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CONCEPTOS EN UROLOGÍA

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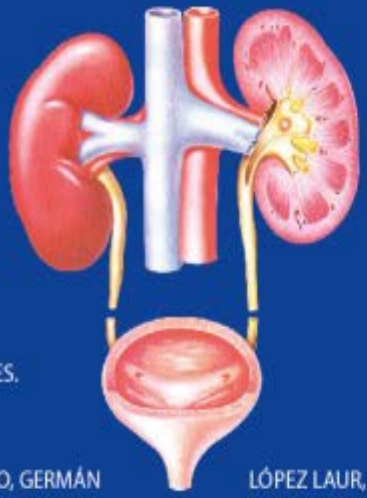

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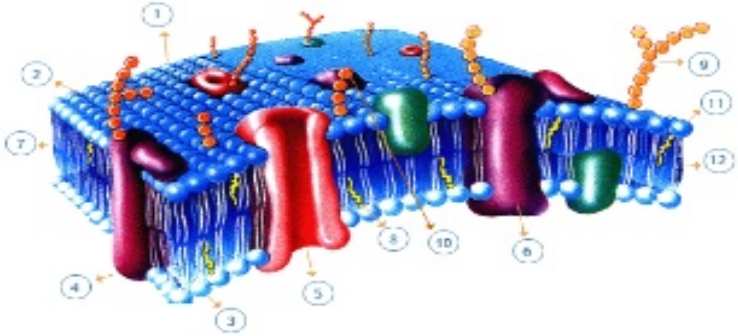
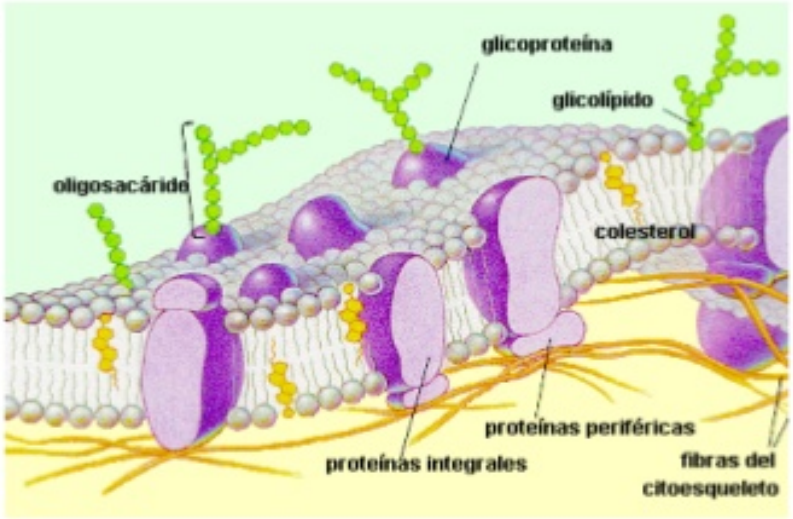
ISLAS LIPIDICAS: UN NUEVO CONCEPTO EN CaP

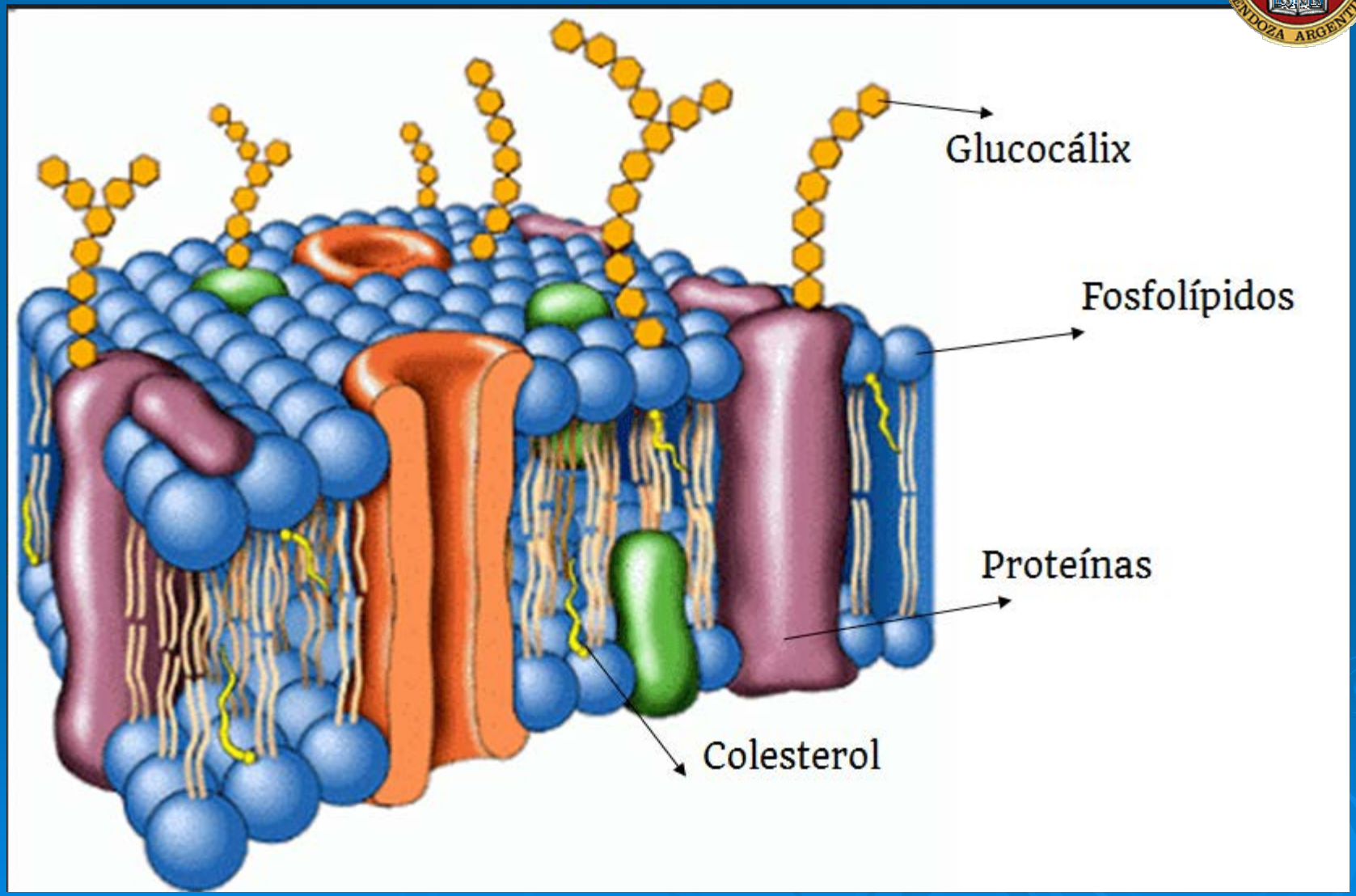
CLINICA ANDINA DE UROLOGIA
CATEDRA UROLOGIA FCM UNCUYO

Membrana plasmática

- Protege y da forma a la célula, puede "comunicarse" con el exterior para alimentarse y desechar toxinas
- Función: paso de substancias de un lado a otro de la célula
- Formación: lípidos, proteínas y carbohidratos






