vs* 35,40±6,17; $p=0.0037$), infoldings (10,90±6,40 *vs* 32,27±7,21; $p=0,0047$) and branching (2,99±1,34 *vs* 47,94±5,11; $p=0.0003$). These features are indicative of follicle growth, rather than follicle formation. No significant differences were observed in END-treated females. Previously, we reported that END exposure reduced triiodothyronine (T3) levels in males, but increased thyrox-

ine (T4) levels in females. Thus, our findings suggest that in males, follicular hyperplasia could arise from increased TSH stimulation due to diminished T3 levels. In females, elevated levels of biologically less active T4 would likely have a less pronounced impact on TSH levels and thyroid histofunctional characteristics. In conclusion, in ovo exposure to END appears to exert a persistent disruptive influence on thyroid homeostasis, which differentially affects peripheral conversion of T4 into T3 in males and females.

598. 427. **INHIBITORY POTENTIAL OF TWO ORGANIC MOLECULES AGAINST THE PROTEOLYTIC ACTIVITY OF BOTHROPS ALTERNATUS SNAKE VENOM**

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Bothrops snakebites cause hemorrhage and myotoxicity at the site of envenomation and lead to progressive tissue damage. Snake venom metalloproteinases (SVMPs) are responsible for major local symptoms. Thus, the SVMP inhibition by natural or low-cost compounds may represent a valuable complement to serotherapy mainly to abrogate the local effects. The ability of two organic molecules (OM), a dye (alizarin: Az) and an antioxidant vitamin (ascorbic acid: AA), to neutralize the proteolytic activity of the *Bothrops alternatus* venom was studied. The substrate-agarose plate and substrate-gel methods were carried out to quantitatively and qualitatively assess such activity. Different mass ratios of OM:venom (0.025:1-4:1 for Az and 1:1-16:1 for AA) were incubated for 30 min at 37°C (in 0.1 M Tris-HCl, 0.5 mM $CaCl_2$, pH 8 buffer). Wells of 1% defatted bovine milk (DBM)–1% agarose plates were filled with 10 µL of mixtures. After 24 h of incubation at 37 °C, the diameters of proteolytic halos were measured. Mixtures were also incubated with DBM for 1 h at 37°C (20:1 substrate:venom ratio), resolved by 12% SDS-PAGE and analyzed with *GelAnalyzer 19.1* software. Both OM were able to neutralize the proteolytic activity of the venom in a dose-dependent manner. The highest ratios of OM:venom assayed, 0.29 and 9.5 μg/μg, significantly reduced ($p<0.05$) the venom proteolytic halo to 33 and 40% with inhibitory concentrations 50 of 3.6 and 188.2 mM for Az and AA, respectively. Electrophoretic pattern analysis showed cleavage of major milk proteins by venom: the casein (CN) bands (αs2-, αs1- and β-CN) and, a protein band of ~18 kDa compatible with β-lactoglobulin (β-Lg). The hydrolysis of CN and β-Lg was partially inhibited by Az, whereas AA was only able to partially inhibit the degradation of CN bands at conditions assayed. OM can find potential use as alternative therapeutic agents in snakebite treatment. Additional *in vitro* and *in vivo* assays will be performed to support these results.

599. 444. **EFFECTS OF HYDROCARBONS ON CELL SURVIVAL AND CELL CYCLE OF BREAST TUMORIGENIC AND NON-TUMORIGENIC CELLS**

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Over the last centuries, contamination with polycyclic aromatic hydrocarbons (PAH) has risen due to the intensified industrial activities. PAH are a group of organic pollutants with adverse effects on humans and the environment, widely distributed in various ecosystems and present in crude oil, drinking water and food. Crude oil toxicity is mainly caused by the water-accommodated fraction (WAF). Our aim was to evaluate the effects of WAF and anthracene, one of the PAH present in WAF, on cell survival and proliferation, cell cycle and the antioxidant system. MCF-7 and MDA-MB-231 human breast tumorigenic cells and/or MCF-10A epithelial non-tumorigenic cells were exposed for 7d to WAF (serial dilutions from 1/500 to 1/25) or anthracene (0 to 28 µM) to perform clonogenic assays, for 72h to study viability (MTT assay), glutathione S-transferase (GST) and catalase (CAT) activities and for 18h to study cell cycle by flow cytometry. Neither WAF nor anthracene affected MCF-7 and MDA-MB-231 clonogenicity. However, anthracene decreased MCF-10A proliferation in a dose-dependent manner ($p<0.05$). A significant decrease in cell viability was observed after exposing MCF-7 cells to the most diluted WAF solutions and MDA-MB-231 cells to intermediate dilutions of WAF ($p<0.05$). No significant differences were observed in the viability of tumorigenic cells exposed to anthracene compared to control. However, 28 µM anthracene significantly decreased MCF-10A viability ($p<0.05$). WAF increased CAT activity of MCF-7 and MDA-MB-231 cells ($p<0.05$) but did not affect GST activity. Anthracene increased G0/G1 phase of MCF-7 and S phase of MDA-MB-231 cell cycle ($p<0.05$). Our results suggest that non-tumorigenic cells are more sensitive to HC exposure. WAF would induce predominant cytotoxic effects at low concentrations, triggering an antioxidant response through CAT. Also, we should evaluate other parameters in order to understand the mechanism of action of the toxicity of PAH and HC.

