Liver Cancer is now the 2nd cause of cancer-related death worldwide
Abstracts of papers presented at the 2016 Cold Spring Harbor Asia Conference

LIVER DISEASES & TUMORIGENESIS: FROM BENCH TO BEDSIDE

April 25–April 29, 2016

Arranged by

Gen-Sheng Feng, University of California San Diego, USA
Hongyang Wang, Eastern Hepatobiliary Surgery Hospital/Institute, China
Jessica Zucman-Rossi, INSERM, France
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![Lilly Logo](image)

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为了进行更为深入的研究，观察到肿瘤中隐藏的所有信息至关重要。借助PERKINELMER肿瘤免疫研究方案，您可以在同一石蜡标本上同时对多种免疫组化反应进行定位观察、检测和对比，从而这些数据完全基于相同的肿瘤组织及其微环境信息，凭借有效的生物标志物评价和更好的统计数据，有望发现新的生物标志物方法，最终形成一套完整的组织原位分析方案。

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| Wednesday | 9:45 pm| Free Open Bar<br>
Sponsored by BioBAY                                                        |
| Thursday  | 9:00 am| 7 Cancer Genomics and Evolution                                       |
| Thursday  | 2:00 pm| 8 Liver Metabolism and Reprogramming                                  |
| Thursday  | 6:00 pm| Poster Awards<br>
Cocktails and Banquet                                                        |
| Friday    | 9:00 am| 9 Animal Models for Liver Disease and Cancer                          |

Oral presentation sessions are located in the Watson Auditorium. 
Poster session and Chinese Tea & Beer Tasting are in the Poster Hall. 
Cocktail social hour is held in the Poster Hall. 
Old Suzhou visits depart from the hotel lobby. 
*optional tour requires additional fee.*

Meal locations and times are as follows: 
Breakfast Octagon 7:00am - 9:00am 
Lunch Octagon 12:00am - 1:30pm 
Dinner Octagon 6:00pm - 7:30pm 
Banquet Suz Garden 7:00pm 
More information will be available at CSHA office. 
(Map at the end of this abstract book)
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PROGRAM

MONDAY, April 25—7:00 PM

Welcome Remarks
Maoyen Chi, CSH-Asia

SESSION 1  KEYNOTE SPEAKERS

Introduction by: Hong-Yang Wang, Eastern Hepatobiliary Hospital / Institute of Shanghai, China

Tumor suppressor and tumor maintenance genes
Scott W. Lowe [35']
Presenter affiliation: Howard Hughes Medical Institute, New York, New York.

Introduction by: Gen-Sheng Feng, University of California San Diego, USA

Revisiting nosology of liver tumors through the scope of genomics
Jessica Zucman-Rossi [35']
Presenter affiliation: INSERM, Paris, France; Université Paris Descartes, Paris, France.

TUESDAY, April 26—9:00 AM

SESSION 2  LIVER FIBROSIS, INFLAMMATION AND CARCINOGENESIS

Chairpersonss: David Brenner, UC San Diego, La Jolla, California, USA
Hongyang Wang, Eastern Hepatobiliary Hospital / Institute of Shanghai, China

Genetics of liver fibrosis
David A. Brenner [20']
Presenter affiliation: UC San Diego, La Jolla, California.
Immunoregulation of liver fibrosis—Novel pathways
Sophie Lotersztajn [20’]
Presenter affiliation: Center for Research on Inflammation, Paris, France; Université Paris Diderot, Paris, France.

The novel potential therapeutic target for liver fibrosis—Intracellular oxidative stress sensor
Jing Liu, Pingsheng Chen [10’]
Presenter affiliation: Southeast University, Nanjing, China.

Coffee / Tea Break

New insights into inflammation regulation and liver cancer
Hongyang Wang [20’]
Presenter affiliation: Eastern Hepatobiliary Surgery Hospital/Institute of Shanghai, Shanghai, China; National Center for Liver Cancer, Shanghai, China.

A bifunctional role for c-Jun in regulating liver homeostasis during fibrosis
Kanaga Sabapathy [20’]
Presenter affiliation: National Cancer Centre Singapore, Singapore; Duke-NUS Graduate Medical School, Singapore.

Targeting nuclear receptor CAR in HCC
Bingning Dong, Ju-Seog Lee, Yun-Yong Park, Feng Yang, Wendong Huang, David D. Moore [10’]
Presenter affiliation: Baylor College of Medicine, Houston, Texas.

SESSION 3 POSTER SESSION

Compensatory roles of CD8+ T cells and dendritic cells in immune regulation in the gut with non-functional CD4+ Tregs
Jaehee Ahn, Hyunjeong Ko
Presenter affiliation: Kangwon National University, Chuncheon, South Korea.
Quantitative succinylome study in liver of non-alcoholic steatohepatitis model in rats
Yang Cheng, Tianlu Hou, Jianjie Chen
Presenter affiliation: Shanghai Hospital for Infectious Diseases of Pudong New Area, Shanghai, China; Shuguang Hospital affiliated to Shanghai University of TCM, Shanghai, China.

