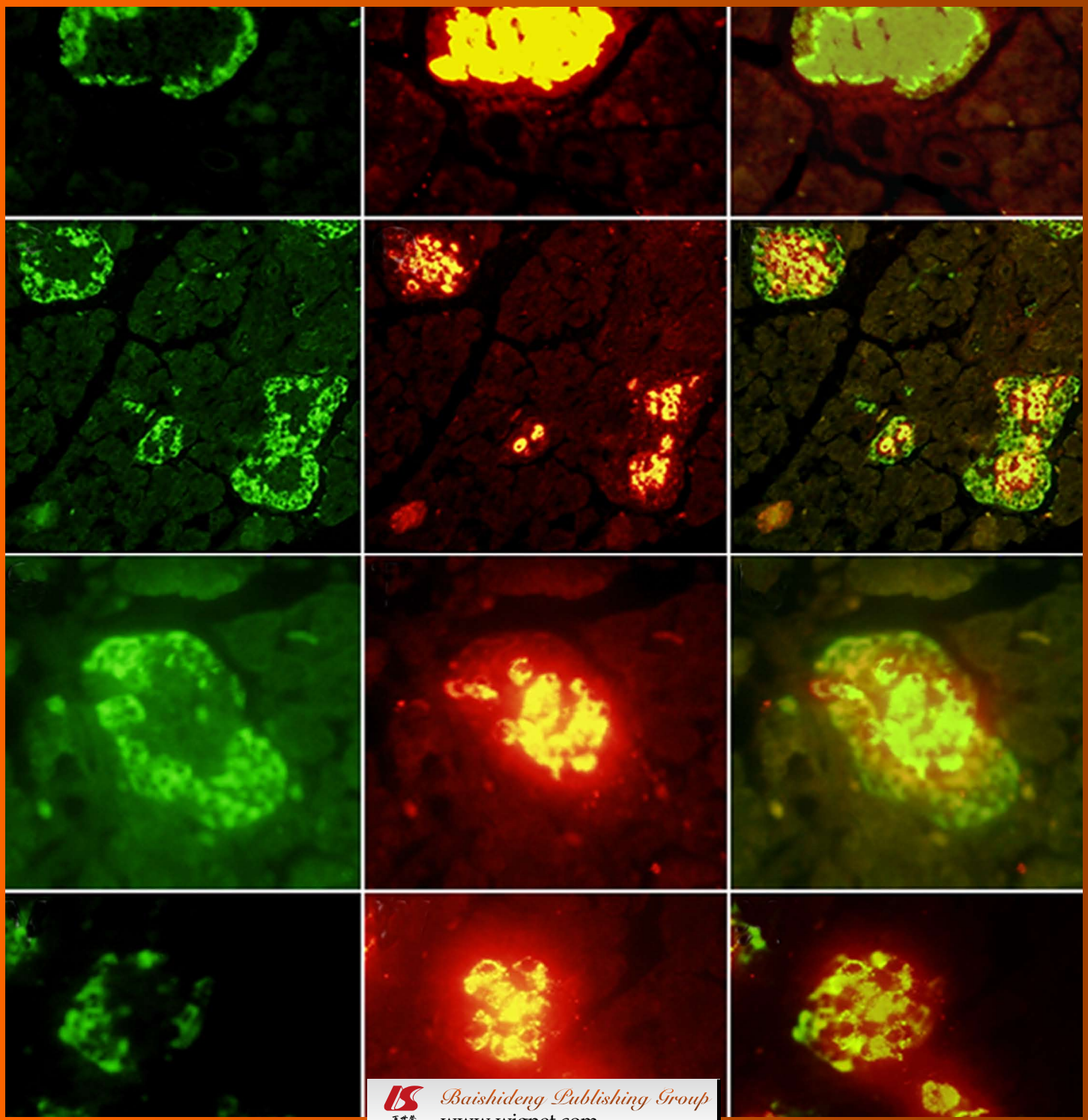
ISSN 1007-9327 (print)
ISSN 2219-2840 (online)

World Journal of *Gastroenterology*

World J Gastroenterol 2011 June 21; 17(23): 2781-2878



Baishideng Publishing Group
www.wjgnet.com

World Journal of Gastroenterology

A peer-reviewed, online, open-access journal of gastroenterology and hepatology

Editorial Board

2010-2013

The *World Journal of Gastroenterology* Editorial Board consists of 1144 members, representing a team of worldwide experts in gastroenterology and hepatology. They are from 60 countries, including Albania (1), Argentina (8), Australia (29), Austria (14), Belgium (12), Brazil (10), Brunei Darussalam (1), Bulgaria (2), Canada (20), Chile (3), China (69), Colombia (1), Croatia (2), Cuba (1), Czech (4), Denmark (8), Ecuador (1), Egypt (2), Estonia (2), Finland (8), France (24), Germany (75), Greece (14), Hungary (10), India (26), Iran (6), Ireland (7), Israel (12), Italy (101), Japan (112), Jordan (1), Kuwait (1), Lebanon (3), Lithuania (2), Malaysia (1), Mexico (10), Moldova (1), Netherlands (29), New Zealand (2), Norway (11), Pakistan (2), Poland (11), Portugal (4), Romania (3), Russia (1), Saudi Arabia (3), Serbia (3), Singapore (10), South Africa (2), South Korea (32), Spain (38), Sweden (18), Switzerland (11), Thailand (1), Trinidad and Tobago (1), Turkey (24), United Arab Emirates (2), United Kingdom (82), United States (249), and Uruguay (1).

Robert JL Fraser, *Daw Park*
Jacob George, *Westmead*
Mark D Gorrell, *Sydney*
Alexander G Heriot, *Melbourne*
Michael Horowitz, *Adelaide*
John E Kellow, *Sydney*
William Kemp, *Melbourne*
Finlay A Macrae, *Victoria*
Daniel Markovich, *Brisbane*
Vance Matthews, *Melbourne*
Phillip S Oates, *Perth*
Shan Rajendra, *Tasmania*
Rajvinder Singh, *Elizabeth Vale*
Ross C Smith, *Sydney*
Kevin J Spring, *Brisbane*
Nathan Subramaniam, *Brisbane*
Phil Sutton, *Melbourne*
Cuong D Tran, *North Adelaide*
Debbie Trinder, *Fremantle*
David Ian Watson, *Bedford Park*

Austria

Herwig R Cerwenka, *Graz*
Ashraf Dahaba, *Graz*
Peter Ferenci, *Vienna*
Valentin Fuhrmann, *Vienna*
Alfred Gangl, *Vienna*
Alexander M Hirschl, *Wien*
Kurt Lenz, *Linz*
Dietmar Öfner, *Salzburg*
Markus Peck-Radosavljevic, *Vienna*
Markus Raderer, *Vienna*
Stefan Riss, *Vienna*
Georg Roth, *Vienna*
Michael Trauner, *Graz*
Thomas Wild, *Kapellerfeld*

Belgium

Rudi Beyaert, *Gent*
Benedicte Y De Winter, *Antwerp*
Inge I Depoortere, *Leuven*
Olivier Detry, *Liège*
Philip Meuleman, *Ghent*
Marc Peeters, *De Pintelaan*
Freddy Penninckx, *Leuven*
Jean-Yves L Reginster, *Liège*
Mark De Ridder, *Brussels*
Etienne M Sokal, *Brussels*
Kristin Verbeke, *Leuven*
Eddie Wisse, *Keerbergen*

Brazil

José LF Caboclo, *São José do Rio Preto*
Roberto J Carvalho-Filho, *São Paulo*
Jaime Natan Eisig, *São Paulo*
Andre Castro Lyra, *Salvador*
Marcelo Lima Ribeiro, *Braganca Paulista*
Joao Batista Teixeira Rocha, *Santa Maria*
Heitor Rosa, *Goiania*
Damiao C Moraes Santos, *Rio de Janeiro*
Ana Cristina Simões e Silva, *Belo Horizonte*
Eduardo Garcia Vilela, *Belo Horizonte*

Brunei Darussalam

Vui Heng Chong, *Bandar Seri Begawan*

Bulgaria

Zahariy Krastev, *Sofia*
Mihaela Petrova, *Sofia*

Canada

Alain Bitton, *Montreal*
Michael F Byrne, *Vancouver*
Kris Chadee, *Calgary*
Wangxue Chen, *Ottawa*
Ram Prakash Galwa, *Ottawa*
Philip H Gordon, *Montreal*
Waliul Khan, *Ontario*
Qiang Liu, *Saskatoon*
John K Marshall, *Ontario*
Andrew L Mason, *Alberta*
Kostas Pantopoulos, *Quebec*
Nathalie Perreault, *Sherbrooke*
Baljinder Singh Salh, *Vancouver*
Eldon Shaffer, *Calgary*
Martin Storr, *Calgary*
Pingchang Yang, *Hamilton*
Eric M Yoshida, *Vancouver*
Claudia Zwingmann, *Montreal*

Chile

Marcelo A Beltran, *La Serena*
Xabier De Aretxabala, *Santiago*
Silvana Zanlungo, *Santiago*

China

Hui-Jie Bian, *Xi'an*
San-Jun Cai, *Shanghai*
Guang-Wen Cao, *Shanghai*
Xiao-Ping Chen, *Wuhan*
Chi-Hin Cho, *Hong Kong*
Zong-Jie Cui, *Beijing*
Jing-Yuan Fang, *Shanghai*
De-Liang Fu, *Shanghai*
Ze-Guang Han, *Shanghai*
Chun-Yi Hao, *Beijing*
Ming-Liang He, *Hong Kong*
Ching-Lung Lai, *Hong Kong*
Simon Law, *Hong Kong*
Yuk-Tong Lee, *Hong Kong*
En-Min Li, *Shantou*
Fei Li, *Beijing*
Yu-Yuan Li, *Guangzhou*
Zhao-Shen Li, *Shanghai*
Xing-Hua Lu, *Beijing*
Yi-Min Mao, *Shanghai*
Qin Su, *Beijing*
Paul Kwong-Hang Tam, *Hong Kong*
Yuk Him Tam, *Hong Kong*
Ren-Xiang Tan, *Nanjing*
Wei-Dong Tong, *Chongqing*
Eric WC Tse, *Hong Kong*
Fu-Sheng Wang, *Beijing*
Xiang-Dong Wang, *Shanghai*
Nathalie Wong, *Hong Kong*
Justin CY Wu, *Hong Kong*
Wen-Rong Xu, *Zhenjiang*
An-Gang Yang, *Xi'an*
Wei-Cheng You, *Beijing*
Chun-Qing Zhang, *Jinan*
Jian-Zhong Zhang, *Beijing*
Xiao-Peng Zhang, *Beijing*
Xuan Zhang, *Beijing*

Colombia

Germán Campuzano-Maya, *Medellín*

Croatia

Tamara Cacev, *Zagreb*
Marko Duvnjak, *Zagreb*

Cuba

Damian C Rodriguez, *Havana*

Czech

Jan Bures, *Hradec Kralove*
Milan Jirsa, *Praha*
Marcela Kopacova, *Hradec Kralove*
Pavel Trunečka, *Prague*

Denmark

Leif Percival Andersen, *Copenhagen*
Asbjørn M Drewes, *Aalborg*
Morten Frisch, *Copenhagen*
Jan Mollenhauer, *Odense*
Morten Hylander Møller, *Holte*
Søren Rafaelsen, *Vejle*
Jorgen Rask-Madsen, *Skodsborg*
Peer Wille-Jørgensen, *Copenhagen*

Ecuador

Fernando E Sempértegui, *Quito*

Egypt

Zeinab Nabil Ahmed, *Cairo*
Hussein M Atta, *El-Minia*

Estonia

Riina Salupere, *Tartu*
Tamara Vorobjova, *Tartu*

Finland

Saila Kauhanen, *Turku*

Thomas Kietzmann, *Oulu*
Kaija-Leena Kolho, *Helsinki*
Jukka-Pekka Mecklin, *Jyvaskyla*
Minna Nyström, *Helsinki*
Pauli Antero Puolakkainen, *Turku*
Juhani Sand, *Tampere*
Lea Veijola, *Helsinki*

France

Claire Bonithon-Kopp, *Dijon*
Lionel Bueno, *Toulouse*
Sabine Colnot, *Paris*
Catherine Daniel, *Lille Cedex*
Alexis Desmoulière, *Limoges*
Thabut Dominique, *Paris*
Francoise L Fabiani, *Angers*
Jean-Luc Faucheron, *Grenoble*
Jean Paul Galmiche, *Nantes cedex*
Boris Guiu, *Dijon*
Paul Hofman, *Nice*
Laurent Huwart, *Paris*
Juan Iovanna, *Marseille*
Abdel-Majid Khatib, *Paris*
Philippe Lehours, *Bordeaux*
Flavio Maina, *Marseille*
Patrick Marcellin, *Paris*
Rene Gerolami Santandera, *Marseille*
Annie Schmid-Alliana, *Nice cedex*
Alain L Servin, *Châtenay-Malabry*
Stephane Supiot, *Nantes*
Baumert F Thomas, *Strasbourg*
Jean-Jacques Tuech, *Rouen*
Frank Zerbib, *Bordeaux Cedex*

Germany

Erwin Biecker, *Siegburg*
Hubert Blum, *Freiburg*
Thomas Bock, *Tuebingen*
Dean Bogoevski, *Hamburg*
Elfriede Bollschweiler, *Köln*
Jürgen Borlak, *Hannover*
Christa Buechler, *Regensburg*
Jürgen Büning, *Lübeck*
Elke Cario, *Essen*
Bruno Christ, *Halle/Saale*
Christoph F Dietrich, *Bad Mergentheim*
Ulrich R Fölsch, *Kiel*
Nikolaus Gassler, *Aachen*
Markus Gerhard, *Munich*
Dieter Glebe, *Giessen*
Ralph Graeser, *Freiburg*
Axel M Gressner, *Aachen*
Nils Habbe, *Marburg*
Thilo Hackert, *Heidelberg*
Wolfgang Hagmann, *Heidelberg*
Dirk Haller, *Freising*
Philip D Hard, *Giessen*
Claus Hellerbrand, *Regensburg*
Klaus R Herrlinger, *Stuttgart*
Eberhard Hildt, *Berlin*
Andrea Hille, *Goettingen*
Joerg C Hoffmann, *Berlin*
Philipe N Khalil, *Munich*
Andrej Khandoga, *Munich*
Jorg Kleeff, *Munich*
Ingmar Königsrainer, *Tübingen*
Peter Konturek, *Erlangen*
Stefan Kubicka, *Hannover*
Joachim Labenz, *Siegen*
Michael Linnebacher, *Rostock*
Jutta Elisabeth Lüttges, *Riegelsberg*
Peter Malfertheiner, *Magdeburg*
Oliver Mann, *Hamburg*
Peter N Meier, *Hannover*
Sabine Mihm, *Göttingen*
Klaus Mönkemüller, *Bottrop*
Jonas Mudter, *Erlangen*
Sebastian Mueller, *Heidelberg*
Robert Obermaier, *Freiburg*
Matthias Ocker, *Erlangen*
Stephan Johannes Ott, *Kiel*
Gustav Paumgartner, *Munich*
Christoph Reichel, *Bad Brückenau*
Markus Reiser, *Bochum*
Steffen Rickes, *Magdeburg*
Elke Roeb, *Giessen*
Christian Rust, *Munich*
Hans Scherubl, *Berlin*
Martin K Schilling, *Homburg*
Joerg F Schlaak, *Essen*
Rene Schmidt, *Freiburg*
Andreas G Schreyer, *Regensburg*
Karsten Schulmann, *Bochum*
Henning Schulze-Bergkamen, *Mainz*
Manfred V Singer, *Mannheim*
Jens Standop, *Bonn*
Jurgen M Stein, *Frankfurt*
Ulrike S Stein, *Berlin*
Wolfgang R Stremmel, *Heidelberg*
Harald F Teutsch, *Ulm*
Hans L Tillmann, *Leipzig*
Christian Trautwein, *Aachen*
Joerg Trojan, *Frankfurt*
Arndt Vogel, *Hannover*
Siegfried Wagner, *Deggendorf*
Frank Ulrich Weiss, *Greifswald*
Fritz von Weizsäcker, *Berlin*
Thomas Wex, *Magdeburg*
Stefan Wirth, *Wuppertal*
Marty Zdichavsky, *Tübingen*

Greece

Helen Christopoulou-Aletra, *Thessaloniki*
T Choli-Papadopoulou, *Thessaloniki*
Tsianos Epameinondas, *Ioannina*
Ioannis Kanellos, *Thessaloniki*
Elias A Kouroumalis, *Heraklion*
Ioannis E Koutroubakis, *Heraklion*
Michael Koutsilieris, *Athens*
Andreas Larentzakis, *Athens*
Emanuel K Manesis, *Athens*
Spilios Manolakopoulos, *Athens*
Konstantinos Mimidis, *Alexandroupolis*
George Papatheodoridis, *Athens*
Spiros Sgouros, *Athens*
Evangelos Tsiambas, *Ag Paraskevi Attiki*

Hungary

György M Buzás, *Budapest*
László Czakó, *Szeged*
Gyula Farkas, *Szeged*
Peter Hegyi, *Szeged*
Peter L Lakatos, *Budapest*
Yvette Mándi, *Szeged*
Zoltan Rakonczay, *Szeged*
Ferenc Sipos, *Budapest*
Zsuzsa Szondy, *Debrecen*
Gabor Veres, *Budapest*

India

Philip Abraham, *Mumbai*
Vineet Ahuja, *New Delhi*
Giriraj Ratan Chandak, *Hyderabad*
Devinder Kumar Dhawan, *Chandigarh*
Radha K Dhiman, *Chandigarh*
Pankaj Garg, *Panchkula*
Pramod Kumar Garg, *New Delhi*
Debidas Ghosh, *Midnpore*
Uday C Ghoshal, *Lucknow*
Bhupendra Kumar Jain, *Delhi*
Ashok Kumar, *Lucknow*
Bikash Medhi, *Chandigarh*
Sri P Misra, *Allahabad*
Gopal Nath, *Varanasi*
Samiran Nundy, *New Delhi*
Jagannath Palepu, *Mumbai*
Vandana Panda, *Mumbai*
Benjamin Perakath, *Tamil Nadu*
Ramesh Roop Rai, *Jaipur*
Nageshwar D Reddy, *Hyderabad*
Barjesh Chander Sharma, *New Delhi*
Virendra Singh, *Chandigarh*
Rupjyoti Talukdar, *Guwahati*
Rakesh Kumar Tandon, *New Delhi*
Jai Dev Wig, *Chandigarh*

Iran

Mohammad Abdollahi, *Tehran*
Peyman Adibi, *Isfahan*
Seyed-Moayed Alavian, *Tehran*
Seyed Mohsen Dehghani, *Shiraz*
Reza Malekzadeh, *Tehran*
Alireza Mani, *Tehran*

Ireland

Billy Bourke, *Dublin*
Ted Dinan, *Cork*
Catherine Greene, *Dublin*
Ross McManus, *Dublin*
Anthony P Moran, *Galway*
Marion Rowland, *Dublin*

Israel

Simon Bar-Meir, *Hashomer*
Alexander Becker, *Afula*
Abraham R Eliakim, *Haifa*
Sigal Fishman, *Tel Aviv*
Boris Kirshtein, *Beer Sheva*
Eli Magen, *Ashdod*
Menachem Moshkowitz, *Tel-Aviv*
Assy Nimer, *Safed*
Shmuel Odes, *Beer Sheva*
Mark Pines, *Bet Dagan*
Ron Shaoul, *Haifa*
Ami D Sperber, *Beer-Sheva*

Italy

Donato F Altomare, *Bari*
Piero Amodio, *Padova*
Angelo Andriulli, *San Giovanni Rotondo*
Paolo Angeli, *Padova*
Bruno Annibale, *Rome*
Paolo Aurello, *Rome*
Salvatore Auricchio, *Naples*
Antonio Basoli, *Rome*
Claudio Bassi, *Verona*
Gabrio Bassotti, *Perugia*
Mauro Bernardi, *Bologna*
Alberto Biondi, *Rome*
Luigi Bonavina, *Milano*
Guglielmo Borgia, *Naples*
Roberto Berni Canani, *Naples*
Maria Gabriella Caruso, *Bari*
Fausto Catena, *Bologna*
Giuseppe Chiarioni, *Valeggio*
Michele Cicala, *Rome*
Dario Conte, *Milano*
Francesco Costa, *Pisa*
Antonio Craxì, *Palermo*
Salvatore Cucchiara, *Rome*
Giuseppe Currò, *Messina*
Mario M D'Elios, *Florence*
Mirko D'Onofrio, *Verona*
Silvio Danese, *Milano*
Roberto de Franchis, *Milano*
Paola De Nardi, *Milan*
Giovanni D De Palma, *Naples*
Giuliana Decorti, *Trieste*
Gianlorenzo Dionigi, *Varese*
Massimo Falconi, *Verona*
Silvia Fargion, *Milan*
Giammarco Fava, *Ancona*
Francesco Feo, *Sassari*
Alessandra Ferlini, *Ferrara*
Alessandro Ferrero, *Torino*
Mirella Fraquelli, *Milan*
Luca Frulloni, *Verona*
Giovanni B Gaeta, *Napoli*
Antonio Gasbarrini, *Rome*
Edoardo G Giannini, *Genoa*
Alessandro Granito, *Bologna*
Fabio Grizzi, *Milan*
Salvatore Gruttadauria, *Palermo*
Pietro Invernizzi, *Milan*
Achille Iolascon, *Naples*
Angelo A Izzo, *Naples*
Ezio Laconi, *Cagliari*
Giovanni Latella, *L'Aquila*
Massimo Levrero, *Rome*
Francesco Luzza, *Catanzaro*
Lucia Malaguarnera, *Catania*
Francesco Manguso, *Napoli*
Pier Mannuccio Mannucci, *Milan*
Giancarlo Mansueto, *Verona*
Giulio Marchesini, *Bologna*
Mara Massimi, *Coppito*
Giovanni Milito, *Rome*
Giuseppe Montalto, *Palermo*
Giovanni Monteleone, *Rome*
Luca Morelli, *Trento*
Giovanni Musso, *Torino*
Mario Nano, *Torino*
Gerardo Nardone, *Napoli*
Riccardo Nascimbeni, *Brescia*
Valerio Nobili, *Rome*
Fabio Pace, *Milan*
Nadia Peparini, *Rome*
Marcello Persico, *Naples*
Mario Pescatori, *Rome*
Raffaele Pezzilli, *Bologna*
Alberto Piperno, *Monza*
Anna C Piscaglia, *Rome*
Piero Portincasa, *Bari*
Michele Reni, *Milan*
Vittorio Ricci, *Pavia*
Oliviero Riggio, *Rome*
Mario Rizzetto, *Torino*
Ballarin Roberto, *Modena*
Gerardo Rosati, *Potenza*
Franco Roviello, *Siena*
Cesare Ruffolo, *Treviso*
Massimo Rugge, *Padova*
Marco Scarpa, *Padova*
C armelo Scarpignato, *Parma*
Giuseppe Sica, *Rome*
Marco Silano, *Rome*
Pierpaolo Sileri, *Rome*
Vincenzo Stanghellini, *Bologna*
Fiorucci Stefano, *Perugia*
Giovanni Tarantino, *Naples*
Alberto Tommasini, *Trieste*
Guido Torzilli, *Rozzano Milan*
Cesare Tosetti, *Porretta Terme*
Antonello Trecca, *Rome*
Vincenzo Villanacci, *Brescia*
Lucia Ricci Vitiani, *Rome*
Marco Vivarelli, *Bologna*

Japan

Kyoichi Adachi, *Izumo*
Yasushi Adachi, *Sapporo*
Takafumi Ando, *Nagoya*
Akira Andoh, *Otsu*
Masahiro Arai, *Tokyo*
Hitoshi Asakura, *Tokyo*
Kazuo Chijiiwa, *Miyazaki*
Yuichiro Eguchi, *Saga*
Itaru Endo, *Yokohama*
Munechika Enjoji, *Fukuoka*
Yasuhiro Fujino, *Akashi*
Mitsuhiro Fujishiro, *Tokyo*
Kouhei Fukushima, *Sendai*
Masanori Hatakeyama, *Tokyo*
Keiji Hirata, *Kitakyushu*
Toru Hiyama, *Higashihiroshima*
Masahiro Iizuka, *Akita*
Susumu Ikehara, *Osaka*
Kenichi Ikejima, *Bunkyo-ku*
Yutaka Inagaki, *Kanagawa*
Hiromi Ishibashi, *Nagasaki*
Shunji Ishihara, *Izumo*
Toru Ishikawa, *Niigata*
Toshiyuki Ishiwata, *Tokyo*
Hajime Isomoto, *Nagasaki*
Yoshiaki Iwasaki, *Okayama*
Satoru Kakizaki, *Gunma*
Terumi Kamisawa, *Tokyo*
Mototsugu Kato, *Sapporo*
Naoya Kato, *Tokyo*
Takumi Kawaguchi, *Kurume*
Yohei Kida, *Kainan*
Shogo Kikuchi, *Aichi*
Tsuneo Kitamura, *Chiba*
Takashi Kobayashi, *Tokyo*
Yasuhiro Koga, *Isehara*
Takashi Kojima, *Sapporo*
Norihiro Kokudo, *Tokyo*
Masatoshi Kudo, *Osaka*
Shin Maeda, *Tokyo*
Satoshi Mamori, *Hyogo*
Atsushi Masamune, *Sendai*
Yasushi Matsuzaki, *Tsukuba*
Kenji Miki, *Tokyo*
Toshihiro Mitaka, *Sapporo*
Hiroto Miwa, *Hyogo*
Kotaro Miyake, *Tokushima*
Manabu Morimoto, *Yokohama*
Yoshiharu Motoo, *Kanazawa*
Yoshiaki Murakami, *Hiroshima*
Yoshiki Murakami, *Kyoto*
Kunihiko Murase, *Tusima*
Akihito Nagahara, *Tokyo*
Yuji Naito, *Kyoto*
Atsushi Nakajima, *Yokohama*
Hisato Nakajima, *Tokyo*
Hiroki Nakamura, *Yamaguchi*
Shotaro Nakamura, *Fukuoka*
Akimasa Nakao, *Nagogya*
Shuhei Nishiguchi, *Hyogo*
Mikio Nishioka, *Niihama*
Keiji Ogura, *Tokyo*
Susumu Ohmada, *Maebashi*
Hirohide Ohnishi, *Akita*
Kenji Okajima, *Nagoya*
Kazuichi Okazaki, *Osaka*
Morikazu Onji, *Ehime*
Satoshi Osawa, *Hamamatsu*
Hidetsugu Saito, *Tokyo*
Yutaka Saito, *Tokyo*
Naoaki Sakata, *Sendai*
Yasushi Sano, *Chiba*
Tokihiko Sawada, *Tochigi*
Tomohiko Shimatan, *Hiroshima*
Yukihiro Shimizu, *Kyoto*
Shinji Shimoda, *Fukuoka*
Yoshio Shirai, *Niigata*
Masayuki Sho, *Nara*
Shoichiro Sumi, *Kyoto*
Hidekazu Suzuki, *Tokyo*
Masahiro Tajika, *Nagoya*
Yoshihisa Takahashi, *Tokyo*
Toshinari Takamura, *Kanazawa*
Hiroaki Takeuchi, *Kochi*
Yoshitaka Takuma, *Okayama*
Akihiro Tamori, *Osaka*
Atsushi Tanaka, *Tokyo*
Shinji Tanaka, *Hiroshima*
Satoshi Tanno, *Hokkaido*
Shinji Togo, *Yokohama*
Hitoshi Tsuda, *Tokyo*
Hiroyuki Uehara, *Osaka*
Masahito Uemura, *Kashihara*
Yoshiyuki Ueno, *Sendai*
Mitsuyoshi Urashima, *Tokyo*
Takuya Watanabe, *Niigata*
Satoshi Yamagiwa, *Niigata*
Taketo Yamaguchi, *Chiba*
Mitsunori Yamakawa, *Yamagata*
Takayuki Yamamoto, *Yokkaichi*
Yutaka Yata, *Maebashi*
Hiroshi Yoshida, *Tokyo*
Norimasa Yoshida, *Kyoto*
Yuichi Yoshida, *Osaka*
Kentaro Yoshika, *Toyoake*
Hitoshi Yoshiji, *Nara*
Katsutoshi Yoshizato, *Higashihiroshima*
Tomoharu Yoshizumi, *Fukuoka*

Jordan

Ismail Matalka, *Irbid*

Kuwait

Islam Khan, *Safat*

Lebanon

Bassam N Abboud, *Beirut*
Ala I Sharara, *Beirut*
Rita Slim, *Beirut*

Lithuania

Giedrius Barauskas, *Kaunas*
Limas Kupcinskas, *Kaunas*

Malaysia

Andrew Seng Boon Chua, *Ipoh*

Mexico

Richard A Awad, *Mexico*
Aldo Torre Delgadillo, *Mexico*
Diego Garcia-Compean, *Monterrey*
Paulino M Hernández Magro, *Celaya*
Miguel Angel Mercado, *Distrito Federal*
Arturo Panduro, *Jalisco*
Omar Vergara-Fernandez, *Tlalpan*
Saúl Villa-Trevio, *Mexico*

Moldova

Igor Mishin, *Kishinev*

Netherlands

Ulrich Beuers, *Amsterdam*
Lee Bouwman, *Leiden*
Albert J Bredenoord, *Nieuwegein*
Lodewijk AA Brosens, *Utrecht*
J Bart A Crusius, *Amsterdam*
Wouter de Herder, *Rotterdam*
Pieter JF de Jonge, *Rotterdam*
Robert J de Knegt, *Rotterdam*
Wendy W Johanna de Leng, *Utrecht*
Annemarie de Vries, *Rotterdam*
James CH Hardwick, *Leiden*
Frank Hoentjen, *Haarlem*
Misha Luyer, *Sittard*
Jeroen Maljaars, *Maastricht*
Gerrit A Meijer, *Amsterdam*
Servaas Morré, *Amsterdam*
Chris JJ Mulder, *Amsterdam*
John Plukker, *Groningen*
Albert Frederik Pull ter Gunne, *Tilburg*
Paul E Sijens, *Groningen*
BW Marcel Spanier, *Arnhem*
Shiri Sverdlov, *Maastricht*
Maarten Tushuizen, *Amsterdam*
Jantine van Baal, *Heidelberglaan*
Astrid van der Velde, *The Hague*
Karel van Erpecum, *Utrecht*
Loes van Keimpema, *Nijmegen*
Robert Christiaan Verdonk, *Groningen*
Erwin G Zoetendal, *Wageningen*

New Zealand

Andrew S Day, *Christchurch*

Norway

Olav Dalgard, *Oslo*
Trond Peder Flaten, *Trondheim*
Reidar Fossmark, *Trondheim*
Rasmus Goll, *Tromso*
Ole Høie, *Arendal*
Asle W Medhus, *Oslo*
Espen Melum, *Oslo*
Trine Olsen, *Tromso*
Eyvind J Paulssen, *Tromso*
Jon Arne Søreide, *Stavanger*
Kjetil Soreide, *Stavanger*

Pakistan

Shahab Abid, *Karachi*
Syed MW Jafri, *Karachi*

Poland

Marek Bebenek, *Wroclaw*
Tomasz Brzozowski, *Cracow*
Halina Cichoż-Lach, *Lublin*
Andrzej Dabrowski, *Bialystok*
Hanna Gregorek, *Warsaw*
Marek Hartleb, *Katowice*
Beata Jolanta Jabłońska, *Katowice*
Stanislaw J Konturek, *Krakow*
Jan Kulig, *Krakow*
Dariusz M Lebensztejn, *Bialystok*
Julian Swierczynski, *Gdansk*

Portugal

Raquel Almeida, *Porto*
Ana Isabel Lopes, *Lisboa Codex*
Ricardo Marcos, *Porto*
Guida Portela-Gomes, *Estoril*

Romania

Dan L Dumitrascu, *Cluj*
Adrian Saftoiu, *Craiova*
Andrada Seicean, *Cluj-Napoca*

Russia

Vasiliy I Reshetnyak, *Moscow*

Saudi Arabia

Ibrahim A Al Mofleh, *Riyadh*
Abdul-Wahed Meshikhes, *Qatif*
Faisal Sanai, *Riyadh*

Serbia

Tamara M Alempijevic, *Belgrade*
Dusan M Jovanovic, *Sremska Kamenica*
Zoran Krivokapic, *Belgrade*

Singapore

Madhav Bhatia, *Singapore*
Kong Weng Eu, *Singapore*
Brian Kim Poh Goh, *Singapore*
Khek-Yu Ho, *Singapore*
Kok Sun Ho, *Singapore*
Fock Kwong Ming, *Singapore*
London Lucien Ooi, *Singapore*
Nagarajan Perumal, *Singapore*
Francis Seow-Choen, *Singapore*

South Africa

Rosemary Joyce Burnett, *Pretoria*
Michael Kew, *Cape Town*

South Korea

Sang Hoon Ahn, *Seoul*
Sung-Gil Chi, *Seoul*
Myung-Gyu Choi, *Seoul*
Hoon Jai Chun, *Seoul*
Yeun-Jun Chung, *Seoul*
Young-Hwa Chung, *Seoul*
Kim Donghee, *Seoul*
Ki-Baik Hahm, *Incheon*
Sun Pyo Hong, *Geonggi-do*
Seong Gyu Hwang, *Seongnam*
Hong Joo Kim, *Seoul*
Jae J Kim, *Seoul*
Jin-Hong Kim, *Suwon*
Nayoung Kim, *Seongnam-si*
Sang Geon Kim, *Seoul*
Seon Hahn Kim, *Seoul*
Sung Kim, *Seoul*
Won Ho Kim, *Seoul*
Jeong Min Lee, *Seoul*
Kyu Taek Lee, *Seoul*
Sang Kil Lee, *Seoul*
Sang Yeoup Lee, *Gyeongsangnam-do*
Yong Chan Lee, *Seoul*
Eun-Yi Moon, *Seoul*
Hyoung-Chul Oh, *Seoul*
Seung Woon Paik, *Seoul*
Joong-Won Park, *Goyang*
Ji Kon Ryu, *Seoul*
Si Young Song, *Seoul*
Marie Yeo, *Suwon*
Byung Chul Yoo, *Seoul*
Dae-Yeul Yu, *Daejeon*

Spain

Maria-Angeles Aller, *Madrid*
Raul J Andrade, *Málaga*
Luis Aparisi, *Valencia*
Gloria González Aseguinolaza, *Navarra*
Matias A Avila, *Pamplona*

Fernando Azpiroz, *Barcelona*
Ramon Bataller, *Barcelona*
Belén Beltrán, *Valencia*
Adolfo Benages, *Valencia*
Josep M Bordas, *Barcelona*
Lisardo Boscá, *Madrid*
Luis Bujanda, *San Sebastián*
Juli Busquets, *Barcelona*
Matilde Bustos, *Pamplona*
José Julián calvo Andrés, *Salamanca*
Andres Cardenas, *Barcelona*
Antoni Castells, *Barcelona*
Fernando J Corrales, *Pamplona*
J E Domínguez-Muñoz, *Santiago de Compostela*
Juan Carlos Laguna Egea, *Barcelona*
Isabel Fabregat, *Barcelona*
Antoni Farré, *Barcelona*
Vicente Felipo, *Valencia*
Laureano Fernández-Cruz, *Barcelona*
Luis Grande, *Barcelona*
Angel Lanas, *Zaragoza*
Juan-Ramón Larrubia, *Guadalajara*
María IT López, *Jaén*
Juan Macías, *Seville*
Javier Martin, *Granada*
José Manuel Martin-Villa, *Madrid*
Julio Mayol, *Madrid*
Mireia Miquel, *Sabadell*
Albert Parés, *Barcelona*
Jesús M Prieto, *Pamplona*
Pedro L Majano Rodriguez, *Madrid*
Joan Roselló-Catafau, *Barcelona*
Eva Vaquero, *Barcelona*

Sweden

Lars Erik Agréus, *Stockholm*
Mats Andersson, *Stockholm*
Roland Andersson, *Lund*
Mauro D'Amato, *Huddinge*
Evangelos Kalaitzakis, *Gothenburg*
Greger Lindberg, *Stockholm*
Annika Lindblom, *Stockholm*
Sara Lindén, *Göteborg*
Hanns-Ulrich Marschall, *Stockholm*
Pär Erik Myrelid, *Linköping*
Åke Nilsson, *Lund*
Helena Nordenstedt, *Stockholm*
Kjell Öberg, *Uppsala*
Lars A Pahlman, *Uppsala*
Stefan G Pierzynowski, *Lund*
Sara Regnér, *Malmö*
Bobby Tingstedt, *Lund*
Zongli Zheng, *Stockholm*

Switzerland

Pascal Bucher, *Geneva*
Michelangelo Foti, *Geneva*
Jean L Frossard, *Geneva*
Andreas Geier, *Zürich*
Pascal Gervaz, *Geneva*
Gerd A Kullak-Ublick, *Zürich*
Fabrizio Montecucco, *Geneva*
Paul M Schneider, *Zürich*
Felix Stickel, *Berne*
Bruno Stieger, *Zürich*
Inti Zlobec, *Basel*

Trinidad and Tobago

Shivananda Nayak, *Mount Hope*

Turkey

Sinan Akay, *Tekirdag*
Metin Basaranoglu, *Istanbul*
Yusuf Bayraktar, *Ankara*
A Mithat Bozdayi, *Ankara*
Hayrullah Derici, *Balıkesir*
Eren Ersoy, *Ankara*
Mukaddes Esrefoglu, *Malatya*
Can Goen, *Kutahya*
Selin Kapan, *Istanbul*
Aydin Karabacakoglu, *Konya*
Cuneyt Kayaalp, *Malatya*
Kemal Kismet, *Ankara*
Seyfettin Köklü, *Ankara*
Mehmet Refik Mas, *Etlik-Ankara*
Osman C Ozdogan, *Istanbul*
Bülent Salman, *Ankara*
Orhan Sezgin, *Mersin*
Ilker Tasci, *Ankara*
Müge Tecder-Ünal, *Ankara*
Ahmet Tekin, *Mersin*
Mesut Tez, *Ankara*
Ekmel Tezel, *Ankara*
Özlem Yilmaz, *Izmir*

United Arab Emirates

Fikri M Abu-Zidan, *Al-Ain*
Sherif M Karam, *Al-Ain*

United Kingdom

Simon Afford, *Birmingham*
Navneet K Ahluwalia, *Stockport*
Mohamed H Ahmed, *Southampton*
Basil Ammori, *Salford*
Lesley A Anderson, *Belfast*
Chin Wee Ang, *Liverpool*
Yeng S Ang, *Wigan*
Anthony TR Axon, *Leeds*
Kathleen B Bamford, *London*
Jim D Bell, *London*
John Beynon, *Swansea*
Chris Briggs, *Sheffield*
Geoffrey Burnstock, *London*
Alastair D Burt, *Newcastle*
Jeff Butterworth, *Shrewsbury*
Jeremy FL Cobbold, *London*
Jean E Crabtree, *Leeds*
Tatjana Crnogorac-Jurcevic, *London*
William Dickey, *Londonderry*
Sunil Dolwani, *Cardiff*
Emad M El-Omar, *Aberdeen*
A M El-Tawil, *Birmingham*
Charles B Ferguson, *Belfast*
Andrew Fowell, *Southampton*
Piers Gatenby, *London*
Daniel R Gaya, *Edinburgh*
Anil George, *London*
Rob Glynne-Jones, *Northwood*
Jason CB Goh, *Birmingham*
Gianpiero Gravante, *Leicester*
Brian Green, *Belfast*
William Greenhalf, *Liverpool*
Indra N Guha, *Nottingham*
Stefan G Hübscher, *Birmingham*
Robin Hughes, *London*
Pali Hungin, *Stockton*
Nawfal Hussein, *Nottingham*
Clement W Imrie, *Glasgow*
Janusz AZ Jankowski, *Oxford*
Sharad Karandikar, *Birmingham*
Peter Karayiannis, *London*
Shahid A Khan, *London*
Patricia F Lalor, *Birmingham*
John S Leeds, *Sheffield*
Ian Lindsey, *Oxford*
Hong-Xiang Liu, *Cambridge*
Dileep N Lobo, *Nottingham*
Graham MacKay, *Glasgow*
Mark Edward McAlindon, *Sheffield*
Anne McCune, *Bristol*
Donald Campbell McMillan, *Glasgow*
Giorgina Mieli-Vergani, *London*
Jamie Murphy, *London*
Guy Fairbairn Nash, *Poole*
James Neuberger, *Birmingham*
Patrick O'Dwyer, *Glasgow*
Christos Paraskeva, *Bristol*
Richard Parker, *North Staffordshire*
Thamara Perera, *Birmingham*
Kondragunta Rajendra Prasad, *Leeds*
D Mark Pritchard, *Liverpool*
Alberto Quaglia, *London*
Akhilesh B Reddy, *Cambridge*
Kevin Robertson, *Glasgow*
Sanchoy Sarkar, *Liverpool*
John B Schofield, *Kent*
Marco Senzolo, *Padova*
Venkatesh Shanmugam, *Derby*
Paul Sharp, *London*
Chew Thean Soon, *Manchester*
Aravind Suppiah, *East Yorkshire*
Noriko Suzuki, *Middlesex*
Simon D Taylor-Robinson, *London*
Frank I Tovey, *London*
A McCulloch Veitch, *Wolverhampton*
Vamsi R Velchuru, *Lowestoft*
Sumita Verma, *Brighton*
Catherine Walter, *Cheltenham*
Julian RF Walters, *London*
Roger Williams, *London*

United States

Kareem M Abu-Elmagd, *Pittsburgh*
Sami R Achem, *Florida*
Golo Ahlenstiel, *Bethesda*
Bhupinder S Anand, *Houston*
M Ananthanarayanan, *New York*
Balamurugan N Appakalal, *Minneapolis*
Dimitrios V Avgerinos, *New York*
Shashi Bala, *Worcester*
Anthony J Bauer, *Pittsburgh*
Kevin E Behrns, *Gainesville*
Roberto Bergamaschi, *New York*
Henry J Binder, *New Haven*
Edmund J Bini, *New York*
Wojciech Blonski, *Philadelphia*
Mark Bloomston, *Columbus*
Edward L Bradley III, *Sarasota*
Carla W Brady, *Durham*

World Journal of Gastroenterology

Contents

Weekly Volume 17 Number 23 June 21, 2011

Contents

World Journal of Gastroenterology
Volume 17 Number 23 June 21, 2011

World Journal of Gastroenterology
Volume 17 Number 23 June 21, 2011

Contents

NAME OF JOURNAL
World Journal of Gastroenterology

LAUNCH DATE
October 1, 1995

RESPONSIBLE INSTITUTION
Department of Science and Technology of Shanxi Province

SPONSOR
Taiyuan Research and Treatment Center for Digestive Diseases, 77 Shuangta Xijie, Taiyuan 030001, Shanxi Province, China

EDITING
Editorial Board of *World Journal of Gastroenterology*
Room 903, Building D, Ocean International Center,
No. 62 Dongsihuan Zhonglu, Chaoyang District,
Beijing 100025, China
Telephone: +86-10-5908-0039
Fax: +86-10-8538-1893
E-mail: wjg@wjgnet.com
http://www.wjgnet.com

PUBLISHING
Baishideng Publishing Group Co., Limited
Room 1701, 17/F, Henan Building,
No.90 Jaffe Road, Wanchai, Hong Kong, China
Fax: +852-3115-8812
Telephone: +852-5804-2046
E-mail: baishideng@wjgnet.com
http://www.wjgnet.com

SUBSCRIPTION
Beijing Baishideng BioMed Scientific Co., Ltd.
Room 903, Building D, Ocean International Center,
No. 62 Dongsihuan Zhonglu, Chaoyang District,
Beijing 100025, China
Telephone: +86-10-8538-1892
Fax: +86-10-8538-1893
E-mail: baishideng@wjgnet.com
http://www.wjgnet.com

PRINT SUBSCRIPTION
RMB 245 Yuan for each issue, RMB 11760 Yuan for one year.

PUBLICATION DATE
June 21, 2011

ISSN AND EISSN
ISSN 1007-9327 (print)
ISSN 2219-2840 (online)

EDITORIAL OFFICE
Jian-Xia Cheng, Director
World Journal of Gastroenterology
Room 903, Building D, Ocean International Center,
No. 62 Dongsihuan Zhonglu, Chaoyang District,
Beijing 100025, China
Telephone: +86-10-5908-0039
Fax: +86-10-8538-1893
E-mail: wjg@wjgnet.com
http://www.wjgnet.com

INSTRUCTIONS TO AUTHORS
Full instructions are available online at http://www.wjgnet.com/1007-9327/g_info_20100315215714.htm.

ONLINE SUBMISSION
http://www.wjgnet.com/1007-9327office

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/1007-9327office
wjg@wjgnet.com
doi:10.3748/wjg.v17.i23.2781

World J Gastroenterol 2011 June 21; 17(23): 2781-2790
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

EDITORIAL

Targeting key signalling pathways in oesophageal adenocarcinoma: A reality for personalised medicine?

Richard R Keld, Yeng S Ang

Richard R Keld, Yeng S Ang, Department of Gastroenterology, Royal Albert Edward Infirmary, Wigan Lane, Wigan, Greater Manchester, WN1 2NN, United Kingdom
Richard R Keld, Yeng S Ang, School of Translational Medicine, Faculty of Medical and Human Sciences, The University of Manchester, Manchester, M13 9PL, United Kingdom
Author contributions: Keld RR reviewed and searched the literature; Ang YS conceived the idea and revised this paper.
Supported by UK National Institute of Health Research/Cancer Research Network (UK NIHR/UKCRN) and Research and Development Department of Wrightington Wigan and Leigh NHS Foundation Trust (to Ang YS); R Keld Wrightington Wigan and Leigh NHS Foundation Trust Cancer Therapy Fund (to Keld RR, in part)
Correspondence to: Dr. Yeng S Ang, MD, FRCP, FRCPI, FEBG, Consultant Gastroenterologist/Honorary Senior Lecturer, Department of Gastroenterology, Royal Albert Edward Infirmary, Wigan Lane, Wigan, Greater Manchester WN1 2NN, United Kingdom. yeng.ang@wwl.nhs.uk
Telephone: +44-1942-773119 Fax: +44-1942-822340
Received: April 17, 2010 Revised: July 20, 2010
Accepted: July 27, 2010
Published online: June 21, 2011

Abstract

Cancer treatments are rapidly changing. Curative treatment for oesophageal adenocarcinoma currently involves surgery and cytotoxic chemotherapy or chemoradiotherapy. Outcomes for both regimes are generally poor as a result of tumor recurrence. We have reviewed the key signalling pathways associated with oesophageal adenocarcinomas and discussed the recent trials of novel agents that attempt to target these pathways. There are many trials underway with the aim of improving survival in oesophageal cancer. Currently, phase 2 and 3 trials are focused on MAP kinase inhibition, either through inhibition of growth factor receptors or signal transducer proteins. In order to avoid tumor resistance, it appears to be clear that targeted therapy will be needed to combat the multiple signalling pathways that are in operation in oesophageal adenocarcinomas. This may be achievable in the future with the advent of gene signatures and a combinatorial approach.

Key words: Oesophageal adenocarcinoma; Signalling pathways; MAP and PI3 Kinase pathways; Wnt signalling; Transforming growth factor-β pathway; Nuclear factor-κB pathways; Transcription factors; Tyrosine kinase receptors

Peer reviewers: David Ian Watson, Professor, Head, Flinders University Department of Surgery, Room 3D211, Flinders Medical Center, Bedford Park, South Australia 5042, Australia; Marco Giuseppe Patti, MD, Professor of Surgery, Director, Center for Esophageal Diseases, University of Chicago Pritzker School of Medicine, 5841 S. Maryland Avenue, MC 5095, Room G 201, Chicago, IL 60637, United States

Keld RR, Ang YS. Targeting key signalling pathways in oesophageal adenocarcinoma: A reality for personalised medicine? *World J Gastroenterol* 2011; 17(23): 2781-2790 Available from: URL: http://www.wjgnet.com/1007-9327/full/v17/i23/2781.htm DOI: http://dx.doi.org/10.3748/wjg.v17.i23.2781

INTRODUCTION

Oesophageal adenocarcinoma is the 10th commonest malignancy in the UK yet it is the 5th commonest cause of cancer death[1]. This poor prognosis is partly attributable to a disease afflicting an elderly population. All too often the disease presents with symptoms of dysphagia which usually heralds advanced disease, typically with lymph node or distant metastases[2]. The 5 year survival, despite recent advances in neo-adjuvant chemotherapy, radiotherapy and surgery is approximately 25%. The incidence has been steadily increasing over the past 30 years[3-5]; this is thought to be due to the trend of an aging and increasingly obese population in combination with *Helicobacter pylori* eradication[1,6,7]. Barrett's oesophagus has been established

as a clear risk factor for oesophageal adenocarcinoma[8]. It has been demonstrated that surveillance of patients with Barrett's oesophagus can identify early stage adenocarcinomas[9,10]. If diagnosed at an early stage, with the disease confined to the submucosa, 5 year survival rates are as high as 90%[11]. Unfortunately current strategies for surveillance of Barrett's oesophagus are insufficient to reduce the incidence of oesophageal adenocarcinoma and most cases are diagnosed in patients that are not on Barrett's surveillance programs[10]. This may be accounted for by the fact that a significant proportion of patients with Barrett's oesophagus are asymptomatic. Currently, it is not economically viable to screen the whole population for Barrett's oesophagus[12]. Until this is addressed, there does not seem to be a solution to providing an early diagnosis of oesophageal adenocarcinoma for the majority of patients. This indicates the importance of developing improved treatments for advanced disease.

CURRENT MEDICAL TREATMENTS

The medical therapies in mainstream use for the treatment of oesophageal and junctional adenocarcinomas are cytotoxic and antimetabolite agents. They target rapidly dividing cells in an non cancer cell specific manner[13]. 5-Fluorouracil (5-FU) inhibits DNA synthesis through inhibition of thymidylate synthetase[14]. The platinum agents cisplatin and oxaliplatin form DNA adducts and cross-links which prevents DNA transcription and replication[15]. The anthracyclines epirubicin and doxorubicin induce DNA damage and inhibit DNA transcription through inhibition of topoisomerase Ⅱ and DNA helicase activity[16]. The cytotoxic action of taxanes are predominantly due to disruption of microtubules[17].

Cytotoxic chemotherapy is generally not very effective and side effects are common. The agents are usually contraindicated in severe cardiac and liver disease, a common occurrence in the affected elderly population. Recent advances have been made with the route of administration. A tablet form is now available, capecitabine, which is an effective alternative to infusing 5-FU. This reduces the morbidity associated with central venous catheterisation. Furthermore, oxaliplatin appears to be less toxic and more potent than cisplatin, and it can be infused over a shorter period of time[18,19]. Approximately 30% of patients with oesophageal adenocarcinoma are offered palliative chemotherapy and radiotherapy[1,20]. Prognosis is only 6-11 mo[21,22], with a 5 year survival of 4%[22]. Surgery is beneficial in patients that present with disease localised to the oesophagus or with localised lymph node metastases. Neo-adjuvant chemotherapy modestly improves survival compared to surgery alone; 5 year survival is 23% compared to 36% with neo-adjuvant chemotherapy[23]. On subgroup analysis, patients with tumors at the gastro-oesophageal junction seemed to benefit the most and this regimen is offered in the UK[24]. In the USA, the protocol of neo-adjuvant chemo-radiotherapy is favoured[25]. 5 year survival is 8%-20% in selected patients. Curative chemo-radiation is an alternative strategy to surgery and prognosis is similar[2]. This may be due to the avoidance of postoperative mortality and morbidity. Whatever regimen is used, the poor prognosis for oesophageal adenocarcinoma is largely a result of disease recurrence and the morbidity surrounding major surgery[2]. Treatment failure is thought to be a consequence of the blanket therapy approach due to the nature of the non-specific or non-targeted mechanism of action of the medical agents described earlier. Recent evidence suggests that standard chemotherapy and radiotherapy activate signalling pathways that stimulate growth and resistance of cancer cells[26]. Prognosis may improve with agents that specifically target mitogenic signalling pathways and intense research is currently underway.

The aim of this review is to explore the key signalling pathways that are associated with oesophageal adenocarcinoma and review the clinical trials of novel therapeutic agents that are in progress. We will draw parallels from breast and colon cancer. We will address the question: by targeting key signalling pathways is personalised medicine a reality in oesophageal adenocarcinoma?

DEFINING SIGNALLING PATHWAYS

Signalling pathways are essential components in all cells; they are important to stimulate cell growth, proliferation, differentiation, invasion and apoptosis. Certain pathways are specifically important in embryonic development, inflammation and carcinogenesis. Signalling pathways have common mechanisms of action. They convey extra-cellular stimuli, usually *via* cell surface receptors, onto a chain of signal transducer proteins which subsequently enter the nucleus. In the nucleus the signalling proteins activate transcriptional machinery on gene promoters. Gene expression and cell phenotype are altered. In the context of cancer cells, phenotypic change may include cell growth, cell division, increased cell motility, evasion of apoptosis and sustained angiogenesis. These changes constitute the hallmarks of cancer[27]. Signalling pathways in cancer cells are usually unregulated and resistant to feedback inhibition, and this usually occurs as a consequence of sustained activation from their components. The components are commonly known as oncogenes or tumor suppressor genes[28]. Oncogenes and tumor suppressor genes are usually expressed as a result of genetic mutations. In oesophageal cancer, mutations occur as a consequence of DNA damage from bile or acid reflux, nitric oxide, alcohol and cigarette smoking. Mutations usually involve chromosomal translocations of oncogenes or tumor suppressor genes onto housekeeping genes or other genes undergoing active transcription[29,30]. This culminates in persistent activation or inhibition of specific signalling pathways. Components of signalling pathways can be potentially inhibited at a variety of levels. Inhibitors can target the cell surface receptor, signal transducer proteins or even transcription factors. Unfortunately multiple pathways and receptors are associated with oesophageal cancer and the complexity that exists between different pathways is likened to a computer circuit (Figure 1). Blockade of one pathway or component may not be sufficient.

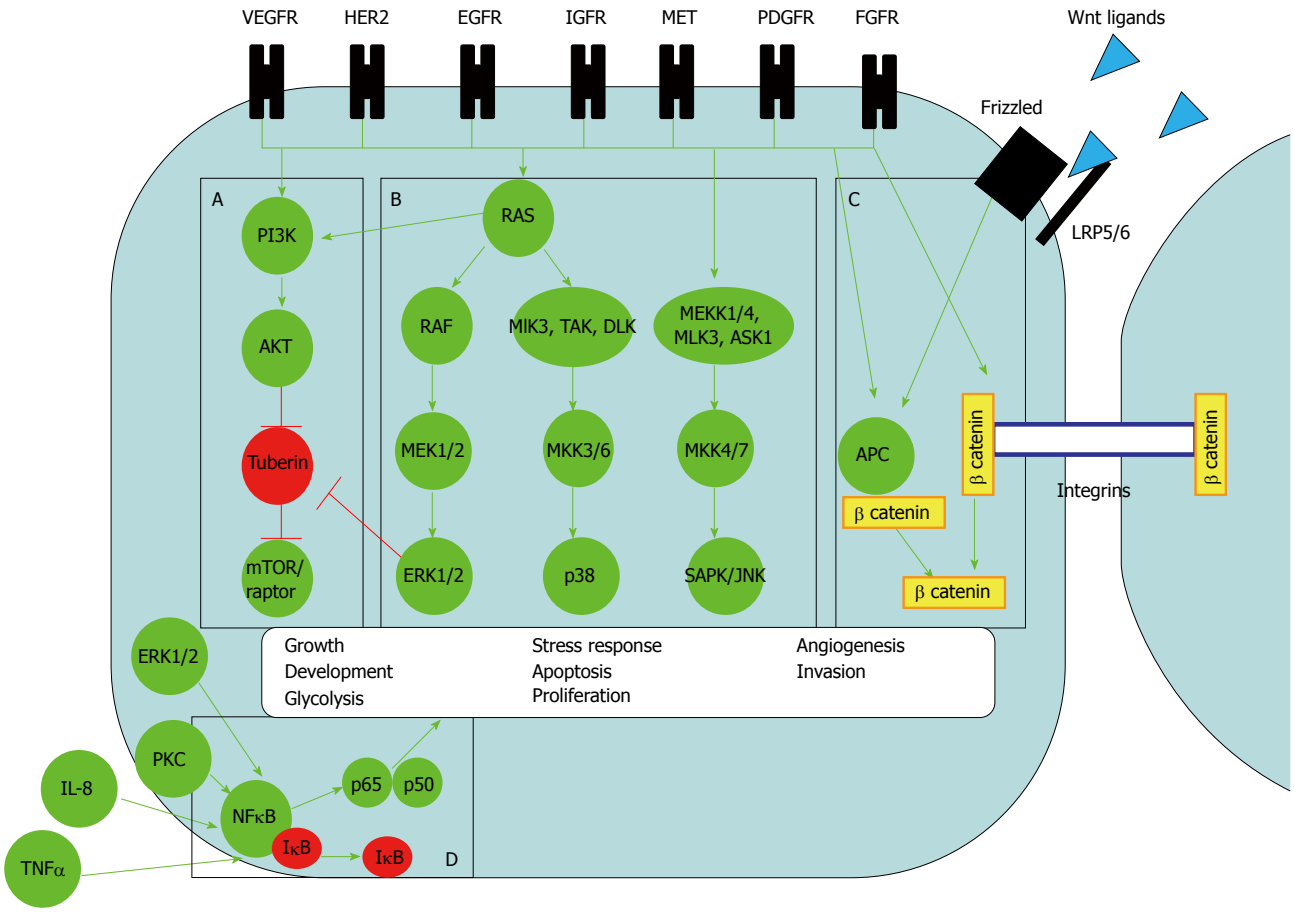


Figure 1 Signalling pathways in oesophageal adenocarcinoma. The pathways known to be operative in oesophageal adenocarcinoma are A: PI3-Kinase, B: Mitogen activated protein (MAP) Kinase, C: Wnt signalling and D: Nuclear factor-κB (NF-κB). The green arrows indicate activation, the red arrows indicate inhibition. Vascular epidermal growth factor (VEGF), human epidermal growth factor receptor 2 (HER2), epidermal growth factor receptor 1 (EGFR), insulin growth factor receptor (IGFR), hepatocyte growth factor receptor (MET), platelet derived growth factor receptor (PDGFR) and fibroblast growth factor receptor (FGFR) are the known receptor tyrosine kinase (RTK) associated with oesophageal adenocarcinomas. The pathways are complex and inter-connected. For example RTKs can activate all 4 known pathways. The ultimate biological response varies from cell proliferation, development, apoptosis, differentiation, inflammatory response, angiogenesis and invasion depending on which specific pathway or receptor is activated. A: PI3-Kinase pathway. RTK activates PI3-Kinase directly or through RAS. As a consequence of phosphorylation, PI3K recruits AKT. AKT inhibits tuberin, this allows activation of the Raptor/mTOR complex to activate transcriptional machinery. mTOR/raptor activates transcription factors and co-activator proteins cMyc and hypoxia inducible factor (HIF)-1α that drive the biological response of growth, proliferation, glycolysis, angiogenesis and invasion; B: MAP Kinase pathways. The 3 main MAP Kinase pathways are extracellular related kinase (ERK), p38 and JUN kinase (JNK). They are all activated by similar RTKs. The signalling pathways have a common feature of a cascade of phospho-proteins. A MAPKK Kinase (e.g. RAF), a MAPK Kinase [e.g. MAP ERK kinase (MEK)1/2] and a MAP Kinase (eg. ERK) transfer the signal onto multiple transcription factors on gene promoters[31]. ERK signalling for example can alter the gene expression of over 200 genes[32]. The subsequent biological response varies from cell proliferation, development, apoptosis, differentiation and inflammatory response, depending on the specific pathway activated; C: Wnt signalling. Wnt signalling exerts a biological response through release of β catenin into the nucleus with subsequent action on gene promoters. β catenin is released directly from RTK phosphorylation of Axin. Alternatively Wnt ligands bind to Frizzled receptors and form a complex with the LRP5/6 membrane receptor. The membrane receptor complex recruits dishevelled and axin from a cytoplasmic complex of dishevelled, Axin, adenomatous polyposis coli protein (APC) and β catenin. This allows release of β catenin; D: NF-κB pathway. NF-κB (p65p50) activates gene promoters only when released from IκB. Interleukin-8 (IL-8), tumour necrosis factor (TNF)α and radiation activate the pathway. Adding to complexity, ERK MAP Kinase and Wnt, through protein Kinase C, can also potentially activate NF-κB although this link has not been investigated in oesophageal adenocarcinoma.

KEY SIGNALLING PATHWAYS IN OESOPHAGEAL ADENOCARCINOMA

MAP-Kinase and PI3 kinase pathways

MAP-Kinase (MAPK) pathways are the most well described pathways in carcinogenesis. They are made up of three distinct pathways: ERK, SAP/JNK and p38[31] (Figure 1[31,32]). The pathways are normally activated by growth factors, temperature changes, cytokines and hypoxia *via* a variety of cell surface receptors[33]. In oesophageal cancer cells, gastric and bile acid[34,35] and the cytotoxic agent etoposide[36] are known to activate MAPK pathways. Cell surface receptors known to activate MAPK include receptor tyrosine kinase (RTK), G protein linked receptors and integrins[33]. Following activation of cell surface receptors a cascade of phospho-proteins is initiated *via* GTPase signal transducer proteins. RAS and RAF are examples of GTPase signal transducer proteins which act as a hub, receiving signals from many different cell surface receptors[28] (Figure 1). GTPase signal transducer proteins also amplify signals onward through multiple signalling pathways (Figure 1). MAPK pathways are often up-regulated in breast[37,38], ovarian[39] and prostate cancer[40]; however the impact on prognosis of MAPK signalling is sometimes conflicting. ERK MAPK is active in 60% of ERK MAPK oesophageal adenocarcinomas[36]. Tumors with active ERK MAPK signalling frequently have metastases and a worse prognosis. This suggests that blockade of ERK MAPK in oesophageal adenocarcinomas may have an important therapeutic role.

The PI3-Kinase (PI3K) pathway is activated by RTKs and RAS (Figure 1). Following RAS and/or RTK activation, AKT is phosphorylated by PI3K. Activation of the PI3K pathway stimulates cell growth, glycolysis, and proliferation[41] mainly through cMyc and hypoxia inducible factor 1α (HIF-1α) stimulation. Components of the PI3K pathway are up-regulated in oesophageal adenocarcinoma. The expression of phosphorylated AKT is increased in oesophageal adenocarcinoma tissue compared to normal epithelial and Barrett's tissue[34]. PI3K pathway mutations are thought to occur in 6% of oesophageal adenocarcinomas[42]. Crosstalk exists between the MAPK and PI3K pathways at the levels of RAS and ERK (Figure 1). This is likely to play a role in drug resistance seen in therapies that target signal transducer proteins. Crosstalk indicates that inhibition of multiple pathways may be needed for effective anti-cancer therapy.

Mechanisms of sustained MAPK and PI3K activation

RTKs on the cell surface are key activators of MAPK and PI3K pathways. RTKs can be activated constitutively by dimerisation, by ligand activation or by receptor over-expression[43]. Alternatively RAS mutations can render the GTPase in its active form so that the signal is permanently switched on, resistant to the activity of cell surface receptors. RAS mutations occur in only 10% of oesophageal adenocarcinomas. Aberrant expressions of RTK are frequently associated with oesophageal adenocarcinoma and there are many different family members (Figure 1). Receptor over-expression is usually associated with disease recurrence and a poor prognosis. Human epidermal growth factor receptor 1 (EGFR) and human epidermal growth factor receptor 2 (HER2) are over-expressed in 50% of oesophageal adenocarcinomas and positive expression is associated with a poor prognosis and cytotoxic drug resistance[30,44]. High expression of the hepatocyte growth factor receptor (Met) predicts metastases and recurrence in resectable oesophageal adenocarcinoma[45]. Vascular endothelial growth factor receptors (VEGF) are commonly associated with oesophageal adenocarcinoma, VEGF is thought to be important in angiogenesis and correlates with tumor microvessel density, crucial for tumor growth. VEGF A and C expression indicates a poor prognosis[46]. The significance of insulin like growth factor receptors (IGFR) has not been studied in oesophageal adenocarcinoma[47,48], however low IFGR expression correlates with an improved survival in metastatic gastric adenocarcinoma. The platelet derived growth factor receptor (PDGF) has also been shown to be expressed in oesophageal adenocarcinoma[49]. In oesophageal squamous cell carcinoma, fibroblast growth factor receptors[50] and tropomyosin-related kinase receptors[51] are indicators of tissue invasion and chemo-resistance respectively. Each member of an RTK family may have up to 20 subtypes[52] and it has been demonstrated that oesophageal adenocarcinoma cells often co-express the different subtypes of RTKs[49]. Therefore a therapy that targets only one receptor may not be effective. Furthermore cancers are known to be heterogeneous, made up of a population of genetically different cells. Gene expression at the invasive site of a tumor is different from the centre[53] and the gene expression of primary tumors is different to those at metastatic sites[54]. The variety of RTKs and downstream MAPK signalling pathways indicates that complete blockade of such a complex and diverse system may be impossible. Growth inhibition is more pronounced in oesophageal adenocarcinoma cells treated with combined inhibition of the EGFR and IGFR compared to inhibition of either receptor in isolation[55]. Furthermore patients with co-expression of HER-2 and EGFR also have a worse outcome[56]. Even if drug inhibition of both IGFR and EGFR is successful, resistance will prevail if alternative receptors or signal transducer proteins are active. Tumor heterogeneity may explain the modest improvement in response and survival seen with agents directed towards a solitary receptor, discussed in more detail below.

Targeting the epidermal growth factor receptor 1

Gefitinib and Erlotinib: Gefitinib and Erlotinib are small molecular inhibitors of tyrosine kinase phosphorylation of EGFR (Figure 2). Gefitinib therapy has been investigated in metastatic oesophageal adenocarcinoma[57]. Two thirds of patients had prior standard cytotoxic chemotherapy of which half had received surgery. Partial response and stable disease (according to Response Evaluation Criteria of Solid Tumors) was achieved in 37% and the median survival was 4.5 mo[57]. It is difficult to compare small phase 2 clinical trials but results were not significantly different compared to treatment with combined cytotoxic chemotherapy in a similar cohort of patients. Partial response was 12.5% and a median survival of 5 mo was seen in patients treated with irinotecan with docetaxel[58]. Partial response was 29% and median survival was 6.4 mo in patients treated with irinotecan with 5-FU/leucovorin[59]. To understand the poor results seen with gefitinib, ERK MAPK and PI3K pathway activation was determined by immunohistochemistry. Staining for phospho-ERK and phospho-AKT was assessed before and after treatment in 7 patients. No differences in staining patterns were seen in tumors treated with gefitinib, suggesting that the two pathways were not inhibited by the drug. This result is mirrored in a larger study of 70 gastric adenocarcinomas treated with gefitinib[60]. This indicates ERK MAPK and PI3K pathway resistance to EGFR blockade. A further study conducted in 43 metastatic adenocarcinomas at the gastro-oesophageal junction treated with gefitinib[61] also had similar survival and response rates to the study by Ferry *et al*[57]. Trends for favourable outcome were more likely in tumors with expression of EGFR, ERK MAPK and PI3K signalling activation. This was assessed by immunohistochemistry prior to treatment although assessment was not made post treatment. The differences in outcome did not meet statistical significance, but this is likely due to the small sample size. Of the poor responders, 2 (9%) had k-RAS mutations. This study indicates the importance of patient selection with targeted therapy. On the contrary in gastric and oesophageal adenocarcinomas treated with a similar EGFR inhibitor, erlotinib (Figure 2), EGFR expression

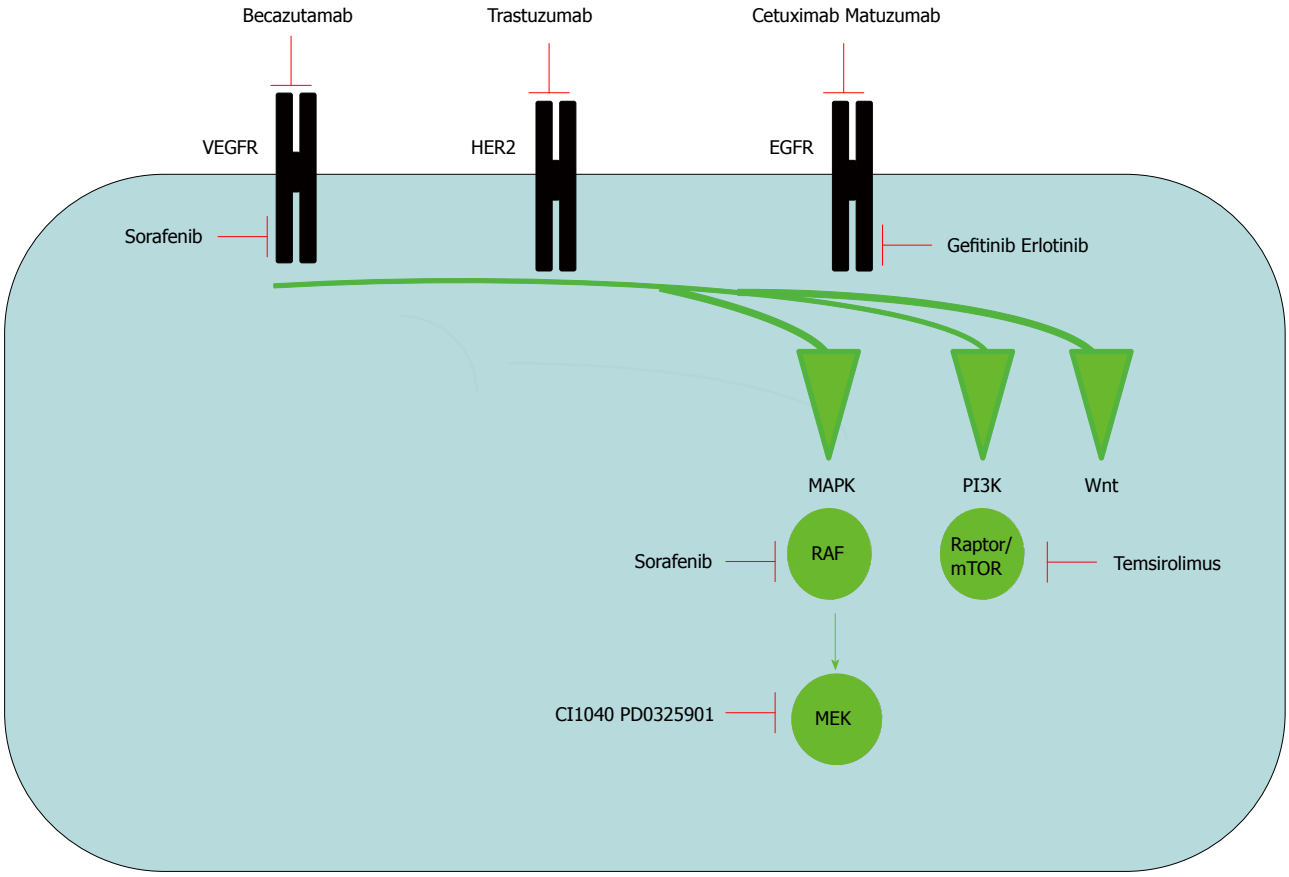


Figure 2 Drug inhibition of signalling pathways. Drugs that target signalling pathways can be divided into two groups. Antibodies [Becazutumab, trastuzumab, cetuximab and matuzumab are antibodies that target the receptor tyrosine kinase (RTK), vascular epidermal growth factor receptor (VEGFR), human epidermal growth factor receptor 2 (HER2) and epidermal growth factor receptor 1 (EGFR) respectively] and small molecular inhibitors (The small molecular inhibitors gefitinib and erlotinib target EGFR. Temsirolimus specifically inhibits the PI3-kinase pathway at the level of Raptor/mTOR. CI1040 and PD0325901 inhibits ERK MAP kinase pathway at the level of MEK. Sorafenib is a dual inhibitor of the VEGF receptor and ERK MAP kinase at the level of RAF).

and PI3K signalling activation was not found to influence drug response[62]. The difference may be explained by different receptor specificity between gefitinib and erlotinib or it may indicate the activity of additional RTKs or other cell surface receptors

Matuzumab: Matuzumab is a humanised monoclonal antibody that binds with the EGFR (Figure 2). Phase 1 trials have been conducted in metastatic oesophageal adenocarcinoma treated with conventional therapy with matuzumab[63]. EGFR was evident in 80%-100% of tumor specimens; however MAPK activity was not measured post treatment. This makes it difficult to assess the efficacy of the medication in the absence of survival data from this phase 1 study.

Cetuximab: Cetuximab is a monoclonal antibody directed against the EGFR (Figure 2), utilised in the treatment of advanced colorectal adenocarcinoma[64]. Trials have shown an improvement in average survival to 9 mo. RAS mutations occur commonly in colon cancer and account for resistance seen with cetuximab. When taken into account, colorectal carcinoma patients without k-RAS mutations have a significantly improved response to cetuximab compared to patient with k-RAS mutations which have survival times comparative to that of supportive care alone[65]. RAS mutations are less commonly seen in oesophageal adenocarcinomas and occur in less than 10% of cases so this is unlikely to account for the poor response seen with tyrosine kinase receptor inhibition[66]. Phase 2 clinical trials with cetuximab in advanced oesophageal adenocarcinoma have shown modest results similar to that seen with gefitinib[67].

Targeting the epidermal growth factor receptor 2

A Phase Ⅱ trial has been conducted with trastuzumab, a monoclonal antibody targeted to targeting the epidermal growth factor receptor 2 (HER2) (Figure 2). Trastuzumab was tested in combination with cisplatin, paclitaxel and radiotherapy in locally advanced oesophageal adenocarcinoma[68]. Patients were selected and included those with HER2 expression on immunohistochemistry. 74% of patients had positive HER2 expression. Median survival was 24 mo and 50% survived for 2 years. The patient population was different to the patients treated with gefitinib and cetuximab. None of the patients had organ metastases and distant lymph node metastases were present in only 37%, which makes it difficult to make direct comparisons.

Targeting the vascular epidermal growth factor receptor

Becazutumab is a monoclonal antibody directed against

the targeting of vascular epidermal growth factor receptor (VEGFR) (Figure 2). A phase 2 trial in metastatic gastric adenocarcinomas with 23 oesophageal junctional adenocarcinomas showed a response rate of 65% and median survival time of 12.3 mo[69]. Most patients were inoperable and the results were an improvement on standard cytotoxic therapies. Although VEGFR is frequently over-expressed in oesophageal adenocarcinomas[49], an assessment of VEGFR expression was not made prior to treatment. This may suggest that an improved outcome could be achieved by selecting tumors with high VEGFR expression.

Taken together, this may indicate that tailored RTK inhibition has a role in the treatment of selected patients with oesophageal adenocarcinomas. Initial trials have yet to make a significant impact and this may be down to poor patient selection and the use of growth factor receptor inhibitors in isolation.

Wnt signalling

Wnt signalling is important in cell growth, motility, angiogenesis, differentiation and other important phenotypic characteristics of cancer cells. Wnt ligands activate the Frizzled cell membrane receptor; Wnt is under feedback control from Wnt ligand inhibitors. Once activated, Frizzled forms a complex with another receptor LRP5/6 and recruits Dishevelled and Axin. The complex of APC, Axin and GSK and β-catenin is disrupted releasing unphosphorylated β-catenin (Figure 1). β-catenin can then enter the nucleus, and activate genes that stimulate growth, angiogenesis, invasion and cell cycle progression (*c-Myc, COX2, MMP7* and *Cyclin D1)*. Alternatively β-catenin can also be released by RTK phosphorylation of E-cadherin or Axin (Figure 1). Furthermore Wnt ligands can also directly activate calmodium kinase Ⅱ and protein kinase C in turn releasing intracellular calcium or increasing JNK. Components of the pathway are altered in oesophageal adenocarcinoma. APC, Axin and Wnt ligand inhibitors are silenced by loss of heterozygosity or DNA methylation and collectively these events increase β-catenin activity. Although APC mutations are less commonly seen than in colorectal cancer, β-catenin or Wnt ligands are over-expressed in up to 77% of oesophageal adenocarcinomas[70,71]. This makes components of this pathway a potential target for drug inhibition.

Transforming growth factor-β pathway

Transforming growth factor-β (TGF-β) is a tumor suppressor gene and a potent inhibitor of cell growth. TGF-β binds to serine/threonine kinase type 1 and type 2 receptors. Upon binding to receptors, TGF-β forms a complex and phosphorylates intracellular signalling mediators called SMAD2/3. SMAD2/3 dissociates from the receptors and forms a complex with SMAD4 allowing it to enter the nucleus and regulate a large number of target genes. One target is *SMAD7* which targets ubiquitin to the membrane receptor complex resulting in feedback inhibition of the pathway. Down regulation of SMAD4 has been shown in the progression of Barrett's oesophagus to adenocarcinoma. TGF-β is anti-proliferative in some oesophageal cancer cell lines[72]. In contrast TGF-β expression has been demonstrated at the invasive margin of oesophageal adenocarcinomas and promotes cell invasion[73]. This may be explained by cross talk between the TGF-β pathway with PI3K, Wnt, PKC and the MAP-Kinase pathways. One potential mechanism is *via* SMAD7 inhibition leading to loss/diminished feedback inhibition of the pathway.

Nuclear factor-κB pathways

Nuclear factor-κB (NF-κB) is a proinflammatory transcription factor. It exists as a heterodimer p50/p65, situated in the cytoplasm under inhibitory control by IκB (Figure 1). Numerous activators have been identified including ERK MAPK signalling, cytokines (IL-8, TNF-α) and radiation. Specifically for oesophageal adenocarcinoma, bile salts and gastric acid have been shown to activate NF-κB. Gastrin has been shown to activate NF-κB through PKC signalling in gastric cancer cells[74]. Once activated, NF-κB enters the nucleus and through chromatin re-modelling it becomes a central regulator of many genes including cell cycle regulators (*cyclin D1*, *cMyc*, *p53*), inhibitors of apoptosis (*Bcl-2*), cytokines (*interleukins*, *TNF-α*), angiogenic mediators (*COX2*) and the growth factor receptor *EGFR*. Increased NF-κB expression is seen in Barrett's oesophagus and adenocarcinoma. In oesophageal adenocarcinoma the expression correlates with chemo-radiation resistance[75].

Wnt signalling, TGF-β pathway and NF-κB pathways all activate important mediators in oesophageal adenocarcinoma. However there are no trials investigating the impact of specific inhibitors of these pathways outside the laboratory setting. RTK inhibitors have been investigated in oesophageal adenocarcinoma; however their role in Wnt signalling inhibition of β catenin has not been evaluated. The development of agents that inhibit alternative components of the Wnt, TGF-β and NF-κB signalling pathways are needed to avoid resistance and improve the modest responses seen with current therapies in clinical trials in oesophageal adenocarcinoma that focus on RTKs and MAPK.

POTENTIAL FUTURE TREATMENT TARGETS IN OESOPHAGEAL ADENOCARCINOMA

ERK MAPK inhibition by targeting MEK

An alternative approach is to target signal transducer proteins which may be downstream of many different RTKs. Theoretically this may reduce resistance of RTK co-expression. MEK is a downstream signal transducer protein of the ERK MAP Kinase pathway (Figure 1). No clinical trials have explored the role of MEK inhibition in oesophageal adenocarcinoma but lessons may be learned from trials in other cancers. Phase 2 clinical trials of the MEK inhibitor CI1040 (Figure 2) in advanced pancreatic, breast and non small cell lung cancer failed to make an impact on tumor progression[76]. Parallels can be drawn from colon cancer where RAS mutations reduced the efficacy of ce-

tuximab. Patients treated with MEK inhibition were tested for ERK MAPK and PI3K signalling activation in archived samples, sometimes many months preceding treatment. This did not influence recruitment into the study and patients were enrolled if ERK or PI3K activation was judged to be low. Better patient selection may have resulted in a better response to treatment. ERK MAPK signalling activity was not assessed post treatment which may mean that the dosage was insufficient. More potent MEK inhibitors, such as PD0325901, have been evaluated in hepatocellular carcinomas[77]. Alternatively the poor responses with MEK inhibition may be explained by "cross talk" between different signalling pathways (Figure 2). Resistance to MEK inhibition may be explained by PI3-Kinase activation. The combination of MEK inhibition and PI3-Kinase inhibition is superior to treatments in isolation for inhibiting the growth of breast cancer cells[78]. This suggest that dual therapy is needed to combat both pathways.

ERK MAPK inhibition by targeting RAF and VEGF

A combined targeted approach may be beneficial in oesophageal adenocarcinoma. Sorafenib, a multifunctional kinase inhibitor, which acts on several growth regulatory pathways including VEGF and RAF (Figure 2), has been shown to be of benefit in renal cell carcinoma and hepatocellular carcinomas[78,79]. Sorafenib has been shown to inhibit key signalling pathways in SEG-1 lung adenocarcinoma cells[80]. This method of inhibition of both receptor and signalling protein such as RAF may prove beneficial due the diversity of growth factor receptors displayed by tumors and this approach may have a future role in the treatment of oesophageal adenocarcinoma.

