



ISHSR
International Society for
HEPATIC SINUSOIDAL RESEARCH



THE 2022 LIVER SINUSOID MEETING

The 21st International Symposium on Cells of the Hepatic Sinusoid

the role of sinusoidal cells in the hepatobiliary diseases

1-2 September, 2022

Venue: Hotel Equatorial Shanghai

Conference manual

Organizer: International Society for Hepatic Sinusoidal Research

Shanghai Medical Association

Digestive Diseases Society of Shanghai Medical Association

Undertaker: Department of gastroenterology, Changzheng hospital, Shanghai

Department of Infectious Diseases, Fifth Medical Center of Chinese PLA General Hospital, Beijing

Chinese Journal of Digestion



Welcome Message



Welcome Message

On behalf of the International Society of Hepatic Sinusoidal Research (ISHSR), the local organising committee looks forward to extending you a very warm welcome to the 2022 Liver Sinusoid Meeting: 21st International Symposium on Cells of the Hepatic Sinusoid (ISCHS 2022), which will be held in Shanghai, China on September 1-2 2022. The meeting will be held as hybrid meeting, with live and interactive sessions, both onsite and on-line.

As with previous symposia, the main aim of ISCHS 2022 is to provide a forum for basic scientists and clinical researchers to meet and discuss recent developments in research on the biology of the cells of the hepatic sinusoid in health and disease. A second and equally important aim is to provide of forum for young investigators to present their work in a friendly and supportive environment. This time, the Society adapts the meeting to the current situation offering an intuitive and evolving online platform to all those not able to attend in person to the meeting.

One of the main themes of ISCHS 2022 will be the integral role of the liver sinusoids in disease and how we can harness the unique properties of the sinusoid to prevent and treat these conditions.

We look forward to seeing you in Shanghai, or online, in September!

Meeting Chair

Executive Chair

Executive Chair

Jisheng Wang

Weifen Xie

TLH



Meeting schedule

Thursday 01/09/2022 (UTC+8)			
Opening Ceremony			
08:00 -08:30	Fu-Sheng Wang (President of ISHSR)		CHAIRS Jordi Gracia-Sancho Wei-Fen Xie
	Yu-Zhang Wu (President of Chinese Society of Immunology)		
	Jian-Guang Xu (President of Shanghai Medical Association)		
Keynote presentation			
Time	Title	Speaker	Chairs
08:30 -08:55	Immune Exhaustion & Dynamics Thereof	Yu-Zhang Wu (Chongqing, China)	Fu-Sheng Wang Jun Yu
08:55 -09:20	Liver-gut signaling in alcoholic liver disease	Natalia Nieto (Chicago, USA)	
09:20 -09:45	Heterogeneity and function of liver-resident NK cells	Hui Peng (Hefei, China)	
09:45 -10:10	Sinusoidal cells as target for ageing	Victoria Cogger (Sydney, Australia)	
10:10 - 10:20	Discussion		
SYMPOSIUM 1 Sinusoidal cells (patho)biology in liver disease			
Time	Title	Speaker	Chairs
10:20 - 10:40	Sinusoidal cells as target for ALD	Laura Nagy (Cleveland, USA)	Anna Mae Diehl Hong-Yan Qin
10:40 - 11:00	Sinusoidal cells in DILI	Hartmut Jaeschke (Kansas, USA)	
11:00 - 11:20	Tutorial talk: Macrophages	Bin Gao (MD, USA)	
11:20 - 11:40	Tutorial talk: Stellate cells	Norifumi Kawada (Osaka, Japan)	
11:40 - 11:50	Discussion		
11:50 - 14:00	Lunch, meetings and poster presentations		



Meeting schedule

Thursday 01/09/2022 (UTC+8)			
SYMPOSIUM 2 Sinusoids as a therapeutic target			
Time	Title	Speaker	Chairs
14:00 - 14:20	The emerging concept and pathogenesis of portal sinusoidal vascular disorder	Ji-Dong Jia (Beijing, China)	Frank Tacke Xiong Ma
14:20 - 14:40	Sinusoidal cells as target for liver fibrosis	Hong You (Beijing, China)	
14:40 - 15:00	Sinusoidal cells as target for NASH	Sven Francque (Antwerp, Belgium)	
15:00 - 15:20	Sinusoidal cells as target for portal hypertension	Jordi Gracia-Sancho (Barcelona, Spain)	
15:20 - 15:30	Discussion		
15:30 - 15:50	Tutorial talk: Biology of LSECs	Bartłomiej Zapotoczny (Krakow, Poland)	Hong You Jordi Gracia-Sancho
15:50 - 16:10	LSECs, Kupffer cells and Chronic HBV infection	Zheng-Hong Yuan (Shanghai, China)	
16:10 - 16:30	Dysfunction of NK cells in hepatocellular carcinoma microenvironment	Hai-Ming Wei (Hefei, China)	
16:30 - 16:50	Germinal center immune response in chronic HBV infection	Yong-Yin Li (Guangzhou, China)	
16:50 - 17:00	Discussion		

Meeting schedule

Friday 02/09/2022 (UTC+8)			
Keynote presentation			
Time	Title	Speaker	Chairs
08:30 - 08:55	Gut microbiota in liver disease	Amany Zekry (Sydney, Australia)	Victoria Cogger Hua Wang
08:55 - 09:20	Gut microbiome and metabolomics in liver cancer	Jun Yu (Hongkong, China)	
09:20 - 09:45	Mechanisms and regression of cirrhotic portal hypertension	Wei-Fen Xie (Shanghai, China)	
09:45 - 10:15	Discussion & Break		
SYMPOSIUM 3 Sinusoidal communication in liver disease			
Time	Title	Speaker	Chairs
10:15 - 10:35	Bile acids and Sphingolipids in cholestatic liver disease	Hui-Ping Zhou (VA, USA)	Laura Nagy Jin Ding
10:35 - 10:55	Lymphatics in liver disease	Yasuko Iwakiri (New Haven, USA)	
10:55 - 11:15	Kupffer cells, microbiome and liver diseases	Bernd Schnabl (San Diego, USA)	
11:15 - 11:35	Activated Neutrophils Inhibit Pro-Inflammatory Macrophage Responses Attenuate Liver Injury	Li-Ying Li (Beijing, China)	
11:35 - 11:45	Discussion		
11:45 - 14:00	Lunch, meetings and poster presentations		
SYMPOSIUM 4 Novel methods/technologies			
Time	Title	Speaker	Chairs
14:00 - 14:20	Methods to study liver sinusoidal mechanobiology	Sergi Guixé (Barcelona, Spain)	Nicholas Hunt Yong-Zhan Nie
14:20 - 14:40	Novel in vitro models to study the liver sinusoid	Savneet Kaur (New Delhi, India)	
14:40 - 15:00	scRNA seq, snRNA seq, spatial transcriptomics	Prakash Ramachandran (Edinburgh, UK)	
15:00 - 15:10	Discussion		



Meeting schedule

Friday 02/09/2022 (UTC+8)			
Short communications			
Time	Title	Speaker	Chairs
15:10 - 15:25	Increased sinusoidal pressure impairs liver endothelial mechanosensing, uncovering novel biomarkers of portal hypertension	Albert Gibert-Ramos Jordi Gracia-Sancho (Barcelona, Spain)	Anabel Fernández-Iglesias Xin Zhang
15:25 - 15:40	Activation of hepatic HNF1α signaling by suppressing gut microbiome related deoxycholic acid upon rifaximin treatment improves NASH in mice	Mei-Tong Nie Wei-Fen Xie (Shanghai, China)	
15:40 - 15:55	Saturated fatty acid-enriched extracellular vesicles promote a negative crosstalk involved in liver inflammation and hepatocyte insulin resistance	Rosa Alen Ángela M. Valverde (Madrid, Spain)	
15:55 - 16:10	Identification of a matrisome signature of liver fibrosis progression and resolution	Wei Chen Hong You (Beijing, China)	
16:10 - 16:25	Endogenous and exogenous globin proteins presenting in Hepatic stellate cell suppress its activation and inhibit liver fibrosis via scavenging reactive oxygen species	Le Thi Thanh Thuy Norifumi Kawada (Osaka, Japan)	
16:25 - 16:35	Discussion		
16:35 - 16:50	Intestinal osteopontin protects from alcohol-induced liver injury by preserving gut microbiome and intestinal barrier	Sukanta Das Natalia Nieto (Chicago, USA)	Sergi Guixé Wen-Ping Xu
16:50 - 17:05	Alcohol binge exacerbates liver damage in a novel cholestatic model of acute-on-chronic liver failure	Martí Ortega-Ribera Gyongyi Szabo (MA, USA)	
17:05 - 17:20	Spatial mapping of endothelial cells using single-cell RNA sequencing during the injury and recovery phases of acetaminophen hepatotoxicity	David S. Umbaugh Hartmut Jaeschke (KS, USA)	
17:20 - 17:35	microRNA-223 attenuates hepatocarcinogenesis by blocking hypoxia-driven angiogenesis and immunosuppression	Yao-Jie Fu Bin Gao (MD, USA)	
17:35 - 17:50	Lack of Neutrophil Hmgb1 in Liver Transplant Recipients Exacerbates Early Allograft Injury	Zhuo-Lun Song Natalia Nieto (Chicago, USA)	
17:50 - 18:00	Discussion		



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Closing Ceremony			
Time	Title	Speaker	Chairs
18:00 - 18:05	Announcement of awards and the ISCHS 2023	Jordi Gracia-Sancho (Secretary of ISHSR)	Wei-Fen Xie
18:05 - 18:10	Closing remarks	Fu-Sheng Wang (President of ISHSR)	



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Chairs & Speakers



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Fu-Sheng Wang
(Beijing, China)

Fu-Sheng Wang, M.D., Ph.D., Academician of Chinese Academy of Sciences, the Director of both National Clinical Research Center for Infectious Diseases and the Department of Infectious Diseases, the Fifth Medical Center of Chinese PLA General Hospital.

He has been worked in management and translational study of infectious diseases for more than 30 years, and has been engaged in the clinical treatment of several major epidemics, including SARS, Ebola and COVID-19. His research is focused on clinical immunology and cellular therapy for the patients with chronic HBV/HCV/HIV infections.

He is an expert of the 8th Discipline Review Group of the Academic Degrees Committee of the State Council, a member of the national and military biosafety expert groups, and the next chairman of the Society of Infectious Diseases, Chinese Medical Association. He is the winner of the national fund for Distinguished Young Scholars, the leader of the innovation research group of the National Natural Science Foundation, the winner of the National Innovation Award, the national outstanding scientific and technological worker, the national advanced individual in the fight against COVID-19, and the national outstanding communist party member.



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Jun Yu
(Hongkong, China)

Assistant Dean, Faculty of Medicine, the Chinese University of Hong Kong
Director, Institute of Digestive Diseases, Chinese University of Hong Kong
Director, State Key Laboratory of Digestive Diseases Research

Vice Chairman, Tumor and Intestinal Microbiome Professional Committee of Chinese Anti-Cancer Association

Vice chairman, the Digestive Committee of Chinese Women Medical Doctor Association

Chang Jiang Scholar Chair Professor, Ministry of Education

Executive Director, Microecology, American Gastroenterological Association (AGA)

Executive Director of Oncology, American Gastroenterological Association (AGA) (2017-2019)



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Yu-Zhang Wu
(Chongqing, China)

Title: Immune Exhaustion & Dynamics Thereof

President, Chinese Society for Immunology

Research Areas

Human immunity atlas
T cell recognition of antigen
Regulation of T cell function
Antigen engineering
Therapeutic vaccine development

Achievements

More than 300 SCI papers
More than 100 patents
More than 20 rewards
2 first-in-class new drugs in phase III clinical trial
16 immune diagnostic kits in market



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Natalia Nieto
(Chicago, USA)

Title: Liver-gut signaling in alcoholic liver disease

During my Ph.D., I acquired solid knowledge on the role of oxidative stress, inflammation and fatty acids in inflammatory bowel disease. In my first period of Postdoctoral training in Dr. Marcos Rojkind Laboratory at the Albert Einstein College of Medicine (New York), an expert in hepatic stellate cells and liver fibrosis, I worked on extracellular matrix biology and on the pathophysiology of liver fibrosis. During my second period of Postdoctoral training in Dr. Arthur I. Cederbaum Laboratory at the Icahn School of Medicine at Mount Sinai (New York), an expert in oxidative stress and liver toxicity, I studied the mechanisms involved in the up-regulation of collagen-I gene expression under oxidative stress conditions and the role of oxidant stress in hepatic fibrosis. Through my training, I developed broad expertise on the crosstalk among liver cells on the fibrogenic response to liver injury, which continues to be the major focus of my research. Thus, I have a long-standing interest in understanding the pathophysiology of liver fibrosis. I have significant experience with various models of liver fibrosis, primary cell isolation, cocultures of hepatocytes, Kupffer cells and hepatic stellate cells and the molecular biology and signaling pathways involved in collagen-I regulation. Upon my arrival to The University of Illinois at Chicago, I started the Extracellular Matrix Biology and Liver Research Program that built a network of scientists within our institution with common research interests.



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Title: Heterogeneity and function of liver-resident NK cells



Hui Peng
(Hefei, China)

Professor Hui Peng's work focuses on NK cells and in particular liver NK cell development and roles in liver diseases. She began the study of NK cells 16 years ago, and has made significant achievements in this field. In 2013, she and colleagues reported a novel CD49a⁺ NK cell subset, termed the liver-resident NK cell, which is currently categorized as the type one innate lymphoid cell (ILC1). Later, she and colleagues found immune-regulatory roles and extramedullary development of this population. They also identified transcription factors specifically regulating liver-resident NK cells. The studies have been published in many high impact journals, including Science, Immunity, Nature Communications, Hepatology, Journal of Clinical Investigation, etc, and the citation is more than 1,400 times.



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Title: Sinusoidal cells as target for ageing



Victoria Cogger
(Sydney, Australia)

Victoria completed a BSc (Hons) in 1999 followed by a PhD on the Ultrastructure of the Ageing Liver, graduating from the University of Sydney in 2003. She was awarded a Healthy Ageing Postdoctoral Fellowship and travelled to the National Institutes of Health, Bethesda MD USA to complete postdoctoral studies. Victoria now leads research investigating the biology of ageing; with particular focus on the liver and targeted interventions for treating age related disease using nanomedicines.

Victoria was elected President of the International Society of Hepatic Sinusoidal Research (2017-2019) and will host the Society's International Symposium at the University of Sydney in September 2019. She is an Associate Editor of the Journals of Gerontology: Biological Sciences.

In July 2018, Victoria was appointed Associate Dean (Research Education) in the Faculty of Medicine and Health. This role involves overseeing and developing strategies to assist in the successful research training for over 1000 enrolled Higher Degree Research students within the Faculty.



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Anna Mae Diehl

Anna Mae Diehl, MD, FAASLD is currently the Florence McAlister Professor of Medicine at Duke University. She is a physician scientist and academic hepatologist. Her lab-based research activities focus on basic mechanisms of liver repair and complement her translational/clinical research programs in alcoholic and nonalcoholic fatty liver disease. In the past two years, Dr. Diehl has co-authored 10 peer-reviewed manuscripts on these topics.

Dr. Diehl has been an associate editor/editorial board member for major journals such as HEPATOLOGY, Gastroenterology, GUT, The American Journal of Physiology, The Journal of Clinical Investigation, Nature Reviews, and eLife. In addition, she has served as a standing member of several NIH study sections and scientific advisory councils for NIDDK, NCI, NIAAA, the American Liver Foundation, the Alcoholic Beverage Medical Research Foundation, NIDDK Digestive Disease Centers at Baylor, USC and U Pittsburgh, and NIAAA Alcohol Research Centers at U Louisville and the Cleveland Clinic. Dr. Diehl is a past-president of the Gastroenterology Research Group, former AASLD Governing Board Councilor-at-Large. Dr. Diehl is currently member of the Executive Governing Council for the International Association for the Study of Cells of the Hepatic Sinusoid, as well as Co-Chair of the NAFLD Special Interest Group for the AASLD.



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Hong-Yan Qin

Director of the Department of Medical Genetics and Developmental Biology in Fourth Military Medical University. She is a vice-chairman of Genetics Association of Shaanxi Province, the Scientific and Technological Innovation Talent in Young People of Shaanxi Province (2020). She majors in the innate immune cells development and their role in the transformation of inflammation to cancer, and as a Postdoc in Department of Immunology and Genomic Medicine of Kyoto University followed with Prof. Tasuku Honjo. She took charge of 8 grants supported by National Natural Science Foundation of China (NSFC) including key program, general program and youth program. 2 grants supported by PLA and one sub-project supported by the National High Technology Research and Development Program of China. Since 2004, she has published 53 international papers on SCI journals, such as Nat Immunol, Immunity, Hepatology, J Hepatology and Cancer Research and so on. She gained the Second Awards for National Scientific and Technological Progress in 2012 (9th place), the First Award for Scientific and Technology of Shaanxi province in 2010 and 2007 (4th and 2nd place).



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Laura Nagy
(Cleveland, USA)

Title: Sinusoidal cells as target for ALD

PhD, Professor

Molecular Medicine, Case Western Reserve

University Staff, Inflammation & Immunity,

Gastroenterology/Hepatology Director,

Northern Ohio Alcohol Center/Cleveland Clinic, Cleveland OH USA



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Hartmut Jaeschke
(Kansas, USA)

Title: Sinusoidal cells in DILI

PhD, ATS, FAASLD

University Distinguished Professor and Chair

Department of Pharmacology, Toxicology & Therapeutics

University of Kansas Medical Center

Kansas City, KS USA



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Bin Gao
(MD, USA)

Title: Tutorial talk: Macrophages

Dr. Bin Gao received both his M.D. and Ph.D. from China, respectively. Following postdoctoral training at the NIAAA, National Institutes of Health (NIH) and Medical College of Virginia, he served as a tenure track Assistant Professor at Medical College of Virginia from 1995 to 2000. In 2000, he accepted a tenure track position and established the Section of Liver Biology at the NIAAA, NIH. In 2009, Dr. Gao established the Laboratory of Liver Diseases and became the Laboratory Chief of Liver Diseases at NIAAA, NIH. In 2007, Dr. Gao was elected to the American Society for Clinical Investigation (ASCI).

Dr. Gao's group is studying basic liver immunology and liver biology and investigating the pathogenesis of fatty liver diseases and their associated liver cancer and metabolic syndrome. Dr. Gao's group has introduced novel, more robust mouse models of alcohol-associated liver disease, nonalcoholic fatty liver disease, acute-on-chronic liver failure, and cell-specific ablation. By using these models, Dr. Gao's group has made major contributions to our understanding of the molecular mechanisms of liver injury and repair, alcohol-associated liver disease, nonalcoholic fatty liver disease, and liver failure and their potential treatment.



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Norifumi Kawada
(Osaka, Japan)

Title: Tutorial talk: Stellate cells

Norifumi KAWADA, M.D., Ph.D., FAASLD

Dean

Graduate School of Medicine

Osaka Metropolitan University, Osaka, JAPAN

Professor of Medicine

Chairman, Department of Hepatology

Graduate School of Medicine

Osaka Metropolitan University, Osaka, JAPAN



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Frank Tacke

Director Medical Department of Hepatology and Gastroenterology, Charité – Campus Mitte and Charité – Campus Virchow-Klinikum, Certified Intestinal Cancer Center, ENETS Center of Excellence (ENETS CoE)
1994 – 2001 Human medicine studies, Hannover Medical School & Baylor College of Medicine, Houston, TX, USA (clinical electives)
2001 – 2004 Assistant physician, department Gastroenterology, Hepatology & Endocrinology, Hannover Medical School
2001 – 2004 MD/PhD postgraduate studies „Molecular Medicine“, Hannover Medical School
2002 Doctorate degree Dr. med.
2004 Doctorate degree PhD (summa cum laude)
2006 – 2019 Department of Internal Medicine III, University Clinic Aachen
2007 Habilitation, RWTH Aachen
2008 Specialist Internal Medicine
2008 – 2019 Senior physician, department of Internal Medicine III, University Clinic Aachen, lastly executive senior physician
2011 Specialist Internal Medicine and Gastroenterology
2012 – 2019 W2 Professor for „Hepatology and Gastroenterology“, at RWTH Aachen
2014 Master of Health Business Administration (MHBA), University Nürnberg-Erlangen
2019 Specialist Internal medicine and Endocrinology and Diabetology
Since 2019 W3 Professor Director Medical Department of Hepatology and Gastroenterology at Charité



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Xiong Ma

M.D., Ph.D

The distinguished Professor, Shanghai Jiao Tong University School of Medicine

Director of Division of Infectious Diseases, Vice-Director of Division of Gastroenterology and Hepatology, Shanghai Ren Ji Hospital;

Vice-Director, Shanghai Institute of Digestive Disease;

The National Distinguished Young Scholar.

Co-Chairman, Consortium of the Complex Liver Diseases (CCLD)

Membership: Globe PBC Study Group, International Autoimmune

Hepatitis Group (IAIHG), Chinese Society of Hepatology.



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Title: The emerging concept and pathogenesis of portal sinusoidal vascular disorder



Ji-Dong Jia
(Beijing, China)

MD, PhD, Professor of Medicine,
Liver Research Center,
Beijing Friendship Hospital, Capital Medical University
National Clinical Research Center for Digestive Diseases Beijing,
China.
Past President, the IASL (2013-2016)
Past president, the APASL (2009-2010)
Past president, the Chinese Society of Hepatology (2006 -2012)
Has been served as associated editor of several international
journals, including Liver Int, JGH, Hepatol Int, and JCTH.



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Title: Sinusoidal cells as target for liver fibrosis



Hong You
(Beijing, China)

MD, PhD, professor at the Liver Research Center, Beijing Friendship Hospital, Capital Medical University, in Beijing (China). She is one of the Executive Committee member of the Asian Pacific Association of the Study of Liver Diseases (APASL), and Vice President of Chinese Society of Hepatology, Chinese Medical Association. She has been the principal investigator in 3 recent studies involving hepatitis and cirrhosis, and has numerous articles and book chapters in medical journals addressing viral hepatitis and liver fibrosis.