Glucocálix
Fosfolípidos
Proteínas
Colesterol

MEMBRANA PLASMÁTICA

• La membrana plasmática es un modelo de mosaico fluido, donde los lípidos son un líquido desordenado en el cual las proteínas flotan libremente.

SINGER Y NICHOLSON (1972)

• Se observa la aparición de dominios ordenados de lípidos a los que se denomino “Rafts lipídicos” o “Balsas lipídicas”

SIMONS K. AND VAN MEER G. (1988)

• Se caracterizan por presentar niveles elevados de colesterol y esfingolípidos.

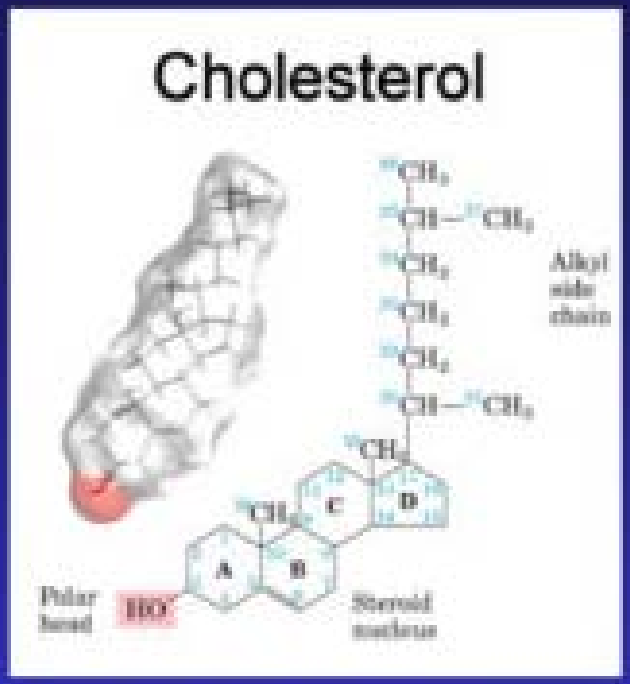
ANTECEDENTES

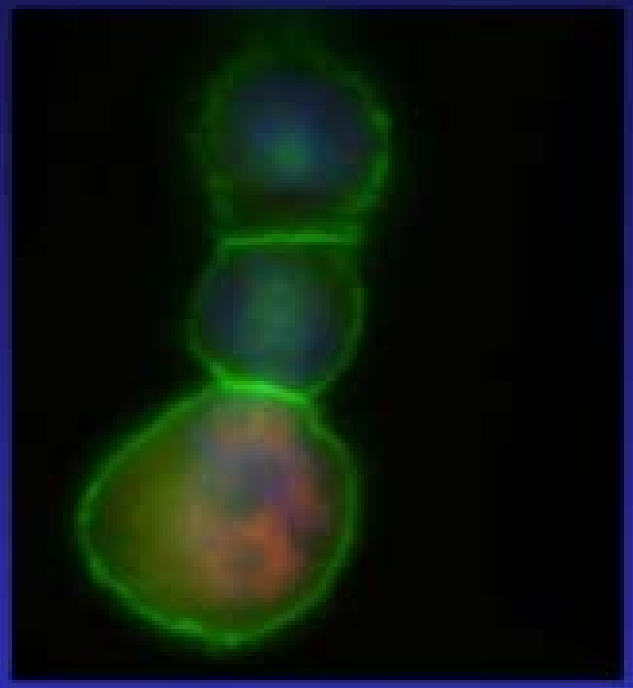
Algunos tumores sólidos tienden a acumular colesterol.

• En los tumores se detecta un aumento en la actividad de la vía del colesterol.

• Estudios en modelos celulares y animales mostraron que una reducción en el Col genera una disminución en el volumen y agresividad tumoral.

Liyan Zhuang, et al; Cholesterol targeting alters lipid raft composition and cell survival in prostate cancer cells and xenografts; The Journal of Clinical investigation http://www.jci.org Volume 115 Number 4 April 2005

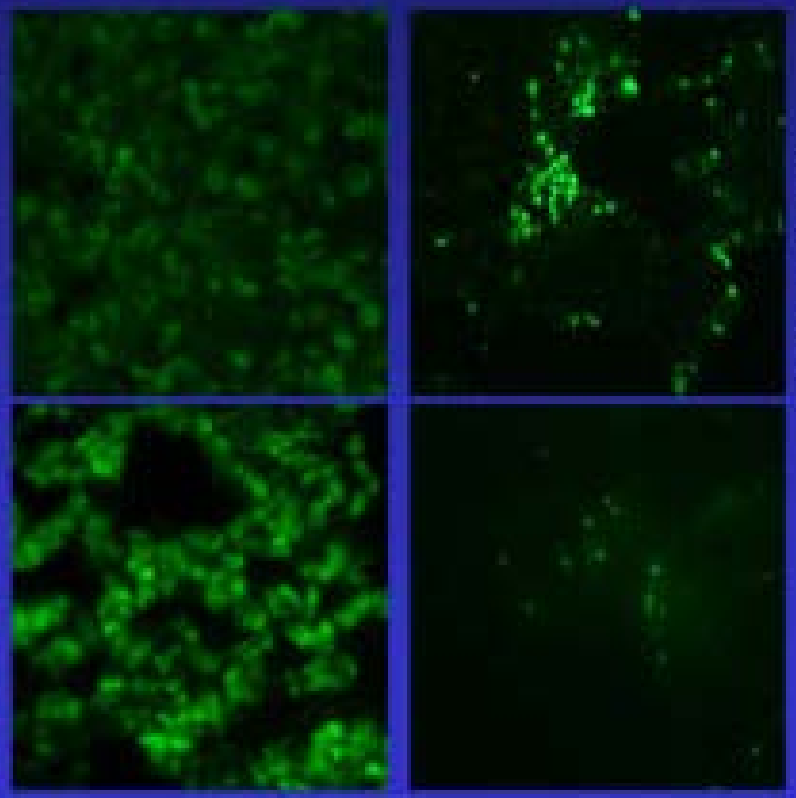




White, R.M. On the occurrence of crystals in tumors *J. Pathol. Bacteriol.* 13:3-10, 1909

"…the presence of crystals in the proliferating areas of cancers…suggests that cholesterol may…be associated with the regulation of proliferation."