PI3 kinase by targeting mTOR

No inhibitors of the PI3 kinase have been evaluated in oesophageal adenocarcinoma. Cell line studies in oesophageal adenocarcinoma have identified that the PI3 kinase pathway is important for cell growth. Mutations of the PI3 kinase pathway occur infrequently in oesophageal adenocarcinoma. However, activation of the pathway is known to occur from RTK, a common occurrence in oesophageal adenocarcinomas. Indeed in breast cancer, the PI3 kinase pathway has been proposed as a mechanism of drug resistance to MEK inhibition[55]. Inhibition of the PI3 kinase pathway has been used with success in metastatic renal cell carcinoma. Analogues of rapamycin have been developed to target mTOR (Figure 2). The agent temsirolimus has been evaluated in stage 3 clinical trials[81] (Figure 2). In this trial, 626 patients were divided into 3 groups; temsirolimus alone, interferon alone, and interferon in combination with temsirolimus. Overall survival was 10.9, 7.3 and 8.4 mo respectively in favour of temsirolimus. In view of this, a strategy of mTOR inhibition may have a future role in oesophageal adenocarcinoma.

Targeting transcription factors

Targeting an activated transcription factor or central regulator such as NF-κB would theoretically reduce the chance of the development of drug resistance from the activity of multiple surface receptors and multiple signalling pathways. This is not without problems. Firstly transcription factors are difficult to target. Interference RNA technology involves the insertion of an oligonucleotide into the nucleus of a cancer cell, usually using a viral vector. Oligonucleotides can be manufactured to complement the sequence and therefore dimerize with any RNA of interest such as NF-κB. This allows the targeting of transcription factor RNA with the prevention of protein translation. Interference RNA technology may be the answer to transcription factor inhibition but the technology remains in its infancy. The major hurdle appears to be the development of an efficient delivery system of oligonucleotides into cancer cells. Phase 1 trials are currently underway targeting VEGF in macular degeneration using direct ocular injection[82,83]. If this technology is developed in oesophageal cancer then gene expression profiling of tumors would be required to ensure that specific targeted therapy is delivered. The identification of more central regulators of carcinogenesis, such as HIF-1α and PEA3/ETV4 transcription factors, is likely to increase treatment options. The advent of gene expression profiling will certainly increase the number of potential targets.

CONCLUSION

An international effort is underway with the aim of improving survival in oesophageal adenocarcinoma by targeting key signalling pathways. Clinical trials using receptor tyrosine kinase inhibitors in oesophageal adenocarcinomas have so far only recruited patients with advanced or metastatic cancer. Studies utilised agents that inhibit solitary receptor tyrosine kinase, sometimes in an unselected manner. This strategy is problematic. At an advanced stage the heterogeneity within the tumor is extensive, which increases the likelihood of alternative signalling pathways resistant to receptor blockade. Secondly the pathway or receptor of interest may not be active or expressed, culminating in ineffective treatment. Tumor growth is immensely complex and this is emphasised in a study of 75 oesophageal adenocarcinoma specimens. Micro array studies identified 4 genes important in disease progression[84]. The genes independently predicted prognosis independent from traditional radiological methods. Unfortunately a further 115 genes were also indicators of survival. It is not clear what role the genes play in carcinogenesis; however this study indicates the complexity and diversity of the factors implicated in oesophageal adenocarcinoma development. Taken together, this suggests that a tailored combinatorial approach for treatment that inhibits multiple genes may be useful; therapies targeting either receptors, hub signalling proteins or even transcription factors, is likely to be necessary to deliver effective responses. By tailoring therapy to tumors that express specific gene and protein signatures and prescribing a regimen of treatments that act in fundamentally different

mechanisms, then further improvements in survival are likely to be possible in oesophageal adenocarcinoma.

REFERENCES

1 **Office for National Statistics**. Cancer Statistics registrations: Registrations of cancer diagnosed in 2006, England. Series MB1 No. 37. 2008
2 **Adams R**, Morgan M, Mukherjee S, Brewster A, Maughan T, Morrey D, Havard T, Lewis W, Clark G, Roberts S, Vachtsevanos L, Leong J, Hardwick R, Carey D, Crosby T. A prospective comparison of multidisciplinary treatment of oesophageal cancer with curative intent in a UK cancer network. *Eur J Surg Oncol* 2007; **33**: 307-313
3 **Devesa SS**, Blot WJ, Fraumeni JF Jr. Changing patterns in the incidence of esophageal and gastric carcinoma in the United States. *Cancer* 1998; **83**: 2049-2053
4 **el-Serag HB**. The epidemic of esophageal adenocarcinoma. *Gastroenterol Clin North Am* 2002; **31**: 421-440, viii
5 **Newnham A**, Quinn MJ, Babb P, Kang JY, Majeed A. Trends in oesophageal and gastric cancer incidence, mortality and survival in England and Wales 1971-1998/1999. *Aliment Pharmacol Ther* 2003; **17**: 655-664
6 **Hampel H**, Abraham NS, El-Serag HB. Meta-analysis: obesity and the risk for gastroesophageal reflux disease and its complications. *Ann Intern Med* 2005; **143**: 199-211
7 **Rokkas T**, Pistiolas D, Sechopoulos P, Robotis I, Margantinis G. Relationship between Helicobacter pylori infection and esophageal neoplasia: a meta-analysis. *Clin Gastroenterol Hepatol* 2007; **5**: 1413-1417, 1417.e1-2
8 **Solaymani-Dodaran M**, Logan RF, West J, Card T, Coupland C. Risk of oesophageal cancer in Barrett's oesophagus and gastro-oesophageal reflux. *Gut* 2004; **53**: 1070-1074
9 **Rubenstein JH**, Sonnenberg A, Davis J, McMahon L, Inadomi JM. Effect of a prior endoscopy on outcomes of esophageal adenocarcinoma among United States veterans. *Gastrointest Endosc* 2008; **68**: 849-855
10 **Cooper GS**, Kou TD, Chak A. Receipt of previous diagnoses and endoscopy and outcome from esophageal adenocarcinoma: a population-based study with temporal trends. *Am J Gastroenterol* 2009; **104**: 1356-1362
11 **Wang VS**, Hornick JL, Sepulveda JA, Mauer R, Poneros JM. Low prevalence of submucosal invasive carcinoma at esophagectomy for high-grade dysplasia or intramucosal adenocarcinoma in Barrett's esophagus: a 20-year experience. *Gastrointest Endosc* 2009; **69**: 777-783
12 **Yousef F**, Cardwell C, Cantwell MM, Galway K, Johnston BT, Murray L. The incidence of esophageal cancer and high-grade dysplasia in Barrett's esophagus: a systematic review and meta-analysis. *Am J Epidemiol* 2008; **168**: 237-249
13 **Webb A**, Cunningham D, Scarffe JH, Harper P, Norman A, Joffe JK, Hughes M, Mansi J, Findlay M, Hill A, Oates J, Nicolson M, Hickish T, O'Brien M, Iveson T, Watson M, Underhill C, Wardley A, Meehan M. Randomized trial comparing epirubicin, cisplatin, and fluorouracil versus fluorouracil, doxorubicin, and methotrexate in advanced esophagogastric cancer. *J Clin Oncol* 1997; **15**: 261-267
14 **Pinedo HM**, Peters GF. Fluorouracil: biochemistry and pharmacology. *J Clin Oncol* 1988; **6**: 1653-1664
15 **Raymond E**, Faivre S, Woynarowski JM, Chaney SG. Oxaliplatin: mechanism of action and antineoplastic activity. *Semin Oncol* 1998; **25**: 4-12
16 **Sinha BK**, Politi PM. Anthracyclines. *Cancer Chemother Biol Response Modif* 1990; **11**: 45-57
17 **Rowinsky EK**, Onetto N, Canetta RM, Arbuck SG. Taxol: the first of the taxanes, an important new class of antitumor agents. *Semin Oncol* 1992; **19**: 646-662
18 **Cunningham D**, Starling N, Rao S, Iveson T, Nicolson M, Coxon F, Middleton G, Daniel F, Oates J, Norman AR. Capecitabine and oxaliplatin for advanced esophagogastric cancer. *N Engl J Med* 2008; **358**: 36-46
19 **Sumpter K**, Harper-Wynne C, Cunningham D, Rao S, Tebbutt N, Norman AR, Ward C, Iveson T, Nicolson M, Hickish T, Hill M, Oates J. Report of two protocol planned interim analyses in a randomised multicentre phase III study comparing capecitabine with fluorouracil and oxaliplatin with cisplatin in patients with advanced oesophagogastric cancer receiving ECF. *Br J Cancer* 2005; **92**: 1976-1983
20 **2nd Annual Report of the National Oesophago-Gastric Cancer Audit 2009**. The NHS Information Centre IC23090209
21 **Kulke MH**, Wu B, Clark JW, Enzinger PC, Lynch TJ, Vincitore M, Michelini A, Fuchs CS. A phase II study of doxorubicin, cisplatin, and 5-fluorouracil in patients with advanced adenocarcinoma of the stomach or esophagus. *Cancer Invest* 2006; **24**: 229-234
22 **Polee MB**, Hop WC, Kok TC, Eskens FA, van der Burg ME, Splinter TA, Siersema PD, Tilanus HW, Stoter G, van der Gaast A. Prognostic factors for survival in patients with advanced oesophageal cancer treated with cisplatin-based combination chemotherapy. *Br J Cancer* 2003; **89**: 2045-2050
23 **Cunningham D**, Allum WH, Stenning SP, Thompson JN, Van de Velde CJ, Nicolson M, Scarffe JH, Lofts FJ, Falk SJ, Iveson TJ, Smith DB, Langley RE, Verma M, Weeden S, Chua YJ, MAGIC Trial Participants. Perioperative chemotherapy versus surgery alone for resectable gastroesophageal cancer. *N Engl J Med* 2006; **355**: 11-20
24 Surgical resection with or without preoperative chemotherapy in oesophageal cancer: a randomised controlled trial. *Lancet* 2002; **359**: 1727-1733
25 **Macdonald JS**, Smalley SR, Benedetti J, Hundahl SA, Estes NC, Stemmermann GN, Haller DG, Ajani JA, Gunderson LL, Jessup JM, Martenson JA. Chemoradiotherapy after surgery compared with surgery alone for adenocarcinoma of the stomach or gastroesophageal junction. *N Engl J Med* 2001; **345**: 725-730
26 **Brozovic A**, Osmak M. Activation of mitogen-activated protein kinases by cisplatin and their role in cisplatin-resistance. *Cancer Lett* 2007; **251**: 1-16
27 **Hanahan D**, Weinberg RA. The hallmarks of cancer. *Cell* 2000; **100**: 57-70
28 **Alberts BJ**, Lewis J, Raff M, Roberts K, Walter P. Molecular Biology of the Cell. 4 ed. Garland Science, 2002
29 **Chaganti RS**. Significance of chromosome change to hematopoietic neoplasms. *Blood* 1983; **62**: 515-524
30 **Tomlins SA**, Laxman B, Dhanasekaran SM, Helgeson BE, Cao X, Morris DS, Menon A, Jing X, Cao Q, Han B, Yu J, Wang L, Montie JE, Rubin MA, Pienta KJ, Roulston D, Shah RB, Varambally S, Mehra R, Chinnaiyan AM. Distinct classes of chromosomal rearrangements create oncogenic ETS gene fusions in prostate cancer. *Nature* 2007; **448**: 595-599
31 **Dunn KL**, Espino PS, Drobic B, He S, Davie JR. The Ras-MAPK signal transduction pathway, cancer and chromatin remodeling. *Biochem Cell Biol* 2005; **83**: 1-14
32 **Mirzoeva OK**, Das D, Heiser LM, Bhattacharya S, Siwak D, Gendelman R, Bayani N, Wang NJ, Neve RM, Guan Y, Hu Z, Knight Z, Feiler HS, Gascard P, Parvin B, Spellman PT, Shokat KM, Wyrobek AJ, Bissell MJ, McCormick F, Kuo WL, Mills GB, Gray JW, Korn WM. Basal subtype and MAPK/ERK kinase (MEK)-phosphoinositide 3-kinase feedback signaling determine susceptibility of breast cancer cells to MEK inhibition. *Cancer Res* 2009; **69**: 565-572
33 **Zhang W**, Liu HT. MAPK signal pathways in the regulation of cell proliferation in mammalian cells. *Cell Res* 2002; **12**: 9-18
34 **Beales IL**, Ogunwobi OO. Leptin synergistically enhances the anti-apoptotic and growth-promoting effects of acid in OE33 oesophageal adenocarcinoma cells in culture. *Mol Cell Endocrinol* 2007; **274**: 60-68
35 **Soma T**, Kaganoi J, Kawabe A, Kondo K, Tsunoda S, Imamura M, Shimada Y. Chenodeoxycholic acid stimulates the

progression of human esophageal cancer cells: A possible mechanism of angiogenesis in patients with esophageal cancer. *Int J Cancer* 2006; **119**: 771-782
36 **Keld R**, Guo B, Downey P, Gulmann C, Ang YS, Sharrocks AD. The ERK MAP kinase-PEA3/ETV4-MMP-1 axis is operative in oesophageal adenocarcinoma. *Mol Cancer* 2010; **9**: 313
37 **Gee JM**, Robertson JF, Ellis IO, Nicholson RI. Phosphorylation of ERK1/2 mitogen-activated protein kinase is associated with poor response to anti-hormonal therapy and decreased patient survival in clinical breast cancer. *Int J Cancer* 2001; **95**: 247-254
38 **Milde-Langosch K**, Bamberger AM, Rieck G, Grund D, Hemminger G, Müller V, Löning T. Expression and prognostic relevance of activated extracellular-regulated kinases (ERK1/2) in breast cancer. *Br J Cancer* 2005; **92**: 2206-2215
39 **Givant-Horwitz V**, Davidson B, Lazarovici P, Schaefer E, Nesland JM, Tropé CG, Reich R. Mitogen-activated protein kinases (MAPK) as predictors of clinical outcome in serous ovarian carcinoma in effusions. *Gynecol Oncol* 2003; **91**: 160-172
40 **Malik SN**, Brattain M, Ghosh PM, Troyer DA, Prihoda T, Bedolla R, Kreisberg JI. Immunohistochemical demonstration of phospho-Akt in high Gleason grade prostate cancer. *Clin Cancer Res* 2002; **8**: 1168-1171
41 **Yuan TL**, Cantley LC. PI3K pathway alterations in cancer: variations on a theme. *Oncogene* 2008; **27**: 5497-5510
42 **Phillips WA**, Russell SE, Ciavarella ML, Choong DY, Montgomery KG, Smith K, Pearson RB, Thomas RJ, Campbell IG. Mutation analysis of PIK3CA and PIK3CB in esophageal cancer and Barrett's esophagus. *Int J Cancer* 2006; **118**: 2644-2646
43 **Scaltriti M**, Baselga J. The epidermal growth factor receptor pathway: a model for targeted therapy. *Clin Cancer Res* 2006; **12**: 5268-5272
44 **Gibson MK**, Abraham SC, Wu TT, Burtness B, Heitmiller RF, Heath E, Forastiere A. Epidermal growth factor receptor, p53 mutation, and pathological response predict survival in patients with locally advanced esophageal cancer treated with preoperative chemoradiotherapy. *Clin Cancer Res* 2003; **9**: 6461-6468
45 **Tuynman JB**, Lagarde SM, Ten Kate FJ, Richel DJ, van Lanschot JJ. Met expression is an independent prognostic risk factor in patients with oesophageal adenocarcinoma. *Br J Cancer* 2008; **98**: 1102-1108
46 **Kleespies A**, Bruns CJ, Jauch KW. Clinical significance of VEGF-A, -C and -D expression in esophageal malignancies. *Onkologie* 2005; **28**: 281-288
47 **Iravani S**, Zhang HQ, Yuan ZQ, Cheng JQ, Karl RC, Jove R, Coppola D. Modification of insulin-like growth factor 1 receptor, c-Src, and Bcl-XL protein expression during the progression of Barrett's neoplasia. *Hum Pathol* 2003; **34**: 975-982
48 **Matsubara J**, Yamada Y, Nakajima TE, Kato K, Hamaguchi T, Shirao K, Shimada Y, Shimoda T. Clinical significance of insulin-like growth factor type 1 receptor and epidermal growth factor receptor in patients with advanced gastric cancer. *Oncology* 2008; **74**: 76-83
49 **Gockel I**, Moehler M, Frerichs K, Drescher D, Trinh TT, Duenschede F, Borschitz T, Schimanski K, Biesterfeld S, Herzer K, Galle PR, Lang H, Junginger T, Schimanski CC. Co-expression of receptor tyrosine kinases in esophageal adenocarcinoma and squamous cell cancer. *Oncol Rep* 2008; **20**: 845-850
50 **Yoshino M**, Ishiwata T, Watanabe M, Matsunobu T, Komine O, Ono Y, Yamamoto T, Fujii T, Matsumoto K, Tokunaga A, Naito Z. Expression and roles of keratinocyte growth factor and its receptor in esophageal cancer cells. *Int J Oncol* 2007; **31**: 721-728
51 **Tanaka K**, Mohri Y, Nishioka J, Ohi M, Yokoe T, Miki C, Tonouchi H, Nobori T, Kusunoki M. Neurotrophic receptor, tropomyosin-related kinase B, as a chemoresistant marker in oesophageal cancer. *Clin Oncol* (R Coll Radiol) 2009; **21**: 362-363
52 **Robinson DR**, Wu YM, Lin SF. The protein tyrosine kinase family of the human genome. *Oncogene* 2000; **19**: 5548-5557
53 **Tan X**, Egami H, Abe M, Nozawa F, Hirota M, Ogawa M. Involvement of MMP-7 in invasion of pancreatic cancer cells through activation of the EGFR mediated MEK-ERK signal transduction pathway. *J Clin Pathol* 2005; **58**: 1242-1248
54 **Albino AP**, Le Strange R, Oliff AI, Furth ME, Old LJ. Transforming ras genes from human melanoma: a manifestation of tumour heterogeneity? *Nature* 1984; **308**: 69-72
55 **Sutter AP**, Höpfner M, Huether A, Maaser K, Scherübl H. Targeting the epidermal growth factor receptor by erlotinib (Tarceva) for the treatment of esophageal cancer. *Int J Cancer* 2006; **118**: 1814-1822
56 **Kawaguchi Y**, Kono K, Mimura K, Mitsui F, Sugai H, Akaike H, Fujii H. Targeting EGFR and HER-2 with cetuximab- and trastuzumab-mediated immunotherapy in oesophageal squamous cell carcinoma. *Br J Cancer* 2007; **97**: 494-501
57 **Ferry DR**, Anderson M, Beddard K, Tomlinson S, Atherfold P, Obszynska J, Harrison R, Jankowski J. A phase II study of gefitinib monotherapy in advanced esophageal adenocarcinoma: evidence of gene expression, cellular, and clinical response. *Clin Cancer Res* 2007; **13**: 5869-5875
58 **Lordick F**, von Schilling C, Bernhard H, Hennig M, Bredenkamp R, Peschel C. Phase II trial of irinotecan plus docetaxel in cisplatin-pretreated relapsed or refractory oesophageal cancer. *Br J Cancer* 2003; **89**: 630-633
59 **Assersohn L**, Brown G, Cunningham D, Ward C, Oates J, Waters JS, Hill ME, Norman AR. Phase II study of irinotecan and 5-fluorouracil/leucovorin in patients with primary refractory or relapsed advanced oesophageal and gastric carcinoma. *Ann Oncol* 2004; **15**: 64-69
60 **Rojo F**, Tabernero J, Albanell J, Van Cutsem E, Ohtsu A, Doi T, Koizumi W, Shirao K, Takiuchi H, Ramon y Cajal S, Baselga J. Pharmacodynamic studies of gefitinib in tumor biopsy specimens from patients with advanced gastric carcinoma. *J Clin Oncol* 2006; **24**: 4309-4316
61 **Janmaat ML**, Gallegos-Ruiz MI, Rodriguez JA, Meijer GA, Vervenne WL, Richel DJ, Van Groeningen C, Giaccone G. Predictive factors for outcome in a phase II study of gefitinib in second-line treatment of advanced esophageal cancer patients. *J Clin Oncol* 2006; **24**: 1612-1619
62 **Dragovich T**, McCoy S, Fenoglio-Preiser CM, Wang J, Benedetti JK, Baker AF, Hackett CB, Urba SG, Zaner KS, Blanke CD, Abbruzzese JL. Phase II trial of erlotinib in gastroesophageal junction and gastric adenocarcinomas: SWOG 0127. *J Clin Oncol* 2006; **24**: 4922-4927
63 **Rao S**, Starling N, Cunningham D, Benson M, Wotherspoon A, Lüpfert C, Kurek R, Oates J, Baselga J, Hill A. Phase I study of epirubicin, cisplatin and capecitabine plus matuzumab in previously untreated patients with advanced oesophagogastric cancer. *Br J Cancer* 2008; **99**: 868-874
64 **Cunningham D**, Humblet Y, Siena S, Khayat D, Bleiberg H, Santoro A, Bets D, Mueser M, Harstrick A, Verslype C, Chau I, Van Cutsem E. Cetuximab monotherapy and cetuximab plus irinotecan in irinotecan-refractory metastatic colorectal cancer. *N Engl J Med* 2004; **351**: 337-345
65 **Karapetis CS**, Khambata-Ford S, Jonker DJ, O'Callaghan CJ, Tu D, Tebbutt NC, Simes RJ, Chalchal H, Shapiro JD, Robitaille S, Price TJ, Shepherd L, Au HJ, Langer C, Moore MJ, Zalcberg JR. K-ras mutations and benefit from cetuximab in advanced colorectal cancer. *N Engl J Med* 2008; **359**: 1757-1765
66 **Hollstein MC**, Peri L, Mandard AM, Welsh JA, Montesano R, Metcalf RA, Bak M, Harris CC. Genetic analysis of human esophageal tumors from two high incidence geographic areas: frequent p53 base substitutions and absence of ras mutations. *Cancer Res* 1991; **51**: 4102-4106
67 **Pinto C**, Di Fabio F, Siena S, Cascinu S, Rojas Llimpe FL, Ceccarelli C, Mutri V, Giannetta L, Giaquinta S, Funaioli C, Berardi R, Longobardi C, Piana E, Martoni AA. Phase II study of cetuximab in combination with FOLFIRI in patients with

untreated advanced gastric or gastroesophageal junction adenocarcinoma (FOLCETUX study). *Ann Oncol* 2007; **18**: 510-517
68 **Safran H**, Dipetrillo T, Akerman P, Ng T, Evans D, Steinhoff M, Benton D, Purviance J, Goldstein L, Tantravahi U, Kennedy T. Phase I/II study of trastuzumab, paclitaxel, cisplatin and radiation for locally advanced, HER2 overexpressing, esophageal adenocarcinoma. *Int J Radiat Oncol Biol Phys* 2007; **67**: 405-409
69 **Shah MA**, Ramanathan RK, Ilson DH, Levnor A, D'Adamo D, O'Reilly E, Tse A, Trocola R, Schwartz L, Capanu M, Schwartz GK, Kelsen DP. Multicenter phase II study of irinotecan, cisplatin, and bevacizumab in patients with metastatic gastric or gastroesophageal junction adenocarcinoma. *J Clin Oncol* 2006; **24**: 5201-5206
70 **Clément G**, Jablons DM, Benhattar J. Targeting the Wnt signaling pathway to treat Barrett's esophagus. *Expert Opin Ther Targets* 2007; **11**: 375-389
71 **Clément G**, Braunschweig R, Pasquier N, Bosman FT, Benhattar J. Alterations of the Wnt signaling pathway during the neoplastic progression of Barrett's esophagus. *Oncogene* 2006; **25**: 3084-3092
72 **Onwuegbusi BA**, Aitchison A, Chin SF, Kranjac T, Mills I, Huang Y, Lao-Sirieix P, Caldas C, Fitzgerald RC. Impaired transforming growth factor beta signalling in Barrett's carcinogenesis due to frequent SMAD4 inactivation. *Gut* 2006; **55**: 764-774
73 **Peters CR**, Hardwick J, Hardwick R, Zhang J, Vowler C, Save S, O'donovan V, Caldas M, Alderson C, Fitzgerald D. On Behalf Of The OCCAMS Study Group, A seven gene signiture outperforms clinical features at predicting survival in oesophageal adenocarcinoma World Congress of Gastroenterology/UEGW, London, 2009
74 **Ogasa M**, Miyazaki Y, Hiraoka S, Kitamura S, Nagasawa Y, Kishida O, Miyazaki T, Kiyohara T, Shinomura Y, Matsuzawa Y. Gastrin activates nuclear factor kappaB (NFkappaB) through a protein kinase C dependent pathway involving NFkappaB inducing kinase, inhibitor kappaB (IkappaB) kinase, and tumour necrosis factor receptor associated factor 6 (TRAF6) in MKN-28 cells transfected with gastrin receptor. *Gut* 2003; **52**: 813-819
75 **Abdel-Latif MM**, Kelleher D, Reynolds JV. Potential role of NF-kappaB in esophageal adenocarcinoma: as an emerging molecular target. *J Surg Res* 2009; **153**: 172-180
76 **Rinehart J**, Adjei AA, Lorusso PM, Waterhouse D, Hecht JR, Natale RB, Hamid O, Varterasian M, Asbury P, Kaldjian EP, Gulyas S, Mitchell DY, Herrera R, Sebolt-Leopold JS, Meyer MB. Multicenter phase II study of the oral MEK inhibitor, CI-1040, in patients with advanced non-small-cell lung, breast, colon, and pancreatic cancer. *J Clin Oncol* 2004; **22**: 4456-4462
77 **Wentz SC**, Wu H, Yip-Schneider MT, Hennig M, Klein PJ, Sebolt-Leopold J, Schmidt CM. Targeting MEK is effective chemoprevention of hepatocellular carcinoma in TGF-alpha-transgenic mice. *J Gastrointest Surg* 2008; **12**: 30-37
78 **Zhang T**, Ding X, Wei D, Cheng P, Su X, Liu H, Wang D, Gao H. Sorafenib improves the survival of patients with advanced hepatocellular carcinoma: a meta-analysis of randomized trials. *Anticancer Drugs* 2010; **21**: 326-332
79 **Gollob JA**, Rathmell WK, Richmond TM, Marino CB, Miller EK, Grigson G, Watkins C, Gu L, Peterson BL, Wright JJ. Phase II trial of sorafenib plus interferon alfa-2b as first- or second-line therapy in patients with metastatic renal cell cancer. *J Clin Oncol* 2007; **25**: 3288-3295
80 **Delgado JS**, Mustafi R, Yee J, Cerda S, Chumsangsri A, Dougherty U, Lichtenstein L, Fichera A, Bissonnette M. Sorafenib triggers antiproliferative and pro-apoptotic signals in human esophageal adenocarcinoma cells. *Dig Dis Sci* 2008; **53**: 3055-3064
81 **Hudes G**, Carducci M, Tomczak P, Dutcher J, Figlin R, Kapoor A, Staroslawska E, Sosman J, McDermott D, Bodrogi I, Kovacevic Z, Lesovoy V, Schmidt-Wolf IG, Barbarash O, Gokmen E, O'Toole T, Lustgarten S, Moore L, Motzer RJ. Temsirolimus, interferon alfa, or both for advanced renal-cell carcinoma. *N Engl J Med* 2007; **356**: 2271-2281
82 **Kleinman ME**, Yamada K, Takeda A, Chandrasekaran V, Nozaki M, Baffi JZ, Albuquerque RJ, Yamasaki S, Itaya M, Pan Y, Appukuttan B, Gibbs D, Yang Z, Karikó K, Ambati BK, Wilgus TA, DiPietro LA, Sakurai E, Zhang K, Smith JR, Taylor EW, Ambati J. Sequence- and target-independent angiogenesis suppression by siRNA *via* TLR3. *Nature* 2008; **452**: 591-597
83 **Grünweller A**, Hartmann RK. RNA interference as a gene-specific approach for molecular medicine. *Curr Med Chem* 2005; **12**: 3143-3161
84 **Peters CJ**, Rees JR, Hardwick RH, Hardwick JS, Vowler SL, Ong CA, Zhang C, Save V, O'Donovan M, Rassl D, Alderson D, Caldas C, Fitzgerald RC. A 4-gene signature predicts survival of patients with resected adenocarcinoma of the esophagus, junction, and gastric cardia. *Gastroenterology* 2010; **139**: 1995-2004.e15

S- Editor Wang JL **L- Editor** O'Neill M **E- Editor** Ma WH

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/1007-9327office
wjg@wjgnet.com
doi:10.3748/wjg.v17.i23.2791

World J Gastroenterol 2011 June 21; 17(23): 2791-2800
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

REVIEW

Need for a comprehensive medical approach to the neuro-immuno-gastroenterology of irritable bowel syndrome

Pejman Katiraei, Gilberto Bultron

Pejman Katiraei, Gilberto Bultron, Department of Pediatrics, Loma Linda University, 11175 Campus Street, Coleman Pavilion A1121, Loma Linda, CA 92350, United States
Author contributions: Katiraei P is the primary author and major contributor to this paper; Bultron G is the secondary author and is a substantial contributor; both Katiraei P and Bultron G performed literature research, drafted the article, contributed to the intellectual content, and wrote the article.
Supported by A Generous Grant from the Riverside Community Health Foundation
Correspondence to: Pejman Katiraei, DO, Department of Pediatrics, Loma Linda University, 11175 Campus Street, Coleman Pavilion A1121, Loma Linda, CA 92350, United States. pkatiraei@llu.edu
Telephone: +1-909-5588142 Fax: +1-909-5584184
Received: December 23, 2010 Revised: March 8, 2011
Accepted: March 15, 2011
Published online: June 21, 2011

Abstract

Irritable bowel syndrome (IBS) is defined by the Rome Ⅲ criteria as symptoms of recurrent abdominal pain or discomfort with the onset of a marked change in bowel habits with no evidence of an inflammatory, anatomic, metabolic, or neoplastic process. As such, many clinicians regard IBS as a central nervous system problem of altered pain perception. Here, we review the recent literature and discuss the evidence that supports an organic based model, which views IBS as a complex, heterogeneous, inter-dependent, and multi-variable inflammatory process along the neuronal-gut axis. We delineate the organic pathophysiology of IBS, demonstrate the role of inflammation in IBS, review the possible differences between adult and pediatric IBS, discuss the merits of a comprehensive treatment model as taught by the Institute of Functional Medicine, and describe the potential for future research for this syndrome.

Key words: Irritable bowel syndrome; Abdominal pain; Inflammation; Probiotics; Stress

Peer reviewer: Dr. Wei-Dong Tong, MD, PhD, Associate Professor, Daping Hospital, Third Military Medical University, Chongqing 400042, China

Katiraei P, Bultron G. Need for a comprehensive medical approach to the neuro-immuno-gastroenterology of irritable bowel syndrome. *World J Gastroenterol* 2011; 17(23): 2791-2800 Available from: URL: http://www.wjgnet.com/1007-9327/full/v17/i23/2791.htm DOI: http://dx.doi.org/10.3748/wjg.v17.i23.2791

INTRODUCTION

Functional abdominal pain (FAP) and irritable bowel syndrome (IBS) are debilitating and common conditions. IBS is defined by the Rome Ⅲ criteria as, "symptoms of recurrent abdominal pain or discomfort and a marked change in bowel habit for at least six months, with symptoms experienced on at least three days of at least three months, with two of the three following findings: (1) Pain is relieved by a bowel movement; (2) Onset of pain is related to a change in frequency of stool; and (3) Onset of pain is related to a change in the appearance of stool"[1].

FAP occurs in 10%-15% of school-aged children, of which 17%-24% have pain significant enough to disrupt their activity, and 13%-15% awaken from their sleep due to the pain[2,3]. Up to 53% of children with abdominal pain continue to have abdominal pain as adults, and 18% are ultimately diagnosed with IBS[4].

Chronic abdominal pain is associated with significant morbidity, including depression[2], decreased quality of life measures[5], and disability leading to inability to work[6]. Adults with abdominal pain have higher rates of potentially unnecessary surgeries[7-10]. Patients with IBS and FAP are costly to the medical system[11,12]. Both children and adults with IBS frequently visit the offices of primary care physicians and gastroenterologists[13]. Adults with IBS

have significantly more hospitalizations, outpatient visits, diagnostic testing, and overall medication use than well patients[14]. A large percentage of the medical costs associated with IBS are related to hospitalizations and inpatient diagnostic testing, such as endoscopies[15]. Antidepressants and other neuropharmacological agents help the symptoms of IBS[16], but these treatments have their own limitations and potential adverse effects.

IBS is thought to be just a functional problem that is "without demonstrable evidence of a pathological condition such as an anatomic, metabolic, infectious, inflammatory, or neoplastic disorder"[17]. IBS is seen as a non-organic syndrome, primarily involving altered perception and processing of pain. As a result, the majority of current therapies for IBS revolve around stress reduction, alteration of pain pathways, and alleviation of symptoms[16].

In this literature review, we delineate the gastrointestinal-neuro-immune pathophysiology of IBS and discuss the link between inflammation and pain. We believe that more effective treatment models are possible through a patient-centered approach that simultaneously treats the multiple variables that lead to IBS, as addressed in this review. The integration of this IBS treatment model may improve patient outcomes while reducing the medical cost burden of IBS. This paper will also discuss the possible differences between adult and pediatric IBS and present potential areas of future research.

STRESS AND THE GASTROINTESTINAL-NEURO-IMMUNE AXIS

Stress in various forms predisposes individuals to developing IBS[18-20] and increases IBS symptoms in children[21]. Abuse or other significant stressors change the neurobiology of stress and alters the levels of corticotropin-releasing factor (CRF) or hormone (CRH)[22], a hypothalamic stress hormone. CRF activates the pituitary-adrenal axis and mediates behavioral, autonomic, immune, and visceral responses to stress[23]. Patients with IBS have enhanced stress responses and release higher amounts of CRF in response to stress[24].

Stress changes the physiology of the gastrointestinal tract. Maternal separation of rat pups causes CRF-mediated mucosal barrier dysfunction with macromolecular permeability and increased bacterial adherence/penetration of the gastrointestinal mucosa with translocation to the spleen[25]. These animals also have mitochondrial swelling of the gut epithelial cells, immune cell infiltration, mucus depletion, and mast cell degranulation[23,26-29]. Stressed human beings show similar findings[30].

Stress compromises the integrity of the gut and induces inflammation through numerous pathways, as demonstrated by several published papers[22,28,31]. CRF released from the hypothalamus can directly influence human colonic mast cells[32,33], which then induce intestinal epithelial pathophysiology and mucosal barrier defects[34-37]. Substance P (SP) and calcitonin gene-related peptide (CGRP)-containing gastrointestinal efferent neurons can also influence mast cells[38-41] and result in degranulation[42] and release of TNF-α[43]. These compounds, in turn, result in gut inflammation and intestinal permeability[44].

These stress-induced changes in the gastrointestinal tract "persist after the stressor is removed from the animal"[37]. This is likely to be due to the ability of mast cells to influence their environment. In rats, inflammation results in increased mast cell-neuronal contacts and mucosal nerve cell density that last well beyond the initial insult[43,45]. Gastrointestinal inflammation in humans also results in neuron proliferation[46-48]. Stress and inflammation modulate nerve growth factor (NGF), which then affects mucosal nerve remodeling[49,50], sprouting, and synaptogenesis[51]. Mast cells, in close contact with neurons, synthesize and release NGF, and thus, can alter neuronal density and synaptogenesis[49,52].

Furthermore, inflammation preceding a psychological stress can alter the epithelial response to stress signals and make the gut more susceptible to stress[53]. In addition, inflammation can change the morphology of mast cells and their intracellular contents, further changing the susceptibility of the gut to various future stressors[54-57].

Inflammation can play an important part in the manifestation of IBS symptoms[58]. Once the inflammatory cascade is activated, this immune response can create a vicious cycle of self-perpetuating inflammation. Activated mast cells can directly release CRF[59]. Patients with inflammatory bowel disease (IBD) and IBS have CRF-immunoreactive macrophages, enterochromaffin cells, lymphocytes, neutrophils, and eosinophils, which are present in higher concentrations than in healthy controls[60-63]. CRF induces lymphocyte proliferation[64] and macrophage release of pro-inflammatory cytokines (TNF-α, IL-1, and IL-6)[65]. These activated immune cells, in turn, locally release CRF and other immune peptides[61,66], which then activate mast cells[22]. Mast cell-derived tryptase is another compound that recruits lymphocytes, eosinophils, and macrophages[67], and can further perpetuate inflammation.

INFLAMMATION INDUCED NEUROLOGICAL TONE

Patients with IBS have central processing abnormalities associated with the perception of pain[68-71]. Colonic irritation can lead to visceral hypersensitivity[72]. Patients with IBS have inflammatory changes in their gut mucosa, which can only be identified by quantitative histopathology, immunohistochemistry, and electron microscopy[73]. These patients have increased numbers of mast cells in the mucosa of the colon[30,74,75]. Mast cell concentrations and their distance from mucosal nerve cells are positively associated with various IBS symptoms[75]. The tryptase released from these mast cells can directly activate gastrointestinal neurons in animals and humans, and can cause visceral hypersensitivity[76-78]. Tryptase cleaves and activates transmembrane proteins called proteinase-activated receptor-2 (PAR2), which are found on the primary afferent neurons of the gastrointestinal tract[79]. Activation of PAR2 receptors leads to neuronal activation, which then creates the experience of

chronic pain.

In addition to central nervous system activation, patients with IBS also have sensitization and upregulation of the dorsal horn[70,71,80], which explains the cutaneous hyperalgesia found in the lower extremities, rather than upper extremities, due to viscerosomatic convergence of nociceptive afferent neurons from the colon/rectum and lower extremities[81]. Seybold *et al*[82] review the mechanisms by which gastrointestinal inflammation leads to gastrointestinal primary afferent neuronal activation and spinal cord activation/sensitization and inflammation.

If true, the perpetual mild mast-cell mediated inflammation can trigger the "excessive or prolonged stimulation of extrinsic afferents (that) may also result in the development of neuronal sensitization, at peripheral, spinal, or higher CNS levels, such that perception of sensations from the bowel is heightened, resulting in symptoms of urgency, bloating, and pain"[83]. This subclinical inflammation may also influence gastrointestinal serotonin pathways.

IBS, NEUROLOGICAL TONE, AND SEROTONIN

Serotonin (5-HT) can influence the motor function and sensitivity of the gastrointestinal tract[84-89]. Serotonin exerts a range of effects *via* its seven receptor subtypes (5-HT_1 to 5HT-7). Serotonin receptor $5HT_5$, $5HT_6$, $5HT_7$ are found in the brain, whereas $5HT_1$, $5HT_2$, $5HT_3$, $5HT_4$, and $5HT_7$ are the gastrointestinal serotonin receptors[90]. A large majority of the body's serotonin is stored in gastrointestinal enterochromaffin cells (EC)[85]. Patients with diarrhea predominant IBS have increased EC cells[91-93], which are activated by inflammation to release serotonin and may result in the elevated serotonin levels found in patients with IBS[94,95]. Tegaserod, a partial $5HT_4$ agonist has been used for constipation dominant IBS and Alosetron, a $5HT_3$ antagonist, in diarrhea dominant IBS.

Serotonin reuptake transporters (SERT) in the gut epithelial cells terminate the effects of serotonin[96,97] and influence serotonin concentrations and symptoms of IBS[85]. Patients with IBS have genetic polymorphisms that lead to lower expression of transport proteins and less serotonin reuptake[83,98,99]. The noted inflammation may also alter SERT expression and decrease its function in patients with IBS[100]. Further studies on the modulation of the gastrointestinal tract serotonin pathways may help further define and treat IBS.

INTESTINAL PERMEABILITY, CHRONIC INFLAMMATION, AND ANTIGENS

The presence and activity of mast cells, along with other inflammatory cells, alone are not likely result in chronic inflammation. Other intestinal antigens, such as food, bacteria, and fungi, are likely to be needed to perpetuate the inflammation in the presence of an impaired gastrointestinal epithelial barrier.

Healthy individuals have tight junctions that help to form the gastrointestinal epithelial barrier along with mucous, SIgA, and other peptides. This epithelial barrier controls the interaction between luminal bacteria and antigens and the mucosal immune system[22,101]. It also allows immune tolerance of food antigens and bacteria. Activation of PAR2 not only leads to neuronal activation, but also to epithelial barrier defects in patients with IBS[102,103].

Low level PAR2 activation of the myosin light chain kinase (MLCK), causes phosphorylation of the myosin light chain, which then leads to contraction of the actin-myosin ring. Tight junction protein zona occludens-1 (ZO-1) relocalizes into the cytoplasm and disrupts the tight junctions, which increases paracellular permeability. High level PAR2 activation in the rat colon results in localized inflammation and increased production of TNF-α and IFN-γ. INF-γ decreases ZO-1 expression and alters the actin cytoskeleton organization[104]. TNF-α activates MLCK and results in tight junction protein relocation[105,106]. A more detailed discussion of these pathways can be found in articles by Gareau *et al*[23] and Cenac *et al*[103].

Children and adults with IBS have increased intestinal permeability[107,108]. Increased intestinal permeability results in "mucosal barrier defects (that) allow the passage of an increased load of luminal antigens of dietary and bacterial origin which, in turn, elicit the activation of mucosal immune responses"[109].

Various triggers can activate mast cells. Bacteria are powerful antigens for the gastrointestinal immune system[110-115]. Stress can result in increased bacterial adherence and penetration into the gastrointestinal mucosa[23,25-27], which may increase the interaction between the luminal bacteria and local immune response. This may explain why patients with IBS have higher antibody titers to specific bacterial flagella than healthy controls[116]. The DNA of these bacteria can interact with toll-like receptors[117], which then influence the immune system through regulation of tumor necrosis factor alpha and interferon gamma[118].

Escherichia coli, Campylobacter, and other bacteria can negatively influence the GI immune system and result in gastrointestinal inflammation and intestinal permeability[46,91,119-121]. Conversely, commercially available beneficial bacteria, in the form of probiotics, can reduce gastrointestinal inflammation[122-125], reverse or prevent intestinal permeability[120], and stop bacterial adhesion[126] and translocation[27,127]. Probiotics can also reverse visceral hypersensitivity from various causes[128,129], including stress[130]. Probiotics attenuate the upregulation of pain pathways at the spinal and supraspinal levels[131], and induce epithelial cells to express micro-opiate receptor 1 (MOR1) and cannabinoid 2 (CB2) opioid receptors[132]. Probiotics can reduce the symptoms of IBS[133,134].

Adults with IBS have gastrointestinal microflora that are significantly different than those of healthy populations[135]. Children with IBS are also likely to have significant alterations in their gastrointestinal microflora. We speculate that there may be a subset of children who are predisposed to developing IBS through repeated or

prolonged exposure to antibiotics for various reasons (recurrent otitis media, sepsis, meningitis, osteomyelitis, vesicoureteral reflux, acne, *etc*). Various antibiotics, including Augmentin, the macrolides, and amoxicillin significantly alter the composition of the bacteria in the GI tract[136-138]. Antibiotic use has been related to increased rates of IBS and functional abdominal pain[139,140].

Gastrointestinal bacteria are also influenced by the diet. Dietary soluble fiber encourages the growth of beneficial species like lactobacilli and bifidobacteria[141-143]. In mice, a white bread diet significantly prolonged antibiotic induced bacterial perturbations[136]. It is common knowledge that the standard American diet lacks fiber, and thus may predispose human beings to have prolonged antibiotic induced bacterial perturbations.

Prebiotics are short chain carbohydrates that help some of the beneficial bacteria or probiotics in the intestines to grow more effectively[142,143]. Prebiotics may decrease IBS symptoms[144-146]. Prebiotics are fermented by probiotics and metabolized into short chain fatty acids (SCFA). SCFAs can decrease inflammation and are used in maintaining the intestinal epithelial lining[147]. While breast milk naturally contains prebiotics[148], up until a few years ago, most infant formulas did not contain prebiotics. Thus, there may be a population of children who were formula fed and required several courses of antibiotics that now have perturbed gastrointestinal flora, as well as intestinal epithelial barriers. We believe that these children may be at risk of developing IBS.

Food proteins are other significant antigens for the gut immune system. Food antigens induce mast cell activation[149] and degranulation, which can lead to visceral hypersensitivity. In children, certain foods may exacerbate intestinal permeability and the elimination of the foods help resolve the IBS symptoms[150]. Elimination of certain foods may decrease immune activation by removing the allergic antigenic load to the local immune system. In patients with IBS, sodium cromoglycate can eliminate IBS symptoms[151-153] by preventing the degranulation of mast cells and inhibiting the release of inflammatory mediators, following contact with an allergen[154].

Over 60% of patients believe that certain foods worsen their IBS symptoms and that elimination of these foods can reduce their symptoms[155-157]. Some believe that these food reactions are psychological in origin[158-160]. Blinded food challenges have raised many questions about the validity of elimination diets for IBS treatment[161-163]. There is also a growing body of evidence to support the use of elimination diets as part of a treatment protocol for IBS[164-167]. Milk, wheat, and eggs are the most commonly identified food triggers[163].

Another potential antigen for the gastrointestinal immune system is *Candida albicans*. Adult studies have shown that *Candida* does not play a significant role in patients with IBS[168,169]. To our knowledge, the role of candida in pediatric IBS has not been determined. Some children who have received numerous courses of antibiotics, such as amoxicillin, can have disruption of the bacterial balance and have overgrowth of the commensal *Candida*[137,170-173]. *Candida* induces inflammation. It produces alcohols and glycoproteins that stimulate mast cells to produce histamine and prostaglandins[174,175]. *Candida* also produces inflammatory prostaglandins that affect mammalian cells[176], as well as proteases that degrade the gastrointestinal IgA and, thus, allow candida to overcome the local immune defense mechanisms[177]. Candidal proteases can induce a B-cell response and result in increased inflammation[174]. In animals and humans, *Candida* perpetuates intestinal inflammation[169].

SMALL INTESTINAL BACTERIAL OVERGROWTH

Another possible contributing factor to IBS signs and symptoms is small intestinal bacterial overgrowth (SIBO), defined as bacterial counts greater than 10^5 cf/mL from small intestinal aspirates[178]. Controversy exists over the ideal method of assessing SIBO[178-181]. A significant number of patients with IBS complain of bloating and pain. SIBO may explain this bloating and pain, as well as other IBS-like-symptoms[182-184]. Several studies have shown antibiotics to be helpful in reducing the symptoms of IBS[185-188].

Patients with IBS who have delayed gastric emptying have a higher risk of developing SIBO[189-192]. Stress is one cause of delayed gastric emptying[193-196]. Once SIBO is present, it can trigger an inflammatory response. SIBO, through abnormal gastrointestinal flora fermentation, may be another cause of IBS symptoms and must be considered in the evaluation of the patient. Furthermore, proton pump inhibitors can also increase the risk of SIBO by decreasing gastric acidity and further perturbations of the gastrointestinal flora species[170,197-200]. We speculate that SIBO may play a larger role in adults with IBS than in children. Further studies are required to elucidate the various differences between adult and pediatric IBS.

CONCLUSION

The evidence presented in our review suggests that IBS is an organic disease with a complex pathophysiology (Figure 1) that is difficult to identify by standard diagnostic tools. The pathophysiology of IBS varies from person to person and from children to adults. The underlying mast cell mediated inflammation of IBS, along with serotonin signaling, can drive the chronic nociceptive input from the periphery to dynamically maintain the altered central processing defects and perception of pain[70,80,201].

In addition to the pathophysiology, clinicians must focus more attention on the well known and less well characterized risk factors that may predispose individuals to developing IBS (Table 1). It is our belief that clinicians should further use the field of neurogastroenterology to better understand the effects of stress on the gastrointestinal tract. Clinicians and researchers must work to develop and adopt models to help us better predict and prevent this condition in susceptible individuals. For chil-

Table 1 Risk factors for irritable bowel syndrome

Genetics/family history
Stress/high academic performance/parental psychiatric disorders
Recurrent or chronic antibiotic use
Bacterial or viral enteritis
Unrecognized food sensitivities
Low fiber diet/diet high in simple carbohydrates
Formula feeding
Chronic acid suppression

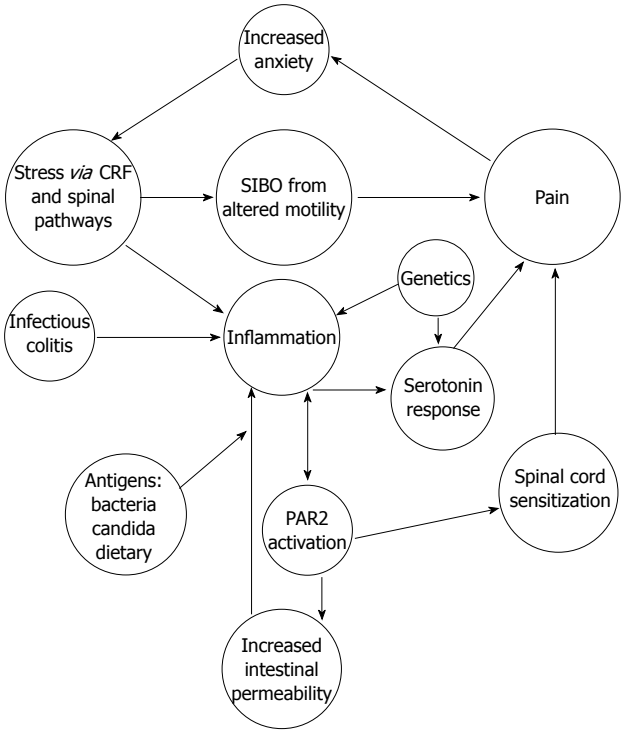


Figure 1 Proposed pathophysiology of irritable bowel syndrome. CRF: Corticotropin-releasing factor; SIBO: Small intestinal bacterial overgrowth; PAR2: Proteinase-activated receptor-2.

dren, these models will require additional studies to evaluate the impact of recurrent antibiotic use and resultant overgrowth of candida on the development of IBS.

Effective treatment models for IBS must reflect the complex physiology of IBS and simultaneously address multiple pathophysiological factors to break the vicious cycle of inflammation and ultimately allow for cessation of symptoms. The Institute of Functional Medicine (IFM)[202] has created such a model of care for IBS. The IFM model has the potential to provide significant improvement in patient care, while reducing healthcare costs and deserves further consideration and evaluation. Please refer to the IFM website and various publications for a more detailed discussion on treatment options.

REFERENCES

1 Appendix A Rome III Diagnostic Criteria for Functional Gastrointestinal Disorders, pg 889 accessed 8/22/09. Available from: URL: http://www.romecriteria.org

2 **Hyams JS**, Burke G, Davis PM, Rzepski B, Andrulonis PA. Abdominal pain and irritable bowel syndrome in adolescents: a community-based study. *J Pediatr* 1996; **129**: 220-226

3 **Hyams JS**, Treem WR, Justinich CJ, Davis P, Shoup M, Burke G. Characterization of symptoms in children with recurrent abdominal pain: resemblance to irritable bowel syndrome. *J Pediatr Gastroenterol Nutr* 1995; **20**: 209-214

4 **Christensen MF**, Mortensen O. Long-term prognosis in children with recurrent abdominal pain. *Arch Dis Child* 1975; **50**: 110-114

5 **Frank L**, Kleinman L, Rentz A, Ciesla G, Kim JJ, Zacker C. Health-related quality of life associated with irritable bowel syndrome: comparison with other chronic diseases. *Clin Ther* 2002; **24**: 675-689; discussion 674

6 **Creed F**, Ratcliffe J, Fernandez L, Tomenson B, Palmer S, Rigby C, Guthrie E, Read N, Thompson D. Health-related quality of life and health care costs in severe, refractory irritable bowel syndrome. *Ann Intern Med* 2001; **134**: 860-868

7 **Longstreth GF**, Yao JF. Irritable bowel syndrome and surgery: a multivariable analysis. *Gastroenterology* 2004; **126**: 1665-1673

8 **Hasler WL**, Schoenfeld P. Systematic review: Abdominal and pelvic surgery in patients with irritable bowel syndrome. *Aliment Pharmacol Ther* 2003; **17**: 997-1005

9 **Talley NJ**. Unnecessary abdominal and back surgery in irritable bowel syndrome: time to stem the flood now? *Gastroenterology* 2004; **126**: 1899-1903

10 **Longstreth GF**. Avoiding unnecessary surgery in irritable bowel syndrome. *Gut* 2007; **56**: 608-610

11 **Talley NJ**, Gabriel SE, Harmsen WS, Zinsmeister AR, Evans RW. Medical costs in community subjects with irritable bowel syndrome. *Gastroenterology* 1995; **109**: 1736-1741

12 **Leong SA**, Barghout V, Birnbaum HG, Thibeault CE, Ben-Hamadi R, Frech F, Ofman JJ. The economic consequences of irritable bowel syndrome: a US employer perspective. *Arch Intern Med* 2003; **163**: 929-935

13 **Lane MM**, Weidler EM, Czyzewski DI, Shulman RJ. Pain symptoms and stooling patterns do not drive diagnostic costs for children with functional abdominal pain and irritable bowel syndrome in primary or tertiary care. *Pediatrics* 2009; **123**: 758-764

14 **Longstreth GF**, Wilson A, Knight K, Wong J, Chiou CF, Barghout V, Frech F, Ofman JJ. Irritable bowel syndrome, health care use, and costs: a U.S. managed care perspective. *Am J Gastroenterol* 2003; **98**: 600-607

15 **Maxion-Bergemann S**, Thielecke F, Abel F, Bergemann R. Costs of irritable bowel syndrome in the UK and US. *Pharmacoeconomics* 2006; **24**: 21-37

16 **Ford AC**, Talley NJ, Schoenfeld PS, Quigley EM, Moayyedi P. Efficacy of antidepressants and psychological therapies in irritable bowel syndrome: systematic review and meta-analysis. *Gut* 2009; **58**: 367-378

17 Chronic abdominal pain in children. *Pediatrics* 2005; **115**: 812-815

18 **White DL**, Savas LS, Daci K, Elserag R, Graham DP, Fitzgerald SJ, Smith SL, Tan G, El-Serag HB. Trauma history and risk of the irritable bowel syndrome in women veterans. *Aliment Pharmacol Ther* 2010; **32**: 551-561

19 **Spiller R**, Garsed K. Postinfectious irritable bowel syndrome. *Gastroenterology* 2009; **136**: 1979-1988

20 **Alonso C**, Guilarte M, Vicario M, Ramos L, Ramadan Z, Antoln M, Martnez C, Rezzi S, Saperas E, Kochhar S, Santos J, Malagelada JR. Maladaptive intestinal epithelial responses to life stress may predispose healthy women to gut mucosal inflammation. *Gastroenterology* 2008; **135**: 163-172.e1

21 **Walker LS**, Garber J, Smith CA, Van Slyke DA, Claar RL. The relation of daily stressors to somatic and emotional symptoms in children with and without recurrent abdominal pain. *J Consult Clin Psychol* 2001; **69**: 85-91

22 **Kiank C**, Tach Y, Larauche M. Stress-related modulation of inflammation in experimental models of bowel disease and

post-infectious irritable bowel syndrome: role of corticotropin-releasing factor receptors. *Brain Behav Immun* 2010; **24**: 41-48
23 **Gareau MG**, Silva MA, Perdue MH. Pathophysiological mechanisms of stress-induced intestinal damage. *Curr Mol Med* 2008; **8**: 274-281
24 **Posserud I**, Agerforz P, Ekman R, Bjrnsson ES, Abrahamsson H, Simrn M. Altered visceral perceptual and neuroendocrine response in patients with irritable bowel syndrome during mental stress. *Gut* 2004; **53**: 1102-1108
25 **Gareau MG**, Jury J, Yang PC, MacQueen G, Perdue MH. Neonatal maternal separation causes colonic dysfunction in rat pups including impaired host resistance. *Pediatr Res* 2006; **59**: 83-88
26 **Mazzon E**, Sturniolo GC, Puzzolo D, Frisina N, Fries W. Effect of stress on the paracellular barrier in the rat ileum. *Gut* 2002; **51**: 507-513
27 **Zareie M**, Johnson-Henry K, Jury J, Yang PC, Ngan BY, McKay DM, Soderholm JD, Perdue MH, Sherman PM. Probiotics prevent bacterial translocation and improve intestinal barrier function in rats following chronic psychological stress. *Gut* 2006; **55**: 1553-1560
28 **Barreau F**, Cartier C, Leveque M, Ferrier L, Moriez R, Laroute V, Rosztoczy A, Fioramonti J, Bueno L. Pathways involved in gut mucosal barrier dysfunction induced in adult rats by maternal deprivation: corticotrophin-releasing factor and nerve growth factor interplay. *J Physiol* 2007; **580**: 347-356
29 **Gareau MG**, Jury J, Perdue MH. Neonatal maternal separation of rat pups results in abnormal cholinergic regulation of epithelial permeability. *Am J Physiol Gastrointest Liver Physiol* 2007; **293**: G198-G203
30 **Piche T**, Saint-Paul MC, Dainese R, Marine-Barjoan E, Iannelli A, Montoya ML, Peyron JF, Czerucka D, Cherikh F, Filippi J, Tran A, Hbuterne X. Mast cells and cellularity of the colonic mucosa correlated with fatigue and depression in irritable bowel syndrome. *Gut* 2008; **57**: 468-473
31 **Rijnierse A**, Nijkamp FP, Kraneveld AD. Mast cells and nerves tickle in the tummy: implications for inflammatory bowel disease and irritable bowel syndrome. *Pharmacol Ther* 2007; **116**: 207-235
32 **Farhadi A**, Fields JZ, Keshavarzian A. Mucosal mast cells are pivotal elements in inflammatory bowel disease that connect the dots: stress, intestinal hyperpermeability and inflammation. *World J Gastroenterol* 2007; **13**: 3027-3030
33 **Wallon C**, Yang PC, Keita AV, Ericson AC, McKay DM, Sherman PM, Perdue MH, Sderholm JD. Corticotropin-releasing hormone (CRH) regulates macromolecular permeability *via* mast cells in normal human colonic biopsies in vitro. *Gut* 2008; **57**: 50-58
34 **Santos J**, Saunders PR, Hanssen NP, Yang PC, Yates D, Groot JA, Perdue MH. Corticotropin-releasing hormone mimics stress-induced colonic epithelial pathophysiology in the rat. *Am J Physiol* 1999; **277**: G391-G399
35 **Saunders PR**, Santos J, Hanssen NP, Yates D, Groot JA, Perdue MH. Physical and psychological stress in rats enhances colonic epithelial permeability *via* peripheral CRH. *Dig Dis Sci* 2002; **47**: 208-215
36 **Pothoulakis C**, Castagliuolo I, Leeman SE. Neuroimmune mechanisms of intestinal responses to stress. Role of corticotropin-releasing factor and neurotensin. *Ann N Y Acad Sci* 1998; **840**: 635-648
37 **Castagliuolo I**, Lamont JT, Qiu B, Fleming SM, Bhaskar KR, Nikulasson ST, Kornetsky C, Pothoulakis C. Acute stress causes mucin release from rat colon: role of corticotropin releasing factor and mast cells. *Am J Physiol* 1996; **271**: G884-G892
38 **Miceli PC**, Jacobson K. Cholinergic pathways modulate experimental dinitrobenzene sulfonic acid colitis in rats. *Auton Neurosci* 2003; **105**: 16-24
39 **Stead RH**, Tomioka M, Quinonez G, Simon GT, Felten SY, Bienenstock J. Intestinal mucosal mast cells in normal and nematode-infected rat intestines are in intimate contact with peptidergic nerves. *Proc Natl Acad Sci USA* 1987; **84**: 2975-2979
40 **Santos J**, Guilarte M, Alonso C, Malagelada JR. Pathogenesis of irritable bowel syndrome: the mast cell connection. *Scand J Gastroenterol* 2005; **40**: 129-140
41 **De Jonge F**, De Laet A, Van Nassauw L, Brown JK, Miller HR, van Bogaert PP, Timmermans JP, Kroese AB. In vitro activation of murine DRG neurons by CGRP-mediated mucosal mast cell degranulation. *Am J Physiol Gastrointest Liver Physiol* 2004; **287**: G178-G191
42 **Janiszewski J**, Bienenstock J, Blennerhassett MG. Picomolar doses of substance P trigger electrical responses in mast cells without degranulation. *Am J Physiol* 1994; **267**: C138-C145
43 **Stead RH**, Colley EC, Wang B, Partosoedarso E, Lin J, Stanisz A, Hillsley K. Vagal influences over mast cells. *Auton Neurosci* 2006; **125**: 53-61
44 **Wang L**, Stanisz AM, Wershil BK, Galli SJ, Perdue MH. Substance P induces ion secretion in mouse small intestine through effects on enteric nerves and mast cells. *Am J Physiol* 1995; **269**: G85-G92
45 **Stead RH**, Kosecka-Janiszewska U, Oestreicher AB, Dixon MF, Bienenstock J. Remodeling of B-50 (GAP-43)- and NSE-immunoreactive mucosal nerves in the intestines of rats infected with Nippostrongylus brasiliensis. *J Neurosci* 1991; **11**: 3809-3821
46 **Wang LH**, Fang XC, Pan GZ. Bacillary dysentery as a causative factor of irritable bowel syndrome and its pathogenesis. *Gut* 2004; **53**: 1096-1101
47 **Di Sebastiano P**, Fink T, Weihe E, Friess H, Beger HG, Bchler M. Changes of protein gene product 9.5 (PGP 9.5) immunoreactive nerves in inflamed appendix. *Dig Dis Sci* 1995; **40**: 366-372
48 **Leonard N**, Hourihane DO, Whelan A. Neuroproliferation in the mucosa is a feature of coeliac disease and Crohn's disease. *Gut* 1995; **37**: 763-765
49 **Barreau F**, Salvador-Cartier C, Houdeau E, Bueno L, Fioramonti J. Long-term alterations of colonic nerve-mast cell interactions induced by neonatal maternal deprivation in rats. *Gut* 2008; **57**: 582-590
50 **Stead RH**. Nerve remodelling during intestinal inflammation. *Ann N Y Acad Sci* 1992; **664**: 443-455
51 **Burgos I**, Cuello AC, Liberini P, Pioro E, Masliah E. NGF-mediated synaptic sprouting in the cerebral cortex of lesioned primate brain. *Brain Res* 1995; **692**: 154-160
52 **Leon A**, Buriani A, Dal Toso R, Fabris M, Romanello S, Aloe L, Levi-Montalcini R. Mast cells synthesize, store, and release nerve growth factor. *Proc Natl Acad Sci USA* 1994; **91**: 3739-3743
53 **Saunders PR**, Miceli P, Vallance BA, Wang L, Pinto S, Tougas G, Kamath M, Jacobson K. Noradrenergic and cholinergic neural pathways mediate stress-induced reactivation of colitis in the rat. *Auton Neurosci* 2006; **124**: 56-68
54 **Lantz CS**, Huff TF. Differential responsiveness of purified mouse c-kit mast cells and their progenitors to IL-3 and stem cell factor. *J Immunol* 1995; **155**: 4024-4029
55 **Rennick D**, Hunte B, Holland G, Thompson-Snipes L. Cofactors are essential for stem cell factor-dependent growth and maturation of mast cell progenitors: comparative effects of interleukin-3 (IL-3), IL-4, IL-10, and fibroblasts. *Blood* 1995; **85**: 57-65
56 **Nakahata T**, Toru H. Cytokines regulate development of human mast cells from hematopoietic progenitors. *Int J Hematol* 2002; **75**: 350-356
57 **Blennerhassett MG**, Bienenstock J. Sympathetic nerve contact causes maturation of mast cells in vitro. *J Neurobiol* 1998; **35**: 173-182
58 **Barbara G**, De Giorgio R, Stanghellini V, Cremon C, Corinaldesi R. A role for inflammation in irritable bowel syndrome? *Gut* 2002; **51** Suppl 1: i41-i44
59 **Theoharides TC**, Donelan JM, Papadopoulou N, Cao J, Kempuraj D, Conti P. Mast cells as targets of corticotropin-

releasing factor and related peptides. *Trends Pharmacol Sci* 2004; **25**: 563-568
60 **Saito-Nakaya K**, Hasegawa R, Nagura Y, Ito H, Fukudo S. Corticotropin-releasing hormone receptor 1 antagonist blocks colonic hypersensitivity induced by a combination of inflammation and repetitive colorectal distension. *Neurogastroenterol Motil* 2008; **20**: 1147-1156
61 **Gross KJ**, Pothoulakis C. Role of neuropeptides in inflammatory bowel disease. *Inflamm Bowel Dis* 2007; **13**: 918-932
62 **Chadwick VS**, Chen W, Shu D, Paulus B, Bethwaite P, Tie A, Wilson I. Activation of the mucosal immune system in irritable bowel syndrome. *Gastroenterology* 2002; **122**: 1778-1783
63 **Cremon C**, Gargano L, Morselli-Labate AM, Santini D, Cogliandro RF, De Giorgio R, Stanghellini V, Corinaldesi R, Barbara G. Mucosal immune activation in irritable bowel syndrome: gender-dependence and association with digestive symptoms. *Am J Gastroenterol* 2009; **104**: 392-400
64 **Singh VK**. Stimulatory effect of corticotropin-releasing neurohormone on human lymphocyte proliferation and interleukin-2 receptor expression. *J Neuroimmunol* 1989; **23**: 257-262
65 **Agelaki S**, Tsatsanis C, Gravanis A, Margioris AN. Corticotropin-releasing hormone augments proinflammatory cytokine production from macrophages in vitro and in lipopolysaccharide-induced endotoxin shock in mice. *Infect Immun* 2002; **70**: 6068-6074
66 **Black PH**. Stress and the inflammatory response: a review of neurogenic inflammation. *Brain Behav Immun* 2002; **16**: 622-653
67 **He S**, Peng Q, Walls AF. Potent induction of a neutrophil and eosinophil-rich infiltrate in vivo by human mast cell tryptase: selective enhancement of eosinophil recruitment by histamine. *J Immunol* 1997; **159**: 6216-6225
68 **Verne GN**, Himes NC, Robinson ME, Gopinath KS, Briggs RW, Crosson B, Price DD. Central representation of visceral and cutaneous hypersensitivity in the irritable bowel syndrome. *Pain* 2003; **103**: 99-110
69 **Silverman DH**, Munakata JA, Ennes H, Mandelkern MA, Hoh CK, Mayer EA. Regional cerebral activity in normal and pathological perception of visceral pain. *Gastroenterology* 1997; **112**: 64-72
70 **Verne GN**, Price DD. Irritable bowel syndrome as a common precipitant of central sensitization. *Curr Rheumatol Rep* 2002; **4**: 322-328
71 **Price DD**, Zhou Q, Moshiree B, Robinson ME, Verne GN. Peripheral and central contributions to hyperalgesia in irritable bowel syndrome. *J Pain* 2006; **7**: 529-535
72 **Al-Chaer ED**, Kawasaki M, Pasricha PJ. A new model of chronic visceral hypersensitivity in adult rats induced by colon irritation during postnatal development. *Gastroenterology* 2000; **119**: 1276-1285
73 **De Giorgio R**, Barbara G. Is irritable bowel syndrome an inflammatory disorder? *Curr Gastroenterol Rep* 2008; **10**: 385-390
74 **Park JH**, Rhee PL, Kim HS, Lee JH, Kim YH, Kim JJ, Rhee JC. Mucosal mast cell counts correlate with visceral hypersensitivity in patients with diarrhea predominant irritable bowel syndrome. *J Gastroenterol Hepatol* 2006; **21**: 71-78
75 **Barbara G**, Stanghellini V, De Giorgio R, Cremon C, Cottrell GS, Santini D, Pasquinelli G, Morselli-Labate AM, Grady EF, Bunnett NW, Collins SM, Corinaldesi R. Activated mast cells in proximity to colonic nerves correlate with abdominal pain in irritable bowel syndrome. *Gastroenterology* 2004; **126**: 693-702
76 **Barbara G**, Wang B, Stanghellini V, de Giorgio R, Cremon C, Di Nardo G, Trevisani M, Campi B, Geppetti P, Tonini M, Bunnett NW, Grundy D, Corinaldesi R. Mast cell-dependent excitation of visceral-nociceptive sensory neurons in irritable bowel syndrome. *Gastroenterology* 2007; **132**: 26-37
77 **Buhner S**, Li Q, Vignali S, Barbara G, De Giorgio R, Stanghellini V, Cremon C, Zeller F, Langer R, Daniel H, Michel K, Schemann M. Activation of human enteric neurons by supernatants of colonic biopsy specimens from patients with irritable bowel syndrome. *Gastroenterology* 2009; **137**: 1425-1434
78 **Cenac N**, Andrews CN, Holzhausen M, Chapman K, Cottrell G, Andrade-Gordon P, Steinhoff M, Barbara G, Beck P, Bunnett NW, Sharkey KA, Ferraz JG, Shaffer E, Vergnolle N. Role for protease activity in visceral pain in irritable bowel syndrome. *J Clin Invest* 2007; **117**: 636-647
79 **Ossovskaya VS**, Bunnett NW. Protease-activated receptors: contribution to physiology and disease. *Physiol Rev* 2004; **84**: 579-621
80 **Moshiree B**, Zhou Q, Price DD, Verne GN. Central sensitisation in visceral pain disorders. *Gut* 2006; **55**: 905-908
81 **Verne GN**, Robinson ME, Price DD. Hypersensitivity to visceral and cutaneous pain in the irritable bowel syndrome. *Pain* 2001; **93**: 7-14
82 **Seybold VS.** The role of peptides in central sensitization. *Handb Exp Pharmacol* 2009; 451-491
83 **Yeo A**, Boyd P, Lumsden S, Saunders T, Handley A, Stubbins M, Knaggs A, Asquith S, Taylor I, Bahari B, Crocker N, Rallan R, Varsani S, Montgomery D, Alpers DH, Dukes GE, Purvis I, Hicks GA. Association between a functional polymorphism in the serotonin transporter gene and diarrhoea predominant irritable bowel syndrome in women. *Gut* 2004; **53**: 1452-1458
84 **Spiller R**. Serotonin, inflammation, and IBS: fitting the jigsaw together? *J Pediatr Gastroenterol Nutr* 2007; **45** Suppl 2: S115-S119
85 **Kim DY**, Camilleri M. Serotonin: a mediator of the brain-gut connection. *Am J Gastroenterol* 2000; **95**: 2698-2709
86 **Lesurtel M**, Soll C, Graf R, Clavien PA. Role of serotonin in the hepato-gastroIntestinal tract: an old molecule for new perspectives. *Cell Mol Life Sci* 2008; **65**: 940-952
87 **Gershon MD**. Review article: roles played by 5-hydroxytryptamine in the physiology of the bowel. *Aliment Pharmacol Ther* 1999; **13** Suppl 2: 15-30
88 **Crowell MD**. The role of serotonin in the pathophysiology of irritable bowel syndrome. *Am J Manag Care* 2001; **7**: S252-S260
89 **Crowell MD**. Role of serotonin in the pathophysiology of the irritable bowel syndrome. *Br J Pharmacol* 2004; **141**: 1285-1293
90 **Sikander A**, Rana SV, Prasad KK. Role of serotonin in gastrointestinal motility and irritable bowel syndrome. *Clin Chim Acta* 2009; **403**: 47-55
91 **Spiller RC**. Postinfectious irritable bowel syndrome. *Gastroenterology* 2003; **124**: 1662-1671
92 **Spiller RC**, Jenkins D, Thornley JP, Hebden JM, Wright T, Skinner M, Neal KR. Increased rectal mucosal enteroendocrine cells, T lymphocytes, and increased gut permeability following acute Campylobacter enteritis and in post-dysenteric irritable bowel syndrome. *Gut* 2000; **47**: 804-811
93 **Dunlop SP**, Jenkins D, Neal KR, Spiller RC. Relative importance of enterochromaffin cell hyperplasia, anxiety, and depression in postinfectious IBS. *Gastroenterology* 2003; **125**: 1651-1659
94 **Wang H**, Steeds J, Motomura Y, Deng Y, Verma-Gandhu M, El-Sharkawy RT, McLaughlin JT, Grencis RK, Khan WI. CD4T cell-mediated immunological control of enterochromaffin cell hyperplasia and 5-hydroxytryptamine production in enteric infection. *Gut* 2007; **56**: 949-957
95 **Atkinson W**, Lockhart S, Whorwell PJ, Keevil B, Houghton LA. Altered 5-hydroxytryptamine signaling in patients with constipation- and diarrhea-predominant irritable bowel syndrome. *Gastroenterology* 2006; **130**: 34-43
96 **Chen JX**, Pan H, Rothman TP, Wade PR, Gershon MD. Guinea pig 5-HT transporter: cloning, expression, distribution, and function in intestinal sensory reception. *Am J Physiol* 1998; **275**: G433-G448
97 **Wade PR**, Chen J, Jaffe B, Kassem IS, Blakely RD, Gershon MD. Localization and function of a 5-HT transporter in crypt epithelia of the gastrointestinal tract. *J Neurosci* 1996;