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Title: Sinusoidal cells as target for NASH



Sven Francque
(Antwerp, Belgium)

Sven M.A. Francque, MD, PhD

Chair, Department of Gastroenterology and Hepatology

Antwerp University Hospital, Belgium

Senior Full Professor of Hepatology

Chair, Translational Sciences in Inflammation and Immunology (TWI²N)

University of Antwerp, Belgium

Educational Councillor-Elect

Governing Board of the European Association for the Study of the Liver



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Title: Sinusoidal cells as target for portal hypertension



Jordi Gracia-Sancho
(Barcelona, Spain)

Jordi's research focuses on liver vascular pathobiology with special interest in the role of sinusoidal cells, and their interactions, in acute and chronic liver diseases, and in aging. He (co-)authored more than 115 peer-reviewed original papers and reviews, and currently serves as Associate Editor for the Journal of Hepatology. In addition, he is invited professor in different universities within Spain and Europe and co-leads diverse educational initiatives including the Liver Seminars Program (www.liverseminars.eu), the 2018 EASL-Basic School of Hepatology, the 2020 APASL-Symposium on Regression of Portal Hypertension, the 2020 AASLD-Symposium on Senescence and Liver Diseases, and the 2021 EASL-Symposium on Sinusoidal Cells in Liver Diseases. Dr Gracia has been invited speaker in different national and international conferences, including the annual meetings of the European, American and Asia-Pacific associations of Hepatology. He has been member of the Scientific Committee of the Spanish Association for the Study of the Liver, and the Steering Committee of the Portal Hypertension Special Interest Group of the American Association for the Study of Liver Diseases (AASLD), being now member of the Basic Research Committee of AASLD. In 2016 he received the Emerging Leader Award from European Association for the Study of the Liver (EASL). He served as Scientific Secretary of the International Society for Hepatic Sinusoidal Research (ISHSR) from 2017-2022, being now the President-Elect of this Society.



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Wei Chen
(Beijing, China)

Wei Chen, PhD. is the associate researcher fellow from Beijing Friendship Hospital, Capital Medical University. He devotes to explore the potential therapeutic targets and mechanisms involved in liver disease, particularly focusing on extracellular matrix of liver fibrosis. In recent three years, he has hosted 2 projects from National Natural Science Foundation of China and published over 10 SCI papers as the first or co-first author in Hepatology, Hepatology Communications, etc. He was awarded the "Young Investigator Award" of EASL in 2019, the "Early Career Investigator Award" of AASLD in 2020 and the "Young Investigator Award" of APASL STC in 2022.



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Jordi Gracia-Sancho
(Barcelona, Spain)

Jordi's research focuses on liver vascular pathobiology with special interest in the role of sinusoidal cells, and their interactions, in acute and chronic liver diseases, and in aging. He (co-)authored more than 115 peer-reviewed original papers and reviews, and currently serves as Associate Editor for the Journal of Hepatology. In addition, he is invited professor in different universities within Spain and Europe and co-leads diverse educational initiatives including the LiverSeminars Program (www.liverseminars.eu), the 2018 EASL-Basic School of Hepatology, the 2020 APASL-Symposium on Regression of Portal Hypertension, the 2020 AASLD-Symposium on Senescence and Liver Diseases, and the 2021 EASL-Symposium on Sinusoidal Cells in Liver Diseases. Dr Gracia has been invited speaker in different national and international conferences, including the annual meetings of the European, American and Asia-Pacific associations of Hepatology. He has been member of the Scientific Committee of the Spanish Association for the Study of the Liver, and the Steering Committee of the Portal Hypertension Special Interest Group of the American Association for the Study of Liver Diseases (AASLD), being now member of the Basic Research Committee of AASLD. In 2016 he received the Emerging Leader Award from European Association for the Study of the Liver (EASL). He served as Scientific Secretary of the International Society for Hepatic Sinusoidal Research (ISHSR) from 2017-2022, being now the President-Elect of this Society.



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Bartłomiej Zapotoczny
(Krakow, Poland)

Title: Tutorial talk: Biology of LSECs

Bartłomiej Zapotoczny is an adjunct at the Institute of Nuclear Physics PAS in Krakow, Poland. He has seven years of experience in the field of liver sinusoidal endothelial cells (LSEC). Bartłomiej specializes in multiparametric cell imaging focusing on LSEC fenestration. He uses atomic force microscopy (AFM) in correlation with other microscopy and nanoscopy modalities. He is the author of an AFM-based methodology allowing for the imaging of fenestration in live LSEC for the first time. He tracks cell dynamics in response to drugs. Bartłomiej has always recognised the interdisciplinary approach to science—biology-educated, PhD in physics; he realized his education in several facilities in Poland, Germany, and Greece. Recently Bartłomiej worked as a Postdoc at UiT, the University of Tromsø. From 2021, he realizes his own scientific project in Krakow. His scientific goals relate to the pharmacological regulation of liver transport in liver pathology. Bartłomiej uses his positive attitude and tireless energy to encourage others to work hard and develop. Bartłomiej is inspired daily by her wife and their two daughters.



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Zheng-Hong Yuan
(Shanghai, China)

Title: LSECs, Kupffer cells and Chronic HBV infection

Director of Key Laboratory of Medical Molecular Virology, MOE, MOH & CAM,
Shanghai Medical College, Fudan University, China

Secretary of the Party Committee of Shanghai Medical College, Fudan University,
Dean of the School of Basic Medicine

Honorary Chairman of Chinese Society of Medical Virology

Vice Chairman of Virology Committee of Chinese Society of Microbiology

Chairman of Shanghai Society of Microbiology

Member of Discipline Evaluation Group of Academic Degree Committee of the State
Council



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Hai-Ming Wei
(Hefei, China)

Title: Dysfunction of NK cells in hepatocellular carcinoma microenvironment

Haiming Wei, PhD, is Professor and Director in Division of Life Sciences and Medicine, University of Science and Technology of China, Hefei, P.R. China. He is mainly engaged in innate immunity research, in particular on Tumor immunity and Reproductive immunity. He has published 226 scientific research articles as corresponding author in Nature Immunology, Immunity, Cell Metabolism, Science Translational Medicine, Nat Commun, and PNAS. He was awarded the Second Class Prize of National Natural Science Award in 2008, and the First prize of Chinese medical science and technology in 2007 and 2010 respectively. In 2016, he was awarded the First Prize of Natural Science of Anhui Province. In 2020, he was awarded the National Advanced Individual in Fighting COVID-19. In 2021, he was awarded the "Wuyang Award" of the National Health Commission, and as the leader of the team, he was awarded "Huang Danian Type Teacher Team of National Universities" awarded by the Ministry of Education.



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Yong-Yin Li
(Guangzhou, China)

Title: Germinal center immune response in chronic HBV infection

Hepatology Unit of Nanfang Hospital, Southern Medical University

PI of State Key Laboratory of Organ Failure Research

Deputy Director of Guangdong Institute of Liver Diseases

Deputy Director of Guangdong Provincial Key Laboratory of Viral Hepatitis

Member of Youth Committee of Hepatology Branch of Chinese Medical Association

Member of Asia Pacific Medical Society of Biological Immunology

She is an expert in the field of viral hepatitis with a specific interest in the immunopathogenesis of HBV infection. her current research is focus on the function of germinal center reaction and humoral immunity in chronic HBV infection. She has hosted 4 projects from National Natural Science Foundation of China, including a project of Excellent Young Scholar, and published over 20 papers on SCI journals as the first author or corresponding author, including Journal of Hepatology, Hepatology, Cellular and Molecular Immunology, and other well-known journals of infectious diseases. She was nominated by the 17th Chinese Young Female Scientist of Chinese Medical Association, and was also awarded the "Early Career Investigator Award" of AASLD in 2018 and 2019.



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Victoria Cogger
(Sydney, Australia)

Victoria completed a BSc (Hons) in 1999 followed by a PhD on the Ultrastructure of the Ageing Liver, graduating from the University of Sydney in 2003. She was awarded a Healthy Ageing Postdoctoral Fellowship and travelled to the National Institutes of Health, Bethesda MD USA to complete postdoctoral studies. Victoria now leads research investigating the biology of ageing; with particular focus on the liver and targeted interventions for treating age related disease using nanomedicines.

Victoria was elected President of the International Society of Hepatic Sinusoidal Research (2017-2019) and will host the Society's International Symposium at the University of Sydney in September 2019. She is an Associate Editor of the Journals of Gerontology: Biological Sciences.

In July 2018, Victoria was appointed Associate Dean (Research Education) in the Faculty of Medicine and Health. This role involves overseeing and developing strategies to assist in the successful research training for over 1000 enrolled Higher Degree Research students within the Faculty.



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Hua Wang

Dr. Hua Wang is currently a professor in the Institute for Liver Disease at Anhui Medical University. He is also Chief, Division of Tumor Immunotherapy in the Department of Oncology, and also Director of biobank of the First Affiliated Hospital of Anhui Medical University.

His main interest is liver injury and repair. He is the author of more than 100 peer-reviewed publications in the highly prestigious journal including Gastroenterology, Hepatology, Journal of Hepatology and PNAS.

In the past several years, he received several grants supporting from national and provincial natural science foundation of China, including Outstanding Youth Fund of National Natural Science Foundation of China



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Amany Zekry
(Sydney, Australia)

Title : Gut microbiota in liver disease

Associate Professor Amany Zekry is a Clinical Academic at St George and Sutherland Clinical School. She is a Gastroenterologist and Hepatologist at St George Hospital. A/Professor Amany Zekry is part of the research team at the Microbiome Research Centre (MRC) She leads a research group investigating the role of the micro biome in the immunopathogenesis of liver disease and liver cancer in obesity. Our research group has published novel data on the role of adipocytokines in mediating liver injury and impairing the immune response. Presently, we are using ex-vivo experiments and animal models of obesity and liver cancer to study the effect of the microbiome on the immune/inflammatory responses. In addition, we are commencing animal and human interventional studies to investigate the effect of manipulating the gut microbiome on the immune/inflammatory responses, and associated liver injury.



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Jun Yu
(Hongkong, China)

Title: Gut microbiome and metabolomics in liver cancer

Assistant Dean, Faculty of Medicine, the Chinese University of Hong Kong
Director, Institute of Digestive Diseases, Chinese University of Hong Kong
Director, State Key Laboratory of Digestive Diseases Research

Vice Chairman, Tumor and Intestinal Microbiome Professional Committee of Chinese Anti-Cancer Association

Vice chairman, the Digestive Committee of Chinese Women Medical Doctor Association

Chang Jiang Scholar Chair Professor, Ministry of Education

Executive Director, Microecology, American Gastroenterological Association (AGA)

Executive Director of Oncology, American Gastroenterological Association (AGA) (2017-2019)



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Title: Mechanisms and regression of cirrhotic portal hypertension



Wei-Fen Xie
(Shanghai, China)

Chief Scientist of Changzheng Hospital, Naval Medical University

Director of Department of Gastroenterology Changzheng Hospital

Editor-in-chief of Chinese Journal of Digestive Diseases

Associate editor of Journal of Digestive Diseases



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Laura Nagy

Laura E. Nagy, PhD, Professor,

Molecular Medicine, Case Western Reserve

University Staff, Inflammation & Immunity,

Gastroenterology/Hepatology Director,

Northern Ohio Alcohol Center/Cleveland Clinic, Cleveland OH USA



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Jin Ding

Director, Clinical Cancer Institute, Center for
Translational Medicine, Naval Medical University,
Shanghai, China

Professor of Liver Cancer Stem Cell and Targeted
Therapy

Visiting Scholar at New York University School of
Medicine, NY, USA



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Hui-Ping Zhou
(VA, USA)

Title: Bile acids and Sphingolipids in cholestatic liver disease

Dr. Huiping Zhou is a tenured Full Professor at the Department of Microbiology and Immunology, Virginia Commonwealth University and a Research Career Scientist at McGuire VA Medical Center of Richmond. Dr. Zhou received her BS and MS from China Pharmaceutical University and Ph.D. from the College of Pharmacy, University of Kentucky. Dr. Zhou's research interest focuses on the study of ER stress, inflammation, bile acids, and sphingosine 1-phosphate signaling in liver diseases, including non-alcoholic fatty liver disease, alcoholic liver diseases, drug-induced liver injury, and biliary diseases. She is a fellow of AGA and AASLD and a member of ASBMB, ASIP, APS, APET, and CALS. She served as the Basic Science Research Committee of AASLD and steering committee of the cholestatic liver disease interest group of AASLD, APS Award Committee, AASLD Foundation research award committee, DDW, and AASLD abstract review committee chair. Dr. Zhou is the past President of the Chinese American Liver Society. She has been continuously funded by NIH, Veteran Merit Review Award and other funding agencies since 2005. She is currently serving as a member of the study section of NIH HBPP, AGA award committee and National VA promotion committee. She serves as an Associate Editor for Cell & Bioscience, editorial board member for Hepatology, World Journal of Gastroenterology, Liver Research, Digestive Liver Disease, etc. She has published more than 160 peer-reviewed papers and review articles in top journals, including Hepatology, Gastroenterology, JBC, Molecular Pharmacology, and AJP-GI, and more than 200 meeting abstracts. She has trained more than 50 graduate students and post-doctoral fellows. Dr. Zhou received the 2018 WISDM (Women in Science, Dentistry & Medicine) Professional Achievement Award from the School of Medicine, Virginia Commonwealth University, the Research Career Scientist Award from the Department of Veterans Affairs in 2019, Distinguished Research Award from the American Physiology Society-GI-Liver section in 2020 and Distinguished Service Award from the Society of Chinese Bioscientists in America, Hepatology Division in 2021.



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Yasuko Iwakiri
(New Haven, USA)

Title: Lymphatics in liver disease

Professor of Medicine

Department of Internal Medicine, Section of Digestive Diseases,
Yale School of Medicine, New Haven, Connecticut, U.S.A.

Yasuko.iwakiri@yale.edu

Dr. Iwakiri conducts basic and translational research in various liver diseases with particular interest in liver fibrosis, portal hypertension and lymphatic vascular systems. She is currently an Associate Editor for Hepatology Communications, has served as an Associate Editor for Hepatology twice and as an editorial board member on major Hepatology journals including Hepatology, Journal of Hepatology, Hepatology International, Liver International, Liver Research and JHEP Reports. She has been serving as members for numerous grant review committees, including NIDDK, NIAAA, DoD and international grant agencies. She has served as a mentor for more than 50 lab members over 16 years since she started her independent career.



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Bernd Schnabl
(San Diego, USA)

Title: Kupffer cells, microbiome and liver diseases

Dr. Schnabl is a trained gastroenterologist and physician-scientist. He received his MD degree from the University Freiburg in Germany. After finishing his residency in internal medicine, he completed a gastroenterology fellowship at Columbia University in New York City. He joined the Division of Gastroenterology at UC San Diego in 2008 and he is currently Professor of Medicine. He is staff physician and attending at the VA San Diego Medical Center in La Jolla and the UCSD Medical Center. He is the Director of the NIH-funded San Diego Digestive Diseases Research Center (SDDRC). His research focus is to understand the complex multi-directional interactions that occur between the gut microbiota and the liver. Dr. Schnabl has published extensively in such highly-regarded journals as Nature, Nature Communications, Cell Host & Microbe, Journal of Clinical Investigation and Proceedings of the National Academy of Sciences and he has authored multiple reviews and book chapters. Dr. Schnabl is the principal investigator of a VA Merit Award, several NIH, foundation and industry-sponsored grants. He serves as Associate Editor for Journal of Hepatology.



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Li-Ying Li
(Beijing, China)

Title: Activated Neutrophils Inhibit Pro-Inflammatory Macrophage Responses Attenuate Liver Injury

Li Liying, professor of the Department of Cell Biology in Capital Medical University, received a bachelor's degree from Peking University in 1986, a master's degree from Peking University in 1995, and a doctorate degree from University of Paris 11, France in 2005. She mainly engaged in the research on the mechanism of liver injury/repair and liver fibrosis, has presided over the National Natural Science Foundation of China key projects, general projects, Beijing Natural Science Foundation, etc., and has published more than 50 SCI papers (40 Correspondence author). Many of these articles have been published in high-impact journals in the field (Hepatology, Journal of Hepatology, Am J Pathol, J Immunol, J Molecular Medicine (Berl)). She was selected as a candidate for the New Century Hundreds and Tens of Thousands of Talents Project in Beijing, and was awarded the title of "Beijing's Higher Education Institutions and Education Deepening Program-Innovative Talents", "Beijing Excellent Doctoral Dissertation Instructor", and was approved by the State Council to enjoy the government Special allowance, as the leader of the innovation team, she presided over the innovation team building and teacher professional development plan projects in Beijing-affiliated colleges and universities.

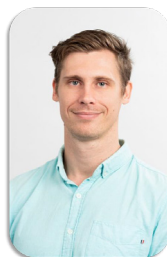


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Nicholas Hunt

Nicholas J. Hunt PhD

Lecturer

Concord Clinical School

Faculty of Medicine and Health

The University of Sydney



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Yong-Zhan Nie

Yongzhan Nie MD, PhD
Professor and Chief Physician
nieyongzhan@qq.com
Department of Gastroenterology, Xijing Hospital; State key Lab of Cancer Biology
Fourth Military Medical University

Research of Interest: (1) Molecular signaling of NAFLD (2) Identification of Cancer Bio-marker. (3) Molecular and Cellular Mechanism of Cancer Metastasis.

Education:

A.B. Fourth Military Medical University, China 1994
Ph.D. Fourth Military Medical University, China 2002
Post-doctoral, Yale University School of Medicine (2002-2005)
Associate Research Scientist, Yale University School of Medicine (2006-2009)
Chief Physician, Professor of Cancer Biology
Vice chief of the Section of Gastroenterology
Vice director of State key Lab of Cancer Biology



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Sergi Guixé
(Barcelona, Spain)

Title: Methods to study liver sinusoidal mechanobiology

Sergi Guixé-Muntet studied Biochemistry and took his master in Translational Medicine at the University of Barcelona.

In 2017, he obtained his PhD in Medicine by the same University under the direction of Prof. Jordi Gracia-Sancho and Prof. Jaime Bosch. During his PhD period, he described mechanisms of hepatic damage and regulation of sinusoidal cells during cirrhosis and ischemia/reperfusion, proposing new therapeutic strategies for the treatment of these hepatic diseases.

In 2017, he moved to Bern (Switzerland) as a postdoctoral researcher in the group of Prof. Jordi Gracia-Sancho and Prof. Annalisa Berzigotti. During this period, he continued his research in vascular liver biology focusing on the study of mechanical forces modulating the phenotype of liver cells.

In 2021 he moved back to Barcelona and started a second Postdoc, following his studies on the vascular biology of liver cells in response to mechanical stimulation.



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Savneet Kaur
(New Delhi, India)

Title: Novel in vitro models to study the liver sinusoid

Dr. Savneet Kaur is an Assistant Professor in the Department of Molecular and Cellular Medicine, Institute of liver and Biliary Sciences (ILBS), New Delhi. The focus and interests of her lab revolve around studying liver vascular biology and pathology and developing novel in vitro model systems of liver. She has a specialization in endothelial cell biology and angiogenesis. Her work has immensely contributed towards understanding the role of endothelial progenitor cell-mediated angiogenesis in liver disease. She has received prestigious awards including 'young investigator award' from the 'American Association for the Study of Liver Disease (AASLD)' and 'International Research Experience award' from 'Science and Engineering Research Board', Govt of India. She has authored more than 50 publications in peer-reviewed indexed journals and 5 book chapters.



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Prakash
Ramachandran
(Edinburgh, UK)

Title: scRNA seq, snRNA seq, spatial transcriptomics

MRC Senior Clinical Fellow and Honorary Consultant Hepatologist
University of Edinburgh Centre for Inflammation Research



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Anabel Fernández-Iglesias

Anabel Fernández got her Bachelor's degree in Biochemistry in 2005 by University Rovira i Virgili, Tarragona, Spain. Next she received the MSc in Nutrition and Metabolism from the same university and she obtained her PhD in Biochemistry-Nutrition & Metabolism in 2013.

Nowadays and since 2015 she is part of the Liver Vascular Biology Research Group led by Dr. Jordi Gracia in at IDIBAPS, Hospital Clinic Barcelona.

Dr Fernández Iglesias' research line focuses on the study of the mechanisms involved in LSECs deregulation during the progression of liver diseases, mainly in advanced liver disease such as cirrhosis to ultimately propose new and potential therapeutic options with the final objective of improving the health and well-being status of patients.



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Xin Zhang

Research Professor

Department of Gastroenterology, Changzheng Hospital,
Naval Medical University; Shanghai, China.

Dr. Zhang's research interest focuses on the study of
chronic liver disease, liver regeneration and liver cancer.

Her research studies have been published in 75 peer-reviewed articles, including Cell Stem Cell, Gut, Hepatology and Cancer Research.



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Sergi Guixé
(Barcelona, Spain)

Sergi Guixé-Muntet studied Biochemistry and took his master in Translational Medicine at the University of Barcelona.

In 2017, he obtained his PhD in Medicine by the same University under the direction of Prof. Jordi Gracia-Sancho and Prof. Jaime Bosh. During his PhD period, he described mechanisms of hepatic damage and regulation of sinusoidal cells during cirrhosis and ischemia/reperfusion, proposing new therapeutic strategies for the treatment of these hepatic diseases.

In 2017, he moved to Bern (Switzerland) as a postdoctoral researcher in the group of Prof. Jordi Gracia-Sancho and Prof. Annalisa Berzigotti. During this period, he continued his research in vascular liver biology focusing on the study of mechanical forces modulating the phenotype of liver cells.

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Wen-Ping Xu

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Abstracts



Lack of Neutrophil Hmgb1 in Liver Transplant Recipients Exacerbates Early Allograft Injury

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Introduction: early allograft dysfunction (EAD) is a severe event leading to graft failure after liver transplant (LT). Extracellular High-mobility group box-1 (HMGB1) is a damage-associated molecular pattern that contributes to hepatic ischemia-reperfusion injury and is released during organ preservation. However, the specific contribution of intracellular HMGB1 to LT graft injury remains elusive.