600. 490. **CHLORPYRIFOS AND CYPERMETHRIN INSECTICIDES INDUCE PROLIFERATION AND MIGRATION IN A HUMAN ENDOMETRIAL CANCER CELL LINE**

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Chlorpyrifos (CPF) and cypermethrin (CPM) are insecticides widely used in our country, both individually and in combination. We previously demonstrated that CPF acts as an endocrine disruptor in rats inducing uterine endometrial cell proliferation. Our objective was to evaluate whether CPF, CPM or the combination of both of them affects human endometrial cancer ECC-1 cell proliferation and/or migration and to elucidate the role of estrogen receptor α (ERα) in these actions. ECC-1 cells were exposed to CPF or CPM environmental concentrations ranged from 0.05 to 50 μM. Clonogenic assays were performed in the presence or absence of the specific ERα inhibitor ICI 182,780 (ICI). To assess migration we used the wound healing assay. The estrogen receptor α (*ESR-1*) mRNA expression by qRT-PCR and cell cycle by flow cytometry were also studied. CPF and CPM at 0.05 μM induced proliferation ($p<0.001$ vs C, respectively) similarly to E2 ($p<0.001$ vs C). This effect was reversed when cells were pre-incubated with ICI 1 μM. We also observed that exposure to 50 μM of CPF and CPM decreased proliferation in ECC-1 cells ($p<0.01$ vs C). Cell cycle analysis showed that CPF 25 μM induces G2/M phase arrest ($p<0.05$), while CPM 0.05 μM increases G0/G1 phase cells ($p<0.001$). The *ERS-1* expression was significantly reduced when cells were exposed to CPF such as CPM ($p<0.001$). Simultaneous exposure to different concentrations of CPF and CPM showed no significant differences in proliferation with respect to the individual effects of each pesticide. These results demonstrate that environmental concentrations of CPF or CPM can induce proliferation or migration in human endometrial cancer cells, warning of potential health risks associated with exposure to these pesticides. Since simultaneous exposure to CPF plus CPM constitutes a real exposure scenario, we need to conduct further studies to evaluate the effect of this mixture.

601. 541. **EARLY ENDOCRINE DISRUPTION IN FEMALE RATS EXPOSED TO CHLORPYRIFOS DURING GESTATION AND LACTATION**

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We previously demonstrated the endocrine disrupting action of chlorpyrifos (CPF) in a chronic exposure model in female rats, where we observed the appearance of irregular cycles and proliferative alterations in breast and uterus of exposed animals. The aim of this work was to evaluate whether environmentally relevant concentrations of CPF (NOAEL: 0.1 mg/Kg/day - CPF 0.1) and ADI: 0.001 mg/Kg/day - CPF 0.001) affect the normal development of exposed female offspring from gestational day 1 (GD1) to weaning in an intrauterine exposure model in Sprague-Dawley rats. We focused particularly on organs with great hormonal influence such as breast, uterus, and ovary. We evaluated different developmental parameters such as weight, ano-genital distance (AGD), age of vaginal opening (AVO) and age at first estrus by microscopic evaluation of vaginal lavage at 8- and 21-days postnatal life (DPN) of female offspring exposed to CPF or vehicle (castor oil). We also performed histopathological studies of the uterus, ovary, and breast. We found that body weight was significantly higher from PND 34 ($p<0.01$) and PND 44 ($p<0.05$) of intrauterine exposed rats to CPF 0.001 and CPF 0.1, respectively. AGD increased at PND 21 in rats exposed to both CPF concentrations (CPF 0.001: $p<0.05$; CPF 0.1: 0.001). Intrauterine exposure showed an advance in the AVO (CPF 0.1: $p<0.05$). At PND 21, the uteri showed an epithelium with a certain degree of atypia and abundant intracellular edema in the stroma, the ovaries showed a significant reduction of primordial follicles and an increase of follicular cysts and an increase of typical mammary intraductal hyperplasia in exposed rats. These results demonstrate that early exposure to CPF induces both developmental and histological changes in hormonally regulated tissues, which ratifies CPF endocrine disrupting role, with serious consequences in the health of the offspring of exposed individuals.