Circulating cholesterol activates oncogenic pathways and inhibits tumor cell apoptosis



Zhuang *Cancer Res* 2002

Kim *Endocrinol* 2004

Zhuang *J Clin Invest* 2005

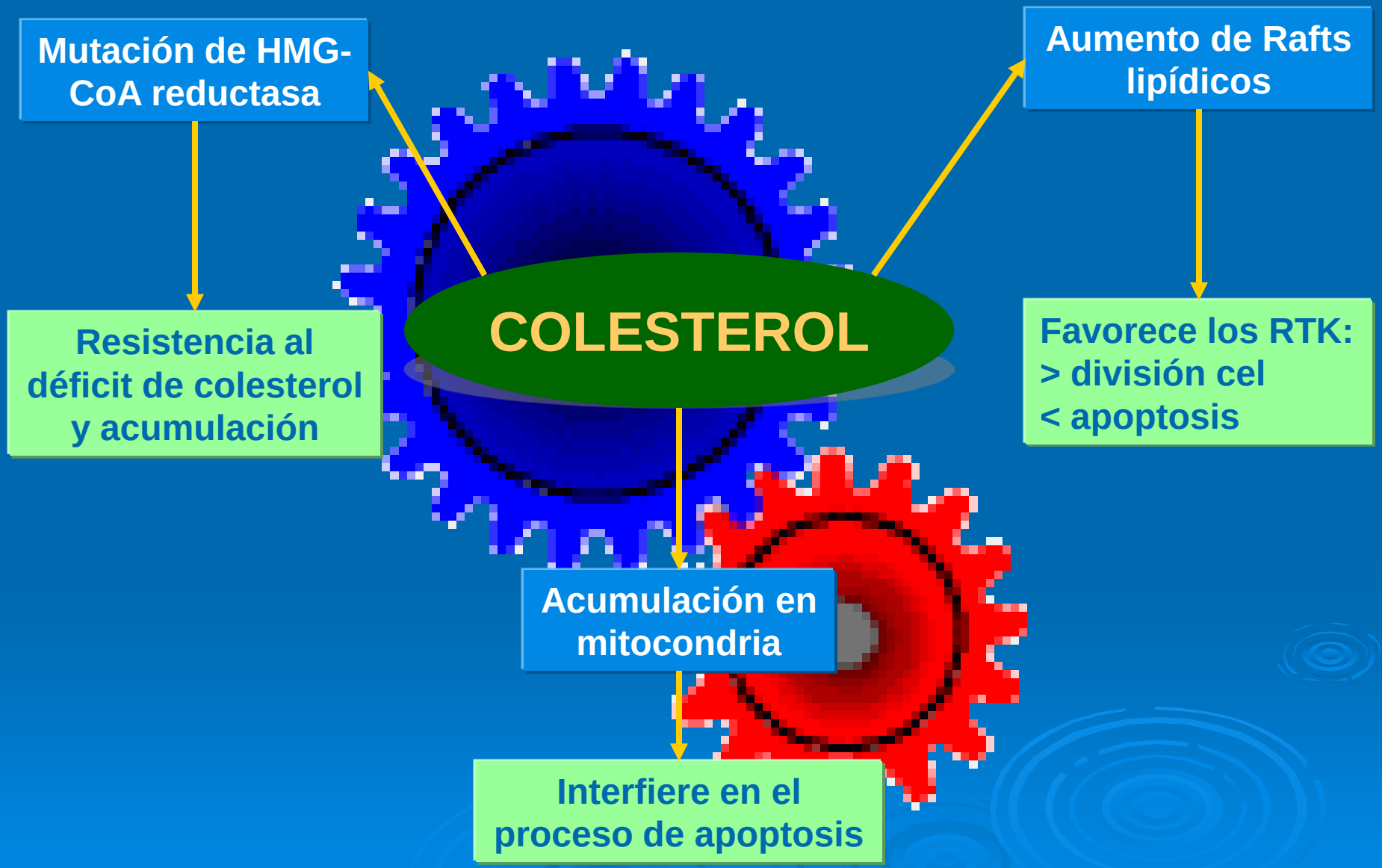
Adam *Cancer Res* 2007

Cinar *J Biol Chem* 2007

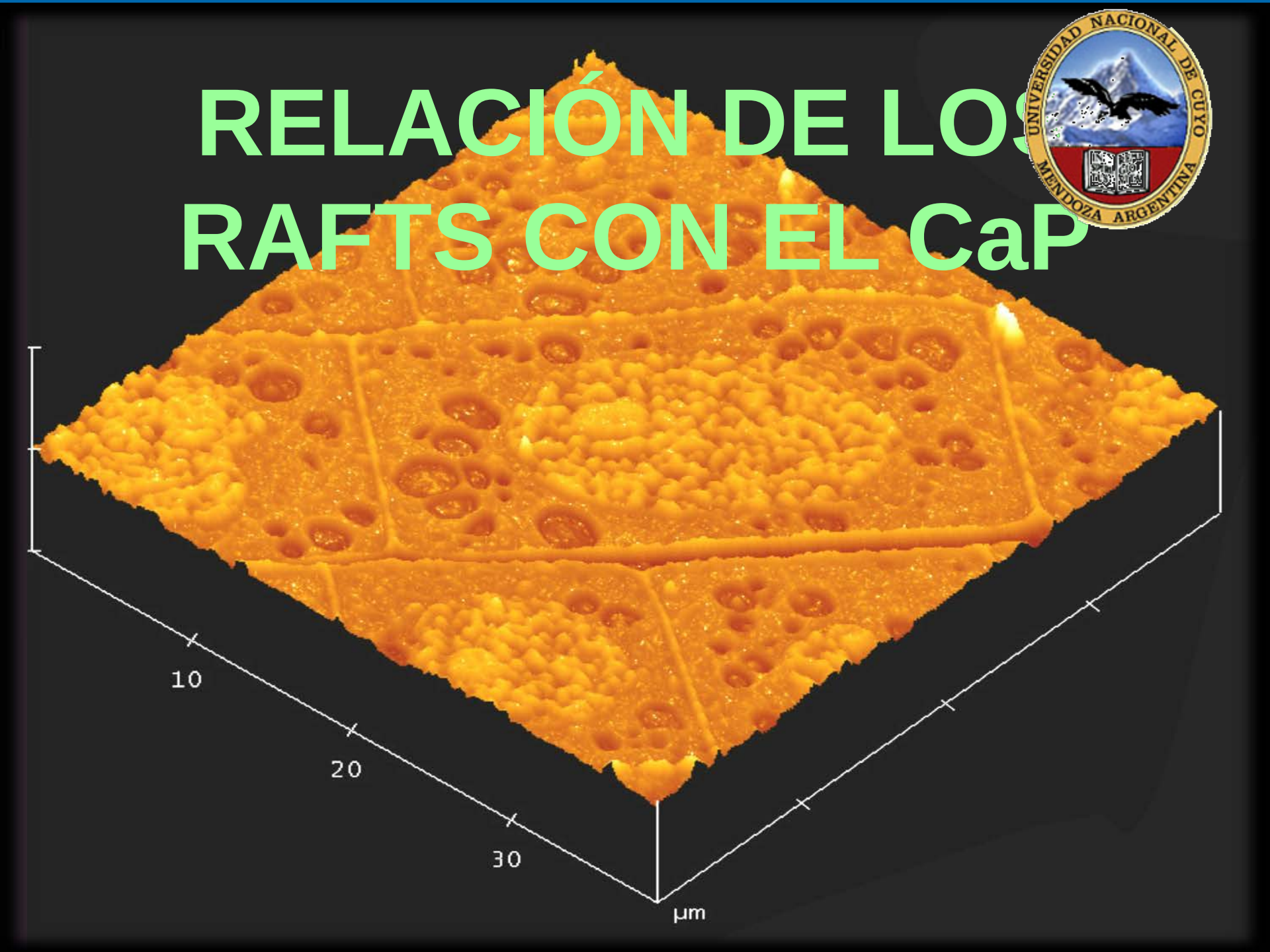
Cinar *EMBO J* 2007

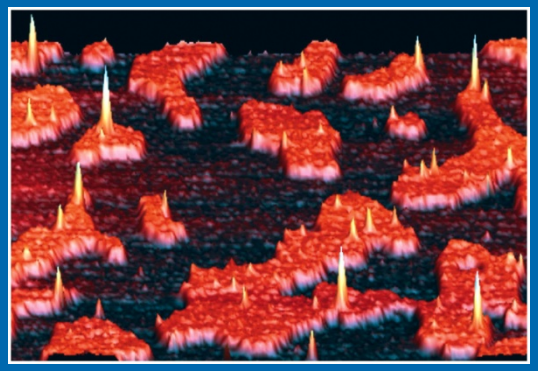
DiVizio *Cell Cycle* 2008

Solomon *Am J Pathol* 2009


Mutación de HMG-CoA reductasa
Aumento de Rafts lipídicos
COLESTEROL
Resistencia al déficit de colesterol y acumulación