Gene networks activated in cultivated primary human and mouse hepatocytes represent in vivo disease states
Steven Dooley, Patricio Godoy, Wolfgang Schmidt-Heck, Jan G. Hengster
Presenter affiliation: Medical Faculty Mannheim, Heidelberg University, Mannheim, Germany.

Hepatocyte-specific Smad7 deletion accelerates DEN induced HCC via activation of STAT3 signaling in mice
Steven Dooley, Teng Feng, Thorsten Maass, Andreas Teufel, Silke Marhenke, Arndt Vogel, Nadja M. Meindl-Beinker
Presenter affiliation: Medical Faculty Mannheim, Heidelberg University, Mannheim, Germany.

Metabolic alterations of clinical significance in liver cancer
Steven Dooley, Zeribe Nwosu, Christoph Meyer
Presenter affiliation: Medical Faculty Mannheim, Heidelberg University, Mannheim, Germany.

Vessels that encapsulated tumor clusters—Implication in hepatocellular carcinoma metastasis and its regulation mechanism
Jian-Hong Fang, Hui-Chao Zhou, Shi-Mei Zhuang
Presenter affiliation: Sun Yat-sen University, Guangzhou, China.

Differential role of hepatic insulin receptor/PTEN and IGF-1 receptor/PTEN signaling in liver metabolism and inter-organ communication
Dorothea Portius, Cyril Sobolewski, Anne-Sophie Ay, Jean-Luc Pitetti, Nicolas Calo, Flavien Berthou, Laurent Vinet, Xavier Montet, Serge Nef, Michelangelo Foti
Presenter affiliation: Faculty of Medicine, University of Geneva, Geneva, Switzerland.

The role of biomechanics in HCC microenvironment
Jian Gao, Youguang Wang, Jing Du, Congying Wu
Presenter affiliation: Peking University, Beijing, China.
HCCDB—Hepatocellular carcinoma gene expression atlas  
Dongfang Wang, Guchao Zhang, Jin Gu  
Presenter affiliation: Tsinghua University, Beijing, China.  

Non-cell-autonomous function of YAP in initiating liver tumors by recruiting tumor associated macrophages  
Xiaocan Guo, Bin Zhao  
Presenter affiliation: Life Sciences Institute, Hangzhou, China.  

Inhibitory role of WW45/MST1 on PI3K pathway in liver disease  
Sun-Hye Jeong, Han-Byul Kim, Dae-Sik Lim  
Presenter affiliation: KAIST, Daejeon, South Korea.  

CCR2 antagonist ameliorates non-alcoholic fatty liver disease in type 2 diabetes  
Hyun-Jeong Ko, Jae-Hee Ahn, Hong-Min Kim, Choon Hee Chung  
Presenter affiliation: Kangwon National University, Chuncheon, South Korea.  

DJ-1 promotes expansion of liver progenitor cells in HBV patients and in a DDC-induced murine model of chronic liver injury  
Lili Chen, Meng Luo, Yankai Wen, Jinyang Gu, Qiang Xia, Xiaoni Kong  
Presenter affiliation: Shanghai Jiao Tong University, Shanghai, China.  

Loss of ARID1A expression potentiates angiogenesis of liver cancer by up-regulating ANG2  
Chaobo Hu, Weiping Li, Feng tian, Yuan Ji, Zhong Wang, Junhao Hu, Lijian Hui  
Presenter affiliation: Shanghai Institute of Biochemistry and Cell Biology, Shanghai, China.  

Differential roles of YAP activity in regulation of hepatocyte dedifferentiation  
Jae Oh Park, Da-Hye Lee, Dae-Sik Lim  
Presenter affiliation: Korea Advanced Institute of Science and Technology(KAIST), Daejeon, South Korea.  

High levels of peroxiredoxin 1 in liver cancer predict poor prognosis  
YuLin Sun, Fang Liu, LanPing Zhou, XiaoHang Zhao  
Presenter affiliation: Cancer Hospital, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing, China.
Role of c-Jun in hepatic fibrosis
Min Xie, Derrick Chia, Kanaga Sabapathy
Presenter affiliation: National Cancer Centre Singapore, Singapore.

Identification of metabolic alterations in hepatitis B virus core protein transfected hepatocellular carcinoma cell by integrative multi-omics analysis
Qi Xie, Fengxu Fan, Wei Wei, Ping Xu
Presenter affiliation: National Center for Protein Sciences, Beijing, China.

Essential roles of Myc in mouse hepatocarcinogenesis induced by the activation of AKT and RAS pathways
Bing Xin, Masahiro Yamamoto, Kiyonaga Fujii, Takako Ooshio, Xi Chen, Yoko Okada, Kenji Watanabe, Yuji Nishikawa
Presenter affiliation: Asahikawa Medical University, Asahikawa, Japan.

Direct reversion of HCC cell to functional liver-like cell with the loss of tumorigenecity potential by small-molecule compounds
Xu Zhang, Guoxu Fang, Liping Li, Peilin Zhang, Hongyang Wang
Presenter affiliation: Eastern Hepatobiliary Surgery Institute, Shanghai, China; National Center for Liver Cancer, Shanghai, China.