16: 2352-2364
98 **Coates MD**, Mahoney CR, Linden DR, Sampson JE, Chen J, Blaszyk H, Crowell MD, Sharkey KA, Gershon MD, Mawe GM, Moses PL. Molecular defects in mucosal serotonin content and decreased serotonin reuptake transporter in ulcerative colitis and irritable bowel syndrome. *Gastroenterology* 2004; **126**: 1657-1664
99 **Kohen R**, Jarrett ME, Cain KC, Jun SE, Navaja GP, Symonds S, Heitkemper MM. The serotonin transporter polymorphism rs25531 is associated with irritable bowel syndrome. *Dig Dis Sci* 2009; **54**: 2663-2670
100 **Mawe GM**, Coates MD, Moses PL. Review article: intestinal serotonin signalling in irritable bowel syndrome. *Aliment Pharmacol Ther* 2006; **23**: 1067-1076
101 **Kraehenbuhl JP**, Corbett M. Immunology. Keeping the gut microflora at bay. *Science* 2004; **303**: 1624-1625
102 **Kong W**, McConalogue K, Khitin LM, Hollenberg MD, Payan DG, Bhm SK, Bunnett NW. Luminal trypsin may regulate enterocytes through proteinase-activated receptor 2. *Proc Natl Acad Sci USA* 1997; **94**: 8884-8889
103 **Cenac N**, Chin AC, Garcia-Villar R, Salvador-Cartier C, Ferrier L, Vergnolle N, Buret AG, Fioramonti J, Bueno L. PAR2 activation alters colonic paracellular permeability in mice *via* IFN-gamma-dependent and -independent pathways. *J Physiol* 2004; **558**: 913-925
104 **Ferrier L**, Mazelin L, Cenac N, Desreumaux P, Janin A, Emilie D, Colombel JF, Garcia-Villar R, Fioramonti J, Bueno L. Stress-induced disruption of colonic epithelial barrier: role of interferon-gamma and myosin light chain kinase in mice. *Gastroenterology* 2003; **125**: 795-804
105 **Ye D**, Ma I, Ma TY. Molecular mechanism of tumor necrosis factor-alpha modulation of intestinal epithelial tight junction barrier. *Am J Physiol Gastrointest Liver Physiol* 2006; **290**: G496-G504
106 **Ma TY**, Boivin MA, Ye D, Pedram A, Said HM. Mechanism of TNF-{alpha} modulation of Caco-2 intestinal epithelial tight junction barrier: role of myosin light-chain kinase protein expression. *Am J Physiol Gastrointest Liver Physiol* 2005; **288**: G422-G430
107 **Dunlop SP**, Hebden J, Campbell E, Naesdal J, Olbe L, Perkins AC, Spiller RC. Abnormal intestinal permeability in subgroups of diarrhea-predominant irritable bowel syndromes. *Am J Gastroenterol* 2006; **101**: 1288-1294
108 **Barau E**, Dupont C. Modifications of intestinal permeability during food provocation procedures in pediatric irritable bowel syndrome. *J Pediatr Gastroenterol Nutr* 1990; **11**: 72-77
109 **Barbara G**. Mucosal barrier defects in irritable bowel syndrome. Who left the door open? *Am J Gastroenterol* 2006; **101**: 1295-1298
110 **Rautava S**, Walker WA. Commensal bacteria and epithelial cross talk in the developing intestine. *Curr Gastroenterol Rep* 2007; **9**: 385-392
111 **Tlaskalova-Hogenova H**, Tuckova L, Mestecky J, Kolinska J, Rossmann P, Stepankova R, Kozakova H, Hudcovic T, Hrncir T, Frolova L, Kverka M. Interaction of mucosal microbiota with the innate immune system. *Scand J Immunol* 2005; **62** Suppl 1: 106-113
112 **Kelly D**, Conway S. Bacterial modulation of mucosal innate immunity. *Mol Immunol* 2005; **42**: 895-901
113 **Taurog JD**, Richardson JA, Croft JT, Simmons WA, Zhou M, Fernndez-Sueiro JL, Balish E, Hammer RE. The germfree state prevents development of gut and joint inflammatory disease in HLA-B27 transgenic rats. *J Exp Med* 1994; **180**: 2359-2364
114 **Sellon RK**, Tonkonogy S, Schultz M, Dieleman LA, Grenther W, Balish E, Rennick DM, Sartor RB. Resident enteric bacteria are necessary for development of spontaneous colitis and immune system activation in interleukin-10-deficient mice. *Infect Immun* 1998; **66**: 5224-5231
115 **Rath HC**, Herfarth HH, Ikeda JS, Grenther WB, Hamm TE, Balish E, Taurog JD, Hammer RE, Wilson KH, Sartor RB. Normal luminal bacteria, especially Bacteroides species, mediate chronic colitis, gastritis, and arthritis in HLA-B27/human beta2 microglobulin transgenic rats. *J Clin Invest* 1996; **98**: 945-953
116 **Schoepfer AM**, Schaffer T, Seibold-Schmid B, Mller S, Seibold F. Antibodies to flagellin indicate reactivity to bacterial antigens in IBS patients. *Neurogastroenterol Motil* 2008; **20**: 1110-1118
117 **Rachmilewitz D**, Katakura K, Karmeli F, Hayashi T, Reinus C, Rudensky B, Akira S, Takeda K, Lee J, Takabayashi K, Raz E. Toll-like receptor 9 signaling mediates the anti-inflammatory effects of probiotics in murine experimental colitis. *Gastroenterology* 2004; **126**: 520-528
118 **Jijon H**, Backer J, Diaz H, Yeung H, Thiel D, McKaigney C, De Simone C, Madsen K. DNA from probiotic bacteria modulates murine and human epithelial and immune function. *Gastroenterology* 2004; **126**: 1358-1373
119 **Yuhan R**, Koutsouris A, Savkovic SD, Hecht G. Enteropathogenic Escherichia coli-induced myosin light chain phosphorylation alters intestinal epithelial permeability. *Gastroenterology* 1997; **113**: 1873-1882
120 **Mangell P**, Nejdfors P, Wang M, AhrnS, Westrm B, Thorlacius H, Jeppsson B. Lactobacillus plantarum 299v inhibits Escherichia coli-induced intestinal permeability. *Dig Dis Sci* 2002; **47**: 511-516
121 **Spitz J**, Yuhan R, Koutsouris A, Blatt C, Alverdy J, Hecht G. Enteropathogenic Escherichia coli adherence to intestinal epithelial monolayers diminishes barrier function. *Am J Physiol* 1995; **268**: G374-G379
122 **McCarthy J**, O'Mahony L, O'Callaghan L, Sheil B, Vaughan EE, Fitzsimons N, Fitzgibbon J, O'Sullivan GC, Kiely B, Collins JK, Shanahan F. Double blind, placebo controlled trial of two probiotic strains in interleukin 10 knockout mice and mechanistic link with cytokine balance. *Gut* 2003; **52**: 975-980
123 **Zoumpopoulou G**, Foligne B, Christodoulou K, Grangette C, Pot B, Tsakalidou E. Lactobacillus fermentum ACA-DC 179 displays probiotic potential in vitro and protects against trinitrobenzene sulfonic acid (TNBS)-induced colitis and Salmonella infection in murine models. *Int J Food Microbiol* 2008; **121**: 18-26
124 **Peran L**, Sierra S, Comalada M, Lara-Villoslada F, Bailn E, Nieto A, Concha A, Olivares M, Zarzuelo A, Xaus J, Glvez J. A comparative study of the preventative effects exerted by two probiotics, Lactobacillus reuteri and Lactobacillus fermentum, in the trinitrobenzenesulfonic acid model of rat colitis. *Br J Nutr* 2007; **97**: 96-103
125 **Madsen K**, Cornish A, Soper P, McKaigney C, Jijon H, Yachimec C, Doyle J, Jewell L, De Simone C. Probiotic bacteria enhance murine and human intestinal epithelial barrier function. *Gastroenterology* 2001; **121**: 580-591
126 **Gopal PK**, Prasad J, Smart J, Gill HS. In vitro adherence properties of Lactobacillus rhamnosus DR20 and Bifidobacterium lactis DR10 strains and their antagonistic activity against an enterotoxigenic Escherichia coli. *Int J Food Microbiol* 2001; **67**: 207-216
127 **Mangell P**, Lennerns P, Wang M, Olsson C, Ahrn S, Molin G, Thorlacius H, Jeppsson B. Adhesive capability of Lactobacillus plantarum 299v is important for preventing bacterial translocation in endotoxemic rats. *APMIS* 2006; **114**: 611-618
128 **Verd EF**, Bercik P, Verma-Gandhu M, Huang XX, Blennerhassett P, Jackson W, Mao Y, Wang L, Rochat F, Collins SM. Specific probiotic therapy attenuates antibiotic induced visceral hypersensitivity in mice. *Gut* 2006; **55**: 182-190
129 **Liebregts T**, Adam B, Bertel A, Jones S, Schulze J, Enders C, Sonnenborn U, Lackner K, Holtmann G. Effect of E. coli Nissle 1917 on post-inflammatory visceral sensory function in a rat model. *Neurogastroenterol Motil* 2005; **17**: 410-414
130 **Eutamene H**, Lamine F, Chabo C, Theodorou V, Rochat F, Bergonzelli GE, Corthsy-Theulaz I, Fioramonti J, Bueno L. Synergy between Lactobacillus paracasei and its bacterial

products to counteract stress-induced gut permeability and sensitivity increase in rats. *J Nutr* 2007; **137**: 1901-1907
131 **Ait-Belgnaoui A**, Eutamene H, Houdeau E, Bueno L, Fioramonti J, Theodorou V. Lactobacillus farciminis treatment attenuates stress-induced overexpression of Fos protein in spinal and supraspinal sites after colorectal distension in rats. *Neurogastroenterol Motil* 2009; **21**: 567-573, e18-e19
132 **Rousseaux C**, Thuru X, Gelot A, Barnich N, Neut C, Dubuquoy L, Dubuquoy C, Merour E, Geboes K, Chamaillard M, Ouwehand A, Leyer G, Carcano D, Colombel JF, Ardid D, Desreumaux P. Lactobacillus acidophilus modulates intestinal pain and induces opioid and cannabinoid receptors. *Nat Med* 2007; **13**: 35-37
133 **Hoveyda N**, Heneghan C, Mahtani KR, Perera R, Roberts N, Glasziou P. A systematic review and meta-analysis: probiotics in the treatment of irritable bowel syndrome. *BMC Gastroenterol* 2009; **9**: 15
134 **Barbara G**, Stanghellini V, Cremon C, De Giorgio R, Gargano L, Cogliandro R, Pallotti F, Corinaldesi R. Probiotics and irritable bowel syndrome: rationale and clinical evidence for their use. *J Clin Gastroenterol* 2008; **42** Suppl 3 Pt 2: S214-S217
135 **Kassinen A**, Krogius-Kurikka L, Mkivuokko H, Rinttil T, Paulin L, Corander J, Malinen E, Apajalahti J, Palva A. The fecal microbiota of irritable bowel syndrome patients differs significantly from that of healthy subjects. *Gastroenterology* 2007; **133**: 24-33
136 **Dubos R**, Schaedler RW, Stephens M. The effect of antibacterial drugs on the fecal flora of mice. *J Exp Med* 1963; **117**: 231-243
137 **Sullivan A**, Edlund C, Nord CE. Effect of antimicrobial agents on the ecological balance of human microflora. *Lancet Infect Dis* 2001; **1**: 101-114
138 **Edlund C**, Nord CE. Effect on the human normal microflora of oral antibiotics for treatment of urinary tract infections. *J Antimicrob Chemother* 2000; **46** Suppl 1: 41-48; discussion 63-65
139 **Mendall MA**, Kumar D. Antibiotic use, childhood affluence and irritable bowel syndrome (IBS). *Eur J Gastroenterol Hepatol* 1998; **10**: 59-62
140 **Maxwell PR**, Rink E, Kumar D, Mendall MA. Antibiotics increase functional abdominal symptoms. *Am J Gastroenterol* 2002; **97**: 104-108
141 **Blaut M**. Relationship of prebiotics and food to intestinal microflora. *Eur J Nutr* 2002; **41** Suppl 1: I11-I16
142 **Delzenne NM**. Oligosaccharides: state of the art. *Proc Nutr Soc* 2003; **62**: 177-182
143 **Gibson GR**. Dietary modulation of the human gut microflora using the prebiotics oligofructose and inulin. *J Nutr* 1999; **129**: 1438S-1441S
144 **Giannini EG**, Mansi C, Dulbecco P, Savarino V. Role of partially hydrolyzed guar gum in the treatment of irritable bowel syndrome. *Nutrition* 2006; **22**: 334-342
145 **Silk DB**, Davis A, Vulevic J, Tzortzis G, Gibson GR. Clinical trial: the effects of a trans-galactooligosaccharide prebiotic on faecal microbiota and symptoms in irritable bowel syndrome. *Aliment Pharmacol Ther* 2009; **29**: 508-518
146 **Paineau D**, Payen F, Panserieu S, Coulombier G, Sobaszek A, Lartigau I, Brabet M, Galmiche JP, Tripodi D, Sacher-Huvelin S, Chapalain V, Zourabichvili O, Respondek F, Wagner A, Bornet FR. The effects of regular consumption of short-chain fructo-oligosaccharides on digestive comfort of subjects with minor functional bowel disorders. *Br J Nutr* 2008; **99**: 311-318
147 **Macfarlane S**, Macfarlane GT, Cummings JH. Review article: prebiotics in the gastrointestinal tract. *Aliment Pharmacol Ther* 2006; **24**: 701-714
148 **Newburg DS**. Oligosaccharides in human milk and bacterial colonization. *J Pediatr Gastroenterol Nutr* 2000; **30** Suppl 2: S8-S17
149 **Marshall JS**. Repeated antigen challenge in rats induces a mucosal mast cell hyperplasia. *Gastroenterology* 1993; **105**: 391-398
150 **Barau E**, Dupont C. Modifications of intestinal permeability during food provocation procedures in pediatric irritable bowel syndrome. *J Pediatr Gastroenterol Nutr* 1990; **11**: 72-77
151 **Grazioli I**, Melzi G, Balsamo V, Castellucci G, Castro M, Catassi C, Rtsch JM, Scotta S. [Food intolerance and irritable bowel syndrome of childhood: clinical efficacy of oral sodium cromoglycate and elimination diet]. *Minerva Pediatr* 1993; **45**: 253-258
152 **Lunardi C**, Bambara LM, Biasi D, Cortina P, Peroli P, Nicolis F, Favari F, Pacor ML. Double-blind cross-over trial of oral sodium cromoglycate in patients with irritable bowel syndrome due to food intolerance. *Clin Exp Allergy* 1991; **21**: 569-572
153 **Stefanini GF**, Prati E, Albini MC, Piccinini G, Capelli S, Castelli E, Mazzetti M, Gasbarrini G. Oral disodium cromoglycate treatment on irritable bowel syndrome: an open study on 101 subjects with diarrheic type. *Am J Gastroenterol* 1992; **87**: 55-57
154 **Zar S**, Kumar D, Benson MJ. Food hypersensitivity and irritable bowel syndrome. *Aliment Pharmacol Ther* 2001; **15**: 439-449
155 **Monsbakken KW**, Vandvik PO, Farup PG. Perceived food intolerance in subjects with irritable bowel syndrome-- etiology, prevalence and consequences. *Eur J Clin Nutr* 2006; **60**: 667-672
156 **Dainese R**, Galliani EA, De Lazzari F, Di Leo V, Naccarato R. Discrepancies between reported food intolerance and sensitization test findings in irritable bowel syndrome patients. *Am J Gastroenterol* 1999; **94**: 1892-1897
157 **Nanda R**, James R, Smith H, Dudley CR, Jewell DP. Food intolerance and the irritable bowel syndrome. *Gut* 1989; **30**: 1099-1104
158 **Teufel M**, Biedermann T, Rapps N, Hausteiner C, Henningsen P, Enck P, Zipfel S. Psychological burden of food allergy. *World J Gastroenterol* 2007; **13**: 3456-3465
159 **Heizer WD**, Southern S, McGovern S. The role of diet in symptoms of irritable bowel syndrome in adults: a narrative review. *J Am Diet Assoc* 2009; **109**: 1204-1214
160 **Rief W**, Barsky AJ. Psychobiological perspectives on somatoform disorders. *Psychoneuroendocrinology* 2005; **30**: 996-1002
161 **Farah DA**, Calder I, Benson L, MacKenzie JF. Specific food intolerance: its place as a cause of gastrointestinal symptoms. *Gut* 1985; **26**: 164-168
162 **Bernstein M**, Day JH, Welsh A. Double-blind food challenge in the diagnosis of food sensitivity in the adult. *J Allergy Clin Immunol* 1982; **70**: 205-210
163 **Niec AM**, Frankum B, Talley NJ. Are adverse food reactions linked to irritable bowel syndrome? *Am J Gastroenterol* 1998; **93**: 2184-2190
164 **Atkinson W**, Sheldon TA, Shaath N, Whorwell PJ. Food elimination based on IgG antibodies in irritable bowel syndrome: a randomised controlled trial. *Gut* 2004; **53**: 1459-1464
165 **Zar S**, Mincher L, Benson MJ, Kumar D. Food-specific IgG4 antibody-guided exclusion diet improves symptoms and rectal compliance in irritable bowel syndrome. *Scand J Gastroenterol* 2005; **40**: 800-807
166 **Stefanini GF**, Saggioro A, Alvisi V, Angelini G, Capurso L, di Lorenzo G, Dobrilla G, Dodero M, Galimberti M, Gasbarrini G. Oral cromolyn sodium in comparison with elimination diet in the irritable bowel syndrome, diarrheic type. Multicenter study of 428 patients. *Scand J Gastroenterol* 1995; **30**: 535-541
167 **Jones VA**, McLaughlan P, Shorthouse M, Workman E, Hunter JO. Food intolerance: a major factor in the pathogenesis of irritable bowel syndrome. *Lancet* 1982; **2**: 1115-1117
168 **Middleton SJ**, Coley A, Hunter JO. The role of faecal Candida albicans in the pathogenesis of food-intolerant irritable bowel syndrome. *Postgrad Med J* 1992; **68**: 453-454
169 **Zwolinska-Wcislo M**, Brzozowski T, Budak A, Kwiecien

S, Sliwowski Z, Drozdowicz D, Trojanowska D, Rudnicka-Sosin L, Mach T, Konturek SJ, Pawlik WW. Effect of Candida colonization on human ulcerative colitis and the healing of inflammatory changes of the colon in the experimental model of colitis ulcerosa. *J Physiol Pharmacol* 2009; **60**: 107-118
170 **O'May GA**, Reynolds N, Smith AR, Kennedy A, Macfarlane GT. Effect of pH and antibiotics on microbial overgrowth in the stomachs and duodena of patients undergoing percutaneous endoscopic gastrostomy feeding. *J Clin Microbiol* 2005; **43**: 3059-3065
171 **Loy CE**. Antibiotic-associated diarrhoea: an overlooked aetiology? *Br J Biomed Sci* 2005; **62**: 166-169
172 **Song HJ**, Shim KN, Jung SA, Choi HJ, Lee MA, Ryu KH, Kim SE, Yoo K. Antibiotic-associated diarrhea: candidate organisms other than Clostridium difficile. *Korean J Intern Med* 2008; **23**: 9-15
173 **Maraki S**, Mouzas IA, Kontoyiannis DP, Chatzinikolaou I, Tselentis Y, Samonis G. Prospective evaluation of the impact of amoxicillin, clarithromycin and their combination on human gastrointestinal colonization by Candida species. *Chemotherapy* 2001; **47**: 215-218
174 **Santelmann H**, Howard JM. Yeast metabolic products, yeast antigens and yeasts as possible triggers for irritable bowel syndrome. *Eur J Gastroenterol Hepatol* 2005; **17**: 21-26
175 **Nosál R**, Novotný J, Sikl D. The effect of glycoprotein from Candida albicans on isolated rat mast cells. *Toxicon* 1974; **12**: 103-108
176 **Noverr MC**, Phare SM, Toews GB, Coffey MJ, Huffnagle GB. Pathogenic yeasts Cryptococcus neoformans and Candida albicans produce immunomodulatory prostaglandins. *Infect Immun* 2001; **69**: 2957-2963
177 **Reinholdt J**, Krogh P, Holmstrup P. Degradation of IgA1, IgA2, and S-IgA by Candida and Torulopsis species. *Acta Pathol Microbiol Immunol Scand C* 1987; **95**: 265-274
178 **Simrén M**, Stotzer PO. Use and abuse of hydrogen breath tests. *Gut* 2006; **55**: 297-303
179 **Khoshini R**, Dai SC, Lezcano S, Pimentel M. A systematic review of diagnostic tests for small intestinal bacterial overgrowth. *Dig Dis Sci* 2008; **53**: 1443-1454
180 **Corazza GR**, Menozzi MG, Strocchi A, Rasciti L, Vaira D, Lecchini R, Avanzini P, Chezzi C, Gasbarrini G. The diagnosis of small bowel bacterial overgrowth. Reliability of jejunal culture and inadequacy of breath hydrogen testing. *Gastroenterology* 1990; **98**: 302-309
181 **Ghoshal UC**, Ghoshal U, Das K, Misra A. Utility of hydrogen breath tests in diagnosis of small intestinal bacterial overgrowth in malabsorption syndrome and its relationship with oro-cecal transit time. *Indian J Gastroenterol* 2006; **25**: 6-10
182 **Lin HC**. Small intestinal bacterial overgrowth: a framework for understanding irritable bowel syndrome. *JAMA* 2004; **292**: 852-858
183 **Parodi A**, Dulbecco P, Savarino E, Giannini EG, Bodini G, Corbo M, Isola L, De Conca S, Marabotto E, Savarino V. Positive glucose breath testing is more prevalent in patients with IBS-like symptoms compared with controls of similar age and gender distribution. *J Clin Gastroenterol* 2009; **43**: 962-966
184 **Lin HC**, Pimentel M. Bacterial concepts in irritable bowel syndrome. *Rev Gastroenterol Disord* 2005; **5** Suppl 3: S3-S9
185 **Majewski M**, Reddymasu SC, Sostarich S, Foran P, McCallum RW. Efficacy of rifaximin, a nonabsorbed oral antibiotic, in the treatment of small intestinal bacterial overgrowth. *Am J Med Sci* 2007; **333**: 266-270
186 **Majewski M**, McCallum RW. Results of small intestinal bacterial overgrowth testing in irritable bowel syndrome patients: clinical profiles and effects of antibiotic trial. *Adv Med Sci* 2007; **52**: 139-142
187 **Pimentel M**, Chow EJ, Lin HC. Eradication of small intestinal bacterial overgrowth reduces symptoms of irritable bowel syndrome. *Am J Gastroenterol* 2000; **95**: 3503-3506
188 **Pimentel M**, Chow EJ, Lin HC. Normalization of lactulose breath testing correlates with symptom improvement in irritable bowel syndrome. a double-blind, randomized, placebo-controlled study. *Am J Gastroenterol* 2003; **98**: 412-419
189 **Pimentel M**, Soffer EE, Chow EJ, Kong Y, Lin HC. Lower frequency of MMC is found in IBS subjects with abnormal lactulose breath test, suggesting bacterial overgrowth. *Dig Dis Sci* 2002; **47**: 2639-2643
190 **Husebye E**. The pathogenesis of gastrointestinal bacterial overgrowth. *Chemotherapy* 2005; **51** Suppl 1: 1-22
191 **Nieuwenhuijs VB**, Verheem A, van Duijvenbode-Beumer H, Visser MR, Verhoef J, Gooszen HG, Akkermans LM. The role of interdigestive small bowel motility in the regulation of gut microflora, bacterial overgrowth, and bacterial translocation in rats. *Ann Surg* 1998; **228**: 188-193
192 **Reddymasu SC**, McCallum RW. Small intestinal bacterial overgrowth in gastroparesis: are there any predictors? *J Clin Gastroenterol* 2010; **44**: e8-e13
193 **Nakade Y**, Fukuda H, Iwa M, Tsukamoto K, Yanagi H, Yamamura T, Mantyh C, Pappas TN, Takahashi T. Restraint stress stimulates colonic motility *via* central corticotropin-releasing factor and peripheral 5-HT3 receptors in conscious rats. *Am J Physiol Gastrointest Liver Physiol* 2007; **292**: G1037-G1044
194 **Martnez V**, Wang L, Rivier JE, Vale W, Tach Y. Differential actions of peripheral corticotropin-releasing factor (CRF), urocortin II, and urocortin III on gastric emptying and colonic transit in mice: role of CRF receptor subtypes 1 and 2. *J Pharmacol Exp Ther* 2002; **301**: 611-617
195 **Martinez V**, Wang L, Million M, Rivier J, Tach Y. Urocortins and the regulation of gastrointestinal motor function and visceral pain. *Peptides* 2004; **25**: 1733-1744
196 **Million M**, Maillot C, Saunders P, Rivier J, Vale W, Tach Y. Human urocortin II, a new CRF-related peptide, displays selective CRF(2)-mediated action on gastric transit in rats. *Am J Physiol Gastrointest Liver Physiol* 2002; **282**: G34-G40
197 **Guarner F**, Malagelada JR. Gut flora in health and disease. *Lancet* 2003; **361**: 512-519
198 **Thorens J**, Froehlich F, Schwizer W, Saraga E, Bille J, Gyr K, Duroux P, Nicolet M, Pignatelli B, Blum AL, Gonvers JJ, Fried M. Bacterial overgrowth during treatment with omeprazole compared with cimetidine: a prospective randomised double blind study. *Gut* 1996; **39**: 54-59
199 **Lewis SJ**, Franco S, Young G, O'Keefe SJ. Altered bowel function and duodenal bacterial overgrowth in patients treated with omeprazole. *Aliment Pharmacol Ther* 1996; **10**: 557-561
200 **Spiegel BM**, Chey WD, Chang L. Bacterial overgrowth and irritable bowel syndrome: unifying hypothesis or a spurious consequence of proton pump inhibitors? *Am J Gastroenterol* 2008; **103**: 2972-2976
201 **Gracely RH**, Lynch SA, Bennett GJ. Painful neuropathy: altered central processing maintained dynamically by peripheral input. *Pain* 1992; **51**: 175-194
202 http: //www.functionalmedicine.org/about/whatis.asp accessed 9/12/2010

S- Editor Tian L **L- Editor** Stewart GJ **E- Editor** Ma WH

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/1007-9327office
wjg@wjgnet.com
doi:10.3748/wjg.v17.i23.2801

World J Gastroenterol 2011 June 21; 17(23): 2801-2811
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

REVIEW

Adiponectin, a key adipokine in obesity related liver diseases

Christa Buechler, Josef Wanninger, Markus Neumeier

Christa Buechler, Josef Wanninger, Markus Neumeier, Department of Internal Medicine I, University Hospital of Regensburg, D-93042 Regensburg, Germany
Author contributions: Buechler C wrote the review article and Neumeier M and Wanninger J revised the manuscript and participated in the design of the figures.
Supported by The Faculty of Medicine of the University of Regensburg (ReForM C); The Deutsche Forschungsgemeinschaft
Correspondence to: Christa Buechler, PhD, Department of Internal Medicine I, University Hospital of Regensburg, D-93042 Regensburg, Germany. christa.buechler@klinik.uni-regensburg.de
Telephone: +49-941-9447147 Fax: +49-941-9447019
Received: August 17, 2010 Revised: November 17, 2010
Accepted: November 24, 2010
Published online: June 21, 2011

Abstract

Non-alcoholic fatty liver disease (NAFLD) comprising hepatic steatosis, non-alcoholic steatohepatitis (NASH), and progressive liver fibrosis is considered the most common liver disease in western countries. Fatty liver is more prevalent in overweight than normal-weight people and liver fat positively correlates with hepatic insulin resistance. Hepatic steatosis is regarded as a benign stage of NAFLD but may progress to NASH in a subgroup of patients. Besides liver biopsy no diagnostic tools to identify patients with NASH are available, and no effective treatment has been established. Visceral obesity is a main risk factor for NAFLD and inappropriate storage of triglycerides in adipocytes and higher concentrations of free fatty acids may add to increased hepatic lipid storage, insulin resistance, and progressive liver damage. Most of the adipose tissue-derived proteins are elevated in obesity and may contribute to systemic inflammation and liver damage. Adiponectin is highly abundant in human serum but its levels are reduced in obesity and are even lower in patients with hepatic steatosis or NASH. Adiponectin antagonizes excess lipid storage in the liver and protects from inflammation and fibrosis. This review aims to give a short survey on NAFLD and the hepatoprotective effects of adiponectin.

Key words: Hepatic steatosis; Non-alcoholic steatohepatitis; Adiponectin; Obesity; Adipose tissue

Peer reviewer: Christopher O'Brien, MD, Professor of Clinical Medicine, Chief of Clinical Hepatology, Center for Liver Diseases, Divisions of Liver and GI Transplantation, University of Miami School of Medicine, 1500 Northwest 12th Ave., Suite #1101, Miami, FL 33136, United States

Buechler C, Wanninger J, Neumeier M. Adiponectin, a key adipokine in obesity related liver diseases. *World J Gastroenterol* 2011; 17(23): 2801-2811 Available from: URL: http://www.wjgnet.com/1007-9327/full/v17/i23/2801.htm DOI: http://dx.doi.org/10.3748/wjg.v17.i23.2801

INTRODUCTION

Obesity is associated with insulin resistance, a common risk factor for type 2 diabetes, cardiovascular disease, hepatic steatosis and non-alcoholic steatohepatitis (NASH)[1,2]. Hypertrophied adipocytes in obesity fail to appropriately store excess triglycerides and excessive ectopic accumulation of lipids in skeletal muscle and liver disturbs insulin signalling[3]. Body fat distribution appears to be even more important than the total amount of adipose tissue, and visceral fat mass is strongly linked to insulin resistance and non-alcoholic fatty liver disease (NAFLD)[4]. Visceral fat released free fatty acids are transported to the liver by the portal vein and may contribute to hepatic steatosis, production of triglyceride rich very low density lipoproteins (VLDL) and elevated β-oxidation[5,6] (Figure 1). Metabolically healthy but obese (MHO) individuals are insulin sensitive and hepatic fat accumulation is significantly lower compared to similarly overweight subjects that develop

insulin resistance[7,8]. Despite comparable fatness between MHO and control cohorts that develop insulin resistance, MHO subjects have 49% less visceral fat which further emphasizes the unfavourable characteristics of this fat depot[9]. Lean body mass may be associated with a higher insulin sensitivity and is significantly lower in MHO subjects[9]. A recent study even describes an independent association of lean body mass with impaired glucose disposal and systemic C-reactive protein (CRP) levels in centrally obese postmenopausal women that may exacerbate the harmful effects of visceral fat mass[10]. These studies further point to the highly complex interplay of various factors associated with metabolic diseases like the metabolic syndrome.

Various epidemiological studies have identified central obesity as an independent risk factor for metabolic diseases and highlight the crucial role of impaired production or activity of adipose tissue released proteins[6,11]. Most of the adipokines identified so far are elevated in obesity and raised chemokine C-C motif ligand 2 (CCL2) contributes to the increasing number of adipose tissue resident macrophages[11,12]. They produce inflammatory proteins like interleukin-6 (IL-6) and tumour necrosis factor (TNF) whose circulating levels are increased in obesity, a state of low-grade, chronic inflammation[13]. TNF impairs insulin signalling and plays a crucial role in non-alcoholic steatohepatitis (NASH) progression[14,15]. Visceral fat released proteins are directly transported to the liver by the portal vein and the anatomical feature of this fat depot may explain the harmful metabolic effects of visceral adiposity[6]. IL-6 is preferentially released from visceral fat and upregulates suppressor of cytokine signalling 3 (SOCS3) in the liver that causes hepatic insulin resistance[16-18]. Furthermore, IL-6 is a well known inducer of CRP, a marker protein for systemic inflammation[19] (Figure 1). Leptin is mainly produced by adipocytes, and obesity is characterized by elevated systemic levels and central and peripheral leptin resistance[6]. Leptin prevents lipid accumulation in non-adipose tissues like the liver. Leptin lowers stearoyl-CoA desaturase that catalyzes the rate-limiting reaction of monounsaturated fatty acid synthesis and thereby may ameliorate hepatic insulin sensitivity[20]. Animal studies have proven that leptin directly promotes fibrogenesis. Leptin induces transforming growth factor β (TGF-β) and connective tissue growth factor (CTGF) production in hepatic stellate cells through indirect effects on Kupffer cells[21]. In humans, a direct association of circulating leptin and liver fibrosis has not been confirmed yet and locally produced leptin and/or leptin resistance may have to be taken into account[22].

The adipokine adiponectin is highly abundant in human serum and is secreted by adipose tissue in inverse proportion to the body mass index[23]. Adiponectin circulates as trimer, hexamer and higher order multimer in serum and isoform-specific effects have been described[24-26]. Adiponectin may also form hetero-oligomers with additional members of the C1q/TNF-related protein (CTRP) family like the recently described CTRP9[27]. Early studies indicate

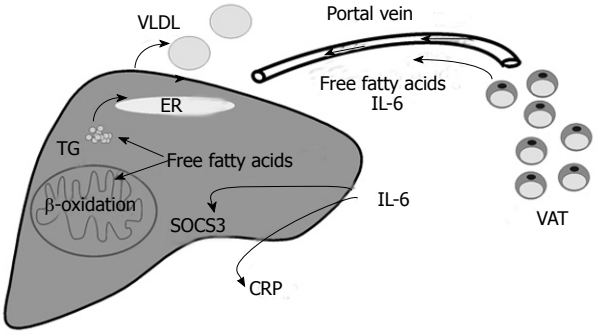


Figure 1 Crosstalk of visceral adipose tissue and the liver. Free fatty acids and Interleukin (IL)-6 released by visceral adipose tissue (VAT) are transported to the liver by the portal vein. Free fatty acids promote steatosis, enhance β-oxidation and the release of very low density lipoproteins (VLDL) contributing to dyslipidemia. IL-6 induces hepatic C-reactive protein (CRP) synthesis and suppressor of cytokine signalling 3 (SOCS3), and thereby is linked to systemic inflammation and hepatic insulin resistance, respectively (adapted from Schaffler A, Scholmerich J, Buchler C. Mechanisms of disease: adipocytokines and visceral adipose tissue--emerging role in nonalcoholic fatty liver disease. *Nat Clin Pract Gastroenterol Hepatol* 2005; 2: 273-280[6]). ER: Endoplasmatic reticulum; TG: Triglycerides.

that globular adiponectin, the globular C1q domain of this protein generated by proteolysis of the full-length protein, may also exist in serum[28]. However, circulating levels seem to be rather low, questioning the biological significance of this protein[29] that may nevertheless be of therapeutic relevance. Epidemiological studies revealed that low adiponectin levels are associated with NASH independent of insulin resistance and body mass index, and hepatoprotective effects of adiponectin have been identified in animal studies or with isolated liver cells[30-32]. MHO individuals are insulin sensitive and have adiponectin levels similar to normal-weight controls despite excessive weight and body fat, and this association may further underline the protective effects of this adipokine[33].

NAFLD not only compromises the hepatic manifestation of the metabolic syndrome but is linked to a higher risk of develop metabolic disorders like type 2 diabetes or cardiovascular disease[34,35]. Fatty liver is even associated with dyslipidemia, metabolic syndrome and low adiponectin independent of body mass index (BMI), waist to hip ratio and visceral fat mass[36]. NAFLD has been predicted to increase along with the growing epidemic of obesity[37] and understanding of its pathophysiology is a prerequisite to develop non-invasive diagnostic tools and to establishing effective treatment regimes. Rising adiponectin levels may be beneficial in liver disease and its protective effects in hepatic steatosis and NASH[38] are summarized in the current review article.

EPIDEMIOLOGY OF NAFLD

Diagnosis of NAFLD requires a careful anamnesis to exclude other liver diseases or drug-mediated liver damage. Moderate alcohol intake, defined by most physicians as 20 to 40 g/d in men and 20 g/d in women, has to be en-

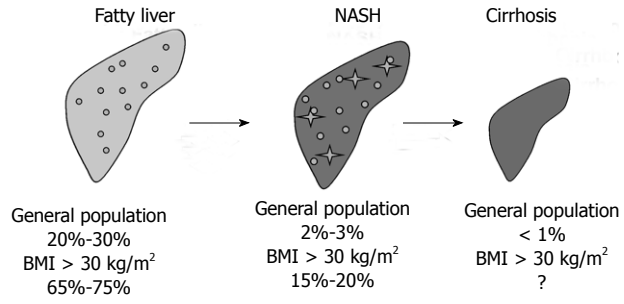


Figure 2 Epidemiology of non-alcoholic fatty liver disease. Current estimates of the prevalence of fatty liver, non-alcoholic steatohepatitis (NASH) and obesity-related liver cirrhosis in the general population and in obesity defined as body mass index (BMI) above 30 kg/m^2.

quired about[39]. Liver biopsy is essential for diagnosis and staging but the use of this invasive method is limited to a subgroup of patients[39].

When NAFLD is defined as elevation of aspartate aminotransferase (AST) or alanine aminotransferase (ALT) and transferrin saturation of less than 50% the frequency is 5.4% in the general population of the United States[40]. When elevated gamma-glutamyltranspeptidase (GGT) is included and lower cut-off values are used the prevalence is 24%[41]. Studies performed in gastroenterology units restricted cohorts identified 11% of the patients as having NAFLD[42]. In bariatric surgery patients hepatic steatosis ranges from 65% to 90%[43,44]; NASH has been diagnosed in 15 to 55% and fibrosis in 34% to 47%[41,45].

Current estimates based on different studies in unselected and selected populations indicate that about 20% to 30% of adults in Western countries have excess fat accumulation in the liver, 2% to 3% of adults are thought to meet current diagnostic criteria for NASH and eventually up to one third of those with NASH suffer from progressive fibrosis or even cirrhosis[41,45,46]. In obesity defined as BMI above 30 kg/m^2 and in morbidly obese patients these values are much higher and patients with NASH are overrepresented in these populations[41,45] (Figure 2).

GENETICS OF NAFLD

Visceral adiposity and insulin resistance are clearly related to NAFLD[47] and genetic variations associated with obesity and disproportionate body fat distribution may predispose to development of steatotic liver. Chemerin is a recently identified adipokine and a common genetic variation is associated with increased visceral fat mass in non-obese subjects but epidemiological studies to link chemerin alleles with NAFLD are still lacking[48]. The adiponectin 45T →G variant contributes to overall fatness and abdominal obesity but is not an important determinant of NAFLD at least in Chinese people[49,50].

Gene variations may influence NAFLD stage, progression and even occurrence. NAFLD is much more likely in Hispanic Americans than among whites[51] and African Americans have a lower degree of hepatic steatosis relative to whites[52]. Familial clustering of NAFLD has been demonstrated, and fatty liver is more common in siblings and parents of children with NAFLD indicating that NAFLD, similar to type 2 diabetes, is a multifactorial disease[53]. Environmental and genetic factors define the individual risk of developing NAFLD and may also explain why only a subgroup of patients develop more progressive liver damage.

Studies in small cohorts have identified genetic associations of microsomal triglyceride transfer protein, an enzyme regulating hepatic VLDL release, the antioxidant mitochondrial enzyme superoxide dismutase 2, the inflammatory cytokine TNF and the main profibrotic cytokine TGF-β with NAFLD[54,55]. Genome-wide association studies find that variations of patatin-like phospholipase domain containing 3 (PNPLA3, adiponectin), a protein with close homology to adipose triglyceride lipase but so far unknown function, contributes to ethnic and inter-individual differences in hepatic steatosis and susceptibility to NAFLD[56,57].

This association has not been confirmed in a recent study in non-Hispanic, Caucasian, women with liver biopsy proven NAFLD, where an association between NASH activity score and single nucleotide polymorphisms (SNPs) within the squalene synthase (FDFT1) gene, a key regulator of cholesterol biosynthesis, is described. Polymorphisms of the pregnancy zone protein, a proteinase involved in clearance of TGF-β, are linked to systemic AST levels, and variants of platelet-derived growth factor α are linked to liver fibrosis[58].

Genetic variations of adiponectin are found to be associated with NAFLD[50,59], and single nucleotide polymorphisms in adiponectin receptor 1 (AdipoR1) and AdipoR2 contribute to variations in hepatic fat accumulation in humans[60,61].

SYSTEMIC ADIPONECTIN IN NAFLD

Systemic adiponectin concentrations are in the μg/mL-range indicating that adiponectin constitutes a substantial fraction of plasma proteins, and these high levels are remarkably constant. Despite its abundant presence in plasma, adiponectin is cleared rapidly by the liver with a half-life of about 75 min[62].

Visceral adiposity is associated with elevated circulating free fatty acids and higher concentrations of most adipose tissue released proteins[63]. Adiponectin differs from the adipokines described so far because its systemic levels are decreased in obesity[23]. In high fat diet induced obese rodents and in ob/ob mice adiponectin levels are reduced in plasma and clearance is significantly prolonged, indicating markedly impaired adiponectin synthesis in obesity[62,64,65]. Visceral fat accumulation is associated with hypoadiponectinemia and negative associations of visceral fat with systemic adiponectin have been identified[66,67].

Besides adiponectin, circulating levels of omentin predominantly released from the stromovascular cells of visceral fat are found reduced in obesity and serum omentin levels are increased in patients with NAFLD and independently predict hepatocyte ballooning[68].

In healthy Caucasians, BMI and adiponectin, but not insulin resistance, predict serum concentrations of both ALT and GGT[69]. Low adiponectin levels are even found associated with NASH independent of insulin resistance and BMI[30]. Multivariate regression analysis identifies decreased adiponectin as an independent predictor of liver steatosis and elevated ALT and GGT levels in healthy obese individuals[32]. In NAFLD patients low adiponectin levels are closely associated with the degree of hepatic steatosis, necroinflammation and fibrosis[30,32]. Shimada *et al*[70] reported that 90% of patients with early-stage NASH can be predicted by a combined evaluation of the serum adiponectin level, homeostasis assessment model-insulin resistance (HOMA-IR) score, and serum type Ⅳ collagen 7S level.

Circulating adiponectin levels in the μg/mL range by far exceed concentrations commonly required for receptor-dependent signalling. This may indicate receptor independent functions of adiponectin and binding to growth factors like platelet derived growth factor (PDGF), extracellular matrix proteins, low density lipoprotein (LDL) and opsonization of apoptotic cells to stimulate phagocytosis have been described[71-74].

Systemic adiponectin is about 20% to 60% lower in NAFLD than healthy controls[75-77] but considering the high levels in the circulation and a half-maximal effective dose of 0.85 μg/mL full-length adiponectin for AdipoR2 stimulated fatty acid oxidation[78] the question arises whether impaired receptor-mediated signalling due to reduced concentrations is a reasonable explanation for metabolic complications associated with hypoadiponectinemia. Therefore, it is likely that adiponectin receptor signal transduction pathways are also impaired in NAFLD.

ADIPONECTIN RECEPTORS IN NAFLD

Two 7-transmembrane proteins, AdipoR1 and AdipoR2, have been identified to function as adiponectin receptors[78]. Although initial studies using rodent tissues reveal preferential expression of AdipoR2 in the liver, in human tissues AdipoR1 and AdipoR2 mRNAs are most abundant in skeletal muscle and both are moderately expressed in the liver[78]. AdipoR1 protein is easily detected in human hepatocytes indicating that both receptors may play a role in liver physiology[79].

Although there is a well documented relationship between low adiponectin and liver disease, an association of NAFLD and reduced expression of hepatic adiponectin receptors is not consistently reported. Furthermore, mainly mRNA expression has been analysed and this may not necessarily predict protein abundance[80,81].

In animal models of obesity, hepatic adiponectin receptor mRNAs are found unchanged or even increased[65,82,83]. In human biopsies, hepatic adiponectin receptor mRNAs are increased in biopsy-proven NASH compared to steatotic livers[84]. Other studies, however, describe similar levels of adiponectin receptor mRNA in normal liver, steatotic liver and NASH[85,86]. There are also reports on reduced AdipoR2 mRNA in NASH compared to simple steatosis or lower AdipoR2 mRNA in fatty liver with no further reduction in NASH[87,88].

Data on AdipoR2 proteins are sparse and one study demonstrates reduced AdipoR2 protein in human NASH compared to steatotic liver[88]. Treatment of hepatocytes with palmitate is used as an in vitro model for hepatocyte steatosis and 200 μmol/L of this fatty acid reduce AdipoR2 protein in Huh7 cells[89]. Activating transcription factor 3 (ATF3) is induced upon endoplasmic reticulum stress and in the liver of ob/ob mice, and suppresses AdipoR2 in HepG2 cells[90]. Therefore, besides low circulating adiponectin, AdipoR2 may be reduced in hepatic steatosis and NASH indicating a possible adiponectin resistant state.

ANTISTEATOTIC EFFECTS OF ADIPONECTIN

Dyslipidemia is characterized by high circulating triglycerides[91] and low high density lipoprotein (HDL) cholesterol levels, and is frequently accompanied by hepatic steatosis[92]. Adiponectin negatively correlates with serum triglycerides and apolipoprotein B (ApoB), the main apolipoprotein of the triglyceride rich VLDL[93,94]. Hepatocyte ApoB and triglycerides are reduced by adiponectin indicating lower hepatic VLDL release[28,95,96]. Furthermore, VLDL catabolism is enhanced by an increased skeletal muscle lipoprotein lipase and VLDL receptor expression[97]. This more favourable lipid profile may be linked to lower hepatic lipid storage.

A choline and L-amino acid deficient diet induces more severe hepatic steatosis in adiponectin deficient mice compared to wild type animals[14]. Adenoviral expression of adiponectin ameliorates lipid deposition in the liver[95]. SREBP-1c is a central regulator of fatty acid synthesis, and is suppressed by adiponectin in hepatocytes and in the liver of db/db mice[95]. AMP-activated protein kinase (AMPK) is physiologically activated by low energy status, and switches on ATP-producing catabolic pathways (such as fatty acid oxidation and glycolysis), and switches off ATP-consuming anabolic pathways (such as lipogenesis)[98]. Adiponectin activates AMPK by binding to AdipoR1[78]. Suppression of SREBP-1c by adiponectin is mediated through AdipoR1/LKB1, an upstream kinase of AMPK, and AMPK pathway[95]. AMPK in addition phosphorylates acetyl-CoA carboxylase (ACC) and this is subsequently associated with a higher activity of carnitine palmitoyltransferase 1 (CPT-1), a rate limiting enzyme in fatty acid oxidation[98].

Signalling *via* AdipoR2 enhances peroxisome-proliferator activated receptor α (PPARα) activity[78,99]. PPARα upregulates CPT-1, stimulates β-oxidation, reduces lipid synthesis and thereby prevents excess triglyceride storage[100].

ANTIINFLAMMATORY AND ANTIAPOPTOTIC EFFECTS OF ADIPONECTIN

Lipopolysaccharide (LPS) is involved in the pathogenesis

of NAFLD and elevated levels of circulating LPS are found in obesity[101,102]. Increased gut permeability and a higher prevalence of small intestinal bacterial overgrowth correlates with the severity of steatosis but not with NASH[103]. Besides age, inflammation was identified as an independent predictor of progression to advanced fibrosis in NASH patients[104]. Hepatic steatosis may be accompanied by inflammatory cell infiltrates composed of neutrophils and mononuclear cells. In several mouse models of immune mediated hepatitis, adiponectin reduces TNF and induces interleukin-10 (IL-10) release from Kupffer cells[105]. Adiponectin lowers CRP synthesis in cytokine stimulated rat hepatocytes, and an inverse correlation of systemic adiponectin and CRP has been identified in obese patients[106,107]. Adiponectin may exert its anti-inflammatory activity by lowering nuclear factor kappa B (NFκB) action in preactivated cells or by inducing tolerance to inflammatory stimuli by a rapid and transient activation of NFκB that subsequently renders the cells inert to further activation[108-111].

Nevertheless, in patients suffering from chronic inflammatory diseases like inflammatory bowel disease or type 1 diabetes that are not associated with adiposity elevated circulating adiponectin levels that even correlate with inflammatory markers are found[112-114], and an induction of inflammatory proteins and activation of NFκB by recombinant adiponectin is described in several studies[25,108,113,115]. Therefore, adiponectin seems to be regulated in the opposite direction in classic versus obesity-associated chronic inflammatory diseases and may even exert opposite activities in resting compared to activated cells[116].

NFκB promotes cell survival and NEMO-mediated NFκB activation in hepatocytes has an essential physiological function to prevent the spontaneous development of steatohepatitis and hepatocellular carcinoma[117]. Adiponectin activates NFκB in human hepatocytes, and thereby may prevent hepatocyte apoptosis. Adiponectin further upregulates the chemokine interleukin 8 (CXCL8) *via* AdipoR1 and NFκB dependent pathways in primary human hepatocytes[118]. CXCL8 is an antiapoptotic protein[119] and overexpression of the rodent CXCL8 homologous protein protects the liver from galactosamine and endotoxin induced damage[120].

Adiponectin further antagonizes hepatocyte death by blocking fatty acid-induced activation of c-Jun NH2 terminal kinase[121], by reducing TNF levels[105] and by inhibiting fatty acid mediated upregulation of CD95[122].

ANTIOXIDATIVE EFFECTS OF ADIPONECTIN

Fatty liver is thought to represent the first incident towards the subsequent development of liver fibrosis[6]. Accelerated β-oxidation of fatty acids in hepatic steatosis is associated with excess reactive oxygen species (ROS), lipid peroxidation, the release of inflammatory cytokines, death of hepatocytes and activation of hepatic stellate cells[1]. ROS and lipid peroxidation are thought to contribute to the progression of liver injury partly by accelerating inflammation that in turn causes ROS production[1]. Oxidative stress is enhanced in human hypoadiponectinemia and in adiponectin knock-out mice fed a choline-deficient L-amino acid deficient diet[123,124]. Hepatic cytochrome P450 2E1 (CYP2E1) is elevated in these animals and in human NASH and may contribute to higher ROS levels[125,126].

Aldehyde oxidase 1 (AOX1) is a xenobiotic metabolizing protein whose physiological role has not been evaluated in detail so far[127]. AOX1 activity has been identified as an important source of ROS[128] and is reduced in hepatocytes by adiponectin *via* activation of PPARα[31] Adiponectin also increases ROS detoxifying enzymes and AdipoR2 is involved in the induction of superoxide dismutase 1 and catalase[129].

ANTIFIBROTIC EFFECTS OF ADIPONECTIN

Liver injury causes activation of otherwise "quiescent" hepatic stellate cells (HSC) and activated cells proliferate, synthesize CTGF and extracellular matrix proteins[130]. TGF-β is the main profibrotic factor in fibrosis and induces CTGF synthesis. CTGF stimulates binding of TGF-β to its receptor and thereby enhances TGF-β activity[130]. CTGF is induced by TGF-β indicating an autocrine or paracrine loop that mutually enhances synthesis of both proteins[130]. Knock-down of AdipoR2 in mice fed a methionine-choline deficient diet to cause progressive fibrosing steatohepatitis is associated with higher levels of steatosis, inflammation and fibrosis[131]. Overexpression of AdipoR2 is protective, and this mechanistically includes inhibition of TGF-β signaling and stimulation of PPARα activity[131].

Expression of recombinant adiponectin in activated HSC reduces proliferation and lowers α-smooth muscle actin that is induced in activated HSC[132]. Furthermore, apoptotic cell death of activated HSC is augmented[132]. Exogenously added recombinant adiponectin suppresses PDGF-stimulated HSC proliferation by activation of AMPK[133]. Adiponectin may also bind to growth factors like PDGF and thereby inhibits binding to their corresponding receptors[71]. Leptin is a well described profibrotic adipokine and several studies have shown that adiponectin antagonizes leptin bioactivity[134,135]. Adiponectin blocks leptin-induced STAT3 phosphorylation in activated HSC and leptin-mediated upregulation of TIMP-1 release and these in-vitro findings have been confirmed *in-vivo*[134].

DIET, EXERCISE AND PHARMACOLOGICAL INTERVENTIONS

Studies analysing the impact of changes in life style and medications in NAFLD have been performed in small patient groups sometimes even lacking suitable controls. Currently weight loss and exercise are recommended as initial strategies to improve NASH[136]. Diet and diet in conjunction with exercise for 6 mo cause a similar reduction in body weight and intrahepatic fat[137]. In 19 sedentary obese

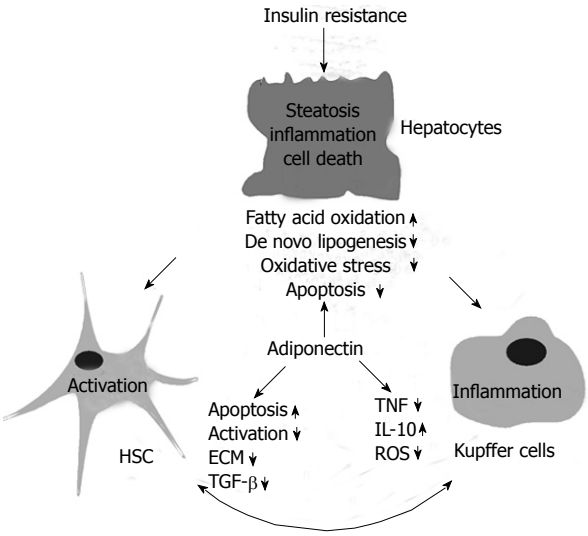


Figure 3 Hepatoprotective effects of adiponectin. Hepatic insulin resistance correlates with liver fat content, and is currently thought to represent the first incident in metabolic liver diseases. Insulin resistance and steatosis may also promote inflammation and fibrosis although the factors leading to advanced liver damage have not been identified so far. Major pathophysiological alterations of hepatocytes, hepatic stellate cells (HSC) and Kupffer cells in hepatic steatosis and/or non-alcoholic steatohepatitis are indicated. The protective activities of adiponectin are listed and arrows indicate an induction or repression of these pathways/proteins by adiponectin. IL: Interleukin; TGF: Transforming growth factor; TNF: Tumor necrosis factor; ECM: extracellular matrix; ROS: Reactive oxygen species.

men and women four weeks of aerobic exercise improved hepatic steatosis even in the absence of weight loss[138]. In a randomized controlled trial enrolling 31 patients with biopsy-proven NASH intensive changes in life style with the objective of at least 7% weight loss and educational training without weight reduction have been compared[139]. Weight loss significantly correlates with improvement in NASH histological activity score and weight loss of 7% or even more is recommended as a treatment strategy for these patients[139]. Another study also reports improvements of histological and laboratory parameters when body weight is reduced by 10% in NASH patients[140]. Adiponectin concentrations increase by about 36% in type 2 diabetic patients by 13% weight loss[141], and this may partly contribute to the metabolic improvements observed in these patients.

Clinical trials using fibrates have revealed inconsistent results so far. Treatment of sixteen NASH patients with clofibrate did not ameliorate biochemical or histological parameters[142], whereas a second study demonstrated biochemical and ultrasound improvements with fenofibrate[143]. Emerging data on thiazolidinediones have demonstrated improvement in both liver enzymes and histology[144,145]. These drugs activate PPARγ and thereby inhibit growth of HSC and TGF-β mediated induction of CTGF, respectively[146]. PPARγ is the main adipogenic transcription factor and its agonists stimulate adipogenesis[147]. Thiazolidinediones strongly stimulate adiponectin synthesis and elevate systemic adiponectin[147]. Increase of adiponectin by pioglitazone is related to histological improvement of steatosis, inflammation and fibrosis confirming the crucial role of adiponectin in NAFLD[148]. The PPARγ agonist rosiglitazone even induces AdipoR2 in hepatocytes[146]. A recent study reports that pioglitazone therapy improves adipose tissue insulin sensitivity and this correlates with a reduction in hepatic fat and necroinflammation[149]. Activation of PPARγ primes human monocytes into alternative M2 macrophages with anti-inflammatory properties and patients may also benefit from reduced inflammation[150]. In line with this hypothesis pentoxifylline with multiple pharmacological effects including antioxidant and anti-inflammatory activity[151] has been tested in small clinical trials, and biochemical and histological improvements have been reported[151,152]. Vitamin E therapy decreases AST and ALT levels and hepatic steatosis but does not improve necroinflammation and fibrosis[153]. Antioxidants may even prevent health-promoting effects of physical exercise namely insulin sensitivity and rise of systemic adiponectin in untrained and pre-trained individuals, and therefore, may be more effective in patients with low physical activity[154]. In summary to date no pharmacologic treatment has been reliably shown to be effective for the treatment of NASH patients.

CONCLUSION

Adiponectin has emerged as a protective adipokine in insulin resistance and obesity related liver diseases (Figure 3), and drugs that elevate systemic adiponectin may be useful as therapeutics for NAFLD. Adiponectin receptor signalling pathways and potential hepatic adiponectin resistance in NASH, however, have been poorly investigated so far. Identification of molecules downstream of AdipoR1/2 and strategies to enhance adiponectin receptor activity may constitute promising approaches towards treatment of NAFLD.

ACKNOWLEDGEMENTS

The authors thank Professor Charalampos Aslanidis for helpful suggestions.

REFERENCES

1 **Browning JD**, Horton JD. Molecular mediators of hepatic steatosis and liver injury. *J Clin Invest* 2004; **114**: 147-152
2 **Gil-Campos M**, Cañete RR, Gil A. Adiponectin, the missing link in insulin resistance and obesity. *Clin Nutr* 2004; **23**: 963-974
3 **Goossens GH**. The role of adipose tissue dysfunction in the pathogenesis of obesity-related insulin resistance. *Physiol Behav* 2008; **94**: 206-218
4 **Calamita G**, Portincasa P. Present and future therapeutic strategies in non-alcoholic fatty liver disease. *Expert Opin Ther Targets* 2007; **11**: 1231-1249
5 **Jensen MD**. Role of body fat distribution and the metabolic complications of obesity. *J Clin Endocrinol Metab* 2008; **93**: S57-S63
6 **Schäffler A**, Schölmerich J, Büchler C. Mechanisms of disease: adipocytokines and visceral adipose tissue--emerging role in nonalcoholic fatty liver disease. *Nat Clin Pract Gastroenterol Hepatol* 2005; **2**: 273-280

7 **Messier V**, Karelis AD, Robillard ME, Bellefeuille P, Brochu M, Lavoie JM, Rabasa-Lhoret R. Metabolically healthy but obese individuals: relationship with hepatic enzymes. *Metabolism* 2010; **59**: 20-24
8 **Stefan N**, Kantartzis K, Machann J, Schick F, Thamer C, Rittig K, Balletshofer B, Machicao F, Fritsche A, Häring HU. Identification and characterization of metabolically benign obesity in humans. *Arch Intern Med* 2008; **168**: 1609-1616
9 **Brochu M**, Tchernof A, Dionne IJ, Sites CK, Eltabbakh GH, Sims EA, Poehlman ET. What are the physical characteristics associated with a normal metabolic profile despite a high level of obesity in postmenopausal women? *J Clin Endocrinol Metab* 2001; **86**: 1020-1025
10 **Brochu M**, Mathieu ME, Karelis AD, Doucet E, Lavoie ME, Garrel D, Rabasa-Lhoret R. Contribution of the lean body mass to insulin resistance in postmenopausal women with visceral obesity: a Monet study. *Obesity* (Silver Spring) 2008; **16**: 1085-1093
11 **Catalán V**, Gómez-Ambrosi J, Rodríguez A, Salvador J, Frühbeck G. Adipokines in the treatment of diabetes mellitus and obesity. *Expert Opin Pharmacother* 2009; **10**: 239-254
12 **Kanda H**, Tateya S, Tamori Y, Kotani K, Hiasa K, Kitazawa R, Kitazawa S, Miyachi H, Maeda S, Egashira K, Kasuga M. MCP-1 contributes to macrophage infiltration into adipose tissue, insulin resistance, and hepatic steatosis in obesity. *J Clin Invest* 2006; **116**: 1494-1505
13 **Zeyda M**, Stulnig TM. Adipose tissue macrophages. *Immunol Lett* 2007; **112**: 61-67
14 **Kamada Y**, Takehara T, Hayashi N. Adipocytokines and liver disease. *J Gastroenterol* 2008; **43**: 811-822
15 **Borst SE**. The role of TNF-alpha in insulin resistance. *Endocrine* 2004; **23**: 177-182
16 **Fontana L**, Eagon JC, Trujillo ME, Scherer PE, Klein S. Visceral fat adipokine secretion is associated with systemic inflammation in obese humans. *Diabetes* 2007; **56**: 1010-1013
17 **Sabio G**, Das M, Mora A, Zhang Z, Jun JY, Ko HJ, Barrett T, Kim JK, Davis RJ. A stress signaling pathway in adipose tissue regulates hepatic insulin resistance. *Science* 2008; **322**: 1539-1543
18 **Wiest R**, Weigert J, Wanninger J, Neumeier M, Bauer S, Schmidhofer S, Farkas S, Scherer MN, Schäffler A, Schölmerich J, Buechler C. Impaired hepatic removal of interleukin-6 in patients with liver cirrhosis. *Cytokine* 2011; **53**: 178-183
19 **Heinrich PC**, Castell JV, Andus T. Interleukin-6 and the acute phase response. *Biochem J* 1990; **265**: 621-636
20 **Biddinger SB**, Miyazaki M, Boucher J, Ntambi JM, Kahn CR. Leptin suppresses stearoyl-CoA desaturase 1 by mechanisms independent of insulin and sterol regulatory element-binding protein-1c. *Diabetes* 2006; **55**: 2032-2041
21 **Wang J**, Leclercq I, Brymora JM, Xu N, Ramezani-Moghadam M, London RM, Brigstock D, George J. Kupffer cells mediate leptin-induced liver fibrosis. *Gastroenterology* 2009; **137**: 713-723
22 **Lanthier N**, Horsmans Y, Leclercq IA. The metabolic syndrome: how it may influence hepatic stellate cell activation and hepatic fibrosis. *Curr Opin Clin Nutr Metab Care* 2009; **12**: 404-411
23 **Arita Y**, Kihara S, Ouchi N, Takahashi M, Maeda K, Miyagawa J, Hotta K, Shimomura I, Nakamura T, Miyaoka K, Kuriyama H, Nishida M, Yamashita S, Okubo K, Matsubara K, Muraguchi M, Ohmoto Y, Funahashi T, Matsuzawa Y. Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. *Biochem Biophys Res Commun* 1999; **257**: 79-83
24 **Wang Y**, Lam KS, Yau MH, Xu A. Post-translational modifications of adiponectin: mechanisms and functional implications. *Biochem J* 2008; **409**: 623-633
25 **Neumeier M**, Weigert J, Schäffler A, Wehrwein G, Müller-Ladner U, Schölmerich J, Wrede C, Buechler C. Different effects of adiponectin isoforms in human monocytic cells. *J Leukoc Biol* 2006; **79**: 803-808
26 **Schober F**, Neumeier M, Weigert J, Wurm S, Wanninger J, Schäffler A, Dada A, Liebisch G, Schmitz G, Aslanidis C, Buechler C. Low molecular weight adiponectin negatively correlates with the waist circumference and monocytic IL-6 release. *Biochem Biophys Res Commun* 2007; **361**: 968-973
27 **Wong GW**, Krawczyk SA, Kitidis-Mitrokostas C, Ge G, Spooner E, Hug C, Gimeno R, Lodish HF. Identification and characterization of CTRP9, a novel secreted glycoprotein, from adipose tissue that reduces serum glucose in mice and forms heterotrimers with adiponectin. *FASEB J* 2009; **23**: 241-258
28 **Fruebis J**, Tsao TS, Javorschi S, Ebbets-Reed D, Erickson MR, Yen FT, Bihain BE, Lodish HF. Proteolytic cleavage product of 30-kDa adipocyte complement-related protein increases fatty acid oxidation in muscle and causes weight loss in mice. *Proc Natl Acad Sci USA* 2001; **98**: 2005-2010
29 **Kusminski CM**, McTernan PG, Schraw T, Kos K, O'Hare JP, Ahima R, Kumar S, Scherer PE. Adiponectin complexes in human cerebrospinal fluid: distinct complex distribution from serum. *Diabetologia* 2007; **50**: 634-642
30 **Hui JM**, Hodge A, Farrell GC, Kench JG, Kriketos A, George J. Beyond insulin resistance in NASH: TNF-alpha or adiponectin? *Hepatology* 2004; **40**: 46-54
31 **Neumeier M**, Weigert J, Schäffler A, Weiss TS, Schmidl C, Büttner R, Bollheimer C, Aslanidis C, Schölmerich J, Buechler C. Aldehyde oxidase 1 is highly abundant in hepatic steatosis and is downregulated by adiponectin and fenofibric acid in hepatocytes in vitro. *Biochem Biophys Res Commun* 2006; **350**: 731-735
32 **Targher G**, Bertolini L, Scala L, Poli F, Zenari L, Falezza G. Decreased plasma adiponectin concentrations are closely associated with nonalcoholic hepatic steatosis in obese individuals. *Clin Endocrinol* (Oxf) 2004; **61**: 700-703
33 **Aguilar-Salinas CA**, García EG, Robles L, Riaño D, Ruiz-Gomez DG, García-Ulloa AC, Melgarejo MA, Zamora M, Guillen-Pineda LE, Mehta R, Canizales-Quinteros S, Tusie Luna MT, Gómez-Pérez FJ. High adiponectin concentrations are associated with the metabolically healthy obese phenotype. *J Clin Endocrinol Metab* 2008; **93**: 4075-4079
34 **Rubinstein E**, Lavine JE, Schwimmer JB. Hepatic, cardiovascular, and endocrine outcomes of the histological subphenotypes of nonalcoholic fatty liver disease. *Semin Liver Dis* 2008; **28**: 380-385
35 **Bellentani S**, Bedogni G, Tiribelli C. Liver and heart: a new link? *J Hepatol* 2008; **49**: 300-302
36 **Speliotes EK**, Massaro JM, Hoffmann U, Vasan RS, Meigs JB, Sahani DV, Hirschhorn JN, O'Donnell CJ, Fox CS. Fatty liver is associated with dyslipidemia and dysglycemia independent of visceral fat: the Framingham Heart Study. *Hepatology* 2010; **51**: 1979-1987
37 **Roberts EA**. Non-alcoholic steatohepatitis in children. *Clin Liver Dis* 2007; **11**: 155-172, x
38 **Wang Y**, Zhou M, Lam KS, Xu A. Protective roles of adiponectin in obesity-related fatty liver diseases: mechanisms and therapeutic implications. *Arq Bras Endocrinol Metabol* 2009; **53**: 201-212
39 **Neuschwander-Tetri BA**, Caldwell SH. Nonalcoholic steatohepatitis: summary of an AASLD Single Topic Conference. *Hepatology* 2003; **37**: 1202-1219
40 **Clark JM**, Brancati FL, Diehl AM. The prevalence and etiology of elevated aminotransferase levels in the United States. *Am J Gastroenterol* 2003; **98**: 960-967
41 **Clark JM**. The epidemiology of nonalcoholic fatty liver disease in adults. *J Clin Gastroenterol* 2006; **40** Suppl 1: S5-S10
42 **Byron D**, Minuk GY. Clinical hepatology: profile of an urban, hospital-based practice. *Hepatology* 1996; **24**: 813-815
43 **Andersen T**, Christoffersen P, Gluud C. The liver in consecutive patients with morbid obesity: a clinical, morphological, and biochemical study. *Int J Obes* 1984; **8**: 107-115

44 **Angulo P**. Nonalcoholic fatty liver disease. *N Engl J Med* 2002; **346**: 1221-1231
45 **Preiss D**, Sattar N. Non-alcoholic fatty liver disease: an overview of prevalence, diagnosis, pathogenesis and treatment considerations. *Clin Sci* (Lond) 2008; **115**: 141-150
46 **Erickson SK**. Nonalcoholic fatty liver disease. *J Lipid Res* 2009; **50** Suppl: S412-S416
47 **Marchesini G**, Marzocchi R, Agostini F, Bugianesi E. Nonalcoholic fatty liver disease and the metabolic syndrome. *Curr Opin Lipidol* 2005; **16**: 421-427
48 **Müssig K**, Staiger H, Machicao F, Thamer C, Machann J, Schick F, Claussen CD, Stefan N, Fritsche A, Häring HU. RARRES2, encoding the novel adipokine chemerin, is a genetic determinant of disproportionate regional body fat distribution: a comparative magnetic resonance imaging study. *Metabolism* 2009; **58**: 519-524
49 **Loos RJ**, Ruchat S, Rankinen T, Tremblay A, Pérusse L, Bouchard C. Adiponectin and adiponectin receptor gene variants in relation to resting metabolic rate, respiratory quotient, and adiposity-related phenotypes in the Quebec Family Study. *Am J Clin Nutr* 2007; **85**: 26-34
50 **Wang ZL**, Xia B, Shrestha U, Jiang L, Ma CW, Chen Q, Chen H, Hu ZG. Correlation between adiponectin polymorphisms and non-alcoholic fatty liver disease with or without metabolic syndrome in Chinese population. *J Endocrinol Invest* 2008; **31**: 1086-1091
51 **Weston SR**, Leyden W, Murphy R, Bass NM, Bell BP, Manos MM, Terrault NA. Racial and ethnic distribution of nonalcoholic fatty liver in persons with newly diagnosed chronic liver disease. *Hepatology* 2005; **41**: 372-379
52 **Mohanty SR**, Troy TN, Huo D, O'Brien BL, Jensen DM, Hart J. Influence of ethnicity on histological differences in non-alcoholic fatty liver disease. *J Hepatol* 2009; **50**: 797-804
53 **Schwimmer JB**, Celedon MA, Lavine JE, Salem R, Campbell N, Schork NJ, Shiehmorteza M, Yokoo T, Chavez A, Middleton MS, Sirlin CB. Heritability of nonalcoholic fatty liver disease. *Gastroenterology* 2009; **136**: 1585-1592
54 **Wilfred de Alwis NM**, Day CP. Genes and nonalcoholic fatty liver disease. *Curr Diab Rep* 2008; **8**: 156-163
55 **Wilfred de Alwis NM**, Day CP. Genetics of alcoholic liver disease and nonalcoholic fatty liver disease. *Semin Liver Dis* 2007; **27**: 44-54
56 **Romeo S**, Kozlitina J, Xing C, Pertsemlidis A, Cox D, Pennacchio LA, Boerwinkle E, Cohen JC, Hobbs HH. Genetic variation in PNPLA3 confers susceptibility to nonalcoholic fatty liver disease. *Nat Genet* 2008; **40**: 1461-1465
57 **Rotman Y**, Koh C, Zmuda JM, Kleiner DE, Liang TJ. The association of genetic variability in patatin-like phospholipase domain-containing protein 3 (PNPLA3) with histological severity of nonalcoholic fatty liver disease. *Hepatology* 2010; **52**: 894-903
58 **Chalasani N**, Guo X, Loomba R, Goodarzi MO, Haritunians T, Kwon S, Cui J, Taylor KD, Wilson L, Cummings OW, Chen YD, Rotter JI. Genome-wide association study identifies variants associated with histologic features of nonalcoholic Fatty liver disease. *Gastroenterology* 2010; **139**: 1567-1576, 1576.e1-e6
59 **Musso G**, Gambino R, De Michieli F, Durazzo M, Pagano G, Cassader M. Adiponectin gene polymorphisms modulate acute adiponectin response to dietary fat: Possible pathogenetic role in NASH. *Hepatology* 2008; **47**: 1167-1177
60 **Kotronen A**, Yki-Järvinen H, Aminoff A, Bergholm R, Pietiläinen KH, Westerbacka J, Talmud PJ, Humphries SE, Hamsten A, Isomaa B, Groop L, Orho-Melander M, Ehrenborg E, Fisher RM. Genetic variation in the ADIPOR2 gene is associated with liver fat content and its surrogate markers in three independent cohorts. *Eur J Endocrinol* 2009; **160**: 593-602
61 **Stefan N**, Machicao F, Staiger H, Machann J, Schick F, Tschritter O, Spieth C, Weigert C, Fritsche A, Stumvoll M, Häring HU. Polymorphisms in the gene encoding adiponectin receptor 1 are associated with insulin resistance and high liver fat. *Diabetologia* 2005; **48**: 2282-2291
62 **Halberg N**, Schraw TD, Wang ZV, Kim JY, Yi J, Hamilton MP, Luby-Phelps K, Scherer PE. Systemic fate of the adipocyte-derived factor adiponectin. *Diabetes* 2009; **58**: 1961-1970
63 **Phillips LK**, Prins JB. The link between abdominal obesity and the metabolic syndrome. *Curr Hypertens Rep* 2008; **10**: 156-164
64 **Kadowaki T**, Yamauchi T, Kubota N, Hara K, Ueki K. Adiponectin and adiponectin receptors in obesity-linked insulin resistance. *Novartis Found Symp* 2007; **286**: 164-176; discussion 176-182, 200-203
65 **Neumeier M**, Hellerbrand C, Gäbele E, Buettner R, Bollheimer C, Weigert J, Schäffler A, Weiss TS, Lichtenauer M, Schölmerich J, Buechler C. Adiponectin and its receptors in rodent models of fatty liver disease and liver cirrhosis. *World J Gastroenterol* 2006; **12**: 5490-5494
66 **Matsuzawa Y**. The role of fat topology in the risk of disease. *Int J Obes* (Lond) 2008; **32** Suppl 7: S83-S92
67 **Nakamura Y**, Sekikawa A, Kadowaki T, Kadota A, Kadowaki S, Maegawa H, Kita Y, Evans RW, Edmundowicz D, Curb JD, Ueshima H. Visceral and subcutaneous adiposity and adiponectin in middle-aged Japanese men: the ERA JUMP study. *Obesity* (Silver Spring) 2009; **17**: 1269-1273
68 **Yilmaz Y**, Yonal O, Kurt R, Alahdab YO, Eren F, Ozdogan O, Celikel CA, Imeryuz N, Kalayci C, Avsar E. Serum levels of omentin, chemerin and adipsin in patients with biopsy-proven nonalcoholic fatty liver disease. *Scand J Gastroenterol* 2011; **46**: 91-97
69 **López-Bermejo A**, Botas P, Funahashi T, Delgado E, Kihara S, Ricart W, Fernández-Real JM. Adiponectin, hepatocellular dysfunction and insulin sensitivity. *Clin Endocrinol* (Oxf) 2004; **60**: 256-263
70 **Shimada M**, Kawahara H, Ozaki K, Fukura M, Yano H, Tsuchishima M, Tsutsumi M, Takase S. Usefulness of a combined evaluation of the serum adiponectin level, HOMA-IR, and serum type IV collagen 7S level to predict the early stage of nonalcoholic steatohepatitis. *Am J Gastroenterol* 2007; **102**: 1931-1938
71 **Wang Y**, Lam KS, Xu JY, Lu G, Xu LY, Cooper GJ, Xu A. Adiponectin inhibits cell proliferation by interacting with several growth factors in an oligomerization-dependent manner. *J Biol Chem* 2005; **280**: 18341-18347
72 **Okamoto Y**, Arita Y, Nishida M, Muraguchi M, Ouchi N, Takahashi M, Igura T, Inui Y, Kihara S, Nakamura T, Yamashita S, Miyagawa J, Funahashi T, Matsuzawa Y. An adipocyte-derived plasma protein, adiponectin, adheres to injured vascular walls. *Horm Metab Res* 2000; **32**: 47-50
73 **Kobayashi K**, Inoguchi T, Sonoda N, Sekiguchi N, Nawata H. Adiponectin inhibits the binding of low-density lipoprotein to biglycan, a vascular proteoglycan. *Biochem Biophys Res Commun* 2005; **335**: 66-70
74 **Takemura Y**, Ouchi N, Shibata R, Aprahamian T, Kirber MT, Summer RS, Kihara S, Walsh K. Adiponectin modulates inflammatory reactions *via* calreticulin receptor-dependent clearance of early apoptotic bodies. *J Clin Invest* 2007; **117**: 375-386
75 **Bugianesi E**, Pagotto U, Manini R, Vanni E, Gastaldelli A, de Iasio R, Gentilcore E, Natale S, Cassader M, Rizzetto M, Pasquali R, Marchesini G. Plasma adiponectin in nonalcoholic fatty liver is related to hepatic insulin resistance and hepatic fat content, not to liver disease severity. *J Clin Endocrinol Metab* 2005; **90**: 3498-3504
76 **Pagano C**, Soardo G, Esposito W, Fallo F, Basan L, Donnini D, Federspil G, Sechi LA, Vettor R. Plasma adiponectin is decreased in nonalcoholic fatty liver disease. *Eur J Endocrinol* 2005; **152**: 113-118
77 **Vuppalanchi R**, Marri S, Kolwankar D, Considine RV, Chalasani N. Is adiponectin involved in the pathogenesis of nonalcoholic steatohepatitis? A preliminary human study. *J Clin Gastroenterol* 2005; **39**: 237-242

78 **Yamauchi T**, Kamon J, Ito Y, Tsuchida A, Yokomizo T, Kita S, Sugiyama T, Miyagishi M, Hara K, Tsunoda M, Murakami K, Ohteki T, Uchida S, Takekawa S, Waki H, Tsuno NH, Shibata Y, Terauchi Y, Froguel P, Tobe K, Koyasu S, Taira K, Kitamura T, Shimizu T, Nagai R, Kadowaki T. Cloning of adiponectin receptors that mediate antidiabetic metabolic effects. *Nature* 2003; **423**: 762-769

79 **Neumeier M**, Weigert J, Schäffler A, Weiss T, Kirchner S, Laberer S, Schölmerich J, Buechler C. Regulation of adiponectin receptor 1 in human hepatocytes by agonists of nuclear receptors. *Biochem Biophys Res Commun* 2005; **334**: 924-929

80 **Bauer S**, Weigert J, Neumeier M, Wanninger J, Schäffler A, Luchner A, Schnitzbauer AA, Aslanidis C, Buechler C. Low-abundant adiponectin receptors in visceral adipose tissue of humans and rats are further reduced in diabetic animals. *Arch Med Res* 2010; **41**: 75-82

81 **Weigert J**, Neumeier M, Wanninger J, Wurm S, Kopp A, Schober F, Filarsky M, Schäffler A, Zeitoun M, Aslanidis C, Buechler C. Reduced response to adiponectin and lower abundance of adiponectin receptor proteins in type 2 diabetic monocytes. *FEBS Lett* 2008; **582**: 1777-1782

82 **Inukai K**, Nakashima Y, Watanabe M, Takata N, Sawa T, Kurihara S, Awata T, Katayama S. Regulation of adiponectin receptor gene expression in diabetic mice. *Am J Physiol Endocrinol Metab* 2005; **288**: E876-E882

83 **Tsuchida A**, Yamauchi T, Ito Y, Hada Y, Maki T, Takekawa S, Kamon J, Kobayashi M, Suzuki R, Hara K, Kubota N, Terauchi Y, Froguel P, Nakae J, Kasuga M, Accili D, Tobe K, Ueki K, Nagai R, Kadowaki T. Insulin/Foxo1 pathway regulates expression levels of adiponectin receptors and adiponectin sensitivity. *J Biol Chem* 2004; **279**: 30817-30822

84 **Nannipieri M**, Cecchetti F, Anselmino M, Mancini E, Marchetti G, Bonotti A, Baldi S, Solito B, Giannetti M, Pinchera A, Santini F, Ferrannini E. Pattern of expression of adiponectin receptors in human liver and its relation to nonalcoholic steatohepatitis. *Obes Surg* 2009; **19**: 467-474

85 **Ma H**, Gomez V, Lu L, Yang X, Wu X, Xiao SY. Expression of adiponectin and its receptors in livers of morbidly obese patients with non-alcoholic fatty liver disease. *J Gastroenterol Hepatol* 2009; **24**: 233-237

86 **Uribe M**, Zamora-Valdés D, Moreno-Portillo M, Bermejo-Martínez L, Pichardo-Bahena R, Baptista-González HA, Ponciano-Rodríguez G, Uribe MH, Medina-Santillán R, Méndez-Sánchez N. Hepatic expression of ghrelin and adiponectin and their receptors in patients with nonalcoholic fatty liver disease. *Ann Hepatol* 2008; **7**: 67-71

87 **Shimizu A**, Takamura T, Matsuzawa N, Nakamura S, Nabemoto S, Takeshita Y, Misu H, Kurita S, Sakurai M, Yokoyama M, Zen Y, Sasaki M, Nakanuma Y, Kaneko S. Regulation of adiponectin receptor expression in human liver and a hepatocyte cell line. *Metabolism* 2007; **56**: 1478-1485

88 **Kaser S**, Moschen A, Cayon A, Kaser A, Crespo J, Pons-Romero F, Ebenbichler CF, Patsch JR, Tilg H. Adiponectin and its receptors in non-alcoholic steatohepatitis. *Gut* 2005; **54**: 117-121

89 **Rahman SM**, Qadri I, Janssen RC, Friedman JE. Fenofibrate and PBA prevent fatty acid-induced loss of adiponectin receptor and pAMPK in human hepatoma cells and in hepatitis C virus-induced steatosis. *J Lipid Res* 2009; **50**: 2193-2202

90 **Koh IU**, Lim JH, Joe MK, Kim WH, Jung MH, Yoon JB, Song J. AdipoR2 is transcriptionally regulated by ER stress-inducible ATF3 in HepG2 human hepatocyte cells. *FEBS J* 2010; **277**: 2304-2317

91 **Tietge UJ**, Böker KH, Manns MP, Bahr MJ. Elevated circulating adiponectin levels in liver cirrhosis are associated with reduced liver function and altered hepatic hemodynamics. *Am J Physiol Endocrinol Metab* 2004; **287**: E82-E89

92 **Qureshi K**, Abrams GA. Metabolic liver disease of obesity and role of adipose tissue in the pathogenesis of nonalcoholic fatty liver disease. *World J Gastroenterol* 2007; **13**: 3540-3553

93 **Matsubara M**, Maruoka S, Katayose S. Inverse relationship between plasma adiponectin and leptin concentrations in normal-weight and obese women. *Eur J Endocrinol* 2002; **147**: 173-180

94 **Zietz B**, Herfarth H, Paul G, Ehling A, Müller-Ladner U, Schölmerich J, Schäffler A. Adiponectin represents an independent cardiovascular risk factor predicting serum HDL-cholesterol levels in type 2 diabetes. *FEBS Lett* 2003; **545**: 103-104

95 **Awazawa M**, Ueki K, Inabe K, Yamauchi T, Kaneko K, Okazaki Y, Bardeesy N, Ohnishi S, Nagai R, Kadowaki T. Adiponectin suppresses hepatic SREBP1c expression in an AdipoR1/LKB1/AMPK dependent pathway. *Biochem Biophys Res Commun* 2009; **382**: 51-56

96 **Xu A**, Wang Y, Keshaw H, Xu LY, Lam KS, Cooper GJ. The fat-derived hormone adiponectin alleviates alcoholic and nonalcoholic fatty liver diseases in mice. *J Clin Invest* 2003; **112**: 91-100

97 **Qiao L**, Zou C, van der Westhuyzen DR, Shao J. Adiponectin reduces plasma triglyceride by increasing VLDL triglyceride catabolism. *Diabetes* 2008; **57**: 1824-1833

98 **Hardie DG**, Pan DA. Regulation of fatty acid synthesis and oxidation by the AMP-activated protein kinase. *Biochem Soc Trans* 2002; **30**: 1064-1070

99 **Yamauchi T**, Kadowaki T. Physiological and pathophysiological roles of adiponectin and adiponectin receptors in the integrated regulation of metabolic and cardiovascular diseases. *Int J Obes* (Lond) 2008; **32** Suppl 7: S13-S18

100 **Fruchart JC**, Duriez P, Staels B. Peroxisome proliferator-activated receptor-alpha activators regulate genes governing lipoprotein metabolism, vascular inflammation and atherosclerosis. *Curr Opin Lipidol* 1999; **10**: 245-257

101 **Al-Attas OS**, Al-Daghri NM, Al-Rubeaan K, da Silva NF, Sabico SL, Kumar S, McTernan PG, Harte AL. Changes in endotoxin levels in T2DM subjects on anti-diabetic therapies. *Cardiovasc Diabetol* 2009; **8**: 20

102 **Yang SQ**, Lin HZ, Lane MD, Clemens M, Diehl AM. Obesity increases sensitivity to endotoxin liver injury: implications for the pathogenesis of steatohepatitis. *Proc Natl Acad Sci USA* 1997; **94**: 2557-2562

103 **Miele L**, Valenza V, La Torre G, Montalto M, Cammarota G, Ricci R, Mascianà R, Forgione A, Gabrieli ML, Perotti G, Vecchio FM, Rapaccini G, Gasbarrini G, Day CP, Grieco A. Increased intestinal permeability and tight junction alterations in nonalcoholic fatty liver disease. *Hepatology* 2009; **49**: 1877-1887

104 **Argo CK**, Northup PG, Al-Osaimi AM, Caldwell SH. Systematic review of risk factors for fibrosis progression in non-alcoholic steatohepatitis. *J Hepatol* 2009; **51**: 371-379

105 **Matsumoto H**, Tamura S, Kamada Y, Kiso S, Fukushima J, Wada A, Maeda N, Kihara S, Funahashi T, Matsuzawa Y, Shimomura I, Hayashi N. Adiponectin deficiency exacerbates lipopolysaccharide/D-galactosamine-induced liver injury in mice. *World J Gastroenterol* 2006; **12**: 3352-3358

106 **Ouchi N**, Walsh K. Adiponectin as an anti-inflammatory factor. *Clin Chim Acta* 2007; **380**: 24-30

107 **Devaraj S**, Torok N, Dasu MR, Samols D, Jialal I. Adiponectin decreases C-reactive protein synthesis and secretion from endothelial cells: evidence for an adipose tissue-vascular loop. *Arterioscler Thromb Vasc Biol* 2008; **28**: 1368-1374

108 **Tsao TS**, Murrey HE, Hug C, Lee DH, Lodish HF. Oligomerization state-dependent activation of NF-kappa B signaling pathway by adipocyte complement-related protein of 30 kDa (Acrp30). *J Biol Chem* 2002; **277**: 29359-29362

109 **Park PH**, McMullen MR, Huang H, Thakur V, Nagy LE. Short-term treatment of RAW264.7 macrophages with adiponectin increases tumor necrosis factor-alpha (TNF-alpha) expression *via* ERK1/2 activation and Egr-1 expression: role of TNF-alpha in adiponectin-stimulated interleukin-10 production. *J Biol Chem* 2007; **282**: 21695-21703

110 **Saijo S**, Nagata K, Nakano Y, Tobe T, Kobayashi Y. Inhibition by adiponectin of IL-8 production by human macrophages upon coculturing with late apoptotic cells. *Biochem Biophys Res Commun* 2005; **334**: 1180-1183

111 **Tsatsanis C**, Zacharioudaki V, Androulidaki A, Dermitzaki E, Charalampopoulos I, Minas V, Gravanis A, Margioris AN. Adiponectin induces TNF-alpha and IL-6 in macrophages and promotes tolerance to itself and other pro-inflammatory stimuli. *Biochem Biophys Res Commun* 2005; **335**: 1254-1263

112 **Weigert J**, Obermeier F, Neumeier M, Wanninger J, Filarsky M, Bauer S, Aslanidis C, Rogler G, Ott C, Schäffler A, Schölmerich J, Buechler C. Circulating levels of chemerin and adiponectin are higher in ulcerative colitis and chemerin is elevated in Crohn's disease. *Inflamm Bowel Dis* 2010; **16**: 630-637

113 **Abke S**, Neumeier M, Weigert J, Wehrwein G, Eggenhofer E, Schäffler A, Maier K, Aslanidis C, Schölmerich J, Buechler C. Adiponectin-induced secretion of interleukin-6 (IL-6), monocyte chemotactic protein-1 (MCP-1, CCL2) and interleukin-8 (IL-8, CXCL8) is impaired in monocytes from patients with type I diabetes. *Cardiovasc Diabetol* 2006; **5**: 17

114 **Karmiris K**, Koutroubakis IE, Xidakis C, Polychronaki M, Voudouri T, Kouroumalis EA. Circulating levels of leptin, adiponectin, resistin, and ghrelin in inflammatory bowel disease. *Inflamm Bowel Dis* 2006; **12**: 100-105

115 **Rovin BH**, Song H. Chemokine induction by the adipocyte-derived cytokine adiponectin. *Clin Immunol* 2006; **120**: 99-105

116 **Fantuzzi G**. Adiponectin and inflammation: consensus and controversy. *J Allergy Clin Immunol* 2008; **121**: 326-330

117 **Luedde T**, Beraza N, Kotsikoris V, van Loo G, Nenci A, De Vos R, Roskams T, Trautwein C, Pasparakis M. Deletion of NEMO/IKKgamma in liver parenchymal cells causes steatohepatitis and hepatocellular carcinoma. *Cancer Cell* 2007; **11**: 119-132

118 **Wanninger J**, Neumeier M, Weigert J, Bauer S, Weiss TS, Schäffler A, Krempl C, Bleyl C, Aslanidis C, Schölmerich J, Buechler C. Adiponectin-stimulated CXCL8 release in primary human hepatocytes is regulated by ERK1/ERK2, p38 MAPK, NF-kappaB, and STAT3 signaling pathways. *Am J Physiol Gastrointest Liver Physiol* 2009; **297**: G611-G618

119 **Aggarwal BB**, Shishodia S, Sandur SK, Pandey MK, Sethi G. Inflammation and cancer: how hot is the link? *Biochem Pharmacol* 2006; **72**: 1605-1621

120 **Hanson JC**, Bostick MK, Campe CB, Kodali P, Lee G, Yan J, Maher JJ. Transgenic overexpression of interleukin-8 in mouse liver protects against galactosamine/endotoxin toxicity. *J Hepatol* 2006; **44**: 359-367

121 **Jung TW**, Lee YJ, Lee MW, Kim SM, Jung TW. Full-length adiponectin protects hepatocytes from palmitate-induced apoptosis *via* inhibition of c-Jun NH2 terminal kinase. *FEBS J* 2009; **276**: 2278-2284

122 **Wedemeyer I**, Bechmann LP, Odenthal M, Jochum C, Marquitan G, Drebber U, Gerken G, Gieseler RK, Dienes HP, Canbay A. Adiponectin inhibits steatotic CD95/Fas upregulation by hepatocytes: therapeutic implications for hepatitis C. *J Hepatol* 2009; **50**: 140-149

123 **Fujita K**, Nishizawa H, Funahashi T, Shimomura I, Shimabukuro M. Systemic oxidative stress is associated with visceral fat accumulation and the metabolic syndrome. *Circ J* 2006; **70**: 1437-1442

124 **Kamada Y**, Tamura S, Kiso S, Matsumoto H, Saji Y, Yoshida Y, Fukui K, Maeda N, Nishizawa H, Nagaretani H, Okamoto Y, Kihara S, Miyagawa J, Shinomura Y, Funahashi T, Matsuzawa Y. Enhanced carbon tetrachloride-induced liver fibrosis in mice lacking adiponectin. *Gastroenterology* 2003; **125**: 1796-1807

125 **Leclercq IA**, Farrell GC, Field J, Bell DR, Gonzalez FJ, Robertson GR. CYP2E1 and CYP4A as microsomal catalysts of lipid peroxides in murine nonalcoholic steatohepatitis. *J Clin Invest* 2000; **105**: 1067-1075

126 **Weltman MD**, Farrell GC, Liddle C. Increased hepatocyte CYP2E1 expression in a rat nutritional model of hepatic steatosis with inflammation. *Gastroenterology* 1996; **111**: 1645-1653

127 **Garattini E**, Fratelli M, Terao M. Mammalian aldehyde oxidases: genetics, evolution and biochemistry. *Cell Mol Life Sci* 2008; **65**: 1019-1048

128 **Kundu TK**, Hille R, Velayutham M, Zweier JL. Characterization of superoxide production from aldehyde oxidase: an important source of oxidants in biological tissues. *Arch Biochem Biophys* 2007; **460**: 113-121

129 **Yamauchi T**, Nio Y, Maki T, Kobayashi M, Takazawa T, Iwabu M, Okada-Iwabu M, Kawamoto S, Kubota N, Kubota T, Ito Y, Kamon J, Tsuchida A, Kumagai K, Kozono H, Hada Y, Ogata H, Tokuyama K, Tsunoda M, Ide T, Murakami K, Awazawa M, Takamoto I, Froguel P, Hara K, Tobe K, Nagai R, Ueki K, Kadowaki T. Targeted disruption of AdipoR1 and AdipoR2 causes abrogation of adiponectin binding and metabolic actions. *Nat Med* 2007; **13**: 332-339

130 **Gressner AM**, Weiskirchen R. Modern pathogenetic concepts of liver fibrosis suggest stellate cells and TGF-beta as major players and therapeutic targets. *J Cell Mol Med* 2006; **10**: 76-99

131 **Tomita K**, Oike Y, Teratani T, Taguchi T, Noguchi M, Suzuki T, Mizutani A, Yokoyama H, Irie R, Sumimoto H, Takayanagi A, Miyashita K, Akao M, Tabata M, Tamiya G, Ohkura T, Hibi T. Hepatic AdipoR2 signaling plays a protective role against progression of nonalcoholic steatohepatitis in mice. *Hepatology* 2008; **48**: 458-473

132 **Ding X**, Saxena NK, Lin S, Xu A, Srinivasan S, Anania FA. The roles of leptin and adiponectin: a novel paradigm in adipocytokine regulation of liver fibrosis and stellate cell biology. *Am J Pathol* 2005; **166**: 1655-1669

133 **Adachi M**, Brenner DA. High molecular weight adiponectin inhibits proliferation of hepatic stellate cells *via* activation of adenosine monophosphate-activated protein kinase. *Hepatology* 2008; **47**: 677-685

134 **Handy JA**, Saxena NK, Fu P, Lin S, Mells JE, Gupta NA, Anania FA. Adiponectin activation of AMPK disrupts leptin-mediated hepatic fibrosis *via* suppressors of cytokine signaling (SOCS-3). *J Cell Biochem* 2010; **110**: 1195-1207

135 **Jardé T**, Caldefie-Chézet F, Goncalves-Mendes N, Mishellany F, Buechler C, Penault-Llorca F, Vasson MP. Involvement of adiponectin and leptin in breast cancer: clinical and in vitro studies. *Endocr Relat Cancer* 2009; **16**: 1197-1210

136 **Sanyal AJ**. AGA technical review on nonalcoholic fatty liver disease. *Gastroenterology* 2002; **123**: 1705-1725

137 **Shah K**, Stufflebam A, Hilton TN, Sinacore DR, Klein S, Villareal DT. Diet and exercise interventions reduce intrahepatic fat content and improve insulin sensitivity in obese older adults. *Obesity* (Silver Spring) 2009; **17**: 2162-2168

138 **Johnson NA**, Sachinwalla T, Walton DW, Smith K, Armstrong A, Thompson MW, George J. Aerobic exercise training reduces hepatic and visceral lipids in obese individuals without weight loss. *Hepatology* 2009; **50**: 1105-1112

139 **Promrat K**, Kleiner DE, Niemeier HM, Jackvony E, Kearns M, Wands JR, Fava JL, Wing RR. Randomized controlled trial testing the effects of weight loss on nonalcoholic steatohepatitis. *Hepatology* 2010; **51**: 121-129

140 **Palmer M**, Schaffner F. Effect of weight reduction on hepatic abnormalities in overweight patients. *Gastroenterology* 1990; **99**: 1408-1413

141 **Pasarica M**, Tchoukalova YD, Heilbronn LK, Fang X, Albu JB, Kelley DE, Smith SR, Ravussin E. Differential effect of weight loss on adipocyte size subfractions in patients with type 2 diabetes. *Obesity* (Silver Spring) 2009; **17**: 1976-1978

142 **Laurin J**, Lindor KD, Crippin JS, Gossard A, Gores GJ, Ludwig J, Rakela J, McGill DB. Ursodeoxycholic acid or clofibrate in the treatment of non-alcohol-induced steatohepatitis: a pilot study. *Hepatology* 1996; **23**: 1464-1467

143 **Athyros VG**, Mikhailidis DP, Didangelos TP, Giouleme OI, Liberopoulos EN, Karagiannis A, Kakafika AI, Tziomalos K,

Burroughs AK, Elisaf MS. Effect of multifactorial treatment on non-alcoholic fatty liver disease in metabolic syndrome: a randomised study. *Curr Med Res Opin* 2006; **22**: 873-883

144 **Neuschwander-Tetri BA**, Brunt EM, Wehmeier KR, Oliver D, Bacon BR. Improved nonalcoholic steatohepatitis after 48 weeks of treatment with the PPAR-gamma ligand rosiglitazone. *Hepatology* 2003; **38**: 1008-1017

145 **Neuschwander-Tetri BA**, Brunt EM, Wehmeier KR, Sponseller CA, Hampton K, Bacon BR. Interim results of a pilot study demonstrating the early effects of the PPAR-gamma ligand rosiglitazone on insulin sensitivity, aminotransferases, hepatic steatosis and body weight in patients with non-alcoholic steatohepatitis. *J Hepatol* 2003; **38**: 434-440

146 **Sun K**, Wang Q, Huang XH. PPAR gamma inhibits growth of rat hepatic stellate cells and TGF beta-induced connective tissue growth factor expression. *Acta Pharmacol Sin* 2006; **27**: 715-723

147 **Maeda N**, Takahashi M, Funahashi T, Kihara S, Nishizawa H, Kishida K, Nagaretani H, Matsuda M, Komuro R, Ouchi N, Kuriyama H, Hotta K, Nakamura T, Shimomura I, Matsuzawa Y. PPARgamma ligands increase expression and plasma concentrations of adiponectin, an adipose-derived protein. *Diabetes* 2001; **50**: 2094-2099

148 **Gastaldelli A**, Harrison S, Belfort-Aguiar R, Hardies J, Balas B, Schenker S, Cusi K. Pioglitazone in the treatment of NASH: the role of adiponectin. *Aliment Pharmacol Ther* 2010; **32**: 769-775

149 **Gastaldelli A**, Harrison SA, Belfort-Aguilar R, Hardies LJ, Balas B, Schenker S, Cusi K. Importance of changes in adipose tissue insulin resistance to histological response during thiazolidinedione treatment of patients with nonalcoholic steatohepatitis. *Hepatology* 2009; **50**: 1087-1093

150 **Bouhlel MA**, Derudas B, Rigamonti E, Dièvart R, Brozek J, Haulon S, Zawadzki C, Jude B, Torpier G, Marx N, Staels B, Chinetti-Gbaguidi G. PPARgamma activation primes human monocytes into alternative M2 macrophages with anti-inflammatory properties. *Cell Metab* 2007; **6**: 137-143

151 **Satapathy SK**, Sakhuja P, Malhotra V, Sharma BC, Sarin SK. Beneficial effects of pentoxifylline on hepatic steatosis, fibrosis and necroinflammation in patients with non-alcoholic steatohepatitis. *J Gastroenterol Hepatol* 2007; **22**: 634-638

152 **Lee YM**, Sutedja DS, Wai CT, Dan YY, Aung MO, Zhou L, Cheng CL, Wee A, Lim SG. A randomized controlled pilot study of Pentoxifylline in patients with non-alcoholic steatohepatitis (NASH). *Hepatol Int* 2008; **2**: 196-201

153 **Yakaryilmaz F**, Guliter S, Savas B, Erdem O, Ersoy R, Erden E, Akyol G, Bozkaya H, Ozenirler S. Effects of vitamin E treatment on peroxisome proliferator-activated receptor-alpha expression and insulin resistance in patients with non-alcoholic steatohepatitis: results of a pilot study. *Intern Med J* 2007; **37**: 229-235

154 **Ristow M**, Zarse K, Oberbach A, Klöting N, Birringer M, Kiehntopf M, Stumvoll M, Kahn CR, Blüher M. Antioxidants prevent health-promoting effects of physical exercise in humans. *Proc Natl Acad Sci USA* 2009; **106**: 8665-8670

S- Editor Tian L **L- Editor** O'Neill M **E- Editor** Ma WH

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/1007-9327office
wjg@wjgnet.com
doi:10.3748/wjg.v17.i23.2812

World J Gastroenterol 2011 June 21; 17(23): 2812-2820
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL ARTICLE

Streptozotocin-induced expression of Ngn3 and Pax4 in neonatal rat pancreatic α-cells

Xiao-Di Liang, Yuan-Yuan Guo, Ming Sun, Ying Ding, Ning Wang, Li Yuan, Wei De

Xiao-Di Liang, Yuan-Yuan Guo, Ming Sun, Ying Ding, Ning Wang, Li Yuan, Wei De, Department of Biochemistry and Molecular Biology, Nanjing Medical University, Nanjing 210029, Jiangsu Province, China

Author contributions: De W and Liang XD contributed to the conception and design; Liang XD, Guo YY and Sun M contributed to the acquisition of data; Liang XD and Wang N analyzed and interrelated the data; Liang XD and Guo YY drafted the manuscript; Yuan L revised the manuscript critically for important intellectual content; all the authors have read and approved the final version of the manuscript.