602. 552. **SYNERGISTIC IMPACTS OF UNDERNUTRITION AND AIR POLLUTION ON PULMONARY HEALTH**

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Inadequate nutrition and air pollution (gases and particulate matter-PM) are two major nongenetic environmental factors known to cause serious public health problems worldwide. The nutritional status may modify the susceptibility to PM exposure and cause a wide range of acute and chronic diseases. PM depending on the concentration, composition and time of exposure may, through the release of pro-inflammatory and pro-oxidant mediators, exert its adverse impact on the individual's health. The aim of this study was to evaluate in a nutritional growth retardation (NGR) animal model, the subchronic exposure of *Residual Oil Fly Ash* (ROFA, an ambient air PM surrogate) on lung oxidative metabolism. Furthermore, we analyze *in vitro* the alveolar macrophage (AM) response to virus-like particles. Wistar male weanling rats were divided in two groups: NGR animals were fed during 4 weeks a restricted diet 20% compared to *ad libitum* intake of Control (C) animals. NGR and C rats were intranasally instilled with either 0.17mg/kg BW of ROFA or its vehicle 3 times a week during 1 month. Lung was isolated and the oxidative metabolism was assessed by means of antioxidant enzymes activity (Catalase-CAT and Superoxide dismutase-SOD). AM obtained by bronchoalveolar lavage were isolated and cultured. After 24h AM cultures were exposed to 50 μg/ml PolyIC virus-like particle and cell viability was assayed. In lung, only a significative CAT response to ROFA instillation was observed in C animals. Although NGR animals displayed higher levels of both antioxidant enzymes, no changes were observed despite ROFA exposure. In vitro, only AM from NGR animals exposed to ROFA and treated with PolyIC showed a significantly decrease on cell viability. In conclusion, exploring lung antioxidant response and alveolar macrophage (AM) functionality offers valuable insights into the synergistic alterations of pulmonary response to both relevant environmental stressors: air pollution and chronic undernutrition.

603. 583. **ASSESSMENT OF REPRODUCTIVE EFFECTS IN A NATIVE GASTROPOD EXPOSED TO WATER FROM LUGANO LAKE (BUENOS AIRES CITY, ARGENTINA)**

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Lugano lake is situated within the Lugano Lake Ecological Reserve in the lower Matanza-Riachuelo basin, one of the most urbanized and industrialized areas in Argentina. It is a refuge for biodiversity, retains rainfall water, and has a high recreational value for the community. *Biomphalaria straminea*, a native aquatic gastropod in Argentina, is a suitable model in reproductive assays due to its high egg production, rapid development and growth, and short life cycle. The aim of this work was to assess the reproductive effects of *B. straminea* following a 6-day exposure to water samples from Lugano lake (L1, L2, and L3) over 5 sampling (2022-2023). A control group was exposed to tap water (TW). The egg masses deposited on the 6th day were individually separated, observed for 14 days, and the number of eggs and embryonated eggs per mass, hatching success and, embryo development abnormalities were recorded. Exposure to water samples did not lead to alteration in the number of eggs and embryonated eggs per mass. Significant differences were observed in the % of hatching between the treatments and the TW during the spring 2022 (L1: 43%, L2 and L3: 68%), in summer 2023 (L1: 53%) and in autumn 2023 (L1: 60% and L3: 61%). Egg masses in TW showed hatching rates ranging from 76% to 100%. In autumn 2022 and 2023, alterations in embryonic development were observed, with a maximum of 15% arrested embryos in autumn 2023 in L1, and 5% delayed embryos in autumn 2022 in L3, both significantly higher than in TW (0.4% arrested and 1% delayed embryos). The water samples caused a significant decrease in the % of normal embryos, with a minimum of 85% in autumn 2023 in L1 compared to TW (97%). Our results suggest that exposure to compounds present in the lake caused negative effects on embryo development and hatching of *B. straminea*. In this sense, embryotoxicity proved to be a sensitive parameter that could serve as a tool for water quality biomonitoring and the protection of aquatic life.

604. 639. **PESTICIDE EXPOSURE IN PREGNANT WOMEN FROM SANTA FE: A BIOMONITORING STUDY**

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The exposome represents lifestyle factors, endogenous systems and environmental exposures from gestation and throughout life that may affect reproductive health. Epidemiological studies have suggested associations between the environmental exposome and adverse reproductive outcomes. However, few have focused on biomonitoring environmental toxicants in samples of pregnant women. This study aims to determine which pesticides are present in urine samples of pregnant women living in the Litoral region. Participants (first, second and third trimester, aged 18-40) were enrolled at several health centers in Santa Fe, Reconquista and San Justo. After written informed consent, urine samples were collected and stored at -20°C until analysis. According to the World's Health Organization recommendations for the assessment of chemicals in human samples, urine samples were excluded if the creatinine concentration was outside the range of 30-300 mg/dl. Sample extraction and clean-up were performed using solid phase extraction with Oasis HLB Prime cartridges, and residue identification and quantification was performed using gas chromatography-tandem mass spectrometry. Twenty-six samples were included and 23 residues were identified. Among them, hexachlorobenzene (0.1-0.2 ppb), vinclozolin (0.1-0.7 ppb) and propazine (0.1-0.2 ppb) were more frequently quantified. For these compounds, the detection and quantification limits of the method were 0.03 and 0.1 ppb, respectively. In addition, more than one residue was detected in 85% (22/26) and quantified in 58% (15/26) of the samples. We demonstrate the importance of measuring chemicals in human samples after detecting several agrochemicals reported to impair human health. This is the first report of pesticide biomonitoring in urine samples during pregnancy in Argentina. Further analysis will include measurements on a larger group of pregnant women, and the risk assessment for adverse pregnancy outcomes.