Aims: to investigate the effect of intracellular HMGB1 from hepatocytes and myeloid cells in the context of LT and elucidate the mechanism involved in EAD.

Methods: to investigate the role of intracellular HMGB1 in LT, we generated mice with conditional ablation of Hmgb1 in hepatocytes and myeloid cells (Hmgb1 Δ Hep Δ Mye) from donors and recipients. We studied the role of HMGB1 in hepatocytes or myeloid cells alone using Hmgb1 knock out mice in hepatocytes (Hmgb1 Δ Hep) as well as knock out and knock in mice in myeloid cells (Hmgb1 Δ Mye and Hmgb1KI Mye). To evaluate early allograft injury, we performed mouse orthotopic LT.

Results: ablation of Hmgb1 from hepatocytes and myeloid cells did not affect liver graft injury during 1, 6 and 16 hours of cold storage. Hmgb1 Δ Hep Δ Mye recipients exhibited aggravated early allograft injury when they received grafts from Hmgb1 Δ Hep Δ Mye donors. Ablation of Hmgb1 from hepatocytes and myeloid cells of liver grafts did not worsen allograft injury. However, recipients lacking Hmgb1 in myeloid cells showed higher activity of serum transaminases, larger necrotic areas and higher expression of inflammatory cytokines in the liver graft 6 h after LT. Absence of Hmgb1 in recipient myeloid cells did not promote immune cell mobilization from the bone marrow and infiltration into liver grafts but increased the production of reactive oxygen species and inflammatory cytokines in the liver grafts, which exacerbated allograft injury. Neutrophils significantly infiltrated into the liver graft after LT and produced more reactive oxygen species and proinflammatory cytokines after an LPS challenge when intracellular Hmgb1 was ablated. Depletion of neutrophils using anti-Ly6G antibody attenuated allograft injury in recipients with ablation of myeloid cell Hmgb1.

Discussion: neutrophil HMGB1 from recipients is central for limiting the production of reactive oxygen species and proinflammatory cytokines and protects from early liver allograft injury.



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Role of Osteopontin in Biliary Atresia

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Introduction: biliary atresia (BA) is a devastating neonatal cholangiopathy that leads to cholestasis and progressive hepatic failure. The poor understanding of the pathogenesis and progression of BA results in compromised transplant-free survival. Osteopontin (OPN), encoded by the SPP1 gene, is highly expressed in biliary epithelial cells (BECs) and is involved in chronic liver disease; however, whether OPN participates in BA is unknown.

Aim: to investigate the role of BEC-derived OPN in the onset and progression of BA.

Methods: the SPP1 gene expression profile in BA patients was analyzed using publicly available datasets of scRNA-seq, RNA-seq and microarrays. OPN protein expression in BA livers was analyzed by immunohistochemistry. To induce BA in mice, we inoculated i.p. rhesus rotavirus (RRV) to BALB/c pups within 24 hours of birth, which resulted in BEC infection and bile duct obstruction. Control mice were injected with saline solution. Symptoms of BA were monitored after RRV inoculation and mice were sacrificed at day 11. The activity of transaminases and the levels of total bilirubin were measured in serum, histopathological changes were assessed and OPN levels in serum, urine and liver were determined.

Results: in humans, SPP1 is predominantly expressed in BECs in healthy individuals and is highly increased in cirrhosis. Notably, SPP1 gene expression is significantly higher in livers from BA patients compared to non-BA cholestatic liver disease or control pediatric liver donors (GSE122340, GSE46960). SPP1 gene expression highly correlates with KRT7, KRT19 and MMP7, all markers of BECs. BA patients with fibrosis exhibit higher SPP1 expression than those with inflammation only (GSE15235). Mice with BA show delayed development, jaundice and acholic stools. Moreover, they have increased activity of transaminases and levels of total bilirubin. The H&E staining reveals massive ductular reaction and hepatic inflammation. The concentration of OPN in serum and urine and the hepatic expression of OPN are significantly increased in mice with BA compared to controls.

Discussion: OPN expression is significantly increased in patients and mice with BA and correlates with BEC markers. To dissect if OPN participates in the pathogenesis and progression of BA, our laboratory has generated a mouse model with ablation or overexpression of Spp1 in BECs. These mice will be inoculated with RRV to elucidate the role of BEC-derived OPN in BA progression.



Saturated fatty acid-enriched extracellular vesicles promote a negative crosstalk involved in liver inflammation and hepatocyte insulin resistance

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Introduction and aims: Lipotoxicity triggers non-alcoholic fatty liver disease (NAFLD) progression due to the accumulation of toxic lipids species in hepatocytes including saturated free fatty acids (SFAs), which activate pro-inflammatory pathways. This study investigates the impact of hepatocyte-or circulating-derived extracellular vesicles (EVs) secreted during NAFLD on liver inflammation and insulin signaling.

Methods: Primary hepatocyte-derived EVs (PH-EV) from mice under lipotoxic stress were characterized and analyzed by lipidomics. PH-EV were added to mouse macrophages/Kupffer cells (KCs) to monitor internalization and pro-inflammatory effects. Conditioned medium (CM) from PH-EV-loaded macrophages was used to analyze hepatocyte insulin signaling. In vivo injections of PH-EV were conducted to study biodistribution, liver inflammation and hepatocyte insulin signaling. The crosstalk macrophages-hepatocytes was also evaluated using circulating EVs from mice (Circ-EV) and humans (h-EV) with NAFLD.

Results: The release of PH-EV, Circ-EV and h-EV was increased under NAFLD conditions. Lipotoxic PH-EV were internalized by macrophages through the endosomal pathway and triggered



pro-inflammatory responses. These effects were ameliorated by TLR4 inhibition. Moreover, CM from lipotoxic PH-EV-loaded macrophages impaired insulin signaling in hepatocytes. Both lipotoxic PH-EV and the recipient macrophages were enriched in SFAs, such as palmitic acid (C16:0) and stearic acid (C18:0), both TLR4 activators. In vivo injection of PH-EV identified KCs as early targets of lipotoxic PH-EV leading to increased JNK phosphorylation, NF- κ B nuclear translocation and pro-inflammatory cytokine expression; these effects being attenuated by TLR4 inhibition. Macrophage inflammation and subsequent hepatocyte insulin resistance was also induced by circulating EVs from mice and humans with NAFLD.

Conclusion: We identified a novel hepatocyte-macrophage/KC-hepatocyte interactome during NAFLD where EVs are SFAs transporters which target macrophages/KC triggering TLR4-mediated inflammatory response enough to induce insulin resistance in hepatocytes.

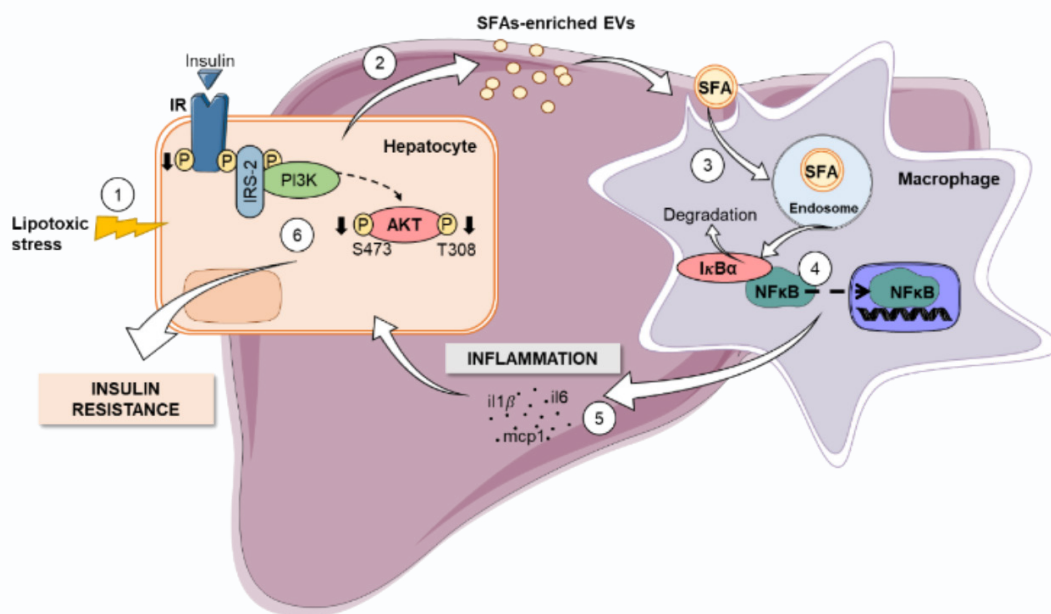


Figure 1. Graphical abstract



Ablation of Spp1 in hepatocytes protects non-alcoholic steatohepatitis

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Introduction: Osteopontin (OPN, encoded by the SPP1 gene) is an extracellular matrix protein involved in chronic liver disease. We have previously shown that increasing the expression of OPN in hepatocytes enhances CCl₄-induced fibrosis but worsens alcohol-associated liver injury in mice.

Aims: In this study our aim was to determine the role of hepatocyte-derived OPN in the development of NASH.

Methods: we analyzed three publicly available datasets of RNA-seq and scRNA-seq from patients with NASH. We generated conditional Spp1 knock-in and knock-out mice in hepatocytes (Spp1 Δ Hep and Spp1KI Hep) and fed them a control or NASH-inducing diet for 6 months.

Results and Discussion: we identified positive correlation of 73 genes and negative correlation of 7 genes with SPP1 in human NASH. ScRNA-seq data revealed that 89% of the genes with positive correlation were expressed in non-parenchymal cells (mainly fibroblasts, stellate cells and biliary epithelial cells). In contrast, 86% the genes with negative correlation were expressed by hepatocytes and are known to be hepatoprotective (HAAO, ACOX2 and AGXT2). Both genders of Spp1 Δ Hep mice fed a NASH-inducing diet showed lower NAFLD activity score, liver-to-body ratio, liver triglycerides (TGs), cholesterol (CHO), serum ALT, total bilirubin and chicken-wire fibrosis compared to control mice. In addition, qPCR analysis revealed that they had a decrease in the mRNA expression of Col1a1, Col1a2 and Pdgfa in the liver. Compared to Spp1 Δ Hep mice, Spp1KI Hep mice fed a NASH-inducing diet also showed reduction in liver TGs and CHO but the changes in liver-to-body ratio and the NAFLD activity score were minor due to increased inflammation, hepatocyte ballooning degeneration, chicken wire fibrosis, and collagen type I expression. In sum, ablation of hepatocyte-derived Spp1 protects from NASH, opening the possibility of a new therapeutic intervention to treat NASH.



Myeloid cell-derived HMGB1 protects from the development of hepatocellular carcinoma

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Background: HMGB1 is a non-histone chromatin-associated protein involved in the pathogenesis of chronic liver disease. HMGB1 is expressed in myeloid cells, including conventional dendritic cells (cDC) and tumor-associated macrophages (TAMs), which play a major role in the tumor microenvironment. However, whether intracellular myeloid cell-derived HMGB1 is involved in HCC is unknown. Our hypothesis is that intracellular HMGB1 drives cDC maturation towards LAMP3+ DCs and decreases immunosuppressive TAMs in the tumor; hence, allowing effective cytotoxic CD8+ T cell responses to reduce HCC. **Methods:** we analyzed publicly available scRNA-seq datasets from human HCC for the expression of HMGB1 in all subsets of DCs and TAMs in HCC tumor and non-tumor tissue and in hepatic draining lymph nodes (dLNs). We generated mice with conditional ablation or overexpression of Hmgb1 in myeloid cells (Hmgb1ΔMye and Hmgb1KI Mye). To induce HCC, 14-days-old male mice were injected i.p. with diethylnitrosamine (DEN) and were sacrificed at 5, 6, 8 and 10 months. **Results:** mature LAMP3+ DCs increase in human and mouse HCC tumor tissue and hepatic dLNs. TAMs, with low expression of HMGB1, are proangiogenic and immunosuppressive and increase in human and mouse HCC tumor tissue. Hmgb1KI Mye mice are fully protected from HCC, whereas control mice develop HCC after 8 months and Hmgb1ΔMye mice start developing HCC at 5 months. Macroscopic analysis and H&E staining of the livers from Hmgb1ΔMye mice shows more tumors and higher tumor volume compared to control and Hmgb1KI Mye mice. Immunohistochemistry of HCC tumor sections reveals that Hmgb1ΔMye mice have increased infiltration of TAMs. Analysis of immune cell populations by flow cytometry shows that Hmgb1ΔMye mice have less mature LAMP3+ DCs in liver and hepatic dLNs compared to control and Hmgb1KI Mye mice, suggesting less CD8+ T cell activation. In addition, there is enhanced CD8+ T cell apoptosis in the HCC tumor tissue from Hmgb1ΔMye mice. **Conclusion:** ablation of myeloid derived HMGB1 accelerates HCC development in mice. Therefore, increasing HMGB1 expression in specific myeloid cell subsets (cDCs and TAMs) could be a therapeutic approach to protect from HCC.



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Identification of a matrisome signature of liver fibrosis progression and resolution

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Background & Aims: excessive deposition and crosslinking of extracellular matrix proteins increases liver stiffness and the risk of developing fibrosis. The composition of the pathological extracellular matrix is etiology specific. Unfortunately, there are no approved anti-fibrotic therapies to prevent fibrosis progression or promote resolution. Our aim was to unveil the matrisome genes during liver fibrosis progression and resolution. **Methods:** using the database from the in-silico matrisome project, we analyzed the matrisome genes in publicly available datasets of transcriptomics from patients with liver fibrosis. We performed RNA-seq of 54 liver biopsies from 28 patients obtained from our prospective HBV-related fibrosis/cirrhosis cohort studies (NCT01938781 and NCT01938820) that were treatment naive or on antiviral therapy for 78 and/or 260 weeks. The dysregulation pattern and cellular landscape of the liver fibrosis matrisome genes were further explored in the CCl₄ mouse model of liver fibrosis progression and regression by RNA-seq and accessing publicly available scRNA-seq datasets. **Results:** we identified 35 liver fibrosis matrisome genes that were independent of etiology, which could be used as a combined signature to identify the risk of developing liver fibrosis. Histological analysis proved that the expression of the liver fibrosis matrisome genes increased with disease severity and decreased during fibrosis regression both in humans and mice. The decrease in the liver fibrosis matrisome genes during fibrosis regression was not etiology specific. In addition, we revealed the cellular landscape of the dysregulated liver fibrosis matrisome genes during fibrosis progression and resolution in mice. The liver fibrosis matrisome genes sub-classified liver fibrosis patients with distinct clinical characteristics. Subgroups of liver fibrosis patients were identified based on the liver fibrosis matrisome gene signature and exhibited different cellular and molecular hallmarks. **Conclusions:** we provide a characterization of the genetic landscape and sub-classification of liver fibrosis patients from the point of view of the liver fibrosis matrisome genes to help develop targeted therapeutics.

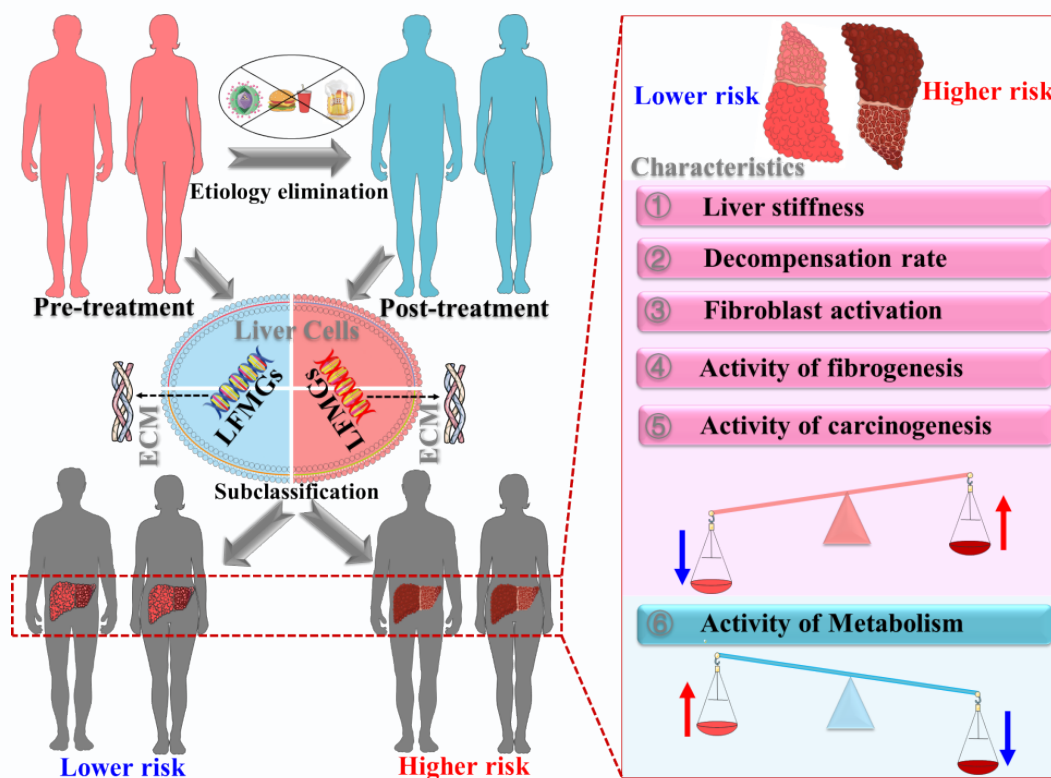


Figure. Liver fibrosis-specific matrisome genes clearly separated patients pre- or post-etiological treatment. Higher risk subgroups showed more aggravated liver stiffness, decompensation rate, fibroblast activation, fibrogenesis and carcinogenesis signaling activation and reduced metabolic activity, in contrast to lower risk subgroups.



INTESTINAL OSTEOPONTIN PROTECTS FROM ALCOHOL-INDUCED LIVER INJURY BY PRESERVING GUT MICROBIOME AND INTESTINAL BARRIER

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Introduction: the gut-liver axis plays a key role in the pathogenesis of alcohol-associated liver disease (ALD). We demonstrated that *Opn*^{-/-} mice develop worse ALD than WT mice; however, the role of intestinal Osteopontin (OPN) in ALD remains unknown. We hypothesized that overexpression of OPN in intestinal epithelial cells (IECs) could ameliorate ALD by preserving the gut microbiome and the intestinal barrier function.

im: to investigate the role of IEC-derived OPN in the development and progression of ALD.

Methods: IEC-specific knock in (*Opn*KI IEC) and knock out (*Opn*ΔIEC) mice were generated and fed control or ethanol Lieber-DeCarli diet for 6 wk to provoke alcohol-induced liver injury. Fecal microbiome transplant (FMT) from *Opn*KI IEC to WT and vice-versa were performed and then mice were fed control or ethanol Lieber-DeCarli diet for 6 wk. WT mice were given oral administration of milk OPN with control or ethanol Lieber-DeCarli diet for 6 wk. Liver injury, intestinal permeability and fecal microbiota were analyzed. Expression of intestinal antimicrobial peptides (AMPs), aryl hydrocarbon receptor (Ahr) and tight-junction (TJ) protein were measured in IECs from jejunum. Portal serum tryptophan (Trp) metabolites and short-chain fatty acids (SCFAs) were measured.

Results: *Opn*KI IEC but not *Opn*ΔIEC mice showed improved intestinal barrier function and protection from ALD. There were less pathogenic bacteria and more beneficial bacteria in ethanol-fed *Opn*KI IEC than in WT mice. Fecal microbiome transplant (FMT) from *Opn*IEC KI to WT mice protected from ALD. FMT from ethanol-fed WT to *Opn*KI IEC mice did not causes ALD. AMPs, Il33, pSTAT3, Ahr and TJ expression were higher in IECs from jejunum of ethanol-fed *Opn*KI IEC than in WT mice. Ethanol-fed *Opn*KI IEC showed more tryptophan (Trp) metabolites and short-chain fatty acids (SCFAs) in portal serum than WT mice. FMT from *Opn*KI IEC to WT mice enhanced IECs Ahr and TJ protein expression. Oral administration of milk OPN (mOPN) replicated the protective effect of *Opn*KI IEC mice in ALD.



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Conclusion: overexpression of OPN in IECs or administration of mOPN maintain the intestinal microbiome by intestinal AMPs. The increase in Trp metabolites and SCFAs signaling through the Ahr in IECs, preserve the intestinal barrier function and protect from ALD.



ABLATION OF OSTEOPONTIN IN HEPATOCYTES AMELIORATES ALCOHOLIC LIVER DISEASE BY PROMOTING FATTY ACID DEGRADATION IN THE LIVER

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Introduction: we previously showed that Opn^{-/-} develop more alcohol-induced liver injury than WT mice. Alcohol drinking induces the expression of osteopontin (OPN) in hepatocytes; however, whether it plays a protective role in alcoholic-associated liver disease (ALD) is unknown. We hypothesized that hepatocyte-derived OPN could protect from ALD by reducing steatosis.

Aim: to investigate the role of hepatocyte-derived OPN in alcohol-induced liver injury.

Methods: we analyzed publicly available sequencing datasets (GSE167308, GSE142530, GSE28619 and GSE155907) from healthy controls (HC) or patients with alcoholic hepatitis (AH) for the expression of OPN mRNA in the liver. In addition, we analyzed the expression of Opn mRNA in wild-type (WT) mice fed the Leiber-DeCarli control or ethanol diet for 6 weeks. Then, we determined the correlation between OPN and significantly dysregulated genes in AH compared to HC. We performed gene set enrichment analysis (GSEA) of the genes with significant correlation to identify pathways up- or down-regulated. We validated the main findings in hepatocyte-specific Opn knockout (Opn Δ Hep) mice fed the Leiber-DeCarli control or ethanol diet for 6 weeks.