Supported by The National Natural Science Foundation of China, No. 81070620

Correspondence to: Wei De, MD, PhD, Professor of Molecular Biology, Department of Biochemistry and Molecular Biology, Nanjing Medical University, Nanjing 210029, Jiangsu Province, China. dewei@njmu.edu.cn
Telephone: +86-25-86862728 Fax: +86-25-86862728

Received: January 14, 2010 Revised: March 7, 2011
Accepted: March 14, 2011
Published online: June 21, 2011

Abstract

AIM: To investigate the mechanism behind β-cell regeneration in neonatal rat pancreas treated with streptozotocin (STZ).

METHODS: Neonatal Sprague Dawley rats were intraperitoneally injected with 70 mg/kg STZ. Body weight, pancreas weight and blood glucose were recorded every two days after the treatment. To identify the expression and location of transcription factors in the rat pancreas, double immunofluorescent staining was performed using antibodies to specific cell markers and transcription factors.

RESULTS: Expression of Neurogenin 3 (Ngn3), a marker for endocrine precursor cells, was observed by immunofluorescence in a few β-cells and many α-cells. The expression reached a peak 12 d after treatment. Pax4, a transcription factor that lies downstream of Ngn3 and plays an important role in β-cell differentiation, was also expressed in the α-cells of STZ-treated rats. We did not observe significant changes in Nkx6.1, which is essential for β-cell maturation in the treated rats.

CONCLUSION: α-cells dedifferentiated into endocrine precursor cells and acquired the ability to dedifferentiate in the neonatal rat pancreas after STZ treatment.

Key words: Pancreatic remodeling; Dedifferentiation; Endocrine precursor cells; Streptozotocin; Transcription factors

Peer reviewer: Dr. Thiruvengadam Muniraj, University of Pittsburgh Medical Center, 100 Chatham Park Drive, Apt 511, Pittsburgh, PA 15220, United States

Liang XD, Guo YY, Sun M, Ding Y, Wang N, Yuan L, De W. Streptozotocin-induced expression of Ngn3 and Pax4 in neonatal rat pancreatic α-cells. *World J Gastroenterol* 2011; 17(23): 2812-2820 Available from: URL: http://www.wjgnet.com/1007-9327/full/v17/i23/2812.htm DOI: http://dx.doi.org/10.3748/wjg.v17.i23.2812

INTRODUCTION

The pancreas originates from gut endoderm. During development, the rat pancreas undergoes two transitions in embryonic days[1]. After birth, many major developmental changes occur, including β-cell apoptosis, replication, and exogenesis[2]. This stage is referred to as the remodeling of pancreas. In our previous work, we found alpha-fetoprotein and Mesothelin in embryonic rat pancreases but not in adult rat pancreases, nevertheless, we observed the expression of these two proteins during the remodeling of the rat pancreas[3,4]. These studies indicate that during the remodeling phase, the neonatal pancreas is not fully matured. Furthermore, after treatment with Streptozotocin

(STZ) during this stage, the ontogeny of regeneration can be observed[5]. Conversely, after treatment with STZ during adulthood, little regeneration of β-cells was found[6]. Extensive studies have been reported on the model of STZ-induced depletion of β-cells in the neonatal rat pancreas, which showed that this model can be used to study β-cell replacement therapy for diabetes[5,7,8].

The development of β-cells is regulated by a series of transcription factors[9,10]. However, few studies have focused on the expression of these transcription factors during the regeneration of β-cells in STZ-treated neonatal rats. One of the most important upstream transcription factors is pancreas-duodenal homeobox 1 (Pdx1)[11]. The initial expression of Pdx1 (E8.5-E9.0) marks the prepancreatic endoderm before it is visibly thickened[12-14], and corresponds to the classically defined period of pancreatic specification[15]. Following the expression of Pdx1 is the Neurogenin 3 (Ngn3), a basic helix-loop-helix transcription factor[16] that marks endocrine pancreatic precursor cells. Among a series of transcription factors that differentiate endocrine precursors into β-cells, paired domain homeobox gene 4 (Pax4)[17] and NK family member Nkx6.1[18] lie downstream of Ngn3.

Although these factors are essential for the development and maturation of β-cells, it is unknown whether Ngn3, Pax4 and Nkx6.1 participate in the regeneration of β-cells after STZ treatment during the remodeling phase of the pancreas. Especially, there is little information on the differentiation factors that are involved in the remodeling of the rat pancreas. This study was designed to determine the expression and location of these transcription factors in the STZ-treated neonatal rat pancreas.

MATERIAL AND METHODS

Animals

Pregnant Sprague Dawley rats from the Animal Center of Nanjing Medical University, Nanjing, China, were kept under conventional conditions and provided with a 12:12 h light-dark cycle. Litters were reduced to 12 pups at birth. Four days after birth, half of the pups in each litter was intraperitoneally injected with 70 mg/kg STZ freshly dissolved in citrate buffer (0.05 mol/L, pH 4.5). The remaining pups received vehicle only. Blood glucose was measured with a OneTouch Ultra blood glucose meter (LifeScan Inc. Milpitas, CA, USA) in blood obtained by lancing the tail vein. Body weight was recorded every two days. On the day of treatment and days 4, 8, 12, 16 and 20 after treatment, animals were killed by decapitation or by overdose of anesthesia (sodium amobarbital, amytal sodium, Sigma-Aldrich 200 mg/kg body weight). Pancreases were collected immediately and frozen in liquid nitrogen or fixed. Three to five pups from at least three separate litters were studied at each time point. All experiments were conducted in accordance with the Chinese Law for Animal Protection and were approved by Nanjing Medical University Ethics Review Committee (approval No. 200913).

Fluorescence immunohistochemistry

Tissues were fixed in 4% paraformaldehyde for 24-36 h followed by a standard protocol of dehydration and paraffin embedding. Sections (5 μm) were cut and mounted on glass slides (Fisher Scientific, Pittsburgh, PA, USA). The paraffin sections were deparaffinized in xylene and dehydrated in graded ethanol and distilled water. The tissue sections were blocked in 1% bovine serum albumin for 1 h. For double fluorescence immunohistochemical localization of glucagon and insulin, the mouse anti-glucagon (1:100, Sigma-Aldrich, St. Louis, MO, USA) antibody was applied after blocking and revealed using goat anti-mouse IgG-FITC (1:400, Santa Cruz, Santa Cruz, CA, USA). Rabbit anti-insulin polyclonal antibody (1:100, Santa Cruz, Santa Cruz, CA, USA) was then applied and revealed by Cy3-labeled anti-rabbit IgG (1:400, Santa Cruz, Santa Cruz, CA, USA). For dual fluorescence immunohistochemical localization of Ngn3, Pax4 or Nkx6.1 and insulin, rabbit anti-neurogenin 3 (1:100, Santa Cruz, Santa Cruz, CA, USA), goat anti-Pax4 (1:100, Santa Cruz, Santa Cruz, CA, USA) or goat anti-Nkx6.1 (1:100, Santa Cruz, Santa Cruz, CA, USA) antibody were added, respectively, and revealed by rabbit anti-goat IgG-FITC (1:400, Chemicon, Temecula, CA, USA) or mouse anti-rabbit IgG-FITC (1:400, Chemicon, Temecula, CA, USA). Mouse anti-insulin (1:100, Sigma-Aldrich, St. Louis, MO, USA) was applied and revealed by Cy3 conjugated anti-mouse (1:400, Chemicon, Temecula, CA, USA) antibody. For co-localizations of Ngn3, Pax4 or Nkx6.1 and glucagon, rabbit anti-neurogenin 3 (1:100), goat anti-Pax4 (1:100) or goat anti-Nkx6.1 (1:100) antibodies were added and revealed by rabbit anti-goat IgG-FITC (1:400) or mouse anti-rabbit IgG-FITC (1:400). Mouse anti-glucagon (1:100) was applied and revealed by Cy3 conjugated anti-mouse (1:400) antibody. Sections were placed in Gel Mount Aqueous Mounting Medium (G0918, Sigma) with a cover glass, and were examined under an Olympus BX51 microscope (Olympus Optical, Tokyo, Japan) at a magnification of × 200 or × 400.

β-cell mass

β-cell mass was measured by point counting morphometry on the same stained sections as described above. Each section was covered systematically at a magnification of × 400 using a 48-point grid to obtain the number of grid intercepts over β-cells, endocrine non-β-cells, exocrine pancreatic tissues, and non-pancreatic tissues. The relative β-cell area was calculated by dividing the number of intercepts over β-cells by the number of intercepts over total pancreatic tissue; the β-cell mass was then estimated by multiplying the relative β-cell volume by the corrected pancreatic weight. Non-β-cell mass was similarly calculated. A correction factor for pancreas weight was obtained by multiplying the pancreas weight by the ratio of intercepts over non- pancreatic tissues to intercepts over total tissues. Actual pancreas weight was then calculated by subtracting this correction factor from total pancreas weight. A monogram related to the number of points, the

Table 1 Body and pancreas weight in control and streptozotocin-treated animals after streptozotocin treatment on postnatal day 4 (mean ± SD)

	Days after STZ treatment				
	4	8	12	16	20
Body weight (g)					
Control	13.6 ± 0.3	24.4 ± 1.5	30.5 ± 1.3	34.5 ± 1.5	50.0 ± 1.5
STZ	12.4 ± 0.3	22.3 ± 1.4	26.4 ± 1.3	32.4 ± 1.7	43.9 ± 1.5
Pancreas weight (g)					
Control	24.4 ± 2.3	49.8 ± 3.2	59.8 ± 1.4	60.5 ± 3.4	187.5 ± 6.9
STZ	18.7 ± 1.4	46.8 ± 3.3	57.1 ± 1.9	57.3 ± 3.2	162.5 ± 8.4

n = 3 litters/18 animals per group. STZ: Streptozotocin.

volume density and the expected relative standard error of the mean (< 10%) was used to determine the number of intercepts needed for a representative sampling.

Statistical analysis

The experimental data was analyzed by paired Student *t* test using the SPSS 17.0 software. $P < 0.05$ was considered statistically significant. Data were presented as mean ± SD.

RESULTS

Body and pancreatic weight, blood glucose and islets in STZ-treated neonatal rat pancreases

After STZ treatment, body and pancreas weight did not change significantly (Table 1). Blood glucose concentrations significantly increased within 2 d after STZ treatment (Figure 1A). However, on day 20 after treatment, there was no longer a difference in blood glucose concentrations between the two groups.

Histological analysis showed that approximately 60% of insulin immunoreactive cells within the islets were lost 4 d after STZ treatment (Figure 1B). On day 8 after treatment, an increased number of small islets was observed (Figure 1C). On day 20 after treatment, more large islets were found, which may indicate that islet function had also recovered. Similarly, calculation of β-cell mass showed a reduction in β-cell mass from 4 d after STZ treatment onwards (Figure 1D). While β-cell mass was still reduced in STZ-treated rats on day 20 after treatment, blood glucose levels were not significantly different.

Expression and location of Ngn3

We used double immunofluorescence to stain Ngn3 and insulin or glucagon at different time points after STZ treatment. We did not find Ngn3 co-located with insulin in either treated or control rats (Figure 2A). By analyzing the coexpression of Ngn3 and glucagon, we observed abundant expression of Ngn3 in the treated rat islet α-cells (Figure 2B). In the STZ group, expression of Ngn3 could be detected on day 8 and reached a peak on day 12 after treatment (Figure 2C). However, no significant changes were observed in the signal from Ngn3 in α-cells 20 d after treatment compared with the control rats. In contrast, few α-cells expressed Ngn3 in control rats at each time point.

Expression and location of Nkx6.1

We stained Nkx6.1 and glucagon or insulin by immunofluorescence. Consistent with previous work, we found coexpression of Nkx6.1 and insulin in both the controls and the treated group (Figure 3A), while no Nkx6.1 expression was found in α-cells at any time point (Figure 3B) when we studied the coexpression of glucagon and Nkx6.1.

Expression and location of Pax4

We studied the colocation of Pax4 and insulin or glucagon by dual immunofluorescence. Consistent with previous work, we observed coexpression of insulin and Pax4 in both the control group and the treated group (Figure 4A). We also found enhanced expression of Pax4 in STZ-treated rat pancreases compared with control rats (Figure 4A). Eight days after treatment, we observed expression of Pax4 in α-cells of the treated rats but little expression in the control rats. However, we found coexpression of glucagon and Pax4 in both treated and control rats on day 12 after treatment (Figure 4B). On day 20 after STZ treatment, we could still observe a signal of Pax4 in the α-cells. However, in the control rats, few α-cells expressed Pax4 on day 20.

DISCUSSION

It is established that neonatal β-cells are able to regenerate after subtotal β-cell damage by STZ treatment. Regeneration of neonatal β-cells after destruction mainly relies on replication of pre-existing β-cells and heterogenesis of new cells[19]. In this article, we demonstrated a series of transcription factors expressed in pancreatic α-cells, which suggested that α-cells may be a source of β-cells during the regeneration of the STZ-treated neonatal rat pancreas.

We found that β-cells were damaged 4 d after STZ treatment. On day 8 after treatment, β-cell numbers were recovered in STZ-treated rats. By day 20 after treatment, there was still a reduction in β-cell mass but the blood glucose concentrations had reverted to normal. Although the model resulted in transient hyperglycemia, no difference in the mean body weight or pancreatic weight was seen between the two groups.

The study of pancreatic development has focused on transcription factors and transcription factor hierarchies during development. A central and heavily studied transcription factor in pancreatic development is Pdx1. Although Pdx1 is a key component of pancreatic specification, we found no significant difference in α or β-cells between STZ-treated rats and control animals. This indicates that Pdx1 is not involved in the regeneration of β-cells in STZ-treated animals.

After pancreatic formation, which is mediated by Pdx1, Ngn3 regulates the differentiation of endocrine pancreas. Lack of Ngn3 leads to an absence of islets[20]; and the ectopic expression of Ngn3 in other cells converts these

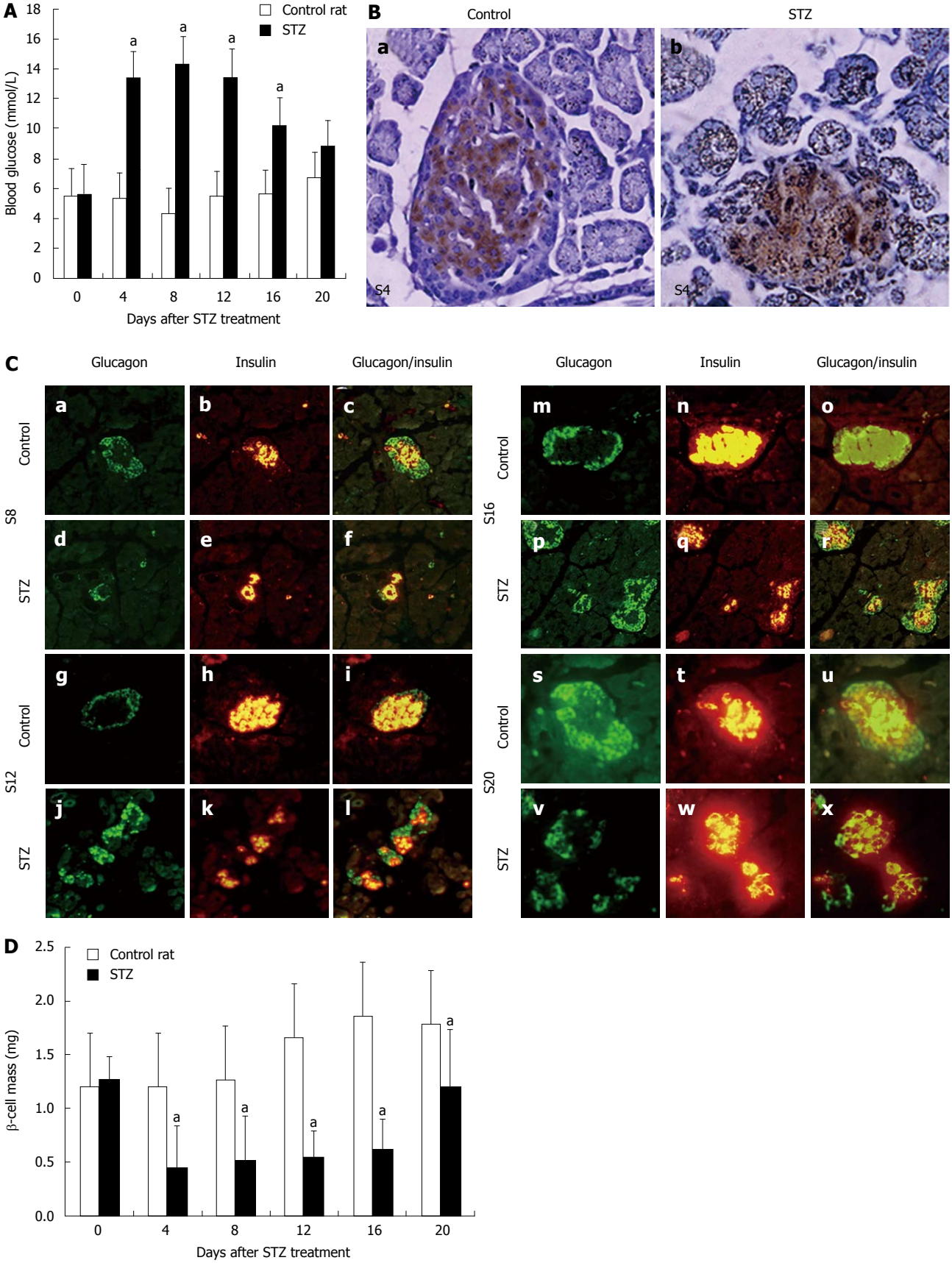


Figure 1 Body and pancreatic weight, blood glucose, islets and β-cell mass in streptozotocin-treated neonatal rat pancreas. A: Concentrations of fasting blood glucose in control rats or rats treated with streptozotocin (STZ) between day 0 and day 20 after STZ treatment. Data were obtained from 12-18 animals at each time point. $^{a}P < 0.005$ *vs* control; B: Immunohistochemical location of insulin in sections of rat pancreas in the control (a) and 4 d after STZ treatment; (b). Original magnification, × 400; C: Structure of islets in STZ-treated rats (d-f, j-l, p-r and v-x) and control rats (a-c, g-i, m-o and s-u) on d 8 (a-f), 12 (g-l), 16 (m-r) and 20 (s-x) after STZ treatment. Pancreatic sections were immunostained with anti-glucagon antibody (green) and anti-insulin antibody (red). Original magnification, × 400. Mean ± SD; D: β-cell mass in control rats or rats treated with STZ between day 0 and day 20 after STZ treatment. Data were obtained from three rats per time point. $^{a}P < 0.05$ *vs* control rats.

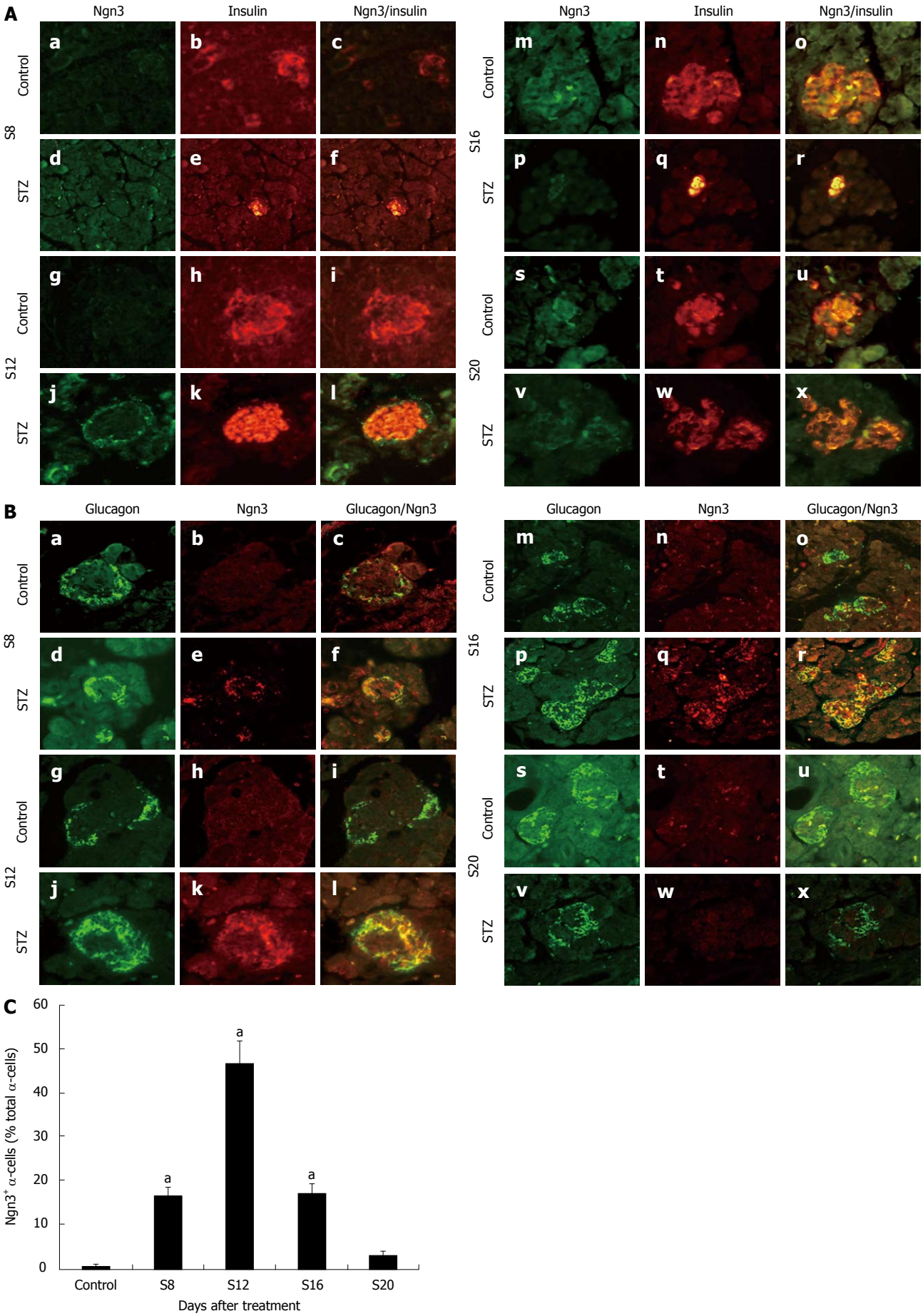


Figure 2 Expression and location of Ngn3. A: Immunofluorescent colocalization of Ngn3 and insulin in streptozotocin (STZ)-treated rats (d-f, j-l, p-r and v-x) and

control rats (a-c, g-i, m-o and s-u) on d 8 (a-f), 12 (g-l), 16 (m-r) and 20 (s-x) after STZ treatment. Pancreatic sections were immunostained with anti-Ngn3 antibody (green) and anti-insulin antibody (red). Original magnification, × 400; B: Immunofluorescent colocalization of Ngn3 and glucagon in STZ-treated rats (d-f, j-l, p-r and v-x) and control rats (a-c, g-i, m-o and s-u) on d 8 (a-f), 12 (g-l), 16 (m-r) and 20 (s-x) after STZ treatment. Pancreatic sections were immunostained with anti-glucagon antibody (green) and anti-Ngn3 antibody (red). Original magnification, × 400; C: Proportion of Ngn3+ / glucagon+ cells in total α-cells after treatment (Y). $^{a}P < 0.05$ *vs* control rats.

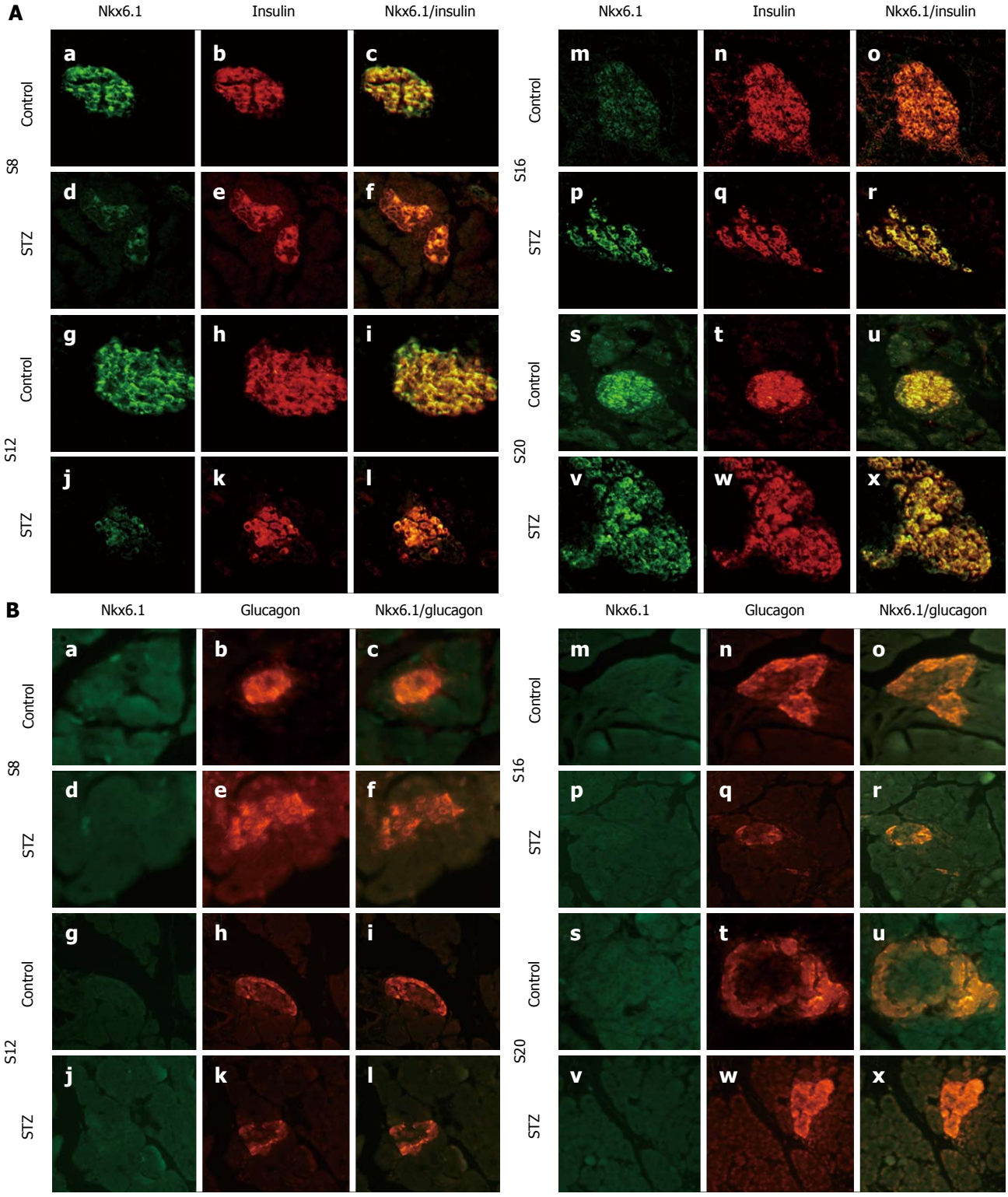


Figure 3 Expression and location of Nkx6.1. A: Immunofluorescent colocalization of Nkx6.1 and insulin in streptozotocin (STZ)- treated rats (d-f, j-l, p-r and v-x) and control rats (a-c, g-i, m-o and s-u) on d 8 (a-f), 12 (g-l), 16 (m-r) and 20 (s-x) after STZ treatment. Pancreatic sections were immunostained with anti-Nkx6.1 antibody (green) and anti-insulin antibody (red); B: Immunofluorescent colocalization of Nkx6.1 and glucagon in STZ-treated rats (d-f, j-l, p-r and v-x) and control rats (a-c, g-i, m-o and s-u) on d 8 (a-f), 12 (g-l), 16 (m-r) and 20 (s-x) after STZ treatment. Pancreatic sections were immunostained with anti-Nkx6.1 antibody (green) and anti-glucagon antibody (red). Original magnification, × 400.

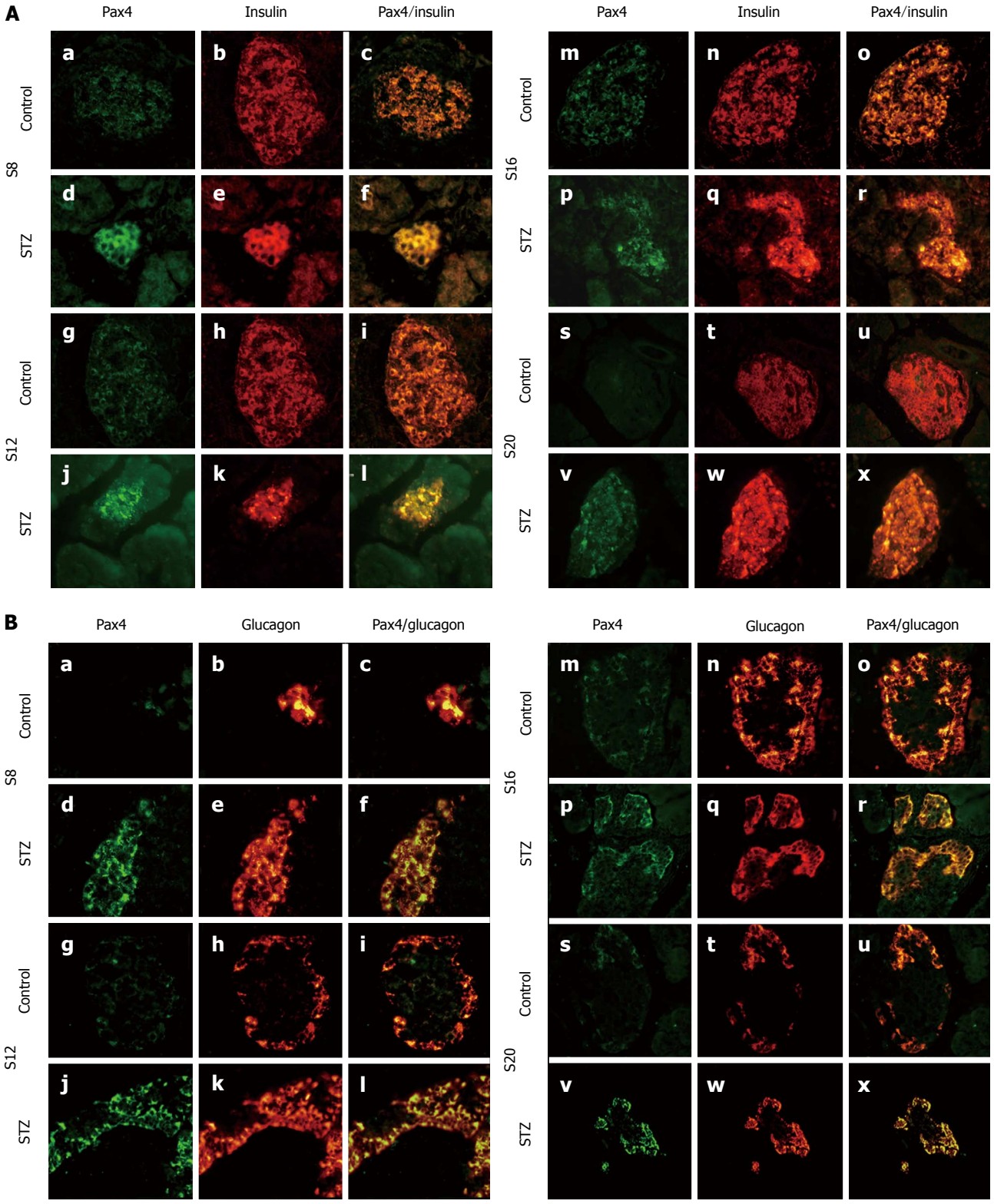


Figure 4 Expression and location of Pax4. A: Immunofluorescent colocalization of Pax4 and insulin in streptozotocin (STZ)-treated rats (d-f, j-l, p-r and v-x) and control rats (a-c, g-i, m-o and s-u) on d 8 (a-f), 12 (g-l), 16 (m-r) and 20 (s-x) after STZ treatment. Pancreatic sections were immunostained with anti-Pax4 antibody (green) and anti-insulin antibody (red); B: Immunofluorescent colocalization of Pax4 and glucagon in STZ-treated rats (d-f, j-l, p-r and v-x) and control rats (a-c, g-i, m-o and s-u) on d 8 (a-f), 12 (g-l), 16 (m-r) and 20 (s-x) after STZ treatment. Pancreatic sections were immunostained with anti-Pax4 antibody (green) and anti-glucagon antibody (red). Original magnification, × 400.

cells into endocrine cells[21]. We observed Ngn3 expression in the α-cells of treated rats, which reached a peak on day 12 after STZ treatment. It has been reported that Ngn3 is activated through partial duct ligation in the adult mouse pancreas[22]. However, Ngn3 is absent in the β-cells of mice that underwent partial pancreatectomy[23]. The dif-

ferent results among these three models indicate that the mechanism of β-cell regeneration in different pathological situations can be varied. Interestingly, we observed abundant expression of Ngn3 in STZ-treated rats but not in control rats. The expression of Ngn3 in α-cells after STZ treatment indicated that α-cells dedifferentiated into precursor cells and may be candidates for β-cell formation. It has been suggested that high Ngn3 expression at an inappropriately early time in the developing mouse pancreas may result in a pancreas entirely consisting of small clusters of glucagon-positive cells[21,24]. Furthermore, by overexpression of Ngn3 in human[25] or mouse[26,27] pancreatic duct cells, the pancreatic duct cells could become endocrine cells. Apparently, the expression of Ngn3 is necessary for the transdifferentiation of α-cells to β-cells.

Next to Ngn3 induction, a complex network of transcription factors, including Pax4, progressively and differentially promotes the particular endocrine fates[28,29]. The expression of Pax4 is first observed around E9.5 in dorsal pancreatic buds of mouse embryos and vanishes shortly after birth[29,30]. Pax4 specifies the β-cell lineage into β and δ precursor cells[31]. In mice lacking Pax4, mature pancreatic and δ-cells were absent[29]. Conversely, ectopic expression of Pax4 in the mouse pancreas converted α-cells into β-cells. Moreover, the transgenic adult mice could survive after STZ-induced hyperglycemia[32]. Activation of Pax4 in endocrine progenitor cells may be mediated by Ngn3 since it binds to the Pax4 regulatory region and is necessary for Pax4 expression[33]. Ngn3 is required for ectopic Pax4 expressing α-cells to acquire a β-cell phenotype[34]. Interestingly, we observed expression of Pax4 in α-cells of both control and STZ-treated rats. In the STZ-treated rats, the expression of Pax4 reached a peak on day 16 after the treatment. The expression of Pax4 in the control animals suggests that Pax4 expression is characteristic of the pancreatic remodeling phase. Both cell differentiation and maturation occur during remodeling of the pancreas, which explains the presence of transcription factors in islet cells. After STZ treatment, the expression of Pax4 increased and a Pax4 signal could still be observed in α-cells 20 d after the treatment. This suggests that STZ treatment exaggerates and extends the period of remodeling.

Another transcript factor which lies downstream of Ngn3 is Nkx6.1, and it is associated with the development and maturation of β-cells. Nkx6.1 appears to be a marker for multipotent pancreatic progenitor cells[35]. At later developmental stages and in the adult pancreas, Nkx6.1 becomes completely restricted to insulin-expressing cells. Consistent with previous researches, we observed coexpression of Nkx6.1 and insulin in both STZ-treated and control rats. However, we did not find any Nkx6.1-positive α-cells. Immunoblotting revealed that the expression of Nkx6.1 decreased 4 d after STZ treatment, and reached normal levels on day 12 after treatment. Although Nkx6.1 is critical for the development of β-cells, it does not affect the generation of β-cells from α-cells.

It has been established that regeneration of neonatal rat β-cells after subtotal destruction by STZ occurs by two mechanisms of equal significance. The first mechanism is the replication of surviving β-cells in the islet compartment, the second mechanism is the replication of cells from a β-cell pool outside the islet compartment. In this article, we have demonstrated that α-cells may also be a source for β-cell regeneration. Mature α-cells converted to β-cells after partial duct ligation plus alloxan treatment, and the contribution of α-cells to the emergence of new β-cells was proportional to the degree of β-cell ablation[36]. However, α-cells could only convert to β-cells when the proportion of β-cell loss reached 99%[37].

In conclusion, during the period of pancreatic remodeling, the islets are not completely matured and the dedifferentiation of α-cells into endocrine precursor cells contributes to the recovery of β-cell mass after impairment by STZ.

COMMENTS

Background

Streptozotocin-induced β-cell loss in neonatal rat pancreas can trigger transient hyperglycemia. β-cell mass recovers 20 d after treatment. It is unknown whether Ngn3, Pax4 and Nkx6.1 participate in the regeneration of β-cells after streptozotocin (STZ) treatment during the remodeling phase of the pancreas.

Research frontiers

It has been shown that mature α-cells converted to β-cells after partial duct ligation plus alloxan treatment, and that the contribution of α-cells to the emergence of new β-cells was proportional to the degree of β-cell ablation.

Innovations and breakthroughs

There is little information on the differentiation factors that are involved in the remodeling of the rat pancreas. This study was designed to determine the expression and location of these transcription factors in the STZ-treated neonatal rat pancreas. The authors for the first time found the expression of Ngn3 and Pax4 in α-cells during remodeling of rat pancreas after STZ treatment.

Applications

Insulin deficiency caused by a reduced pancreatic islet β-cell number underlies the progression of both type 1 and type 2 diabetes, prompting efforts to develop β-cell replacement therapies. This study demonstrated the dedifferentiation of α-cells into endocrine precursor cells may contributes to the recovery of β-cell mass after impairment by STZ and this may provide a alternative way for β-cell replacement therapies.

Terminology

After birth, many major developmental changes occur, including β-cell apoptosis, replication, and exogenesis. This stage is referred to as the remodeling of pancreas.

Peer review

The study is well conducted and the results are interesting. This paper puts up the fact that α-cells dedifferentiate to endocrine precursor cells.

REFERENCES

1 **Bernardo AS**, Hay CW, Docherty K. Pancreatic transcription factors and their role in the birth, life and survival of the pancreatic beta cell. *Mol Cell Endocrinol* 2008; **294**: 1-9

2 **Scaglia L**, Cahill CJ, Finegood DT, Bonner-Weir S. Apoptosis participates in the remodeling of the endocrine pancreas in the neonatal rat. *Endocrinology* 1997; **138**: 1736-1741

3 **Liu L**, Guo J, Yuan L, Cheng M, Cao L, Shi H, Tong H, Wang N, De W. Alpha-fetoprotein is dynamically expressed in rat pancreas during development. *Dev Growth Differ* 2007; **49**: 669-681

4 **Hou LQ**, Wang YH, Liu LJ, Guo J, Teng LP, Cao LH, Shi H, Yuan L, De W. Expression and localization of mesothelin in developing rat pancreas. *Dev Growth Differ* 2008; **50**: 531-541

5 **Thyssen S**, Arany E, Hill DJ. Ontogeny of regeneration of beta-cells in the neonatal rat after treatment with strepto-

zotocin. *Endocrinology* 2006; **147**: 2346-2356
6 **Srinivasan K**, Viswanad B, Asrat L, Kaul CL, Ramarao P. Combination of high-fat diet-fed and low-dose streptozotocin-treated rat: a model for type 2 diabetes and pharmacological screening. *Pharmacol Res* 2005; **52**: 313-320
7 **Kodama S**, Toyonaga T, Kondo T, Matsumoto K, Tsuruzoe K, Kawashima J, Goto H, Kume K, Kume S, Sakakida M, Araki E. Enhanced expression of PDX-1 and Ngn3 by exendin-4 during beta cell regeneration in STZ-treated mice. *Biochem Biophys Res Commun* 2005; **327**: 1170-1178
8 **Nicholson JM**, Arany EJ, Hill DJ. Changes in islet microvasculature following streptozotocin-induced beta-cell loss and subsequent replacement in the neonatal rat. *Exp Biol Med* (Maywood) 2010; **235**: 189-198
9 **Zaret KS**, Grompe M. Generation and regeneration of cells of the liver and pancreas. *Science* 2008; **322**: 1490-1494
10 **Gittes GK**. Developmental biology of the pancreas: a comprehensive review. *Dev Biol* 2009; **326**: 4-35
11 **Ohlsson H**, Thor S, Edlund T. Novel insulin promoter- and enhancer-binding proteins that discriminate between pancreatic alpha- and beta-cells. *Mol Endocrinol* 1991; **5**: 897-904
12 **Guz Y**, Montminy MR, Stein R, Leonard J, Gamer LW, Wright CV, Teitelman G. Expression of murine STF-1, a putative insulin gene transcription factor, in beta cells of pancreas, duodenal epithelium and pancreatic exocrine and endocrine progenitors during ontogeny. *Development* 1995; **121**: 11-18
13 **Ahlgren JD**. Epidemiology and risk factors in pancreatic cancer. *Semin Oncol* 1996; **23**: 241-250
14 **Offield MF**, Jetton TL, Labosky PA, Ray M, Stein RW, Magnuson MA, Hogan BL, Wright CV. PDX-1 is required for pancreatic outgrowth and differentiation of the rostral duodenum. *Development* 1996; **122**: 983-995
15 **Wessells NK**. Differentiation of epidermis and epidermal derivatives. *N Engl J Med* 1967; **277**: 21-33
16 **Schwitzgebel VM**, Scheel DW, Conners JR, Kalamaras J, Lee JE, Anderson DJ, Sussel L, Johnson JD, German MS. Expression of neurogenin3 reveals an islet cell precursor population in the pancreas. *Development* 2000; **127**: 3533-3542
17 **Liew CG**, Shah NN, Briston SJ, Shepherd RM, Khoo CP, Dunne MJ, Moore HD, Cosgrove KE, Andrews PW. PAX4 enhances beta-cell differentiation of human embryonic stem cells. *PLoS One* 2008; **3**: e1783
18 **Hald J**, Sprinkel AE, Ray M, Serup P, Wright C, Madsen OD. Generation and characterization of Ptf1a antiserum and localization of Ptf1a in relation to Nkx6.1 and Pdx1 during the earliest stages of mouse pancreas development. *J Histochem Cytochem* 2008; **56**: 587-595
19 **Wang RN**, Bouwens L, Klöppel G. Beta-cell proliferation in normal and streptozotocin-treated newborn rats: site, dynamics and capacity. *Diabetologia* 1994; **37**: 1088-1096
20 **Gradwohl G**, Dierich A, LeMeur M, Guillemot F. neurogenin3 is required for the development of the four endocrine cell lineages of the pancreas. *Proc Natl Acad Sci USA* 2000; **97**: 1607-1611
21 **Apelqvist A**, Li H, Sommer L, Beatus P, Anderson DJ, Honjo T, Hrabe de Angelis M, Lendahl U, Edlund H. Notch signalling controls pancreatic cell differentiation. *Nature* 1999; **400**: 877-881
22 **Xu X**, D'Hoker J, Stangé G, Bonné S, De Leu N, Xiao X, Van de Casteele M, Mellitzer G, Ling Z, Pipeleers D, Bouwens L, Scharfmann R, Gradwohl G, Heimberg H. Beta cells can be generated from endogenous progenitors in injured adult mouse pancreas. *Cell* 2008; **132**: 197-207
23 **Lee CS**, De León DD, Kaestner KH, Stoffers DA. Regeneration of pancreatic islets after partial pancreatectomy in mice does not involve the reactivation of neurogenin-3. *Diabetes* 2006; **55**: 269-272
24 **Sista AK**, Knebel RJ, Tavri S, Johansson M, DeNardo DG, Boddington SE, Kishore SA, Ansari C, Reinhart V, Coakley FV, Coussens LM, Daldrup-Link HE. Optical imaging of the peri-tumoral inflammatory response in breast cancer. *J Transl Med* 2009; **7**: 94
25 **Heremans Y**, Van De Casteele M, in't Veld P, Gradwohl G, Serup P, Madsen O, Pipeleers D, Heimberg H. Recapitulation of embryonic neuroendocrine differentiation in adult human pancreatic duct cells expressing neurogenin 3. *J Cell Biol* 2002; **159**: 303-312
26 **Gasa R**, Mrejen C, Leachman N, Otten M, Barnes M, Wang J, Chakrabarti S, Mirmira R, German M. Proendocrine genes coordinate the pancreatic islet differentiation program in vitro. *Proc Natl Acad Sci USA* 2004; **101**: 13245-13250
27 **Mellitzer G**, Bonné S, Luco RF, Van De Casteele M, Lenne-Samuel N, Collombat P, Mansouri A, Lee J, Lan M, Pipeleers D, Nielsen FC, Ferrer J, Gradwohl G, Heimberg H. IA1 is NGN3-dependent and essential for differentiation of the endocrine pancreas. *EMBO J* 2006; **25**: 1344-1352
28 **Collombat P**, Mansouri A, Hecksher-Sorensen J, Serup P, Krull J, Gradwohl G, Gruss P. Opposing actions of Arx and Pax4 in endocrine pancreas development. *Genes Dev* 2003; **17**: 2591-2603
29 **Sosa-Pineda B**, Chowdhury K, Torres M, Oliver G, Gruss P. The Pax4 gene is essential for differentiation of insulin-producing beta cells in the mammalian pancreas. *Nature* 1997; **386**: 399-402
30 **Wang J**, Elghazi L, Parker SE, Kizilocak H, Asano M, Sussel L, Sosa-Pineda B. The concerted activities of Pax4 and Nkx2.2 are essential to initiate pancreatic beta-cell differentiation. *Dev Biol* 2004; **266**: 178-189
31 **Collombat P**, Hecksher-Sørensen J, Broccoli V, Krull J, Ponte I, Mundiger T, Smith J, Gruss P, Serup P, Mansouri A. The simultaneous loss of Arx and Pax4 genes promotes a somatostatin-producing cell fate specification at the expense of the alpha- and beta-cell lineages in the mouse endocrine pancreas. *Development* 2005; **132**: 2969-2980
32 **Collombat P**, Hecksher-Sørensen J, Serup P, Mansouri A. Specifying pancreatic endocrine cell fates. *Mech Dev* 2006; **123**: 501-512
33 **Smith SB**, Gasa R, Watada H, Wang J, Griffen SC, German MS. Neurogenin3 and hepatic nuclear factor 1 cooperate in activating pancreatic expression of Pax4. *J Biol Chem* 2003; **278**: 38254-38259
34 **Collombat P**, Xu X, Ravassard P, Sosa-Pineda B, Dussaud S, Billestrup N, Madsen OD, Serup P, Heimberg H, Mansouri A. The ectopic expression of Pax4 in the mouse pancreas converts progenitor cells into alpha and subsequently beta cells. *Cell* 2009; **138**: 449-462
35 **Schisler JC**, Fueger PT, Babu DA, Hohmeier HE, Tessem JS, Lu D, Becker TC, Naziruddin B, Levy M, Mirmira RG, Newgard CB. Stimulation of human and rat islet beta-cell proliferation with retention of function by the homeodomain transcription factor Nkx6.1. *Mol Cell Biol* 2008; **28**: 3465-3476
36 **Chung CH**, Hao E, Piran R, Keinan E, Levine F. Pancreatic β-cell neogenesis by direct conversion from mature α-cells. *Stem Cells* 2010; **28**: 1630-1638
37 **Thorel F**, Népote V, Avril I, Kohno K, Desgraz R, Chera S, Herrera PL. Conversion of adult pancreatic alpha-cells to beta-cells after extreme beta-cell loss. *Nature* 2010; **464**: 1149-1154

S- Editor Sun H **L- Editor** Ma JY **E- Editor** Ma WH

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/1007-9327office
wjg@wjgnet.com
doi:10.3748/wjg.v17.i23.2821

World J Gastroenterol 2011 June 21; 17(23): 2821-2828
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL ARTICLE

Pathological and MR-DWI study of the acute hepatic injury model after stem cell transplantation

Quan-Liang Shang, En-Hua Xiao, Qi-Chang Zhou, Jian-Guang Luo, Hai-Jun Wu

Quan-Liang Shang, En-Hua Xiao, Jian-Guang Luo, Hai-Jun Wu, Department of Radiology, Second Xiangya Hospital, Central South University, Changsha 410011, Hunan Province, China
Qi-Chang Zhou, Department of Ultrasound, Second Xiangya Hospital, Central South University, Changsha 410011, Hunan Province, China
Author contributions: Shang QL and Xiao EH designed the research; Shang QL, Xiao EH, Zhou QC, Luo JG and Wu HJ performed the research; Shang QL analyzed the data; Shang QL and Xiao EH wrote the paper.
Supported by The National Natural Science Foundation of China, No. 30070235, No. 30470508 and No. 30870695; The Natural Science Foundation of Hunan Province, No. 06JJ2008, 07JJ6040
Correspondence to: Dr. En-Hua Xiao, Department of Radiology, Second Xiangya Hospital, Central South University, Changsha 410011, Hunan Province, China. cjr.xiaoenhua@vip.163.com
Telephone: +86-731-85292116 Fax: +86-731-85533525
Received: December 30, 2010 Revised: March 17, 2011
Accepted: March 24, 2011
Published online: June 21, 2011

Abstract

AIM: To investigate apparent diffusion coefficient (ADC) values as an indication of reconditioning of acute hepatic injury (AHI) after allogeneic mononuclear bone marrow cell (MBMC) transplantation.

METHODS: Three groups were used in our study: a cell transplantation group (n = 21), transplantation control group (n = 21) and normal control group (n = 10). AHI model rabbits in the cell transplantation group were injected with 5 mL of MBMC suspension at multiple sites in the liver and the transplantation controls were injected with 5 mL D-Hanks solution. At the end of the 1st, 2nd and 4th wk, 7 rabbits were randomly selected from the cell transplantation group and transplantation control group for magnetic resonance diffusion-weighted imaging (MR-DWI) and measurement of the mean ADC values of injured livers. After MR-DWI examination, the rabbits were sacrificed and the livers subjected to pathological examination. Ten healthy rabbits from the normal control group were used for MR-DWI examination and measurement of the mean ADC value of normal liver.

RESULTS: At all time points, the liver pathological scores from the cell transplantation group were significantly lower than those in the transplantation control group (27.14 ± 1.46 *vs* 69.29 ± 6.16, 22.29 ± 2.29 *vs* 57.00 ± 1.53, 19.00 ± 2.31 *vs* 51.86 ± 6.04, P = 0.000). The mean ADC values of the cell transplantation group were significantly higher than the transplantation control group ((1.07 ± 0.07) × 10^{-3} mm^2/s *vs* (0.69 ± 0.05) × 10^{-3} mm^2/s, (1.41 ± 0.04) × 10^{-3} mm^2/s *vs* (0.84 ± 0.06) × 10^{-3} mm^2/s, (1.68 ± 0.04) × 10^{-3} mm^2/s *vs* (0.86 ± 0.04) × 10^{-3} mm^2/s, P = 0.000). The pathological scores of the cell transplantation group and transplantation control group gradually decreased. However, their mean ADC values gradually increased to near that of the normal control. At the end of the 1st wk, the mean ADC values of the cell transplantation group and transplantation control group were significantly lower than those of the normal control group [(1.07 ± 0.07) × 10^{-3} mm^2/s *vs* (1.76 ± 0.03) × 10^{-3} mm^2/s, (0.69 ± 0.05) × 10^{-3} mm^2/s *vs* (1.76 ± 0.03) × 10^{-3} mm^2/s, P = 0.000]. At any 2 time points, the pathological scores and the mean ADC values of the cell transplantation group were significantly different (P = 0.000). At the end of the 1st wk, the pathological scores and the mean ADC values of the transplantation control group were significantly different from those at the end of the 2nd and 4th wk (P = 0.000). However, there was no significant difference between the 2nd and 4th wk (P = 0.073 and 0.473, respectively). The coefficient of correlation between the pathological score and the mean ADC value in the cell transplantation group was -0.883 (P = 0.000) and -0.762 (P = 0.000) in the transplantation control group.

CONCLUSION: Tracking the longitudinally dynamic

change in the mean ADC value of the AHI liver may reflect hepatic injury reconditioning after allogeneic MBMC transplantation.

Key words: Stem cells; Transplantation; Hepatic injury; Magnetic resonance imaging; Diffusion weighted imaging

Peer reviewer: Christopher Christophi, Professor and Head of The University of Melbourne Department of Surgery, Austin Hospital, Melbourne, 145 Studley Road, Victoria 3084, Australia

Shang QL, Xiao EH, Zhou QC, Luo JG, Wu HJ. Pathological and MR-DWI study of the acute hepatic injury model after stem cell transplantation. *World J Gastroenterol* 2011; 17(23): 2821-2828 Available from: URL: http://www.wjgnet.com/1007-9327/full/v17/i23/2821.htm DOI: http://dx.doi.org/10.3748/wjg.v17.i23.2821

INTRODUCTION

Mononuclear bone marrow cells (MBMCs) are adult stem cells that have multi-potential differentiation capabilities and low immunogenicity. Direct allogeneic MBMC transplantation can repair various organ injuries such as hepatic injury without obvious immune rejection[1]. There are many studies evaluating the therapeutic effect of MBMC transplantation therapy for hepatic injury. However, few studies have been undertaken to determine the feasibility of magnetic resonance diffusion-weighted imaging (MR-DWI) to evaluate the therapeutic effect of MBMC transplantation therapy on models of acute hepatic injury (AHI).

MR-DWI is an atraumatic and functional imaging technique that is very sensitive to molecular diffusion from the random and microscopic translational motion of molecules known as Brownian motion[2]. MR-DWI can image the difference in microscopic diffusion movements of water molecules in various tissues. The most noticeable merit of MR-DWI is providing an apparent diffusion coefficient (ADC) value that can distinguish the microscopic diffusion movement of water molecules in different tissues *in vivo* by assigning numerical values[3-7]. When a tissue has a pathological change, the microscopic diffusion movement of water molecules changes and the mean ADC value should also change. It has been generally accepted that MR-DWI is valuable in qualitatively and quantitatively diagnosing cerebral ischemia in the hyper-inchoate period[8]. During recent years, many studies of hepatic pathological changes using MR-DWI have been reported[3-7]. These showed that MR-DWI of the liver seems promising for the characterization of many diseases (especially focal liver lesions) by calculating ADC values.

Similarly, after MBMC transplantation therapy, there should be a dynamic change in the microscopic diffusion movement of water molecules in hepatic tissue during the repair process of AHI. Thus, the aim of our study was to evaluate the contribution of the mean ADC value in reflecting the repair process of AHI after MBMC transplantation therapy by comparison with the pathological change. The pathological mechanisms behind the dynamic change of the mean ADC value from injured hepatic tissue will be discussed in further detail.

MATERIALS AND METHODS

Material and instruments

Experiments were performed using 57 healthy, male New Zealand White rabbits weighing ~2.5 kg with an average age of ~2 mo. All animal work was conducted in accordance with the guidelines provided by the Institutional Animal Control and Utilization Committee. Five rabbits were randomly selected and used to isolate MBMCs. Dulbecco's modified Eagle's medium and fetal bovine serum were purchased from Gibco (New York, USA). Mononuclear cell separation medium was purchased from Tianjin Haoyang Company (Tianjin, China). D-Hanks solution, an electronic balance, 3% pentobarbital sodium, sterile surgical instruments, an optical microscope, cell separation tools and 2% trypan blue were supplied by the Second Xiangya Hospital. D-galactosamine (D-GaIN) was purchased from Jiangsu Nantong Tonglu Co. Ltd. (Nantong, China). Imaging was performed using a 1.5-Tesla Signa Twinspeed MR scanner (General Electron Medical Systems, USA) with a small diameter cylindrical brain radiofrequency coil.

Study groups and the establishment of AHI models

Acute hepatic injury was induced by D-galactosamine (D-GalN). D-GalN was dissolved in sterile 0.9% NaCl at a concentration of 10 g/100 mL (w/v). Forty-two rabbits were randomly selected to establish the AHI models. According to the weight of each rabbit, D-GalN solution was injected into the upper abdomen at a dosage of 1.0 g/kg. This amount was determined by preliminary experiments. The rabbits' weight, drug dosage and detailed administration times were recorded. The 42AHI rabbits were randomly and equally divided into 2 groups: a cell transplantation group and a transplantation control group. Liver function assays were performed 24 h after drug administration and pathological examinations of liver sections taken during cell transplantation were performed to verify the establishment of the AHI model. The remaining 10 healthy rabbits were assigned to the normal control group, and only MR-DWI examination was performed for measurement of the normal liver mean ADC value.

Isolation and transplantation of MBMCs

After being sacrificed by air injection into the ear vein, the bodies of 5 healthy male rabbits were sterilized by incubation in 75% ethanol. Their limb bones were then isolated and bone marrow was repetitively flushed using D-Hanks solution containing heparin. Soft tissue clumps were removed with 100 pore filters. Recovered cell suspensions were aliquoted into multiple centrifuge tubes. MBMCs were then obtained through density gradient centrifugation. Cell number was counted and the viabil-

Table 1 Criteria for the acute injured liver pathological score

Score	Cellular necrosis and liver hyperplasia	Inflammatory cell infiltration in the area of the header and lobule	Injury of vascular endothelium and thrombus
0	Without	Without	Without
1	Spotty liver cell degeneration, necrosis without change of hepatic sinusoid and lobule shape	Infiltration area < 1/3 lobule or low inflammatory cell infiltration in the area of the header	Hyperemia of hepatic sinusoid or thrombus
2	Scattering severe liver cell degeneration, necrosis of whole lobule or unobvious liver cell hyperplasia	Infiltration area: 1/3-2/3 of lobule or comparatively wide-bound inflammatory cell infiltration	Injury of vascular endothelium or inflammatory cell infiltration under vascular endothelium
3	Large sheet liver cell degeneration and necrosis involving multiple lobules	Infiltration area: > 2/3 of lobule or inflammatory cells surround header	Extensive injury of vascular endothelium and thrombus

Obvious liver cell hyperplasia score: -2; Not obvious liver cell hyperplasia score: +2.

ity examined using trypan blue. The percentage of live cells had to be > 95%. The MBMCs were resuspended in D-Hanks solution at a density of 4×10^6/mL. Five milliliters of cell suspension was aliquoted into 10 mL glass syringes and kept in an incubator at 37℃ with 5% CO_2 for a short time before transplantation.

MBMC transplantations were performed between 24 and 48 h after establishing the AHI models. Each AHI rabbit from the cell transplantation group was properly anesthetized and immobilized on an operating table. The skin of the upper abdomen was prepared and sterilized, then the liver was exposed with a 2 cm cut beneath the xiphoid process. After slowly injecting 5 mL of the MBMC suspension at multiple sites in the liver, the needle was withdrawn and the wound sutured to stop bleeding. The wound was treated with penicillin and covered with a sterile dressing. After transplantation, each model rabbit was given intramuscular injections of penicillin in the buttocks over a 3-d period with normal feeding. In addition to 5 mL of D-Hanks solution substituted for the MBMC suspension, manipulations of the rabbits in the transplantation control group were the same as those in the cell transplantation group.

Pathological management of the liver

At the end of the 1st, 2nd and 4th wk after transplantation, 7 rabbits were randomly selected for MR-DWI examination of the liver at each time point in the cell transplantation group and transplantation control group. Then they were sacrificed for histological examination. Rabbit liver tissue blocks were fixed in 4% paraformaldehyde and were processed for paraffin embedding. Microsections were prepared and stained with hematoxylin and eosin, then examined under an optical microscope. The *criteria* for the liver pathological scores were established according to the characteristic of this study, histology activity index and previous studies[9] (Table 1). Six pathological microsections from each model rabbit were randomly selected to count the pathological scores by 2 experienced pathologists using a double-blind method. The sum of the pathological scores from 10 random high power fields (400 ×) from each microsection was regarded as the pathological score of that microsection.

MR-DWI protocol

Rabbits in the normal control group were subjected only to liver MR-DWI examination. At the end of the 1st, 2nd and 4th wk after transplantation, 7 rabbits from the cell transplantation group and from the transplantation control group were randomly selected at each time point for liver MR-DWI examination.

Rabbits were anesthetized and immobilized, then MR-DWI (axial) was carried out with a 1.5-Tesla Signa Twinspeed MR scanner equipped with a small diameter cylindrical brain radiofrequency coil. The scanning parameters of the MR-DWI included a spin echo echoplanar imaging series, b value 0 and 400 s/mm^2, repetition time 6000 ms, echo time 45 ms, all diffusion directions, frequency coding direction R/L, field of view 20 cm × 15 cm, number of excitations 8, thickness layer 3 mm, 0.5 mm space, and matrix 128 × 128.

ADC values were obtained using Function Software on a GE workstation. Three different regions of interest (ROIs) (~50 mm^2 each) were chosen in the liver parenchyma in every clear axial slice of each liver and their ADC values were measured. The mean value of the above was considered to be the ADC value of each liver.

Statistical analysis

Based on the mean ADC values from ROIs and the pathological scores, the differences between the various groups and time points, and the correlation between the mean ADC value and pathological score were assessed. The statistical significance was calculated by an independent sample *t*-test, analysis of variance and linear correlation using SPSS 11.0 software. P values < 0.05 were considered to indicate statistical significance.

RESULTS

Mean ADC values from each group and analysis

The mean ADC value of the normal control group was $(1.76 \pm 0.03) \times 10^{-3}$ mm^2/s. At all time points after transplantation, the mean liver ADC values from the cell transplantation group were significantly higher than those of the transplantation control group ($P = 0.000$) (Table 2, Figure 1).

The mean liver ADC values from the cell transplantation group and transplantation control group gradually increased to near those of the normal control group over time. At the end of the 1st wk after transplantation,

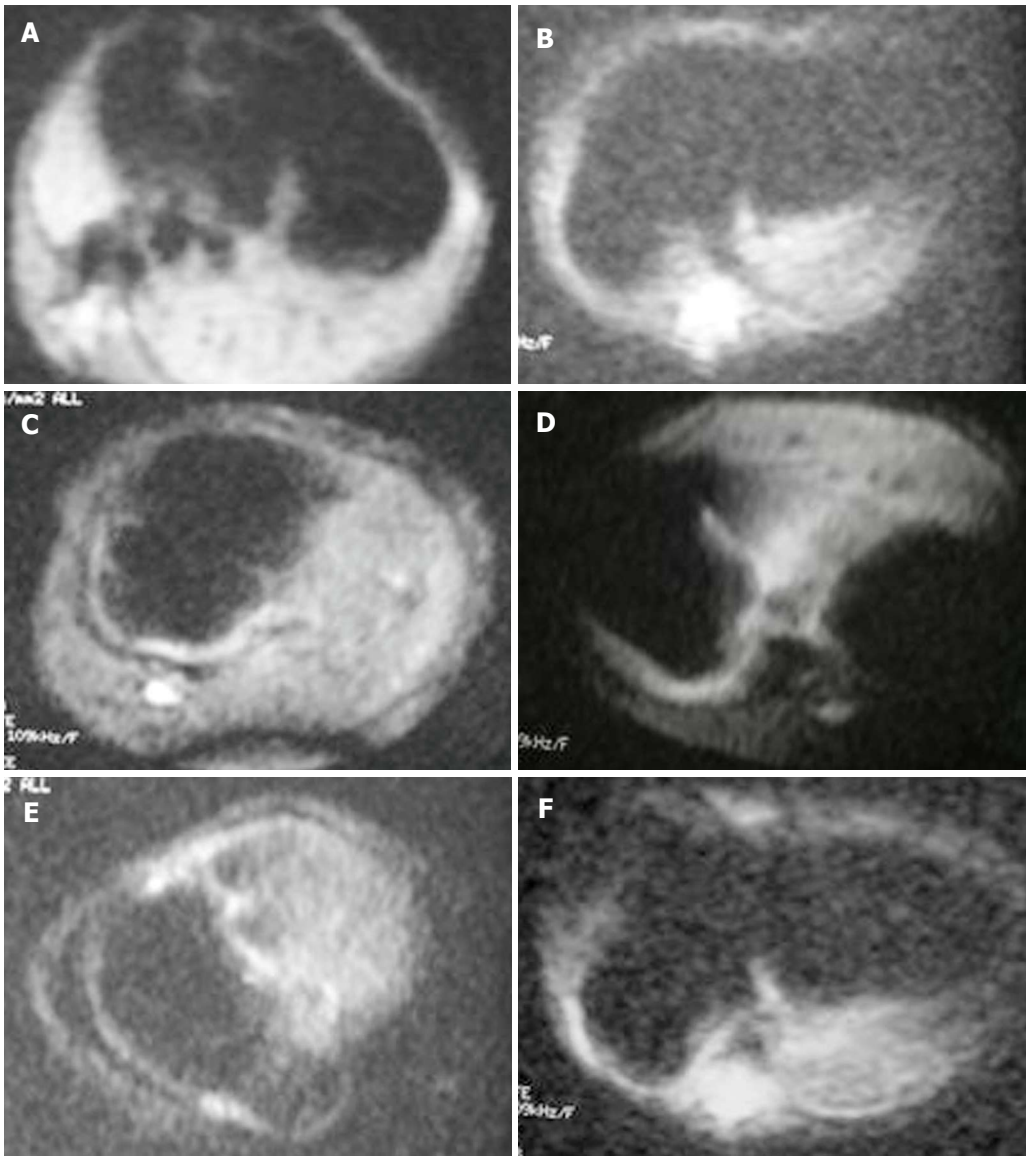


Figure 1 Diffusion-weighted imaging of hepatic injury at different time point. A: At the end of the 1st wk after transplantation, the mean apparent diffusion coefficient value of the acute hepatic injury liver in the transplantation control was (0.69 ± 0.05) × 10^{-3} mm^2/s; B: At the end of the 1st wk after transplantation, the mean apparent diffusion coefficient value of the acute hepatic injury liver in the cell transplantation group was (1.07 ± 0.07) × 10^{-3} mm^2/s; C: At the end of the 2nd wk after transplantation, the mean apparent diffusion coefficient value of the acute hepatic injury liver in the transplantation control group was (0.84 ± 0.06) × 10^{-3} mm^2/s; D: At the end of the 2nd wk after transplantation, the mean apparent diffusion coefficient value of the acute hepatic injury liver in the cell transplantation group was (1.41 ± 0.04) × 10^{-3} mm^2/s; E: At the end of the 4th wk after transplantation, the mean apparent diffusion coefficient value of the acute hepatic injury liver in the transplantation control group was (0.86 ± 0.04) × 10^{-3} mm^2/s; F: At the end of the 4th wk after transplantation, the mean apparent diffusion coefficient value of the acute hepatic injury liver in the cell transplantation group was (1.68 ± 0.04) × 10^{-3} mm^2/s.

Table 2 Mean apparent diffusion coefficient values from the cell transplantation group and transplantation control group at each time point (mean ± SD) × 10^{-3} mm^2/s

Group	1st wk	2nd wk	4th wk
Cell transplantation group	1.07 ± 0.07	1.41 ± 0.04	1.68 ± 0.04
Transplantation control	0.69 ± 0.05	0.84 ± 0.06	0.86 ± 0.04
t	11.452	21.735	37.876
P	0.000	0.000	0.000

they were both significantly lower than those of the normal control group (t = 23.612, P = 0.000; t = 52.416, P = 0.000).

Between any 2 time points, the differences in the mean liver ADC values from the cell transplantation group were statistically significant (P = 0.000). At the end of the 1st wk after transplantation, the mean ADC values from the transplantation control group were significantly lower than those at the end of the 2nd and 4th wk (P = 0.000). However, there was no significant difference between the end of the 2nd and 4th wk (P = 0.473).