Favorece los RTK:
> división cel
< apoptosis
Acumulación en mitocondria
Interfiere en el proceso de apoptosis

RELACIÓN DE LOS RAFTS CON EL CaP





“Rafts” Lipídicos

- **Están enriquecidos en muchos tipos de prot. incluidos receptores transmembrana.**
- **SON DETERMINANTES DE FACTORES DE CRECIMIENTO**

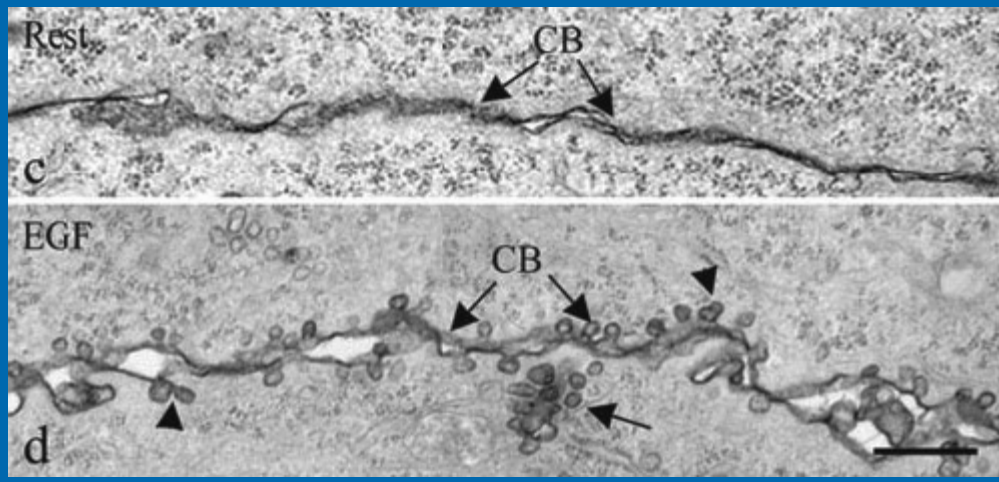
- **Tipos Rafts Lipídicos:**

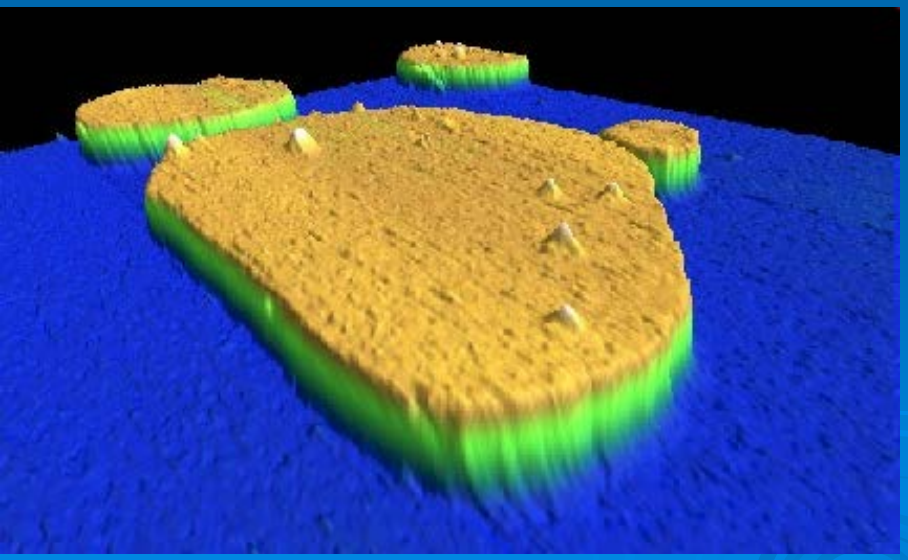
  - Rafts morfológicamente planos (identificados por extracto bioquímico).