Results: alcohol consumption up-regulated the expression of OPN in human and mouse liver. OPN showed positive correlation with 1,070 (GSE167308), 1,056 (GSE142530), 1,874 (GSE28619) and 1,559 (GSE155907) genes and negative correlation with 1,503 (GSE167308), 376 (GSE142530), 1,042 (GSE28619) and 405 (GSE155907) genes. GSEA revealed enrichment in the fatty acid degradation (FAD) pathway (enrichment score \geq 60%, FDR<0.000). The genes linked to FAD were significantly downregulated in AH compared to HC. Of these, 18 FAD genes present in the four datasets showed negative correlation with OPN. Ethanol-fed Opn Δ Hep were protected from ALD and had significant up-regulation of the 18 FAD genes compared to WT mice. Serum free fatty acids (FFAs) were significantly higher in ethanol-fed mice compared to controls. Hepatic



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FFAs were significantly lower in ethanol-fed Opn Δ Hep compared to WT mice. The expression of fatty acid transporters was high in livers from ethanol-fed WT and Opn Δ Hep mice. Adipocytes were smaller and the expression of genes linked with lipolysis was high in adipose tissue from ethanol-fed mice compared to controls.

Conclusion: alcohol drinking induces the expression of OPN in hepatocytes, which results in impaired FAD and contributes to ALD.



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Background: Hepatocellular carcinoma (HCC), the third leading cause of mortality attributed to cancer, arises in the context of liver fibrosis (LF). Although HCC is generally poorly fibrogenic, some patients harbor focal intratumor deposits of extracellular matrix (ECM) proteins (a.k.a. fibrous nest). ECM remodeling can be studied using proteomics, referred to as matrisome analysis. To date, the matrisome of HCC and LF has not been fully characterized.

Methods: We performed quantitative matrisome analysis by tandem mass tag mass spectrometry in 40 human HCCs and matched non-tumor (NT) samples with high-grade or low-grade intratumor fibrosis. We validated the results by matrisome analysis of 12 mouse samples with LF or HCC as well as by transcriptomics of publicly available data of HCC and/or matched NT liver (n=2,285 patients).

Results: 94 ECM proteins were showing different abundance between high-grade and low-grade intratumor fibrosis in HCCs (74 up, 20 down), including fibrillar and basement membrane collagens, fibrillins, laminins, Elastin, proteoglycans and Lysyl oxydase. Analysis of the whole proteomics identified a metabolic switch in high-grade fibrosis, with more glycolysis and less oxidative phosphorylation. By transcriptomics, HCCs patients expressing a fibrous nest phenotype were associated with worst outcome and with the transcriptomic program of the Wnt/TGF β (S1) subclass, while patients expressing ECM genes highly expressed in LF were associated with better outcome and with non-proliferative transcriptomic subclasses. By overlapping proteomics and transcriptomics, we isolated a list of 27 fibrous nest proteins highly expressed both at the mRNA and protein levels in HCCs with high-grade fibrosis.

Conclusion: the matrisome analysis of HCC and LF highlighted a cancer-specific ECM remodeling in patients expressing high-grade intratumor fibrosis, typical of a subtype of proliferative tumors matching the Wnt/TGF β subclass and associated with poor outcome. Hence, the histological analysis of intratumor fibrosis is of clinical interest.



Increased sinusoidal pressure impairs liver endothelial mechanosensing, uncovering novel biomarkers of portal hypertension

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Introduction: Portal hypertension (PH) is the most frequent and severe clinical syndrome associated to chronic liver disease (CLD) and is defined by a pathological increase in the hepatic venous pressure gradient (HVPG). Considering that endothelial cells can sense changes in hydrostatic pressure and shear stress, we hypothesized that PH could not only be a consequence of liver sinusoidal endothelial cells (LSEC) alteration during CLD, but also a key factor in the deregulation of their phenotype and secretome.

Aims: The aim of this study was to investigate the effects of pathological hydrodynamic pressure on LSECs and to identify endothelial-derived biomarkers of PH.

Methods: Primary LSECs were cultured under normal or increased hydrodynamic pressure within a pathophysiological range (1 vs 12 mmHg) using a microfluidic liver-on-a-chip device. RNAseq was used to identify pressure-sensitive genes, which were validated in liver biopsies from two independent cohorts of CLD patients with PH (n=19; n=40) vs subjects without PH (n=12; n=11). Biomarker discovery was performed in plasma from a third independent cohort of 64 patients (46 with PH vs 18 w/o).

Results: Pathological hydrodynamic pressure was deleterious to LSECs, as evidenced by profound alterations in important transcriptomic pathways including regulation of eNOS and antioxidant capacity. Chromobox 7 (CBX7) was identified as a key transcription factor diminished by pressure. CBX7 downregulation was validated in three pre-clinical models of CLD and, importantly, in patients with PH, where it negatively correlated with HVPG. MicroRNA 181a was identified as a



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pressure-induced upstream downregulator of CBX7, showing an increase in CBX7 when inhibited in vitro. Two transcriptional targets of CBX7, ECAD and SPINK1, were increased in the plasma of patients with PH, and their combination was highly predictive of PH and clinically significant PH (CSPH), with a sensitivity of 91.3% and 91.4%, respectively.

Discussion: We describe the detrimental effects of increased hydrodynamic pressure on the sinusoidal endothelium phenotype. CBX7 is identified as a pressure-sensitive transcription factor, which is diminished in preclinical models of CLD and in human biopsies of cirrhotic patients with PH, and showed its regulation by miR181a. Finally, we propose that the combination of ECAD and SPINK1, reported products of CBX7, could be used as non-invasive biomarkers of PH and CSPH in ACLD patients.



Preclinical statin treatment improves liver function and reduces severity of advanced liver injury via the modulation of cell death

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Background and Aims: Statins are often used for the treatment of patients with advanced liver fibrosis. However, the action mechanism of statins in liver cells remains unclear. We hypothesized that statins (eg: Simvastatin, Atorvastatin) can modulate how cell death thereby protecting against liver dysfunction and advanced liver fibrosis. **Methods:** Biopsy of twelve obese undergoing bariatric surgery patients with NAFLD activity score (NAS) score between 0 to 3 were analyzed by Western blot. Furthermore, a microarray analysis of chronic liver failure (ACLF) and healthy patients was analyzed. Wistar and Sprague Dawley rats were subjected to liver fibrosis using carbon tetrachloride (CCl₄) and treated with Simvastatin and Atorvastatin, respectively. Upon sacrifice, livers were analyzed by qRT-PCR, Western blot and IF. Human hepatocyte cell lines (HepG2) were treated with CCl₄ and/or TNF/D-GalN in presence or absence of statins, and functional assays were performed. **Results:** In human samples, cell death markers were overexpressed in patients with higher NAS score and ACLF. In rodents with advanced liver fibrosis, statins decreased CCl₄-induced inflammation, fibrotic markers and cell death. Moreover, the high expression of cleaved caspase 3 (CC3) and caspase 8 (CC8) decreased after statins treatment. Furthermore, statin treatment protected against acute (CCl₄/TNF)-induced cell death in hepatic cell lines. **Conclusions:** Statin treatment of rodent models of advanced liver fibrosis and human cell lines, lowered systemic inflammation and improved liver function, reducing disease severity. Specifically, we unveil a novel mechanism by which statins protect against cell death.

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Alcohol binge exacerbates liver damage in a novel cholestatic model of acute-on-chronic liver failure

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Introduction & Aims: Acute-on-chronic liver failure (ACLF) is a clinical condition defined by systemic inflammation, organ failure and high short-term mortality. ACLF can arise in patients with advanced chronic liver disease undergoing an acute decompensation leading to severe liver dysfunction. Alcohol abuse is reported as one of the most frequent precipitating factors in ACLF; nevertheless, preclinical models mimicking this understudied clinical setting are scarce. The aim of this study was to develop an alcohol-induced ACLF model to further dissect molecular mechanisms underlying this syndrome.

Methods: Liver fibrosis was induced in 10-12 weeks old male C57BL/6 mice by common bile duct ligation (BDL) for 28 days (n=5 per group). Alcohol binge (5g/Kg) was given to a subgroup of animals to induce ACLF. Sham surgery groups receiving either alcohol or water binge were used as controls and tissue collection was performed 9 hours after binge.

Results: BDL-induced liver fibrosis was indicated by increased α -SMA levels and Sirius red staining. Hepatocellular damage was increased in BDL mice and further aggravated in ACLF group as shown by higher ALT (p=0.0002) and decreased hepatic albumin expression (p=0.035) compared to BDL. Alcohol binge in ACLF mice resulted in increased blood ammonia (p=0.0003) and blood urea nitrogen (p<0.0001) suggesting kidney dysfunction. Circulating neutrophil (p=0.001) and monocyte (p=0.008) numbers increased in ACLF mice compared to BDL mice. Analysis of liver sinusoidal endothelial cell markers by qPCR revealed a further capillarized [increased CD34 (p=0.009) & vWF (p=0.04)] and activated [increased VCAM1 (p=0.03), ICAM1 (p=0.09) & CXCL2 (p=0.04)] phenotype in ACLF. Furthermore, alcohol binge induced CD68+ macrophages infiltration in the liver (p=0.045) and neutrophil activation as shown by increased liver citrullinated H3 histone (p=0.0003). BDL surgery triggered inflammasome activation and induction of pyroptosis shown as an increase in cleaved Gasdermin D (p<0.0001). IL-18 in the serum was further increased (p=0.02) in the ACLF mice when compared to BDL.

Discussion: Our results suggest that alcohol binge in advanced liver disease induced by BDL shows characteristics of ACLF including acute liver failure, kidney injury and systemic inflammation. Compared to BDL-fibrotic livers, alcohol-induced ACLF further induces endothelial dysfunction, immune cell recruitment, infiltration and liver damage through inflammasome activation.



GTX-011 improves portal hypertension, liver fibrosis and endothelial function in a rat model of Non-Alcoholic Steatohepatitis

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Introduction. Non-alcoholic steatohepatitis (NASH) is a hepatic metabolic disease that can lead to cirrhosis and the need for liver transplantation in more advanced stages. Without a current treatment for NASH, its prevalence continues to rise, thus intensifying the need to identify an effective treatment. GTX-011 is a first-in-class drug with a novel mechanism of action: a non-steroidal allosteric modulator of nuclear receptor subfamily 3C (NR3C) and has previously shown anti-fibrotic properties.

Aims. Considering that liver fibrosis and portal hypertension are the strongest prognostic markers in advanced NASH, the present study aimed at evaluating GTX-011 effects on hepatic hemodynamics and fibrosis, as well as its underlying mechanisms, in a pre-clinical model of NASH.

Methods. Male Wistar rats with NASH, induced by a 10-week protocol that combines high-fat high-cholesterol diet with CCl₄ and phenobarbital, were randomly assigned to receive two different concentrations of GTX-011 (1 and 10 mg/kg/day p.o.) or vehicle, for 14 days (n=15/group). In vivo systemic and hepatic hemodynamic parameters (mean arterial pressure; portal pressure, PP; portal blood flow, PBF) were assessed and a transcriptomic analysis of liver tissue was performed. Hepatic fibrosis (Sirius red staining) and liver sinusoidal endothelial cell (LSEC) phenotype (p-eNOS/eNOS; von Willebrand Factor, vWF; fenestrae frequency and porosity) were analysed in GTX-011 10 mg/kg and vehicle-treated rats. Immortalized human-activated hepatic stellate cells (HSC) LX-2 and primary HSC from male Wistar rats with CCl₄-induced cirrhosis were cultured to evaluate their phenotype (α -SMA, Col1 α 1 and PDGFR β) after treatment with either GTX-011 (0.1 μ M, 1 μ M and 10 μ M) or vehicle for 24 and 72 hours.

Results. NASH rats receiving GTX-011 showed significantly lower PP compared with vehicle-treated animals, in a dose-dependent manner (-8.4% p=0.05 and -11.7% p=0.005 for 1 and 10 mg/kg respectively) without changes in PBF or in their systemic hemodynamics. These results, together with bulk RNA sequencing, showed a greater response and higher target engagement of GTX-011 10mg/kg than 1mg/kg. For these reasons, we further explored the effects of GTX-011 10 mg/kg. Transcriptomic analysis manifested HSC de-activation (α -sma, Desmin and Tnf) and increased expression of pro-inflammatory markers (IL18, IL7, IL6, Cd40, Cd14, Tgf- β 1, Trem2 and Nos2) as well as a significant increase in genes regulating the lipid metabolism (Ppara



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and Sirt1) in response to GTX-011. Furthermore, they exhibited hepatic fibrosis regression (-28.1% $p=0.009$), suggesting that the observed hemodynamic effects might respond to a reduced intrahepatic vascular resistance in GTX-treated rats. Interestingly, primary rat HSC and LX-2 cells treated in vitro with GTX-011 showed a de-activation associated with a significant reduction in pro-fibrogenic markers after 24 and 72 hours of treatment, suggesting a direct effect of GTX-011 on HSC phenotype. Moreover, LSEC phenotype was enhanced (kdr, Stab1, Stab2 and Mrc1) and adhesion molecules (E-Selectin, Icam1 and Vcam1) and vasoconstrictor molecules (Ptgs2) showed a significant decrease in response to GTX-011 by transcriptomics data. Molecular and histological analysis revealed that LSEC from animals receiving GTX-011 showed an improved phenotype as evidenced by a significant increase in p-eNOS/eNOS ratio (+80% $p=0.009$), a reduction in vWF expression (-32% $p=0.050$) and a trend toward reversing their capillarization, as suggested by a marked increment in both fenestrae frequency and porosity (+32% $p=0.056$ and +33% $p=0.063$ respectively). Altogether, these results suggest that the beneficial effects derived from GTX-011 might also rely on endothelial phenotype restoration.

Discussion. This study shows for the first time the beneficial effects of GTX-011 in portal hypertension and liver fibrosis in pre-clinical NASH, possibly mediated by HSC de-activation and endothelial phenotype restoration. These results encourage its clinical evaluation as a possible new treatment for this disease.



Non-heat-stressed method to isolate hepatic stellate cells from highly steatotic tumor-bearing liver using CD49a

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Introduction: Obesity is becoming prevalent worldwide, and the number of cases of obesity-associated cancers, including obesity-associated liver cancer, has recently increased. We previously showed that hepatic stellate cells (HSCs) play key roles in tumor microenvironment (TME) of obesity-associated hepatocellular carcinoma (HCC) by secreting senescence-associated secretory factors that suppress anti-tumor immunity. Therefore, development of a refined method to characterize HSCs in TME under various conditions is necessary to understand their roles in HCC progression. However, the efficient isolation method of HSCs from tumor tissues in highly steatotic liver in less heat-stressed condition have not been established yet.

Aims: Here, we aimed to develop a method to isolate HSCs at a low temperature (6°C) to minimize over digestion by enzymes and heat-associated stress after dissociation.

Methods: The primary HSCs were isolated through cannulation via portal-vein (PV) and digestion using ice-cold enzymatic solution, and were concentrated using Nycodenz gradient medium. Subsequent experiments including exclusion of hepatocytes and erythrocytes, flow cytometry and cell sorting were performed. The CD31-/CD45- cells, excluding LSECs and immune cells, were sorted and performed single-cell RNA-sequencing (scRNA-seq) analysis to screen HSC-specific cell surface markers. The HSCs from murine and human tumor tissues were analyzed by cell culture analysis, flow cytometry, bulk RNA-sequencing, and scRNA-seq.

Results and Discussion: In conclusion, we developed a method to isolate HSCs with high yield and high purity from highly steatotic liver and steatohepatic HCC tissues. The PV-mediated enzymatic cold perfusion method can minimize the artifact gene expression induced by the heat-stress response in enzyme digestion at 37°C. Furthermore, we identified CD49a as a reliable HSC marker under various conditions in this procedure. These results lay a foundation for investigating the role of HSCs in liver under both normal and steatotic HCC conditions.



Precise regulation of fenestration diameter in liver sinusoidal endothelial cells

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Introduction: Liver sinusoidal endothelial cells (LSECs) facilitate the efficient transport of macromolecules and solutes between blood and hepatocytes. The efficiency of this transport is realized via transcellular pores, called fenestrations. The size and number of fenestrations respond to changes in the environment and can be altered pharmacologically in vitro. Reports of multiple agents tested on the fenestration morphology of LSEC suggest that the phosphorylation of the myosin light chain (MLC) can be the core of a cellular mechanism used for the control of LSEC porosity. Adverse changes to fenestration-mediated transport are associated with chronic liver diseases such as hepatitis, steatosis, and cirrhosis as well as aging. Understanding the mechanisms responsible for the opening of new fenestrations and controlling their size could be used to design treatments for these conditions.

Aims: In this study, we investigated both ROCK and MLCK-dependent phosphorylation of MLC using a combination of several cellular inhibitors. We verify whether ROCK or MLCK pathways are involved in the regulation of fenestration diameter. We investigated colocalization of sieve plates and fenestration with monophosphorylated (pMLC) and diphosphorylated (ppMLC).

Methods: To quantify the morphology of LSEC we used modern high throughput scanning electron microscopy (SEM), a well-established microscopic technique in LSEC morphology research. We also used data acquired with structured illumination microscopy (SIM), a method with resolution better than those imposed by the diffraction limit of light that allowed us to colocalize pMLC and ppMLC to sieve plates and fenestrations. The selected methodology and data analysis methods were carefully selected based on our recent reports. We supported our data with the assessment of LSEC functions, by performing the following: a viability test, a mitochondrial activity test, and an assessment of endocytic activity.

Results and Discussion: We analysed the effects elicited by various inhibitors on LSEC, describing parameters such as porosity (the fraction of cell area covered by fenestrations), fenestration size (distribution of fenestration diameter), the distribution and shape of long fibres and actin mesh, the level and localisation of pMLC and ppMLC myosin regulatory light chain. To better understand the correlation between pMLC and actin cytoskeleton, we used blebbistatin to prevent the contraction of activated myosin. Moreover, to connect the role of MLC with actin cytoskeleton we



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used an actin depolymerising agent – cytochalasin B, alongside the inhibitors. We conclude that the regulation of pMLC/ppMLC is divergent at ROCK and MLCK and controls not only the size but also the number of open fenestrations in LSECs. We demonstrated precise, dose-dependent, and reversible regulation of the mean fenestration diameter within a wide range from 120 nm to 220 nm. Moreover, our findings indicate that MLCK is involved in the formation of new fenestrations. Closed fenestrations, after blocking MLCK, cannot re-open. We therefore conclude that the Rho-ROCK pathway is responsible for the control of fenestration diameter, while inhibition of MLCK prevents the formation of new fenestrations.

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Hepatocyte derived microRNAs embedded in extracellular vesicles negatively impact on the liver sinusoidal endothelium in chronic liver disease

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Introduction: Liver cellular components work in synergy through paracrine mechanisms to maintain liver homeostasis. Cells' secretome, mainly composed by extracellular vesicles (EVs) and soluble factors, may influence neighbouring cells through active communication via microRNAs (miRNAs).

Aims: The aim of this study was to investigate the role of hepatocyte derived miRNAs embedded in EVs modulating endothelial de-differentiation in chronic liver disease (CLD).

Methods: Small EVs were purified from hepatocytes supernatant (hepEVs) isolated from healthy (CT) and cirrhotic (CH) human and rat liver tissues for subsequent experiments. In vivo experiments: Healthy rats received either hepEVs-CTrat, hepEVs-CHrat (200µg/day for 3 days, i.v.) or vehicle (PBS) (n=10 per group). hepEVs biodistribution was evaluated using PKH26 fluorescent labelled hepEVs and visualizing them using the In Vivo Imaging Software (IVIS). Specific sinusoidal uptake was confirmed through VE-Cadherin co-localization in liver slices. Effects of hepEVs on LSECs phenotype upon 72h of treatment was analyzed through von Willebrand factor (vWF) expression by immunofluorescence. In vitro experiments: human hepEVs miRNAs profile was characterized using microarray cards and those significantly dysregulated were validated in rat hepEVs by qPCR (n=3-9). Finally, primary LSECs isolated from healthy rats were transfected for 48h with miR-A or miR-B mimic or their corresponding negative control. The RNA extracted was sequenced using Illumina platform HiSeq250 and the transcriptomic data were analysed using Ingenuity Pathway Analysis (IPA) software (n=5).

Results: Upon in vivo administration, liver tissue was significantly enriched in EVs and this was associated with increased expression of the endothelial dysfunction marker vWF in rats treated with hepEVs-CH whereas in those who received hepEVs-CT treatment no changes were observed. Analysis of miRNAs embedded in human hepEVs revealed twenty-two miRNAs significantly dysregulated in hepEVs-CH in comparison to hepEVs-CT. From those, miR-A and miR-B were validated in rat hepEVs-CH and, interestingly, correlated with a marked down-regulation in the expression of their target genes in primary LSECs isolated from CCl₄-induced cirrhotic rats. Transcriptome analysis of LSECs transfected with miR-A or miR-B revealed significant changes in



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70 and 171 genes, respectively, compared with the corresponding control. miR-B transfected cells shared 70% of their transcriptome with that from primary cirrhotic LSECs, thus supporting its role in LSECs de-differentiation. Indeed, IPA confirmed the detrimental effects of miR-B on LSECs phenotype, promoting dysregulation in key molecular processes such as NF- κ B signalling, among others.

Discussion: Our study suggests that EVs released by hepatocytes actively contribute to endothelial de-differentiation in CLD. Changes in the content of the hepEVs during the progression of the disease, such as miR-A and miR-B up-regulation, reveals their key pathobiological role in this paracrine process. Specifically, miR-B shows an important role in processes related with liver pathobiology in CLD.