Pathological scores and analysis

The livers' pathological scores from the cell transplantation group and transplantation control group gradually decreased over time (Table 3, Figure 2). At all time

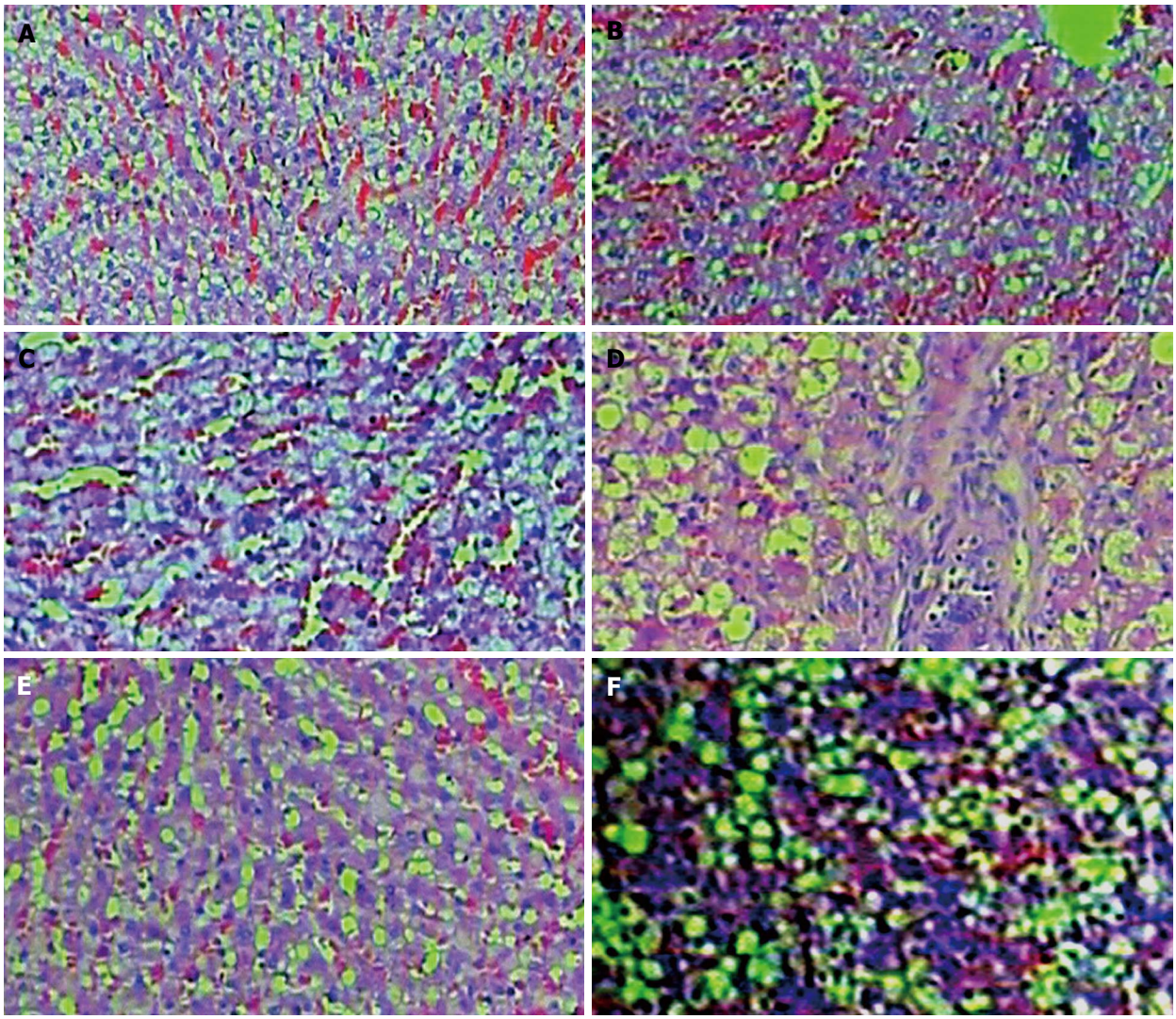


Figure 2 Pathological change of hepatic injury at different time point (Hematoxylin and eosin × 100). A: At the end of the 1st wk after transplantation, the pathological sections of the cell transplantation group showed extensive hepatic cell degenerations, few binucleate cells, abnormal hepatic cords and local inflammatory cell infiltration; B: At the end of the 1st wk after transplantation, the pathological sections of the transplantation control group showed obvious and extensive edema of hepatic cells, spotty hepatolysis, abnormal hepatic cords, local inflammatory cell infiltration and injured vascular endothelium; C: At the end of the 2nd wk after transplantation, the pathological sections of the cell transplantation group showed extensive edema of hepatic cells, abnormal hepatic cords, imperceptible inflammatory cell infiltration and few scattered binucleate cells. (Hematoxylin and eosin ×100); D: At the end of the 2nd wk after transplantation, the pathological sections of the transplantation control group showed obvious and extensive edema of hepatic cells, an increased number of binucleate cells and obvious inflammatory cell infiltration around the header; E: At the end of the 4th wk after transplantation, the pathological sections of the cell transplantation group showed scattering edema of the hepatic cells, hepatic cords, sinusoid near normal and hyperemia in local sinusoid; F: At the end of the 4th wk after transplantation, the pathological sections of the transplantation control group showed scattering and sheet edema of the hepatic cells, hyperemia in local sinusoids and an increased number of binucleate cells.

Table 3 Pathological scores from the cell transplantation group and transplantation control at each time point (mean ± SD)

Group	1st wk	2nd wk	4th wk
Cell transplantation group	27.14 ± 1.46	22.29 ± 2.29	19.00 ± 2.31
Transplantation control	69.29 ± 6.16	57.00 ± 1.53	51.86 ± 6.04
t	-17.619	-33.379	-13.444
P	0.000	0.000	0.000

points after transplantation, the pathological scores of the livers from the cell transplantation group were significantly lower than those from the transplantation control group ($P = 0.000$) (Table 3, Figure 2).

Between any 2 time points, the differences in pathological scores from the cell transplantation group were statistically significant ($P = 0.000$). At the end of the 1st wk after transplantation, the pathological scores from the transplantation control group were significantly higher than those at the end of the 2nd and 4th wk after transplantation ($P = 0.000$). However, there was no significant difference between the end of 2nd and 4th wk ($P = 0.073$).

Correlation analysis

When the *b* value was 400 s/mm^2, there was a significant negative correlation between the pathological score and

mean ADC value in the cell transplantation group and transplantation control group (r = -0.883, P = 0.000; r = -0.762, P = 0.000).

DISCUSSION

The efficacy of allogeneic MBMC transplantation therapy for AHI has already been proven[10,11]. Our results showed that the pathological changes such as cellular edema and inflammatory cell infiltration in the hepatic tissue of the transplantation control group were more obvious than those in the cell transplantation group at any similar time point. The pathological scores from the cell transplantation group were significantly lower than those of the transplantation control group at all time points. These results imply that allogeneic MBMC transplantation into an acute injury of the liver could improve hepatic injury reconditioning. This trend is similar to that found in other studies[10,11].

Because of the high resolution and sensitivity, recent studies have focused on *in vivo* real-time tracking and detecting the fate of transplanted stem cells with MRI[11,12]. However, there are few studies using MR-DWI to evaluate the therapeutic efficacy of MBMC transplantation for acute hepatic injuries.

When the b value is more than 300 s/mm^2, physiological factors such as perfusion have little influence on the mean ADC value of hepatic tissue[13-15]. Therefore, in our study we performed the MR-DWI examination of rabbit liver with 400 s/mm^2 for the b value, as determined by preliminary experiments.

Our study showed that at the end of the 1st wk after transplantation, the mean liver ADC values from the cell transplantation group and transplantation control group were much lower than the normal control group and both gradually increased to near the normal control over time. The correlation between the pathological scores and mean ADC values in the cell transplantation group or transplantation control group was significantly negative. This suggests the possibility of determining the reconditioning of an injured liver after allogeneic MBMC transplantation by tracking the longitudinally dynamic change of the mean ADC value from the AHI liver tissue.

By observing the pathological sections from the cell transplantation group and transplantation control group, we found the main pathological change was varying degrees of cytotoxic edema of the hepatocytes in all pathological sections. Therefore, we hypothesized that the pathological mechanism behind the change of the mean ADC value in hepatic tissue was mainly connected with cytotoxic edema of the hepatocytes in our study. Because the D-GalN injected into the peritoneal cavity was absorbed to injure the hepatic tissue causing Na-K pumps to be dysfunctional, the concentration of intracellular electrolytes increases. This causes water molecules inside cells to increase significantly, while extracellular water molecules decrease significantly. The cytotoxic edema of hepatocytes causes a decrease in gaps between hepatocytes, so that the space where extracellular water molecules can randomly move decreases. Therefore, the mean ADC value of hepatic tissue in the AHI model started to decrease[13-18] and was lower than that of the normal hepatic tissue.

In our study, the mean ADC values from the cell transplantation group and transplantation control group gradually increased over time and their pathological scores gradually decreased. The pathological sections showed their hepatic tissue injury gradually healed. At any similar time point, the mean ADC values from the cell transplantation group were significantly higher than those from the transplantation control group, and the pathological scores of the cell transplantation group were significantly better than those of the transplantation control group. According to previous studies[19-22] and our pathological sections, we hypothesized that there were 2 main hepatic reconditioning mechanisms causing these results. Firstly, the transplanted MBMCs promoted hepatic tissue reconditioning. Secondly, the hepatic injury caused the autologous hepatic reconditioning mechanism to activate. Because of the hepatic reconditioning, the hepatic cells' cytotoxic edema gradually decreased, and the number of extracellular water molecules and the space where extracellular water molecules could move randomly both gradually recovered. Thus, the macroscopic mean ADC values from the cell transplantation group and transplantation control group gradually increased[23,24]. Because of the 2 mentioned hepatic reconditioning mechanisms, the reconditioning in the cell transplantation group was faster than the transplantation control group without transplanted MBMCs. Therefore, at any time point, the mean ADC values from the cell transplantation group were significantly higher than those from the transplantation control group.

Of course, if there were extensive hepatolysis and necrosis, the number of extracellular water molecules and the space where extracellular water molecules randomly move in the AHI model liver would greatly increase. This would cause the mean ADC value of the hepatic tissue in the AHI model to exceed that of the normal hepatic tissue[25,26]. However, we did not observe this phenomenon in our study. Through observing pathological sections from all time points, we found sporadic scattering but no extensive hepatolysis and necrosis. The dominant pathological change in the hepatic tissue was varying degrees of hepatocellular cytotoxic edema. Perhaps the reason was that the dosage of D-GalN used was not high enough to cause extensive hepatolysis in our study. Considering the influence of hepatolysis on the ADC value of hepatic tissue, we chose many ROIs in all clear images from each AHI model's liver to measure ADC values. The mean ADC value was calculated from all ROIs and ADC values from each AHI liver. Thus, we could increase the accuracy of the ADC value of each AHI model's liver to the utmost extent.

In conclusion, when the b value is equal to 400 s/mm^2, tracking the longitudinally dynamic change of the mean ADC value of the AHI liver could determine injured he-

patic tissue reconditioning after allogeneic MBMC transplantation.

COMMENTS

Background

Many studies have proven that allogeneic mononuclear bone marrow cell (MBMC) transplantation therapy is an effective way to repair liver injury. The most notable merit of magnetic resonance diffusion-weighted imaging (MR-DWI) is the provision of an apparent diffusion coefficient (ADC) value that can distinguish the microscopic diffusion movement of water molecules in various tissues in vivo by assigning a numerical value. After MBMC transplantation therapy, there should be a dynamic change in the microscopic diffusion movement of water molecules in the hepatic tissue during the repair process of acute hepatic injury. Thus, the authors attempted to use MR-DWI to evaluate the contribution of the acute injured liver's mean ADC value in reflecting the repair process after MBMC transplantation therapy.

Research frontiers

Allogeneic MBMC transplantation therapy can accelerate the repair of liver injury. Many studies are focusing on the mechanisms behind the transplanted MBMCs repair of the liver injury. Recently, some researchers have been attempting to track and detect the fate of transplanted MBMCs with magnetic resonance imaging.

Innovations and breakthroughs

It has been proven that allogeneic MBMC transplantation can repair liver injury. There are many studies on evaluating the therapeutic effect of MBMC transplantation therapy for hepatic injury, but few studies have determined the feasibility of MR-DWI to evaluate the therapeutic effect of MBMC transplantation therapy on models of acute hepatic injury.

Applications

The results of this study suggest that the dynamic change of the mean ADC value of the acute hepatic injury model's liver can determine the injured liver's reconditioning after allogeneic MBMCs transplantation.

Terminology

MR-DWI is a functional imaging technique that can image the difference in microscopic diffusion movements of water molecules in various tissues. This technique only requires the examinee to lie still on the examining table while the examination is carried out. It is atraumatic and very safe.

Peer review

This article investigates the role of MR diffusion-weighted images for detection of acute liver injury following allogeneic bone marrow cell transplantation in a rabbit model. The conclusion reached confirmed the value and usefulness of this modality. The method is non-traumatic and easily performed. The methodology and design of the study was sound. This study is of significance in the field of bone marrow transplantation.

REFERENCES

1 **Akihama S**, Sato K, Satoh S, Tsuchiya N, Kato T, Komatsuda A, Hirokawa M, Sawada K, Nanjo H, Habuchi T. Bone marrow-derived cells mobilized by granulocyte-colony stimulating factor facilitate vascular regeneration in mouse kidney after ischemia/reperfusion injury. *Tohoku J Exp Med* 2007; **213**: 341-349

2 **Koike N**, Cho A, Nasu K, Seto K, Nagaya S, Ohshima Y, Ohkohchi N. Role of diffusion-weighted magnetic resonance imaging in the differential diagnosis of focal hepatic lesions. *World J Gastroenterol* 2009; **15**: 5805-5812

3 **Kamel IR**, Liapi E, Reyes DK, Zahurak M, Bluemke DA, Geschwind JF. Unresectable hepatocellular carcinoma: serial early vascular and cellular changes after transarterial chemoembolization as detected with MR imaging. *Radiology* 2009; **250**: 466-473

4 **Schaudt A**, Kriener S, Schwarz W, Wullstein C, Zangos S, Vogl T, Mehrabi A, Fonouni H, Bechstein WO, Golling M. Role of transarterial chemoembolization for hepatocellular carcinoma before liver transplantation with special consideration of tumor necrosis. *Clin Transplant* 2009; **23** Suppl 21: 61-67

5 **Kamel IR**, Bluemke DA, Eng J, Liapi E, Messersmith W, Reyes DK, Geschwind JF. The role of functional MR imaging in the assessment of tumor response after chemoembolization in patients with hepatocellular carcinoma. *J Vasc Interv Radiol* 2006; **17**: 505-512

6 **Chen CY**, Li CW, Kuo YT, Jaw TS, Wu DK, Jao JC, Hsu JS, Liu GC. Early response of hepatocellular carcinoma to transcatheter arterial chemoembolization: choline levels and MR diffusion constants--initial experience. *Radiology* 2006; **239**: 448-456

7 **Muhi A**, Ichikawa T, Motosugi U, Sano K, Matsuda M, Kitamura T, Nakazawa T, Araki T. High-b-value diffusion-weighted MR imaging of hepatocellular lesions: estimation of grade of malignancy of hepatocellular carcinoma. *J Magn Reson Imaging* 2009; **30**: 1005-1011

8 **Kloska SP**, Wintermark M, Engelhorn T, Fiebach JB. Acute stroke magnetic resonance imaging: current status and future perspective. *Neuroradiology* 2010; **52**: 189-201

9 **Scheuer PJ**, Standish RA, Dhillon AP. Scoring of chronic hepatitis. *Clin Liver Dis* 2002; **6**: 335-347, v-vi

10 **Jin SZ**, Meng XW, Han MZ, Sun X, Sun LY, Liu BR. Stromal cell derived factor-1 enhances bone marrow mononuclear cell migration in mice with acute liver failure. *World J Gastroenterol* 2009; **15**: 2657-2664

11 **Ju S**, Teng GJ, Lu H, Zhang Y, Zhang A, Chen F, Ni Y. In vivo MR tracking of mesenchymal stem cells in rat liver after intrasplenic transplantation. *Radiology* 2007; **245**: 206-215

12 **Modo M**, Cash D, Mellodew K, Williams SC, Fraser SE, Meade TJ, Price J, Hodges H. Tracking transplanted stem cell migration using bifunctional, contrast agent-enhanced, magnetic resonance imaging. *Neuroimage* 2002; **17**: 803-811

13 **Erdem G**, Erdem T, Muammer H, Mutlu DY, Firat AK, Sahin I, Alkan A. Diffusion-weighted images differentiate benign from malignant thyroid nodules. *J Magn Reson Imaging* 2010; **31**: 94-100

14 **Dale BM**, Braithwaite AC, Boll DT, Merkle EM. Field strength and diffusion encoding technique affect the apparent diffusion coefficient measurements in diffusion-weighted imaging of the abdomen. *Invest Radiol* 2010; **45**: 104-108

15 **Eiber M**, Beer AJ, Holzapfel K, Tauber R, Ganter C, Weirich G, Krause BJ, Rummeny EJ, Gaa J. Preliminary results for characterization of pelvic lymph nodes in patients with prostate cancer by diffusion-weighted MR-imaging. *Invest Radiol* 2010; **45**: 15-23

16 **Choi JS**, Kim MJ, Choi JY, Park MS, Lim JS, Kim KW. Diffusion-weighted MR imaging of liver on 3.0-Tesla system: effect of intravenous administration of gadoxetic acid disodium. *Eur Radiol* 2010; **20**: 1052-1060

17 **Eccles CL**, Haider EA, Haider MA, Fung S, Lockwood G, Dawson LA. Change in diffusion weighted MRI during liver cancer radiotherapy: preliminary observations. *Acta Oncol* 2009; **48**: 1034-1043

18 **Sun X**, Wang H, Chen F, De Keyzer F, Yu J, Jiang Y, Feng Y, Li J, Marchal G, Ni Y. Diffusion-weighted MRI of hepatic tumor in rats: comparison between in vivo and postmortem imaging acquisitions. *J Magn Reson Imaging* 2009; **29**: 621-628

19 **Higashiyama R**, Inagaki Y, Hong YY, Kushida M, Nakao S, Niioka M, Watanabe T, Okano H, Matsuzaki Y, Shiota G, Okazaki I. Bone marrow-derived cells express matrix metalloproteinases and contribute to regression of liver fibrosis in mice. *Hepatology* 2007; **45**: 213-222

20 **Shinya S**, Sasaki T, Nakagawa Y, Guiquing Z, Yamamoto F, Yamashita Y. The efficacy of diffusion-weighted imaging for the detection of colorectal cancer. *Hepatogastroenterology* 2009; **56**: 128-132

21 **Kandpal H**, Sharma R, Madhusudhan KS, Kapoor KS. Respiratory-triggered versus breath-hold diffusion-weighted MRI of liver lesions: comparison of image quality and apparent diffusion coefficient values. *AJR Am J Roentgenol*

2009; **192**: 915-922
22 **Yu JS**, Kim JH, Chung JJ, Kim KW. Added value of diffusion-weighted imaging in the MRI assessment of perilesional tumor recurrence after chemoembolization of hepatocellular carcinomas. *J Magn Reson Imaging* 2009; **30**: 153-160
23 **Sandrasegaran K**, Akisik FM, Lin C, Tahir B, Rajan J, Saxena R, Aisen AM. Value of diffusion-weighted MRI for assessing liver fibrosis and cirrhosis. *AJR Am J Roentgenol* 2009; **193**: 1556-1560
24 **Maniam S**, Szklaruk J. Magnetic resonance imaging: Review of imaging techniques and overview of liver imaging. *World J Radiol* 2010; **2**: 309-322
25 **Wu X**, Wang H, Chen F, Jin L, Li J, Feng Y, DeKeyzer F, Yu J, Marchal G, Ni Y. Rat model of reperfused partial liver infarction: characterization with multiparametric magnetic resonance imaging, microangiography, and histomorphology. *Acta Radiol* 2009; **50**: 276-287
26 **Sandrasegaran K**, Akisik FM, Lin C, Tahir B, Rajan J, Aisen AM. The value of diffusion-weighted imaging in characterizing focal liver masses. *Acad Radiol* 2009; **16**: 1208-1214

S- Editor Sun H **L- Editor** Cant MR **E- Editor** Ma WH

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/1007-9327office
wjg@wjgnet.com
doi:10.3748/wjg.v17.i23.2829

World J Gastroenterol 2011 June 21; 17(23): 2829-2837
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

BRIEF ARTICLE

NOD2 and *ATG16L1* polymorphisms affect monocyte responses in Crohn's disease

Dylan M Glubb, Richard B Gearry, Murray L Barclay, Rebecca L Roberts, John Pearson, Jacqui I Keenan, Judy McKenzie, Robert W Bentley

Dylan M Glubb, Department of Medicine, University of Chicago, IL 60637-1470, United States
Richard B Gearry, Murray L Barclay, Rebecca L Roberts, Department of Medicine, University of Otago, Christchurch, 8140, New Zealand
Richard B Gearry, Murray L Barclay, Department of Gastroenterology, Christchurch Hospital, Christchurch, 8140, New Zealand
Rebecca L Roberts, Department of Biochemistry, University of Otago, Dunedin, 9054, New Zealand
John Pearson, Department of Pathology, University of Otago, Christchurch, 8140, New Zealand
Jacqui I Keenan, Department of Surgery, University of Otago, Christchurch, 8140, New Zealand
Judy McKenzie, Department of Haematology, University of Otago, Christchurch, 8140, New Zealand
Robert W Bentley, Department of Paediatrics, University of Otago, Christchurch, 8140, New Zealand
Author contributions: Glubb DM, McKenzie J and Bentley RW performed the research; Glubb DM, Roberts RL, Bentley RW and Gearry RB designed the research; Pearson J, Glubb DM, Roberts RL and Bentley RW analyzed the data; Barclay ML and Gearry RB provided patient samples; all authors contributed to the writing of the manuscript.
Supported by Broad Medical Research Program of The Broad Foundation, Inflammatory Bowel Disease Grant IBD-0236. RLR is the recipient of a Sir Charles Hercus Health Research Fellowship from the Health Research Council of New Zealand
Correspondence to: Robert W Bentley, PhD, Department of Paediatrics, University of Otago, PO Box 4345, Christchurch 8140, New Zealand. robert.bentley@otago.ac.nz
Telephone: +64-3-3641558 Fax: +64-3-3640009
Received: July 1, 2010 Revised: September 30, 2010
Accepted: October 7, 2010
Published online: June 21, 2011

Abstract

AIM: To assess whether polymorphisms in *NOD2* and *ATG16L1* affect cytokine responses and mycobacterium avium subspecies paratuberculosis (MAP) survival in monocytes from Crohn's disease (CD) patients.

METHODS: Monocytes were isolated from peripheral blood of CD patients of known genotype for common single nucleotide polymorphisms of *NOD2* and *ATG16L1*. Monocytes were challenged with MAP and bacterial persistence assessed at subsequent time-points. Cytokine responses were assayed using a Milliplex multi-analyte profiling assay for 13 cytokines.

RESULTS: Monocytes heterozygous for a *NOD2* polymorphism (R702W, P268S, or 1007fs) were more permissive for growth of MAP (P = 0.045) than those without. There was no effect of *NOD2* genotype on subsequent cytokine expression. The T300A polymorphism of *ATG16L1* did not affect growth of MAP in our model (P = 0.175), but did increase expression of cytokines interleukin (IL)-10 (P = 0.047) and IL-6 (P = 0.019).

CONCLUSION: CD-associated polymorphisms affected the elimination of MAP from *ex vivo* monocytes (*NOD2*), or expression of certain cytokines (*ATG16L1*), implying independent but contributory roles in the pathogenesis of CD.

Key words: Inflammatory bowel disease; Mycobacterium avium subspecies paratuberculosis; Cytokine; CARD15; Autophagy

Peer reviewers: Shashi Bala, PhD, Post Doctoral Associate, Department of Medicine, LRB 270L, 364 Plantation street, UMass Medical School, Worcester, MA 01605, United States; Giuliana Decorti, MD, PhD, Department of Life Sciences, University of Trieste, Via L. Giorgieri n° 7, Trieste 34127, Italy

Glubb DM, Gearry RB, Barclay ML, Roberts RL, Pearson J, Keenan JI, McKenzie J, Bentley RW. *NOD2* and *ATG16L1* polymorphisms affect monocyte responses in Crohn's disease. *World J Gastroenterol* 2011; 17(23): 2829-2837 Available from: URL:

http://www.wjgnet.com/1007-9327/full/v17/i23/2829.htm DOI: http://dx.doi.org/10.3748/wjg.v17.i23.2829

INTRODUCTION

Crohn's disease (CD) has been proposed as being the product of chronic inflammation caused by a dysfunctional interaction between the intestinal immune system and commensal gut microbiota[1]. The inflammation seen in CD is characterized by pronounced Th1 and Th17/23 responses involving the cytokines interleukin (IL)-1, IL-6, tumor necrosis factor (TNF)-α, IFN-γ[1-3], IL-23 and IL-17[4-6].

Although the commensal intestinal microflora appear to play an important role in the etiology of CD, certain bacterial species have also been implicated as putative causal agents of CD. These include Pseudomonas maltophilia[7], Chlamydia trachomatis[8], Bacteroides fragilis[9] Yersinia species[10], adherent invasive *Escherichia coli* (*E. coli*)[11] and Mycobacterium avium subspecies paratuberculosis (MAP)[12]. The role of MAP in the etiology of CD is unclear. However, MAP causes chronic intestinal inflammation in ruminants (Johne's disease) with similar pathophysiology to CD[13] and it has been reported as a causative agent of regional enteritis with similarities to CD in a number of monogastrics, including two primate species[14,15]. MAP has also been cultured from both resected tissue[16] and peripheral blood of CD patients[17], and has been visualized inside macrophages from CD patients[18]. Meta-analyses of epidemiological studies confirm an association of MAP with CD[19,20], although it remains unknown whether MAP is pathogenic in humans, or whether this association reflects a defective host immune system permissive for the survival of MAP.

Twin and family studies have demonstrated a significant genetic component to the development and progression of CD[21-23]. Linkage analysis, candidate gene approaches and, most recently, genome-wide association studies (GWAS) have identified over 30 risk genes for CD[24-26], many of which are involved with bacterial recognition (e.g. *NOD2*) or processing and elimination of bacteria through the autophagy pathway (e.g. *IRGM*[27] and *ATG16L1*[25]).

In this study, we developed an *ex vivo* monocyte model to assess the impact of the CD-associated single nucleotide polymorphisms (SNPs) in *NOD2* (rs2066842, P268S; rs2066844, R702W; and 1007fs, rs2066847) and *ATG16L1* (rs2241880, T300A) on cytokine responses to the putative pathogen MAP. MAP can survive and replicate within phagocytic cells, and consequently we also evaluated the impact of these polymorphisms on the intracellular persistence of MAP. The use of *ex vivo* monocytes allows functional evaluation of SNPs associated with CD, and may provide a more realistic insight into the impact that genotype has on CD compared to studies which involve abrogation of protein expression or whole gene deletion.

MATERIALS AND METHODS

Patient recruitment

Patients for the current study were selected from a New Zealand population-based Caucasian inflammatory bowel disease (IBD) cohort recruited to investigate genetic and environmental factors that contribute to IBD etiology[28-32]. Inclusion criteria for the current study were a confirmed diagnosis of CD and negative MAP status as ascertained by IS900 PCR in peripheral blood[28].

The genotype combinations and patient phenotype information are detailed in Table 1. Briefly, the potential impact of *ATG16L1* and *NOD2* polymorphisms were assessed separately. For analysis of *ATG16L1*, monocytes were collected from CD patients who had a wild-type *NOD2* genotype and were homozygous for either the major (G) allele (n = 6) or minor (A) allele (n = 6) of *ATG16L1* 1138G > A (rs2241880). Conversely, for experiments evaluating the effect of *NOD2* genotype, monocytes were collected from patients who were *ATG16L1* 1138G homozygotes and were heterozygous for one of the three *NOD2* SNPs previously associated with CD; 2104C > T (R702W, rs2066844), 2722G > C (G908R, rs2066845), or 3020insC (1007fs, rs2066847), and were heterozygous for the background variant 802C > T (P268S, rs2066842). A total of 12 patients were recruited, 6 carried the polymorphisms described, and 6 were homozygous at these *NOD2* loci (Table 1). None of the patients included in this study had the SNPs rs13361189 and rs4958847. These SNPs are in complete linkage disequilibrium with a 20 kb insertion/deletion polymorphism which has been shown to alter expression of the autophagy gene *IRGM*[33].

Preparation of monocytes from peripheral blood

Blood (40 mL) was drawn into heparin tubes (Sigma-Aldrich), divided into 20 mL aliquots, and 15 mL of Phosphate Buffered Saline (PBS) was added to each. Ficoll-Paque™ PREMIUM (10 mL) (GE Healthcare Bio-Sciences Uppsala, Sweden) was layered under each aliquot and the samples centrifuged (1000 × *g*, 20 min). Mononuclear cells collected from the interface were added to 30 mL of PBS, centrifuged (350 × *g*, 10 min) and resuspended in 15 mL of PBS. In order to standardize the number of monocytes used in experiments, mononuclear cells were enumerated using a hemocytometer and a 200 μL aliquot was analyzed on a Beckman Coulter FC500 MPL flow cytometer to determine the percentage of monocytes based on forward and side scatter characteristics. After centrifugation (350 × *g*, 10 min), cells were resuspended in RPMI medium 1640 supplemented with 10% fetal bovine serum (FBS) (Invitrogen, Carlsbad, CA, USA) to a concentration of 4 × 10^5 monocytes/mL. Then 500 μL of this preparation were plated per well in a 24-well tissue culture plate (Nunc, Roskilde, Denmark). Monocytes were left to adhere for 1 h in a humidified incubator at 37℃ with 5% CO_2 and non-adherent cells were removed by washing three times with 1 mL of room temperature (RT) PBS.

Table 1 ***IRGM*, *ATG16L1*, and *NOD2* genotypes of Crohn's disease patients from whom monocytes were collected for *ex vivo* experiments**

	IRGM		*ATG16L1*	*NOD2*				Clinical characteristics			
Patient	rs13361189[1]	rs4958847[1]	T300A 1138G > A rs2241880	R702W 2104C > T rs2066844	P268S 802C > T rs2066842	G908R 2722G > C rs2066845	1007fs 3020insC rs2066847	Gender	Time since diagnosis (yr)	Disease location	Harvey bradshaw index[2]
1	TT	GG	AA	CC	CC	GG	00	M	53	Ileal	0
2	TT	GG	AA	CC	CC	GG	00	M	5	Ileo-colonic	0
3	TT	GG	AA	CC	CC	GG	00	F	5	Colonic	0
4	TT	GG	GG	CC	CC	GG	00	M	25	Ileo-colonic	2
5	TT	GG	AA	CC	CC	GG	00	F	6	Ileo-colonic	1
6	TT	GG	AA	CC	CC	GG	00	F	9	Ileal	3
7	TT	GG	AA	CC	CC	GG	00	M	1	Ileal	0
8	TT	GG	GG	CC	CC	GG	00	M	6	Colonic	2
9	TT	GG	GG	CC	CC	GG	00	F	10	Colonic	5
10	TT	GG	GG	CC	CC	GG	00	M	24	Ileo-colonic	1
11	TT	GG	GG	CC	CC	GG	00	F	11	Colonic	0
12	TT	GG	GG	CC	CC	GG	00	F	7	Ileo-colonic	4
13	TT	GG	GG	CT	CT	GG	00	F	5	Colonic	4
14	TT	GG	GG	CT	CT	GG	00	F	9	Ileo-colonic	0
15	TT	GG	GG	CT	CT	GG	00	M	17	Ileal	0
16	TT	GG	GG	CT	CT	GG	00	F	18	Colonic	4
17	TT	GG	GG	CC	CT	GG	0C	M	7	Ileal	1
18	TT	GG	GG	CT	CT	GG	00	F	16	Ileo-colonic	4

[1]SNPs located upstream (5') to *IRGM*; [2]Non-invasive clinical index used to assess disease activity in patients with Crohn's disease. A score of ⩾ 7 indicates active disease.

Adherent monocytes were incubated overnight in 500 μL of RPMI 1640/10% FBS.

Culture of MAP

MAP strain Dominic (ATCC 43545) was inoculated into 10 mL volumes of Difco™ Middlebrook 7H9 broth (BD Biosciences, Sparks, MD, USA) supplemented with 10% v/v BBL™ Middlebrook OADC Enrichment (BD Biosciences), 0.05% v/v Tween 80 (Sigma-Aldrich, St Louis, MO, USA) and 2 mg/mL Mycobactin J (Allied Monitor, Fayette, MO, USA) and grown at 37℃. A standard growth curve of MAP was obtained by measuring the optical density at 600 nm (OD_{600}) of an aliquot of bacterial suspension during the log phase, which had been passed repeatedly through a 25-gauge needle, and enumerating the bacteria by plating on the same medium supplemented with 1.5% agar (Invitrogen, Carlsbad, CA, USA).

Monocyte challenge experiments

Bacteria were grown to mid log phase (approximately 1×10^8 cells/mL, with reference to previously obtained growth curve data) and harvested by centrifugation (13000 × *g*, 5 min). Bacteria were resuspended in 500 μL of PBS and passed ten times through a 25-gauge needle to break up clumps of cells. One 500 μL aliquot of MAP was heat-inactivated at 90℃ for 5 min and then both aliquots were diluted to 4×10^6 cells/mL in RPMI 1640/10% FBS. The optimal temperature and incubation time for heat-inactivation had been previously confirmed by plating heat-treated MAP cells onto agar. Growth medium was removed from the monocytes and 500 μL of the MAP suspension was added to each well. Challenge experiments were performed in triplicate for each time-point/genotype combination. After incubation for 4 h at 37℃, 5% CO_2, the supernatant was removed and stored at -80℃ as a zero time-point sample for cytokine assays. Monolayers were washed three times with 1 mL of PBS to remove extracellular bacteria. A zero time-point lysate to assess bacterial uptake and persistence was removed by incubating 500 μL of a 0.1% sodium deoxycholate (Sigma-Aldrich, St Louis, MO, USA) solution with the monolayer for 5 min at RT. Lysates were centrifuged (13000 × *g*, 5 min) and resuspended in 200 μL of PBS. Serial dilutions of the lysates were made with PBS and 50 μL were spread on to a Middlebrook agar plate. The agar plates were left to incubate for four weeks at 37℃ before counting MAP colonies. Subsequent samples at time-points of 24, 48, 72 and 96 h were similarly processed.

Confirmation of genotypes of study participants

ATG16L1 genotypes were confirmed at recruitment by direct DNA sequencing of PCR products. Briefly, genomic DNA was extracted from fresh peripheral blood using GenElute™ (Sigma-Aldrich, St Louis, MO, USA) spin columns according to manufacturer's protocols. A 480 bp fragment containing the *ATG16L1* 1138G > A SNP was amplified for DNA sequencing from patient genomic DNA using the following primers: 5'-CCACAGGTTAGTGTGCAGGA-3' (forward primer) and 5'-CACAGCTGACAGAGCCAAAA-3' (reverse primer). PCR was carried out in a 20 μL volume containing 1 μL of genomic DNA, 0.3 μmol/L of each primer, 200 μmol/L dNTPs, 0.75 mmol/L $MgCl_2$, 1 × TAQ-Ti reaction buffer (Thermo Fisher Scientific, Pittsburgh, PA, USA) and 0.25 U of TAQ-Ti DNA polymerase (Thermo Fisher Scientific). After an initial denaturation step of 94℃ for 2 min, 35 cycles were performed at 94℃ for 30 s, 60℃ for 30 s and 72℃ for 30 s. Five microlitres of each PCR product was checked on 1% agarose. Another 5 μL aliquot was

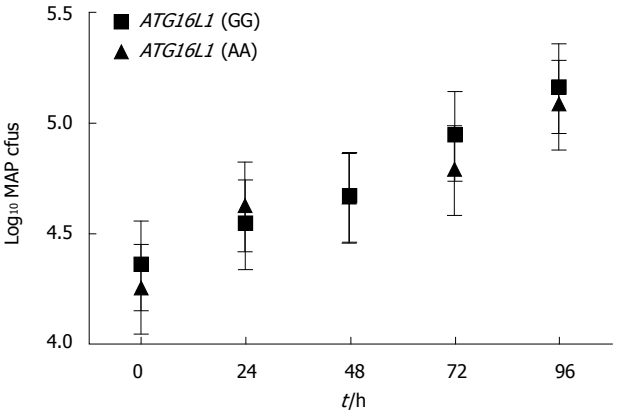


Figure 1 Effect of *ATG16L1* T300A variant on mycobacterium avium subspecies paratuberculosis numbers. Time course of mycobacterium avium subspecies paratuberculosis (MAP) growth in the context of the *ATG16L1* 1138 G > A variant. Monocytes were derived from subjects homozygous for either *ATG16L1* allele and carrying *NOD2* wild-type alleles (n = 6 for each group). MAP growth is expressed as colony forming units (cfu).

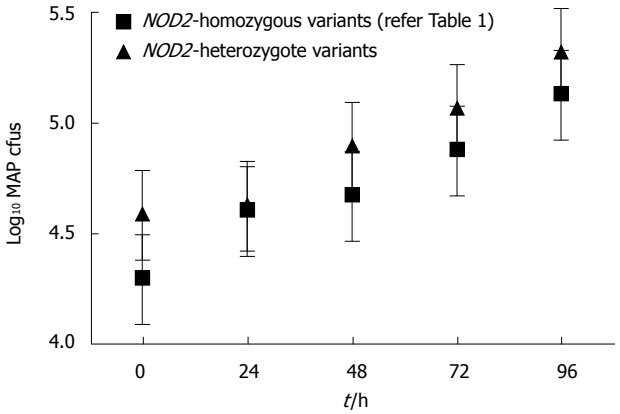


Figure 2 Effect of *NOD2* variants on mycobacterium avium subspecies paratuberculosis numbers. Time course of mycobacterium avium subspecies paratuberculosis (MAP) growth in the context of *NOD2* genetic variation. Monocytes were derived from subjects homozygous for the *ATG16L1* 1138G allele and with/without *NOD2* genetic variants (n = 6 for each group). MAP growth is expressed as colony forming units (cfu).

purified with Exo-SAP-IT (USB Corporation, Ohio, USA) and sequenced using BigDye chemistry (Applied Biosystems, California, USA) on an ABI 3730 Genetic Analyzer (Foster City, California, USA). The *NOD2* and *IRGM* genotypes of study participants were established as previously described using allele-specific PCR and pre-designed TaqMan SNP genotyping assays, respectively[34,35].

Multiplex cytokine analysis

Cytokine analysis was performed using a 13-plex MILLIPLEX™ MAP human cytokine kit according to manufacturer's recommendations (Millipore) for the following: IFNγ, IL-10, IL-12p40, IL-12p70, IL-17, IL-1b, IL-2, IL-4, IL-5, IL-6, IL-8, TNFα, TNFβ.

Statistical analysis

Data from the five time-points were analyzed by repeated measures ANOVA, with fixed effects for time (as categorical variable) and random effects for subject i.e. genotype. For cytokine analyses, where readings were below the threshold for detection (3.2 pg/mL) they were included as 3.2 pg/mL. Results were considered significant at $P \leqslant 0.05$.

Ethical considerations

Informed written consent was obtained from all participants in this study and ethical approval for this work was granted by the Upper South B Regional Ethics Committee of New Zealand.

RESULTS

Bacterial persistence ATG16L1

Numbers of MAP increased from a mean $\log_{10}$ colony forming units (cfu) of 4.24 at 0 h to 5.08 at 96 h for the AA genotype, and from log10 cfu of 4.47 to 5.29 for the GG genotype (Figure 1). There was no evidence (T-testing) for the effect of the *ATG16L1* T300A polymorphism on MAP numbers at any individual time-point, or overall (pooled time-points ANOVA, $P = 0.175$) (Figure 1).

NOD2

MAP also grew in the monocytes with different *NOD2* genotypes, from a mean $\log_{10}$cfu of 4.30 at 0 h to 5.12 for the cells homozygous for the major alleles of the *NOD2* variants, and from $\log_{10}$cfu of 4.58 to 5.42 for cells heterozygous for any *NOD2* variant (Figure 2). There was no significant effect (*T*-testing) of *NOD2* genotype on MAP numbers at any individual time-point. However, analysis of all time-points indicated that monocytes heterozygous for a *NOD2* polymorphism were more permissive for growth of MAP (ANOVA, $P = 0.045$) (Figure 2).

Multiplex cytokine panels

Thirteen cytokines were evaluated from *ex vivo* monocyte supernatants using a MILLIPLEX™ MAP human cytokine kit. Each time-point/genotype combination was assayed in triplicate. All assays passed quality controls and r^2 values for the standard curves were ⩾ 0.99.

Of the thirteen cytokines analyzed in the multiplex format, only four, IL-10, IL-6, IL-8 and TNFα, had measurable responses above the detection threshold (> 3.2 pg/mL) on the multiplex ELISA platform used. Where samples had values of < 3.2 pg/mL, they were considered to be 3.2 pg/mL for statistical purposes.

Effect of ATG16L1 genotype

Cytokine expression results are shown in Figure 3. The AA genotype of *ATG16L1* was associated with greater expression of cytokines IL-10 and IL-6 in response to challenge with MAP ($P = 0.047$ and $P = 0.019$, respectively). No significant difference was seen between *ATG16L1* genotypes AA and GG for expression of either IL-8 or TNFα ($P = 0.758$ and $P = 0.289$, respectively) (Figure 3).

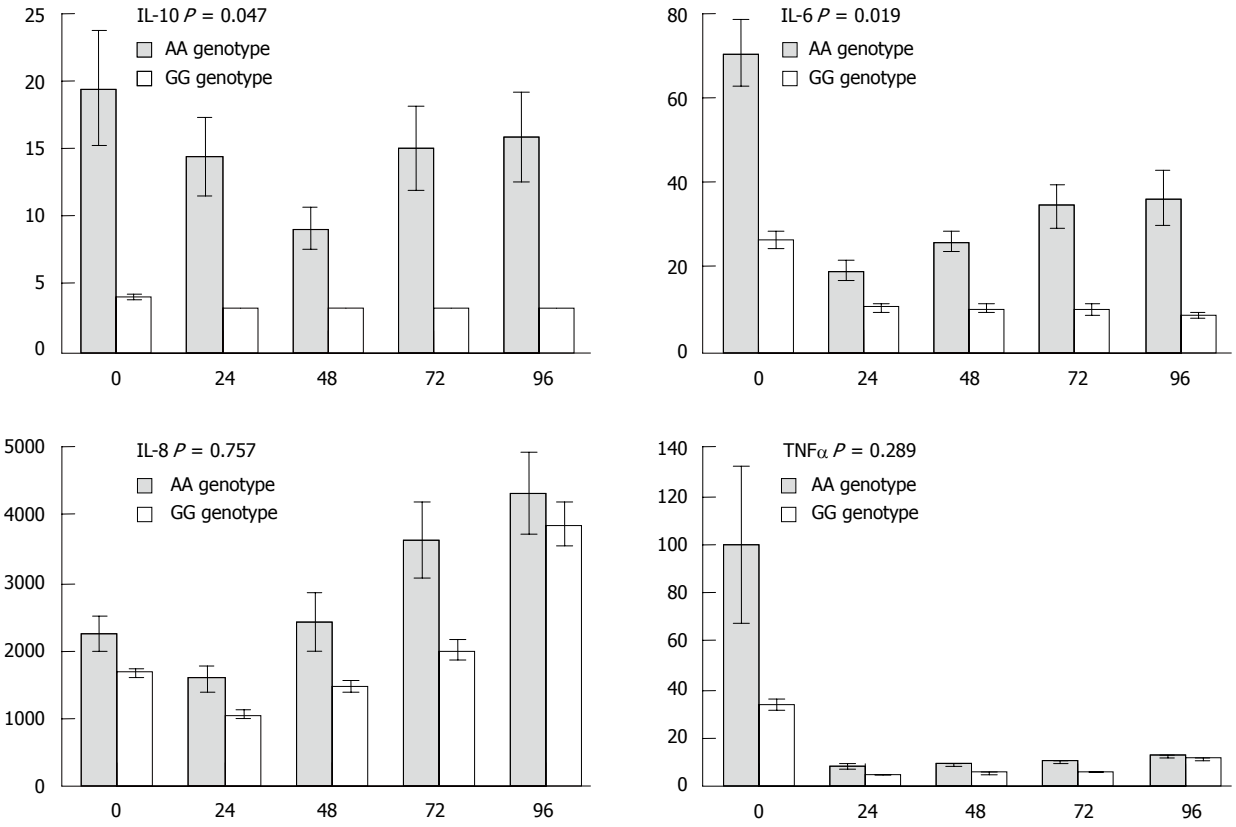


Figure 3 Effect of the *ATG16L1* T300A polymorphism on expression of cytokines following challenge with mycobacterium avium subspecies paratuberculosis. Cytokine concentrations are in pg/mL. Results are the means (and standard error of the mean) of triplicate samples from patients in Table 1. *P*-values are derived from repeated measures ANOVA testing for the effect of genotype on cytokine expression. Fixed effects for time (categorical variable) and *ATG16L1* genotype, random effect for subject. IL: Interleukin; TNF: Tumor necrosis factor.

Effect of NOD2 genotype

Cytokine expression changes for IL-10, IL-6, IL-8 and TNFα by cells of different *NOD2* genotypes are shown in Figure 4. In general, the presence of a *NOD2* polymorphism resulted in a trend of lower expression of these four cytokines throughout the time-course, compared to monocytes without *NOD2* variants.

However, this effect was not significant, with P-values of 0.56, 0.32, 0.41 and 0.97 for cytokines IL-10, IL-6, IL-8 and TNFα, respectively (Figure 4).

DISCUSSION

Numerous genetic association studies have indicated a strong role for *NOD2* and *ATG16L1* in the etiology of CD. However, functional studies have yet to fully elucidate whether, and to what extent, polymorphic variation in these genes affects bacterial clearance and inflammation in CD. Models of gene/environment interaction have mostly used epithelial or monocyte cell lines in vitro with bacterial pathogens such as Salmonella typhimurium that are not generally associated with CD. Also, the use of gene silencing or ablation techniques in these models may assist our understanding of the function of these genes, but may not be representative of the effects of SNPs which have been associated with CD. In this study we developed an *ex vivo* cellular model using monocytes from CD patients and a putative CD pathogen, MAP, in order to assess the effect of SNPs in *NOD2* and *ATG16L1* on bacterial survival and subsequent inflammatory response.

NOD2 is located in the cytoplasm, and plays an important role in cellular responses to bacterial infection[36] through recognition of muramyl dipeptide (MDP), a subunit of bacterial peptidoglycan (PGN)[37]. Furthermore, the R702W, G908R and 1007fs *NOD2* variants, which confer susceptibility to CD, have been shown to impair responses to the bacterial antigen lipopolysaccharide[38].

We found the effect of *NOD2* polymorphisms appeared to be primarily on bacterial persistence/growth, with heterozygosity at R702W or 1007fs making monocytes from CD patients significantly more permissive to growth of MAP. Indeed, a recent study has observed that these variants are associated with an impairment of monocyte phagocytosis and the development of bacteremia in intensive care unit patients[39]. Despite the increased bacterial load, no differences were seen in subsequent cytokine responses for the different host *NOD2* genotypes. Whilst there has been no prior published research with respect to MAP in this type of model of CD, the functional effects of *NOD2* SNPs have been examined for adherent invasive *E. coli* (AIEC). No differences were found in the persistence or growth of AIEC in monocytes from patients who were heterozygous or homozygous for the minor allele of *NOD2* variants R702W, G908R and 1007fs compared to

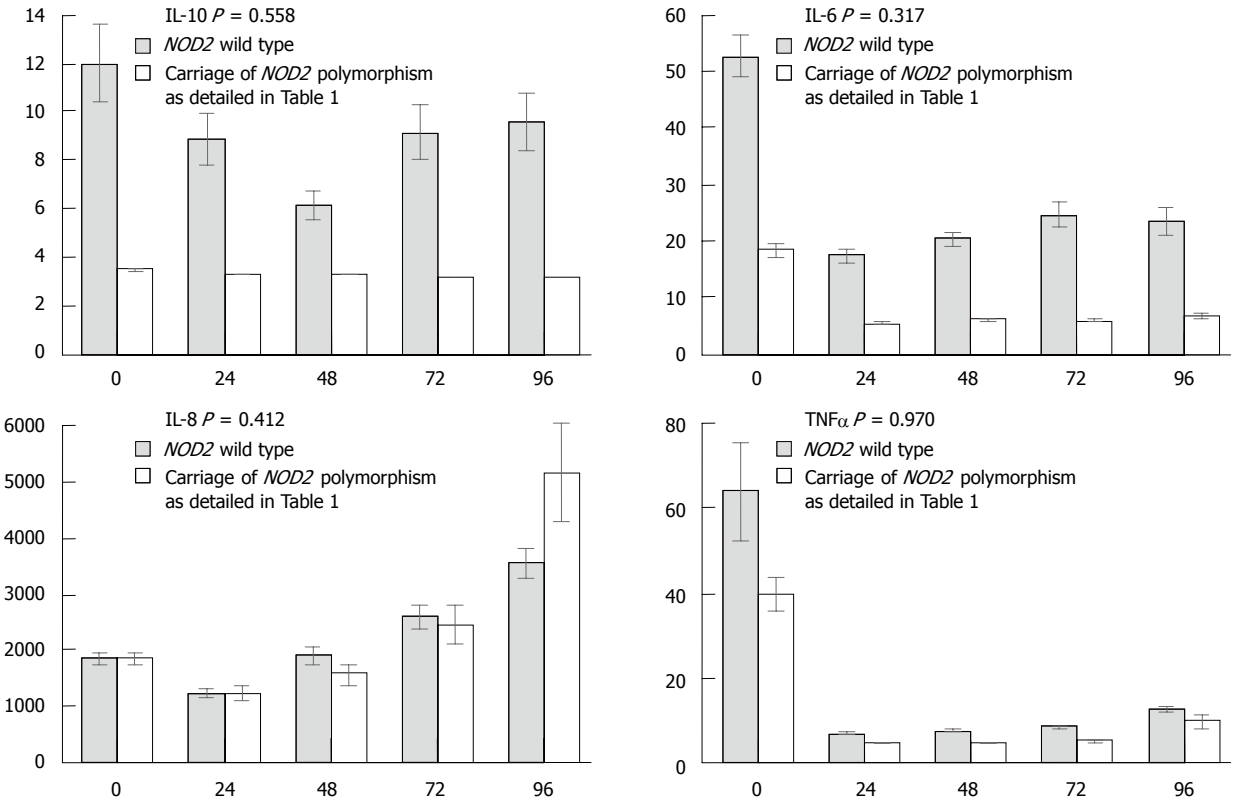


Figure 4 Effect of *NOD2* polymorphisms on expression of cytokines following challenge with mycobacterium avium subspecies paratuberculosis. Cytokine concentrations are in pg/mL. Results are the means (and standard error of the mean) of triplicate samples from patients in Table 1. *P*-values are derived from repeated measures ANOVA testing for the effect of genotype on cytokine expression. Fixed effects for time (categorical variable) and *NOD2* genotype, random effect for subject. IL: Interleukin; TNF: Tumor necrosis factor.

NOD2 major allele homozygotes[40]. It has also been shown that in peripheral blood mononuclear cells from CD patients, homozygosity for R702W does not affect the cytokine response to Gram-negative (*Helicobacter pylori*) bacterial peptidoglycan[41], although homozygosity for the 1007 fs mutation did. In our study we recruited no 1007fs homozygotes and only a single 1007 fs heterozygote so were unable to make any direct comparisons with this previous study. Comparative studies for a broader range of bacterial species in *ex vivo* cells from patients who are homozygous for these *NOD2* SNPs will help clarify the role of this genotype in disease pathogenesis.

Both MAP and AIEC are capable of intracellular survival and growth in monocytes and macrophages, and it appears that the presence of *NOD2* polymorphisms may influence their respective intracellular survival and growth in different ways[42].

Autophagy has been identified as a mechanism for clearing intracellular pathogens, and two autophagy genes, *ATG16L1* and *IRGM*, have been associated with CD[25,27]. In our study, we investigated the effect of the *ATG16L1* T300A polymorphism, and controlled for genotypic variation in *IRGM* (Table 1). *ATG16L1* T300A (rs2241880; 1138G > A) is a common non-synonymous SNP where the G major allele confers greater disease risk and results in a threonine-to-alanine substitution at amino acid position 300 of the *ATG16L1* protein (T300A). This SNP appears to account for all of the disease risk conferred by this locus[43], and functionally, this polymorphism has been proposed to contribute to defective macrophage killing of bacteria[44]. This assertion is supported by the results of two in vitro studies. Kuballa *et al*[45] found that the T300A variant impaired handling and autophagy of Salmonella within human epithelial cells, and Lapaquette *et al*[46] showed that siRNA knockdown of *ATG16L1* led to loss of autophagy of intracellular AIEC bacteria by HeLa cells. Transfection of affected HeLa cells with wild-type *ATG16L1* restored autophagic function, whereas transfection with the T300A polymorphic form did not. In contrast, *ATG16L1* T300A had no effect on the survival of either S. typhimurium or group A Streptococcus in mouse embryonic fibroblasts[47]. Although direct comparison of different model systems is complex and potentially misleading, our results with MAP in *ex vivo* monocytes generate the question as to whether the T300A polymorphism of *ATG16L1* affects autophagic clearance of certain intracellular bacteria as profoundly as indicated by knockdown or silencing models of gene function.

In our study, the T300A polymorphism was associated with significant changes in production of the cytokines IL-6 and IL-10 in response to bacterial challenge with MAP. These two cytokines are components of the Th1 (pro-inflammatory) and Th2 (modulatory) pathways of inflammation, respectively, and it is likely that CD results from an imbalance between these two pathways. It is tempting to speculate that the relative levels of the two

cytokines that were induced are indicative of an imbalance, but extrapolating from a very specific model to describe a complex disease state would be misleading and clearly further comparative work is required in this area in disease-relevant models.

Our study is the first to investigate the effect of *NOD2* and *ATG16L1* genotype on the response of *ex vivo* human monocytes to the putative CD pathogen MAP. Although our results are preliminary and need to be replicated in a larger sample, they provide novel insights into the effect of disease-associated SNPs in innate immunity genes on detection, handling, and elimination of bacteria, and ultimately CD pathogenesis. Our observations indicate that *NOD2* SNPs R702W, P268S, or 1007 fs impair the elimination of MAP yet do not impact on cytokine production. They may, therefore, increase susceptibility to prolonged intracellular bacterial infection. Conversely, the *ATG16L1* T300A polymorphism significantly alters the expression of certain Th1 and Th2 cytokines after MAP challenge, but does not seem to affect the autophagic clearance of this putative CD pathogen.

ACKNOWLEDGMENTS

We thank the people of Canterbury with IBD who generously gave of their time to take part in the study. We also thank Rhondda Brown and Judy Hoar for their assistance in coordinating the recruitment of patients to the Canterbury IBD cohort.

COMMENTS

Background

Polymorphisms of the genes *NOD2* and *ATG16L1* have been associated with susceptibility to Crohn's disease (CD). These genes are important for an effective innate immune response against potential bacterial pathogens [such as *Mycobacterium avium* subspecies *paratuberculosis* (MAP)] which may trigger or exacerbate inflammation. Monocytes from CD patients of known genotype were used to determine whether polymorphisms in *NOD2* and *ATG16L1* alter cytokine responses and bacterial survival following challenge with MAP.

Research frontiers

Previous research has investigated the role of polymorphisms in *NOD2* and *ATG16L1* in various model systems. In general, these have used gene silencing strategies that may not realistically reflect the biological consequences of single nucleotide changes in these genes, or model bacterial pathogens that have little relevance to CD. None have reported the functional consequences of the naturally occurring single nucleotide polymorphisms using patient-derived cells and bacteria such as MAP that have been implicated in CD etiology.

Innovations and breakthroughs

The authors findings indicate that monocytes heterozygous for a *NOD2* polymorphism were more permissive for the intracellular growth of MAP than those without. However, these polymorphisms did not affect subsequent cytokine expression. The T300A polymorphism of *ATG16L1* did not affect growth of MAP in our monocyte model but did result in increased expression of certain cytokines - interleukin (IL)-10 and IL-6.

Applications

By understanding how naturally occurring disease-related polymorphisms of *NOD2* and *ATG16L1* influence bacterial survival and also the production of inflammatory mediators, the authors may gain insight into the contribution of these genetic changes to the function of the host innate immune system. Development of this model system that utilizes patient cells with known single nucleotide changes in key CD-susceptibility genes will provide another research tool to assist better understanding of disease pathogenesis related to bacterial handling.

Terminology

NOD2 (CARD15) - nucleotide oligomerization domain 2 - is a cytosolic pattern recognition receptor that recognizes muramyl dipeptide, a component of bacterial peptidoglycan. Polymorphisms in *NOD2* have been associated with altered susceptibility to CD in many genetic studies. *ATG16L1* - autophagy-related 16-like 1 - is a key component of the autophagic apparatus that is involved with uptake and digestion of intracellular bacteria. The T300A polymorphism of *ATG16L1* has also been associated with altered susceptibility to CD. MAP is an intracellular bacterium that has been cited in several studies as a putative causal agent of CD.

Peer review

This is a very well-designed and well-written study, with interesting and important scientific merit. Not just simple polymorphism descriptions, but their effect on human monocyte cytokine production and intracellular pathogen survival were examined with a very functional methodology. Their *ex vivo* model is much closer to the real pathogenesis of CD than any earlier one. Using the author's concept, more descriptive polymorphism analysis of CD and other diseases may be placed into functional analysis.

REFERENCES

1 **Packey CD**, Sartor RB. Interplay of commensal and pathogenic bacteria, genetic mutations, and immunoregulatory defects in the pathogenesis of inflammatory bowel diseases. *J Intern Med* 2008; **263**: 597-606
2 **Reimund JM**, Wittersheim C, Dumont S, Muller CD, Kenney JS, Baumann R, Poindron P, Duclos B. Increased production of tumour necrosis factor-alpha interleukin-1 beta, and interleukin-6 by morphologically normal intestinal biopsies from patients with Crohn's disease. *Gut* 1996; **39**: 684-689
3 **Hisamatsu T**, Suzuki M, Reinecker HC, Nadeau WJ, McCormick BA, Podolsky DK. CARD15/*NOD2* functions as an antibacterial factor in human intestinal epithelial cells. *Gastroenterology* 2003; **124**: 993-1000
4 **Annunziato F**, Cosmi L, Santarlasci V, Maggi L, Liotta F, Mazzinghi B, Parente E, Filì L, Ferri S, Frosali F, Giudici F, Romagnani P, Parronchi P, Tonelli F, Maggi E, Romagnani S. Phenotypic and functional features of human Th17 cells. *J Exp Med* 2007; **204**: 1849-1861
5 **Kamada N**, Hisamatsu T, Okamoto S, Chinen H, Kobayashi T, Sato T, Sakuraba A, Kitazume MT, Sugita A, Koganei K, Akagawa KS, Hibi T. Unique CD14 intestinal macrophages contribute to the pathogenesis of Crohn disease *via* IL-23/IFN-gamma axis. *J Clin Invest* 2008; **118**: 2269-2280
6 **Kobayashi K**, Blaser MJ, Brown WR. Immunohistochemical examination for mycobacteria in intestinal tissues from patients with Crohn's disease. *Gastroenterology* 1989; **96**: 1009-1015
7 **Graham DY**, Yoshimura HH, Estes MK. DNA hybridization studies of the association of Pseudomonas maltophilia with inflammatory bowel diseases. *J Lab Clin Med* 1983; **101**: 940-954
8 **Orda R**, Samra Z, Levy Y, Shperber Y, Scapa E. Chlamydia trachomatis and inflammatory bowel disease--a coincidence? *J R Soc Med* 1990; **83**: 15-17
9 **Darfeuille-Michaud A**, Boudeau J, Bulois P, Neut C, Glasser AL, Barnich N, Bringer MA, Swidsinski A, Beaugerie L, Colombel JF. High prevalence of adherent-invasive Escherichia coli associated with ileal mucosa in Crohn's disease. *Gastroenterology* 2004; **127**: 412-421
10 **Kallinowski F**, Wassmer A, Hofmann MA, Harmsen D, Heesemann J, Karch H, Herfarth C, Buhr HJ. Prevalence of enteropathogenic bacteria in surgically treated chronic inflammatory bowel disease. *Hepatogastroenterology* 1998; **45**: 1552-1558
11 **Rolhion N**, Darfeuille-Michaud A. Adherent-invasive Escherichia coli in inflammatory bowel disease. *Inflamm Bowel Dis* 2007; **13**: 1277-1283
12 **McFadden JJ**, Butcher PD, Chiodini R, Hermon-Taylor J.

Crohn's disease-isolated mycobacteria are identical to Mycobacterium paratuberculosis, as determined by DNA probes that distinguish between mycobacterial species. *J Clin Microbiol* 1987; **25**: 796-801
13 **Greenstein RJ**. Is Crohn's disease caused by a mycobacterium? Comparisons with leprosy, tuberculosis, and Johne's disease. *Lancet Infect Dis* 2003; **3**: 507-514
14 **McClure HM**, Chiodini RJ, Anderson DC, Swenson RB, Thayer WR, Coutu JA. Mycobacterium paratuberculosis infection in a colony of stumptail macaques (Macaca arctoides). *J Infect Dis* 1987; **155**: 1011-1019
15 **Zwick LS**, Walsh TF, Barbiers R, Collins MT, Kinsel MJ, Murnane RD. Paratuberculosis in a mandrill (Papio sphinx). *J Vet Diagn Invest* 2002; **14**: 326-328
16 **Chiodini RJ**, Van Kruiningen HJ, Merkal RS, Thayer WR Jr, Coutu JA. Characteristics of an unclassified Mycobacterium species isolated from patients with Crohn's disease. *J Clin Microbiol* 1984; **20**: 966-971
17 Naser SA, Ghobrial G, Romero C, Valentine JF. Culture of Mycobacterium avium subspecies paratuberculosis from the blood of patients with Crohn's disease. *Lancet* 2004; **364**: 1039-1044
18 **Gearry RB**, Aitken JM, Roberts RL, Ismail S, Keenan J, Barclay ML. Images of interest. Gastrointestinal: Mycobacterium avium paratuberculosis and Crohn's disease. *J Gastroenterol Hepatol* 2005; **20**: 1943
19 **Abubakar I**, Myhill D, Aliyu SH, Hunter PR. Detection of Mycobacterium avium subspecies paratuberculosis from patients with Crohn's disease using nucleic acid-based techniques: a systematic review and meta-analysis. *Inflamm Bowel Dis* 2008; **14**: 401-410
20 **Feller M**, Huwiler K, Stephan R, Altpeter E, Shang A, Furrer H, Pfyffer GE, Jemmi T, Baumgartner A, Egger M. Mycobacterium avium subspecies paratuberculosis and Crohn's disease: a systematic review and meta-analysis. *Lancet Infect Dis* 2007; **7**: 607-613
21 **Orholm M**, Binder V, Sørensen TI, Rasmussen LP, Kyvik KO. Concordance of inflammatory bowel disease among Danish twins. Results of a nationwide study. *Scand J Gastroenterol* 2000; **35**: 1075-1081
22 **Satsangi J**, Grootscholten C, Holt H, Jewell DP. Clinical patterns of familial inflammatory bowel disease. *Gut* 1996; **38**: 738-741
23 **Spehlmann ME**, Begun AZ, Burghardt J, Lepage P, Raedler A, Schreiber S. Epidemiology of inflammatory bowel disease in a German twin cohort: results of a nationwide study. Inflamm *Bowel Dis* 2008; **14**: 968-976
24 **Barrett JC**, Hansoul S, Nicolae DL, Cho JH, Duerr RH, Rioux JD, Brant SR, Silverberg MS, Taylor KD, Barmada MM, Bitton A, Dassopoulos T, Datta LW, Green T, Griffiths AM, Kistner EO, Murtha MT, Regueiro MD, Rotter JI, Schumm LP, Steinhart AH, Targan SR, Xavier RJ, Libioulle C, Sandor C, Lathrop M, Belaiche J, Dewit O, Gut I, Heath S, Laukens D, Mni M, Rutgeerts P, Van Gossum A, Zelenika D, Franchimont D, Hugot JP, de Vos M, Vermeire S, Louis E, Cardon LR, Anderson CA, Drummond H, Nimmo E, Ahmad T, Prescott NJ, Onnie CM, Fisher SA, Marchini J, Ghori J, Bumpstead S, Gwilliam R, Tremelling M, Deloukas P, Mansfield J, Jewell D, Satsangi J, Mathew CG, Parkes M, Georges M, Daly MJ. Genome-wide association defines more than 30 distinct susceptibility loci for Crohn's disease. *Nat Genet* 2008; **40**: 955-962
25 **Rioux JD**, Xavier RJ, Taylor KD, Silverberg MS, Goyette P, Huett A, Green T, Kuballa P, Barmada MM, Datta LW, Shugart YY, Griffiths AM, Targan SR, Ippoliti AF, Bernard EJ, Mei L, Nicolae DL, Regueiro M, Schumm LP, Steinhart AH, Rotter JI, Duerr RH, Cho JH, Daly MJ, Brant SR. Genome-wide association study identifies new susceptibility loci for Crohn disease and implicates autophagy in disease pathogenesis. *Nat Genet* 2007; **39**: 596-604
26 **Hampe J**, Franke A, Rosenstiel P, Till A, Teuber M, Huse K, Albrecht M, Mayr G, De La Vega FM, Briggs J, Günther S, Prescott NJ, Onnie CM, Häsler R, Sipos B, Fölsch UR, Lengauer T, Platzer M, Mathew CG, Krawczak M, Schreiber S. A genome-wide association scan of nonsynonymous SNPs identifies a susceptibility variant for Crohn disease in *ATG16L1*. *Nat Genet* 2007; **39**: 207-211
27 **Singh SB**, Davis AS, Taylor GA, Deretic V. Human *IRGM* induces autophagy to eliminate intracellular mycobacteria. *Science* 2006; **313**: 1438-1441
28 **Bentley RW**, Keenan JI, Gearry RB, Kennedy MA, Barclay ML, Roberts RL. Incidence of Mycobacterium avium subspecies paratuberculosis in a population-based cohort of patients with Crohn's disease and control subjects. *Am J Gastroenterol* 2008; **103**: 1168-1172
29 **Bentley RW**, Pearson J, Gearry RB, Barclay ML, McKinney C, Merriman TR, Roberts RL. Association of higher DEFB4 genomic copy number with Crohn's disease. *Am J Gastroenterol* 2010; **105**: 354-359
30 **Gearry RB**, Richardson A, Frampton CM, Collett JA, Burt MJ, Chapman BA, Barclay ML. High incidence of Crohn's disease in Canterbury, New Zealand: results of an epidemiologic study. *Inflamm Bowel Dis* 2006; **12**: 936-943
31 **Roberts RL**, Gearry RB, Hollis-Moffatt JE, Miller AL, Reid J, Abkevich V, Timms KM, Gutin A, Lanchbury JS, Merriman TR, Barclay ML, Kennedy MA. IL23R R381Q and *ATG16L1* T300A are strongly associated with Crohn's disease in a study of New Zealand Caucasians with inflammatory bowel disease. *Am J Gastroenterol* 2007; **102**: 2754-2761
32 **Roberts RL**, Hollis-Moffatt JE, Gearry RB, Kennedy MA, Barclay ML, Merriman TR. Confirmation of association of *IRGM* and NCF4 with ileal Crohn's disease in a population-based cohort. *Genes Immun* 2008; **9**: 561-565
33 **McCarroll SA**, Huett A, Kuballa P, Chilewski SD, Landry A, Goyette P, Zody MC, Hall JL, Brant SR, Cho JH, Duerr RH, Silverberg MS, Taylor KD, Rioux JD, Altshuler D, Daly MJ, Xavier RJ. Deletion polymorphism upstream of *IRGM* associated with altered *IRGM* expression and Crohn's disease. *Nat Genet* 2008; **40**: 1107-1112
34 **Roberts RL**, Gearry RB, Hollis-Moffatt JE, Miller AL, Reid J, Abkevich V, Timms KM, Gutin A, Lanchbury JS, Merriman TR, Barclay ML, Kennedy MA. IL23R R381Q and *ATG16L1* T300A are strongly associated with Crohn's disease in a study of New Zealand Caucasians with inflammatory bowel disease. *Am J Gastroenterol* 2007; **102**: 2754-2761
35 **Roberts RL**, Gearry RB, Barclay ML, Kennedy MA. Rapid detection of common CARD15 variants in patients with inflammatory bowel disease. *Mol Diagn* 2004; **8**: 101-105
36 **Opitz B**, Püschel A, Schmeck B, Hocke AC, Rosseau S, Hammerschmidt S, Schumann RR, Suttorp N, Hippenstiel S. Nucleotide-binding oligomerization domain proteins are innate immune receptors for internalized Streptococcus pneumoniae. *J Biol Chem* 2004; **279**: 36426-36432
37 **Rosenstiel P**, Sina C, End C, Renner M, Lyer S, Till A, Hellmig S, Nikolaus S, Fölsch UR, Helmke B, Autschbach F, Schirmacher P, Kioschis P, Hafner M, Poustka A, Mollenhauer J, Schreiber S. Regulation of DMBT1 *via NOD2* and TLR4 in intestinal epithelial cells modulates bacterial recognition and invasion. *J Immunol* 2007; **178**: 8203-8211
38 **Bonen DK**, Cho JH. The genetics of inflammatory bowel disease. *Gastroenterology* 2003; **124**: 521-536
39 **Henckaerts L**, Nielsen KR, Steffensen R, Van Steen K, Mathieu C, Giulietti A, Wouters PJ, Milants I, Vanhorebeek I, Langouche L, Vermeire S, Rutgeerts P, Thiel S, Wilmer A, Hansen TK, Van den Berghe G. Polymorphisms in innate immunity genes predispose to bacteremia and death in the medical intensive care unit. *Crit Care Med* 2009; **37**: 192-201, e1-e3
40 **Rutgeerts P**, Goboes K, Peeters M, Hiele M, Penninckx F, Aerts R, Kerremans R, Vantrappen G. Effect of faecal stream diversion on recurrence of Crohn's disease in the neoterminal ileum. *Lancet* 1991; **338**: 771-774
41 **Ferwerda G**, Kullberg BJ, de Jong DJ, Girardin SE, Langen-

berg DM, van Crevel R, Ottenhoff TH, Van der Meer JW, Netea MG. Mycobacterium paratuberculosis is recognized by Toll-like receptors and *NOD2*. *J Leukoc Biol* 2007; **82**: 1011-1018

42 **Chamaillard M**, Philpott D, Girardin SE, Zouali H, Lesage S, Chareyre F, Bui TH, Giovannini M, Zaehringer U, Penard-Lacronique V, Sansonetti PJ, Hugot JP, Thomas G. Gene-environment interaction modulated by allelic heterogeneity in inflammatory diseases. *Proc Natl Acad Sci USA* 2003; **100**: 3455-3460

43 **Grant SF**, Baldassano RN, Hakonarson H. Classification of genetic profiles of Crohn's disease: a focus on the *ATG16L1* gene. *Expert Rev Mol Diagn* 2008; **8**: 199-207

44 **Silverberg MS**, Satsangi J, Ahmad T, Arnott ID, Bernstein CN, Brant SR, Caprilli R, Colombel JF, Gasche C, Geboes K, Jewell DP, Karban A, Loftus Jr EV, Peña AS, Riddell RH, Sachar DB, Schreiber S, Steinhart AH, Targan SR, Vermeire S, Warren BF. Toward an integrated clinical, molecular and serological classification of inflammatory bowel disease: Report of a Working Party of the 2005 Montreal World Congress of Gastroenterology. *Can J Gastroenterol* 2005; **19** Suppl A: 5-36

45 **Kuballa P**, Huett A, Rioux JD, Daly MJ, Xavier RJ. Impaired autophagy of an intracellular pathogen induced by a Crohn's disease associated *ATG16L1* variant. *PLoS One* 2008; **3**: e3391

46 **Lapaquette P**, Glasser AL, Huett A, Xavier RJ, Darfeuille-Michaud A. Crohn's disease-associated adherent-invasive E. coli are selectively favoured by impaired autophagy to replicate intracellularly. *Cell Microbiol* 2010; **12**: 99-113

47 **Fujita H**, Eishi Y, Ishige I, Saitoh K, Takizawa T, Arima T, Koike M. Quantitative analysis of bacterial DNA from Mycobacteria spp., Bacteroides vulgatus, and Escherichia coli in tissue samples from patients with inflammatory bowel diseases. *J Gastroenterol* 2002; **37**: 509-516

S- Editor Sun H **L- Editor** Logan S **E- Editor** Ma WH

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/1007-9327office
wjg@wjgnet.com
doi:10.3748/wjg.v17.i23.2838

World J Gastroenterol 2011 June 21; 17(23): 2838-2843
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

BRIEF ARTICLE

Is the schatzki ring a unique esophageal entity?

Michaela Müller, Ines Gockel, Philip Hedwig, Alexander J Eckardt, Kathrin Kuhr, Jochem König, Volker F Eckardt

Michaela Müller, Philip Hedwig, Alexander J Eckardt, Volker F Eckardt, Department of Gastroenterology, German Clinic for Diagnostics Wiesbaden, 65191 Wiesbaden, Germany
Ines Gockel, Department of General and Abdominal Surgery, University Medical Center of the Johannes Gutenberg University Mainz, 55131 Mainz, Germany
Kathrin Kuhr, Institute of Medical Statistics, Informatics and Epidemiology, University of Cologne, 50924 Cologne, Germany
Jochem König, Institute of Medical Biostatistics, Epidemiology and Informatics, University Medical Center of the Johannes Gutenberg University Mainz, 55131 Mainz, Germany
Author contributions: All authors contributed equally to the preparation, writing, and editing of this article; all authors read and approved the final manuscript.
Correspondence to: Michaela Müller, MD, Department of Gastroenterology, German Diagnostic Clinic Aukammallee 33, D-65191 Wiesbaden, Germany. mueller.gastro@dkd-wiesbaden.de
Telephone: +49-611-577248 Fax: +49-611-577460
Received: October 22, 2010 Revised: November 25, 2010
Accepted: December 2, 2010
Published online: June 21, 2011

Abstract

AIM: To study, whether the association of Schatzki rings with other esophageal disorders support one of the theories about its etiology.

METHODS: From 1987 until 2007, all patients with newly diagnosed symptomatic Schatzki rings (SRs) were prospectively registered and followed. All of them underwent structured interviews with regards to clinical symptoms, as well as endoscopic and/or radiographic examinations. Endoscopic and radiographic studies determined the presence of an SR and additional morphological abnormalities.

RESULTS: One hundred and sixty-seven patients (125 male, 42 female) with a mean age of 57.1 ± 14.6 years were studied. All patients complained of intermittent dysphagia for solid food and 113 (79.6%) patients had a history of food impaction. Patients experienced symptoms for a mean of 4.7 ± 5.2 years before diagnosis. Only in 23.4% of the 64 patients who had endoscopic and/or radiological examinations before their first presentation to our clinic, was the SR previously diagnosed. At presentation, the mean ring diameter was 13.9 ± 4.97 mm. One hundred and sixty-two (97%) patients showed a sliding hiatal hernia. Erosive reflux esophagitis was found in 47 (28.1%) patients. Twenty-six (15.6%) of 167 patients showed single or multiple esophageal webs; five (3.0%) patients exhibited eosinophilic esophagitis; and four (2.4%) had esophageal diverticula. Four (7%) of 57 patients undergoing esophageal manometry had non-specific esophageal motility disorders.

CONCLUSION: Schatzki rings are frequently associated with additional esophageal disorders, which support the assumption of a multifactorial etiology. Despite typical symptoms, SRs might be overlooked.

Key words: Schatzki ring; Dysphagia; Food impaction; Gastroesophageal reflux disease; Esophageal web

Peer reviewer: Piero Marco Fisichella, MD, Assistant Professor of Surgery, Medical Director, Swallowing Center, Loyola University Medical Center, Department of Surgery, Stritch School of Medicine, 2160 South First Avenue, Room 3226, Maywood, IL 60153, United States

Müller M, Gockel I, Hedwig P, Eckardt AJ, Kuhr K, König J, Eckardt VF. Is the schatzki ring a unique esophageal entity? *World J Gastroenterol* 2011; 17(23): 2838-2843 Available from: URL: http://www.wjgnet.com/1007-9327/full/v17/i23/2838.htm DOI: http://dx.doi.org/10.3748/wjg.v17.i23.2838

INTRODUCTION

Lower esophageal (Schatzki) rings are found in 6%-14% of routine barium radiographs[1-4]. Even though most Schatzki rings (SRs) are asymptomatic, they are considered to be the most common cause of episodic dysphagia for solids and food impaction in adults[5,6]. Since their first

description in 1944[7], the etiology and pathogenesis of the SRs has remained obscure and little is known about their association with other structural and functional abnormalities of the esophagus. Theories about their origin include congenital, anatomical, and inflammatory factors as the most likely events that lead to a circular constriction of the esophagogastric junction[8-12].

Therefore, the aims of this study were: (1) to investigate whether the lower esophageal (Schatzki) ring is associated with other esophageal disorders; (2) to determine whether dysphagia is due to the presence of SRs or additional esophageal disorders; and (3) to determine whether one of the pathogenic theories could be supported.

MATERIALS AND METHODS

From 1987 until 2007, all patients with newly diagnosed symptomatic SRs were prospectively registered and followed. The diagnosis of SRs was based on the results of radiographic and/or endoscopic studies. In 119 patients, radiographic and endoscopic studies showed an SR. Fourteen patients had only radiographic and 34 only endoscopic studies. All patients underwent structured interviews to assess clinical symptoms.

Evaluation of symptoms

At the initial investigation and at each follow-up visit, structured interviews were performed. Questions concentrated on clinical symptoms such as the occurrence of food impaction, and frequency of dysphagia, heartburn and regurgitation (less than once a week, weekly, daily, or several times a day).

Radiographic studies

Radiographic studies (n = 133) were performed by senior staff radiologists using the prone-oblique, full-column technique. To distend the lower esophagus maximally, patients were asked to take a deep breath and to perform a Valsalva maneuver during the course of swallowing. Diagnosis of SR (Figure 1A) was based on the presence of a fixed, symmetric, thin (< 4 mm) structure, which intersected the esophagus perpendicular to its long axis, at the squamocolumnar junction[6,13]. The diameter of the lower esophageal ring was measured directly from the radiographic picture in the area of the narrowing. An esophageal web is defined as a thin (< 2 mm) eccentric membrane that can occur anywhere in the esophagus[14]. A sliding hiatal hernia was diagnosed when a pouch was visible between the tubular esophagus and the diaphragmatic narrowing (length of the pouch ⩽ 3 cm = small hernia, > 3 cm = large hernia)[15]. Diagnosis of diverticulum was based on the presence of a pouch in the esophagus (Zenker's diverticulum: pouch in the pharyngoesophageal area; midesophageal diverticulum: pouch in the mid esophagus; epiphrenic diverticulum: pouch just proximal to diaphragm)[14].

Endoscopic procedures

Upper gastrointestinal endoscopy (n = 153) was performed by senior staff gastroenterologists using a variety of upper gastrointestinal endoscopes (Olympus, Hamburg, Germany), which varied in caliber from 8.5 to 9.5 mm. The endoscopic mucosal appearance determined the presence or absence of esophagitis as well as further morphological abnormalities of the upper gastrointestinal tract. SR (Figure 1B) was defined as a thin, symmetric, mucosal structure located at the esophagogastric junction[3]. A sliding hiatal hernia was diagnosed when gastric mucosa extended for > 1.5 cm above the diaphragm[16]. An esophageal web was defined as a thin (no more than 1.5 mm), eccentric membrane, located above the esophagogastric junction[14]. The degree of esophagitis was classified into four stages according to Savary and Miller[17]. If there were mucosal alterations in addition to reflux esophagitis, biopsies were taken from the esophagus. The presence of ⩾ 20 eosinophils per high-power field in the histopathological examination was used as the criterion to diagnose eosinophilic esophagitis[18].

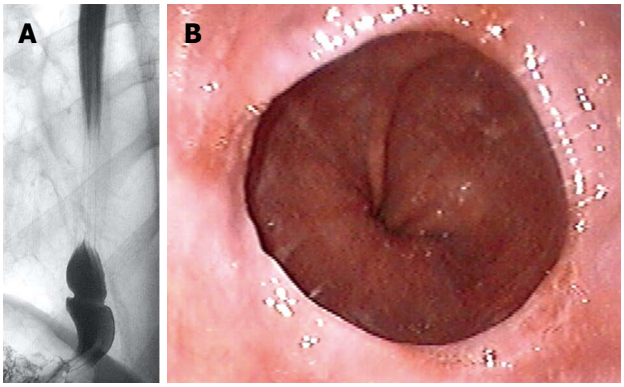


Figure 1 Radiographic (A) and endoscopic (B) image of the lower esophageal (Schatzki) ring.

Esophageal manometry

To exclude an esophageal motility disorder, 57 patients underwent esophageal manometry. Stationary esophageal manometry was performed with the use of a low-compliance capillary perfusion system (Mui Scientific, Mississauga, ON, Canada), using an eight-channel multilumen catheter with a 4.5-mm diameter. The four distal openings were 1 cm apart and the four proximal openings were 5 cm apart. Both sets were radially oriented. The manometric tracings were recorded by a computer polygraph system (Standard Instruments, Karlsruhe, Germany). Manometry was performed using a stationary pull-through method with the catheter introduced transnasally into the stomach. Manometry was carried out and interpreted according to the recommendations of the American Society of Gastroenterology[19]. Non-specific motor disturbance was defined as contractile abnormalities that are insufficient to establish a diagnosis of achalasia, diffuse esophageal spasm or typical scleroderma-like esophageal dysfunction[19,20].