  Caveolas (identificados por ME).

Caveolas:

- Se presentan como pequeñas invaginaciones de la MP visibles con ME, mas estudiadas, estan asociados a la proteína caveolina, y hay de Tres tipos: Cav-1, 2, 3.
- Están implicadas en un gran número de funciones.



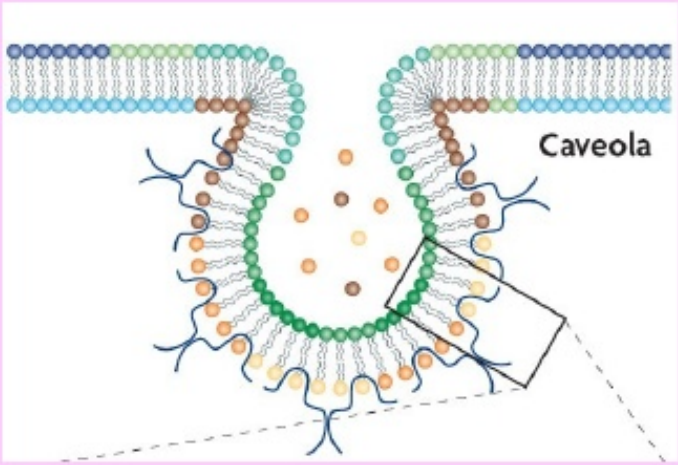


Rafts morfológicamente planos:

- Son los menos estudiados, no están asociados a caveolina y se descubrieron a partir de extractos de MP tratados con Det. no iónicos y posterior análisis de su composición.
- Pueden cumplir las func. de las Caveolas

Endocitosis mediada por caveolas

- Las **caveolinas** son **proteínas estructurales** que están asociadas a **microdominios** en la membrana plasmática, ricos en colesterol y esfingolipidos también llamados **balsas lipídicas**.
- La conformación de la caveola depende de la expresión de la **caveolina-1** en las células no musculares y de la **caveolina-3** en las musculares.
- Estas proteínas forman **olígomeros** que cubren las vesículas.
- Las vesículas son liberadas a estructuras llamadas **caveosomas** presentes en el interior de la célula.
- Estas estructuras representan un **nuevo tipo de organelos**. Y a diferencia de los endosomas estos tienen un **pH neutro**.



ACCIÓN DE LOS RAFTS LIPIDICOS

- Compartimentalización
- Endocitosis mediada por receptores (LDL).
- Activan y concentran receptores de Tirosin quinasa.
- Aumento de la vida media de los receptores TK activados (caveolas).
- Transporte de colesterol.
- Están relacionados en la interacción de Akt y RA

Actúan como antenas parabólicas para la célula, favoreciendo la detección de señales de supervivencia por parte de la célula.

[CANCER RESEARCH 62, 2227–2231, April 15, 2002]

Advances in Brief

Cholesterol-rich Lipid Rafts Mediate Akt-regulated Survival in Prostate Cancer Cells[1]

[CANCER RESEARCH 59, 5719–5723, November 15, 1999]

Caveolin-1 Expression in Clinically Confined Human Prostate Cancer: A Novel Prognostic Marker[1]

Guang Yang, Luan D. Truong, Thomas M. Wheeler, and Timothy C. Thompson[2]

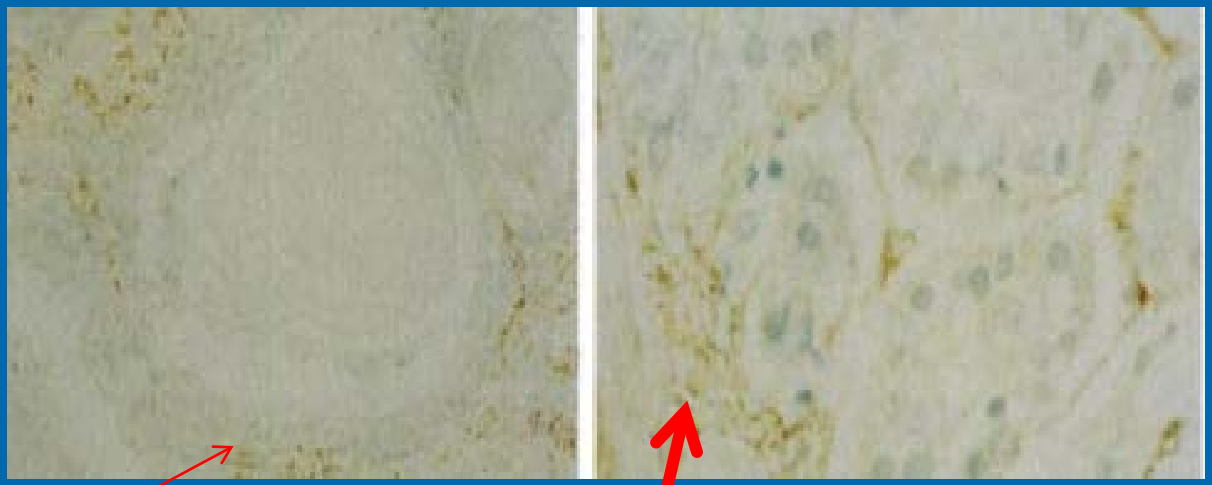
Departments of Urology [G. Y., T. M. W., T. C. T.], Pathology [T. M. W., L. D. T.], Cell Biology [T. C. T.], and Radiology [T. C. T.], Baylor College of Medici Houston, Texas 77030

cer (LNCaP) cells contain cholesterol-rich lipid rafts that mediate epidermal growth factor (EGF)-induced and constitutive signaling through the Akt1 serine-threonine kinase. EGF receptor and Akt1 phosphorylation were inhibited and autonomous cell survival was reduced when the rafts were disrupted. Reconstitution of the rafts with cholesterol restored EGF receptor→Akt1 axis signaling and cytoprotection from a phosphoinositide 3-kinase-dependent apoptotic signal. These results suggest that cholesterol present in membrane microdomains is a prominent mediator of survival in prostate cancer cells.

ing the PTEN phosphatase, a negative regulator of this pathway. Loss of PTEN is a frequent event in human PCa (15, 16), and up-regulation of the PI3K/Akt pathway has been linked to PCa progression (17).