Soluble immune checkpoint protein CD27 is a novel prognostic biomarker of HCC development in HCV-SVR patients

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Background: The development of hepatocellular carcinoma (HCC) even after the sustained virological response (SVR) following anti-HCV therapy is still being investigated. Immune checkpoint proteins (ICPs) are being studied extensively in various cancers such as melanoma, non-small lung cancer, and renal cell cancer, meanwhile, the understanding of their role in hepatocellular carcinoma (HCC) formation is still limited. In this study, we aimed to investigate the role of soluble ICPs in the development of HCC. **Methods:** 168 chronic hepatitis C (HCV) patients achieved sustained virologic response (SVR) after antiviral treatment have enrolled in this study, among them 47 patients developed HCC (HCC group) while 121 patients did not (non-HCC group). Plasma samples were collected following antiviral treatment at baseline, end of treatment, SVR, and the study endpoint (time of HCC occurrence). The concentration of 16 ICPs in plasma samples were measured including soluble (s) form of BTLA, CD27, CD28, TIM-3, HVEM, CD40, GITR, LAG-3, TLR-2, GITRL, PD-1, CTLA-4, CD80, CD86, PD-L1, and ICOS by multiplexed fluorescent bead-based immunoassays. Localization of CD27, its ligand (CD70), and the effect of their axis in HepG2 cells were examined. **Results:** In total patients, following antiviral treatment, 7 and 12 out of 16 ICPs were significantly downregulated at the end of treatment and at SVR, respectively. Interestingly, at baseline, HCC group showed higher levels of sCD27 ($p = 0.005$), sCD28 ($p = 0.007$), sTIM-3 ($p = 0.025$), sHVEM ($p = 0.035$), sCD40 ($p = 0.015$), sTLR-2 ($p = 0.036$), sPD-1 ($p = 0.005$), sCD80 ($p = 0.038$), sCD86 ($p = 0.005$), AFP ($p = 0.022$) compared with non-HCC group. During antiviral treatment, the differences in ICPs were depleted. However, at the study endpoint, levels of sCD27, sCD28, sCD40, sCD86 in HCC group returned higher than non-HCC group ($p = 0.039$, $p = 0.00002$, $p = 0.017$, $p = 0.024$). By Kaplan-Meier analysis, patients with baseline levels of sCD27 ≥ 4104 pg/mL, sCD28 ≥ 1530 pg/mL and sCD40 ≥ 688 pg/mL indicated greater HCC cumulative rate ($p = 0.004$). Next, in HCC tissues, we observed that membrane CD27 was highly accumulated in the tumor and peri-tumoral areas, dominantly in T cells, partly in B cells, and macrophages, but not in epithelial cells. Interestingly, activated T cells produced sCD27 time-dependently by shedding their membrane-bound. Furthermore, CD70, the ligand of CD27, was robustly expressed in the HCC area where CD70 promoter methylation analysis indicated the



hypomethylation compared with the non-tumor pairs. In vitro, recombinant human CD27 treatment induced the proliferation of CD70-bearing HepG2 cells along with the up-regulated of cyclin B1 (1.6-fold), and BCL2 (2-fold) protein levels. The qRT-PCR analysis confirmed the upregulation of genes involved in cell cycle progression including cyclin A2 (1.4 -fold), cyclin B1 (1.4-fold), cyclin E2 (1.5-fold), cyclin-dependent kinase 1 (1.5-fold), cyclin-dependent kinase 2 (1.2-fold and anti-apoptosis genes including cellular inhibitor of apoptosis protein-1 (cIAP-1, 1.2-fold), and -2 (cIAP-2, 2.3-fold), while expression of CDK inhibitor 1A (P21) decreased by 30%. The HepG2 proliferation and cyclin B1 expression were attenuated by the ERK1/2 inhibitor, but not by the NF- κ B or p38 inhibitor. Conclusion: Our data suggest that the immune system could be already affected before antiviral treatment. Baseline sCD27, sCD28, and sCD40 levels could be used as HCC prognostic markers in HCV-SVR patients. sCD27 likely promotes HepG2 cell growth via the CD27-CD70 axis.



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Overexpression of HMGB1 in hepatocytes accelerates hepatocellular carcinoma in Pten Δ Hep mice Dipti Anil Athavale

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Introduction: the incidence of hepatocellular carcinoma (HCC) is raising due to the alarming increase in metabolic diseases such as non-alcoholic steatohepatitis (NASH). High-mobility group box-1 (HMGB1) is a damage-associated molecular pattern that participates in the onset and progression of chronic liver disease.

Aim: In this study, we hypothesised that increasing HMGB1 expression in hepatocytes accelerates the onset of NASH-induced HCC.

Methods: we analyzed publicly available transcriptomics datasets from patients with NASH and NASH-induced HCC for the expression of HMGB1. To provoke NASH-induced HCC, we used mice with liver specific ablation of Pten (Pten Δ Hep). Overexpression of HMGB1 in hepatocytes was achieved by injecting an adeno-associated virus serotype 8 (AAV8) expressing either Hmgb1-EGFP (Hmgb1-AAV8) or EGFP (empty-AAV8) to 6-weeks-old Pten Δ Hep mice, which were followed for the development of tumors.

Results: HMGB1 expression increased in human NASH and NASH-induced HCC compared to healthy liver. Male and female Pten Δ Hep mice injected with Hmgb1-AAV8 showed increased tumor burden with higher tumor size and volume compared to empty-AAV8 injected mice. Approximately 83% (10/12) of male and 45% (8/11) of female Pten Δ Hep mice injected with empty-AAV8 developed liver tumors while 100% of Hmgb1-AAV8 injected male (12/12) and female (9/9) Pten Δ Hep mice developed tumors at 9 and 11 months, respectively. Histological analysis of liver tumors from empty and Hmgb1-AAV8 injected male and female Pten Δ Hep mice revealed mixed HCC-iCCA, HCC, iCCA and benign adenomas. Mechanistically, overexpression of HMGB1 in hepatocytes increased cell proliferation (Ki67+ cells) and inflammation; yet, pAKT remained similar to mice injected with empty-AAV8. Wnt ligands remained similar in Hmgb1-AAV8 compared to empty-AAV8 injected male and female Pten Δ Hep mice. Nuclear YAP and SOX9 were observed in iCCA but not in HCC in Hmgb1-AAV8 injected male Pten Δ Hep mice.

Conclusion: Overexpression of HMGB1 in hepatocytes accelerates HCC in Pten Δ Hep mice.



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Endogenous and exogenous globin proteins presenting in Hepatic stellate cell suppress its activation and inhibit liver fibrosis via scavenging reactive oxygen species

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Background: Anti-fibrotic therapy remains one of unmet medical needs in human chronic liver disease. Cytochrome (CYGB) is a gas-binding hexacoordinated globin which was originally discovered from cytoplasm of hepatic stellate cells (HSCs). We recently reported that CYGB is a crucial molecule to maintain HSCs in a quiescent status. Here we show the promising therapeutic effects of human Cytochrome (CYGB), Myoglobin (MB), and Neuroglobin (NB) but not Hemoglobin (HB) against mouse liver injuries/fibrosis and human HSC activation. **Methods:** Human CYGB, NB open reading frames were cloned into pRSETA vector, following by transformation into BL21-AI host strain. Purified rhCYGB/NB was examined for its safety in human HSC cell line (HHStEC), wild type (WT) mice or humanized hepatocyte chimeric (PXB) mice. HB and MB were obtained from commercial source. IC₅₀ values of reactive oxygen species (ROS)-scavenging activity of these globins were measured. Cellular and organ distribution of administrated proteins were traced by alexa labelling. Anti-fibrotic function of these proteins were assessed in human or mouse HSCs under spontaneous activation or transforming growth factor (TGF)- β 1 challenge in vitro and in vivo mouse models induced by 4 weeks of 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC) diet feeding, 6 weeks of Carbon tetrachloride (CCL₄), or 10 weeks of thioacetamide (TAA) injection. Reactive oxygen species (ROS) scavenger function, and RNA sequencing were investigated. **Results:** Cellular fractionation revealed that extracellularly added MB, NB, and CYGB, but not HB, penetrated human HSCs (HHStECs) and located in membrane, cytoplasm, and cytoskeletal fractions via endocytosis pathway. Except HB, others can scavenge intracellular ROS generated spontaneously or stimulated by H₂O₂ or TGF- β 1 in HHStECs and suppressed ROS induced COL1A1 promoter activity, subsequently inhibited collagen synthesis. RNA-seq analysis of MB, NB and CYGB-treated HHStECs revealed the common downregulation of extracellular matrix-encoding and fibrosis-related genes, and the upregulation of antioxidant genes or inactivated markers of HSCs including GATA, EST2, and PPAR γ . Disruption of disulfide bond in NB or replacement of the iron centre of the heme group with cobalt in CYGB decreased heme activity, superoxide-scavenging activity, and collagen inhibition capacity. In vivo administration of rhCYGB (2-5 mg/kg body weight) exhibited no toxicity to mouse organs and their livers indicated by histology, blood test results and serum levels of AST, ALT, and albumin in WT mice or PXB mice compared to non-treated group. Four, six, or



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10 weeks of DDC, CCl₄, or TAA treatment, respectively, in mice induces liver injuries, collagen deposition indicated by AST, ALT levels, Sirius Red and Fast Green staining, α SMA expression, CD68 macrophage infiltration, and neutrophil populations. Intravenously injected MB, NGB, or CYGB exhibited the clear attenuation of these manifestations without adverse effect.

Conclusion: These findings revealed an unexpected and profound role for MB, NB and CYGB in maintaining HSCs in deactivated status and protect the mouse liver against cirrhosis, proposing the globin therapy as a new strategy to combat fibrotic liver disease.



LSECTin induction by anti-inflammatory cytokines in hepatic antigen presenting cells from cirrhotic mice.

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Introduction: Hepatic Antigen Presenting Cells (hAPCs), mainly Liver Sinusoidal Endothelial Cells (LSECs) and Kupffer Cells (KCs), express C-type lectin receptors, like LSECTin. This protein is known for its interaction with effector T-cell ligands and its consequent role in inflammatory response control during liver damage (1). Previous studies from our laboratory showed a negative transcriptional regulation of LSECTin in models of experimentally induced cirrhosis that resulted in a proinflammatory T-cell expansion (2).

Aims: Our aim in this study is to investigate whether LSECTin expression can be restored via an external intervention in hAPC from cirrhotic mice.

Methods: Progressive liver damage was induced in mice by oral administration of carbon tetrachloride (CCl₄). Laparotomies were performed at week 12 in pools of control and treated mice (n = 6). Liver cirrhosis was assessed by qPCR and IHC staining of different proinflammatory and profibrogenic markers. LSECTin expression in whole livers was studied by qPCR, WB and IHC. FACS-isolated hAPC were treated with and without combinations of anti-inflammatory cytokines (IL-4, IL-13, IL-10 and TGFβ1) for 48 hours. LSECTin expression was assessed in primary cultures by flow cytometry and qPCR.

Results: Livers from treated mice exhibited a strong expression of proinflammatory and profibrogenic markers indicating the onset of cirrhosis. A reduction of LSECTin expression during fibrosis progression was confirmed in our model, not only transcriptionally, but also at the protein level by IHC, WB and flow cytometry (Figure 1). LSECTin gene expression was induced in primary cultures of LSECs and KCs from control and fibrotic mice in the presence of anti-inflammatory cytokines (Table 1). Although IL-10 and IL-13 stood out individually, the combination of all four anti-inflammatory cytokines exhibited a synergistic upregulatory effect on LSECTin expression.

Discussion: LSECTin expression can be exogenously induced in cirrhotic hAPCs by the generation of a tolerogenic microenvironment. Cytokine-induced LSECTin restoration may constitute a new tool in the control of hepatic inflammatory response in advanced liver disease.



	LSEctin Relative Expression			
Treatment (10 ng/mL)	KCs		LSECs	
	Median	σ	Median	σ
None	1.00	0.00	1.00	0.00
IL-4	1.60	0.84	1.17	0.58
IL-10	1.53	0.99	1.68	1.18
IL-13	2.34	0.10	1.88	0.45
TGF-B	1.65	0.41	1.44	0.59
IL-4+IL-10	2.07	0.73	0.95	0.37
IL-4+TGF-B	2.11	0.33	0.94	0.15
IL-10+TGF-B	1.28	0.27	1.34	0.27
IL-4+IL-10+TGF-B	1.01	0.44	1.43	0.48
IL-4+IL-10+IL-13+TGF-B	1.93	0.12	3.05	1.72

(1) Tang, et al. Gastroenterology 137, 1498-508 (2009); (2) Caparros, et al. Cells 5, 9-1227(2020).

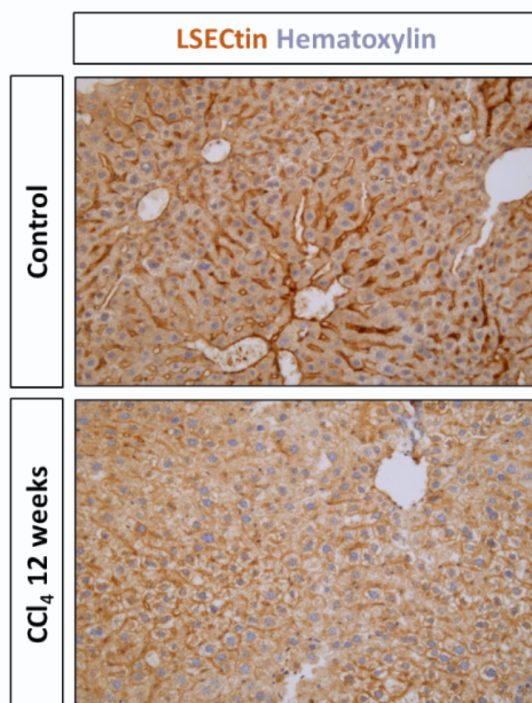


Figure 1.



The role of Intracellular gap formation of the liver sinusoidal endothelial cells in cancer metastasis.

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Introduction and aims: Intracellular gap (iGap) formation in liver sinusoid endothelial cells (LSECs) is caused by the destruction of fenestrae and appears under pathological conditions. Here, we aimed to explore the novel role of LSECs-iGap in liver metastasis of cancer cells.

Methods: Mouse models using acetaminophen or thioacetamide followed by intrasplenic injection of hepatocellular carcinoma (HCC) cell line, Hepa1-6 cells, to assess the LSECs-iGap formation. Functional effects of LSECs-iGap on cancer cells were analyzed using MMPs inducer, monocrotaline (MCT) and inhibitor, doxycycline (DOX). Morphological and molecular features of LSECs-iGap were examined by electron microscopic analyses, RNA-sequencing, cytokine array and endothelial trans-endothelial migration assay in vivo mouse models and in vitro co-culture system. Biopsy specimens from 98 patients with HCC were statistically evaluated using immunohistochemical staining.

Results: Acetaminophen-induced liver injury and thioacetamide-induced fibrotic liver resulted in LSECs-iGap formation, which positively correlated with increased numbers of metastatic foci after Hepa1-6 cells injection. In addition, Hepa1-6 cells induced IL-23-dependent TNF- α secretion by LSECs and triggered LSECs-iGap formation, toward which their processes protruded to transmigrate into the liver parenchyma. TNF- α caused depolymerization of F-actin and increased MMP9, ICAM1, and CXCLs expression in LSECs. Interestingly, the ICAM1 was selectively expressed surrounding the LSEC-iGap. Moreover, high expression of ICAM1 and MMP9 was significantly associated with intrahepatic metastasis and overall survival of patients with HCC. The MCT induced the gap formation in LSECs along with an increasing number of hepatic metastases (52.8 ± 15.3) compared to control (29.9 ± 4.8), but this effect was attenuated when mice treated with DOX (28.8 ± 10.3 , $p < 0.01$).

Conclusion: This study revealed that cancer cells induced LSEC-iGap formation via pro-inflammatory paracrine mechanisms and proposed MMP9 as a novel target for blocking cancer cell metastasis to the liver.



Cirrhosis alters scavenger receptor mediated clearance of therapeutic antibodies by hepatic sinusoidal endothelial cells

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Introduction: Fully human or humanized monoclonal antibodies (mAbs) are increasingly used for immunotherapy; but the design of these molecules is complicated by the need to bypass normal antibody clearance mechanisms. The liver plays a major role in the internalisation and catabolic clearance of biological therapies via interactions with scavenger and Fc-receptors within the hepatic reticuloendothelial system. Liver sinusoidal endothelial cells (LSECs) express the majority of these receptors and play key roles in clearance of immune complexes and regulation of antibody pharmacokinetics. However, changes in the expression of key membrane receptors involved in antibody clearance in chronic disease are not well characterized and may impact on drug efficacy in target patients.

Aims: We wanted to investigate the impact of cirrhosis on the expression of key receptors by human LSEC and assess how this impacts on the uptake and clearance of tool antibody compounds.

Methods: LSEC were isolated from diseased and normal human liver tissue as well as use of whole liver for extraction of RNA and protein lysates. A novel recycling assay was designed using primary cells from both diseased and healthy livers in which the binding and internalisation of IgG-1 formatted wild type, Fc disabled and modified ('LAGA' substitutions L235A and G237A) mAbs were visualised using immunofluorescence confocal imaging, flow cytometry, and quantification of antibody within the cells over 4 and 24hr time periods via meso-scale discovery (MSD) assay. Furthermore, whole liver tissue wedges were used for antibody perfusion over a 4-hour period after which the wedges were either fixed for chromogen staining or mechanically digested to generate cell suspensions for flow cytometric analysis of antibody localisation.

Results and Discussion: Our results confirm that expression of three important receptors known for antibody clearance by human LSEC (CD32b, DC-SIGN and Mannose Receptor) is significantly altered at protein level in cirrhosis. Importantly confocal imaging, MSD, flow cytometry and novel recycling assays on isolated diseased and healthy LSEC confirm that LSEC bind and internalize IgG1-formatted therapeutic mAbs. This binding capacity is altered on cells that originated from cirrhotic livers. Thus, our novel assays using human LSEC have demonstrated that changes in



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LSEC phenotype accompanying chronic disease may explain altered drug pharmacokinetics and toxicity observed in early trials. Therefore, future antibody development pathways should incorporate testing in models representative of the target patient demographic to address issues of poor kinetics, unexpected toxicity and poor predictive ability.



Immune related signalling pathways are affected by the gut microbiota in the early phase of liver regeneration after partial hepatectomy in mice

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Background/Aim. Liver regeneration is a complex biological process orchestrated by redundancy of multiple signalling pathways. Although relevant signalling molecules and pathways have been previously reviewed, the exact mechanism of liver regeneration remains poorly understood. Thus, the aim of this study is to investigate the involvement of immune related signalling pathways and their associated genes, specifically toll-like receptor (TLR), nuclear factor-kappa B (NFB) and tumour necrosis factor (TNF) signalling pathways in the early phase of liver regeneration in normal wild type (WT), germ – free (GF) mice and GF mice reconstituted with normal gut microbiota (XGF).

Methods. Male, 8-10 weeks old, WT(C57BL/6), GF and XGF mice underwent a 70% partial hepatectomy (PHx) under isoflurane anaesthesia (WT and XGF mice) and intraperitoneal Ketamine (100mg/kg)/Xylazine(10mg/kg) (GF mice). Liver regeneration was assessed by liver weight-body weight (LW/BW) ratio and mitotic figures at 3 days after PHx. Liver tissue harvested at 3 hrs after PHx was used for mRNA sequencing. Significantly differentially expressed genes (DEGs) were compared between different mouse groups and were subjected to KEGG pathway enrichment analysis using DAVID. An adjusted p-value < 0.05 was considered significant.

Results. At 3 hrs after PHx, totals of 3338, 1200 and 3647 genes showed significantly different expression in WT vs. GF, WT vs. XGF and GF vs. XGF, respectively. From these DEGs, 36, 13 and 70 pathways were enriched in KEGG pathways in WT vs. GF, WT vs. XGF and GF vs. XGF, respectively. TLR, NFB and TNF signalling pathways were enriched in WT vs. GF and GF vs. XGF but not in WT vs. XGF mice. *Tab2*, *Ikbkg*, *Traf3*, *Ripk1*, *Nfkb* were significantly expressed in these three pathways.

Conclusion. Results of this study have shown that TLR, NFB and TNF signalling pathways were significantly enriched when comparing between WT vs. GF and GF vs. XGF, but not in WT vs. XGF. This indicates that regulation of TLR, NFB and TNF signalling pathways during liver regeneration is dependent on the presence of gut microbiota. Reintroduction of gut microbiota may have activated these immune related signalling pathways, resulting in the improved restoration of liver mass in the XGF mice. In conclusion, these findings denote that early activation of immune related - TLR, NFB and TNF signalling pathways by the gut microbiota may be pivotal in liver regeneration.

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Correlative light, electron and atomic force microscopy for studying the size of liver fenestrations

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Introduction: Fenestrations in liver sinusoidal endothelial cells (LSEC) are transcellular nanopores of 50-300 nm diameter that provide for bidirectional transport of solutes between the blood stream and the interior of the liver. Their number and size are crucial for the maintenance of healthy levels of plasma lipoproteins (e.g., LDL), glucose storage and transport of hepatocyte targeting drugs. Liver diseases, ageing and various substances and medicines can influence this barrier. Over the years, the diameter of fenestrations (mostly below the resolution of a standard light microscopy) remained one of the main challenges in in vitro imaging of the LSEC phenotype.

Aims: Attainment of precise measurement of fenestration size by comparing different microscope modalities in a correlative manner.

Methods: A combination of scanning electron microscopy (SEM), atomic force microscopy (AFM) and optical nanoscopy methods such as structured illumination microscopy (SIM) or stimulated emission depletion (STED) microscopy are applied to gain a better understanding of the previously reported differences in fenestration dimensions. The fenestrations were measured in living, wet fixed and dry fixed isolated LSEC using novel quantitative image analysis methods.

Results: The measured size of the fenestrations varies between the imaging techniques. The measurement of the samples using SIM/SEM and STED/SEM correlative techniques showed around 30% increase in the fenestration diameter after drying the samples (part of the necessary sample preparation for SEM). The combination of AFM/SEM showed similar results. No significant difference was observed in wet-fixed samples measured using AFM/STED combination. The selection of the fixative agent for AFM samples showed an effect on the fenestration measurement.

Discussion: The accurate determination of fenestration size is crucial for understanding LSEC filtration function, as only particles smaller than fenestrations can passively pass between the bloodstream and the space of Disse. Both the selected imaging technique and the fixation/labelling can affect the measured fenestration size and must be taken into consideration. Moreover, correlative imaging shows the advantages of combining different kind of microscope modalities and provides additional information beyond the capabilities of each individual technique.