Statistical analysis

Numerical variables are expressed as mean ± SD, counts

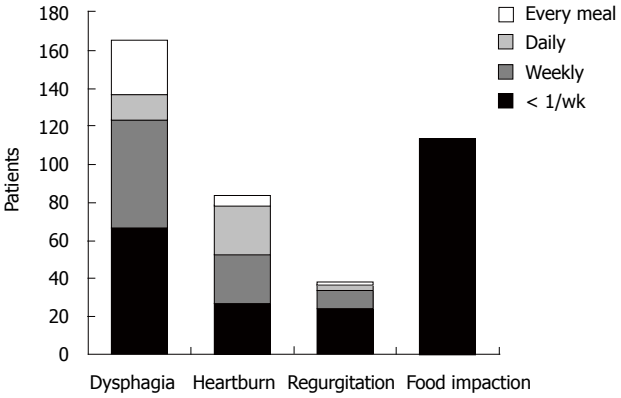


Figure 2 Clinical symptoms of 167 patients with Schatzki rings.

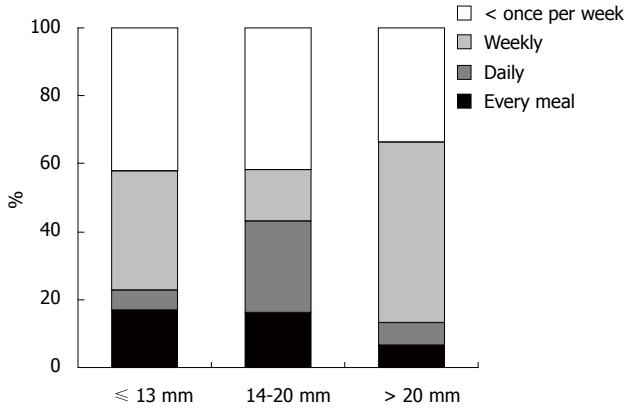


Figure 3 Frequency of dysphagia in relation to the ring size.

and ranges. Categorical variables are described using frequencies and percentages. Statistical significance of the differences between groups was assessed by Mann-Whitney *U* test for metric variables, by the Mantel-Haenszel χ^2 test for trends for ordered categorical variables and by Pearson χ^2 test for binary variables. A two-tailed *P* value < 0.05 was considered statistically significant. The analysis was performed using SPSS for Windows version 15.0 (SPSS, Chicago, IL, USA).

RESULTS

Demographic data and esophageal imaging at initial investigation

One hundred and sixty-seven patients (124 male, 43 female) with a mean age of 57.1 ± 14.6 years were studied. Patients experienced symptoms for a mean of 4.7± 5.2 years before diagnosis. Sixty-four (38.3%) of the 167 patients had endoscopic and/or radiological examinations before their first presentation to our clinic, but only in 15 (23.4%) of these patients was SR was previously diagnosed. In 35 (87.5%) of the 40 patients who received an endoscopic examination, the SR had not been diagnosed, and in 14 (70%) of the 20 patients who underwent radiological examinations, the diagnosis could not be determined.

One hundred and twelve patients received radiological and endoscopic examinations at their initial presentation to our hospital. Endoscopy or a barium swallow accurately diagnosed the SR in all 112 patients in whom both methods were used. In the 34 patients who were only examined by endoscopy, the correct diagnosis was made in all cases, whereas the diagnosis was only made in 14 (66.7%) of the 21 patients who underwent radiological examinations first. In the seven patients without an initial radiological diagnosis, the SR was identified on subsequent endoscopy.

Clinical findings at initial investigation

Clinical symptoms at initial presentation are shown in Figure 2. All patients complained of intermittent dysphagia for solid food: 66 patients (39.5%) less than once per week, 58 (34.7%) weekly, 14 (8.4%) daily, and 29 (16.4%) with every meal. One hundred and thirteen (79.6%) of the 167 patients had a history of food impaction. Forty (23.9%) patients described regurgitation and 87 (52.1%) complained of occasional heartburn.

Table 1 Demographic data, ring size and clinical symptoms of all patients with Schatzki rings, Schatzki rings and additional erosive esophagitis, and with Schatzki rings and additional esophageal webs (mean ± SD)

Variables	SR (*n* = 167)	SR with erosive esophagitis (*n* = 47[1])	SR with esophageal webs (*n* = 26[1])
Age (yr)	57.1 ± 14.6	58.4 ± 13.7	54.4 ± 18.9
Sex (*n*)			
Male	124	33	18
Female	43	14	8
Ring size (mm)	13.9 ± 4.97	14.1 ± 5.3	12.2 ± 3.8
Dysphagia, *n* (%)			
Every meal	29 (17.4)	7 (14.9)	8 (30.8)
Daily	14 (8.4)	3 (6.4)	3 (11.5)
Weekly	58 (34.7)	17 (36.2)	6 (23.1)
< 1 x/wk	66 (39.5)	20 (42.6)	9 (34.6)
Food impaction, *n* (%)	113 (79.6)	34 (72.3)	18 (69.2)
Heartburn, *n* (%)	86 (57.1)	29 (61.7)	12 (46.1)
Regurgitation, *n* (%)	40 (23.9)	11 (23.4)	6 (23.1)

[1]In two patients, erosive esophagitis as well as esophageal webs could be diagnosed. SR: Schatzki ring.

The diameter of the lower esophageal ring was evaluated in 126 patients undergoing radiographic studies, and in 27 patients, endoscopic estimation was used with open biopsy forceps (7 mm) being the reference standard. In 14 patients, no measurement was performed. At initial presentation, the mean ring diameter was 13.9 ± 4.97 mm (range: 5-25 mm). Eighty-three (54.2%) of 153 patients had a ring size ⩽ 13 mm, and 15 (9.8%) had a ring size > 20 mm. There was no correlation between ring size and frequency of dysphagia (*P* = 0.29, Figure 3). Also, sex (*P* = 1.0) and age ⩽ 40 or > 40 years (*P* = 0.93) had no influence on the frequency of dysphagia. Demographic data, ring size and clinical findings of all patients with Schatzki rings are shown in Table 1.

Additional findings and their influence on symptoms

A sliding hiatal hernia was found in 162 (97%) of 167

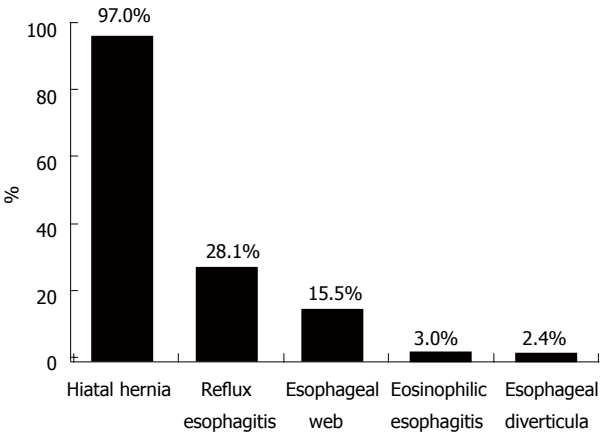


Figure 4 Additional structural abnormalities of the esophagus.

patients (n = 28 radiographic examinations, n = 29 endoscopic examinations, n = 105 radiographic and endoscopic examinations). One hundred and nineteen patients exhibited a small hernia and 43 had a large one. data, ring size and clinical findings of patients with Schatzki rings and additional erosive esophagitis, and /or esophageal webs are demonstrated in Table 1.

Further structural and functional abnormalities of the esophagus were diagnosed in 87 (52%) of 167 patients (Figure 4). The most frequent additional endoscopic finding was erosive reflux esophagitis, which was found in 47 (28.1%) patients; 40 (85.1%) of whom had stage Ⅰ esophagitis, whereas seven (14.9%) presented with stage Ⅱ and Ⅲ, and none with stage Ⅳ. All but one patient with reflux esophagitis showed a hiatal hernia. Twenty-six (15.6%) of 167 patients showed single (n = 15) or multiple ⩾ 2 (n = 11) esophageal webs, and four (2.4%) patients had esophageal diverticula. Two of the four patients with esophageal diverticula showed a Zenker's diverticulum, and in two patients, midesophageal diverticula were diagnosed. Four patients exhibited erosive reflux esophagitis in addition to esophageal webs. Five (16.6%) of the 30 patients in whom biopsies of the esophagus were taken exhibited histopathological signs of eosinophilic esophagitis. All but one patient complained of food impaction, whereas the frequency of dysphagia varied in this subgroup of patients (two patients, every meal; one patient, daily; and two patients, less than once per week).

Four (7%) of 57 patients undergoing esophageal manometry showed pathological results (two with non-specific motor disturbance, one with low contraction amplitudes, and one with diffuse esophageal spasm). Patients with an additional motility disorder of the esophagus showed a higher frequency of dysphagia than patients without (P = 0.03), although there was no difference in ring diameter (patients with motility disorders, 13.53 ± 3.6 mm; patients without, 13.7 ± 4.1 mm; P = 0.92).

The mean ring diameter in patients with additional esophageal webs (12.3 ± 3.8 mm) was smaller than in patients without webs (14.2 ± 5.1 mm) (P = 0.057). However, there were no differences in the frequency of dysphagia in patients with further structural abnormalities of the esophagus (sliding hernia, P = 0.1; erosive esophagitis, P = 0.48; and esophageal webs, P = 0.15) in comparison to the patients with an SR alone.

DISCUSSION

The lower esophageal SR is a common clinical finding and the most common cause of intermittent dysphagia, especially after consumption of solid food[6,21]. However, little is known about its etiology, its pathogenesis, or its association with other esophageal disorders. In addition, the clinical importance of associated disorders has not been described.

In our study, we were able to show that symptomatic SRs cannot always be diagnosed with a single imaging technique, and that the SR is not a unique entity. It is frequently associated with other esophageal disorders, such as hiatal hernias, reflux esophagitis and esophageal webs. In addition, ring size and most other structural abnormalities do not predict symptoms. In contrast, dysphagia was more common in patients with an additional motility disorder, and food impaction was the most common presentation in patients with eosinophilic esophagitis. Whether the frequent association with hiatal hernias and inflammatory esophagitis plays a pathogenic role remains unclear, but a multifactorial etiology is suspected.

In the present study, we confirmed previous observations that patients with symptomatic SRs complain about episodic dysphagia for solid food (mean duration 4.7 years prior to diagnosis), and more than two-thirds develop food impaction. Despite the typical clinical presentation, there was a significant diagnostic delay. Prior to presentation to our hospital, diagnosis of an SR was made in less than half of symptomatic patients; a surprisingly low number of patients. One of the difficulties in detecting lower esophageal rings might be related to the fact that the radiographic and endoscopic visualization depends on proper distension of the esophagogastric region beyond the caliber of the ring, which is often not accomplished[22]. This is especially true for wider rings with a luminal diameter > 13 mm[23]. In such instances, the radiographic examination has been shown to be superior to endoscopy in detecting lower esophageal rings[24]. In contrast, the present study could show a better diagnostic yield of endoscopy as compared with a barium swallow. This is most likely related to our special attention to membranous structures. Our findings suggest that a second imaging study should be performed in patients with typical clinical presentation if the first study fails to make the diagnosis of SR.

The current investigation showed that SRs are not a unique entity, but associated with additional esophageal disorders in 57% of symptomatic patients. Besides the nearly unanimous association with a hiatal hernia[2,24], we found a common association with reflux esophagitis and esophageal webs. In addition, esophageal diverticula were occasionally diagnosed. However the presence of additional structural abnormalities of the esophagus did not change the clinical presentation. Dysphagia was not more common in these patients. Even in patients with addition-

al esophageal webs, whose ring diameters were generally smaller, dysphagia was not more common. These findings suggest that the ring diameter alone may not be responsible for the observed symptoms.

In contrast, dysphagia was more frequently observed in patients with non-specific motility disorders, regardless of ring size, which suggests that the motility disorder may be responsible for the symptoms. Therefore, manometry should be considered in patients with wide ring diameters and symptoms of dysphagia. In addition, we found that intermittent bolus obstructions were a very frequent finding in those patients with an additional diagnosis of eosinophilic esophagitis, and clinicians should keep this clinical entity in mind. Therefore, the presence of an SR should not deter the endoscopist from taking esophageal biopsies in a patient in whom eosinophilic esophagitis remains a possibility. In fact, ring-like structures are common in eosinophilic esophagitis, and we became aware of this entity only a decade ago. Although an association of eosinophilic esophagitis and SRs has been previously suggested, it is not known if this is caused by shared clinical and endoscopic findings, or rather a shared pathogenesis[25,26]. Common clinical features might also explain the frequent coexistence of esophageal webs and eosinophilic esophagitis in our study. However, it is not clear if the additional esophageal disorders occur by chance, or if there is a common pathogenesis. The etiology of SRs remains obscure, and several theories about their etiology and pathogenesis exist. One of these is the developmental theory that holds that the presence of a congenital mucosal ridge at the esophagogastric junction is a rather frequent anatomical phenomenon that could fold in a valve-like fashion to create the ring[3,8]. Arguing against this, is the fact that the majority of symptomatic individuals were over the age of 40 years in the present and previous studies[27]. In the so called plication theory, Stiennon has postulated that longitudinal shortening in the presence of a hiatal hernia may lead to folding of redundant esophageal mucosa[11]. Consistent with this theory is the fact that most of the patients in the present study had a sliding hiatal hernia. However, it still remains unclear why some patients with a hernia develop an SR and others do not. Therefore, the plication theory is unlikely to be the only cause for the development of SRs. Currently, the inflammation theory with gastroesophageal reflux as the main cause of inflammation, is the most popular theory[28], and consequently, some authors have recommended an antireflux regimen to prevent symptomatic recurrence[29,30]. Others have pointed out that, if less than two-thirds of patients are found to have pathological gastroesophageal reflux[31,32], it might not be the main pathogenic factor. Similar to the latter findings, even if reflux esophagitis were one of the most frequent associated esophageal disorders in the present study, less than one-third of all investigated patients showed endoscopic signs of erosive esophagitis, and only half complained of occasional heartburn, which suggests that gastroesophageal reflux is not the only cause for the development and narrowing of the SR. Therefore, we assume that the etiology of SR is multifactorial.

In conclusion, SRs might be overlooked in endoscopic and/or radiological examinations. Therefore, in patients with a typical clinical presentation, a second diagnostic imaging should be considered. Other esophageal disorders are frequently observed and should be kept in mind; most of which do not alter clinical presentation. Non-specific motility disorders and eosinophilic esophagitis should be considered, especially when frequent dysphagia or food impaction is present. With regard to the etiology of SRs, the present findings suggest a multifactorial genesis, which supports the inflammation theory as well as the plication theory.

COMMENTS

Background

The Schatzki ring (SR) is the most common cause of episodic dysphagia to solid food. Nevertheless its etiology and pathogenesis remains unknown and little is known about its association with other structural and functional abnormalities of the esophagus. Theories regarding its origin include inflammatory, developmental, and congenital factors as the most likely events leading to a circular constriction of the esophagogastric junction. In addition, the clinical importance of associated disorders has not been described.

Research frontiers

Currently, the 'inflammation theory' with gastroesophageal reflux disease (GERD) as the main cause of inflammation, is the most popular theory. However, prospective studies have documented an association with GERD in less than two thirds of patients, suggesting that additional pathogenic factors might be responsible for the development of the SR.

Innovations and breakthroughs

It is known that SRs are associated with hiatal hernias and reflux esophagitis, whereas this is the first study that could show the frequent association of SRs with additional esophageal disorders, most of which do not alter clinical presentation. Nonspecific motility disorders and eosinophilic esophagitis should be considered, especially when frequent dysphagia or food impactions are present, respectively. In regards to the etiology of SRs the present findings suggest a multifactorial pathogenesis. Furthermore, despite the typical clinical presentation SRs might be overlooked in endoscopic and /or radiological examinations.

Applications

SRs are frequently associated with other esophageal disorders. These should be sought, especially when frequent dysphagia or food impaction is the presenting symptom. SRs might be overlooked on radiographic or endoscopic examinations, therefore, a second diagnostic modality should be used when suspicion remains high.

Terminology

"Stiennon's plication theory", postulated that longitudinal shortening in the presence of an hiatal hernia may lead to folding of a redundant esophageal mucosa, creating the SR.

Peer review

The manuscript reads very well and flows nicely. The conclusions are supported by the data and the limitations are well addressed. In addition, the study is novel and the topic chosen is original.

REFERENCES

1 **Kramer P**. Frequency of the asymptomatic lower esophageal contractile ring. *N Engl J Med* 1956; **254**: 692-694
2 **Keyting WS**, Baker GM, Mccarver RR, Daywitt AL. The lower esophagus. *Am J Roentgenol Radium Ther Nucl Med* 1960; **84**: 1070-1075
3 **Goyal RK**, Glancy JJ, Spiro HM. Lower esophageal ring. 1. *N Engl J Med* 1970; **282**: 1298-1305
4 **Schatzki R**, GARY JE. The lower esophageal ring. *Am J Roentgenol Radium Ther Nucl Med* 1956; **75**: 246-261
5 **Schatzki R**. The lower esophageal ring. long term follow-up of symptomatic and asymptomatic rings. *Am J Roentgenol*

Radium Ther Nucl Med 1963; **90**: 805-810
6 **Schatzki R**, Gary JE. Dysphagia due to a diaphragm-like localized narrowing in the lower esophagus (lower esophageal ring). *Am J Roentgenol Radium Ther Nucl Med* 1953; **70**: 911-922
7 **Templeton FE**. X-ray examination of the stomach: a description of the roentgenologic anatomy, physiology and pathology of the esophagus, stomach and duodenum. Chicago: University of Chicago Press, 1944
8 **Longstreth GF**. Familial lower esophageal rings. *N Engl J Med* 1982; **307**: 443
9 **Goyal RK**, Bauer JL, Spiro HM. The nature and location of lower esophageal ring. *N Engl J Med* 1971; **284**: 1175-1180
10 **Edelson ZC**, Rosenblatt MS. Hiatal hernia and lower esophageal ring. *Am J Surg* 1962; **104**: 879-882
11 **Stiennon OA**. The anatomic basis for the lower esophageal contraction ring. plication theory and its applications. *Am J Roentgenol Radium Ther Nucl Med* 1963; **90**: 811-822
12 **Rinaldo JA**, Gahagan T. The narrow lower esophageal ring: pathogenesis and physiology. *Am J Dig Dis* 1966; **11**: 257-265
13 **Ott DJ**, Gelfand DW, Wu WC, Castell DO. Esophagogastric region and its rings. *AJR Am J Roentgenol* 1984; **142**: 281-287
14 **Tobin RW**. Esophageal rings, webs, and diverticula. *J Clin Gastroenterol* 1998; **27**: 285-295
15 **Heitmann P**, Wolf BS, Sokol EM, Cohen BR. Simultaneous cineradiographic-manometric study of the distal esophagus: small hiatal hernias and rings. *Gastroenterology* 1966; **50**: 737-753
16 **Wright RA**, Hurwitz AL. Relationship of hiatal hernia to endoscopically proved reflux esophagitis. *Dig Dis Sci* 1979; **24**: 311-313
17 **Savary M**, Miller G. The esophagus: Handbook and atlas of endoscopy. Solothurn, Switzerland: Verlag Gassmann AG, 1978: 135-139
18 **Khan S**, Orenstein SR, Di Lorenzo C, Kocoshis SA, Putnam PE, Sigurdsson L, Shalaby TM. Eosinophilic esophagitis: strictures, impactions, dysphagia. *Dig Dis Sci* 2003; **48**: 22-29
19 **Kahrilas PJ**, Clouse RE, Hogan WJ. American Gastroenterological Association technical review on the clinical use of esophageal manometry. *Gastroenterology* 1994; **107**: 1865-1884
20 **Clouse RE**, Staiano A. Manometric patterns using esophageal body and lower sphincter characteristics. Findings in 1013 patients. *Dig Dis Sci* 1992; **37**: 289-296
21 **Mitre MC**, Katzka DA, Brensinger CM, Lewis JD, Mitre RJ, Ginsberg GG. Schatzki ring and Barrett's esophagus: do they occur together? *Dig Dis Sci* 2004; **49**: 770-773
22 **Frileux C**, Gillot C, Choquart P. [Ischemia or acute vascular stasis of a limb related to psychopathological behavior. Pathominia]. *J Chir* (Paris) 1976; **111**: 163-166
23 **Ott DJ**, Chen YM, Wu WC, Gelfand DW, Munitz HA. Radiographic and endoscopic sensitivity in detecting lower esophageal mucosal ring. *AJR Am J Roentgenol* 1986; **147**: 261-265
24 **Castell DO**, Richter JE. The esophagus. Philadelphia: Lipinoctt Williams & Wilkins, 1999
25 **Nurko S**, Teitelbaum JE, Husain K, Buonomo C, Fox VL, Antonioli D, Fortunato C, Badizadegan K, Furuta GT. Association of Schatzki ring with eosinophilic esophagitis in children. *J Pediatr Gastroenterol Nutr* 2004; **38**: 436-441
26 **Desai TK**, Stecevic V, Chang CH, Goldstein NS, Badizadegan K, Furuta GT. Association of eosinophilic inflammation with esophageal food impaction in adults. *Gastrointest Endosc* 2005; **61**: 795-801
27 **Marshall JB**, Kretschmar JM, Diaz-Arias AA. Gastroesophageal reflux as a pathogenic factor in the development of symptomatic lower esophageal rings. *Arch Intern Med* 1990; **150**: 1669-1672
28 **Ott DJ**, Ledbetter MS, Chen MY, Koufman JA, Gelfand DW. Correlation of lower esophageal mucosal ring and 24-h pH monitoring of the esophagus. *Am J Gastroenterol* 1996; **91**: 61-64
29 **Wills JC**, Hilden K, Disario JA, Fang JC. A randomized, prospective trial of electrosurgical incision followed by rabeprazole versus bougie dilation followed by rabeprazole of symptomatic esophageal (Schatzki's) rings. *Gastrointest Endosc* 2008; **67**: 808-813
30 **Sgouros SN**, Vlachogiannakos J, Karamanolis G, Vassiliadis K, Stefanidis G, Bergele C, Papadopoulou E, Avgerinos A, Mantides A. Long-term acid suppressive therapy may prevent the relapse of lower esophageal (Schatzki's) rings: a prospective, randomized, placebo-controlled study. *Am J Gastroenterol* 2005; **100**: 1929-1934
31 **Eckardt V**, Dagradi AE, Stempien SJ. The esophagogastric (Schatzki) ring and reflux esophagitis. *Am J Gastroenterol* 1972; **58**: 525-530
32 **DeVault KR**. Lower esophageal (Schatzki's) ring: pathogenesis, diagnosis and therapy. *Dig Dis* 1996; **14**: 323-329

S- Editor Tian L **L- Editor** Kerr C **E- Editor** Ma WH

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/1007-9327office
wjg@wjgnet.com
doi:10.3748/wjg.v17.i23.2844

World J Gastroenterol 2011 June 21; 17(23): 2844-2847
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

BRIEF ARTICLE

Factors influencing lower esophageal sphincter relaxation after deglutition

Lita Tibbling, Per Gezelius, Thomas Franzén

Lita Tibbling, Thomas Franzén, Department of Surgery, University of Linköping, SE-581 85 Linköping, Sweden
Per Gezelius, Department of Surgery, University of Linköping, SE-581 85; SynMed, SE-117 43 Stockholm, Sweden
Author contributions: Tibbling L, Gezelius P and Franzén T contributed equally to this work; Tibbling L and Franzén T participated in the development, implementation and management of this project and were involved in drafting the manuscript; Gezelius P participated in the analysis of the high-resolution manometry readings and in drafting this part of the manuscript.
Correspondence to: Dr. Thomas Franzén, Department of Surgery, Linköping University Hospital, SE 581 85 Linköping, Sweden. thomas.franzen@lio.se
Telephone: +46-10-1030000 Fax: +46-10-1043216
Received: December 1, 2010 Revised: March 1, 2011
Accepted: March 8, 2011
Published online: June 21, 2011

Abstract

AIM: To study the relationship between upper esophageal sphincter (UES) relaxation, peristaltic pressure and lower esophageal sphincter (LES) relaxation following deglutition in non-dysphagic subjects.

METHODS: Ten non-dysphagic adult subjects had a high-resolution manometry probe passed transnasally and positioned to cover the UES, the esophageal body and the LES. Ten water swallows in each subject were analyzed for time lag between UES relaxation and LES relaxation, LES pressure at time of UES relaxation, duration of LES relaxation, the distance between the transition level (TL) and the LES, time in seconds that the peristaltic wave was before (negative value) or after the TL when the LES became relaxed, and the maximal peristaltic pressure in the body of the esophagus.

RESULTS: Relaxation of the LES occurred on average 3.5 s after the bolus had passed the UES and in most cases when the peristaltic wave front had reached the TL. The LES remained relaxed until the peristaltic wave faded away above the LES.

CONCLUSION: LES relaxation seemed to be caused by the peristaltic wave pushing the bolus from behind against the LES gate.

Key words: Deglutition; Lower esophageal sphincter; Peristalsis; Relaxation; Upper esophageal sphincter

Peer reviewer: Kevin Michael Reavis, MD, Assistant Clinic Professor, Department of Surgery, Division of Gastrointestinal Surgery, University of California, Irvine Medical Center, 333 City Boulevard West, Suite 850, Orange, CA, United States

Tibbling L, Gezelius P, Franzén T. Factors influencing lower esophageal sphincter relaxation after deglutition. *World J Gastroenterol* 2011; 17(23): 2844-2847 Available from: URL: http://www.wjgnet.com/1007-9327/full/v17/i23/2844.htm DOI: http://dx.doi.org/10.3748/wjg.v17.i23.2844

INTRODUCTION

Swallowing dysfunction with esophageal food retention is a common problem in an adult population with hiatus hernia. Most articles reporting lower esophageal sphincter (LES) studies depict LES relaxation caused by neurogenic mechanisms[1] in gastroesophageal reflux (GER). The ability of the LES to allow esophagogastric transit after deglutition has received little attention. Efforts have been made to prove that LES relaxation is triggered by a neurogenic reflex following deglutition[2], that the LES and the upper esophageal sphincter (UES) relaxations are simultaneous events and that the cervical portion of the vagus nerve mediates inhibitory and excitatory changes in LES pressure[3]. In a recent study, a biodynamic approach was proposed for LES opening when GER takes place in hiatal hernia and the gastric wall tension pulls open the LES[4]. As long ago as 1978[5], LES was suggested to be a biodynamic gate which is forced to open when a bolus is propelled by esophageal peristalsis. We decided to further

challenge the idea of LES as an esophagogastric pressure zone dependent on complex neuro-humoral factors.

The recent development of high-resolution solid-state manometry (HRM) systems with closely spaced circumferential pressure sensors has made it possible to display simultaneous recordings along the entire esophagus in color-coded pressure plots (Figure 1) and has dramatically improved the diagnostic assessment of esophageal function and disease. With conventional esophageal manometry, simultaneous events in the UES and LES have been difficult to display especially since deglutition often will displace the pressure sensors due to shortening of the esophagus. The HRM technique enables, therefore, a unique possibility to study UES and LES pressure during the entire deglutition period and independent of any sphincter dislocation. In order to find out if there is an interplay between pulling forces and LES opening, the aim was therefore to study the relationship between UES relaxation, peristaltic pressure and LES relaxation following deglutition using the HRM technique.

MATERIALS AND METHODS

The HRM system (ManoScan 360 A-100, ManoView analysis software ver. 2.0.1 from Sierra Scientific Instruments Inc., Los Angeles, CA) uses a solid state catheter (Ø 4.2 mm) with closely spaced circumferential pressure sensors with 1 cm intervals over 36 cm. The HRM catheter was passed transnasally and positioned to be recording simultaneously from the hypopharynx, through the body of the esophagus, to the stomach. The catheter was calibrated outside the patient before and immediately after the investigation using the thermal compensation option in the software.

We performed a prospective HRM study in 10 adult patients (median age 45 years, range 38-63 years; 7 women, 3 men) who were admitted to an esophageal laboratory for suspected dyspepsia. It can be claimed that our patients with dyspepsia are not representative for studies of normal LES and UES function. Dyspepsia is a diagnosis without organic lesion and with diffuse symptoms predominantly in the gastric region. The patients did not have any symptoms of dysphagia and reflux, they were free of medication, and the presence of hiatus hernia was excluded at gastroscopy and HRM. It is therefore regarded that confounding factors have been excluded in the study material.

The investigation comprised ten swallows of a 10 mL water bolus in a supine position. The characteristics analyzed were: (1) the time lag between UES relaxation and LES relaxation; (2) the LES pressure at time of UES relaxation; (3) the duration of LES relaxation; (4) the length of the esophagus between UES and LES; (5) the distance between the transition level (TL) and the LES; (6) time in seconds that the peristaltic front wave was before (negative value) the TL or after the TL when the LES pressure dropped to nadir; and (7) the maximal peristaltic pressure in the esophagus. The eight best readable swallow recordings in each subject (a total of 78 swallows) were calculated upon, and the mean values of each individual item are given.

TL is defined as an esophageal zone with striated-to-smooth muscle fiber transition[6] showing as a short loss of peristaltic pressure (Figure 1).

RESULTS

Individual values and the mean of all values are presented in Table 1. The LES relaxed on average 3.5 s after the UES had opened. The mean LES pressure was 26 mmHg at time of UES opening. The mean duration of LES remaining relaxed was 6.7 s. The mean length of the esophagus between UES and LES was 26 cm. The mean distance between the TL and the LES was 18 cm, corresponding to on average 72% of the total length. The mean time that the peristaltic wave was before (negative value) the TL or after the TL when the LES opened was -0.5 s. The mean maximum pressure of the peristaltic wave was 138 mmHg. The time lag between UES and LES relaxation was never over 6.4 s. The individual LES pressure, as well as the maximum peristaltic pressure, varied remarkably from swallow to swallow (Table 1).

DISCUSSION

This study shows quite clearly that the LES becomes relaxed several seconds (on average 3.5 s) after the water bolus has passed the UES. This corresponds with findings by Nguyen *et al*[7] when impedance and manometry techniques were used. They found a mean latency between bolus entry into the esophagus and LES relaxation of 3.6 s. This is also in agreement with the esophageal transit time (3.8 s) as assessed with the biomagnetic technique[8]. The LES pressure did not change in our study at time of UES opening. Therefore, it seems unlikely that LES relaxation is triggered by the start of deglutition.

It seems as if opening of the LES coincides with the peristaltic wave front reaching close to the TL, which is an esophageal level with striated-to-smooth muscle fiber transition. Reasonably, this depends on the premise that the distal end of the water bolus has reached the LES, that the water bolus fills up the esophageal lumen between TL and LES, and that the peristaltic wave pushes the water bolus from behind against the LES gate. Only the combination of manometry and impedance measurement or the combination of HRM and radiography can find out whether LES relaxation and opening take place at the moment when the bolus arrives at the LES. In an impedance study of LES opening and bolus transit, it was found that LES relaxation seemed to be modulated by the bolus transit and occurred predominantly upon arrival of the bolus in the distal esophagus[7]. This discovery clashes with the findings by Pandolfino *et al*[9] who used a combined impedance/manometry technique. They showed that LES relaxation did not necessary coincide with bolus passage or LES opening. The hydrostatic pressure in the esophageal body necessary to open the LES in an upright position, as shown in a combined manometric and radiographic study from 1978[5], was approximately the same as the LES pressure before opening. If no deglutition activ-

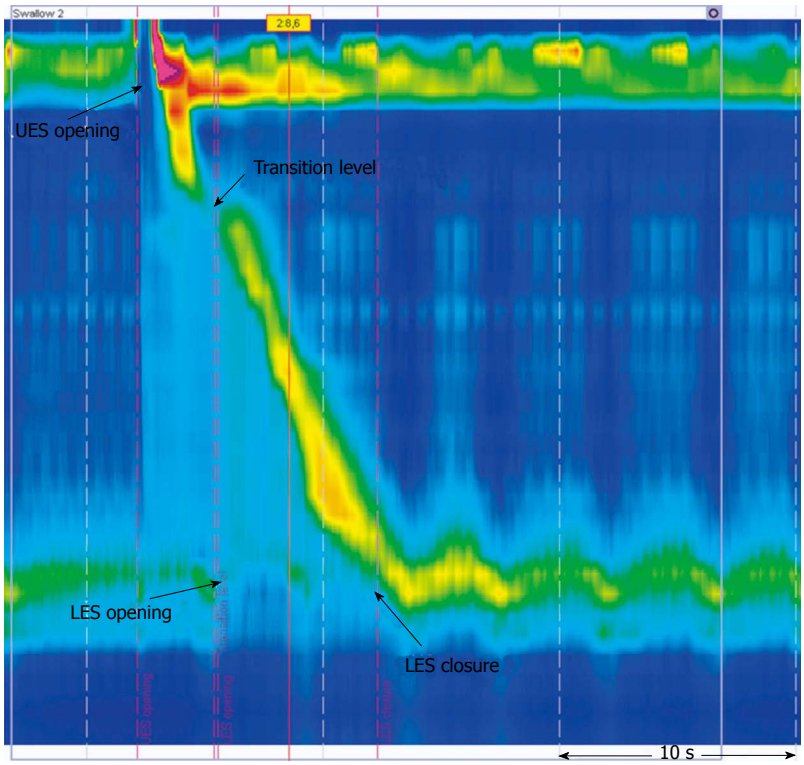


Figure 1 An high-resolution solid-state manometry recorded swallowing event. Transition level = TL, LES relaxation = LES opening to LES closure. Red ⩾ 60 mmHg. Blue ⩽ 0 mmHg. LES fluctuations represent respiration, upwards expiration, downwards inspiration. UES: Upper esophageal sphincter; LES: Lower esophageal sphincter.

Table 1 Different esophageal events in ten subjects

Event	F1	F2	M3	F4	F5	F6	M7	M8	F9	F10	All 10 subjects; mean: ranges
(A) s	2.6	5.2	5.0	3.6	3.8	3.2	3.6	1.7	3.0	3.5	3.5: 1.7-5.2
(B) mmHg	21	19	20	32	13	24	23	52	29	29	26: 13-52
(C) s	5.1	4.2	4.2	6.7	5.5	7.7	10.3	8.5	7.7	7.5	6.7: 4.2-10.3
(D) cm	25	26	28	25	29	23	26	26	22	26	26: 22-29
(E) cm	16	17	20	18	20	17	19	20	16	18	18: 16-20
(F) s	-1.4	0.7	0.9	0.3	0.5	-0.2	-1.4	-2.0	-1.6	-0.3	-0.5: -2.0-0.9
(G) mmHg	109	121	123	71	102	232	187	169	152	116	138: 71-232

Different esophageal events, mean values of ten recordings in ten subjects, and mean values of all ten subjects. A: Time lag between relaxing of UES and LES; B: LES pressure when UES opened; C: Duration of LES remaining relaxed; D: Esophagus length; E: Distance between TL and LES; F: Time in seconds that the peristaltic wave was before (minus value) or after the TL when the LES relaxed; G: maximum pressure of the peristaltic wave. F: Female; M: Male. UES: Upper esophageal sphincter; LES: Lower esophageal sphincter; TL: Transition level defined as the zone when the striated muscle layer transitions into the smooth muscle layer.

ity took place and a contrast medium was instilled into the esophagus, the LES opened when the hydrostatic pressure exceeded the resting LES pressure and closed again when the hydrostatic pressure in an upright position fell short of the LES pressure. Deglutition caused the LES to relax and open when the peristaltic wave reached the upper level of the infused contrast medium. These different findings indicate that the LES is a barrier which is forced to open by the peristaltic pressure. In the clinic, this would explain why, for instance, patients with achalasia cardia or with lack of esophageal peristalsis do not have any LES relaxation after deglutition.

If we look upon the LES as a gate that will be pulled open, it is of interest to compare the pressure of the LES and the maximum pressure of the peristaltic wave in the distal esophagus. In this study, the peristaltic pressure was five times stronger than the LES pressure. HRM and conventional manometry are claimed to be the same in their measurement of LES resting pressure[10]. It has been shown that the longitudinal esophageal muscle is contracted during the peristaltic activity[11] which will decrease esophageal wall compliance. Certainly, the peristaltic force displays an interaction between the longitudinal and circular esophageal muscles[12], and a compliance decrease of the esophageal wall will facilitate bolus transit. The reason for the deglutition-induced pressure overload in the esophageal body is therefore of importance, in order to overcome the stiffness of the esophageal wall. In the study by Babaei *et al*[12], it was

even proposed that the longitudinal esophageal smooth muscle has an important role in the relaxation of LES. In our study, the LES remained relaxed as long as the peristaltic wave was present in the esophagus. This indicates that there is a close interplay between LES function and peristaltic activity. It is reasonable to believe that the LES will be pulled open when the pulling direction is either from the esophagus or from the stomach[12].

In conclusion, LES opening seems to be caused by the peristaltic wave pushing the bolus from behind against the LES gate. Therapeutic attention in patients with dysphagia of non-stricture origin should therefore be focused on esophageal motility function rather than on drugs affecting LES pressure.

COMMENTS

Background

Swallowing problems with food retention in the gullet are present in at least 8% of an adult population. For accurate treatment, it is of importance to know whether transit from the esophagus to the stomach can be treated with drugs aimed at opening the lower esophageal sphincter (LES) or whether transit is due to dynamic properties of gullet muscles. So far, it has been claimed that LES is triggered to open by a neurogenic reflex from the upper esophageal sphincter (UES).

Research frontiers

The newly developed high-resolution manometry (HRM) system is a technical innovation and breakthrough for the understanding of dynamic esophageal events, meaning the interplay between esophageal motility and esophageal sphincter relaxations.

Innovations and breakthroughs

The esophageal HRM probe was used in ten non-dysphagic patients with dyspepsia in order to study the time relationship between UES and LES relaxation and to study where the peristaltic wave front was located when the LES relaxed after deglutition of a 10 mL water bolus in the supine position. These simultaneous activities have previously not been possible to study with conventional esophageal manometry.

Applications

The LES was shown to relax 3.5 s later than the UES after deglutition which is the average time it takes a water bolus front to reach the LES. The LES remained relaxed on average 6.8 s; that is until the propulsive force had faded away. The peristaltic front wave reached a level close to the transition level (TL), either 2 s before or 1 s after the TL.

Terminology

Esophagus-gullet. Dyspepsia-a diagnosis without any specified organic lesion and with diffuse symptoms from the gastric region. Dysphagia-swallowing difficulties. HRM-a pressure catheter with a 36 cm long segment of sensors spaced 1 cm apart giving simultaneous pressure information of sphincter and muscular activity from a total of 432 locations from UES to LES. The pressure can be displayed as a color plot offering visual information of the esophagus and its sphincters very similar to an anatomic picture. LES-lower esophageal sphincter. Manometry-pressure measurement. Smooth muscle-a muscle under autonomic, non-volitional control. Striated muscle-a muscle under volitional control. TL, transition level-the level in the esophagus between the upper striated muscle level and the lower smooth muscle level which is located about 7 cm distal of the UES.

Peer review

More emphasis on why HRM is a great technique and the knowledge it provides compared to conventional esophageal manometry has now been given in the Methods section. The reason why we believe that dyspepsia patients can be regarded as normal people with regard to esophageal transit studies is addressed in the Materials section. The previous conclusion, regarding older theories of a neurogenic reflex causing LES relaxation, has been omitted and basic data for this are given in the discussion.

REFERENCES

1 **Tuch A**, Cohen S. Lower esophageal sphincter relaxation: studies on the neurogenic inhibitory mechanism. *J Clin Invest* 1973; **52**: 14-20

2 **Bardan E**, Saeian K, Xie P, Ren J, Kern M, Dua K, Shaker R. Effect of pharyngeal stimulation on the motor function of the esophagus and its sphincters. *Laryngoscope* 1999; **109**: 437-441

3 **Matarazzo SA**, Snape WJ Jr, Ryan JP, Cohen S. Relationship of cervical and abdominal vagal activity to lower esophageal sphincter function. *Gastroenterology* 1976; **71**: 999-1003

4 **Fein M**, Ritter MP, DeMeester TR, Oberg S, Peters JH, Hagen JA, Bremner CG. Role of the lower esophageal sphincter and hiatal hernia in the pathogenesis of gastroesophageal reflux disease. *J Gastrointest Surg* 1999; **3**: 405-410

5 **Ask P**, Sökjer H, Tibbling L. Mechanisms affecting lower oesophageal sphincter opening and oesophageal retention. A combined X-ray and manometry study. *Scand J Gastroenterol* 1978; **13**: 857-861

6 **Ghosh SK**, Janiak P, Schwizer W, Hebbard GS, Brasseur JG. Physiology of the esophageal pressure transition zone: separate contraction waves above and below. *Am J Physiol Gastrointest Liver Physiol* 2006; **290**: G568-G576

7 **Nguyen HN**, Domingues GR, Winograd R, Lammert F, Silny J, Matern S. Relationship between bolus transit and LES-relaxation studied with concurrent impedance and manometry. *Hepatogastroenterology* 2006; **53**: 218-223

8 **Daghastanli NA**, Braga FJ, Oliveira RB, Baffa O. Oesophageal transit time evaluated by a biomagnetic method. *Physiol Meas* 1998; **19**: 413-420

9 **Pandolfino JE**, Shi G, Zhang Q, Ghosh S, Brasseur JG, Kahrilas PJ. Measuring EGJ opening patterns using high resolution intraluminal impedance. *Neurogastroenterol Motil* 2005; **17**: 200-206

10 **Ayazi S**, Hagen JA, Zehetner J, Ross O, Wu C, Oezcelik A, Abate E, Sohn HJ, Banki F, Lipham JC, DeMeester SR, Demeester TR. The value of high-resolution manometry in the assessment of the resting characteristics of the lower esophageal sphincter. *J Gastrointest Surg* 2009; **13**: 2113-2120

11 **Tibbling L**, Ask P, Pope CE 2nd. Electromyography of human oesophageal smooth muscle. *Scand J Gastroenterol* 1986; **21**: 559-567

12 **Babaei A**, Bhargava V, Korsapati H, Zheng WH, Mittal RK. A unique longitudinal muscle contraction pattern associated with transient lower esophageal sphincter relaxation. *Gastroenterology* 2008; **134**: 1322-1331

S- Editor Sun H **L- Editor** Logan S **E- Editor** Ma WH

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/1007-9327office
wjg@wjgnet.com
doi:10.3748/wjg.v17.i23.2848

World J Gastroenterol 2011 June 21; 17(23): 2848-2854
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

BRIEF ARTICLE

Effects of sargentgloryvine stem extracts on HepG-2 cells *in vitro* and *in vivo*

Ming-Hua Wang, Min Long, Bao-Yi Zhu, Shu-Hui Yang, Ji-Hong Ren, Hui-Zhong Zhang

Ming-Hua Wang, Min Long, Bao-Yi Zhu, Shu-Hui Yang, Ji-Hong Ren, Hui-Zhong Zhang, Department of Laboratory Medicine and Research Center, Tangdu Hospital, Fourth Military Medical University, Xi'an 710038, Shaanxi Province, China
Author contributions: Wang MH and Long M contributed equally to this work; Wang MH and Long M designed the study, prepared the Sargentgloryvine stem extract, performed the statistical analysis and drafted the manuscript; Zhu BY performed the animal model studies; Yang SH performed the protein expression studies; Ren JH conducted the flow cytometry; Zhang HZ conceived the study and also participated in the study design and coordination; all authors have read and approved the final manuscript.
Supported by National Science and Technology Key Project for the Development of New Drugs in China, No. 2009ZX09103-422
Correspondence to: Hui-Zhong Zhang, MD, PhD, Department of Laboratory Medicine and Research Center, Tangdu Hospital, Fourth Military Medical University, Xinsi Road, Xi'an 710038, Shaanxi Province, China. zhz328@yahoo.com.cn
Telephone: +86-29-84777470 Fax: +86-29-84777654
Received: January 24, 2011 Revised: February 24, 2011
Accepted: March 3, 2011
Published online: June 21, 2011

Abstract

AIM: To observe the effects of sargentgloryvine stem extracts (SSE) on the hepatoma cell line HepG-2 *in vitro* and *in vivo* and determine its mechanisms of action.

METHODS: Cultured HepG-2 cells treated with SSE were analysed by 3-(4,5-Dimethyl-thiazol-2-yl)-2,5-Diphenyltetrazolium bromide and clone formation assay. The cell cycle and apoptosis analysis were conducted by flow cytometric, TdT-Mediated dUTP Nick End Labeling and acridine orange/ethidium bromide staining methods, and protein expression was examined by both reverse transcriptase-polymerase chain reaction and Western blotting. The pathological changes of the tumor cells were observed by haematoxylin and eosin staining. Tumor growth inhibition and side effects were determined in a xenograft mouse model.

RESULTS: SSE treatment could not only inhibit HepG-2 cell proliferation in a dose- and time-dependent manner but also induce apoptosis and cell cycle arrest at the S phase. The number of colonies formed by SSE-treated tumor cells was fewer than that of the controls ($P < 0.05$). SSE induced caspase-dependent apoptosis accompanied by a significant decrease in Bcl-xl and Mcl-1 and elevation of Bak expression ($P < 0.05$). Tumor necrosis factor α in the xenograft tumor tissue and the liver functions of SSE-treated mice showed no significant changes at week 8 compared with the control group ($P > 0.05$). Systemic administration of SSE could inhibit the HepG-2 xenograft tumor growth with no obvious toxic side effects on normal tissues.

CONCLUSION: SSE can induce apoptosis of HepG-2 cells in vitro and in vivo through decreasing expression of Bcl-xl and Mcl-1 and increasing expression of Bax.

Key words: Sargentgloryvine stem extract; Apoptosis; Human hepatocellular carcinoma; HepG-2; *Bcl-2* family

Peer reviewer: Fritz von Weizsäcker, Professor, Department of Medicine Schlosspark-Klinik, Humboldt University, Heubnerweg 2, Berlin D-14059, Germany

Wang MH, Long M, Zhu BY, Yang SH, Ren JH, Zhang HZ. Effects of sargentgloryvine stem extracts on HepG-2 cells *in vitro* and *in vivo*. *World J Gastroenterol* 2011; 17(23): 2848-2854 Available from: URL: http://www.wjgnet.com/1007-9327/full/v17/i23/2848.htm DOI: http://dx.doi.org/10.3748/wjg.v17.i23.2848

INTRODUCTION

Although significant progress has been made over the past decades in cancer prevention and treatment, the development of effective treatment regimens remains one of the greatest challenges in the area of cancer che-

motherapy. Recently, plant-derived natural products are becoming important as anti-cancer derivatives, including vincristine, vinblastine, paclitaxel and camptothecin, which are invaluable contributions of nature to modern medicine[1-4]. However, the quest to find novel therapeutic compounds for cancer treatment is a never-ending venture, and diverse plant species are being studied to identify prospective anti-cancer agents[5,6]. Sargentgloryvine stem of *Sargentodoxa cunneata* (Oliv.) has been widely used as an ingredient in Chinese medicine according to the Chinese herbal medicine principles for thousands of years in the treatment of several kinds of diseases, such as chronic pelvic cavity inflammation, rheumatism and appendicitis. Sargentgloryvine stem extract (SSE) as a chemotherapeutic adjuvant can enhance the efficacy and ameliorate the side effects of cancer chemo- or radio-therapy. Because SSE has been used in Chinese herbal medicine as a bioactive constituent in a complex herbal mixture, an important question is whether its biological activity can be largely or exclusively ascribed to one or more individual compounds present in this herb. To address this question, we prepared SSE and studied its effects. Our previous research showed that SSE possesses potent anticancer activities[7]. However, the molecular mechanisms underlying the anticancer effects of SSE are poorly understood and need to be elucidated. To identify potential anticancer mechanisms of SSE in human hepatocellular carcinoma (HCC), the molecular effects of SSE on HepG-2 cells were examined. Down-regulation of the two anti-apoptotic Bcl-2 family proteins Bcl-xl and Mcl-1 may be responsible for antiproliferative and cell apoptotic effects of SSE on HepG-2 cells. Meanwhile, HepG-2 xenograft nude mice treated systemically with SSE were also monitored in tumor growth inhibition and toxicity *in vivo*. The purpose of this study was to observe the effects of SSE on the hepatoma cell line HepG-2 *in vitro* and *in vivo*, and preliminarily analyse its mechanisms of action.

MATERIALS AND METHODS

Materials

SSE was extracted from 10 g dried powder of *Sargentgloryvine* stem in a rotary shaker with 200 mL of 50 mL/L ethanol at 60℃ for 24 h. The extract was then filtered, concentrated using a rotary evaporator to remove the solvent, and finally lyophilised in a freeze-dryer to obtain crude freeze-dried powder. The same batch of SSE was used in all studies in order to keep the results reliable. The powder was dissolved in DMEM culture medium at a stock solution of 5 g/L for further use. The hepatoma cell line HepG-2 was preserved in our laboratory. Specific pathogen-free male athymic BALB/c nude mice of 6-7 wk of age with a body mass of 20-30 g were purchased from the Animal Experimental Centre of the Fourth Military Medical University. HepG-2 cells were cultured in DMEM supplemented with 100 mL/L foetal calf serum, 100 kU/L penicillin, 0.1 g/L streptomycin and 250 μg/L amphotericin B, incubated at 37℃ in a humified atmosphere of 50 mL/L carbon dioxide. The primers for PT-PCR detection of transcript are described in Table 1.

Methods

Cell viability and clone formation assay: HepG-2 and ECV304 cells (control cells) were cultured in 96-well plates at a density of 3×10^8 cells/L with 100 μL per well in DMEM. Cells were treated with 1.5, 15, 45, 60 and 90 mg/L SSE for 6, 12, 24, 36 and 48 h or medium only as a control (DMEM-treated) group. After incubation, cell proliferation was detected by 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, and cytotoxicity was studied with a commercial assay kit (Beyotime Biotechnology, China) following the manufacturer's instructions. Absorbance was measured at 570 nm using the Bio-Rad 550 microplate reader. The protracted cell growth curves and the inhibition of cell growth were calculated based on the absorbance (A) value as follows: inhibitory rate = $(1-A_{\text{treated}}/A_{\text{control group}}) \times 100\%$. For the colony formation assay, a total of 3×10^2 HepG-2 and control cells, and ECV304 tumor cells were plated in 75 mm culture dishes and treated with SSE at a concentration of 30 mg/L. After incubation for an additional 10-14 d, the cells were fixed with methanol and stained with 1 g/L crystal violet (Sigma, USA), and colonies of > 50 cells were manually counted. All experiments were performed in independent triplicates.

Cell apoptosis and cycle analysis: For apoptosis, HepG-2 cells and ECV304 cells were treated with SSE at concentrations of 15, 30 or 60 mg/L, or no drug as a control for 24 h. The cells were harvested and washed twice with cold PBS and resuspended in binding buffer. FITC-labelled annexin V and propidium iodide (PI) were added and incubated for 10 min at room temperature, and the cell suspensions were immediately analysed by flow cytometry. For cell cycle detection, the cells treated with 45 mg/L SSE for 24 h were fixed with 700 mL/L cold ethanol and resuspended in phosphate-buffered saline containing 20 mg/L PI and then analysed for PI fluorescence intensity by flow cytometry to measure the cellular DNA content. The HepG-2 cells were suspended in 75 mm plates and treated with SSE at a concentration of 45 mg/L for 24 h or an equal volume of culture medium as the control. The total volume of each well was one mL. The cells were collected, and a TdT-Mediated dUTP Nick End Labeling assay (Keygen Biotech Co., Ltd., Nanjing, China) was performed as suggested by the manufacturer to detect the incorporation of labelled nucleotides into DNA. At least 300 cells were counted under a light microscope, and apoptotic cells were identified. All experiments were performed in triplicate. The negative control cells were set up with no TdT enzyme added, and positive control cells were pretreated with DNase during the staining process.

Apoptosis quantification and $\Delta\psi$m detection: HepG-2 cells were cultured in 6-well plates and treated with SSE at a concentration of 45 mg/L for 24 h. Acridine orange/ethidium bromide staining was performed following the

Table 1 The primers for detection of transcript

Gene	Sense sequence 5'-3'	Antisense sequence 5'-3'
β-actin	GACTTAGTTGCGTTACACCTTTC	TGCTGTCACCTTCACCGTTC
Bax	ATGGACGGGTCCGGGGAG	TCAGCCCATCTTCTTCCAGAT
Bak	ATGGCTTCGGGGCAAGGC	TCATGATTTGAAGAATCTTCGTACC
Bal-2	ATGGCGCACGCTGGGAGAACG	GTACTCAGTCATCCACAGGGC
Bcl-xl	ATGTCTCAGAGCAACCGGGAGCT	TCATTTCCGACTGAAGAGTGAGC
Mcl-1	TGCCGCTGCTGGAGTTGGT	TTACAGTAAGGCTATCTTATTAGAT
Bcl-w	CTCTGGTGGCAGACTTTGTAG	CCGTCCCCGTATAGAGCTGTGA
Bcl-b	ATGGCCGACTCGCAGGACCCA	TTATAAACGTTTCCATATAAAA
Blf-1	ATGAGTGATCCAGAAACCAG	TTAATCCTCTTCTGAACTTTCA

manufacturer's instructions (Keygen Biotech Co., Ltd., Nanjing, China). Acridine orange is a vital dye and can stain both live and dead cells. Ethidium bromide can only stain the cells that have lost membrane integrity. Live cells appear uniformly green, while early apoptotic cells stain green but contain bright green dots in the nuclei as a consequence of chromatin condensation and nuclear fragmentation. Late apoptotic cells incorporate more ethidium bromides and therefore stain orange and show condensed and often fragmented nuclei. At least 300 cells were counted under a fluorescence microscope to quantify apoptosis. All experiments were performed independently in triplicate. Additionally, HepG-2 cells were harvested following treatment with SSE at 45 mg/L for 12 and 24 h. After washing twice with PBS, 1×10^6 cells were incubated with 10 mg/L of Rh123 (Sigma, USA), a cationic lipophilic fluorochrome that is taken up by mitochondria in proportion to the $\Delta\psi m$, for 30 min at 37℃. Fluorescence intensities were determined by flow cytometry (Becton Dickinson Inc., USA).

Gene expression study: Total RNA from 1×10^6 SSE-treated (45 mg/L for 24 h) HepG-2 cells and control cells were extracted by Trizol™ reagent (Invitrogen, USA), and 1 μg total RNA was used to synthesise cDNA with the superscript first-strand synthesis kit (Takara BioTechnology, Dalian, China) following the manufacturer's instructions. One microliter cDNA was used to amplify the specific genes by reverse transcriptase-polymerase chain reaction (RT-PCR). To normalize cDNA loading, the *β-actin* gene was also amplified from each sample. RT-PCR was performed with the primers listed above. HepG-2 cells treated with SSE (45 mg/L for 24 h) and control cells were harvested by suspension in lysis buffer. The cell extracts were clarified by centrifugation, and the protein concentrations were determined using the Bio-Rad protein assay kit. Each protein extract (25 μg) was electrophoresed on a 100 g/L SDS-polyacrylamide gel, transferred to a membrane and blocked in 50 g/L skimmed milk in tris buffered saline-Tween 20 for 1 h at room temperature. Membranes were probed with anti-Bax, -Bak, -Bcl-xl, -Mcl-1, -caspase-8, -caspase-9, -cytochrome C and -β-actin antibodies overnight at 4℃. Primary antibodies were removed, and the blots were extensively washed with TBS-T three times. Blots were then incubated for 1 h at 37℃ with the secondary antibodies in 10 g/L skimmed milk dissolved in TBS-T. After removal of the secondary antibody, blots were extensively washed and developed using the enhanced chemiluminescence kit in the dark (Santa Cruz Biotechnology). The primary antibodies used in this experiment were monoclonal mouse anti-human Bax, Bcl-xl, Mcl-1, cytochrome C, caspase-8 and-9 (latter two from Abcam, UK) and β-actin and polyclonal goat anti-human Bak (Santa Cruz). Goat anti-mouse and rabbit anti-goat IgG coupled to horseradish peroxidase (Santa Cruz Biotechnology) were used as secondary antibodies for detection of protein expression.

Solid tumor growth assay: Athymic BALB/c nude mice were housed in laminar flow cabinets under specific pathogen-free conditions. All animal studies were performed in compliance with the Institutional Guidelines of the Fourth Military Medical University. Aliquots of 1.0×10^6 HepG-2 cells were suspended 1:1 in PBS and subcutaneously inoculated into the right flank of each mouse. When 300 mm^3 tumors were observed, the mice were randomly assigned to two groups ($n = 5$). The mice of the treatment group received 18 mg/kg SSE suspended in 50 μL DMEM, and the mice of the control group were treated with an equal volume of DMEM *via* vena caudalis injection every 2 d for 14 d. The tumor volume was measured each time before SSE administration with callipers using a standard formula as follows: $width^2 \times length \times 0.5$. An average tumor volume per mouse was used to calculate the group mean tumor volume ± SD ($n = 5$). Mice were sacrificed 24 h after the last administration of SSE to harvest the tissues of tumors and the heart, liver, spleen, kidneys and lungs. All tissues were then fixed in 40 g/L paraformaldehyde overnight and sectioned, and haematoxylin and eosin staining was performed to identify the toxicity of SSE *in vivo*. The supernatants of nude mice xenografts were preserved at -70℃. After protein concentration determination, tumor necrosis factor α (TNFα) levels were detected by ELISA in tumor tissues from nude mice according to the manufacturer's instructions. Liver function (aspartate aminotransferase and alanine aminotransferase) and kidney function (creatinine) were also detected.

Statistical analysis

All statistical analyses were performed using SPSS13.0. Data were expressed as mean ± SD. Comparisons among all groups were performed with the one-way ANOVA analysis of variance test. Differences were considered significant at $P < 0.05$.

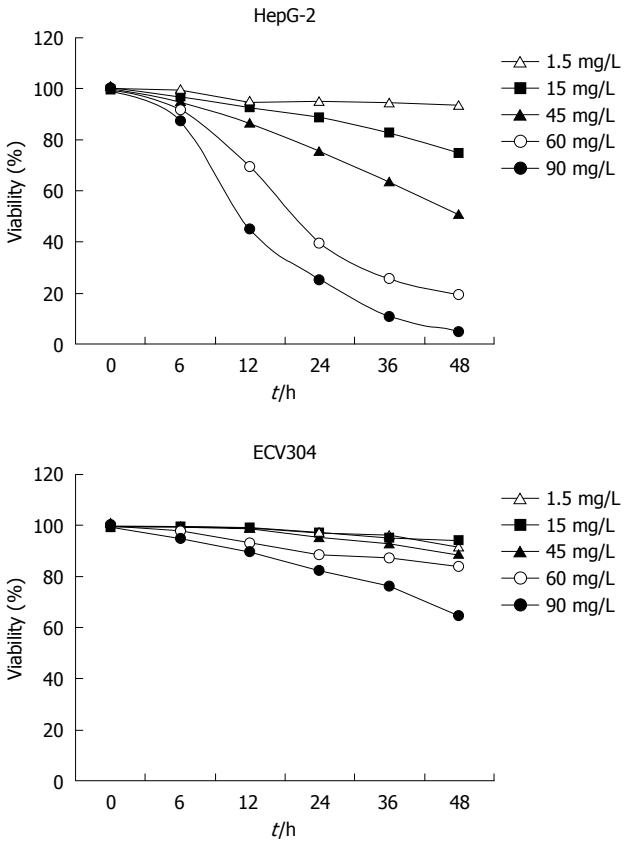


Figure 1 Effects of sargentgloryvine stem extract on HepG-2 viability *in vitro* (MTT assay). HepG-2 and ECV304 cells were treated with sargentgloryvine stem extract (SSE) at five concentrations in five different time points. MTT assay showed that cell proliferation inhibition rates were enhanced with increased SSE concentration and treatment time in HepG-2 cells but not in ECV304 cells. Experiments were repeated in triplicate.

RESULTS

Proliferation and colony formation

SSE-treatment at concentrations of 1.5 and 15 mg/L showed no significant growth inhibition of HepG-2 cells, but higher SSE concentrations of 30, 60 and 90 mg/L significantly inhibited the proliferation of the HepG-2 cells (Figure 1). At an SSE concentration of 30 mg/L, the inhibition rate (mean ± SD) increased with treatment time; the rates were 5.0 ± 1.4, 13.7 ± 2.7, 23.3 ± 6.0, 34.2 ± 5.3 h and 53.7 ± 3 at 6, 12, 24, 36 and 48 h, respectively ($P < 0.05$). All concentrations showed no obvious growth inhibition on ECV304 cells. Additionally, the number of colonies of SSE treated HepG-2 cells was lower than that of the control cells (261 ± 16 *vs* 492 ± 21, Student's *t* test, $P < 0.05$), but no difference was observed on the plates of ECV304 cells. Finally, SSE treatment at 45 mg/L for 24 h significantly increased the proportion of S phase HepG-2 cells from 17.8 ± 1.9 to 63.3 ± 3.3 ($P < 0.05$), but decreased the proportion of G_0/G_1 phase and G_2/M phase cells.

SSE-induced apoptosis

SSE treatment of HepG-2 cells increased the apoptosis rates in a dose-dependent manner (0.2%, 4.7%, 9.5%, 28.7%, as analysed by flow cytometry) (Figure 2). Western blotting analysis showed that SSE treatment (45 mg/L) for 24 h significantly increased caspase-9 but not caspase-8 cleavage (Figure 3), indicating that SSE induces apoptosis through the intrinsic pathway. Additionally, SSE-treated HepG-2 cells lost $\Delta\psi m$, as indicated by a decrease in Rh-123 fluorescence (Figure 4), which was significantly weaker than that of the control cells. Consistent with the results of fluorescence microscopy above, Western blotting analysis demonstrated that the expression of cytochrome C in plasmosin was also altered in SSE-treated HepG-2 cells (Figure 3).

Gene expression

In SSE-treated (45 mg/L) HepG-2 cells, the expression of Bak but not Bax was significantly increased, while the expression levels of Bcl-xl and Mcl-1 were significantly down-regulated compared with the control cells (Figure 5A). Western blotting analysis further confirmed the up-regulation of Bax and down-regulation of Bcl-xl and Mcl-1 (Figure 5B).

Xenograft growth in mice

Compared with DMEM-treated mice, i.v. administration of 18 mg/kg SSE every 2 d for 14 d led to an inhibition of HepG-2 cell growth (Figure 6). No lesions were found in the heart, liver, spleen, lungs or kidneys in SSE-treated xenograft mice, and their functions were also normal. Compared with the control group, the TNFα levels in SSE-treated tumor tissue showed no significant difference at week 8 (9.9 ± 6.8 mg/L *vs* 9.1 ± 5.7 mg/L, $P > 0.05$). In addition, the food intake, mental status and activities were similar in the two groups during the treatment.

DISCUSSION

Recently, the discovery of active compounds from natural products has gained considerable attention as a new source of anticancer drugs[6-10]. The quest to find novel therapeutic compounds for cancer treatment is a never-ending venture. Sargentgloryvine stem, a traditional Chinese medicine, has been used for thousands of years in the treatment of several inflammatory diseases. Recently, we have successfully developed a novel extract from sargentgloryvine stem SSE that has a potent anticancer activity on HCC cells[1]. However, the molecular mechanisms underlying the anticancer effects of SSE have not been elucidated.

Our research shows that SSE can induce apoptosis of the hepatoma cell line HepG-2 *in vitro* and *in vivo*, and its mechanism of action may be through decreasing expression of Bcl-xl and Mcl-1 and increasing expression of Bax. Compared with the control group, TNFα in the tumor tissue and liver function in SSE-treated mice had no significant changes at 8 wk ($P > 0.05$). In addition, this study provides evidence that SSE may be a potent therapeutic agent in the treatment of HCC without obvious toxic side effects.

In this study, the inhibitory effect of SSE on HepG-2 cells was tested, and ECV304 cells as the control were also studied. It was demonstrated that SSE profoundly

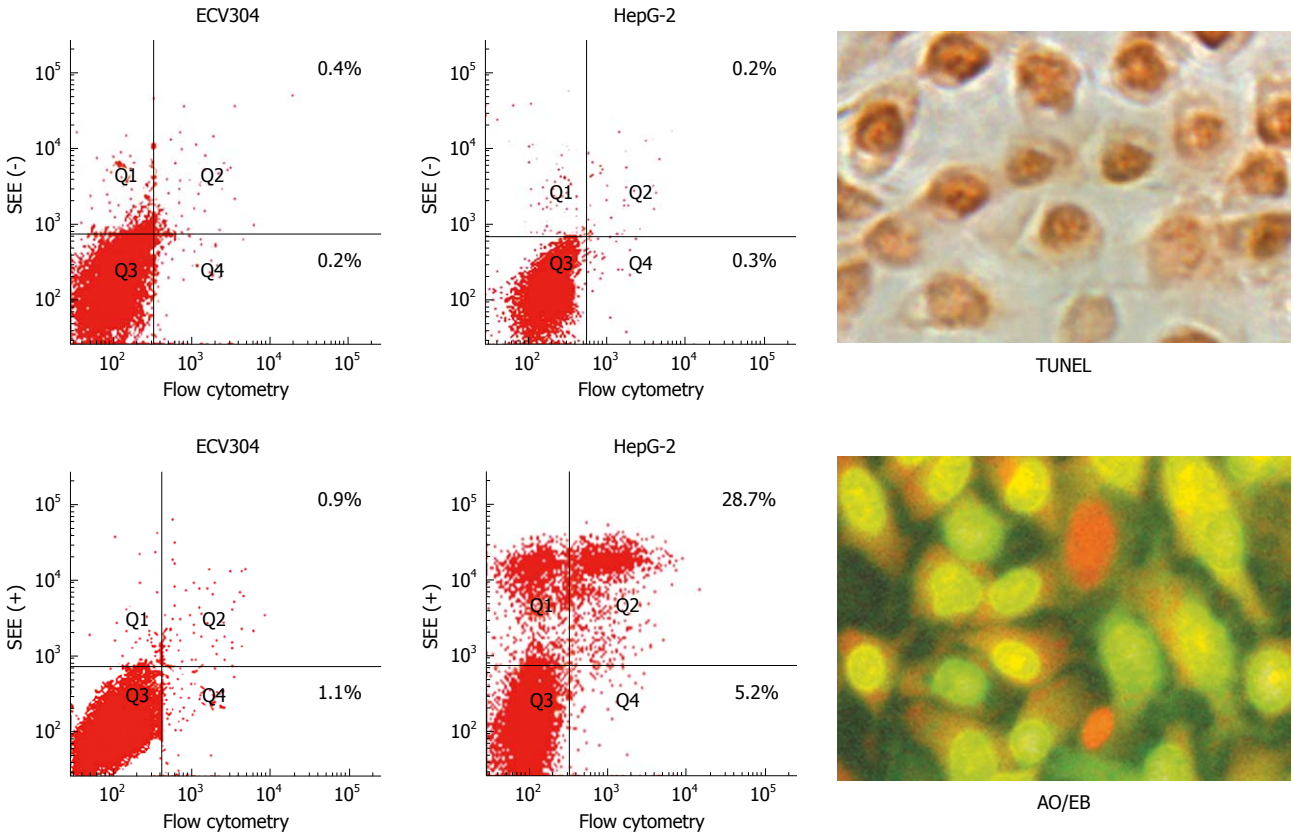


Figure 2 Apoptosis of sargentgloryvine stem extract-treated HepG-2 cells (× 400). HepG-2 and ECV304 cells were treated with sargentgloryvine stem extract (SSE) at a concentration of 45 mg/mL for 24 h. Flow cytometry showed that apoptosis rate was increased obviously compared with the non-treated control cells, $P < 0.05$; while ECV304 cells did not show obvious diversity, $P > 0.05$. Positive signals in nucleus were observed obviously in SSE-treated HepG-2 cells by TdT-Mediated dUTP Nick End Labeling (TUNEL) and acridine orange/ethidium bromide (AO/EB) assays.

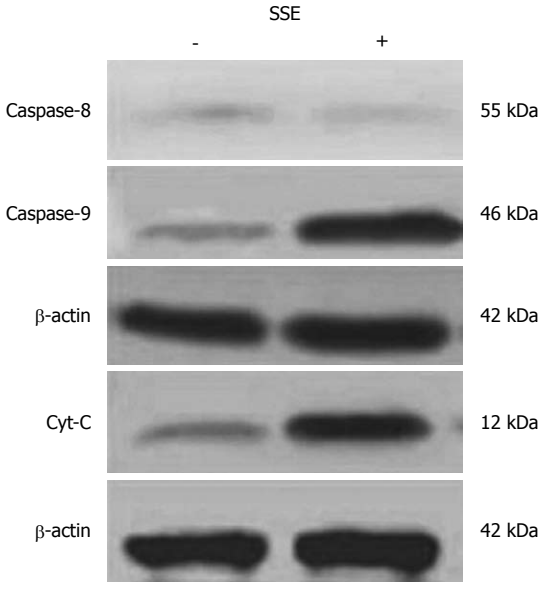


Figure 3 Caspase-8, caspase-9 and cytochrome C in sargentgloryvine stem extract-treated HepG-2 cells. Significantly up-regulated cleavage was found in caspase 9 but not in caspase 8 in HepG-2 cells treated with 45 mg/L sargentgloryvine stem extract (SSE) for 24 h. Cytochrome C in plasmosin was notably increased. $P < 0.05$.

inhibited the growth of HepG-2 cells in a concentration- and time-dependent manner but not in ECV304 cells. MTT and colony formation assays indicate that SSE possesses specific anti-HCC cell activity rather than general cytotoxicity. Additionally, the study suggested that SSE may be safe for normal cells, thus, SSE may have advantages for clinical application.

To reveal the mechanisms of the inhibitory effect of SSE on HepG-2 cells, changes in the cell cycle were analysed. It was demonstrated that the cell cycle of HepG-2 cells was blocked at S phase after 45 mg/L SSE treatment for 24 h, which indicated that the growth inhibition by SSE might be, in part, due to cell cycle arrest. Because cell cycle arrest is always accompanied with cell apoptosis, we analysed apoptosis in the SSE-treated cells. The data showed that SSE treatment induced cell apoptosis, and the apoptosis rates increased with increasing SSE concentrations[11]. More significantly, the induction of apoptosis by SSE in HepG-2 cells was observed at an initial concentration of 30 mg/L within 24 h, further suggesting the safety of SSE for systemic use in the treatment of HCC. It is known that apoptosis is regulated by two main pathways: the extrinsic pathway, which is initiated by the binding of ligands to specific death receptors on the cell surface, and the intrinsic pathway, which is initiated in mitochondria[12,13]. To understand the major *in vivo* pathway through which SSE induces HepG-2 cell growth suppression and apoptosis, the expression of caspase-8 and caspase-9, which play important roles in apoptosis triggered by various proapoptotic signals,

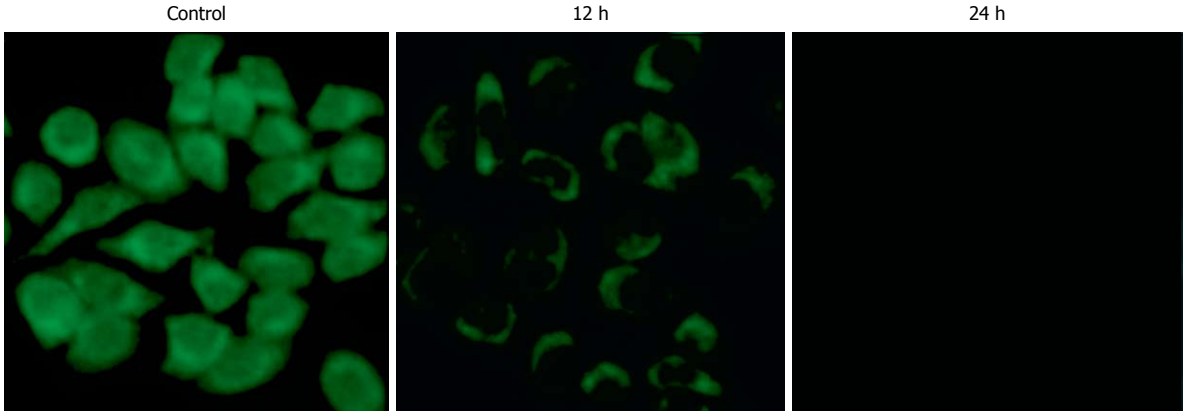


Figure 4 Rh-123 fluorescence in sargentgloryvine stem extract-treated HepG-2 cells (× 400). Flow cytometry showed that fluorescent intensities in sargentgloryvine stem extract (SSE)-treated (45 mg/L) cells for 12 h maintained their $\Delta\psi$m and only displayed minor changes in Rh-123 fluorescence. In contrast, SSE-treated (45 mg/L) cells for 24 h, fluorescent intensities were significantly weaker than control cells.

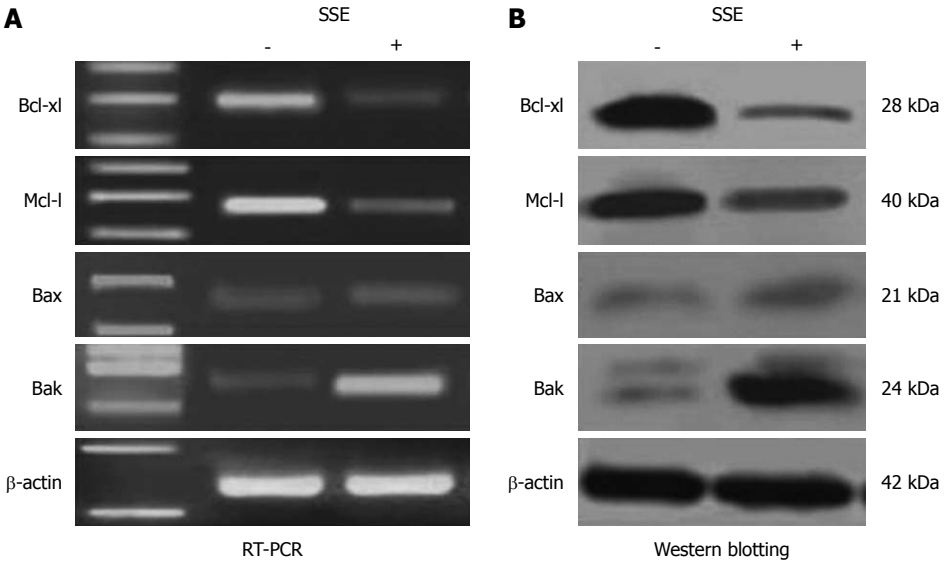


Figure 5 Gene expression in sargentgloryvine stem extract-treated HepG-2 cells. A: Semi-quantitative reverse transcription polymerase chain reaction (RT-PCR) showed that the expression of Bak but not Bax was significantly increased in HepG-2 cells treated with 45 mg/L sargentgloryvine stem extract (SSE) for 24 h, and the expression of Bcl-xl and Mcl-1 was significantly down-regulated; B: Western-blotting confirmed the SSE induced gene expression changes with up-regulated Bak and down-regulated Bcl-xl and Mcl-1 in HepG-2 cells.

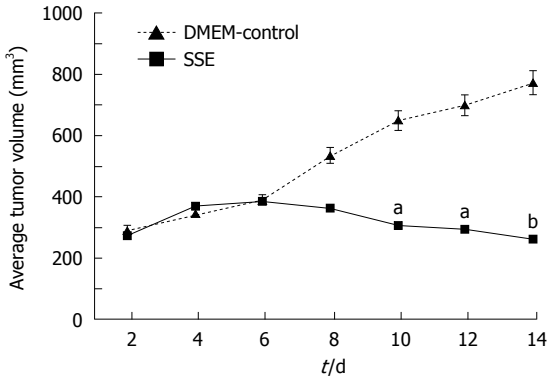


Figure 6 Tumor growth inhibition by systemic sargentgloryvine stem extract-treatment *in vivo*. HepG-2 xenograft tumor (approximately 300 mm^3) growth in Athymic BALB/c nude mice injected with 50 μL of 18 mg/kg sargentgloryvine stem extract (SSE) or DMEM-control every 2 d for 14 d. The tumor size was measured and the tumor volume was calculated as: $width^2$ × length × 0.5. $^aP < 0.05$, $^bP < 0.01$ *vs* DMEM control.

were studied[14]. The results showed that 45 mg/L SSE treatment for 24 h significantly increased caspase-9 but not caspase-8 expression, indicating that SSE-induced apoptosis may be through the intrinsic pathway.

The intrinsic pathway for apoptosis involves several steps including mitochondrial membrane permeabilization and release of cytochrome C, followed by caspase-9 activation[15,16]. Our results showed that treatment with SSE at 45 mg/L for 24 h led to loss of $\Delta\psi$m as indicated by a decrease in Rh-123 fluorescence. SSE also induced cytochrome C release from the mitochondria to cytosol in HepG-2 cells. Taken together, these results further confirmed that SSE may directly trigger the intrinsic pathway to induce the mitochondrial pathway for apoptosis in HCC cells.

Previous studies have shown that in apoptotic cells, anti-apoptotic *bcl-2* members are often inactivated whereas pro-apoptotic members, such as *bax* and *bak*, are activated

and oligomerized in the mitochondria outer membrane. This triggers mitochondrial membrane permeabilization and release of soluble apoptogenic factors such as cytochrome C into the cytosol, which results in caspase activation[17,18]. Further studies revealed that 45 mg/L SSE treatment significantly decreased Bcl-xl and Mcl-1 expression and increased Bak expression and led to mitochondria endomembrane action. Finally, it was confirmed that SSE-induced HepG-2 cell apoptosis is mediated through the *bcl-2* pathway. These results demonstrated that SSE-induced apoptosis is mediated primarily by down-regulated expression of Bcl-xl and Mcl-1, which led to the release of Bak and ultimately activated the intrinsic apoptosis pathway.

In addition to inducing tumor cell apoptosis, systemic injection of SSE into HepG-2 xenografted mice inhibited tumor growth and significantly minimised tumor size but caused no obvious pathological changes in the heart, lungs, liver, spleen or kidneys. The levels of TNFα in the tumor tissue and liver function tests suggest that SEE treatment is relatively safe for the mice.

In conclusion, our research shows that the extract of the Chinese herb sargentgloryvine stem has *in vitro* anticancer effects including inhibition of proliferation and induction of apoptosis in the hepatoma cell line HepG-2 by mechanisms involving expression of Bcl-2 family proteins activating the intrinsic mitochondria apoptosis pathway. Moreover, an *in vivo* solid tumor growth assay further confirmed that systemic administration of the extract could inhibit tumor growth with little cytotoxicity to normal tissues. These *in vitro* and *in vivo* studies provide evidence urging the development of SSE as a novel regimen for human HCC.

ACKNOWLEDGMENTS

We thank our colleagues from the Department of Laboratory Medicine for their technical support.

COMMENTS

Background

Plant-derived natural products have become available such as anticancer derivatives of vincristine, vinblastine, paclitaxel and camptothecin.

Research frontiers

Sargentgloryvine stem extract (SSE) as a chemotherapeutic adjuvant can enhance the efficacy and ameliorate the side effects of cancer chemo- or radiotherapy. However, the effect of SSE on the human hepatocellular carcinoma (HCC) cells remains unknown.

Innovations and breakthroughs

This study showed that SSE treatment was not only able to inhibit the proliferation of human HCC cell HepG-2 cells in a dose and time dependent manner, but also induce apoptosis and cell cycle arrest at S phase.

Applications

SEE is able to inhibit proliferation of human HCC cells and is relatively safe for the mice, and therefore it has a great potential to be a therapeutic agent in the treatment of HCC.

Terminology

SSE: Sargentgloryvine stem is the dried vine stem of *Sargentodoxa cuneata* (Oliv.) and has been widely used as an ingredient in formulated Chinese medicine for thousands of years in the treatment of diseases such as chronic pelvic cavity inflammation, rheumatism and appendicitis.

Peer review

The paper by Wang *et al* addresses an important issue, i.e. novel options for systemic therapy of HCC. In general, the paper is well written, the methods used are sound and the described approach is of potential interest.