Materials and Methods

Cell Culture. Human PCa cell lines LNCaP and PC-3 were purchased from the American Type Culture Collection (Rockville, MD). Both cell lines were cultured in RPMI 1640 supplemented with 10% heat-inactivated FBS. LNCaP cells transfected with the plasmid pcDNA-Cav-1 or with an empty



NORMAL.
ESTROMA

DESPERTAR CAV- 1 CAP

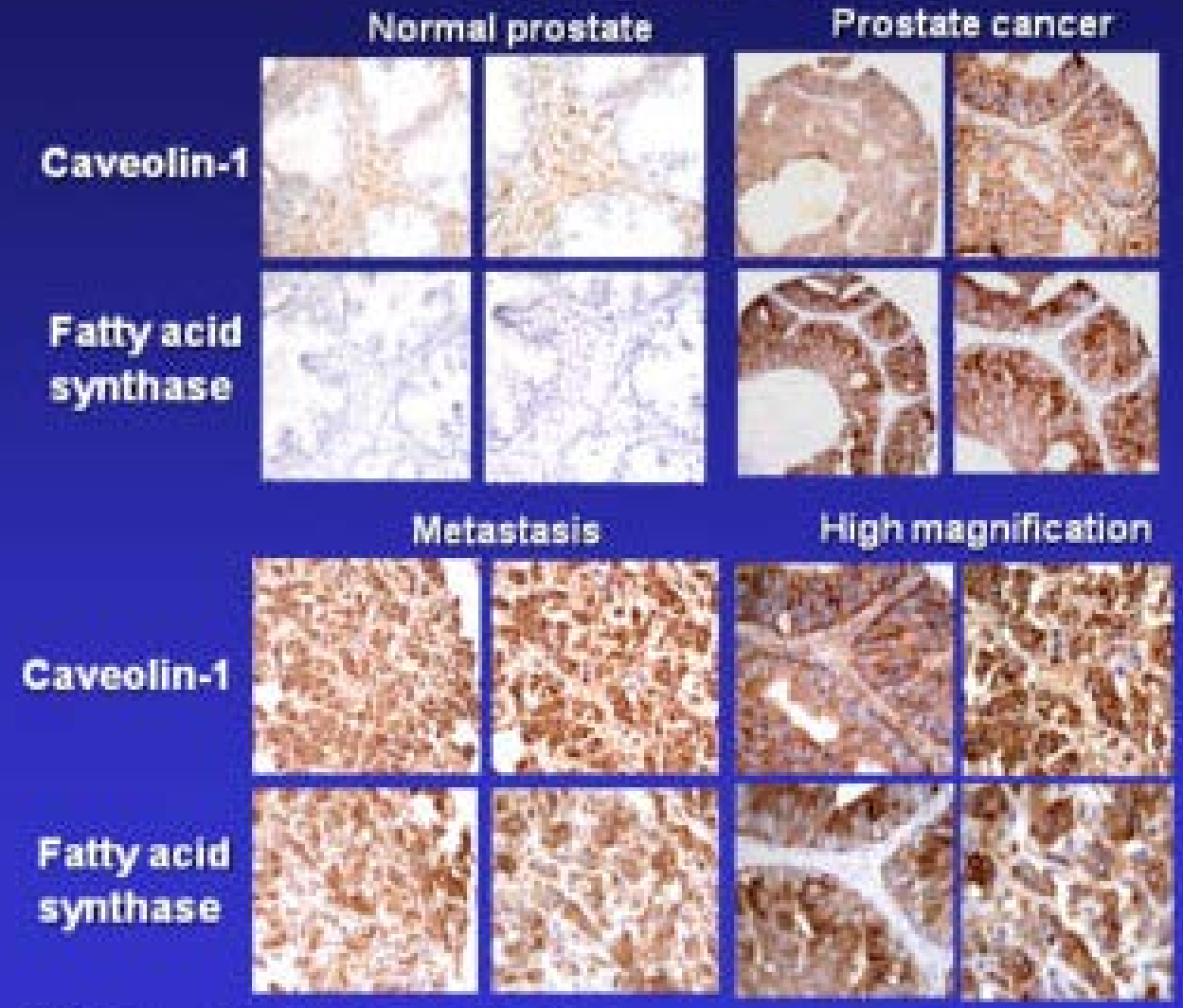
CAVEOLINAS – 1(CAV-1)

- PROMUEVEN LA PROGRESIÓN DEL FENOTIPO METASTÁSICO
- MARCAD ELULAR
- MARCAD GRESIVA

AUMENTA SU EXPRESION EN METASTASIS LINFATICAS, CON GLEASON ELEVADO Y EXTENSION EXTRAPROSTATICA

- SE UNEN A RECEPTORES ANDRÓGENO/ ESTRÓGENOS SUGIRIENDO ACTIVIDAD EN LA HORMONODEPENDENCIA

Lipogenic pathways are upregulated in prostate cancer



Di Vizio et al. *Cell Cycle* (2008)

REGULAN LA RESISTENCIA A

OPEN

Citation: Oncogenesis (2015) **4**, e148; doi:10.1038/oncsis.2015.9

www.nature.com/oncsis

ORIGINAL ARTICLE

Endothelial Caveolin-1 regulates the radiation response of epithelial prostate tumors

D Klein[1], T Schmitz[1], V Verhelst[1], A Panic[1,2], M Schenck[2], H Reis[3], M Drab[4,5], A Sak[6], C Herskind[7], P Maier[7] and V Jendrossek[1]