Correlative image of mouse LSEC obtained using SIM (green) and SEM (grayscale). The white masks represent fenestrations detected for the quantitative image analysis. Image size: 45 x 60 µm.



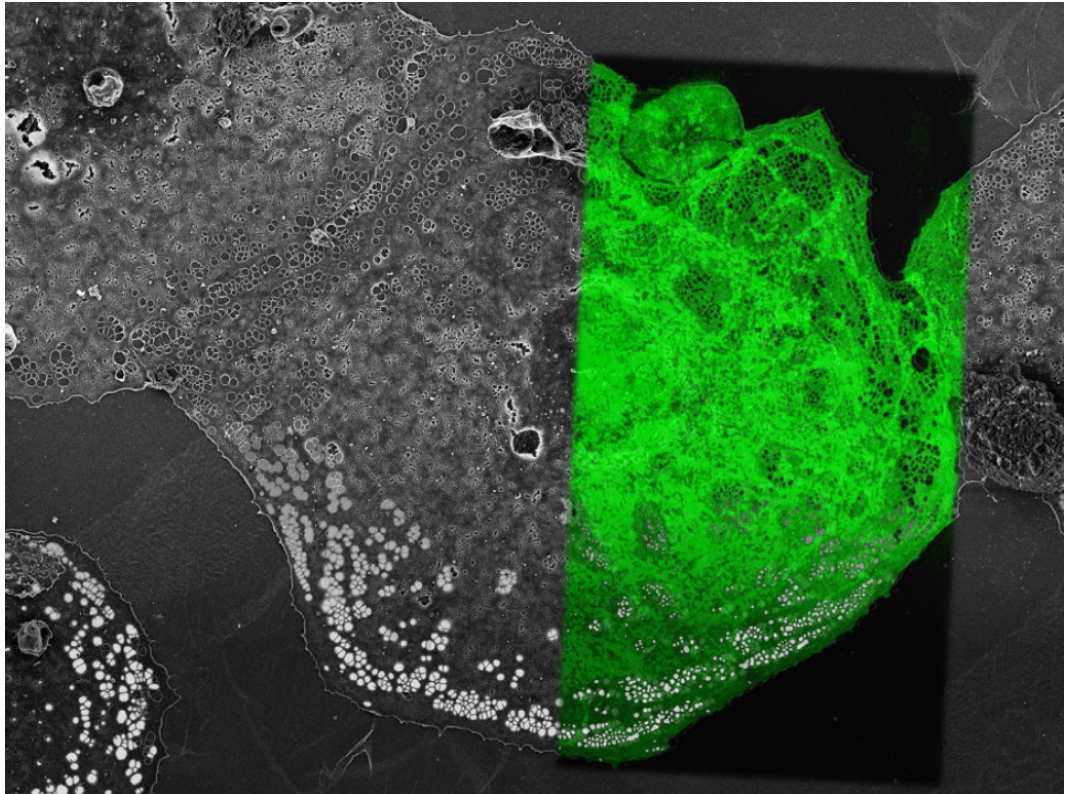
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Correlative image of mouse LSEC obtained using SIM (green)



Sildenafil modifies the porosity of healthy and cirrhotic human LSEC

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Introduction: Human LSEC are strategically placed within the hepatic sinusoid and thus play key gatekeeping[1] roles in both immune cell trafficking and scavenger-mediated clearance of blood proteins and lipids. LSEC membranes contain fenestrations organised into sieve-plates which permit movement of lipoproteins, drug molecules and pathogens to and from the liver parenchyma. Alterations in LSEC porosity as a consequence of ageing, disease or pharmacological compounds can therefore have an impact on hepatic and systemic function. For example, age-related dyslipidaemia[2] has been associated with a reduction in sinusoidal porosity. Studies with rodent LSEC[3] suggest that manipulation of nitric oxide signalling with agents such as sildenafil may improve porosity, but few studies have documented changes in human LSEC fenestration.

Aims: We wished to document the impact of cirrhosis on the expression of key receptors, functional capacity and porosity of human LSEC. We also examined the effects of sildenafil treatment on human LSEC porosity.

Methods: LSEC were isolated from non-diseased and cirrhotic human livers and cultured in vitro. Cells were exposed to 0-25ug/ml Sildenafil citrate and cells were used in a FITC-albumin uptake assay or imaged by dSTORM using the Nanoimager. Fenestration size and number were quantified. Human liver tissue and cultured LSECs were used for RNA extraction and immunochemical staining. mRNA from whole liver and LSEC samples was used for RT-PCR analysis of gene expression.

Results and Discussion: Our results confirm that cirrhotic human LSEC show altered expression of key scavenger receptors in culture which is accompanied by changes in porosity and ability to take up scavenger ligands in vitro. However even cirrhotic LSEC do maintain some functional capacity and fenestration. Exposure of both donor and cirrhotic LSEC to Sildenafil resulted in a dose dependent alteration in expression of proteins such as Caveolin-1, eNOS, VEGFR's, CD31, Stabilin-2 and a change in fenestration size and number. We have demonstrated for the first time in human cells that agents such as Sildenafil can partially restore the differentiated phenotype of even cirrhotic LSEC. Thus, in addition to beneficial effects on hepatic lipid homeostasis, sildenafil treatment may have additional hepatoprotective benefits by maintaining normal LSEC function.



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PRMT3 promotes glycolysis through methylation of PDHK1 to drive tumor immune microenvironment remodeling

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Introduction: Aerobic glycolysis is a classical metabolic phenotype of tumor cells and has been reported to affect tumor microenvironment resulting in tumor immune suppression. Protein arginine methyltransferase 3 (PRMT3) is a type I PRMT family member, which mediates protein asymmetric dimethylarginine (ADMA) modification. Pyruvate dehydrogenase kinase 1 (PDHK1) is the key regulator to keep glycolysis and oxidative phosphorylation balance in cancer cells. However, the role and mechanism of PRMT3 associated with glycolysis in hepatocellular carcinoma (HCC) is not well known.

Aims: To investigate the role of PRMT3 in the progress of HCC and further clarify the molecular mechanism of PRMT3 in the regulation of PDHK1 associated with glycolysis.

Methods: The gain- and loss-of-function assays were carried out to evaluate the effects of PRMT3 on various malignant properties of HCC cell lines in vitro. Prmt3^{HKO} mice generated by crossing Prmt3^{f/f} mice with albumin-Cre mice and the subcutaneously transplanted model of Huh7 cells were conducted to explore the effects of PRMT3 on tumorigenesis in vivo. Immunoprecipitation assay and western blotting were used to test the protein interaction. Further, PDHK1 inhibitors, JX06 treatment were used to explore the underlying role of PDHK1 responsible for PRMT3 function in vitro and in vivo. Human tissue microarray was executed to analyze PRMT3 expression level in patients with HCC.

Results: PRMT3 promoted the malignancy of hepatoma cells, including proliferation, migration, invasion, and glycolytic function. Treatment of SGC707, a selective Prmt3 inhibitor, significantly inhibited the tumor growth in Huh7 cells xenograft model. Knock-out of Prmt3 in hepatocyte reduced the formation of hepatocellular carcinoma in mice treated with DEN plus CCL4. Furthermore, PRMT3 played oncogenic role through interacting with and mediating methylation of PDHK1. As well as JX06 targeting PDHK1 activity abolished PRMT3-induced proliferation, glycolysis and tumorigenicity in vitro and in vivo. Moreover, overexpression PRMT3 induced lactate accumulation and increased PD-L1 expression level compared to the control group, while the treatment of JX06 partially reversed the enhancement of PRMT3 on lactate and PD-L1 expression level in xenograft tumor tissues. Knock-out Prmt3 decreased the level of lactate and PD-L1 expression in mouse tumor tissues. PD-L1 expression level was stimulated by lactate dose-dependently in Huh7 and Hepa1-6 cells. Additionally, human HCC tissue microarray analysis (TMA) showed that PRMT3 protein level was upregulated in HCC, and a higher PRMT3



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expression level was associated with a shorter overall survival of patients with HCC. A positive correlation between PRMT3 and PD-L1 was also observed in TMA.

Conclusion: These results suggested that PRMT3-mediated PDHK1 methylation increased glycolytic metabolism, thereby driving tumor immune escape in liver cancer microenvironment. Thus, targeting PRMT3 combined with PD-(L)1 antibody may be a promising therapeutic strategy for immunotherapy-resistant liver cancer.



Activation of hepatic HNF1 α signaling by suppressing gut microbiome related deoxycholic acid upon rifaximin treatment improves NASH in mice

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Introduction Non-alcoholic steatohepatitis (NASH) is characterized as a metabolic syndrome with hepatic lipid accumulation. Rifaximin serving as a non-absorbable antibiotic with minimal absorption has been reported on beneficial effects for NASH treatment in mice, but the exact mechanisms are not clear.

Aims This study aims to study the underlying mechanisms of the improvement in NASH mice upon rifaximin treatment.

Methods C57BL/6 mice were fed with methionine and choline deficient (MCD) diet or high fat high fructose (HFF) diet for 6 weeks and 23 weeks, respectively. Rifaximin (100 mg/kg) administrated to additional 4 weeks or 8 weeks for two different diet-induced NASH mice. The gut microbiome was analyzed by 16s rRNA sequencing using the mouse stools. Ileum tissues were collected and analyzed by liquid chromatography-mass spectrometry (LC-MC) for bile acids metabolism. Effects of HNF1 α expression on NASH were investigated in HepG2 cells with HNF1 α overexpress or knockdown in vitro and HFF diet-induced NASH mice in vivo.

Results Mice fed with MCD diet or HFF diet developed histologic severe NASH involving hepatic steatosis, ballooning and lobular inflammation. MCD diet induced NASH mice also showed severe hepatic fibrogenesis. NASH mice fed with MCD or HFF diets both showed lower HNF1 α expression in the liver. Rifaximin treatment significantly improved hepatic injuries and HNF1 α expression in NASH mice. The gut microbiome was significantly altered in MCD-induced NASH mice. Bile acids-targeted metabolomics analysis indicated that the MCD diet resulted in the accumulation of primary bile acids and deoxycholic acid (DCA) in ileum samples. Rifaximin significantly decreased DCA level in the mouse ileum by regulating the gut microbiome in MCD-fed mice. In vitro, DCA supplementation also increased the lipogenesis and downregulated HNF1 α expression. Overexpressed HNF1 α revealed decreased lipid accumulation in vitro and in vivo, while knockout HNF1 α expression blunted the beneficial effect of rifaximin in NASH mice.

Discussion Rifaximin treatment ameliorates NASH in mice by activating hepatic HNF1 α expression through regulating gut microbiome related deoxycholic acid.



SOX9 in hepatocytes promotes liver regeneration

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Introduction: The hybrid hepatocytes (HybHPs) coexpressing SOX9 and HNF4 α , existed as liver progenitor cells, contribute to chronic liver regeneration. Previous studies have shown that the expression of SOX9 in periportal hepatocytes is increased during liver injury. Deletion of HNF4 α results in sustained proliferation induced mortality after partial hepatectomy. However, the function of SOX9 during liver regeneration and the relationship of SOX9 and HNF4 α remain unclear.

Aims: This study explored the role of SOX9 and the regulation of HNF4 α on SOX9 expression in hepatocytes during liver regeneration.

Methods: Hepatocyte-specific Sox9 knockout (Sox9HKO) mice and hepatocyte-specific Hnf4 α knockout (Hnf4 α HKO) mice were generated by AAV8-TBG-Cre injection via the tail vein of Sox9^{f/f} mice and Hnf4 α ^{f/f} mice, respectively. AAV8-TBG-Sox9 was used for overexpression of Sox9 in hepatocytes. Standard partial hepatectomy (sHx) and extended partial hepatectomy (eHx) were performed to assess acute liver regeneration. Liver regeneration was evaluated by liver weight/body weight, histology, and immunofluorescence. Proliferation of hepatocyte was analyzed using immunofluorescence, Real-time PCR and Western blot. The potential regulation between HNF4 α and SOX9 was detected in Sox9HKO and Hnf4 α HKO mice. The effect of HNF4 α -related miRNA on SOX9 expression was investigated in HepG2 cells.

Results: The expression of SOX9 in hepatocytes was upregulated at 12 hours accompanied with the decrease of HNF4 α , while the expression of SOX9 and HNF4 α reached the normal level at 7 days post-sHx. Deletion of Sox9 in hepatocytes resulted in decreased liver weight/body weight in sHx model. The hepatocyte proliferation was decreased in Sox9HKO mice compared with Sox9^{f/f} mice. In addition, the mice with overexpression of Sox9 in hepatocytes showed a significantly higher survival rate accompanied with the increased proliferation of hepatocytes after eHx. The expression of SOX9 were increased in Hnf4 α HKO mice as compared with Hnf4 α ^{f/f} mice, while deletion or overexpression of SOX9 in hepatocytes did not influence the level of HNF4 α in mice. Moreover, Hnf4 α HKO mice showed lower expression of HNF4 α -related miR-124/154/381 compared with control mice. Prediction software and luciferase reporter assays identified Sox9 as the direct target of miR-124/154/381. Overexpression of HNF4 α and miR-124/154/381 mimics decreased the level of SOX9 in HepG2 cells. Furthermore, miR-124/154/381 inhibitors partially reversed the effect of HNF4 α on SOX9 expression.

Discussion: In acute liver injury, SOX9 in hepatocytes enhances cell proliferation to promote liver regeneration. HNF4 α regulated the expression of SOX9 through miR-124/154/381. The HNF4 α -miR-124/154/381-SOX9 axis is critical for liver regeneration.



Depletion of Tgfr2 in hepatocytes alleviates liver fibrosis through preventing hepatic function

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Introduction: The activation of TGF- β signaling is required for liver fibrogenesis. Previous studies have demonstrated the pivotal role of TGF- β signaling in the activation of hepatic stellate cells during liver fibrosis. The effect of TGF- β signaling in hepatocytes during hepatic fibrogenesis remains largely unexplored.

Aims: This study aims to demonstrate the effect and underlying mechanism of the TGF- β signaling in hepatocytes on hepatic fibrogenesis.

Methods: Hepatocyte-specific Tgfr2 knockout (Tgfr2HKO) mice were generated by AAV8-TBG-Cre injection via the tail vein of Tgfr2f/f mice. CCl₄ was injected intraperitoneally twice a week for 5 weeks to establish the fibrotic mouse model. The fibrogenesis of the fibrotic livers was evaluated by immunohistochemistry, western-blot and Real-time PCR. RNA-seq analysis was used to detect alterations in the transcriptional profiles of primary hepatocytes isolated from Tgfr2HKO mice and control mice.

Results: The expression of Tgfr2 was markedly upregulated in hepatocytes of the fibrotic liver. Sirius Red staining and the expression of profibrogenic markers, such as Col1a1 and Acta2, were decreased in Tgfr2HKO mice liver treated with CCl₄ compared with Tgfr2f/f mice, suggesting that deletion of Tgfr2 in hepatocytes significantly alleviated the liver fibrosis. Knockout of Tgfr2 in hepatocytes significantly increased the expression of E-cadherin and decreased the level of Vimentin, indicating the inhibition of the epithelial-to-mesenchymal transition of hepatocytes in Tgfr2HKO mice. The expression of Col1a1 also decreased in primary hepatocytes isolated from Tgfr2HKO fibrotic mice. RNA-seq analysis revealed that downregulated genes in Tgfr2HKO fibrotic mice were significantly enriched in inflammatory and immune response pathways compared with Tgfr2f/f mice. KEGG analysis showed that pathways associated with metabolism were decreased in fibrotic livers and further upregulated in hepatocytes with Tgfr2 depletion in the mice after CCl₄ treatment. Consistently, the expression of liver metabolic genes, including Apo4/5, Cyp1a1 and Cyp2e1, were recovered in hepatocytes of Tgfr2HKO mice treated with CCl₄. Notably, the hepatocyte nuclear factors (HNFs) for maintenance liver metabolism and homeostasis, including Hnf4a, Foxa2 and Foxa3, were decreased in fibrotic livers and significantly upregulated in Tgfr2HKO fibrotic mice.

Discussion: Blocking TGF- β signaling pathway in hepatocytes reduces hepatic fibrosis through alleviating hepatocyte damage and restoring hepatic function, suggesting that protecting the hepatic function may be important for alleviating liver fibrosis.



Sulodexide attenuates liver fibrosis in mice by inhibiting liver sinusoidal endothelial cell capillarization and Endothelial-Mesenchymal Transition

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Introduction: Liver sinusoidal endothelial cells (LSECs) are a group of highly specialized endothelial cells with open fenestrae and lack of a basement membrane, which play critical roles in the maintenance of liver homeostasis. During liver fibrosis, LSEC lose its fenestrae, gain basement membrane, thus become capillarized. Sulodexide (SDX) is a heparinoid compound purified from porcine intestine mucosa, which consists of 80% electrophoretically fast-moving heparin and 20% dermatan sulfate, with endothelium protective function.

Aims: In this study, we evaluated the therapeutic potential of SDX in two different models of liver fibrosis as well as the underlying antifibrotic mechanisms.

Methods: We examined the effect of Sulodexide on liver fibrosis in TAA and DDC-induced liver fibrosis mice model. We then employed Sirius red staining, immunohistochemistry, Western blot and qPCR to evaluate the protein and mRNA expression level of Col1a and α -SMA and liver fibrosis. Furthermore, we performed RNA-sequencing in liver tissues to explore the underlying mechanisms. We then isolated LSECs and conducted in vitro culture of primary murine LSECs to examine the effect of SDX on LSECs defenestration and endothelial-mesenchymal transition process.

Results: Sulodexide treatment significantly decreased the ECM deposition, as well as the expression of SMA and COL1 in the livers of both TAA and DDC-induced mice. RNA-sequencing showed that downregulated genes in Sulodexide treated group are enriched in extracellular matrix and cell adhesion pathways compared to TAA group. Moreover, genes involved with LSEC capillarization was significantly upregulated during TAA-induced liver fibrosis and downregulated in Sulodexide treated group. Immunohistochemistry showed that increased expression of LSEC capillarization-related genes, including CD31, CD34 and Laminin in liver fibrotic tissues was reduced in Sulodexide treated group. Immunofluorescence staining of VE-cadherin and SM22 showed that endothelial-mesenchymal transition of LSECs in liver fibrosis was inhibited by the administration of Sulodexide. In vitro study confirmed that the expression level of the LSEC capillarization markers was downregulated with Sulodexide in LSECs cultured for 48 hours. Moreover, Sulodexide also partially reverse the EndMT process in a 7-days LSECs culture experiment.



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Conclusion: Those results demonstrate the protective effect of Sulodexide on LSEC during liver fibrosis progression. Sulodexide ameliorates TAA and DDC-induced liver fibrosis in mice via inhibiting LSECs capillarization and endothelial-mesenchymal transition.

Key words : Liver fibrosis, Sulodexide, LSECs capillarization, Endothelial-mesenchymal transition



Identification of BBS4 as a novel pathogenic gene for autosomal dominant polycystic liver disease

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Introduction : Polycystic liver disease (PLD) refers to a condition characterized by the presence of numerous cholangiocytes-lined and fluid-filled cysts in the liver. PLD includes a group of heterogeneous genetic disorders such as isolated autosomal dominant polycystic liver disease (ADPLD). As a monogenic disorder, ADPLD is caused by pathogenic variants in different genes, which reportedly so far are only responsible for 30-45% of ADPLD cases. Therefore, novel gene variations account for the remaining ADPLD patients deserve research attention.

Aims: To identify novel pathogenic genes for ADPLD, we performed whole-exome sequencing in ADPLD pedigrees and investigated the function of potential pathogenic genes in hepatic cystogenesis.

Methods: Whole-exome sequencing was performed to screen the potential pathogenic genes in the ADPLD families. BBS4 liver-specific knockout mice strain (BBS4LKO mice) was generated by crossing BBS4f/f mice with albumin-Cre mice. The dilation of intrahepatic bile duct was detected by HE staining and IHC of CK19. Retrograde ink injection into the common bile duct was performed to explore the effects of BBS4 depletion on the bile duct morphology. Immunofluorescence analyses were conducted to evaluate the change of proliferation, number of primary cilia and polarity of cholangiocytes in BBS4LKO mice.

Results: BBS4 was identified as a potential pathogenic gene of ADPLD in an ADPLD family. Hepatic cysts began to be observed in 10-month-old BBS4LKO mice by high-resolution X-ray micro-CT scanning. In 18-month-old BBS4LKO mice, the incidence of internal hepatic cysts was 83.3% (5/6) under micro-CT scanning and that of external hepatic cysts was 50% (3/6) in gross observation. In the liver towards the periphery at E18.5 and P1, BBS4LKO mice showed decreased asymmetric primitive ductular structures (PDS) compared with BBS4f/f mice. The number of mature bile duct in the liver near the hilum in P1 BBS4LKO mice also decreased. These phenomena suggested abnormal bile duct morphogenesis, which was confirmed in adult BBS4LKO mice via IHC of CK19 and visualization of the biliary duct system. Moreover, PCNA/CK19 or Ki67/CK19 double-positive cells were detected in BBS4LKO mice, while no PCNA and Ki67 staining was observed in BECs of BBS4f/f mice, suggesting that deletion of BBS4 led to the hyperproliferation of BECs. In addition, the immunofluorescence staining of Ac-tubulin and ARL13B indicated the reduced primary cilia formation of BECs in BBS4LKO mice compared



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with that of BBS4f/f mice. Apico-basal polarity of BECs visualized by osteopontin and laminin displayed perturbation in BBS4LKO mice.

Discussion: BBS4 is a novel pathogenic gene for ADPLD. Depletion of BBS4 enhanced the proliferation of cholangiocytes, inhibited the primary cilia formation and disturbed the polarity of BECs.