REFERENCES

1 **Li X**, Xu W. Recent patents therapeutic agents for cancer. *Recent Pat Anticancer Drug Discov* 2006; **1**: 255-284
2 **Tuma MC**, Malikzay A, Ouyang X, Surguladze D, Fleming J, Mitelman S, Camara M, Finnerty B, Doody J, Chekler EL, Kussie P, Tonra JR. Antitumor Activity of IMC-038525, a Novel Oral Tubulin Polymerization Inhibitor. *Transl Oncol* 2010; **3**: 318-325
3 **Cragg GM**, Newman DJ. Plants as a source of anti-cancer agents. *J Ethnopharmacol* 2005; **100**: 72-79
4 **Kim BK**, Ko YG, Oh S, Kim JS, Kang WC, Jeon DW, Yang JY, Choi D, Hong MK, Ahn T, Jang Y. Comparisons of the effects of stent eccentricity on the neointimal hyperplasia between sirolimus-eluting stent versus paclitaxel-eluting stent. *Yonsei Med J* 2010; **51**: 823-831
5 **Nobili S**, Lippi D, Witort E, Donnini M, Bausi L, Mini E, Capaccioli S. Natural compounds for cancer treatment and prevention. *Pharmacol Res* 2009; **59**: 365-378
6 **Hong YH**, Chao WW, Chen ML, Lin BF. Ethyl acetate extracts of alfalfa (Medicago sativa L.) sprouts inhibit lipopolysaccharide-induced inflammation in vitro and in vivo. *J Biomed Sci* 2009; **16**: 64
7 **Wang X**, Wang R, Hao MW, Dong K, Wei SH, Lin F, Ren JH, Zhang HZ. The BH3-only protein PUMA is involved in green tea polyphenol-induced apoptosis in colorectal cancer cell lines. *Cancer Biol Ther* 2008; **7**: 902-908
8 **Lee DH**, Kim C, Zhang L, Lee YJ. Role of p53, PUMA, and Bax in wogonin-induced apoptosis in human cancer cells. *Biochem Pharmacol* 2008; **75**: 2020-2033
9 **Relja B**, Meder F, Wilhelm K, Henrich D, Marzi I, Lehnert M. Simvastatin inhibits cell growth and induces apoptosis and G0/G1 cell cycle arrest in hepatic cancer cells. *Int J Mol Med* 2010; **26**: 735-741
10 **Kang JX**, Liu J, Wang J, He C, Li FP. The extract of huanglian, a medicinal herb, induces cell growth arrest and apoptosis by upregulation of interferon-beta and TNF-alpha in human breast cancer cells. *Carcinogenesis* 2005; **26**: 1934-1939
11 **Wu WY**, Guo HZ, Qu GQ, Han J, Guo DA. Mechanisms of pseudolaric acid B-induced apoptosis in Bel-7402 cell lines. *Am J Chin Med* 2006; **34**: 887-899
12 **You H**, Pellegrini M, Tsuchihara K, Yamamoto K, Hacker G, Erlacher M, Villunger A, Mak TW. FOXO3a-dependent regulation of Puma in response to cytokine/growth factor withdrawal. *J Exp Med* 2006; **203**: 1657-1663
13 **Soriano ME**, Scorrano L. The interplay between BCL-2 family proteins and mitochondrial morphology in the regulation of apoptosis. *Adv Exp Med Biol* 2010; **687**: 97-114
14 **Alenzi FQ**, Lotfy M, Wyse R. Swords of cell death: caspase activation and regulation. *Asian Pac J Cancer Prev* 2010; **11**: 271-280
15 **Garrido C**, Galluzzi L, Brunet M, Puig PE, Didelot C, Kroemer G. Mechanisms of cytochrome c release from mitochondria. *Cell Death Differ* 2006; **13**: 1423-1433
16 **Gao J**, Jia WD, Li JS, Wang W, Xu GL, Ma JL, Ge YS, Yu JH, Ren WH, Liu WB, Zhang CH. Combined inhibitory effects of celecoxib and fluvastatin on the growth of human hepatocellular carcinoma xenografts in nude mice. *J Int Med Res* 2010; **38**: 1413-1427
17 **Suen DF**, Norris KL, Youle RJ. Mitochondrial dynamics and apoptosis. *Genes Dev* 2008; **22**: 1577-1590
18 **Chipuk JE**, Bouchier-Hayes L, Green DR. Mitochondrial outer membrane permeabilization during apoptosis: the innocent bystander scenario. *Cell Death Differ* 2006; **13**: 1396-1402

S- Editor Tian L **L- Editor** Ma JY **E- Editor** Zheng XM

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/1007-9327office
wjg@wjgnet.com
doi:10.3748/wjg.v17.i23.2855

World J Gastroenterol 2011 June 21; 17(23): 2855-2859
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

BRIEF ARTICLE

Sonographic features of duodenal lipomas in eight clinicopathologically diagnosed patients

Hong-Tan Chen, Guo-Qiang Xu, Li-Jun Wang, Yi-Peng Chen, You-Ming Li

Hong-Tan Chen, Guo-Qiang Xu, Yi-Peng Chen, You-Ming Li, Department of Gastroenterology, The First Affiliated Hospital, Medical School of Zhejiang University, Hangzhou 310003, Zhejiang Province, China
Li-Jun Wang, Department of Pathology, The First Affiliated Hospital, Medical School of Zhejiang University, Hangzhou 310003, Zhejiang Province, China
Author contributions: Xu GQ and Li YM designed the research; Wang LJ and Chen YP collected the data and conducted the literature searches; Chen HT analyzed the data and prepared the manuscript.
Supported by Medical and Health Research Fund of Zhejiang Province, China, No. 491010-W10495
Correspondence to: Dr. Guo-Qiang Xu, Department of Gastroenterology, The First Affiliated Hospital, Medical School of Zhejiang University, 79 Qingchun Road, Hangzhou 310003, Zhejiang Province, China. xgqcht@163.com
Telephone: +86-571-87236718 Fax: +86-571-87236628
Received: January 23, 2011 Revised: April 19, 2011
Accepted: April 26, 2011
Published online: June 21, 2011

Abstract

AIM: To investigate the sonographic features and diagnostic value of endoscopic ultrasonography (EUS) for duodenal lipomas (DLs).

METHODS: A total of eight consecutive patients with DL diagnosed pathologically were included in the study. One EUS expert reviewed the ultrasonic images for all lesions, including the original layer of the duodenal wall, the echo intensity and the echo homogeneity. The size of the lesions and the perifocal structures were also investigated. The diagnosis by EUS was compared with the histological results.

RESULTS: Using routine endoscopy, only one case was correctly diagnosed as DL. Four cases were classified as submucosal tumors, and three cases were mistaken for stromal tumors. All tumors appeared as round or oval intensive hyperechoic lesions with distinct anterior borders that originated from the submucosal layer on EUS. Tumors ranged from 8 to 36 mm in size, with an average size of 16 mm. Homogeneous echogenicity was seen in all cases except one that had a tubular structure inside the tumor. Echo attenuation was observed only in the area behind the tumors in five cases, and it was observed both inside and behind the tumors in three cases in which the posterior border was obscure or invisible. Seven (87.5%) cases were correctly diagnosed as DL, and one (12.5%) was mistaken as Brunner's gland adenoma by EUS. Pathologically, all tumors originated from the submucosal layer and consisted of mature fat cells without heteromorphism. Among the fat cells, there was a small amount of thick-wall vessels infiltrating the lymphocytes, and abundant fibrous connective tissues.

CONCLUSION: On EUS, DL is featured as an intensive homogeneous hyperechoic submucosal lesion with marked echo attenuation and without involvement of the mucosa.

Key words: Duodenum; Lipoma; Endoscopic ultrasonography; Hyperecho; Echo attenuation

Peer reviewer: Alexander Becker, MD, Department of Surgery, Haemek Medical Center, Afula 18000, Israel

Chen HT, Xu GQ, Wang LJ, Chen YP, Li YM. Sonographic features of duodenal lipomas in eight clinicopathologically diagnosed patients. *World J Gastroenterol* 2011; 17(23): 2855-2859 Available from: URL: http://www.wjgnet.com/1007-9327/full/v17/i23/2855.htm DOI: http://dx.doi.org/10.3748/wjg.v17.i23.2855

INTRODUCTION

Gastrointestinal lipomas are uncommon benign tumors that occur anywhere along the gut. The most common location for these lesions is the colon, followed by the il-

eum and the jejunum[1]. Lipomas found in the duodenum are rare and the literature regarding duodenal lipomas (DLs) is only limited to case reports[2-8], and no systematic study of diagnostic means for DLs has been reported. Since preoperative diagnosis of DLs is difficult, and large DLs can mimic malignant tumors on endoscopy, patients could be subjected to extensive surgical procedures that are sometimes destructive. Endoscopic ultrasonography (EUS) is an optimal method for detecting gastrointestinal submucosal tumors (SMTs) such as stromal tumors and leiomyomas; however, the features and diagnostic value of EUS for examining DLs have not been well established because of the rareness of this disease. In this study, we studied the sonographic features and diagnostic value of EUS for identifying DLs.

MATERIALS AND METHODS

Patients

A total of eight consecutive patients with DL were included. The diagnosis of DL for all patients was pathologically established after surgical excision in five patients and endoscopic resection in three patients during the period from June 2000 to December 2010 in the First Affiliated Hospital, School of Medicine, Zhejiang University, China. The patient group was composed of five males and three females, and aged from 42 to 78 years, with a mean of 60 years. Except for one patient who was asymptomatic, DLs presented as bleeding in four patients, dyspepsia in two patients, and epigastric pain in one patient (Table 1). Laboratory examinations, including liver function tests, serum lipids, and tumor markers (carcinoembryonic antigen, carbohydrate antigen 19-9, carbohydrate antigen 125, and alpha-fetoprotein), showed no obvious abnormalities except for anemia in five patients.

Instrument

The EUS system included Olympus EU-M2000 sonogram processing equipment, an Olympus GIF-2T-240 double-cavity electronic gastroscope, Olympus MAJ drive systems with a high-frequency echo probe, UM-DP12-25R miniature ultrasonic probes with a frequency spectrum of 12-15 MHz, and a Daker WP-800 water pump (Olympus Medical System Corp., Tokyo Japan).

Methods

One EUS expert reviewed the ultrasonic image of all lesions, including the original layer of the duodenal wall, the echo intensity, and the echoic homogeneity. The size of the lesions and the perifocal structures were also investigated. The EUS diagnosis was compared with the histological results.

RESULTS

Endoscopic studies

The tumors were located at the bulb in one case and in the descending part of the duodenum in seven cases. The lesions, with a hemispherical or oval shape, were sessile in six cases and had a stalk in two. The surface of the lesions was intact in three cases (Figure 1A) and had an ulcer (Figure 2A) or an erosion in five cases. Prior to EUS, only one case was correctly diagnosed as DL by routine endoscopy because of the yellowish and soft appearance (Figure 3A). Four cases were classified as SMTs, and three cases were mistaken for stromal tumors.

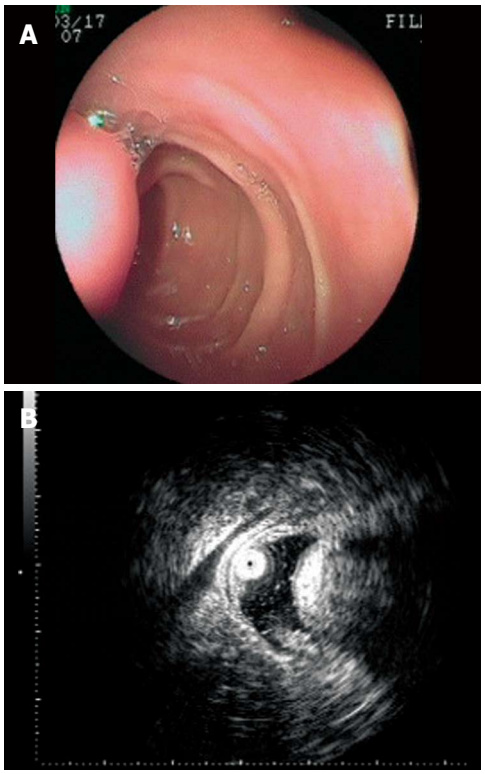


Figure 1 Endoscopic and endoscopic ultrasonography findings in Case 7. A: A submucosal tumors of the descendant duodenum with an intact surface; B: Endoscopic ultrasonography showed a hyperechoic lesion with a distinct anterior border originating from the submucosal layer. There was marked echo attenuation both inside and behind the lesion, and the posterior border was obscure.

Endoscopic ultrasonography

The endoscopic ultrasonography (EUS) findings in all cases are shown in Table 1. All tumors appeared as intensive hyperechoic lesions with a distinct anterior border that originated from the third EUS layer without involvement of the overlying first and second layers (Figures 1B, 2B, 3B and 4). Tumors ranged from 8 to 36 mm in size, with an average size of 16 mm. The margins of the tumors were clear in five cases (Figures 3B and 4), and the posterior borders of three lesions were obscure or invisible because of marked echo attenuation (Figures 1B and 2B). Homogeneous echogenicity was seen in all cases except for one that had a tubular structure inside the tumor (Figure 2B). Echo attenuation was observed only in the area behind the tumors in five cases (Figures 3B and 4) and both inside and behind the tumors in three cases (Figures 1B and 2B). With EUS, seven patients were correctly diagnosed as having DLs, but one

Table 1 Clinical settings and endoscopic ultrasonography observations

Case	Sex	Age	Symptoms	Location	Treatment	EUS features					
						Layer	Size (mm)	Echogenicity	Homogeneity	Border	Echo attenuation
1	M	50	None	Bulb	Endoscopic resection	3rd	12	Hyperecho	Homogenous	Distinct	Behind
2	F	64	Melena	2nd portion	Surgery	3rd	10	Hyperecho	Homogenous	Distinct	Behind
3	M	63	Dyspepsia	2nd portion	Surgery	3rd	25	Hyperecho	Homogenous	Indistinct	Behind and inside
4	F	54	Melena	2nd portion	Surgery	3rd	12	Hyperecho	Homogenous	Distinct	Behind
5	M	67	Dyspepsia	2nd portion	Endoscopic resection	3rd	8	Hyperecho	Homogenous	Distinct	Behind
6	M	78	Melena	2nd portion	Endoscopic resection	3rd	11	Hyperecho	Homogenous	Distinct	Behind
7	F	62	Epigastric pain	2nd portion	Surgery	3rd	15	Hyperecho	Homogenous	Indistinct	Behind and inside
8	M	42	Melena	2nd portion	Surgery	3rd	36	Hyperecho	Heterogeneous	Indistinct	Behind and inside

EUS: Endoscopic ultrasonography; M: Male; F: Female.

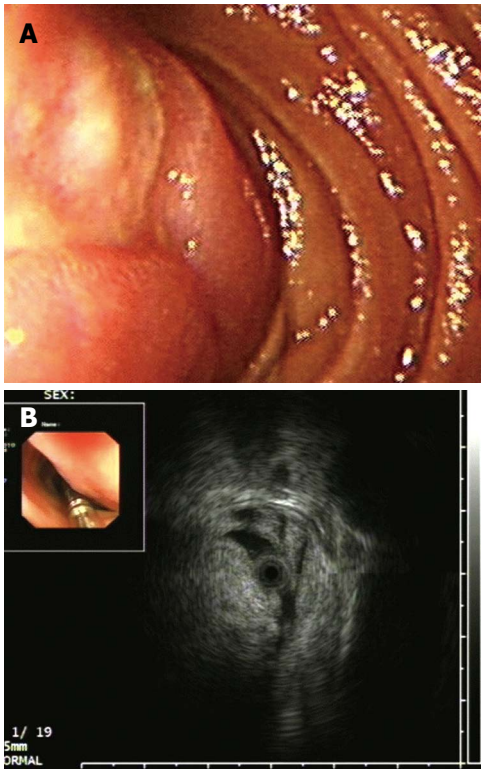


Figure 2 Endoscopic and endoscopic ultrasonography findings in Case 8. A: A lobulated submucosal tumor in the descendant duodenum with an ulcer on its surface; B: Endoscopic ultrasonography showed an intensive hyperechoic lesion with a distinct anterior border originating from the submucosal layer without involvement of the overlying mucosal layer. The posterior border of the tumor was invisible because of the marked echo-attenuation.

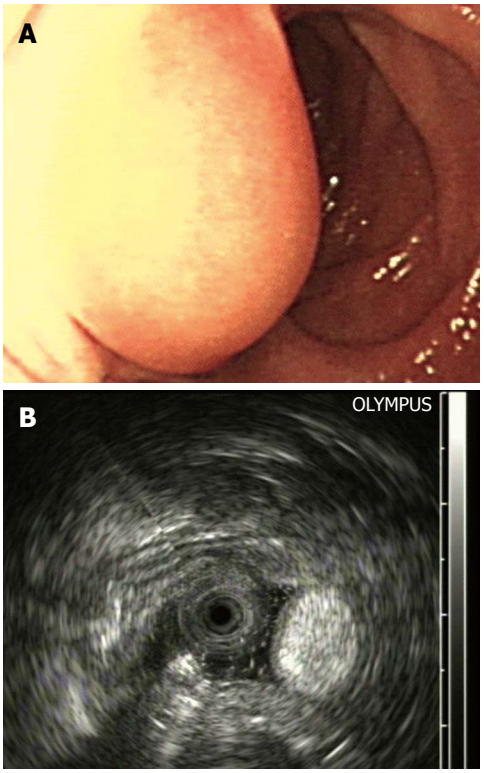


Figure 3 Endoscopic and endoscopic ultrasonography findings in Case 2. A: A submucosal tumor located in the descendant duodenum with a yellowish and soft appearance; B: Endoscopic ultrasonography showed an oval intensive hyperechoic lesion with homogeneous parenchymal echogenicity and a clear margin originating from the third layer without involvement of the overlying mucosal layers. Echo attenuation was seen at the area behind the focus.

patient with DL was mistaken as having Brunner's gland adenoma (BGA) due to the appearance of a tubular structure.

Pathology

The pathological diagnosis after routine endoscopic biopsy was chronic inflammation of the mucosa, whereas the postoperative diagnosis after surgical excision or endoscopic resection was DL. The tumors presented by gross appearance as a node or finger in six cases and as a sub-lobe or cauliflower in two cases. All tumors originated from the submucosal layer, sometimes involving the muscularis propria, with a fully or partially coated fiber peplos. Microscopically, the tumors were composed of mature fat cells without heteromorphism. Among the fat cells, there were small amounts of thick-wall vessels, infiltrating lymphocytes, and abundant fibrous connective tissues (Figures 5-7).

DISCUSSION

Lipomas of the duodenum are rare, with fewer than 230 cases reported in the literature, and most of the described cases are from autopsy records rather than clinical experience[9]. DLs were mostly detected after bleeding or obstruction occurred. Among the patients in

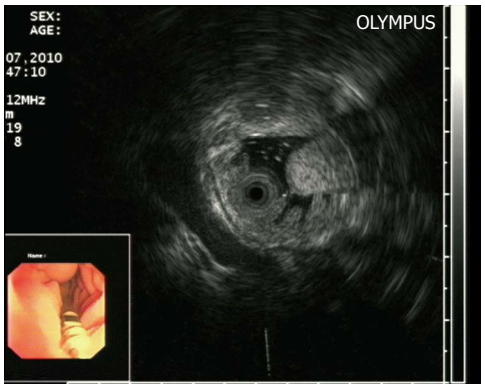


Figure 4 Endoscopic and endoscopic ultrasonography findings in Case 4. A submucosal tumor located in the descendant duodenum. Endoscopic ultrasonography showed a submucosal hyperechoic lesion with marked echo-attenuation behind the focus.

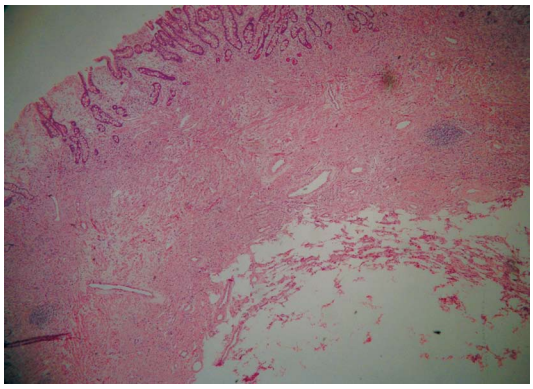


Figure 5 Pathological findings in Case 3. Microscopically, the tumor was located in the submucosa without involvement of the mucosa (HE, × 40).

this study, four (50%) experienced bleeding, two (25%) experienced dyspepsia, one (12.5%) had epigastric pain, and one (12.5%) was asymptomatic. The symptoms of DL are nonspecific, however, and they are not useful for differential diagnosis.

Endoscopy is the preferred means for detecting upper gastrointestinal diseases. However, it can only suggest the presence of submucosal protruding lesions, but cannot provide additional details even though lesions have been detected[10,11]. In this study, four patients were diagnosed as having SMTs by endoscopy, and three patients were mistaken as having stromal tumors. Only one patient was correctly diagnosed with lipoma by routine endoscopy due to the yellowish and soft appearance of the lesion (Figure 2B).

EUS has been reported to be an effective modality for assessing gastrointestinal tumors and evaluating the original layer of the submucosal tumor, the homogeneity of the internal parenchymal echo, and the echogenicity of the lesions[10]. The typical EUS observations of DLs are intensive homogeneous hyperechoic lesions originating from the submucosa, with echo attenuation behind and/or inside the rear area. Thus, the signs of DLs are similar to the ultrasonic features of a fatty liver.

Intensive hyperecho is the most noticeable EUS feature of DLs. A newly-devised echogenicity classification system for SMTs by Okanobu[10] demonstrated the highest echo level of lipomas (levels 5-6). The marked echo-attenuation is another EUS feature of DLs. In our series, there was a more apparent echo decline in the area behind the focus than anywhere else in all cases regardless of the size of the lesions, suggesting a greater attenuation coefficient for DLs than for normal intestinal wall tissues. Furthermore, visible echo decline was seen inside the lesion when the size of the focus was larger than 15 mm. The anterior border was as distinct as the overlying first and second layers of the DLs; however, the appearance of the posterior border depended on the related echo de-

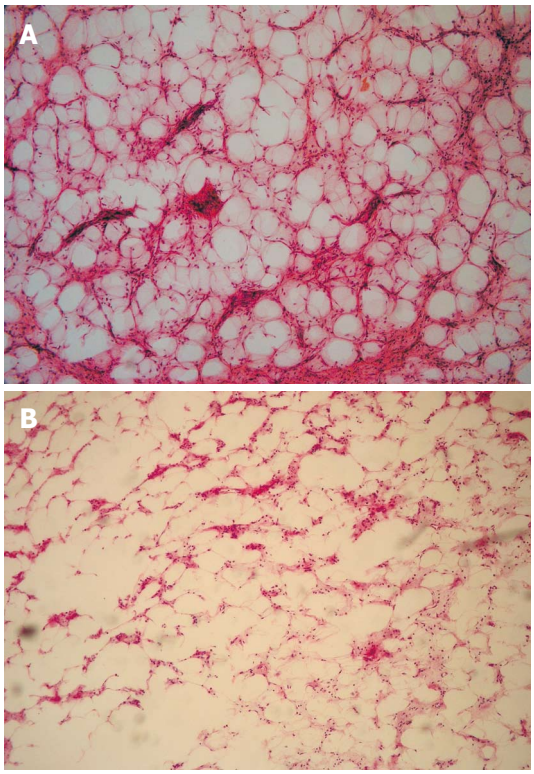


Figure 6 Pathological findings in Cases 2 and 4. A, B: The tumors consisted of mature fat cells. Abundant fibrous connective tissues were found among the fat cells (HE, × 100).

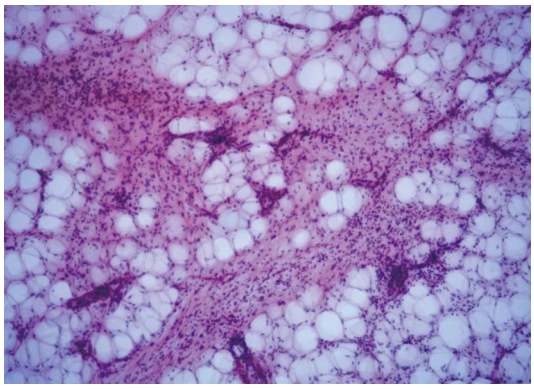


Figure 7 Pathological findings in Case 7. Massive fiber ropes were inside the tumor parenchyma (HE, × 100).

cline. The latter portion and the posterior border of DLs larger than 25 mm are often invisible with the 12-MHz probe. The internal parenchymal echo of DLs is generally homogenous[11]. Occasionally, blood vessels may present as tubular structures inside the focus.

According to two ultrasonic characteristics, we could distinguish DLs from the majority of other SMTs such as leiomyomas, stromal tumors, or cysts. It is noteworthy that Brunner's gland adenomas (BGAs) also show hyperechoic lesions originating from the submucosa, and therefore, DLs may be mistaken for BGAs[11,12]. In our study, one patient was misdiagnosed with duodenal BGA. BGAs appear primarily in the bulb portion or at the junction of the bulb and the descending duodenum, whereas DLs are located primarily in the descending portion of the duodenum. In addition, the echogenicity of BGAs is not as intensive or homogeneous as that of lipomas. Although the second EUS layer often becomes blurry or invisible in BGAs because of involvement of the lamina propria, it is always readable in DLs.

The reason why all gastrointestinal lipomas appeared as hyperechoic is not clear. Interestingly, normal subcutaneous fat tissues appeared as a hypoechoic zone with a small amount of hyperechoic fiber ropes. An earlier report showed that 29% of superficial soft tissue lipomas were hypoechoic, 22% were isoechoic, 29% were hyperechoic, and 20% were of a mixed pattern[13]. Thus, the echo types of lipomas mostly depend on the quantity of the boundary in relation to the mixture of fat and other connective tissues.We found abundant fibrous connective tissues among the fat cells of DLs. The heterogeneous mixture generated innumerable acoustic boundaries that appeared hyperechoic because of the marked acoustic impedance difference between the fat and fibrous tissues.

In summary, EUS has significant value for the differential diagnosis of DLs. The appearance of a round or oval lesion originating from the submucosal layer with intensive homogenous hyperecho and marked echo-attenuation, without involvement of the mucosal layers, suggests a diagnosis of DL. Abundant fibrous connective tissues among the fat cells may be the acoustic basis of this appearance.

COMMENTS

Background

Lipomas located in the duodenum are rare. Because the preoperative diagnosis for duodenal lipomas is difficult to establish and some large lipomas can mimic malignant tumors on endoscopy, patients could be subjected to extensive surgical procedures that sometimes were destructive. Endoscopic ultrasonography (EUS) is the optimal method for the diagnosis of gastrointestinal stromal tumors, but its diagnostic value for duodenal lipomas has not been well established because of its rareness.

Research frontiers

Up to date, less than 230 cases of lipomas have been reported in the literature, but most of them are from autopsy records rather than clinical experience. Clinically, duodenal lipomas are mostly revealed by bleeding or obstruction. Endoscopy can only detect the submucosal lesion but fail to judge its nature. EUS is an effective modality in the assessment of gastrointestinal submucosal tumors and evaluation of the original layer, homogeneity of internal parenchymal echo and echogenicity of the lesions.

Innovations and breakthroughs

EUS is of significant value in the diagnosis and differential diagnosis of duodenal lipomas. The sonographic features were round or oval lesions originated from submucosal layer with intensive homogenous hyperecho and marked echo-attenuation, without involvement of the mucosal layers.

Applications

According to this study, duodenal lipomas can be defined by EUS, thus needless surgery can be avoided. By EUS, they can differentiate duodenal lipomas from other submucosal tumors. The sonographic features of duodenal lipomas can be used for the diagnosis of lipomas in esophagus, stomach and colon.

Peer review

This is an original report on the correlations of the sonographic findings and clinicopathological features of duodenal lipomas. The material is characterized by small case series but the conclusive suggestions are appropriate and interesting. Duodenal lipoma is a rare clinicopathological entity. Therefore, it is interesting to get an eight consecutive duodenal lipoma cases. The EUS is probably the best option to diagnose this pathology.

REFERENCES

1 **Taylor AJ**, Stewart ET, Dodds WJ. Gastrointestinal lipomas: a radiologic and pathologic review. *AJR Am J Roentgenol* 1990; **155**: 1205-1210

2 **Sou S**, Nomura H, Takaki Y, Nagahama T, Matsubara F, Matsui T, Yao T. Hemorrhagic duodenal lipoma managed by endoscopic resection. *J Gastroenterol Hepatol* 2006; **21**: 479-481

3 **Blanchet MC**, Arnal E, Paparel P, Grima F, Voiglio EJ, Caillot JL. Obstructive duodenal lipoma successfully treated by endoscopic polypectomy. *Gastrointest Endosc* 2003; **58**: 938-939

4 **Tung CF**, Chow WK, Peng YC, Chen GH, Yang DY, Kwan PC. Bleeding duodenal lipoma successfully treated with endoscopic polypectomy. *Gastrointest Endosc* 2001; **54**: 116-117

5 **Hizawa K**, Kawasaki M, Kouzuki T, Aoyagi K, Fujishima M. Unroofing technique for the endoscopic resection of a large duodenal lipoma. *Gastrointest Endosc* 1999; **49**: 391-392

6 **Krachman MS**, Dave PB, Gumaste VV. Bleeding duodenal lipoma. *J Clin Gastroenterol* 1992; **15**: 180-181

7 **Kang JY**, Chan-Wilde C, Wee A, Chew R, Ti TK. Role of computed tomography and endoscopy in the management of alimentary tract lipomas. *Gut* 1990; **31**: 550-553

8 **Jennings BS**, Doerr RJ. Duodenal lipoma causing intussusception. *Surgery* 1989; **105**: 560-563

9 **Abu Daff SN**, Abu Daff NS. Laparoscopic enucleation of a duodenal lipoma, with review of the literature. *Saudi Med J* 2008; **29**: 455-457

10 **Okanobu H**, Hata J, Haruma K, Mitsuoka Y, Kunihiro K, Manabe N, Tanaka S, Chayama K. A classification system of echogenicity for gastrointestinal neoplasms. *Digestion* 2005; **72**: 8-12

11 **Xu GQ**, Wu YQ, Wang LJ, Chen HT. Values of endoscopic ultrasonography for diagnosis and treatment of duodenal protruding lesions. *J Zhejiang Univ Sci B* 2008; **9**: 329-334

12 **Xu GQ**, Zhang H, Li YM, Chen HT, Ji F, Chen CX, Ren GP, Ni XY. Analysis on clinical features of duodenal Brunner's gland adenoma. *Zhonghua Xiaohua Zazhi* 2006; **26**: 511-514

13 **Fornage BD**, Tassin GB. Sonographic appearances of superficial soft tissue lipomas. *J Clin Ultrasound* 1991; **19**: 215-220

S- Editor Sun H **L- Editor** Ma JY **E- Editor** Ma WH

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/1007-9327office
wjg@wjgnet.com
doi:10.3748/wjg.v17.i23.2860

World J Gastroenterol 2011 June 21; 17(23): 2860-2866
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

BRIEF ARTICLE

Association between *ITGA2* C807T polymorphism and gastric cancer risk

Jie Chen, Nan-Nan Liu, Jia-Qi Li, Li Yang, Ying Zeng, Xiao-Mei Zhao, Lin-Lin Xu, Xuan Luo, Bin Wang, Xue-Rong Wang

Jie Chen, Nan-Nan Liu, Jia-Qi Li, Ying Zeng, Xiao-Mei Zhao, Lin-Lin Xu, Xuan Luo, Bin Wang, Xue-Rong Wang, Department of Pharmacology, Nanjing Medical University, Nanjing 210029, Jiangsu Province, China
Li Yang, Department of General Surgery, First Affiliated Hospital of Nanjing Medical University, Nanjing 210029, Jiangsu Province, China
Author contributions: Chen J is involved in the study design, conducted polymerase chain reaction-restriction fragment length polymorphism, analyzed the data and wrote the manuscript; Li JQ and Liu NN participated in the study design and extracted genetic DNA from blood samples; Yang L, Zeng Y, Zhao XM, Xu LL and Luo X collected blood samples; Wang B and Wang XR designed and coordinated the study and revised the manuscript.
Supported by The National Natural Science Foundation of China, No. 30873099; Nanjing Medical University start-up research fund for Wang XR; the Natural Science Foundation of education Department, Jiangsu Province, No. 08KJB320004
Correspondence to: Xue-Rong Wang, Professor, Department of Pharmacology, Nanjing Medical University, 140 Hanzhong Road, Nanjing 210029, Jiangsu Province, China. wangxr@njmu.edu.cn
Telephone: +86-25-86862884 Fax: +86-25-86862884
Received: October 9, 2010 Revised: November 5, 2010
Accepted: November 12, 2010
Published online: June 21, 2011

Abstract

AIM: To evaluate the impact of the *ITGA2* gene polymorphism on gastric cancer risk.

METHODS: A hospital-based case-control study was conducted, including 307 gastric cancer patients and 307 age- and gender-matched control subjects. The genotypes were identified by polymerase chain reaction-restriction fragment length polymorphism assay.

RESULTS: The frequencies of the wild and variant genotypes in cases were significantly different from those of controls (P = 0.019). Compared with individuals with the wild genotype CC, subjects with the variant genotypes (CT + TT) had a significantly higher risk of gastric cancer (adjusted odds ratio = 1.57, 95% CI = 1.13-2.17, P = 0.007). In stratified analyses, the elevated gastric cancer risk was especially evident in older individuals aged > 58 years, nonsmokers and rural subjects. Further analyses revealed that the variant genotypes were associated with poor tumor differentiation and adjacent organ invasion in the sub-analysis of gastric cancer patients.

CONCLUSION: The *ITGA2* gene C807T polymorphism may be associated with an increased risk of gastric cancer, differentiation and invasion of gastric cancer.

Key words: Gastric cancer; Integrin; *ITGA2*; Polymorphism; Genotype

Peer reviewer: Ki-Baik Hahm, MD, PhD, Professor, Gachon Graduate School of Medicine, Department of Gastroenterology, Lee Gil Ya Cancer and Diabetes Institute, Lab of Translational Medicine, 7-45 Songdo-dong, Yeonsu-gu, Incheon, 406-840, South Korea

Chen J, Liu NN, Li JQ, Yang L, Zeng Y, Zhao XM, Xu LL, Luo X, Wang B, Wang XR. Association between *ITGA2* C807T polymorphism and gastric cancer risk. *World J Gastroenterol* 2011; 17(23): 2860-2866 Available from: URL: http://www.wjgnet.com/1007-9327/full/v17/i23/2860.htm DOI: http://dx.doi.org/10.3748/wjg.v17.i23.2860

INTRODUCTION

Gastric cancer remains a major public health issue as the fourth most common cancer type and the second leading cause of cancer death worldwide[1,2]. Nearly half of the gastric cancer cases occur in China[3]. Although the

cause of gastric cancer is largely unknown, it has been shown that diet, tobacco smoking, alcohol, gastroesophageal reflux and *Helicobacter pylori* (*H. pylori*) infection are associated with the risk of this cancer[4-7]. As genetic polymorphisms are responsible for the inter-individual variation and diversity, they have been recently considered as the main genetic elements involved in the development of common and complex diseases, including various cancers. Like many malignancies, it is believed that gastric cancer is the result of interactions between environmental factors and genetic factors[8]. Our previous epidemiological studies also provided the evidence that genetic polymorphisms were associated with the risk of gastric cancer[9-12].

Integrins are members of a family of cell-surface heterodimeric proteins that mediate cell-matrix and cell-cell interactions. The 18 α-subunits and 8 β-subunits form together at least 25 different integrins, each pair being specific for a unique set of ligands. It has been demonstrated that integrins may play a crucial role in carcinogenesis, tumor behavior and metastasis[13,14]. Several integrins such as α2β1, α Ⅱ bβ3 and αvβ3 are considered as key factors for cancer development and progression. Integrin α2β1, also known as platelet glycoprotein Ⅰa-Ⅱa, is expressed by epithelial cells, and its level of expression in tumor cells is associated with motility, invasiveness and cellular differentiation[15-17]. Several studies have shown that integrin α2β1 expression is closely associated with invasion and metastasis of gastric cancer[18-21].

The integrin, α2 gene (*ITGA2*) is located on chromosome 5q23-31. A silent change in the coding region at nucleotide 807 (TTT/TTC at codon Phe253) has been identified. The C807T single nucleotide polymorphism (NCBI SNP ID: rs1126643) of the *ITGA2* gene was associated with the integrin α2β1 density. The genotype 807 TT was associated with a higher receptor density and the genotype 807 CC with a lower density, whereas heterozygous individuals expressed intermediate receptor levels[22,23].

Recent studies indicated that the *ITGA2* gene C807T polymorphism was associated with various diseases, including stroke, retinal vein occlusion, acute coronary syndrome, colorectal cancer, and breast cancer[24-29]. To the best of our knowledge, there has been no study that assessed the association between the polymorphism and gastric cancer risk.

Given that the roles of *ITGA2* in the progression of gastric cancer as well as the effect of the polymorphism in *ITGA2* gene on the receptor function, it is plausible that the polymorphism may be associated with the risk of gastric cancer. To test the hypothesis, we performed a hospital-based case-control study in a Chinese population.

MATERIALS AND METHODS

Subjects

This hospital-based case-control study consisted of 307 consecutive inpatients with histologically confirmed gastric cancers without synchronous and/or metachronous secondary malignancy and a population-based and sex- and age-matched 307 cancer-free inpatients as controls. All subjects were recruited between March 2005 and November 2009 from the patients who were admitted to the First Affiliated Hospital of Nanjing Medical University. The most common causes for hospitalization in the control subjects were hernias, appendicitis, hydrocele, cholecystitis and cataract. All subjects were of unrelated Han nationality from Jiangsu Province or its surrounding regions. Information on age, gender, smoking status, residence (urban or rural), body weight and personal medical history was collected by questionnaire. Individuals who formerly or currently smoked ≥ 10 cigarettes per day for at least 2 years were defined as smokers. Depth of tumor invasion and local lymph node status were classified according to the TNM classification criteria of International Union Against Cancer[30]. Differentiation was graded according to World Health Organization classification. The study was approved by the Ethics Committee of Nanjing Medical University First Affiliated Hospital and informed consent was obtained from all the participating subjects.

Genotyping

The protocol for genomic DNA extraction was described in our previous study[9]. The polymerase chain reaction (PCR)-restriction fragment length polymorphism assay was used to identify the *ITGA2* C807T genotypes. The PCR was performed in a total volume of 20 μL reaction mixtures containing 2 μL 10 × PCR buffer (MBI Fermentas), 1.75 mmol/L $MgCl_2$, 0.25 μmol/L each primer (forward 5'-GTGTTTAACTTGAACACATAT-3', reverse 5'-ACCTTGCATATTGAATTGCTT-3'), 0.15 mmol/L dNTP, 1 unit *Taq* polymerase (MBI fermentas) and 150 ng genomic DNA. The amplification protocol is as follows: primary denaturation at 95℃ for 5 min, followed by 35 cycles of denaturation at 94℃ for 30 s, annealing at 55℃ for 30 s, and extension at 72℃ for 1 min, then a final elongation at 72℃ for 5 min. The 115 bp PCR products including the polymorphic site were digested at 65℃ for 12 h, using restriction enzyme *Taq* I (MBI Fermentas) and then separated on a 3% ethidium bromide-stained agarose gel. The wild-type homozygotes (CC) produced two bands at 92 and 23 bp, while the variant homozygotes (TT) produced one band at 115 bp, and the heterozygous (CT) produced three bands at 115, 92 and 23 bp (Figure 1). To control the quality of genotyping, all assays were conducted by two researchers separately in a blind fashion. In addition, a 10% masked samples were randomly selected and retested, and the reproducibility was 100%.

Statistical analysis

Statistical analyses were conducted using SPSS 16.0 software (SPSS Inc., Chicago, IL, USA). All statistical tests were two-tailed and $P < 0.05$ was considered statistically significant. Quantitative variables departing from the normal distribution including age and weight were summarized as median and analyzed by Mann-Whitney rank

Table 1 Demographic information *n* (%)

Characteristics	Cases (*n* = 307)	Controls (*n* = 307)	*P* value
Gender (male)	231 (75.2)	231 (75.2)	1.000
Age[1] (yr), (range)	59 (50-68)	58 (49-66)	0.145
Weight[1] (kg), (range)	62 (55-70)	65 (57-72.75)	0.001
Hypertension	65 (21.17)	59 (19.22)	0.546
Diabetes	17 (5.54)	24 (7.82)	0.262
Smoking	82 (26.71)	53 (17.26)	0.005
Residence			
Rural	139 (45.28)	139 (45.28)	1.000
Urban	168 (54.72)	168 (54.72)	

[1]Median (25th-75th percentiles).

sum test. Pearson's χ^2 test was used to compare the difference in the distribution of categorical variables and genotype frequencies between cases and controls. The Hardy-Weinberg equilibrium of the *ITGA2* genotypes was estimated for cases and controls by a goodness-of-fit χ^2 test. Odds ratio (OR) and 95% CI were calculated to evaluate the association between the polymorphism and the risk of gastric cancer. Carriers of the wild genotype CC were used as the reference. The crude OR was obtained using the Woolf approximation method and the adjusted OR was calculated by unconditional logistic regression method, with adjustment for age, sex, smoking status, residence, hypertension and diabetes.

RESULTS

Demographic information

A total of 614 subjects (307 cases and 307 controls) were analyzed. Baseline demographic characteristics of the study groups are shown in Table 1. The age distribution and proportion of males were quite similar due to the fact that we selected the age- and gender-matched subjects. The two groups were similar with respect to residence, history of hypertension and diabetes. Nevertheless, compared with controls, gastric cancer patients had a lower body-weight (P = 0.001) and more smokers were found among gastric cancer cases than among the controls (26.71% *vs* 17.26%, P = 0.005).

Distribution of ITGA2 genotype in cases and controls and risk estimates

Table 2 shows the frequency distributions of the genotypes and their association with gastric cancer risk by unadjusted OR, adjusted OR and 95% CI. The genotype distributions in cases and controls were consistent with those from the Hardy-Weinberg equilibrium model (P = 0.988, P = 0.675, respectively). The frequencies of the *ITGA2* genotype were significantly different between gastric cancer cases and controls (P = 0.019). Compared with the control group, T allele frequency was significantly higher in the case group (P = 0.024). With the wild genotype CC as reference, we found that the CT genotype was associated with an increased risk of gastric cancer (adjusted OR = 1.54, 95% CI = 1.10-2.18, P = 0.013).

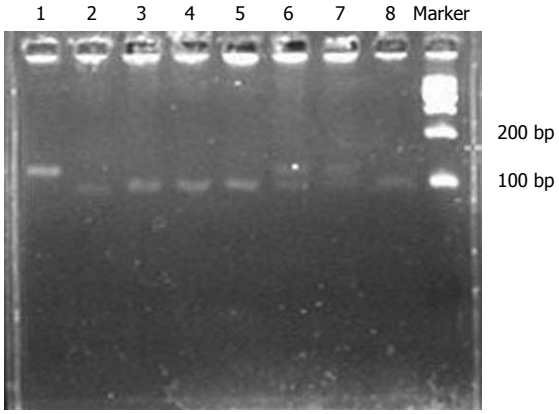


Figure 1 *ITGA2* C807T polymorphism in gastric cancer patients and controls. Amplified polymerase chain reaction products were digested with restriction enzyme *Taq* I and analyzed on a 3% agarose gel. Lane 1: The TT homozygous; Lanes 2-5, 8: The CC homozygous; Lanes 6, 7: The CT homozygous.

Individuals with the variant genotypes (CT + TT) had a 1.57-fold increased risk of developing gastric cancer (adjusted OR = 1.57, 95% CI = 1.13-2.17, P = 0.007).

Stratified analysis of polymorphism and gastric cancer risk

As shown in Table 3, stratified analyses were performed by the median age of controls (58 years), sex, smoking status, and residence. The elevated risk of gastric cancer associated with the variant genotypes was noteworthy in subjects aged > 58 years (adjusted OR = 1.88, 95% CI = 1.17-3.03, P = 0.010), but not in subjects aged ⩽ 58 years. In non-smoking subjects, the variant genotypes were associated with a 51% increased risk of gastric cancer (adjusted OR = 1.51, 95% CI = 1.05-2.18, P = 0.028), whereas the correlation was not statistically significant in smoking subjects. When stratified by residence, the elevated risk was evident in rural subjects (adjusted OR = 2.35, 95% CI = 1.42-3.90, P = 0.001), but not in urban subjects. No statistically significant difference was observed in the association of the polymorphism and susceptibility to gastric cancer between males and females.

Variant genotypes and clinicopathological characteristics of gastric cancer

We also observed the correlations between the *ITGA2* variant genotypes and clinicopathologic features of gastric cancer patients in this study (Table 4). A significantly increased risk was found in individuals with the variant genotypes in both poorly differentiated tumors (adjusted OR = 2.21, 95% CI = 1.12-4.38, P = 0.022) and adjacent invaded organs (adjusted OR = 2.12, 95% CI = 1.10-4.07, P = 0.024) of gastric cancer. However, no significant association was observed between the polymorphism and lymph node metastasis or tumor location.

DISCUSSION

In the present study, we investigated the role of *ITGA2* gene C807T polymorphism in gastric cancer susceptibil-

Table 2 Distributions of *ITGA2* genotype in cases and controls and risk estimates *n* (%)

ITGA2 genotype	Cases[1]	Controls[1]	Crude OR (95% CI)	*P* value	Adjusted OR[2] (95% CI)	*P* value
Overall	307	307				
CC	141 (45.93)	170 (55.37)	1.00		1.00	
CT	135 (43.97)	113 (36.81)	1.44 (1.03-2.01)	0.033	1.54 (1.10-2.18)	0.013
TT	31 (10.10)	24 (7.82)	1.56 (0.87-2.78)	0.133	1.62 (0.90-2.91)	0.112
CT + TT	166 (54.07)	137 (44.63)	1.46 (1.06-2.01)	0.019	1.57 (1.13-2.17)	0.007
C allele	417 (67.92)	453 (73.78)				
T allele	197 (32.08)	161 (26.22)				

[1]Distributions of the *ITGA2* genotype in cases and controls were in Hardy-Weinberg equilibrium ($P = 0.988$, $P = 0.675$, respectively); [2]Adjusted for age, sex, smoking status, residence, hypertension and diabetes. OR: Odds ratio.

Table 3 Stratified analyses for variant *ITGA2* genotypes in cases and controls *n* (%)

Variable	(CT + TT)/CC		Crude OR (95% CI)	*P* value	Adjusted OR[1] (95% CI)	*P* value
	Cases	Controls				
Age (yr), (median)						
≤ 58	81 (26.4)/68 (22.1)	80 (26)/85 (27.7)	1.27 (0.81-1.97)	0.298	1.31 (0.83-2.06)	0.247
> 58	85 (27.7)/73 (23.8)	57 (18.6)/85 (27.7)	1.74 (1.10-2.75)	0.018	1.88 (1.17-3.03)	0.010
Sex						
Females	45 (14.7)/31 (10.1)	38 (12.4)/38 (12.4)	1.45 (0.76-2.76)	0.255	1.52 (0.73-2.93)	0.206
Males	110 (35.8)/121 (39.4)	99 (32.2)/132 (43)	1.21 (0.85-1.73)	0.304	1.29 (0.82-2.01)	0.287
Smoking status						
Smokers	40 (13)/42 (13.7)	19 (6.2)/34 (11.1)	1.70 (0.84-3.46)	0.141	1.87 (0.89-3.94)	0.100
Non-smokers	126 (41)/99 (32.3)	118 (38.4)/136 (44.3)	1.47 (1.02-2.10)	0.037	1.51 (1.05-2.18)	0.028
Residence						
Urban	89 (29)/79 (25.7)	84 (27.4)/84 (27.4)	1.13 (0.73-1.73)	0.585	1.17 (0.76-1.81)	0.479
Rural	77 (25.1)/62 (20.2)	53 (17.2)/86 (28)	2.02 (1.25-3.25)	0.004	2.35 (1.42-3.90)	0.001

[1]Adjusted for age, sex, smoking status, residence, hypertension, and diabetes. OR: Odds ratio.

Table 4 Associations between variant *ITGA2* genotypes and clinicopathological characteristics of gastric cancer[1]

Variable	CT + TT	CC	Crude OR (95% CI)	*P* value	Adjusted OR[2] (95% CI)	*P* value
Tumor differentiation						
Well	42	42	1		1	
Moderate	65	69	0.94 (0.55-1.63)	0.830	0.94 (0.54-1.63)	0.828
Poor	55	27	2.04 (1.09-3.82)	0.027	2.21 (1.12-4.38)	0.022
Depth of tumor infiltration						
T1	24	30	1		1	
T2	21	17	1.54 (0.67-3.56)	0.308	1.76 (0.73-4.25)	0.208
T3	34	40	1.06 (0.53-2.15)	0.866	1.10 (0.52-2.32)	0.797
T4	83	51	2.03 (1.07-3.86)	0.030	2.12 (1.10-4.07)	0.024
Lymph node metastasis						
Negative	59	51	1		1	
Positive	103	87	1.02 (0.64-1.64)	0.923	0.98 (0.61-1.58)	0.942
Localization						
Cardia	38	41	1		1	
Non-cardia	128	100	1.38 (0.83-2.31)	0.217	1.38 (0.81-2.35)	0.231

[1]Data of seven plaintively treated cases were not obtained for the inoperable tumors; [2]Adjusted for age, sex, smoking status, residence, hypertension, and diabetes. OR: Odds ratio.

ity in a Chinese population. We found that the polymorphism may be associated with an increased risk of gastric cancer, differentiation and invasion of gastric cancer.

It has been reported that integrin α2β1 is one of the key factors accelerating tumor progression and metastasis in various types of cancers[15-21,31]. Koike *et al*[20] found that the α2 integrin was expressed in the intestinal-type and diffuse-type gastric carcinoma cells, and invasion through basement membrane and type I collagen gel was inhibited by anti-α2 integrin monoclonal antibody, indicating that the α2 integrin plays an important role in invasion of gastric carcinoma cells. Another study conducted by Lee *et al*[32] elucidated the potential mechanisms underlying the spreading and invasiveness of gastric carcinoma

cells, the integrin transduces signaling directly *via* engagements with extracellular matrix proteins, thereby leading to the regulation of downstream intracellular signaling molecules. It also functions in collaborative (indirect) signaling, in which integrins cosignal with other membrane receptor-mediated signal pathways, e.g. growth factor receptors, G-protein coupled receptors or the transforming growth factor β1 signaling pathway.

The *ITGA2* gene C807T polymorphism is associated with integrin density, but the precise molecular mechanism remains unclear. It is a silent polymorphism in codon 253 (Phe253Phe) and does not cause an altered structure of the integrin molecule, but in linkage disequilibrium with a yet unknown functional polymorphism affecting *ITGA2* expression. Another explanation could be a direct effect on the stability of the *ITGA2* mRNA, which resulted in a change of the amount of integrin protein being expressed.

Limited studies have reported the association between the polymorphism in *ITGA2* gene and cancer risks, although the results remain inconsistent[27-29]. Gerger *et al*[27] found that the *ITGA2* gene C807T polymorphism was associated with reduced colorectal cancer risk (OR = 0.77, 95% CI = 0.64-0.94, P = 0.011). In their another case-control study, they found that carriers of the most common *ITGA2* haplotype (807C_1648G) had a decreased risk for breast cancer (OR = 0.72, 95% CI = 0.53-0.98)[28]. Nevertheless, Ayala *et al*[29] reported that no association was observed between the *ITGA2* gene C807T polymorphism and breast cancer risk.

Based on these studies, we conducted this hospital-based case-control study to investigate the association between the *ITGA2* gene C807T polymorphism and the risk of gastric cancer in a Chinese population. The frequency of the variant T allele in our control group was 26.22%, which was similar to that in another study in a Chinese Taiwanese population (27.1%)[33] and HapMap database (26.7% for Han Chinese). Our results showed that the variant genotypes had a 57% increased risk of developing gastric cancer.

In the subgroup analyses, we found that the polymorphism was associated with the increased risk of gastric cancer in the subgroup of the subjects aged > 58 years, but not in the subjects aged ≤ 58 years. Milne *et al*[34] indicated that carcinogenesis is considered as accumulation of genetic events, and gastric cancer has a steep slope for age-specific increase in incidence. The increased risk observed in older subjects implies that the *ITGA2* genotype effects tend to be age specific. The polymorphism may contribute to elevated integrin α2β1 levels beyond the age of 58, thus representing a significant risk factor in this age group. However, this is just a hypothesis to interpret the results of our study, and further research is warranted to clarify the mechanism underlying the interaction between the polymorphism and age.

Similarly, in statistical analyses stratified by smoking status, a significant association was observed in nonsmokers, but not in smokers. Tobacco smoking has been undoubtedly accepted as a independent risk factor for gastric cancer[3,5,6]. The association between the polymorphism and gastric cancer risk could be masked by the overwhelming accumulated exposure to tobacco carcinogens in smokers so that the association is more evident in nonsmokers.

We also noted that increased risk of gastric cancer associated with the polymorphism was pronounced in rural subjects, but not in urban subjects. It has been suggested that the genetic differences have their strongest effects under conditions of low environmental pollution[9,35]. Our results plausibly agree with the hypothesis that the genetic effects might be more prominent in the better environments of rural areas[9]. However, this result may be found accidentally, further studies are needed to verify it.

In addition, in the stratified analyses by clinicopathological characteristics of gastric cancer, we observed a significant correlation of the variant genotypes with poorly differentiated tumors. Similarly, Langsenlehner *et al*[28] suggested that a histological grade of 3 or 4 was found more often in breast cancer subjects with TT genotype. The result is consistent with our findings. In contrast, Yasoshima *et al*[21] found no correlation between the expression of integrin α2β1 and histopathological features such as the histological grade, stromal type, and infiltrating growth pattern. We also observed the significant association of the variant genotypes with adjacent organ invasions. Several studies have suggested that integrin α2β1 was closely associated with invasion and metastasis in gastric cancer or tumor cells[18-21,31]. These studies might explain the result we observed. However, no correlation between the polymorphism and lymph node metastasis or location of gastric cancer was found in the stratified analyses. Because the number of cases in the subgroups was relatively small and clinicopathological variables were obtained at the time of diagnosis, our findings should be interpreted with caution before being confirmed in further studies. Thus, large-sized studies which prospectively follow up the clinical outcome, especially the survival rate, may be required to elucidate the association between the polymorphism and gastric cancer progression as well as prognosis.

Some limitations may exist in the present study. First, our study is a hospital-based case-control study, so we can not rule out the selection and recall bias. Nevertheless, the T allele frequency in control subjects is quite similar to that reported in HapMap database for Han Chinese in Beijing (0.262 in our study *vs* 0.267 in HapMap database) and the genotype distributions of cases and controls were in Hardy-Weinberg equilibrium. The second limitation is our relatively small sample size, with 307 cases and 307 controls. So gene-environment interactions may have been underpowered in stratified analyses. However, our preliminary data certainly provides some interesting information and valuable guidance for the future studies in this area. Finally, no enough information on *H. pylori* status was available in cases and controls, because of the ethical reasons.

In conclusion, the present study provides evidence that the *ITGA2* gene C807T polymorphism is associated with an increased risk of gastric cancer in a Chinese population. The association is especially evident in older individuals, non-smokers and rural subjects, and the variant genotypes may also play a role in the differentiation and invasion of gastric cancer, indicating that the polymorphism may be a useful diagnostic marker for genetic susceptibility to gastric cancer. Further studies with larger samples and functional studies are needed to elucidate the role of genetic variations in *ITGA2* and the pathogenesis of gastric cancer.

COMMENTS

Background

Integrin α2β1 has been considered as a key factor for cancer development and progression, especially in gastric cancer. Polymorphisms in *ITGA2* gene is responsible for the expression of integrin α2β1. Recent studies indicated that the *ITGA2* gene C807T polymorphism was associated with cancer risk.

Research frontiers

Using polymerase chain reaction-restriction fragment length polymorphism method, this study explored the relationship between *ITGA2* C807T polymorphism and gastric cancer risk.

Innovations and breakthroughs

The results suggest that the polymorphism is associated with the elevated risk of gastric cancer in a Chinese population, especially in older individuals aged > 58 years, nonsmokers, and rural subjects. Further analyses revealed that the polymorphism may play a role in differentiation and invasion of gastric cancer.

Applications

The results of this study could help further understand the genetic determinants of gastric cancer. The polymorphism may be a useful diagnostic marker for genetic susceptibility to gastric cancer.

Terminology

Integrins are members of a family of cell-surface heterodimeric proteins that mediate cell-matrix and cell-cell interactions. Single nucleotide polymorphisms represent a natural genetic variability at a high density in the human genome, which are responsible for the inter-individual variation and diversity. They have been recently considered as the main genetic elements involved in the development of common and complex diseases, including various cancers.

Peer review

The current study was designed, processed and concluded well, deserving publication.

REFERENCES

1 **Crew KD**, Neugut AI. Epidemiology of gastric cancer. *World J Gastroenterol* 2006; **12**: 354-362
2 **Ushijima T**, Sasako M. Focus on gastric cancer. *Cancer Cell* 2004; **5**: 121-125
3 **Parkin DM**. International variation. *Oncogene* 2004; **23**: 6329-6340
4 **Mayne ST**, Navarro SA. Diet, obesity and reflux in the etiology of adenocarcinomas of the esophagus and gastric cardia in humans. *J Nutr* 2002; **132**: 3467S-3470S
5 **Galanis DJ**, Lee J, Kolonel LN. The influence of cigarette smoking, alcohol, and green tea consumption on the risk of carcinoma of the cardia and distal stomach in Shanghai, China. *Cancer* 1997; **79**: 1840-1841
6 **Gammon MD**, Schoenberg JB, Ahsan H, Risch HA, Vaughan TL, Chow WH, Rotterdam H, West AB, Dubrow R, Stanford JL, Mayne ST, Farrow DC, Niwa S, Blot WJ, Fraumeni JF. Tobacco, alcohol, and socioeconomic status and adenocarcinomas of the esophagus and gastric cardia. *J Natl Cancer Inst* 1997; **89**: 1277-1284
7 **Ji BT**, Chow WH, Yang G, McLaughlin JK, Gao RN, Zheng W, Shu XO, Jin F, Fraumeni JF, Gao YT. Body mass index and the risk of cancers of the gastric cardia and distal stomach in Shanghai, China. *Cancer Epidemiol Biomarkers Prev* 1997; **6**: 481-485
8 **Wu MS**, Chen CJ, Lin JT. Host-environment interactions: their impact on progression from gastric inflammation to carcinogenesis and on development of new approaches to prevent and treat gastric cancer. *Cancer Epidemiol Biomarkers Prev* 2005; **14**: 1878-1882
9 **Zhu H**, Yang L, Zhou B, Yu R, Tang N, Wang B. Myeloperoxidase G-463A polymorphism and the risk of gastric cancer: a case-control study. *Carcinogenesis* 2006; **27**: 2491-2496
10 **Gu H**, Yang L, Tang N, Zhou B, Zhu H, Sun Q, Cong R, Wang B. Association of endothelin-converting enzyme-1b C-338A polymorphism with gastric cancer risk: a case-control study. *Eur J Cancer* 2008; **44**: 1253-1258
11 **Yang L**, Gu HJ, Zhu HJ, Sun QM, Cong RH, Zhou B, Tang NP, Wang B. Tissue inhibitor of metalloproteinase-2 G-418C polymorphism is associated with an increased risk of gastric cancer in a Chinese population. *Eur J Surg Oncol* 2008; **34**: 636-641
12 **Gu H**, Yang L, Sun Q, Zhou B, Tang N, Cong R, Zeng Y, Wang B. Gly82Ser polymorphism of the receptor for advanced glycation end products is associated with an increased risk of gastric cancer in a Chinese population. *Clin Cancer Res* 2008; **14**: 3627-3632
13 **Parise LV**, Lee J, Juliano RL. New aspects of integrin signaling in cancer. *Semin Cancer Biol* 2000; **10**: 407-414
14 **Hood JD**, Cheresh DA. Role of integrins in cell invasion and migration. *Nat Rev Cancer* 2002; **2**: 91-100
15 **Zutter MM**, Santoro SA. Widespread histologic distribution of the alpha 2 beta 1 integrin cell-surface collagen receptor. *Am J Pathol* 1990; **137**: 113-120
16 **Gui GP**, Puddefoot JR, Vinson GP, Wells CA, Carpenter R. Altered cell-matrix contact: a prerequisite for breast cancer metastasis? *Br J Cancer* 1997; **75**: 623-633
17 **Felding-Habermann B**, O'Toole TE, Smith JW, Fransvea E, Ruggeri ZM, Ginsberg MH, Hughes PE, Pampori N, Shattil SJ, Saven A, Mueller BM. Integrin activation controls metastasis in human breast cancer. *Proc Natl Acad Sci USA* 2001; **98**: 1853-1858
18 **Matsuoka T**, Yashiro M, Nishimura S, Inoue T, Fujihara T, Sawada T, Kato Y, Seki S, Hirakawa-Ys Chung K. Increased expression of alpha2beta1-integrin in the peritoneal dissemination of human gastric carcinoma. *Int J Mol Med* 2000; **5**: 21-25
19 **Lin MT**, Chang CC, Lin BR, Yang HY, Chu CY, Wu MH, Kuo ML. Elevated expression of Cyr61 enhances peritoneal dissemination of gastric cancer cells through integrin alpha-2beta1. *J Biol Chem* 2007; **282**: 34594-34604
20 **Koike N**, Todoroki T, Komano H, Shimokama T, Ban S, Ohno T, Fukao K, Watanabe T. Invasive potentials of gastric carcinoma cell lines: role of alpha 2 and alpha 6 integrins in invasion. *J Cancer Res Clin Oncol* 1997; **123**: 310-316
21 **Ura H**, Denno R, Hirata K, Yamaguchi K, Yasoshima T. Separate functions of alpha2beta1 and alpha3beta1 integrins in the metastatic process of human gastric carcinoma. *Surg Today* 1998; **28**: 1001-1006
22 **Kritzik M**, Savage B, Nugent DJ, Santoso S, Ruggeri ZM, Kunicki TJ. Nucleotide polymorphisms in the alpha2 gene define multiple alleles that are associated with differences in platelet alpha2 beta1 density. *Blood* 1998; **92**: 2382-2388
23 **Corral J**, González-Conejero R, Rivera J, Ortuño F, Aparicio P, Vicente V. Role of the 807 C/T polymorphism of the alpha2 gene in platelet GP Ia collagen receptor expression and function--effect in thromboembolic diseases. *Thromb Haemost* 1999; **81**: 951-956
24 **Carlsson LE**, Santoso S, Spitzer C, Kessler C, Greinacher A. The alpha2 gene coding sequence T807/A873 of the platelet

collagen receptor integrin alpha2beta1 might be a genetic risk factor for the development of stroke in younger patients. *Blood* 1999; **93**: 3583-3586

25 **Dodson PM**, Haynes J, Starczynski J, Farmer J, Shigdar S, Fegan G, Johnson RJ, Fegan C. The platelet glycoprotein Ia/IIa gene polymorphism C807T/G873A: a novel risk factor for retinal vein occlusion. *Eye* (Lond) 2003; **17**: 772-777

26 **Casorelli I**, De Stefano V, Leone AM, Chiusolo P, Burzotta F, Paciaroni K, Rossi E, Andreotti F, Leone G, Maseri A. The C807T/G873A polymorphism in the platelet glycoprotein Ia gene and the risk of acute coronary syndrome in the Italian population. *Br J Haematol* 2001; **114**: 150-154

27 **Gerger A**, Hofmann G, Langsenlehner U, Renner W, Weitzer W, Wehrschütz M, Wascher T, Samonigg H, Krippl P. Integrin alpha-2 and beta-3 gene polymorphisms and colorectal cancer risk. *Int J Colorectal Dis* 2009; **24**: 159-163

28 **Langsenlehner U**, Renner W, Yazdani-Biuki B, Eder T, Wascher TC, Paulweber B, Clar H, Hofmann G, Samonigg H, Krippl P. Integrin alpha-2 and beta-3 gene polymorphisms and breast cancer risk. *Breast Cancer Res Treat* 2006; **97**: 67-72

29 **Ayala F**, Corral J, González-Conejero R, Sánchez I, Moraleda JM, Vicente V. Genetic polymorphisms of platelet adhesive molecules: association with breast cancer risk and clinical presentation. *Breast Cancer Res Treat* 2003; **80**: 145-154

30 **Sobin LH**, Wittekind CH, editors. TNM classification of malignant tumors. 5th ed. New York: Wiley & Sons Inc., 1997: 59-62

31 **Chan BM**, Matsuura N, Takada Y, Zetter BR, Hemler ME. In vitro and in vivo consequences of VLA-2 expression on rhabdomyosarcoma cells. *Science* 1991; **251**: 1600-1602

32 **Lee MS**, Kim TY, Kim YB, Lee SY, Ko SG, Jong HS, Kim TY, Bang YJ, Lee JW. The signaling network of transforming growth factor beta1, protein kinase Cdelta, and integrin underlies the spreading and invasiveness of gastric carcinoma cells. *Mol Cell Biol* 2005; **25**: 6921-6936

33 **Chen CH**, Lo YK, Ke D, Liu CK, Liou CW, Wu HL, Lai ML. Platelet glycoprotein Ia C807T, Ib C3550T, and IIIa Pl(A1/A2) polymorphisms and ischemic stroke in young Taiwanese. *J Neurol Sci* 2004; **227**: 1-5

34 **Milne AN**, Carvalho R, Morsink FM, Musler AR, de Leng WW, Ristimäki A, Offerhaus GJ. Early-onset gastric cancers have a different molecular expression profile than conventional gastric cancers. *Mod Pathol* 2006; **19**: 564-572

35 **Hung RJ**, Boffetta P, Brennan P, Malaveille C, Gelatti U, Placidi D, Carta A, Hautefeuille A, Porru S. Genetic polymorphisms of MPO, COMT, MnSOD, NQO1, interactions with environmental exposures and bladder cancer risk. *Carcinogenesis* 2004; **25**: 973-978

S- Editor Tian L **L- Editor** Ma JY **E- Editor** Zheng XM

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/1007-9327office
wjg@wjgnet.com
doi:10.3748/wjg.v17.i23.2867

World J Gastroenterol 2011 June 21; 17(23): 2867-2872
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

BRIEF ARTICLE

Log-normal censored regression model detecting prognostic factors in gastric cancer: A study of 3018 cases

Bin-Bin Wang, Cai-Gang Liu, Ping Lu, A Latengbaolide, Yang Lu

Bin-Bin Wang, Cai-Gang Liu, Ping Lu, A Latengbaolide, Yang Lu, Department of Breast Surgery, General surgery, the First Hospital of China Medical University, Shenyang 110001, Liaoning Province, China
Author contributions: Wang BB and Liu CG contributed equally to this work; Wang BB, Liu CG, Lu P, Latengbaolide A and Lu Y designed research; Wang BB and Liu CG performed research and analyzed data; Wang BB, Liu CG, Lu P and Latengbaolide A wrote the paper.
Supported by the Gastric Cancer Laboratory and Pathology Department of Chinese Medical University, Shenyang, China; the Science and Technology Program of Shenyang, No. 1081232-1-00
Correspondence to: Cai-Gang Liu, MD, Department of Breast Surgery, General Surgery, the First Hospital of China Medical University, Shenyang 110001, Liaoning Province, China. angel-s205@163.com
Telephone: +86-24-83282618 Fax: +86-24-22834060
Received: October 23, 2010 Revised: January 11, 2011
Accepted: January 18, 2011
Published online: June 21, 2011

Abstract

AIM: To investigate the efficiency of Cox proportional hazard model in detecting prognostic factors for gastric cancer.

METHODS: We used the log-normal regression model to evaluate prognostic factors in gastric cancer and compared it with the Cox model. Three thousand and eighteen gastric cancer patients who received a gastrectomy between 1980 and 2004 were retrospectively evaluated. Clinic-pathological factors were included in a log-normal model as well as Cox model. The akaike information criterion (AIC) was employed to compare the efficiency of both models. Univariate analysis indicated that age at diagnosis, past history, cancer location, distant metastasis status, surgical curative degree, combined other organ resection, Borrmann type, Lauren's classification, pT stage, total dissected nodes and pN stage were prognostic factors in both log-normal and Cox models.

RESULTS: In the final multivariate model, age at diagnosis, past history, surgical curative degree, Borrmann type, Lauren's classification, pT stage, and pN stage were significant prognostic factors in both log-normal and Cox models. However, cancer location, distant metastasis status, and histology types were found to be significant prognostic factors in log-normal results alone. According to AIC, the log-normal model performed better than the Cox proportional hazard model (AIC value: 2534.72 *vs* 1693.56).

CONCLUSION: It is suggested that the log-normal regression model can be a useful statistical model to evaluate prognostic factors instead of the Cox proportional hazard model.

Key words: Gastric cancer; Log normal regression model; Cox proportional hazard model; Prognostic factors

Peer reviewer: Ki-Baik Hahm, MD, PhD, Professor, Gachon Graduate School of Medicine, Department of Gastroenterology, Lee Gil Ya Cancer and Diabetes Institute, Lab of Translational Medicine, 7-45 Songdo-dong, Yeonsu-gu, Incheon, 406-840, Korea

Wang BB, Liu CG, Lu P, Latengbaolide A, Lu Y. Log-normal censored regression model detecting prognostic factors in gastric cancer: A study of 3018 cases. *World J Gastroenterol* 2011; 17(23): 2867-2872 Available from: URL: http://www.wjgnet.com/1007-9327/full/v17/i23/2867.htm DOI: http://dx.doi.org/10.3748/wjg.v17.i23.2867

INTRODUCTION

The survival of patients with gastric cancer has recently been improved because of early detection, rational lymphadenectomy and several therapeutic modalities[1,2]. However, gastric cancer still remains the second leading cause

of cancer mortality in the world. It is acknowledged that surgery and systemic chemotherapy can clearly improve the survival of patients with gastric cancer[3,4]. However, a sensible treatment option must be fundamentally based on the current evaluation of prognostic factors, so a rational method to evaluate the prognostic factors is very important in establishing therapeutic strategies and evaluate prognosis.

Survival analysis is a branch of statistics which deals with death in biological organisms and failure in mechanical systems. The Cox model is the standard tool for assessing the effect of prognostic factors; however, there may be substantive differences in the estimated prognosis obtained by the Cox model rather than a log-normal model[5]. The Cox model is semiparametric, in that the baseline hazard takes on no particular form[6]. In contrast to Cox, a link to parametric survival models comes through alternative functions for the baseline hazard. In this case, one can let the baseline hazard be a parametric form such as log-normal. It is acknowledged that most of studies used the Cox proportional hazard model to find the relation between survival time and covariates of patients with gastric cancer[7-9]. On the other hand, some studies reported that log-normal regression could estimate the parameter more efficiently than the Cox model[5]. However, the efficiency of log-normal regression was still controversial.

The aim of this retrospective study was to elucidate the factors affecting the survival of patients with GC using log-normal regression, and to compare these results with the Cox model.

MATERIALS AND METHODS

Patients

In this study, three thousand and eighteen cases with gastric cancer were selected on whom an operation was performed at the China Medical University between 1980 and 2004. The selection criteria for inclusion were as follows: (1) an operation was performed; (2) lymph nodes were dissected and then pathologically examined; and (3) the patient medical records were available. All patients were periodically followed up through post letters, and/or telephone interviews with patients and their relatives. Clinical, surgical and pathological findings, and all follow-up information were collected and recorded in a database, and 5-year survival rate was calculated. The study protocol was approved by the Ethics Committee of China Medical University.

Reference standard

Lymph nodes were meticulously dissected from the en bloc specimens, and the classification of the dissected lymph nodes was determined by surgeons who reviewed the excised specimens after surgery based on the Japanese Classification of Gastric Carcinoma[10]. Accordingly, lymphadenectomy was classified as D1, dissection of all the Group 1 lymph nodes; D2, dissection of all Group 1 and Group 2 lymph nodes; and D3, dissection of all the Group 1, Group 2 and Group 3 lymph nodes. pN category was defined as pN0 (no metastatic lymph node), pN1 (1-6 metastatic lymph nodes), pN2 (7-15 metastatic lymph nodes) and pN3 (> 15 metastatic lymph nodes), according to the 5th Edition of UICC[11]. The location of tumors was defined as upper, middle and lower third gastric cancer, according to JCGC[10] and the histological grade was defined as poorly differentiated, moderately differentiated and well differentiated, according to the latest World Health Organization (WHO) classification[12]. The Borrmann type was defined as Borrmann Ⅰ, Borrmann Ⅱ, Borrmann Ⅲ and Borrmann Ⅳ, according to JCGC[10]. The histological type was determined according to Lauren's classification.

Statistical analysis

All data were analyzed using STAT statistics software (Version 10.0, Stata Corp LP). Clinic-pathologic factors were entered to a log-normal censored regression, as well as a Cox proportional hazard model in univariate and multivariate analysis in order to find the prognostic factors. The term of relative risk (RR) was used to interpret the risk of death in parametric results and the term of Akaike Information Criterion (AIC) was employed to compare the efficiency of models. Disease-specific survival was analyzed using the Kaplan-Meier method. The log-rank test was used to analyze survival differences. Lower AIC indicates better likelihood. A P value of less than 0.05 was considered statistically significant.

RESULTS

Clinic-pathological characteristics of patients with gastric cancer

The male-to-female ratio among the 3018 patients enrolled was 2.74:1 and the mean age was 57.54 years (range: 19 to 90 years) at operation. 269, 1362 and 608 cases received D1, D2 and more than D2 lymph node dissection respectively. In addition, six hundred and fifty seven cases received palliative surgery. From 3018 cases, a total of 46081 lymph nodes were removed and examined, and the mean number of examined lymph nodes was 15.27. One thousand six hundred and forty three cases were observed lymph node metastasis. Thus, the incidence of lymph node metastasis was 54.44%. The last follow-up was Jan 1, 2009, with a total follow-up rate of 70.68%. More clinic-pathologic factors are shown in Table 1.

Multivariate analysis of prognostic factors in gastric cancer

Univariate analysis indicated that age at diagnosis, past history, cancer location, distant metastasis status, surgical curative degree, combined other organ resection, Borrmann type, Lauren's classification, pT stage, total dissected nodes and pN stage were prognostic factors in both log-normal and Cox models. In the final multivariate model, age at diagnosis, past history, surgical curative degree, Borrmann type, Lauren's classification pT stage, and pN stage were significant prognostic factors in both

Table 1 Clinicopathological characteristics of 3018 gastric cancers included in the study *n* (%)

Variable	Subgroups	Frequency
Gender ratio	Male	2211 (73.26)
	Female	807 (26.74)
Age at diagnosis (mean ± SD; yr)		57.54 ± 11.24
Past history		
	Without	2234 (74.02)
	With	784 (25.98)
Family history	Without	2467 (81.74)
	With	551 (18.26)
Cancer number	Single	2883 (96.65)
	Multiple	100 (3.35)
Cancer location	Lower stomach	1873 (62.64)
	Middle stomach	492 (16.46)
	Upper stomach	355 (11.87)
	Total stomach	270 (9.03)
Distant metastasis status	Without	2540 (85.04)
	With	447 (14.96)
Maximum tumor diameter (mean ± SD, cm)		5.85 ± 3.30
Surgical curative degree	Absolutely radical	1396 (49.73)
	Relatively radical	809 (28.82)
	Palliative	602 (21.45)
Lymph node dissection	More than D2	608 (20.99)
	D2	1362 (47.03)
	D1	269 (9.29)
	Palliative surgery	657 (22.69)
Combined other organ resection	Without	2016 (76.80)
	With	609 (23.20)
Histological type	Well differentiated	755 (27.43)
	Middle differentiated	382 (13.88)
	Poor differentiated	1615 (58.69)
Borrmann classification	Ⅰ	70 (2.98)
	Ⅱ	426 (18.15)
	Ⅲ	1571 (66.94)
	Ⅳ	280 (11.93)
Lauren classification	Intestinal type	1170 (43.89)
	Diffuse type	1496 (56.11)
pT stage	pT1	328 (11.89)
	pT2	1486 (53.88)
	pT3	737 (26.72)
	pT4	207 (7.51)
Total dissected lymph node (mean ± SD)		15.27 ± 13.11
Pathological lymph node status	pN0	1375 (45.56)
	pN1	1039 (34.43)
	pN2	432 (14.31)
	pN3	172 (5.70)

log-normal and Cox models. However, cancer location, distant metastasis status and histology types were found as significant prognostic factors in log-normal results alone (Table 2). According to AIC, the log-normal model performed better than the Cox proportional hazard model (AIC value: 2534.72 *vs* 1693.56) (Table 3).

Survival outcomes

Overall, the 5-year disease-specific survival rate was 29.57%. The survival was observed significantly different in patients with different cancer locations (5-year disease-specific survival rate, L tumor *vs* M tumor *vs* U tumor *vs* T tumor: 33.11% *vs* 30.46% *vs* 25.66% *vs* 7.59%, χ^2 = 190.27, P = 0.000) (Figure 1). In addition, the cases with distant metastasis received a poorer prognosis than those without distant metastasis (5-year disease-specific survival

Table 2 Univariate model of Cox and log normal regression with prognostic factors

	HR (95% CI)	
	Cox	Log normal
Sex		
Male	0.953 (0.843-1.078)	0.925 (0.818-1.046)
Female	1.00	1.00
Age at diagnosis	1.015[1] (1.009-1.020)	1.016[1] (1.011-1.021)
Past history		
Without	1.00	1.00
With	0.715[1] (0.631-0.831)	0.694[1] (0.612-0.787)
Family history		
Without	1.00	1.00
With	0.871 (0.752-1.009)	0.875 (0.754-1.014)
Cancer number		
Single	1.00	1.00
Multiple	0.870 (0.622-1.218)	0.924 (0.661-1.294)
Cancer location		
Lower third	1.00	1.00
Middle third	1.181[1] (1.017-1.373)	1.302[1] (1.233-1.374)
Upper third	1.436[1] (1.212-1.701)	1.695[1] (1.429-1.897)
Total stomach	2.464[1] (2.062-2.944)	2.207[1] (2.011-2.677)
Distant metastasis		
Absent	1.00	1.00
Present	2.554[1] (2.194-2.973)	2.596[1] (2.227-3.027)
Surgical curative degree		
Absolutely radical	1.00	1.00
Relatively radical	1.835[1] (1.593-2.114)	2.159[1] (2.020-2.308)
Palliative	4.236[1] (3.714-4.832)	4.661[1] (4.214-4.759)
Lymph node dissection		
> D2	1.00	1.00
D2	0.989 (0.859-1.138)	1.536[1] (1.458-1.619)
D1	1.056 (0.853-1.307)	2.359[1] (2.121-2.574)
< D1	3.310[1] (2.854-3.839)	3.624[1] (3.231-3.862)
Combined other organ resection		
Without	1.00	1.00
With	1.981[1] (1.749-2.245)	2.070[1] (1.825-2.348)
Histologic types		
Well differentiated	1.00	1.00
Middle differentiated	0.706[1] (0.592-0.843)	0.976 (0.918-1.039)
Poor differentiated	0.918 (0.814-1.036)	0.952 (0.897-1.011)
Borrmann classification (*n* (%))		
Ⅰ	1.00	1.00
Ⅱ	1.005 (0.892-1.340)	0.981 (0.894-1.019)
Ⅲ	1.247[1] (1.173-1.638)	1.176[1] (1.074-1.293)
Ⅳ	2.512[1] (1.842-3.075)	2.610[1] (2.416-3.153)
Lauren classification (*n* (%))		
Intestinal type	1.00	1.00
Diffuse type	1.245[1] (1.082-1.184)	1.171[1] (1.015-1.384)
pT stage		
pT1	1.00	1.00
pT2	2.936[1] (2.299-3.751)	1.787[1] (1.666-1.916)
pT3	4.305[1] (3.357-5.522)	3.193[1] (3.066-3.321)
pT4	7.697[1] (5.759-10.287)	5.707[1] (5.579-5.833)
Total dissected nodes	0.993[1] (0.988-0.998)	0.994[1] (0.988-0.998)
pN stage		
pN0	1.00	1.00
pN1	1.555[1] (1.372-1.764)	1.633[1] (1.533-1.740)
pN2	2.510[1] (2.133-2.953)	2.667[1] (2.561-2.772)
pN3	3.669[1] (2.901-4.640)	4.355[1] (4.249-4.460)

[1]Statistically significant (P < 0.05). HR: Hazard radio; CI: Confidence interval.