The membrane protein caveolin-1 (Cav1) recently emerged as a novel oncogene involved in prostate cancer progression with opposed regulation in epithelial tumor cells and the tumor stroma. Here we examined the role of stromal Cav1 for growth and radiation response of MPR31-4 prostate cancer xenograft tumors using Cav1-deficient C57Bl/6 mice. Syngeneic MPR31-4 tumors grew faster when implanted into Cav1-deficient mice. Increased tumor growth on Cav1-deficient mice was linked to decreased integration of smooth muscle cells into the wall of newly formed blood vessels and thus with a less stabilized vessel phenotype compared with tumors from Cav1 wild-type animals. However, tumor growth delay of MPR31-4 tumors grown on Cav1 knockout mice to a single high-dose irradiation with 20 Gray was more pronounced compared with tumors grown on wild-type mice. Increased radiation-induced tumor growth delay in Cav1-deficient mice was associated with an increased endothelial cell apoptosis. *In vitro* studies using cultured endothelial cells (ECs) confirmed that the loss of Cav1 expression increases sensitivity of ECs to radiation-induced apoptosis and reduces their clonogenic survival after irradiation. Immunohistochemical analysis of human tissue specimen further revealed that although Cav1 expression is mostly reduced in the tumor stroma of advanced and metastatic prostate cancer, the vascular compartment still expresses high levels of Cav1. In conclusion, the radiation response of MPR31-4 prostate tumors is critically regulated by Cav1 expression in the tumor vasculature. Thus, Cav1 might be a promising therapeutic target for combinatorial therapies to counteract radiation resistance of prostate cancer at the level of the tumor vasculature.

Oncogenesis (2015) **4,** e148; doi:10.1038/oncsis.2015.9; published online 18 May 2015

Table 1 Caveolin immunostaining in human prostate tissues

Prostate specimens	n^a	Caveolin −	Caveolin +	Positivity[b] %
Normal glandular epithelia	13	12	1	7.7
Hyperplastic epithelia	17	14	3	17.6
Pathological stage of cancers				
$T_1/T_{2a}N_0$	29	25	4	13.8
Recurrent	11	9	2	18.2
No recurrence	18	16	2	11.1
T_3N_1				
Primary cancer	17	12	5	29.4
Metastatic site in lymph node	25[c]	11	14	56.0[d]

[a] *n* values denote the number of patients in each group.

[b] Positivity was defined as over one measuring field showing granular immunostaining in cancer.

[c] Tissues from metastases included eight cases of T_3N_1 stage, for which the primary cancer was also stained.

[d] $P < 0.01$ (Fisher's Exact test) as compared with both normal and hyperplastic epithelia as well as the T_1/T_{2a} cancers.

CLIN. CANCER RESEARCH. 2008

[CANCER RESEARCH 59, 5719–5723, November 15, 1999]

Caveolin-1 Expression in Clinically Confined Human Prostate Cancer: A Novel Prognostic Marker[1]

Guang Yang, Luan D. Truong, Thomas M. Wheeler, and Timothy C. Thompson[2]

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ABSTRACT

We demonstrated previously elevated caveolin-1 expression in metastatic mouse and human prostate cancer cells both *in vitro* and *in vivo*. In this study, we analyzed its prognostic value for progression of clinically confined human prostate cancer. Immunohistochemical staining with a caveolin-1-specific antibody was performed on routinely processed paraffin sections from 189 radical prostatectomy specimens. Caveolin-1 immunoreactivity was evaluated in association with patients' age, race, preoperative prostate-specific antigen, clinical stage, and pathological features including Gleason score, extraprostatic extension, status of surgical margins, and time to disease progression after surgery. Positive caveolin-1 immunostaining was detected in 47 of the 189 cancers (25%) and correlated positively with Gleason score, positive surgical margin, as well as lymph node involvement ($P = 0.0071$, 0.0267, and 0.0399, respectively). In lymph node-negative cancers ($n = 162$), caveolin-1 immunoreactivity predicts a shorter time to disease progression after surgery ($P = 0.0033$, univariate analysis). Multivariate analyses that included caveolin-1 and other prognostic pathological markers identified positive caveolin-1 immunostaining as an independent predictor for time to disease progression ($P = 0.0186$). Thus, our study establishes caveolin-1 as a novel prognostic marker for clinically confined human prostate cancer.

metastatic mouse prostate cancer using differential display PCR. In using this system, a number of metastasis-related sequences were identified including a cDNA that encodes caveolin-1 (9). Caveolin-1, a M_r 22,000 protein is a major structural component of caveolae, specialized plasma membrane invaginations that are abundant in smooth muscle cells, adipocytes, and endothelium. Caveolae appear to mediate molecular transport, cell adhesion, and signal transduction activities in a cell- and context-specific fashion (10). Caveolin-1 was found to be overexpressed in both mouse and human metastatic prostate cancer cells (9). Recently, we documented that suppression of *caveolin-1* gene expression with stably transfected antisense caveolin-1 cDNA converted androgen-insensitive metastatic mouse prostate cancer cells to an androgen-sensitive phenotype and that adenovirus-mediated sense caveolin-1 expression blocked androgen sensitivity (11). *Caveolin-1,* therefore, has been shown to be a metastasis-related gene as well as a candidate gene for hormone-resistant human prostate cancer. Interestingly, recent studies also point to a potential role for caveolin-1 in the resistance of various malignances to multiple antineoplastic agents (12, 13).

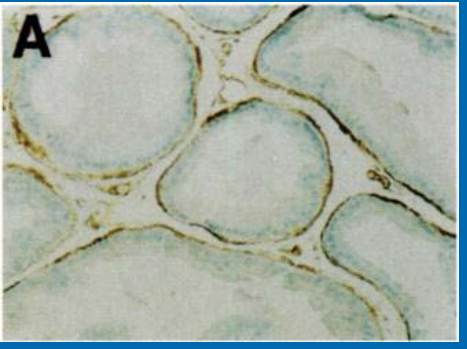
In the present study, we investigated the possibility of using caveo-

Cholesterol targeting alters lipid raft composition and cell survival in prostate cancer cells and xenografts

Liyan Zhuang,[1,2] Jayoung Kim,[1,2] Rosalyn M. Adam,[1,2] Keith R. Solomon,[1,3,4] and Michael R. Freeman[1,2]

[1]The Urological Diseases Research Center, Department of Urology, Children's Hospital Boston, Boston, Massachusetts, USA.
[2]Department of Surgery, and [3]Department of Orthopaedic Surgery, Harvard Medical School, Boston, Massachusetts, USA.
[4]Department of Orthopaedic Surgery, Children's Hospital Boston, Boston, Massachusetts, USA.