Delayed immune response is associated with increased VEGFR2 expression following partial hepatectomy in germ-free mice

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Introduction: Recent studies have demonstrated the dynamic role of the receptor tyrosine kinase VEGFR2 in liver regeneration. As the receptor for VEGF, VEGFR2 expression and protein levels are increased during the remodeling (angiogenic) phase of liver regeneration, promoting LSEC proliferation and restoration of hepatic sinusoids. VEGFR2 expression is also related to angiopoietin-2 function, whereby reduced VEGFR2 expression and liver regeneration was observed following two-thirds partial hepatectomy (PHx) in Ang2-KO mice. Gut microbiota is one of many factors that affects liver regeneration, where restriction of the gut microbiota causes delayed liver regeneration following PHx. The aim of this study was to investigate the impact of gut microbiota restriction on VEGFR2 expression during PHx-induced liver regeneration.

Methods: Male, 8-10 weeks old germ-free (GFM), ex-germ-free (XGFM), and wild-type (WTM) control mice were used in this study. GFM were bred in sterile conditions, and therefore have a sterile gut. XGFM are GFM that had been introduced with normal gut microbiota. Mice underwent 70% PHx and subsequently sacrificed at 3-hr and 72-hr post PHx. Remnant liver was removed and investigated for liver mass restoration and cytokine response. Liver weight-body weight ratio (LW/BW) was used to assess liver mass restoration. Vegfr2, Tnfrsf1a, Il6ra, and Hgf gene expressions were assessed using mRNA sequencing and analysis, and confirmed by qPCR, while VEGFR, TNFR1, IL6R, and HGF protein concentrations were assessed by LUMINEX protein assay.

Results: At 72-hr, LW/BW of GFM was significantly lower than WTM ($p=0.0294$) and XGFM ($p=0.0286$) indicating reduced liver mass restoration in GFM, and improved liver mass restoration in XGFM. At 3-hr, WTM demonstrated increased expression of Tnfrsf1a ($p=0.0006$) and Il6ra ($p=0.0012$) genes; XGFM demonstrated increased expression of Tnfrsf1a ($p=0.0004$), Il6ra ($p=0.0004$) and Hgf ($p=0.0011$); while GFM demonstrated increased expression of Hgf ($p=0.029$). No significant increase in Tnfrsf1a and Il6ra expression was observed in GFM at 3-hr. At 3-hr, mRNA levels of Tnfrsf1a, Il6ra and Hgf was significantly lower in GFM compared to the other groups. WTM demonstrated reduced Vegfr2 expression ($p=0.0012$) at 3-hr, followed by increased expression ($p=0.015$) at 72-hr. In contrast, GFM demonstrated increased Vegfr2 expression ($p=0.0103$) at 3-hr, followed by reduced expression ($p=0.0041$) at 72-hr. Vegfr2 expression in XGFM was similar in trend to the expression pattern of WTM. At 3-hr, Vegfr2 expression was higher in GFM compared to XGFM ($p=0.0005$)



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Discussion and conclusions: Delayed liver regeneration in GFM was evident by the reduced LW/BW ratio following PHx. Conversely, a higher LW/BW following PHx indicated improved liver regeneration in XGFM. These findings further confirm the role of the gut microbiota in liver regeneration. The reduced VEGFR2 expression and protein concentration in WTM at 3-hr was in line with other studies demonstrating suppression of angiogenic activity to allow hepatocyte proliferation during the priming

phase of liver regeneration. The subsequent increase in VEGFR2 expression and protein concentration at 72-hr was also concurrent to literature which demonstrate LSEC proliferation and sinusoidal restoration to occur during the remodeling phase of liver regeneration. In GFM, Vegfr2 expression was upregulated during the priming phase and downregulated during the remodeling phase, which was contrary to the control group. Reduced liver regeneration in GFM is associated with reduced immune response, evident by reduced *Tnfrsf1a*, *Il6ra*, and *Hgf* expression. Therefore, in sterile mice, lack of LPS induced cytokine response during priming phase results in reduced hepatocyte proliferation. While the main cytokines (IL6 and TNF α) and growth factor (HGF) involved in induction of hepatocyte proliferation are affected by the gut microbiota, VEGFR2 expression is independent of the gut microbiota. As VEGF is a known auxiliary mitogen for hepatocyte proliferation, in the absence of gut microbiota, increased VEGFR2 expression may act as a compensatory mechanism to augment hepatocyte proliferation despite low immune response due to the sterile gut. In conclusion, VEGF and VEGFR2 may play a role as a compensatory mechanism in the absence or diminished immune responses during liver regeneration.



Beta-arrestin 2 aggravates non-alcoholic steatohepatitis via the metabolic reprogramming of macrophages

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Abstract

Non-alcoholic fatty liver disease (NAFLD) is a major healthcare burden worldwide, includes a spectrum of liver disorders ranging from simple steatosis to non-alcoholic steatohepatitis (NASH), cirrhosis and liver cancer. Exacerbation of macrophage-mediated inflammation has been implicated in the pathogenesis of NASH, but the immunometabolic program underlying regulation of macrophage activation remains unclear. Beta-arrestin 2 (Arrb2) is a multifunctional adaptor that is strongly expressed in normal human bone marrow tissues and macrophages. However, whether Arrb2 regulates macrophage metabolism and NASH process are still not clear. Here, we observed that Arrb2 expression was significantly increased in the hepatic macrophage of NASH patients and HFD-fed mice. Hepatic steatosis, inflammatory responses and fibrosis were ameliorated in Arrb2 global KO mice fed with HFD, MCD or HFHC diet. Arrb2 in macrophages but not hepatocyte contributed to the development of NASH through promoting M1 polarization of macrophages. Mechanism study showed that Arrb2 could act as an adaptor protein which promoted ubiquitination of IRG1 to inhibit its protein levels. Deletion of Arrb2 in macrophages increased IRG1 expression and promoted further elevation of itaconate, which results in inhibition of SDH activity, thereby enhancing oxidative phosphorylation (OXPHOS), reducing release of mitochondrial reactive oxygen species (mtROS) and inhibiting Hif-1 α /IL-1 β axis. Importantly, up-regulation of Arrb2 expression was also observed in circulating monocytes from NASH and NAFL patients compared with those from healthy controls. Arrb2 levels in monocytes correlated positively with serum ALT, AST and the number of circulating monocytes in NAFLD patients. Conclusively, silencing myeloid Arrb2 signaling may result in beneficial effects on treating NAFLD and other aseptic inflammatory disorders. **Keywords:** beta-Arrestin 2; NAFLD; Macrophage polarization; Metabolic reprogramming.



Notch Signaling Regulates the Phenotypic Switch of Hepatic Macrophages in Liver Fibrosis

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Introduction: Hepatic fibrosis, as a morbid consequence of chronic liver injury, is in a balance of progression and resolution. Hepatic macrophages are a remarkably heterogeneous population of immune cells with different origins, fulfilling pro-fibrotic or anti-fibrotic functions in the liver. When pro-fibrotic macrophages are in a predominant state, fibrosis will continue to progress. While recovery of anti-fibrotic macrophages will greatly contribute to fibrosis resolution. However, how anti-fibrotic macrophages could be recovered still remains unclear.

Aims: We hope to reveal the mechanisms underlying the recovery of anti-fibrotic macrophages and provide new strategies for macrophage-targeted treatment of liver fibrosis.

Methods: The liver fibrosis model was established by carbon tetrachloride or bile duct ligation in myeloid cell-specific Notch signaling activation and control mice. Collagen deposition, liver function, inflammation, activation and apoptosis of hepatic stellate cells were analyzed to identify the severity of liver fibrosis. In-depth analysis of different macrophage subsets was carried out by flow cytometry, combined with RNA-seq and a series of molecular biology experiments.

Results: We found that myeloid-specific Notch signaling activation aggravated the progression of hepatic fibrosis by hindering the phenotypic switch of bone marrow-derived macrophages from Ly6Chi inflammatory macrophages to Ly6Clo restorative macrophages on the one hand, and repressing the recovery of Kupffer-like cells on the other hand. These cellular processes could then result in sustained inflammation, aggravated hepatocyte damage, persistent activation of hepatic stellate cells (HSCs) and impaired matrix degradation ability, leading to the progression of fibrosis. Furthermore, we have also noticed that the phenotypic switch from Ly6Chi inflammatory macrophages to Ly6Clo restorative macrophages was hindered by Notch signaling through inhibiting the expression of C/EBP α .

Discussion: Our research identified novel mechanisms about how Notch signaling regulates hepatic macrophage subsets and their phenotypic switch in liver fibrosis, and the fate of Ly6Chi macrophages need to be further investigated by in vivo tracing strategies.



Dissecting the Role of Stiffness in Regulating Primary Hepatocytes and Non-Parenchymal Cells Communication in Chronic Liver Diseases

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Background: Chronic liver disease is characterized by progressive hepatic fibrosis leading to cirrhosis irrespective of the etiology with no effective treatment currently available. Liver stiffness (LS) is currently the best clinical predictor of this fibrosis progression. Elastography measurements have shown graded increase in LS at various stages of fibrosis (2-4kPa: healthy liver, 8-10kPa: F0-1, 12-25kPa: F2-4 fibrotic liver, and >55kPa: cirrhosis). LS and hepatocytes-nonparenchymal cells (NPC) interactions are two variables known to be important in regulating hepatic function during liver fibrosis, but little is known about the interplay of these cues. It is challenging to control LS in animal models. Using a multidisciplinary approach, we investigated the role of LS in altering cell-cell communication and regulating hepatic function.

Methods: Primary hepatocytes were co-cultured with non-parenchymal cells NPC cells on an innovative biomimetic platform “BEASTS (Bio-Engineered Adhesive Siloxane substrate with Tunable Stiffness)” that recreates physiologic (2 kPa) and pathologic stiffness (8, 15, 25, 55 kPa). Functional/metabolic analysis were performed to determine key hepatocytes metabolic and functional functions by Western Blot and PCR.

Results: Primary hepatocytes on fibrotic stiffness showed decreased urea, albumin production, and expression of drug transporter gene and epithelial cell phenotype marker, hepatocyte nuclear factor 4 alpha (HNF4a). Primary hepatocytes were co-cultured with fibroblasts on soft (2 kPa- physiologic LS) and stiff substrates (55 kPa- cirrhotic LS). Urea synthesis by primary hepatocytes depended on the presence of fibroblast and was independent of the substrate stiffness. However, albumin synthesis and Cytochrome P450 enzyme activity increased in hepatocytes on soft substrates and when in co-culture with fibroblast. Western blot analysis of hepatic markers, E-cadherin, confirmed that hepatocytes on soft substrates in co-culture promoted better maintenance of the hepatic phenotype. Similar functional changes were observed in hepatocytes when co-cultured with liver sinusoidal endothelial cells in fibrotic stiffness.

Conclusion: These data demonstrates that substrate modulus and cell-cell interactions both regulate hepatocyte function. Our findings pointing to the importance of substrate mechanical properties on hepatocyte function inform the critical role of LS just not a consequences but also cause of liver fibrosis and hepatocytes dysfunction.



Signature of gene expression profile of liver sinusoidal endothelial cells in non-alcoholic steatohepatitis

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Abstract

Introduction: There have been emerging evidence that liver sinusoidal endothelial cells (LSECs) play important role in the pathogenesis of non-alcoholic steatohepatitis (NASH). Currently, transcriptomic and microarray analysis has been widely applied to explore new biomarkers in a variety of diseases including cancers, metabolic diseases and auto-immune diseases. In addition, verification of the competitive endogenous RNA (ceRNA) networks can present a deeper mechanism in the transcriptional regulatory network.

Aims: While a remarkable change in transcription is recognized as a defining feature of NASH, the potential key genes and pathway networks of LSECs are worth thorough analysis. Thus in this study we aim to comprehensively analyse the signature of gene expression profile of LSECs in NASH.

Methods: Animal experiments were performed to demonstrate the significant structural damage of LSECs in NASH model. To further understand the functional changes of these damaged LSECs in NASH, we used public GEO database that contained microarray data for the gene expression of LSECs in NASH and normal mouse liver. Differentially expressed genes (DEGs) were analysed, and further Gene Ontology (GO) Enrichment analysis were performed to understand the functional changes. The hub genes were then identified and then validated via external GEO databases. A protein-protein interaction (PPI) network was predicted based on the DEGs to further explore the functional role of of these DEGs by using the online STRING tool. CytoHubba was used to further identify crucial genes in this PPI network as hub genes. We also uploaded the specifically expressed hub genes to Network Analyst to explore the potential miRNA-protein interactions. At last, StarBase version 3.0 was used to predict the interaction of lncRNAs and circRNAs with miRNAs.

Results: There was significant structural damage of LSECs in NASH model, accompanied by remarkable functional changes of LSECs with 174 DEGs (156 upregulated and 18 downregulated genes). The functions of these DEGs were mainly enriched in the following pathways: inflammatory response, immune response, cell adhesion, immune system process, cellular response to interleukin-1, chemokine activity, integrin binding, cell adhesion molecule binding, cytokine activity and identical protein binding. Nine specifically expressed hub genes were identified including CCL4, EPCAM, CXCL2, CCL3, KRT19, CX3CR1, KRT8, ITGAX and CLDN3. Among them, CCL4 and ITGAX showed the most significant correlation with NASH,



with AUROC of 0.77 and 0.86, respectively. A co-expressed network of mRNAs and miRNAs which comprised of 98 target miRNAs, 9 specifically expressed hub genes and 144 mRNA-miRNA connections was obtained. We further explored the lncRNAs and circRNAs related with these target miRNAs and hub genes by using the Starbase tool, and we obtained the networks involving 13 circRNAs, 1 lncRNA related to the miRNAs of CCL4, 15 circRNAs and 1 lncRNA related to the miRNAs of ITGAX.

Conclusion: In this study, we systematically analysed the differentially expressed genes of LSECs in NASH. We identified two immune system-specifically expressed genes, CCL4 and ITAGX expressed by LSECs, as potential key players for the pathogenesis of disease development in NASH at the transcriptome level. Our findings put forward promising therapeutic and prognostic values of the genes expressed by LSECs as well as the ceRNA regulatory networks in NASH.

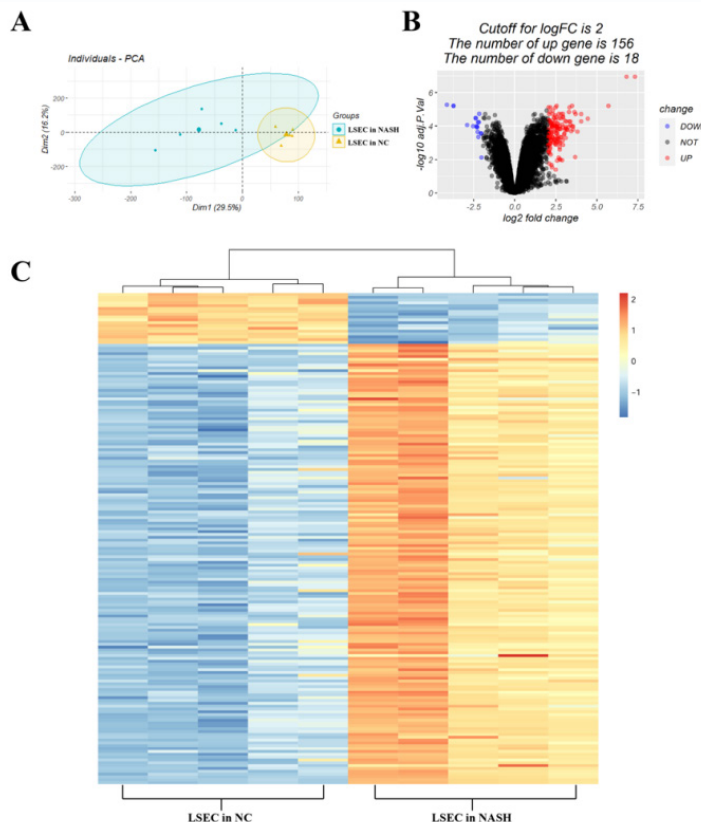


FIGURE 1 | Identification of DEGs. A. principal component analysis between the normal samples and the NASH samples of LSECs; B. Volcano plot of DEGs between the normal samples and the NASH samples of LSECs. The red plots represent upregulated DEGs, the black plots represent non-significant genes, and the blue plots represent downregulated DEGs. C. A heatmap of DEGs between the normal samples and the NASH samples of LSECs. Red rectangles represent high expression, and green rectangles represent low expression.

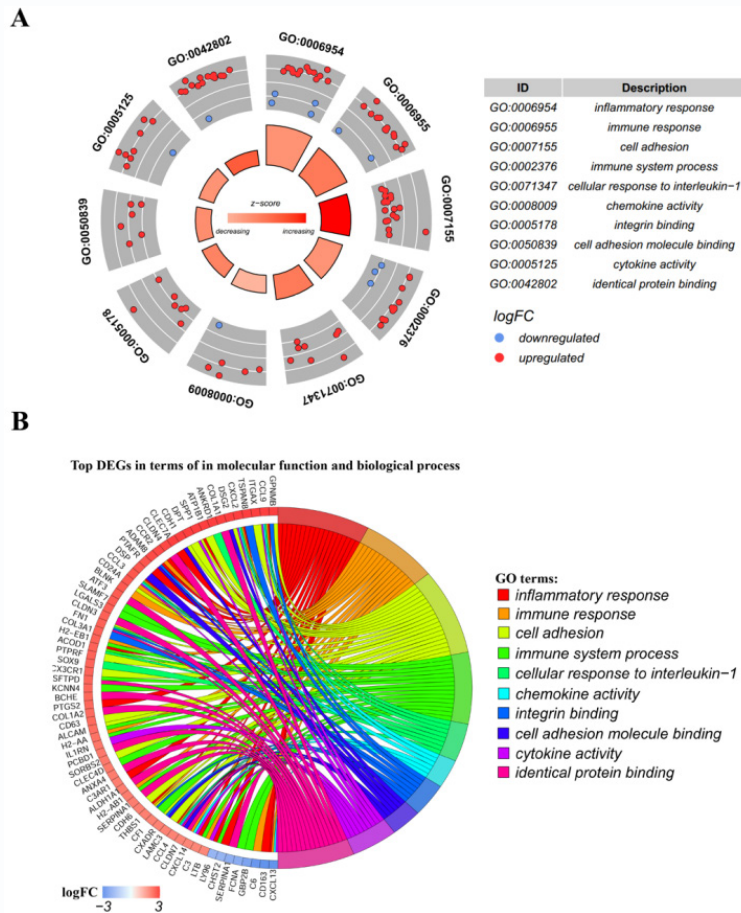


FIGURE 2 | GO (Gene Ontology) terms enrichment analysis. A. GO circle plot; the inner ring was a bar plot where the bar height showed the significance of the term ($-\log_{10}$ P value) and the gradual color showed the z-score. The outer ring displayed scatter plots of the expression levels (logFC) for the genes in each term. The distribution of gene in different database was used to predict different annotations. B. GO chord plot of the relationship between the list of selected genes and their corresponding GO terms, together with the logFC of the genes. Left half of GO chord displayed the up-expression or down-expression DEGs. The right half represented different GO terms with varied colors. A gene was linked to a certain GO term by the colored bands.

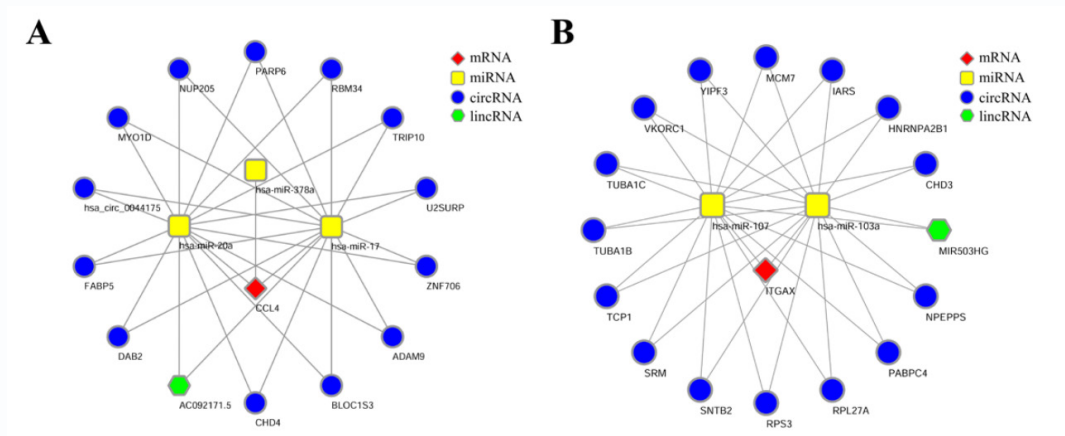


FIGURE 3 | lncRNA/circRNA-miRNA-mRNA ceRNA network. A. Network of CCL4-lncRNAs-miRNA-mRNAs. B. Network of ITGAX-lncRNAs-miRNA-mRNAs.



microRNA-223 attenuates hepatocarcinogenesis by blocking hypoxia-driven angiogenesis and immunosuppression

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Introduction and Aims: Hepatocellular carcinoma (HCC) is a leading cause of cancer-related death worldwide. Although current treatment to block angiogenesis and immunosuppression provides some benefits only for a subsets of HCC patients, optimized therapeutic regimens are unmet needs, which require a thorough understanding of the underlying mechanisms by which tumor cells orchestrate inflamed tumor microenvironment that is associated with significant myeloid cell infiltration. microRNA-223 (miR-223), a neutrophil-specific miRNA, is well known to act as an important anti-inflammatory regulator, thereby inhibiting liver disease progression by controlling neutrophil infiltration and activation; however, whether and how miR-223 affects HCC development remain unclear.

Methods: miR-223 knockout (miR-223KO) mice and wild-type (WT) littermates were generated and subjected to two mouse HCC models induced by injection of diethylnitrosamine (DEN) with chronic carbon tetrachloride (CCl₄) or orthotopic HCC cell implantation with chronic CCl₄ injection. In addition, adenovirus-mediated hepatic miR-223 overexpression was also used to treat HCC.