Table 3 Multivariate model of Cox and log normal regression with prognostic factors (full model and final model)

	Cox HR (95% CI)		Log normal HR (95% CI)	
	Full model (AIC = 1508.49)	Final model (AIC = 2534.72)	Full model (AIC = 913.34)	Final model (AIC = 1693.56)
Sex				
Male	0.91 (0.801-1.034)		0.886 (0.781-1.005)	
Female	1.00			
Age at diagnosis	1.014[1] (1.009-1.02)	1.011[1] (1.006-1.017)	1.015[1] (1.010-1.021)	1.015[1] (1.009-1.020)
Past history				
Without	1.00	1.00	1.00	1.00
With	0.840[1] (0.738-0.955)	0.858[1] (0.755-0.975)	0.813[1] (0.716-0.914)	0.809[1] (0.713-0.919)
Family history				
Without	1.00		1.00	1.00
With	0.957 (0.8254-1.11)		0.967 (0.833-1.234)	
Cancer number				
Single	1.00		1.00	
Multiple	1.21 (0.861-1.701)		1.312 (1.935-1.840)	
Cancer location				
Lower third	1.00		1.00	1.00
Middle third	1.033 (0.885-1.205)		1.135[1] (1.073-1.199)	1.129[1] (1.069-1.194)
Upper third	1.406[1] (1.173-1.686)		1.288[1] (1.224-1.353)	1.277[1] (1.211-1.338)
Total stomach	1.466[1] (1.214-1.771)		1.462[1] (1.365-1.558)	1.439[1] (1.343-1.535)
Distant metastasis				
Absent	1.00		1.00	1.00
Present	1.21[1] (1.013-1.447)		1.205[1] (1.011-1.437)	1.198[1] (1.009-1.424)
Surgical curative degree				
Absolutely radical	1.00	1.00	1.00	1.00
Relatively radical	1.389[1] (1.197-1.611)	1.383[1] (1.194-1.601)	1.724[1] (1.537-1.934)	1.672[1] (1.538-1.817)
Palliative	3.889[1] (2.583-5.855)	2.687[1] (2.316-3.116)	2.972[1] (2.770-3.174)	2.796[1] (2.653-2.938)
Lymph node dissection				
> D2	1.00		1.00	
D2	0.908 (0.784-1.051)		0.967 (0.892-1.049)	
D1	0.901 (0.712-1.14)		0.935 (0.855-1.015)	
< D1	0.607[1] (0.395-0.935)		0.904 (0.784-1.024)	
Combined other organ resection				
Without	1.00		1.00	
With	1.406[1] (1.227-1.61)		1.447[1] (1.264-1.657)	
Histologic types				
Well differentiated	1.00		1.00	1.00
Middle differentiated	1.056 (0.878-1.271)		1.110[1] (1.042-1.183)	1.120[1] (1.051-1.193)
Poor differentiated	1.179[1] (1.035-1.343)		1.232[1] (1.160-1.304)	1.254[1] (1.182-1.327)
Borrmann classification				
Ⅰ	1.00	1.00	1.00	1.00
Ⅱ	1.142 (0.957-1.319)	1.201 (1.068-1.433)	1.018 (0.943-1.106)	1.015 (0.941-1.102)
Ⅲ	1.315[1] (1.113-1.672)	1.394[1] (1.205-1.741)	1.246[1] (1.052-1.539)	1.241[1] (1.047-1.533)
Ⅳ	2.126[1] (1.758-3.119)	2.253[1] (1.827-3.284)	2.530[1] (2.376-2.713)	2.526[1] (2.372-2.708)
Lauren classification				
Intestinal type	1.00		1.00	1.00
Diffuse type	1.131[1] (1.012-1.358)		1.307[1] (1.154-1.528)	1.302[1] (1.148-1.523)
pT stage				
pT1	1.00	1.00	1.00	1.00
pT2	1.851[1] (1.431-2.394)	1.971[1] (1.528-2.542)	1.195[1] (1.102-1.297)	1.193[1] (1.100-1.294)
pT3	1.981[1] (1.511-2.598)	2.19[1] (1.678-2.858)	1.428[1] (1.328-1.527)	1.423[1] (1.324-1.522)
pT4	2.344[1] (1.699-3.235)	2.501[1] (1.821-3.435)	1.706[1] (1.557-1.855)	1.697[1] (1.549-1.847)
Total dissected nodes	0.987[1] (0.981-0.993)		0.988[1] (0.982-0.993)	
pN stage				
pN0	1.00	1.00	1.00	1.00
pN1	1.281[1] (1.123-1.461)	1.266[1] (1.11-1.444)	1.507[1] (1.393-1.620)	1.500[1] (1.387-1.622)
pN2	2.139[1] (1.783-2.566)	2.095[1] (1.749-2.51)	2.271[1] (2.151-2.391)	2.250[1] (2.130-2.370)
pN3	3.24[1] (2.446-4.292)	3.325[1] (2.52-4.386)	3.422[1] (3.242-3.602)	3.375[1] (3.196-3.554)

[1]Statistically significant (< 0.05); HR: Hazard radio; CI: Confidence interval; AIC: Akaike Information Criterion.

rate, 33.50% *vs* 7.56%, χ^2 = 372.21, P = 0.000) (Figure 2). Furthermore, the cases with different histologic types were investigated with a different prognosis (5-year disease-specific survival rate, well differentiated tumors *vs* middle differentiated tumors *vs* poor differentiated tumors: 39.27% *vs* 29.67% *vs* 25.03%, χ^2 = 12.37, P = 0.002) (Figure 3).

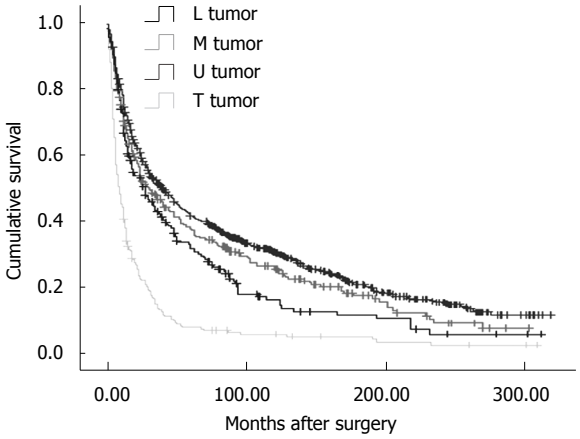


Figure 1 Disease-specific survival analysis according to cancer locations (χ^2 = 190.27, *P* = 0.000, Log Rank test). L tumor: Lower third tumors; M tumor: Middle third tumors; U tumor: Upper third tumors; T tumor: Tumor occupied the total stomach.

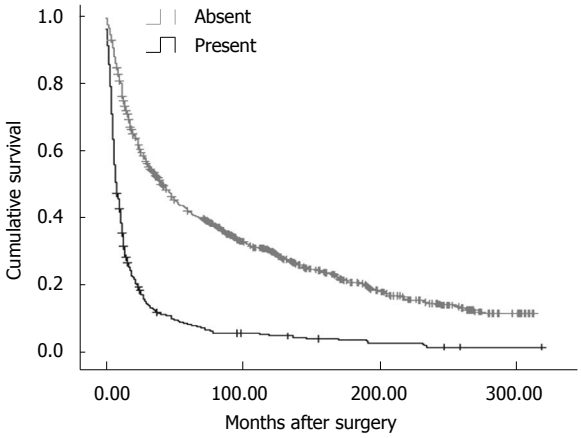


Figure 2 Disease-specific survival analysis according to distant metastasis status (χ^2 = 372.21, *P* = 0.000, Log Rank test).

DISCUSSION

There were several studies that have investigated the factors influencing prognosis[13,14]. The conclusions of the reports were controversial, though most of them used the Cox proportional hazard model to find the relation between survival time and patient characteristics, and clinical and pathological factors in patients with gastric cancer.

After evaluating the clinic-pathological factors of 738 patients, Kulig *et al*[7] reported that patient age, depth of tumor infiltration, tumor location, and metastatic node ratio were identified as independent prognostic factors in a Cox proportional hazards model. In addition, Shiraishi *et al*[15] reported that independent prognostic factors of gastric cancer were serosal invasion, extragastric lymph node metastasis and liver metastasis, but survival was not significantly associated with any of the patient factors or operation factors, including the extent of lymph node dissection. In our study, age at diagnosis, past history, surgical curative degree, Borrmann type, Lauren's classification, pT stage, and pN stage were significant prognostic factors in Cox models. There was a small difference between our study and other reports. In the final model of log-normal analysis, we investigated that cancer location, distant metastasis and histologic types were significantly related to the survival. The outcomes were also verified by disease-specific survival analysis. However, the association between above factors and survival were not observed. In log-normal analysis, Pourhoseingholi *et al*[5] observed that distant metastasis, histology type and pT stage were significant prognostic factors after retrospectively studying 746 Iranian patients. Moreover, distant metastasis was a significant prognostic factor only in log-normal analysis, not in the Cox model.

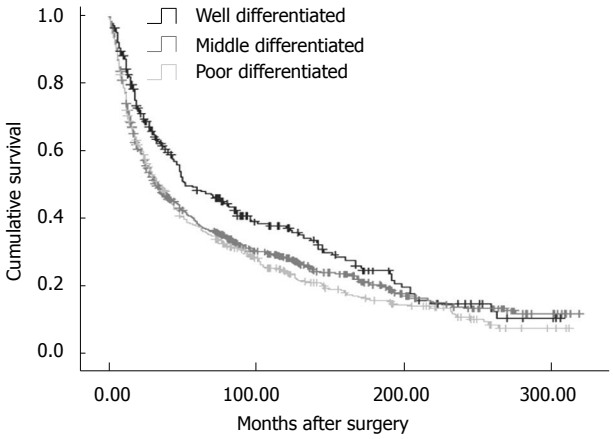


Figure 3 Disease-specific survival analysis according to histologic types (χ^2 = 12.37, *P* = 0.002, Log Rank test).

Compared to the Cox model, the evaluation criteria in our study indicated log-normal regression was more powerful not only in the full model, but also in the final one. In the final model, the selected prognostic factors in the log-normal model were different compared to those in the Cox model. Furthermore, the data strongly supported the log-normal regression in the full and final models, and might lead to more precise results as an alternative for Cox.

In conclusion, according to the results of our study, age at diagnosis, past history, cancer location, distant metastasis status, surgical curative degree, combined other organ resection, histology types, Borrmann type, Lauren's classification, pT stage, total dissected nodes and pN stage were significant prognostic factors of gastric cancer. It is suggested that log-normal regression model can be a useful statistical model to evaluate prognostic factors instead of the Cox proportional hazard model.

ACKNOWLEDGMENTS

The preparation of this manuscript has been assisted by a professional editing company, Medjaden.

COMMENTS

Background

Most of studies used the Cox proportional hazard model to find the relation between survival time and covariates of patients with gastric cancer (GC). On the other hand, some studies reported that log-normal regression could estimate

the parameter more efficiently than the Cox model. However, the efficiency of log-normal regression was still controversial.

Research frontiers

In this retrospective study, the authors elucidated the factors affecting the survival of patients with GC using log-normal regression, and to compare these results with the Cox model.

Applications

It is suggested that log-normal regression model can be a useful statistical model to evaluate prognostic factors instead of the Cox proportional hazard model.

Peer review

Overall the study was well designed, performed, and analyzed. The very minor, but important parameters should be added in analysis.

REFERENCES

1 **Yokota T**, Ishiyama S, Saito T, Teshima S, Shimotsuma M, Yamauchi H. Treatment strategy of limited surgery in the treatment guidelines for gastric cancer in Japan. *Lancet Oncol* 2003; **4**: 423-428

2 **Adachi Y**, Shiraishi N, Kitano S. Modern treatment of early gastric cancer: review of the Japanese experience. *Dig Surg* 2002; **19**: 333-339

3 **Ohdaira H**, Noro T, Terada H, Kameyama J, Ohara T, Yoshino K, Kitajima M, Suzuki Y. New double-stapling technique for esophagojejunostomy and esophagogastrostomy in gastric cancer surgery, using a peroral intraluminal approach with a digital stapling system. *Gastric Cancer* 2009; **12**: 101-105

4 **Vecchione L**, Orditura M, Ciardiello F, De Vita F. Novel investigational drugs for gastric cancer. *Expert Opin Investig Drugs* 2009; **18**: 945-955

5 **Pourhoseingholi MA**, Moghimi-Dehkordi B, Safaee A, Hajizadeh E, Solhpour A, Zali MR. Prognostic factors in gastric cancer using log-normal censored regression model. *Indian J Med Res* 2009; **129**: 262-267

6 **Moghimi-Dehkordi B**, Safaee A, Zali MR. Comparison of colorectal and gastric cancer: survival and prognostic factors. *Saudi J Gastroenterol* 2009; **15**: 18-23

7 **Kulig J**, Sierzega M, Kolodziejczyk P, Popiela T. Ratio of metastatic to resected lymph nodes for prediction of survival in patients with inadequately staged gastric cancer. *Br J Surg* 2009; **96**: 910-918

8 **Marrelli D**, Pedrazzani C, Corso G, Neri A, Di Martino M, Pinto E, Roviello F. Different pathological features and prognosis in gastric cancer patients coming from high-risk and low-risk areas of Italy. *Ann Surg* 2009; **250**: 43-50

9 **Ichikawa D**, Kubota T, Kikuchi S, Fujiwara H, Konishi H, Tsujiura M, Ikoma H, Nakanishi M, Okamoto K, Sakakura C, Ochiai T, Kokuba Y, Otsuji E. Prognostic impact of lymphatic invasion in patients with node-negative gastric cancer. *J Surg Oncol* 2009; **100**: 111-114

10 **Japanese Gastric Cancer Association**. Japanese Classification of Gastric Carcinoma-2nd English Edition. *Gastric Cancer* 1998; **1**: 10-24

11 **International Union Against Cancer**. In: Sobin LH, Wittekind CH, editors. TNM classification of malignant tumors. 5th ed. New York: Wiley, 1997

12 **Hamilton SR**, Aaltonen LA. World Health Organization classification of tumors-pathology and genetics of the digestive system. 2000: 38

13 **Liu X**, Xu Y, Long Z, Zhu H, Wang Y. Prognostic significance of tumor size in T3 gastric cancer. *Ann Surg Oncol* 2009; **16**: 1875-1882

14 **Park JC**, Lee YC, Kim JH, Kim YJ, Lee SK, Hyung WJ, Noh SH, Kim CB. Clinicopathological aspects and prognostic value with respect to age: an analysis of 3,362 consecutive gastric cancer patients. *J Surg Oncol* 2009; **99**: 395-401

15 **Shiraishi N**, Sato K, Yasuda K, Inomata M, Kitano S. Multivariate prognostic study on large gastric cancer. *J Surg Oncol* 2007; **96**: 14-18

S- Editor Tian L **L- Editor** Rutherford A **E- Editor** Ma WH

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/1007-9327office
wjg@wjgnet.com
doi:10.3748/wjg.v17.i23.2873

World J Gastroenterol 2011 June 21; 17(23): 2873-2876
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

CASE REPORT

Diaphragm disease compared with cryptogenic multifocal ulcerous stenosing enteritis

Sook Hee Chung, Yunju Jo, Sang Ryol Ryu, Sang Bong Ahn, Byoung Kwan Son, Seong Hwan Kim, Young Sook Park, Young Ok Hong

Sook Hee Chung, Yunju Jo, Sang Ryol Ryu, Sang Bong Ahn, Byoung Kwan Son, Seong Hwan Kim, Young Sook Park, Department of Internal Medicine, Eulji University School of Medicine, Seoul 139-711, South Korea
Young Ok Hong, Department of Pathology, Eulji University School of Medicine, Seoul 139-711, Korea
Author contributions: Chung SH and Jo Y contributed equally to this work; Ryu SR, Ahn SB, Son BK, Kim SH and Park YS collected and reviewed the patient data; Hong YO analyzed the pathological data.
Correspondence to: Yunju Jo, MD, PhD, Associate Professor, Department of Internal Medicine, Eulji University School of Medicine, Eulji Hospital, 280-1 Hagye 1-dong, Nowon-gu, Seoul 139-711, South Korea. jyj1138@eulji.ac.kr
Telephone: +82-2-9708624 Fax: +82-2-9708621
Received: August 16, 2010 Revised: December 10, 2010
Accepted: December 17, 2010
Published online: June 21, 2011

Key words: Non-steroidal anti-inflammatory agents; Enteritis; Gastrointestinal hemorrhage; Small intestine; Capsule endoscopy

Peer reviewer: Satoru Kakizaki, MD, PhD, Department of Medicine and Molecular Science, Gunma University, Graduate School of Medicine, 3-39-15 Showa-machi, Maebashi, Gunma 371-8511, Japan

Chung SH, Jo Y, Ryu SR, Ahn SB, Son BK, Kim SH, Park YS, Hong YO. Diaphragm disease compared with cryptogenic multifocal ulcerous stenosing enteritis. *World J Gastroenterol* 2011; 17(23): 2873-2876 Available from: URL: http://www.wjgnet.com/1007-9327/full/v17/i23/2873.htm DOI: http://dx.doi.org/10.3748/wjg.v17.i23.2873

Abstract

As the use of drugs such as nonsteroidal anti-inflammatory drugs (NSAIDs) increases, so too do gastrointestinal ulcers, bleeding, perforation and obstruction. Diaphragm disease of the small intestine is formed by submucosal fibrosis and destruction of lamina muscularis due to chronic ulceration, which corresponds to the most severe stage of NSAID enteropathy. It may lead to stricture of the small intestine. If such ulcerations and strictures in the small intestine are multiple, differential diagnosis is between diaphragm disease and cryptogenic multifocal ulcerous stenosing enteritis (CMUSE), because the gross findings of diaphragm disease are similar to those of CMUSE. We report a rare case of diaphragm disease caused by NSAID. It has been finally confirmed by capsule endoscopy and the origin of chronic obscure gastrointestinal bleeding was found to be multiple ulcers and strictures in the small intestine. After operation, we diagnosed the patient with diaphragm disease rather than CMUSE.

INTRODUCTION

The complications of non-steroidal anti-inflammatory drugs (NSAIDs), aspirin, antiplatelet agents, and antithrombotic agents are known to include gastrointestinal ulceration, bleeding, perforation and obstruction[1]. NSAID-induced mucosal injury can occur to the upper and lower gastrointestinal tract. The incidence of NSAID-induced enteropathy is not less than that of gastropathy. Diaphragm disease is the most severe stage of NSAID enteropathy. In ambulatory patients who took NSAIDs for > 3 mo because of arthritis, 70% had small intestinal ulcers and erosions by capsule endoscopy[2]. The diaphragm is formed by chronic fibrosis and destruction of lamina muscularis with chronic ulceration, and causes obstruction of the lumen in the small bowel[3]. However, diaphragm disease is a rare diagnosis.

Cryptogenic multifocal ulcerous stenosing enteritis (CMUSE) has multiple ulcerations and strictures in the small intestine; it is similar to diaphragm disease in terms of gross findings, and it is characterized by a chronic and

relapsing clinical course, and is more common in middle-aged patients.

We report a case of diaphragm disease in the small bowel that presented with obscure overt bleeding due to NSAID use, which was detected by capsule endoscopy. We review the differential diagnosis with CMUSE through histological findings and clinical course after surgery.

CASE REPORT

A 79-year-old woman was hospitalized for a low level of hematochezia. At admission, her hemoglobin level was 8.9 g/dL. She had also taken 20 mg piroxicam, 275 mg naproxen and 25 mg diclofenac daily for 4 years because of degenerative joint diseases. She had been hospitalized for iron deficiency anemia 5 years ago and there was no active bleeding focus upon esophagogatroduodenoscopy (EGD) and colonoscopy at that time. It was recommended that she undergo capsule endoscopy, however, she refused and was discharged.

After hospitalization, she had EGD and abdominal computed tomography (CT) to find the focus of her bleeding. No bleeding foci were revealed by EGD and abdominal CT. She had no more hematochezia after hospitalization. On day 3 of hospitalization, capsule endoscopy was performed. There were multiple, concentric circular ulcerations, with luminal narrowing in the jejunum and ileum, with many variable-sized erosions and bleeding stigmata in the ulcerations (Figure 1). The capsule in the small bowel was not excreted until 8 d after capsule endoscopy, even though she could have a meal. According to the results of capsule endoscopy, we need to diagnose differentially between NSAID-induced enteropathy and CMUSE, because of the gross similarities, such as multiple ulcerations and strictures in the small intestine. Even though the patient was an elderly woman and took NSAIDs, she was not diagnosed with NSAID-induced enteropathy because of the similar endoscopic findings to CMUSE. To confirm the diagnosis, histological examination was needed. She complained of chronic abdominal pain for 2 years, therefore, we could diagnose by capsule endoscopy that she had symptomatic multiple strictures.

To relieve the chronic abdominal pain caused by the multiple strictures in the small bowel, and to remove the retained capsule and evaluate the exact cause of the multiple ulcerations and strictures, we performed segmental resection of the ileum (Figure 2) instead of double balloon enteroscopy. Histological findings in the ileum (Figure 3) revealed typical multiple, circular ulcerations and a mucosal diaphragm, which suggested the most severe complications of chronic NSAID use. These mucosal diaphragms indicate chronic ulcers, submucosal fibrosis, reactive epithelial change, and chronic inflammation. After discharge, she had no more gastrointestinal bleeding and abdominal pain and took misoprostol at the outpatient clinic with close observation for > 1 year.

DISCUSSION

Diaphragm disease is the pathognomic characteristic of NSAID enteropathy, and was named by Bjarnason *et al*[4]. They described the clinicopathological features of NSAID-induced stricture as diaphragm disease. The histopathological characteristics of diaphragm disease are: superficial ulceration at the apex of the villi; circumferential ring-like stricture; multiple, short segment annular strictures; transmural inflammation; and submucosal fibrosis[5].

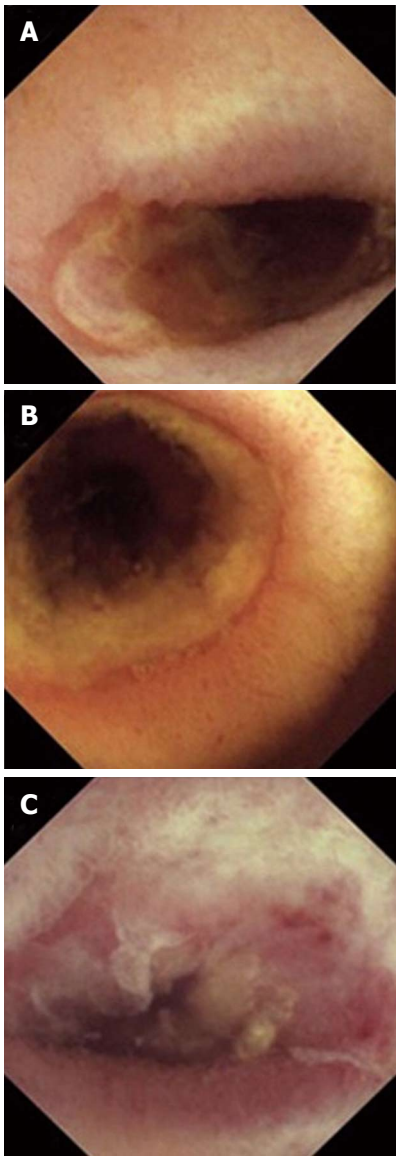


Figure 1 Capsule endoscopic findings. A: There were concentric ulcerations with luminal narrowing; B: There were many variable-sized erosions and bleeding stigmata in the ulcerations; C: There was a totally obstructed lesion in which the capsule was suspected to be retained.

Several other authors have reported diaphragm disease in patients taking NSAIDs, by enteroscopy[6] and after laparotomy[5,7]. The first diagnosis of small intestinal diaphragm disease after use of NSAID through capsule endoscopy was reported by Yousfi *et al*[3]. In their study with capsule endoscopy, there were multiple small-intestinal strictures where the capsule was retained. Thus, exploratory laparoscopy was required to remove the retained capsule, as in our case.

After long-term use of NSAIDs for > 20 years, the small intestine shows fibrotic constriction and thickened

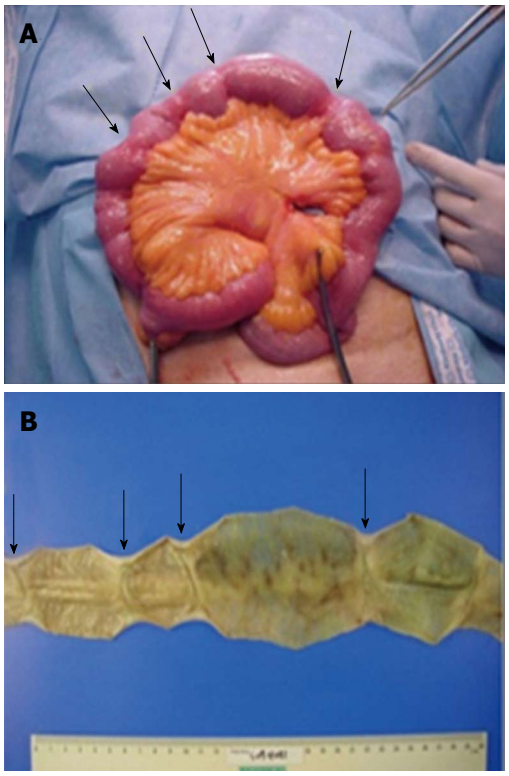


Figure 2 Specimen from segmental resection of the ileum. A: A capsule was located at 60 cm up from the terminal ileum. Forceps and hand point out the location in the ileum where the capsule was retained. There were multiple strictures in the ileum (black arrows); B: In longitudinal section, there were multiple thin, web-like mucosal septa that caused abrupt luminal narrowing in the small intestine (black arrows).

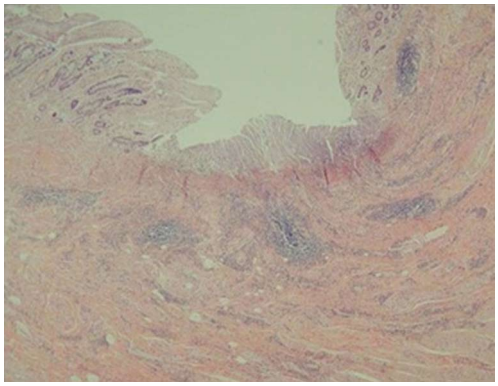


Figure 3 Histological findings of the ileum. The mucosal diaphragm reveals surface erosion or ulceration, submucosal fibrosis, reactive epithelial change, and chronic inflammation (hematoxylin and eosin stain, 40 ×).

hyperemic mucosa that lead to obstruction through local damage and healing process[8].

In NSAID-induced enteropathy, capsule endoscopy shows circumferential ulcerations, erosions and multifocal strictures[3,9]. Capsule endoscopy is a useful option to establish the etiology of obscure overt gastrointestinal bleeding, especially in elderly patients. Diagnostic yield of capsule endoscopy for finding the focus of obscure gastrointestinal bleeding is reported to be 38%-93%[10]. Diagnostic yield of capsule endoscopy for obscure gastrointestinal bleeding is not different for overt and occult bleeding[11,12]. Diagnostic yields of CT angiography and double balloon enteroscopy in finding the focus of obscure gastrointestinal bleeding were 24% and 75.7%, respectively[13,14].

Table 1 Differences between non-steroidal anti-inflammatory drug-induced enteropathy and cryptogenic multifocal ulcerous stenosing enteritis

	NSAID-induced enteropathy	CMUSE
Definition	Ulceration and stricture of small intestine induced by NSAIDs[19,20]	Unexplained small intestinal multiple stricture and ulceration[17,18]
Causes	NSAIDs	Unknown
Age	Old-aged people[26]	Middle-aged people[18]
Treatment	Stop NSAIDs, Prostaglandin (misoprostol)[21], Sulfasalazine[22], Metronidazole[23], Glutamine[24], Glucose/citrate[25]	Steroids[17]

NSAID: Non-steroidal anti-inflammatory drug; CMUSE: Cryptogenic multifocal ulcerous stenosing enteritis.

The origin of gastrointestinal bleeding can be detected in a higher percentage of patients by capsule endoscopy than by CT angiography and double balloon enteroscopy[10]. In our case, we performed capsule endoscopy to find overt obscure bleeding foci after abdominal CT scanning. There was no definite stricture or sign of significant obstruction by abdominal CT. However, the capsule became trapped in the bowel unexpectedly. The important complication of capsule endoscopy is capsule retention, for which the incidence is < 2%[15]. Intestinal stricture might be suspected, especially in patients with chronic NSAID use, ischemic bowel disease, abdominal radiotherapy, and Crohn's disease. For these patients, capsule endoscopy can be performed after abdominal CT or small bowel series to find strictures[10]. Stricture cannot be found completely by abdominal CT or small bowel series, therefore, an M2A patency capsule can be considered, which is biodegradable in the gastrointestinal tract in patients with suspected intestinal stricture[16].

Our patient had multiple ulcerations and strictures in the small intestine, therefore, diaphragm disease was needed to be differentiated from CMUSE, even though the patient was an elderly woman who was taking NSAIDs. The characteristics of NSAID-induced enteropathy and CMUSE using previously published data are summarized in Table 1. CMUSE is defined as unexplained small intestinal multiple strictures and ulcerations of unknown origin. It is characterized as an atypical type of vasculitis[17]. Its clinical features include unexplained stricture and ulceration of the small bowel, without systemic inflammation, which are found in young and middle-aged patients. CMUSE can relapse chronically or after surgery. The symptoms of CMUSE can be improved by steroid treatment[17,18].

In conclusion, NSAID-induced diaphragm disease in the small bowel can be diagnosed efficiently by capsule endoscopy, and the gross endoscopic features of diaphragm disease are similar to those of CMUSE. We

also suggest that NSAID-induced enteropathy, such as diaphragm disease, should be suspected in NSAID users, especially in elderly patients.

REFERENCES

1 **Singh G**. Gastrointestinal complications of prescription and over-the-counter nonsteroidal anti-inflammatory drugs: a view from the ARAMIS database. Arthritis, Rheumatism, and Aging Medical Information System. *Am J Ther* 2000; **7**: 115-121

2 **Graham DY**, Opekun AR, Willingham FF, Qureshi WA. Visible small-intestinal mucosal injury in chronic NSAID users. *Clin Gastroenterol Hepatol* 2005; **3**: 55-59

3 **Yousfi MM**, De Petris G, Leighton JA, Sharma VK, Pockaj BA, Jaroszewski DE, Heigh RI, Ramzan NN, Fleischer DE. Diaphragm disease after use of nonsteroidal anti-inflammatory agents: first report of diagnosis with capsule endoscopy. *J Clin Gastroenterol* 2004; **38**: 686-691

4 **Bjarnason I**, Price AB, Zanelli G, Smethurst P, Burke M, Gumpel JM, Levi AJ. Clinicopathological features of nonsteroidal antiinflammatory drug-induced small intestinal strictures. *Gastroenterology* 1988; **94**: 1070-1074

5 **Scholz FJ**, Heiss FW, Roberts PL, Thomas C. Diaphragmlike strictures of the small bowel associated with use of nonsteroidal antiinflammatory drugs. *AJR Am J Roentgenol* 1994; **162**: 49-50

6 **Abrahamian GA**, Polhamus CD, Muskat P, Karulf RE. Diaphragm-like strictures of the ileum associated with NSAID use: a rare complication. *South Med J* 1998; **91**: 395-397

7 **Matsuhashi N**, Yamada A, Hiraishi M, Konishi T, Minota S, Saito T, Sugano K, Yazaki Y, Mori M, Shiga J. Multiple strictures of the small intestine after long-term nonsteroidal anti-inflammatory drug therapy. *Am J Gastroenterol* 1992; **87**: 1183-1186

8 **Zhao B**, Sanati S, Eltorky M. Diaphragm disease: complete small bowel obstruction after long-term nonsteroidal anti-inflammatory drugs use. *Ann Diagn Pathol* 2005; **9**: 169-173

9 **Manetas M**, O'Loughlin C, Kelemen K, Barkin JS. Multiple small-bowel diaphragms: a cause of obscure GI bleeding diagnosed by capsule endoscopy. *Gastrointest Endosc* 2004; **60**: 848-851

10 **Carretero C**, Fernandez-Urien I, Betes M, Muñoz-Navas M. Role of videocapsule endoscopy for gastrointestinal bleeding. *World J Gastroenterol* 2008; **14**: 5261-5264

11 **Ben Soussan E**, Antonietti M, Hervé S, Savoye G, Ramirez S, Lecleire S, Ducrotté P, Lerebours E. Diagnostic yield and therapeutic implications of capsule endoscopy in obscure gastrointestinal bleeding. *Gastroenterol Clin Biol* 2004; **28**: 1068-1073

12 **Tang SJ**, Haber GB. Capsule endoscopy in obscure gastrointestinal bleeding. *Gastrointest Endosc Clin N Am* 2004; **14**: 87-100

13 **Saperas E**, Dot J, Videla S, Alvarez-Castells A, Perez-Lafuente M, Armengol JR, Malagelada JR. Capsule endoscopy versus computed tomographic or standard angiography for the diagnosis of obscure gastrointestinal bleeding. *Am J Gastroenterol* 2007; **102**: 731-737

14 **Sun B**, Rajan E, Cheng S, Shen R, Zhang C, Zhang S, Wu Y, Zhong J. Diagnostic yield and therapeutic impact of double-balloon enteroscopy in a large cohort of patients with obscure gastrointestinal bleeding. *Am J Gastroenterol* 2006; **101**: 2011-2015

15 **Ho KK**, Joyce AM. Complications of capsule endoscopy. *Gastrointest Endosc Clin N Am* 2007; **17**: 169-178, viii-ix

16 **Fry LC**, De Petris G, Swain JM, Fleischer DE. Impaction and fracture of a video capsule in the small bowel requiring laparotomy for removal of the capsule fragments. *Endoscopy* 2005; **37**: 674-676

17 **Chang DK**, Kim JJ, Choi H, Eun CS, Han DS, Byeon JS, Kim JO. Double balloon endoscopy in small intestinal Crohn's disease and other inflammatory diseases such as cryptogenic multifocal ulcerous stenosing enteritis (CMUSE). *Gastrointest Endosc* 2007; **66**: S96-S98

18 **Perlemuter G**, Guillevin L, Legman P, Weiss L, Couturier D, Chaussade S. Cryptogenetic multifocal ulcerous stenosing enteritis: an atypical type of vasculitis or a disease mimicking vasculitis. *Gut* 2001; **48**: 333-338

19 **Wallace JL**. Nonsteroidal anti-inflammatory drugs and gastroenteropathy: the second hundred years. *Gastroenterology* 1997; **112**: 1000-1016

20 **Maiden L**. Capsule endoscopic diagnosis of nonsteroidal antiinflammatory drug-induced enteropathy. *J Gastroenterol* 2009; **44** Suppl 19: 64-71

21 **Bjarnason I**, Smethurst P, Fenn CG, Lee CE, Menzies IS, Levi AJ. Misoprostol reduces indomethacin-induced changes in human small intestinal permeability. *Dig Dis Sci* 1989; **34**: 407-411

22 **Davies NM**, Saleh JY, Skjodt NM. Detection and prevention of NSAID-induced enteropathy. *J Pharm Pharm Sci* 2000; **3**: 137-155

23 **Bjarnason I**, Hayllar J, Smethurst P, Price A, Gumpel MJ. Metronidazole reduces intestinal inflammation and blood loss in non-steroidal anti-inflammatory drug induced enteropathy. *Gut* 1992; **33**: 1204-1208

24 **Hond ED**, Peeters M, Hiele M, Bulteel V, Ghoos Y, Rutgeerts P. Effect of glutamine on the intestinal permeability changes induced by indomethacin in humans. *Aliment Pharmacol Ther* 1999; **13**: 679-685

25 **Bjarnason I**, Smethurst P, Macpherson A, Walker F, McElnay JC, Passmore AP, Menzies IS. Glucose and citrate reduce the permeability changes caused by indomethacin in humans. *Gastroenterology* 1992; **102**: 1546-1550

26 **Hayashi Y**, Yamamoto H, Taguchi H, Sunada K, Miyata T, Yano T, Arashiro M, Sugano K. Nonsteroidal anti-inflammatory drug-induced small-bowel lesions identified by double-balloon endoscopy: endoscopic features of the lesions and endoscopic treatments for diaphragm disease. *J Gastroenterol* 2009; **44** Suppl 19: 57-63

S- Editor Tian L **L- Editor** Kerr C **E- Editor** Ma WH

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/1007-9327office
wjg@wjgnet.com
doi:10.3748/wjg.v17.i23.2877

World J Gastroenterol 2011 June 21; 17(23): 2877-2878
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

Time for the world to move beyond the percutaneous endoscopic gastrostomy

Ah San Pang

Ah San Pang, LP Surgery, Mount Alvernia Hospital, 820 Thomson Road, Singapore 574623, Singapore
Author contributions: Pang AS wrote this letter.
Correspondence to: Ah San Pang, FRCSEd, LP Surgery, 820 Thomson Road, #02-05 Mount Alvernia Medical Centre A, Singapore 574623, Singapore. pangahsan@gmail.com
Telephone: +65-63563260 Fax: +65-63563261
Received: December 7, 2010 Revised: December 28, 2010
Accepted: January 4, 2011
Published online: June 21, 2011

Abstract

Percutaneous endoscopic gastrostomy (PEG) is a proven feeding tube, just as the nasogastric tube is proven to be able to deliver enteral nutrition. For long-term use, both patient and caregiver want neither. What is desired is the LOOPPEG® 3G tube, more secure than the PEG, and less risky to change than the nasogastric tube. Future clinical research should focus on this high-comfort low-risk tube.

Key words: Tube feeding; Enteral nutrition; Dysphagia; Stroke

Peer reviewer: Damian Casadesus Rodriguez, MD, PhD, Calixto Garcia University Hospital, J and University, Vedado, Havana City, Cuba

Pang AS. Time for the world to move beyond the percutaneous endoscopic gastrostomy. *World J Gastroenterol* 2011; 17(23): 2877-2878 Available from: URL: http://www.wjgnet.com/1007-9327/full/v17/i23/2877.htm DOI: http://dx.doi.org/10.3748/wjg.v17.i23.2877

TO THE EDITOR

I found "Survival of geriatric patients after percutaneous endoscopic gastrostomy in Japan" by Yutaka Suzuki *et al*[1] to be an interesting and timely article.

With an aged population and several centers working together, they were able to recruit a large sample and, consequently, produce robust results. The first lesson to be learnt is that collaboration can produce better results. The second lesson is that, for the Japanese population at least, the percutaneous endoscopic gastrostomy in geriatrics is proven. To the co-authors and their institutions, I offer my heartiest congratulations. My country, Singapore, is ageing rapidly and their experiences can offer valuable lessons.

I emphasize that my subsequent suggestion for future research should not be taken as criticisms of their work, which is an unqualified success. By way of introducing my comments, I pose two questions.

First, since elderly patients in other countries might react differently from Japanese patients, should we not do a similar study of the PEG in Singapore? My answer is no, for the following reason. The difference in year (or mo) is unlikely to be important to the geriatric patient, in whom compassion is more valued than cure.

Second, since the nasogastric tube might give a better survival than the PEG, should we not do a similar study on this feeding tube? Again, my answer is no, for the following reasons. Feeding tube and survival of the elderly have a correlation but not a cause-effect relationship. Both nasogastric tube and PEG can deliver the enteral nutrition, and the choice is determined more by the risk/comfort profile of the tube. Statistical significance does not mean clinical significance. Conversely, lack of the former does not mean that the PEG is not a clinically better tube. The relationship between nasogastric tube and PEG is predictable, independent of age and race, and unlikely to be affected by the study findings, however robust.

This relationship is illustrated in Figure 1. There are enough published data to show that the nasogastric tube is low in risk and low in comfort, whereas the PEG is very comfortable but also high in risk[2]. For long-term use, both patients and caregivers want neither option! What

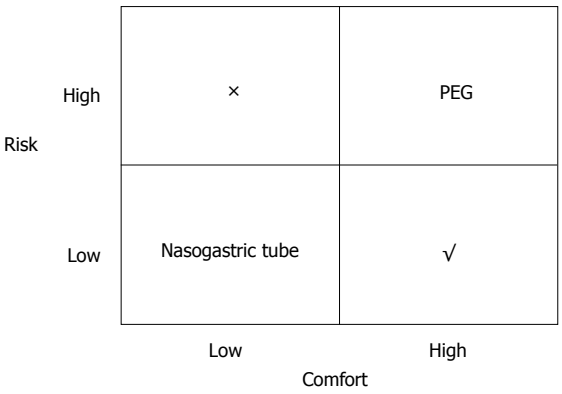


Figure 1 Risk/Comfort Chart. The desired tube is the high-comfort low-risk option (√). PEG: Percutaneous endoscopic gastrostomy.

they need is a very comfortable but low in risk option.

In my opinion, there is only one feeding tube which meets this profile. The LOOPPEG® 3G tube is comfortable because it bypasses the nose and low in risk because it cannot be dislodged[3]. Also, it is less risky to change than the nasogastric tube. Consequently, the 3G tube can be made 15 Fr or smaller because tube blockage is a non-issue, having been negated by easy tube change. Thus, the trauma of changing the tube - physical, psychological and financial - is minimized.

However, no case series has been published. It may take forever for a sizeable sample and robust results to come out from my part of the world. Hence, I hope our Japanese colleagues, having published a solid study, will take up the challenge to move beyond the PEG, research the 3G tube, and report their findings in this fine Journal.

ACKNOWLEDGMENTS

Iruru Maetani, MD, FJSIM, Professor and Chairman, Division of Gastroenterology/Department of Medicine, Toho University Ohashi Medical Center, Tokyo, Japan, encouraged me in my work on the 3G tube.

REFERENCES

1 **Suzuki Y**, Tamez S, Murakami A, Taira A, Mizuhara A, Horiuchi A, Mihara C, Ako E, Muramatsu H, Okano H, Suenaga H, Jomoto K, Kobayashi J, Takifuji K, Akiyama K, Tahara K, Onishi K, Shimazaki M, Matsumoto M, Ijima M, Murakami M, Nakahori M, Kudo M, Maruyama M, Takahashi M, Washizawa N, Onozawa S, Goshi S, Yamashita S, Ono S, Imazato S, Nishiwaki S, Kitahara S, Endo T, Iiri T, Nagahama T, Hikichi T, Mikami T, Yamamoto T, Ogawa T, Ogawa T, Ohta T, Matsumoto T, Kura T, Kikuchi T, Iwase T, Tsuji T, Nishiguchi Y, Urashima M. Survival of geriatric patients after percutaneous endoscopic gastrostomy in Japan. *World J Gastroenterol* 2010; **16**: 5084-5091

2 **Baeten C**, Hoefnagels J. Feeding *via* nasogastric tube or percutaneous endoscopic gastrostomy. A comparison. *Scand J Gastroenterol Suppl* 1992; **194**: 95-98

3 **Pang AS**. A new feeding tube which is secure and easy to change. *Singapore Med J* 2009; **50**: 740-742

S- Editor Tian L **L- Editor** Wang XL **E- Editor** Ma WH

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/1007-9327office
wjg@wjgnet.com
www.wjgnet.com

World J Gastroenterol 2011 June 21; 17(23): I
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

Acknowledgments to reviewers of *World Journal of Gastroenterology*

Many reviewers have contributed their expertise and time to the peer review, a critical process to ensure the quality of *World Journal of Gastroenterology*. The editors and authors of the articles submitted to the journal are grateful to the following reviewers for evaluating the articles (including those published in this issue and those rejected for this issue) during the last editing time period.

Christian Toso PD, MD, PhD, Abdominal and transplant surgery, Department of surgery, Geneva University Hospitals, Rue Gabrielle-Perret-Gentil, 1211 Geneva 14, Switzerland

Shiu-Ming Kuo, MD, University at Buffalo, 15 Farber Hall, 3435 Main Street, Buffalo 14214, United States

Dr. Axel M Gressner, RCH, Professor of Clinical Chemistry and Laboratory Medicine, Clinical Chemist (DGKL), Lutherweg 2, 52074 Aachen, Germany

Eyvind J Paulssen, MD, PhD, Department of Gastroenterology, University Hospital of North Norway, PO Box 83, Tromsø, N-9038, Norway

Ole Haagen Nielsen, MD, DMSc, Professor, Department of Gastroenterology, D112M, Herlev Hospital, University of Copenhagen, Herlev Ringvej 75, DK-2730 Herlev, Denmark

Alan C Moss MD, FACG, Assistant Professor of Medicine, Director of Translational Research, Center for Inflammatory Bowel Disease, Beth Israel Deaconess Medical Center, Harvard Medical School, Rose 1 / East, 330 Brookline Ave, Boston, MA 02215, United States

Gianpiero Gravante, MD, BsC, MBBS, Department of Hepatobiliary and Pancreatic Surgery, Leicester General Hospital, Flat 38, Room 8, Hospital Close, Leicester, LE5 4WU, United Kingdom

José Manuel Martin-Villa, Professor, PhD, Department of Inmunología, Facultad de Medicina, Universidad Complutense de Madrid, Pabellón V. Planta 4ª, Madrid 28040, Spain

Francesco Manguso, Dr., MD, PhD, UOC di Gastroenterologia, AORN A. Cardarelli, Via A. Cardarelli 9, Napoli, 80122, Italy

Ourania M Andrisani, PhD, Professor, B038 Hansen Bldg, Center for Cancer Research, Purdue University, West Lafayette, IN 47907, United States

Cristiano Simone, PhD, Laboratory of Signal-dependent Transcription, Department of Translational Pharmacology, Consorzio Mario Negri Sud, Via Nazionale 8/A, 66030 Santa Maria Imbaro, Italy

Ferenc Sipos, MD, PhD, Cell Analysis Laboratory, 2nd Department of Internal Medicine, Semmelweis University, Szentkirályi u. 46., Budapest 1088, Hungary

Ezio Laconi, MD, PhD, Professor of General Pathology, Department of Sciences and Biomedical Technologies, Unit of Experimental Pathology, University of Cagliari, Via Porcell, 4 - IV Piano, 09125 - Cagliari, Italy

Raquel Almeida, Dr., MS, Institute of Molecular Pathology and Immunology, University of Porto, Rua Dr Roberto Frias s/n, Porto 4200, Portugal

Khaled Jadallah, MD, Assistant Professor of Medicine, Consultant, Gastroenterologist and Hepatologist, Department of Internal Medicine, King Abdullah University Hospital, Jordan University of Science and Technology, Irbid 22110, Jordan

Luis Bujanda, PhD, Professor, Department of Gastroenterology, CIBEREHD, University of Country Basque, Donostia Hospital, Paseo Dr. Beguiristain s/n, 20014 San Sebastián, Spain

Rui Tato Marinho, MD, PhD, Department of Gastroenterology and Hepatology, Hospital Santa Maria, Medical School of Lisbon, Av. Prof. Egas Moniz, 1649-035, Lisboa, Portugal

Christian Toso PD, MD, PhD, Abdominal and transplant surgery, Department of surgery, Geneva University Hospitals, Rue Gabrielle-Perret-Gentil, 1211 Geneva 14, Switzerland

Elfriede Bollschweiler, Professor, Department of Surgery, University of Cologne, Kerpener Straße 62, 50935 Köln, Germany

Dietmar Öfner, MD, MAS, MSc, Professor, Head, Department of Surgery, Paracelsus Private Medical University Salzburg, Müllner Hauptstrasse 48, Salzburg, 5020, Austria

Eric WC Tse, Dr., MB, PhD, Department of Medicine, The University of Hong Kong, Queen Mary Hospital, Pokfulam, Hong Kong, China

Atsushi Masamune, MD, PhD, Division of Gastroenterology, Tohoku University Graduate School of Medicine, 1-1 Seiryo-machi, Aoba-ku, Sendai 980-8574, Japan

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/1007-9327office
wjg@wjgnet.com
www.wjgnet.com

World J Gastroenterol 2011 June 21; 17(23): I
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

Events Calendar 2011

January 14-15, 2011
AGA Clinical Congress of Gastroenterology and Hepatology: Best Practices in 2011 Miami, FL 33101, United States

January 20-22, 2011
Gastrointestinal Cancers Symposium 2011, San Francisco, CA 94143, United States

January 27-28, 2011
Falk Workshop, Liver and Immunology, Medical University, Franz-Josef-Strauss-Allee 11, 93053 Regensburg, Germany

January 28-29, 2011
9. Gastro Forum München, Munich, Germany

February 4-5, 2011
13th Duesseldorf International Endoscopy Symposium, Duesseldorf, Germany

February 13-27, 2011
Gastroenterology: New Zealand CME Cruise Conference, Sydney, NSW, Australia

February 17-20, 2011
APASL 2011-The 21st Conference of the Asian Pacific Association for the Study of the Liver
Bangkok, Thailand

February 22, 2011-March 04, 2011
Canadian Digestive Diseases Week 2011, Vancouver, BC, Canada

February 24-26, 2011
Inflammatory Bowel Diseases 2011-6th Congress of the European Crohn's and Colitis Organisation, Dublin, Ireland

February 24-26, 2011
2nd International Congress on Abdominal Obesity, Buenos Aires, Brazil

February 24-26, 2011
International Colorectal Disease Symposium 2011, Hong Kong, China

February 26-March 1, 2011
Canadian Digestive Diseases Week, Westin Bayshore, Vancouver, British Columbia, Canada

February 28-March 1, 2011
Childhood & Adolescent Obesity: A whole-system strategic approach, Abu Dhabi, United Arab Emirates

March 3-5, 2011
42nd Annual Topics in Internal Medicine, Gainesville, FL 32614, United States

March 7-11, 2011
Infectious Diseases: Adult Issues in the Outpatient and Inpatient Settings, Sarasota, FL 34234, United States

March 14-17, 2011
British Society of Gastroenterology Annual Meeting 2011, Birmingham, England, United Kingdom

March 17-19, 2011
41. Kongress der Deutschen Gesellschaft für Endoskopie und Bildgebende Verfahren e.V., Munich, Germany

March 17-20, 2011
Mayo Clinic Gastroenterology & Hepatology 2011, Jacksonville, FL 34234, United States

March 18, 2011
UC Davis Health Informatics: Change Management and Health Informatics, The Keys to Health Reform, Sacramento, CA 94143, United States

March 25-27, 2011
MedicReS IC 2011 Good Medical Research, Istanbul, Turkey

March 26-27, 2011
26th Annual New Treatments in Chronic Liver Disease, San Diego, CA 94143, United States

April 6-7, 2011
IBS-A Global Perspective, Pfister Hotel, 424 East Wisconsin Avenue, Milwaukee, WI 53202, United States

April 7-9, 2011
International and Interdisciplinary Conference Excellence in Female Surgery, Florence, Italy

April 15-16, 2011
Falk Symposium 177, Endoscopy Live Berlin 2011 Intestinal Disease Meeting, Stauffenbergstr. 26, 10785 Berlin, Germany

April 18-22, 2011
Pediatric Emergency Medicine: Detection, Diagnosis and Developing Treatment Plans, Sarasota, FL 34234, United States

April 20-23, 2011
9th International Gastric Cancer Congress, COEX, World Trade Center, Samseong-dong, Gangnam-gu, Seoul 135-731, South Korea

April 25-27, 2011
The Second International Conference of the Saudi Society of Pediatric Gastroenterology, Hepatology & Nutrition, Riyadh, Saudi Arabia

April 25-29, 2011
Neurology Updates for Primary Care, Sarasota, FL 34230-6947, United States

April 28-30, 2011
4th Central European Congress of Surgery, Budapest, Hungary

May 7-10, 2011
Digestive Disease Week, Chicago, IL 60446, United States

May 12-13, 2011
2nd National Conference Clinical Advances in Cystic Fibrosis, London, England, United Kingdom

May 19-22, 2011
1st World Congress on Controversies in the Management of Viral Hepatitis (C-Hep), Palau de Congressos de Catalunya, Av. Diagonal, 661-671 Barcelona 08028, Spain

May 21-24, 2011
22nd European Society of Gastrointestinal and Abdominal Radiology Annual Meeting and Postgraduate Course, Venise, Italy

May 25-28, 2011
4th Congress of the Gastroenterology Association of Bosnia and Herzegovina with international participation, Hotel Holiday Inn, Sarajevo, Bosnia and Herzegovina

June 11-12, 2011
The International Digestive Disease Forum 2011, Hong Kong, China

June 13-16, 2011
Surgery and Disillusion XXIV SPIGC, II ESYS, Napoli, Italy

June 14-16, 2011
International Scientific Conference on Probiotics and Prebiotics-IPC2011, Kosice, Slovakia

June 22-25, 2011
ESMO Conference: 13th World Congress on Gastrointestinal Cancer, Barcelona, Spain

June 29-2, 2011
XI Congreso Interamericano de Pediatria "Monterrey 2011", Monterrey, Mexico

September 2-3, 2011 Falk Symposium 178, Diverticular Disease, A Fresh Approach to a Neglected Disease, Gürzenich Cologne, Martinstr. 29-37, 50667 Cologne, Germany

September 10-11, 2011
New Advances in Inflammatory Bowel Disease, La Jolla, CA 92093, United States

September 10-14, 2011
ICE 2011-International Congress of Endoscopy, Los Angeles Convention Center, 1201 South Figueroa Street Los Angeles, CA 90015, United States

September 30-October 1, 2011
Falk Symposium 179, Revisiting IBD Management: Dogmas to be Challenged, Sheraton Brussels Hotel, Place Rogier 3, 1210 Brussels, Belgium

October 19-29, 2011
Cardiology & Gastroenterology | Tahiti 10 night CME Cruise, Papeete, French Polynesia

October 22-26, 2011
19th United European Gastroenterology Week, Stockholm, Sweden

October 28-November 2, 2011
ACG Annual Scientific Meeting & Postgraduate Course, Washington, DC 20001, United States

November 11-12, 2011
Falk Symposium 180, IBD 2011: Progress and Future for Lifelong Management, ANA Interconti Hotel, 1-12-33 Akasaka, Minato-ku, Tokyo 107-0052, Japan

December 1-4, 2011
2011 Advances in Inflammatory Bowel Diseases/Crohn's & Colitis Foundation's Clinical & Research Conference, Hollywood, FL 34234, United States

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/1007-9327office
wjg@wjgnet.com
www.wjgnet.com

World J Gastroenterol 2011 June 21; 17(23): I-VI
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

GENERAL INFORMATION

World Journal of Gastroenterology (*World J Gastroenterol*, *WJG*, print ISSN 1007-9327, online ISSN 2219-2840, DOI: 10.3748) is a weekly, open-access (OA), peer-reviewed journal supported by an editorial board of 1144 experts in gastroenterology and hepatology from 60 countries.

The biggest advantage of the OA model is that it provides free, full-text articles in PDF and other formats for experts and the public without registration, which eliminates the obstacle that traditional journals possess and usually delays the speed of the propagation and communication of scientific research results. The open access model has been proven to be a true approach that may achieve the ultimate goal of the journals, i.e. the maximization of the value to the readers, authors and society.

Maximization of personal benefits

The role of academic journals is to exhibit the scientific levels of a country, a university, a center, a department, and even a scientist, and build an important bridge for communication between scientists and the public. As we all know, the significance of the publication of scientific articles lies not only in disseminating and communicating innovative scientific achievements and academic views, as well as promoting the application of scientific achievements, but also in formally recognizing the "priority" and "copyright" of innovative achievements published, as well as evaluating research performance and academic levels. So, to realize these desired attributes of *WJG* and create a well-recognized journal, the following four types of personal benefits should be maximized. The maximization of personal benefits refers to the pursuit of the maximum personal benefits in a well-considered optimal manner without violation of the laws, ethical rules and the benefits of others. (1) Maximization of the benefits of editorial board members: The primary task of editorial board members is to give a peer review of an unpublished scientific article via online office system to evaluate its innovativeness, scientific and practical values and determine whether it should be published or not. During peer review, editorial board members can also obtain cutting-edge information in that field at first hand. As leaders in their field, they have priority to be invited to write articles and publish commentary articles. We will put peer reviewers' names and affiliations along with the article they reviewed in the journal to acknowledge their contribution; (2) Maximization of the benefits of authors: Since *WJG* is an open-access journal, readers around the world can immediately download and read, free of charge, high-quality, peer-reviewed articles from *WJG* official website, thereby realizing the goals and significance of the communication between authors and peers as well as public reading; (3) Maximization of the benefits of readers: Readers can read or use, free of charge, high-quality peer-reviewed articles without any limits, and cite the arguments, viewpoints, concepts, theories, methods, results, conclusion or facts and data of pertinent literature so as to validate the innovativeness, scientific and practical values of their own research achievements, thus ensuring that their articles have novel arguments or viewpoints, solid evidence and correct conclusion; and (4) Maximization of the benefits of employees: It is an iron law that a first-class journal is unable to exist without first-class editors, and only first-class editors can create a first-class academic journal. We insist on strengthening our team cultivation and construction so that every employee, in an open, fair and transparent environment, could contribute their wisdom to edit and publish high-quality articles, thereby realizing the maximization of the personal benefits of editorial board members, authors and readers, and yielding the greatest social and economic benefits.

Aims and scope

The major task of *WJG* is to report rapidly the most recent results in basic and clinical research on esophageal, gastrointestinal, liver, pancreas and biliary tract diseases, *Helicobacter pylori*, endoscopy and gastrointestinal surgery, including: gastroesophageal reflux disease, gastrointestinal bleeding, infection and tumors; gastric and duodenal disorders; intestinal inflammation, microflora and immunity; celiac disease, dyspepsia and nutrition; viral hepatitis, portal hypertension, liver fibrosis, liver cirrhosis, liver transplantation, and metabolic liver disease; molecular and cell biology; geriatric and pediatric gastroenterology; diagnosis and screening, imaging and advanced technology.

Columns

The columns in the issues of *WJG* will include: (1) Editorial: To introduce and comment on major advances and developments in the field; (2) Frontier: To review representative achievements, comment on the state of current research, and propose directions for future research; (3) Topic Highlight: This column consists of three formats, including (A) 10 invited review articles on a hot topic, (B) a commentary on common issues of this hot topic, and (C) a commentary on the 10 individual articles; (4) Observation: To update the development of old and new questions, highlight unsolved problems, and provide strategies on how to solve the questions; (5) Guidelines for Basic Research: To provide guidelines for basic research; (6) Guidelines for Clinical Practice: To provide guidelines for clinical diagnosis and treatment; (7) Review: To review systemically progress and unresolved problems in the field, comment on the state of current research, and make suggestions for future work; (8) Original Article: To report innovative and original findings in gastroenterology; (9) Brief Article: To briefly report the novel and innovative findings in gastroenterology and hepatology; (10) Case Report: To report a rare or typical case; (11) Letters to the Editor: To discuss and make reply to the contributions published in *WJG*, or to introduce and comment on a controversial issue of general interest; (12) Book Reviews: To introduce and comment on quality monographs of gastroenterology and hepatology; and (13) Guidelines: To introduce consensuses and guidelines reached by international and national academic authorities worldwide on basic research and clinical practice gastroenterology and hepatology.

Name of journal

World Journal of Gastroenterology

ISSN and EISSN
ISSN 1007-9327 (print)
ISSN 2219-2840 (online)

Indexed and Abstracted in
Current Contents®/Clinical Medicine, Science Citation Index Expanded (also known as SciSearch®), Journal Citation Reports®, Index Medicus, MEDLINE, PubMed, PubMed Central, Digital Object Identifer, and Directory of Open Access Journals. ISI, Thomson Reuters, 2009 Impact Factor: 2.092 (33/65 Gastroenterology and Hepatology).

Published by
Baishideng Publishing Group Co., Limited

SPECIAL STATEMENT

All articles published in this journal represent the viewpoints of the authors except where indicated otherwise.

Biostatistical editing
Statisital review is performed after peer review. We invite an expert in Biomedical Statistics from to evaluate the statistical method used in the paper, including *t*-test (group or paired comparisons), chi-squared test, Ridit, probit, logit, regression (linear, curvilinear, or stepwise), correlation, analysis of variance, analysis of covariance, *etc*. The reviewing points include: (1) Statistical methods should be described when they are used to verify the results; (2) Whether the statistical techniques are suitable or correct; (3) Only homogeneous data can be averaged. Standard deviations are preferred to standard errors. Give the number of observations and subjects (*n*). Losses in observations, such as drop-outs from the study should be reported; (4) Values such as ED50, LD50, IC50 should have their 95% confidence limits calculated and compared by weighted probit analysis (Bliss and Finney); and (5) The word 'significantly' should be replaced by its synonyms (if it indicates extent) or the *P* value (if it indicates statistical significance).

Conflict-of-interest statement
In the interests of transparency and to help reviewers assess any potential bias, *WJG* requires authors of all papers to declare any competing commercial, personal, political, intellectual, or religious interests in relation to the submitted work. Referees are also asked to indicate any potential conflict they might have reviewing a particular paper. Before submitting, authors are suggested to read "Uniform Requirements for Manuscripts Submitted to Biomedical Journals: Ethical Considerations in the Conduct and Reporting of Research: Conflicts of Interest" from International Committee of Medical Journal Editors (ICMJE), which is available at: http://www.icmje.org/ethical_4conflicts.html.

Sample wording: [Name of individual] has received fees for serving as a speaker, a consultant and an advisory board member for [names of organizations], and has received research funding from [names of organization]. [Name of individual] is an employee of [name of organization]. [Name of individual] owns stocks and shares in [name of organization]. [Name of individual] owns patent [patent identification and brief description].

Statement of informed consent
Manuscripts should contain a statement to the effect that all human studies have been reviewed by the appropriate ethics committee or it should be stated clearly in the text that all persons gave their informed consent prior to their inclusion in the study. Details that might disclose the identity of the subjects under study should be omitted. Authors should also draw attention to the Code of Ethics of the World Medical Association (Declaration of Helsinki, 1964, as revised in 2004).

Statement of human and animal rights
When reporting the results from experiments, authors should follow the highest standards and the trial should conform to Good Clinical Practice (for example, US Food and Drug Administration Good Clinical Practice in FDA-Regulated Clinical Trials; UK Medicines Research Council Guidelines for Good Clinical Practice in Clinical Trials) and/or the World Medical Association Declaration of Helsinki. Generally, we suggest authors follow the lead investigator's national standard. If doubt exists whether the research was conducted in accordance with the above standards, the authors must explain the rationale for their approach and demonstrate that the institutional review body explicitly approved the doubtful aspects of the study.

Before submitting, authors should make their study approved by the relevant research ethics committee or institutional review board. If human participants were involved, manuscripts must be accompanied by a statement that the experiments were undertaken with the understanding and appropriate informed consent of each. Any personal item or information will not be published without explicit consents from the involved patients. If experimental animals were used, the materials and methods (experimental procedures) section must clearly indicate that appropriate measures were taken to minimize pain or discomfort, and details of animal care should be provided.

SUBMISSION OF MANUSCRIPTS

Manuscripts should be typed in 1.5 line spacing and 12 pt. Book Antiqua with ample margins. Number all pages consecutively, and start each of the following sections on a new page: Title Page, Abstract, Introduction, Materials and Methods, Results, Discussion, Acknowledgements, References, Tables, Figures, and Figure Legends. Neither the editors nor the publisher are responsible for the opinions expressed by contributors. Manuscripts formally accepted for publication become the permanent property of Baishideng Publishing Group Co., Limited, and may not be reproduced by any means, in whole or in part, without the written permission of both the authors and the publisher. We reserve the right to copy-edit and put onto our website accepted manuscripts. Authors should follow the relevant guidelines for the care and use of laboratory animals of their institution or national animal welfare committee. For the sake of transparency in regard to the performance and reporting of clinical trials, we endorse the policy of the ICMJE to refuse to publish papers on clinical trial results if the trial was not recorded in a publicly-accessible registry at its outset. The only register now available, to our knowledge, is http://www.clinicaltrials.gov sponsored by the United States National Library of Medicine and we encourage all potential contributors to register with it. However, in the case that other registers become available you will be duly notified. A letter of recommendation from each author's organization should be provided with the contributed article to ensure the privacy and secrecy of research is protected.

Authors should retain one copy of the text, tables, photographs and illustrations because rejected manuscripts will not be returned to the author(s) and the editors will not be responsible for loss or damage to photographs and illustrations sustained during mailing.

Online submissions
Manuscripts should be submitted through the Online Submission

System at: http://www.wjgnet.com/1007-9327office. Authors are highly recommended to consult the ONLINE INSTRUCTIONS TO AUTHORS (http://www.wjgnet.com/1007-9327/g_info_20100315215714.htm) before attempting to submit online. For assistance, authors encountering problems with the Online Submission System may send an email describing the problem to wjg@wjgnet.com, or by telephone: +86-10-5908-0039. If you submit your manuscript online, do not make a postal contribution. Repeated online submission for the same manuscript is strictly prohibited.

MANUSCRIPT PREPARATION

All contributions should be written in English. All articles must be submitted using word-processing software. All submissions must be typed in 1.5 line spacing and 12 pt. Book Antiqua with ample margins. Style should conform to our house format. Required information for each of the manuscript sections is as follows:

Title page

Title: Title should be less than 12 words.

Running title: A short running title of less than 6 words should be provided.

Authorship: Authorship credit should be in accordance with the standard proposed by ICMJE, based on (1) substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; (2) drafting the article or revising it critically for important intellectual content; and (3) final approval of the version to be published. Authors should meet conditions 1, 2, and 3.

Institution: Author names should be given first, then the complete name of institution, city, province and postcode. For example, Xu-Chen Zhang, Li-Xin Mei, Department of Pathology, Chengde Medical College, Chengde 067000, Hebei Province, China. One author may be represented from two institutions, for example, George Sgourakis, Department of General, Visceral, and Transplantation Surgery, Essen 45122, Germany; George Sgourakis, 2nd Surgical Department, Korgialenio-Benakio Red Cross Hospital, Athens 15451, Greece.

Author contributions: The format of this section should be: Author contributions: Wang CL and Liang L contributed equally to this work; Wang CL, Liang L, Fu JF, Zou CC, Hong F and Wu XM designed the research; Wang CL, Zou CC, Hong F and Wu XM performed the research; Xue JZ and Lu JR contributed new reagents/analytic tools; Wang CL, Liang L and Fu JF analyzed the data; and Wang CL, Liang L and Fu JF wrote the paper.

Supportive foundations: The complete name and number of supportive foundations should be provided, e.g. Supported by National Natural Science Foundation of China, No. 30224801

Correspondence to: Only one corresponding address should be provided. Author names should be given first, then author title, affiliation, the complete name of institution, city, postcode, province, country, and email. All the letters in the email should be in lower case. A space interval should be inserted between country name and email address. For example, Montgomery Bissell, MD, Professor of Medicine, Chief, Liver Center, Gastroenterology Division, University of California, Box 0538, San Francisco, CA 94143, United States. montgomery.bissell@ucsf.edu

Telephone and fax: Telephone and fax should consist of +, country number, district number and telephone or fax number, e.g. Telephone: +86-10-59080039 Fax: +86-10-85381893

Peer reviewers: All articles received are subject to peer review. Normally, three experts are invited for each article. Decision for acceptance is made only when at least two experts recommend an article for publication. Reviewers for accepted manuscripts are acknowledged in each manuscript, and reviewers of articles which were not accepted will be acknowledged at the end of each issue. To ensure the quality of the articles published in *WJG*, reviewers of accepted manuscripts will be announced by publishing the name, title/position and institution of the reviewer in the footnote accompanying the printed article. For example, reviewers: Professor Jing-Yuan Fang, Shanghai Institute of Digestive Disease, Shanghai, Affiliated Renji Hospital, Medical Faculty, Shanghai Jiaotong University, Shanghai, China; Professor Xin-Wei Han, Department of Radiology, The First Affiliated Hospital, Zhengzhou University, Zhengzhou, Henan Province, China; and Professor Anren Kuang, Department of Nuclear Medicine, Huaxi Hospital, Sichuan University, Chengdu, Sichuan Province, China.

Abstract

There are unstructured abstracts (no more than 256 words) and structured abstracts (no more than 480). The specific requirements for structured abstracts are as follows:

An informative, structured abstracts of no more than 480 words should accompany each manuscript. Abstracts for original contributions should be structured into the following sections. AIM (no more than 20 words): Only the purpose should be included. Please write the aim as the form of "To investigate/study/…"; MATERIALS AND METHODS (no more than 140 words); RESULTS (no more than 294 words): You should present *P* values where appropriate and must provide relevant data to illustrate how they were obtained, e.g. 6.92 ± 3.86 *vs* 3.61 ± 1.67, $P < 0.001$; CONCLUSION (no more than 26 words).

Key words

Please list 5-10 key words, selected mainly from *Index Medicus*, which reflect the content of the study.

Text

For articles of these sections, original articles and brief articles, the main text should be structured into the following sections: INTRODUCTION, MATERIALS AND METHODS, RESULTS and DISCUSSION, and should include appropriate Figures and Tables. Data should be presented in the main text or in Figures and Tables, but not in both. The main text format of these sections, editorial, topic highlight, case report, letters to the editors, can be found at: http://www.wjgnet.com/1007-9327/g_info_20100315215714.htm.

Illustrations

Figures should be numbered as 1, 2, 3, *etc.*, and mentioned clearly in the main text. Provide a brief title for each figure on a separate page. Detailed legends should not be provided under the figures. This part should be added into the text where the figures are applicable. Figures should be either Photoshop or Illustrator files (in tiff, eps, jpeg formats) at high-resolution. Examples can be found at: http://www.wjgnet.com/1007-9327/13/4520.pdf; http://www.wjgnet.com/1007-9327/13/4554.pdf; http://www.wjgnet.com/1007-9327/13/4891.pdf; http://www.wjgnet.com/1007-9327/13/4986.pdf; http://www.wjgnet.com/1007-9327/13/4498.pdf. Keeping all elements compiled is necessary in line-art image. Scale bars should be

used rather than magnification factors, with the length of the bar defined in the legend rather than on the bar itself. File names should identify the figure and panel. Avoid layering type directly over shaded or textured areas. Please use uniform legends for the same subjects. For example: Figure 1 Pathological changes in atrophic gastritis after treatment. A:...; B:...; C:...; D:...; E:...; F:...; G: …*etc.* It is our principle to publish high resolution-figures for the printed and E-versions.

Tables

Three-line tables should be numbered 1, 2, 3, *etc.*, and mentioned clearly in the main text. Provide a brief title for each table. Detailed legends should not be included under tables, but rather added into the text where applicable. The information should complement, but not duplicate the text. Use one horizontal line under the title, a second under column heads, and a third below the Table, above any footnotes. Vertical and italic lines should be omitted.

Notes in tables and illustrations

Data that are not statistically significant should not be noted. $^{a}P < 0.05$, $^{b}P < 0.01$ should be noted ($P > 0.05$ should not be noted). If there are other series of P values, $^{c}P < 0.05$ and $^{d}P < 0.01$ are used. A third series of P values can be expressed as $^{e}P < 0.05$ and $^{f}P < 0.01$. Other notes in tables or under illustrations should be expressed as ^{1}F, ^{2}F, ^{3}F; or sometimes as other symbols with a superscript (Arabic numerals) in the upper left corner. In a multi-curve illustration, each curve should be labeled with ●, ○, ■, □, ▲, △, *etc.*, in a certain sequence.

Acknowledgments

Brief acknowledgments of persons who have made genuine contributions to the manuscript and who endorse the data and conclusions should be included. Authors are responsible for obtaining written permission to use any copyrighted text and/or illustrations.

REFERENCES

Coding system

The author should number the references in Arabic numerals according to the citation order in the text. Put reference numbers in square brackets in superscript at the end of citation content or after the cited author's name. For citation content which is part of the narration, the coding number and square brackets should be typeset normally. For example, "Crohn's disease (CD) is associated with increased intestinal permeability[1,2]". If references are cited directly in the text, they should be put together within the text, for example, "From references[19,22-24], we know that...".

When the authors write the references, please ensure that the order in text is the same as in the references section, and also ensure the spelling accuracy of the first author's name. Do not list the same citation twice.

PMID and DOI

Pleased provide PubMed citation numbers to the reference list, e.g. PMID and DOI, which can be found at http://www.ncbi.nlm.nih.gov/sites/entrez?db=pubmed and http://www.crossref.org/SimpleTextQuery/, respectively. The numbers will be used in E-version of this journal.

Style for journal references

Authors: the name of the first author should be typed in boldfaced letters. The family name of all authors should be typed with the initial letter capitalized, followed by their abbreviated first and middle initials. (For example, Lian-Sheng Ma is abbreviated as Ma LS, Bo-Rong Pan as Pan BR). The title of the cited article and italicized journal title (journal title should be in its abbreviated form as shown in PubMed), publication date, volume number (in black), start page, and end page [PMID: 11819634 DOI: 10.3748/wjg.13.5396].

Style for book references

Authors: the name of the first author should be typed in boldfaced letters. The surname of all authors should be typed with the initial letter capitalized, followed by their abbreviated middle and first initials. (For example, Lian-Sheng Ma is abbreviated as Ma LS, Bo-Rong Pan as Pan BR) Book title. Publication number. Publication place: Publication press, Year: start page and end page.

Format

Journals

English journal article (list all authors and include the PMID where applicable)

1 **Jung EM**, Clevert DA, Schreyer AG, Schmitt S, Rennert J, Kubale R, Feuerbach S, Jung F. Evaluation of quantitative contrast harmonic imaging to assess malignancy of liver tumors: A prospective controlled two-center study. *World J Gastroenterol* 2007; **13**: 6356-6364 [PMID: 18081224 DOI: 10.3748/wjg.13.6356]

Chinese journal article (list all authors and include the PMID where applicable)

2 **Lin GZ**, Wang XZ, Wang P, Lin J, Yang FD. Immunologic effect of Jianpi Yishen decoction in treatment of Pixudiarrhoea. *Shijie Huaren Xiaohua Zazhi* 1999; **7**: 285-287

In press

3 **Tian D**, Araki H, Stahl E, Bergelson J, Kreitman M. Signature of balancing selection in Arabidopsis. *Proc Natl Acad Sci USA* 2006; In press

Organization as author

4 **Diabetes Prevention Program Research Group**. Hypertension, insulin, and proinsulin in participants with impaired glucose tolerance. *Hypertension* 2002; **40**: 679-686 [PMID: 12411462 PMCID:2516377 DOI:10.1161/01.HYP.0000035706.28494.09]

Both personal authors and an organization as author

5 **Vallancien G**, Emberton M, Harving N, van Moorselaar RJ; Alf-One Study Group. Sexual dysfunction in 1, 274 European men suffering from lower urinary tract symptoms. *J Urol* 2003; **169**: 2257-2261 [PMID: 12771764 DOI:10.1097/01.ju.0000067940.76090.73]

No author given

6 21st century heart solution may have a sting in the tail. *BMJ* 2002; **325**: 184 [PMID: 12142303 DOI:10.1136/bmj.325.7357.184]

Volume with supplement

7 **Geraud G**, Spierings EL, Keywood C. Tolerability and safety of frovatriptan with short- and long-term use for treatment of migraine and in comparison with sumatriptan. *Headache* 2002; **42** Suppl 2: S93-99 [PMID: 12028325 DOI:10.1046/j.1526-4610.42.s2.7.x]

Issue with no volume

8 **Banit DM**, Kaufer H, Hartford JM. Intraoperative frozen section analysis in revision total joint arthroplasty. *Clin Orthop Relat Res* 2002; (**401**): 230-238 [PMID: 12151900 DOI:10.1097/00003086-200208000-00026]

No volume or issue

9 Outreach: Bringing HIV-positive individuals into care. *HRSA Careaction* 2002; 1-6 [PMID: 12154804]

Books

Personal author(s)

10 **Sherlock S**, Dooley J. Diseases of the liver and billiary system. 9th ed. Oxford: Blackwell Sci Pub, 1993: 258-296

Chapter in a book (list all authors)

11 **Lam SK**. Academic investigator's perspectives of medical treatment for peptic ulcer. In: Swabb EA, Azabo S. Ulcer disease: investigation and basis for therapy. New York: Marcel Dekker, 1991: 431-450

Author(s) and editor(s)

12 **Breedlove GK**, Schorfheide AM. Adolescent pregnancy. 2nd ed. Wieczorek RR, editor. White Plains (NY): March of Dimes Education Services, 2001: 20-34

Conference proceedings

13 **Harnden P**, Joffe JK, Jones WG, editors. Germ cell tumours V. Proceedings of the 5th Germ cell tumours Conference; 2001 Sep 13-15; Leeds, UK. New York: Springer, 2002: 30-56

Conference paper

14 **Christensen S**, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. In: Foster JA, Lutton E, Miller J, Ryan C, Tettamanzi AG, editors. Genetic programming. EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming; 2002 Apr 3-5; Kinsdale, Ireland. Berlin: Springer, 2002: 182-191

Electronic journal (list all authors)

15 Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis serial online, 1995-01-03, cited 1996-06-05; 1(1): 24 screens. Available from: URL: http://www.cdc.gov/ncidod/eid/index.htm

Patent (list all authors)

16 **Pagedas AC**, inventor; Ancel Surgical R&D Inc., assignee. Flexible endoscopic grasping and cutting device and positioning tool assembly. United States patent US 20020103498. 2002 Aug 1

Statistical data

Write as mean ± SD or mean ± SE.

Statistical expression

Express *t* test as *t* (in italics), *F* test as *F* (in italics), chi square test as χ^2 (in Greek), related coefficient as *r* (in italics), degree of freedom as υ (in Greek), sample number as *n* (in italics), and probability as *P* (in italics).

Units

Use SI units. For example: body mass, m (B) = 78 kg; blood pressure, p (B) = 16.2/12.3 kPa; incubation time, t (incubation) = 96 h, blood glucose concentration, c (glucose) 6.4 ± 2.1 mmol/L; blood CEA mass concentration, p (CEA) = 8.6 24.5 μg/L; CO_2 volume fraction, 50 mL/L CO_2, not 5% CO_2; likewise for 40 g/L formaldehyde, not 10% formalin; and mass fraction, 8 ng/g, *etc.* Arabic numerals such as 23, 243, 641 should be read 23 243 641.

The format for how to accurately write common units and quantums can be found at: http://www.wjgnet.com/1007-9327/g_info_20100315223018.htm.

Abbreviations

Standard abbreviations should be defined in the abstract and on first mention in the text. In general, terms should not be abbreviated unless they are used repeatedly and the abbreviation is helpful to the reader. Permissible abbreviations are listed in Units, Symbols and Abbreviations: A Guide for Biological and Medical Editors and Authors (Ed. Baron DN, 1988) published by The Royal Society of Medicine, London. Certain commonly used abbreviations, such as DNA, RNA, HIV, LD50, PCR, HBV, ECG, WBC, RBC, CT, ESR, CSF, IgG, ELISA, PBS, ATP, EDTA, mAb, can be used directly without further explanation.

Italics

Quantities: *t* time or temperature, *c* concentration, *A* area, *l* length, *m* mass, *V* volume.
Genotypes: *gyrA*, *arg* 1, *c myc*, *c fos*, *etc.*
Restriction enzymes: *Eco*RI, *Hin*dI, *Bam*HI, *Kbo* I, *Kpn* I, *etc.*
Biology: *H. pylori*, *E coli*, *etc.*

Examples for paper writing

Editorial: http://www.wjgnet.com/1007-9327/g_info_20100315220036.htm

Frontier: http://www.wjgnet.com/1007-9327/g_info_20100315220305.htm

Topic highlight: http://www.wjgnet.com/1007-9327/g_info_20100315220601.htm

Observation: http://www.wjgnet.com/1007-9327/g_info_20100312232427.htm

Guidelines for basic research: http://www.wjgnet.com/1007-9327/g_info_20100315220730.htm

Guidelines for clinical practice: http://www.wjgnet.com/1007-9327/g_info_20100315221301.htm

Review: http://www.wjgnet.com/1007-9327/g_info_20100315221554.htm

Original articles: http://www.wjgnet.com/1007-9327/g_info_20100315221814.htm

Brief articles: http://www.wjgnet.com/1007-9327/g_info_20100312231400.htm

Case report: http://www.wjgnet.com/1007-9327/g_info_20100315221946.htm

Letters to the editor: http://www.wjgnet.com/1007-9327/g_info_20100315222254.htm

Book reviews: http://www.wjgnet.com/1007-9327/g_info_20100312231947.htm

Guidelines: http://www.wjgnet.com/1007-9327/g_info_20100312232134.htm

RESUBMISSION OF THE REVISED MANUSCRIPTS

Please revise your article according to the revision policies of *WJG*. The revised version includes manuscript and high-resolution image figures. The author should re-submit the revised manuscript online, along with printed high-resolution color or black and white photos; Copyright transfer letter, and responses to the reviewers, and science news are sent to us *via* email.

Editorial Office

World Journal of Gastroenterology

Editorial Department: Room 903, Building D,
Ocean International Center,
No. 62 Dongsihuan Zhonglu,

Chaoyang District, Beijing 100025, China
E-mail: wjg@wjgnet.com
http://www.wjgnet.com
Telephone: +86-10-5908-0039
Fax: +86-10-8538-1893

Language evaluation

The language of a manuscript will be graded before it is sent for revision. (1) Grade A: priority publishing; (2) Grade B: minor language polishing; (3) Grade C: a great deal of language polishing needed; and (4) Grade D: rejected. Revised articles should reach Grade A or B.

Copyright assignment form

Please download a Copyright assignment form from http://www.wjgnet.com/1007-9327/g_info_20100315222818.htm.

Responses to reviewers

Please revise your article according to the comments/suggestions provided by the reviewers. The format for responses to the reviewers' comments can be found at: http://www.wjgnet.com/1007-9327/g_info_20100315222607.htm.

Proof of financial support

For paper supported by a foundation, authors should provide a copy of the document and serial number of the foundation.

Links to documents related to the manuscript

WJG will be initiating a platform to promote dynamic interactions between the editors, peer reviewers, readers and authors. After a manuscript is published online, links to the PDF version of the submitted manuscript, the peer-reviewers' report and the revised manuscript will be put on-line. Readers can make comments on the peer reviewer's report, authors' responses to peer reviewers, and the revised manuscript. We hope that authors will benefit from this feedback and be able to revise the manuscript accordingly in a timely manner.

Science news releases

Authors of accepted manuscripts are suggested to write a science news item to promote their articles. The news will be released rapidly at EurekAlert/AAAS (http://www.eurekalert.org). The title for news items should be less than 90 characters; the summary should be less than 75 words; and main body less than 500 words. Science news items should be lawful, ethical, and strictly based on your original content with an attractive title and interesting pictures.

Publication fee

WJG is an international, peer-reviewed, Open-Access, online journal. Articles published by this journal are distributed under the terms of the Creative Commons Attribution Non-commercial License, which permits use, distribution, and reproduction in any medium, provided the original work is properly cited, the use is non commercial and is otherwise in compliance with the license. Authors of accepted articles must pay a publication fee. The related standards are as follows. Publication fee: 1300 USD per article. Editorial, topic highlights, book reviews and letters to the editor are published free of charge.