Lipid rafts are cholesterol- and sphingolipid-enriched microdomains in cell membranes that regulate phosphorylation cascades originating from membrane-bound proteins. In this study, we tested whether alteration of the cholesterol content of lipid rafts in prostate cancer (PCa) cell membranes affects cell survival mechanisms in vitro and in vivo. Simvastatin, a cholesterol synthesis inhibitor, lowered raft cholesterol content, inhibited Akt1 serine-threonine kinase (protein kinase Bα)/protein kinase B (Akt/PKB) pathway signaling, and induced apoptosis in caveolin- and PTEN-negative LNCaP PCa cells. Replenishing cell membranes with cholesterol reversed these inhibitory and apoptotic effects. Cholesterol also potentiated Akt activation in normal prostate epithelial cells, which were resistant to the apoptotic effects of simvastatin. Elevation of circulating cholesterol in SCID mice increased the cholesterol content and the extent of protein tyrosine phosphorylation in lipid rafts isolated from LNCaP/sHB xenograft tumors. Cholesterol elevation also promoted tumor growth, increased phosphorylation of Akt, and reduced apoptosis in the xenografts. Our results implicate membrane cholesterol in Akt signaling in both normal and malignant cells and provide evidence that PCa cells can become dependent on a cholesterol-regulated Akt pathway for cell survival.



UTILIDAD DE LOS RAFTS LIPIDICOS

• **Pronostica: se ha asociado a la expresión elevada de Caveolina con cáncer metastasico, este marcador asociado a Rafts puede ser medido fácilmente por inmunohistoquímica.**

•**Aumento de los Rafts totales en cultivo de cel. hormono-resistentes.**

• **Tratamiento: la reducción de la síntesis celular de colesterol mediante el uso de estatinas podría comprender una nueva terapia en CaP y en otros tumores.**

Caveolin-1 Expression in Clinically Confined Human Prostate Cancer: A Novel Prognostic Marker; Guang Yang et. al.; CANCER RESEARCH 59, 5719–5723, November 15, 1999

MATERIAL Y METODO

38 PACIENTES CON CAP

- 14 GLEASON 3+3
- 16 GLEASON 4+3
- 6 GLEASON 3+ 4
- 12 GLEASON > 8
- 38 c. HIPERCOLESTEROLEMIA
- METODO: DETERMINACION DE CAVEOLINAS POR INMUNOFLUORESCENCIA
- INMUNOHISTOQUIMICA

RESULTADOS

- CON INMUNOFLUORESCENCIA SE LOGRARON DETECTAR SOLO LAS CAVEOLINAS EN LOS PACIENTES CON CAP GLEASON >8
- INMUNO HISTOQUIMICA: SE DESTACO LA RELACION CON MAYORES COMPLEJOS DE ISLAS LIPIDICAS EN LOS CA DE ALTO GRADO
- A MAYOR COLESTEROL > N* DE ISLAS LIPIDICAS DETECTABLES

FUTURO DE LOS RAFTS LIPIDICOS

- **Debe comprobarse en que etapa de la vida tumoral tiene mayor participación.**

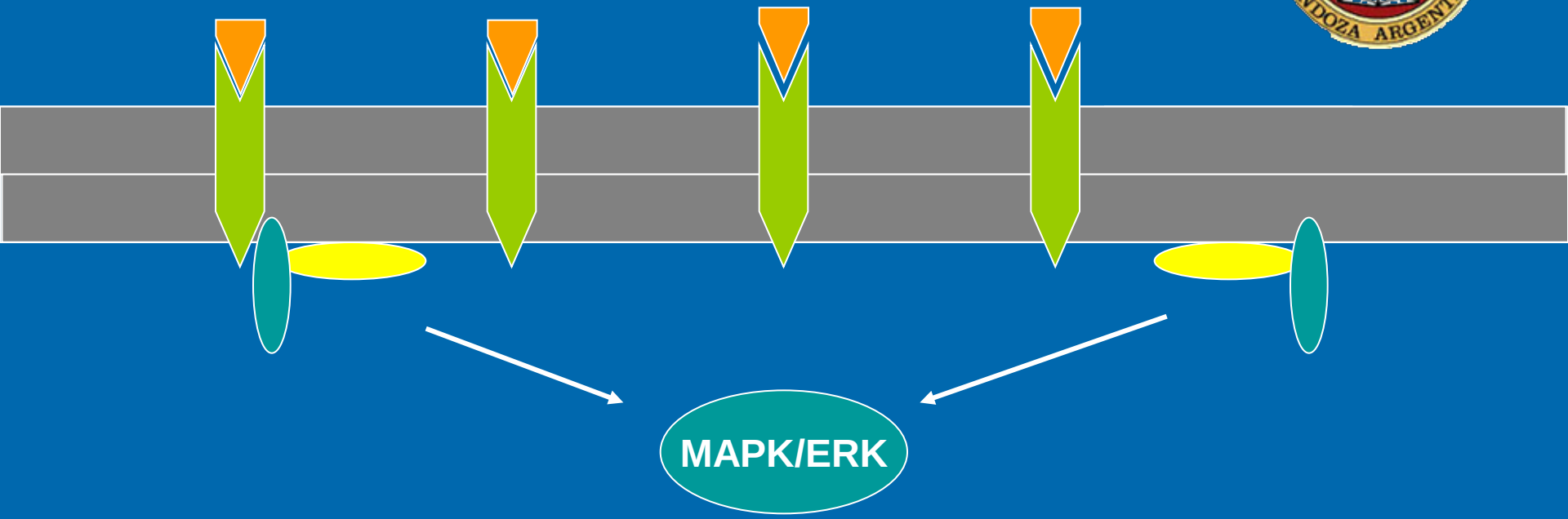
- **Establecer el rango en los niveles de colesterol en el cual el tumor presente su menor crecimiento y el compromiso individual de salud.**

- **Evaluar la efectividad terapéutica de la reducción de colesterol mediante biopsias.**

- **En el CaP debe ser tomado como una herramienta mas en el tratamiento del mismo, por lo que debe evaluarse la efectividad terapéutica conjunta.**

MUCHAS GRACIAS!!!

FELIZ NAVIDAD Y MEJOR AÑO PARA TODOS



Inhibición de la muerte celular.

Aumento de la proliferación y crecimiento celular.