Results: In the current study, we demonstrated that genetic deletion of miR-223 markedly exacerbated tumorigenesis in two mouse models of inflammation-associated HCC induced by injection of DEN with chronic CCl₄ or orthotopic HCC cell implantation with chronic CCl₄ injection as demonstrated that miR-223KO mice had greater tumor masses and number of DEN+CCl₄ HCC compared with WT mice. Interestingly, compared to WT mice, miR-223KO mice had more infiltrated programmed cell death 1 (PD-1+) T cells and programmed cell death



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ligand 1 (PD-L1+) macrophages after DEN+CCl₄ administration. Bioinformatic analyses of RNA-Seq data revealed a strong correlation between miR-223 levels and tumor hypoxia, a condition that is well-documented to regulate PD-1/PD-L1. In vivo and in vitro mechanistic studies demonstrated that miR-223 did not directly inhibit PD-1 and PD-L1 in immune cells rather than indirectly downregulated them by modulating tumor microenvironment in HCC. Our in vivo and in vitro data further suggested that miR-223 targeted hypoxia inducible factor 1 α (HIF-1 α) in HCC tumor cells and subsequently suppressed immune checkpoint PD1/PD-L1 expression, thereby inhibiting HCC. Moreover, gene delivery of miR-223 via adenovirus inhibited angiogenesis and hypoxia-mediated PD-1/PD-L1 axis activation in both HCC models, thereby hindering HCC progression.

Discussion: We identify miR-223 as a key orchestrator for tumor hypoxia and inflammatory tumor microenvironment in controlling HCC progression. Mechanistically, HIF-1 α is a direct target of miR-223 in HCC, and miR-223 ameliorates HCC growth, angiogenesis and PD-1/PD-L1 activation in HCC surrounding regions by limiting HIF-1 α , suggesting that miR-223 plays a critical role in modulating hypoxia-induced tumor immunosuppression and angiogenesis, which may serve as a novel therapeutic strategy for HCC.



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Kupffer Cells Exhibit Phenotypic and Functional Heterogeneity in Mouse Liver Injury Unveiled via Single-Cell Transcriptomics

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Introduction: Kupffer cells reside in liver sinusoids and play key roles in preserving liver homeostasis in both health and injury. In healthy adult, Kupffer cells are immunosuppressive and maintained mainly by self-renewal. While how Kupffer cells behave during damage are still under studying.

Aims: This study is aiming to unveil the heterogeneity and function of Kupffer cells in liver injury.

Methods: Immunofluorescence was applied to unveil the changes of Kupffer cells upon liver injury. Kupffer cell functions were identified in vivo using clodronate liposomes-depleted Kupffer cell combined bile duct ligation liver injury mouse model. Heterogeneity and specific functions of Kupffer cell sub-clusters were studied via single cell RNA sequencing. Cell types were identified via single R and manually annotation.

Results: Liver injury were induced by bile duct ligation surgery. Immunofluorescence for liver tissue sections reflected the expansion of macrophages in liver injury, while liver resident macrophages Kupffer cell percentage was reduced. In order to study the significance and function of Kupffer cells, clodronate liposomes was used to exhaust Kupffer cells, which led to the liver more vulnerable to damage. In livers lacking Kupffer cells, ALT activity were up-regulated, meanwhile, necrotic areas were expanded, indicating the functional damage; Moreover, COL1A1 expression and fibrotic areas were increased suggesting the exacerbation of fibrosis. These results proved that decrease of Kupffer cell percentage resulted in the aggravation of liver injury. Then, single cell RNA sequencing was performed for in-depth study. Transcriptomes of nonparenchymal cells from healthy, bile duct ligation and CCl₄ administrated livers were obtained. 10 600 monocyte/macrophages were identified. After re-clustering, 3565 Kupffer cells and 3187 bone marrow-derived macrophages were confirmed. Functional analysis reflected repair-related and inflammation-related functions of Kupffer cells and bone marrow-derived macrophages, respectively. Therefore, further study was focused on Kupffer cells. Nine sub-clusters (KC-0 to KC-8) were identified in re-clustering analysis, three (KC-1, KC-4, KC-6) of which were enlarged in injury. Gene ontology analysis on top differential expressed genes of KC-1 and KC-4 reveal the repair-related and proliferative potential, respectively. What's more, KC-6 was characterized by highly expressing Mmp12 that regulate extracellular matrix remodeling. Excepted for these, though KC-7 was not expanded in injury, they highly expressed scavenger receptor Cd163 which played key roles in process of phagocytosis. This also suggested the importance of KC-7 in liver



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injury, for Kupffer cell phagocytosis had been considered to contribute to wound healing. The above data reflected the heterogeneity and protective functions of Kupffer cells in liver injury.

Discussion: Herein, we uncovered that the relative shortage of Kupffer cells is one of the reasons for the progression of liver injury. In this process, Kupffer cells showed homeostasis-maintaining function via different features. This finding may expand the knowledge of Kupffer cell function and heterogeneity. Further research might be focused on the importance of each Kupffer cell sub-cluster in vivo. Above all, the current study provides a basis for therapy of liver injury with specific Kupffer cell-supplementing.



Characterization of hepatic sinusoidal endothelium from metabolic syndrome rat model: phenotype and insulin uptake

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Introduction: The hepatic sinusoidal endothelium is highly permeable due to the presence of fenestrae between liver sinusoidal endothelial cells (LSECs) and the absence of basal membrane, thus play an important role in regulating the absorption and metabolism of nutrients and substances between the sinusoidal blood and hepatocytes. During ageing and liver diseases, LSECs undergo phenotypic changes ranging from decreased fenestration number and/or diameter to deposition of basement membrane known as capillarization. The metabolic syndrome (MetS) is defined as a combination of abnormalities including obesity, hypertriglyceridemia, hyperglycemia and has been reported to promote nonalcoholic fatty liver disease (NAFLD). However, the effect of MetS on fenestrations is yet to be investigated.

Aims: To investigate the alteration in liver sinusoidal endothelium and insulin uptake in a rat model of MetS.

Methods: Adult male SD rats were divided into two groups (n=10 each) of control and treatment, where they were given normal drinking water and drinking water containing 20% fructose ad libitum respectively for 8 weeks. The metabolic changes of obesity, dyslipidaemia, and hyperglycaemia were determined. Multiple indicator dilution (MID) method was performed in perfused livers using Evans Blue, 3H-sucrose and 14C-insulin. Livers were then perfusion-fixed and prepared for scanning electron microscopy. Data was analyzed using GraphPad Prism.

Results and discussions: Results showed that fructose has induced MetS characteristics by a significant increase of body weight, hypertriglyceridemia, and hyperglycemia. The MID method has revealed that the hepatic volume of distribution for insulin as a fraction of the extracellular space was significantly decreased ($p=0.02$), indicating reduced insulin uptake and clearance in the MetS liver. This was associated with lipid accumulation in the liver of MetS group compared to control. Metabolic syndrome also caused a reduction of fenestrations diameter ($p=0.02$) and endothelial porosity ($p=0.02$). These findings suggest that metabolic syndrome causes multiple changes at the liver endothelium and influence insulin action, which support the vital role of fenestrations in metabolism homeostasis and potential therapeutic target for liver pathology.



Enhanced secondary bile acids in portal vein escalate angiocrine functions of sinusoidal endothelial cells and liver regeneration during partial hepatectomy

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Background: Gut-microbiota derived signals significantly contribute towards liver injury and regeneration. Here, we aimed to identify important gut microbiota-derived metabolites in the portal circulation associated with liver regeneration after 70% partial hepatectomy (PHx).

Methods: Broad-spectrum antibiotics (Abx) were administered orally for 3 weeks to modulate the gut microbiome of rats, which were thereafter subjected to 70% partial hepatectomy (PHx). Bile acid profiling in peripheral and portal serum was performed by LC-MS/MS in controls (no surgery), 70% PHx and antibiotics plus 70% PHx. Significance among the groups was defined by log₂ (Fold change >1 and P< 0.05). Liver regeneration at day 2 post-PHx was evaluated by studying hepatocyte hyperplasia by PCNA staining, and expression analysis of the cell cycle genes. Primary rat hepatocytes, liver sinusoidal endothelial (LSEC) and hepatic stellate cells (HSC) were isolated by collagenase digestion for gene expression profiling and in vitro studies.

Results: BA pool was significantly different in peripheral and portal serum in both sham and PHx animals. In portal serum, Lithocholic acid (LCA, 1.6-fold, P<0.001), P< 0.05 and Taurolithocholic acid (TLCA, 1.8-fold, P<0.05) were significantly upregulated in PHx as compared to sham. In animals, where Abx were given before PHx, the expression of a few secondary bile acids including Glycoursodeoxycholic acid (1.6-fold, p<0.05) and LCA (2-fold, p<0.05) was significantly reduced in the portal serum that correlated markedly with reduced PCNA-positivity in hepatocytes and a downregulated hepatic gene expression of cyclin B1 (1.5-fold, p<0.0001), cyclin D1 (2-fold, p=0.0022) and cyclin E (2.5-fold, p<0.0001). In terms of BA receptors, the expression of TGR5 was significantly enhanced in both HSCs and LSECs (2-fold, P<0.05 and 2.6-fold, P<0.001) in PHx as compared to sham while it was decreased in Abx-treated PHx animals (4-fold, P<0.0001). In comparison to DMSO treatment, LCA treatment of LSECs enhanced the expression of angiocrine genes, GATA-4 (2-fold), Wnt-2 (6-fold) and CXCR7 (3-fold) (P<0.05 each) and secretion of Wnt2 (3-fold, P<0.01) in vitro. An incubation of hepatocytes with conditioned medium (CM) of LCA-treated LSECs induced a significant proliferation of hepatocytes with increased expression of FoxM1B (2.4-fold, P<0.05) and cyclin genes such as cyclin B1 (1.3-fold, P< 0.05), cyclin E (3.4-fold, p<0.0001) as compared to CM from LSECs alone.

Conclusions: Gut-microbiota derived secondary BAs such as LCA are enhanced in portal serum and may serve as one of the potent metabolic signals that facilitate the regenerative abilities of LSECs and hence hepatocyte proliferation.



Portal vein serotonin 1A receptor as a novel therapeutic target for portal hypertension

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Introduction Portal hypertension is the main non-neoplastic consequence of liver cirrhosis. Although previous studies have evaluated the intrahepatic mechanisms leading to it, no therapies are currently available and therefore further research is highly needed.

Aims This study aimed at expanding the knowledge about the pathophysiology of portal hypertension investigating the role of portal vein (PV) serotonin 1A receptor (HTR1A) as modulator of portal pressure and therefore representing novel therapeutic targets for this syndrome. **Methods** HTR1A modulations using pharmacological (selective HTR1A agonist and antagonist) and genetic (Htr1a knock-out [both non-specific and specific for vascular smooth muscle cells]) approaches were evaluated in three pre-clinical models of portal hypertension (thioacetamide, bile duct ligation and partial portal vein ligation, TAA, BDL and PPVL). PVs and primary smooth muscle cells of PVs (PVSMCs) were isolated from rats to explore the effect and the underlying molecular mechanisms of HTR1A on regulating contraction of PVs.

Results The HTR1A expression was specifically increased in the PVs under portal hypertension conditions both in rats and human samples. Both selective HTR1A antagonism and Htr1a knock-out ameliorated portal hypertension in rats, without any effects on liver fibrosis and systemic hemodynamics. Moreover, both 5-HT and HTR1A agonist led to contraction of the PVs and PVSMCs, which was abrogated by HTR1A antagonism or Htr1a knock-out. In addition, vascular smooth muscle cell-specific Htr1a knock-out mice were protected against portal hypertension. Finally, we demonstrated that HTR1A regulated the contraction of PVSMCs via cAMP pathway.

Discussion Our findings unveil an unknown role of the PV in the pathophysiology of portal hypertension, extending current understanding of pathophysiological mechanisms involved in portal hypertension, and identify HTR1A as a promising therapeutic target for attenuating portal hypertension.



Exacerbation mechanism of steatohepatitis in aged mice through activation of type I and type II NKT cells

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Introduction: Nonalcoholic steatohepatitis (NASH) develops with the background of obesity and metabolic syndrome. Due to its high prevalence, NASH is a major cause of liver-related death and hepatocellular carcinoma in many countries around the world. Several clinical studies have shown that aging is an independent exacerbating factor of NASH. We have previously shown that elder mice develop more severe HFD-induced steatohepatitis following over expression of inflammatory cytokines compared to young mice (J Gastroenterol Hepatol. 2020); however, the mechanism of immunostimulation has not yet been fully elucidated.

Aims: We focused on the role of natural killer T (NKT) cells in the age-related exacerbation mechanism of diet-induced steatohepatitis, and examined using type I NKT cells and all NKT cells-deficient mice.

Methods: Wild C57Bl/6J mice aged 12 or 55 weeks and $V\alpha 14$ knockout (KO) mice (lacking type I NKT cells) and CD1d KO mice (lacking all NKT cells) aged 55 weeks were fed either chow or a high-fat, high-cholesterol diet (HFHC: D09100310N, Research DIETS inc.) for 8 weeks.

Results: Elder wild mice developed much more severe macrovesicular steatohepatitis with many focal necrosis in the lobules than young wild mice. In $V\alpha 14$ KO and CD1dKO mice, HFHC-induced steatosis was clearly milder than in elder wild mice. Serum ALT levels significantly increased to 406 ± 46 IU/L in elder wild mice fed HFHC from 80 ± 32 IU/L in young wild mice fed HFHC, which was significantly decreased to 268 ± 45 IU/L in $V\alpha$ KO fed HFHC, further reduced to 159 ± 23 IU/L in CD1d KO mice fed HFHC. Expression of Toll-like receptor (TLR) 4 and tumor necrosis factor (TNF) α , IL-1 β mRNA in liver tissue was significantly enhanced by HFHC in elder wild mice rather than young wild mice, and was significantly reduced in $V\alpha$ KO mice and CD1dKO mice compared to elder wild mice, although there was no significant difference between elder $V\alpha$ KO and CD1dKO mice fed HFHC. In contrast, the expression of chemokines CC motif chemokine 2 (CCL2) and C-X-C motif chemokine ligand 2 (CXCL2) was significantly increased in wild mice fed HFHC diet compared to mice fed normal chow, which was not significantly decreased in $V\alpha 14$ KO mice whereas significantly reduced in CD1dKO mice.

Discussion: These findings indicated that type I and type II NKT cells are involved in the exacerbation of steatohepatitis in elder mice through different signal enhancement mechanisms. It is considered that type I NKT cells are involved through the TLR4-TNF α /IL1 β -mediated pathways, and in contrast, type II NKT cells are involved through the enhancement of chemokines such as CCL2 in the pathogenesis of steatohepatitis caused by HFHC. In conclusion, type I and type II NKT cells additively exacerbate steatohepatitis caused by high fat- high cholesterol diet. Changes in the regulation of type I/II NKT cells may be a key event exacerbating NASH in the elderly.



Senescence of liver-resident LSEC progenitors (sprocs) in aged rats leads to recruitment of bone marrow sprocs but not to pseudocapillarization

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Introduction: The lack of liver sinusoidal endothelial cell (LSEC) fenestration in aged humans and experimental animals is called pseudocapillarization. Pseudocapillarization is associated with loss of chylomicron remnant clearance with consequent post-prandial hypertriglyceridemia, a risk factor for cardiovascular disease. There is limited characterization of pseudocapillarization.

Methods: LSECs were isolated by elutriation and sprocs identified as the CD133+ fraction of LSECs. Porosity (% cell surface covered by fenestration) of LSEC progenitor cells (sprocs) and LSECs isolated from 24-month old Fischer rats (NIA colony) was determined by ImageJ analysis of scanning electron microscopy. 8-week old Lewis rats were transplanted with bone marrow (BM) from Lew-Tg(CAG-EGFP)ys Lewis rats to track GFP+ BM cells and aged to 24 months; of note, our lab has never observed any evidence of radiation-induced liver disease (RILD) in the protocol used for bone marrow transplantation. LSECs were separated by flow cytometry for GFP (=bone marrow origin), the senescence marker senescence-associated beta-galactosidase (GAL+), and CD133 (=progenitor cells).

Results: 1. Liver-resident sprocs in young rats are fully fenestrated 1. In contrast, intrahepatic sprocs as well as LSECs from aged rat are pseudocapillarized (porosity intrahepatic sprocs in aged rats: $0.38 \pm 0.22\%$; LSECs: $0.43 \pm 0.26\%$; normal LSEC porosity ~6%). 2. 42% of resident sprocs (sprocs of liver origin, CD133+GFP-) and 43% of LSECs of liver origin (CD133-GFP-) were senescent (GAL+. 3. In young rats <3% of sprocs are of BM origin1. In contrast, in aged rats 25% of intrahepatic sprocs (=resident plus BM-derived, CD133+) were BM-derived (GFP+). Whereas in young rats <1% of LSECs are BM-derived1, in aged rats 46% of LSECs are BM-derived. 4. Porosity of resident LSECs (GFP-) was $0.13 \pm 0.08\%$ and BM-derived LSECs (GFP+) was $0.10 \pm 0.06\%$.

Conclusions: 1. The lack of fenestration of intrahepatic sprocs (resident + bone marrow-derived sprocs in the liver) suggests that pseudocapillarization begins at the level of the progenitor cell. 2. Given the prevalence of senescence of resident sprocs and LSECs, the significant percentage of sprocs and LSECs of BM origin indicates that senescence of resident sprocs and LSECs leads to recruitment of BM sprocs to maintain the population of LSECs within the aged liver. 3. BM-derived sprocs recruited to healthy young rat liver after liver injury become fully fenestrated LSECs1, but in healthy aged rats both BM-derived and resident LSECs lack fenestration. The lack of fenestration of both resident and BM-derived LSECs suggests that pseudocapillarization is due to an environmental factor. 4. Given that only 25% of LSECs are senescent, the LSEC porosity



data do not support that pseudocapillarization is a consequence of senescence.

References. 1. Wang L et al. JCI 122: 1567-73, 2012, PMID: 22406533

Separation of BM and resident LSECs and sprocs by flow sprocs (CD133+): cytometry			LSECs (CD133-)
BM-derived, senescent cells	GFP+Gal+	2.5 ± 1.2 %	1.5 ± 0.8 %
BM derived, non-senescent	GFP+Gal -	22.6 ± 4.2 %	44.3 ± 7.8 %
resident cells, senescent	GFP-Gal+	31.2 ± 2.0 %	23.3 ± 7.4 %
resident, non-senescent	GFP-Gal-	43.7 ± 2.0 %	31.5 ± 1.2 %



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the role of sinusoidal cells in the hepatobiliary diseases

Spatial mapping of endothelial cells using single-cell RNA sequencing during the injury and recovery phases of acetaminophen hepatotoxicity

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Introduction: An acetaminophen (APAP) overdose is the leading cause of acute liver failure in the United States. While hepatocytes are the primary cell type affected by APAP toxicity, it is now evident that non-parenchymal cells and infiltrating cells are involved in the injury and recovery phases of APAP overdose. Single-cell RNA sequencing (scRNAseq) allows for unprecedented resolution to explore the transcriptome of individual cell subpopulations and to spatially resolve their location within the hepatic lobule. Liver endothelial cells (ECs) play important physiological roles in scavenging and filtration of the blood before reaching systemic circulation. In particular, the liver sinusoidal endothelial cells (LSECs) play a critical role in antigen presentation and influencing the immune response. How ECs in different spatial regions modulate their functions and influence cell-cell interactions during acute liver injury remains largely unexplored.

Aims: To spatially resolve endothelial cells in the liver lobule and explore their role in cell-cell communication during the APAP hepatotoxicity time course.

Methods: Four published scRNAseq APAP toxicity datasets were combined with unpublished scRNAseq from our lab to capture both the injury and recovery phases (6h, 24h, 48h, 72h, 96h and 120h). Eight- to twelve-week-old C57BL/6J mice were fasted overnight and treated with either 300 mg/kg or 500 mg/kg APAP and sacrificed 6 to 120h later. Seurat v3 was used for data integration and initial quality control which involved filtering cells with fewer than 600 genes or cells with mitochondrial gene expression greater than 20%. Cell types were defined based on established marker genes which in turn resulted in an aggregated dataset of 94,012 cells consisting of neutrophils, hepatic stellate cells, B cells, NK cells, dendritic cells, periportal hepatocytes, pericentral hepatocytes, Kupffer cells, macrophages, and endothelial cells. After initial cell labeling, the ECs were extracted and spatially mapped based on either marker genes or using the AddModuleScore() function in Seurat. Comparisons between APAP and control ECs based on spatial location were carried out in Seurat using a Wilcoxon rank sum test. Top differentially expressed genes (adj. p value < 0.01) were used as inputs into pathway analysis using Metascape. Cell-cell interactions between the spatially mapped ECs and the other cells in the liver were carried out using CellChat. An interactive tool to explore these integrated datasets is available as an R Shiny which can be accessed at <https://github.com/dumbaugh/Single-Cell/tree/main/ShinyProject>.

Results: Endothelial cells were classified into 5 groups based on their spatial characteristics: arterial ECs, periportal ECs (PP-ECs), midzonal ECs (MZ-ECs), pericentral ECs (PC-ECs), pericentral venous ECs (endothelial cells that line the central vein) and a separate group identified



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as proliferating ECs. Vwf readily identified arterial ECs, Ntn4, Msr1, and Efnb2 identified PP-ECs, Stab2 and Lyve identified MZ-ECs, Wnt2, Kit and Thbd identified PC-ECs, pericentral venous ECs by Wnt9b, and Mki67, Pclaf identified proliferating ECs. Pathway analysis revealed an induction of pathways involved in platelet degranulation, regulation of angiogenesis, and blood vessel development in both PC and PP-ECs. However, PC-ECs had a significant induction of pathways related to regulation of cell death, cell adhesion, and receptor-mediated endocytosis. Examination of global cell interactions between ECs and other cell types found that 59.9% were secreted signals, 18.7% were direct cell-cell contact, and 21.4% were ECM-receptor interactions. The CCL signaling network was identified as an important communication system between endothelial cells and macrophages with a restorative phenotype.

Discussion: We provide the first spatial reconstruction of the endothelial cells during the injury and recovery phases of APAP overdose. We uncover a rich network of cell-cell interactions involved in facilitating recovery of the liver. Importantly, to promote ease of access to these rich datasets, we have created a R Shiny application allowing for a user-friendly exploration.



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