Biomarkers of colorectal carcinoma liver metastasis in urine
Meng Cai, YuLin Sun, ZhiXiang Zhou, XiaoHang Zhao
Presenter affiliation: Cancer Hospital, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing, China.

TUESDAY, April 26—4:30 PM
Chinese Tea and Beer Tasting
SESSION 4  LIVER REGENERATION, METABOLISM AND CELL LINEAGES

Chairpersons:  David Cohen, Brigham and Women's Hospital, Boston, Massachusetts, USA
            Athushi Miyajima, University of Tokyo, Japan

Remodeling of the biliary tree during liver regeneration
Atsushi Miyajima, Kenji Kamimoto, Kota Kaneko, Tohru Itoh  [20']
Presenter affiliation: University of Tokyo, Tokyo, Japan.

Hepatocyte plasticity in liver regeneration and cancer
Holger Willenbring  [20']
Presenter affiliation: University of California San Francisco, San Francisco, California.

CRISPR/Cas9-mediated knock-in of large DNA in human cells
Xiangjun He, Bo Feng  [10']
Presenter affiliation: Key Laboratory for Regenerative Medicine, Ministry of Education, Hong Kong, China.

Coffee / Tea Break

Acyl-CoA thioesterase-mediated metabolic regulation
David E. Cohen  [20']
Presenter affiliation: Brigham and Women's Hospital, Boston, Massachusetts.

Liver polyploid—Dr Jekyll or Mr Hide?
Chantal Desdouets  [20']
Presenter affiliation: INSERM U1016, Institut Cochin, Paris, France; CNRS, UMR8104, Paris, France; Université Paris Descartes, Paris, France.

Stress-activated miR-21/miR-21* in hepatocytes promotes lipid and glucose metabolic disorders associated with high-fat diet consumption
Nicolas Calo, Pierluigi Ramadori, Yannick Romero, Christine Maeder, Margot Fournier, Pia Rantakari, Fu-Ping Zhang, Matti Putanen, Jean-François Dufour, Bostjan Humar, Serge Nef, Michelangelo Foti  [10']
Presenter affiliation: Faculty of Medicine, University of Geneva, Geneva, Switzerland.
Comprehensive analysis of The Cancer Genome Atlas defines a unique molecular signature of fibrolamellar carcinoma and identifies novel candidate oncogenes
Timothy A. Dinh, Eliane Wauthier, Eva Vitucci, Reid Reid, Praveen Sethupathy [10’]
Presenter affiliation: UNC Chapel Hill, Chapel Hill, North Carolina. 36

WEDNESDAY, April 27—9:00 AM

SESSION 5 MECHANISMS AND PATHWAYS OF LIVER TUMORIGENESIS

Chairpersons: Robert Schwabe, Columbia University, New York, New York, USA
Jessica Zucman-Rossi, INSERM U674, Paris, France

Contribution of hepatic stellate cells to hepatocarcinogenesis
Robert F. Schwabe [20’]
Presenter affiliation: Columbia University, New York, New York. 37

MicroRNA—Mechanism of deregulation and its implication in hepatocellular carcinoma
Shi-Mei Zhuang [20’]
Presenter affiliation: Sun Yat-sen University, Guangzhou, China. 38

HCC targeted with mTOR inhibitors and biguanides
Xuemei Ge, Sónia R. Veiga, Hala E. Thomas, Carol A. Mercer, George Thomas, Sara C. Kozma [10’]
Presenter affiliation: Catalonian Institute of Oncology/IDIBELL, Barcelona, Spain. 39

Coffee / Tea Break

Cooperating or opposing roles of Pten and Shp2 in hepatocarcinogenesis or leukemogenesis
Gen-Sheng Feng [20’]
Presenter affiliation: University of California San Diego, La Jolla, Armenia. 40
Metabolic reprogramming in beta-catenin-driven liver carcinogenesis in mice
Sabine Colnot [20']
Presenter affiliation: INSERM, Paris, France.

Regulation of hepatocytes oxidative stress by SIRT3
Ligen Lin, Jingxin Liu, Dan Li, Anqi Wang, Qiang Tong, Keyun Chen [10]
Presenter affiliation: University of Macau, Macau, Macau.

WEDNESDAY, April 27—2:00 PM
Visit to Old Suzhou

WEDNESDAY, April 27—7:00 PM

SESSION 6  DRUG TARGETING AND THERAPEUTICS

Chairpersons: Xiao-fan Wang, Duke University Medical Center, Durham, North Carolina, USA
Mathias Heikenwalder, Technische Universität München, Munich, Germany

Inflammation-dependent IL-18 signaling restricts hepatocellular carcinoma growth by systematic modulation of lymphocyte activity
Xiao-Fan Wang, Geoffrey J. Markowitz, Pengyuan Yang, Jing Fu, Qi-Jing Li, Hongyang Wang [20']
Presenter affiliation: Duke University Medical Center, Durham, North Carolina.

Pharmacological targeting of kinases Mst1 and Mst2 augments tissue repair and regeneration
Fuqin Fan, Zhixiang He, Lu-Lu Kong, Qinghua Chen, Quan Yuan, Shihao Zhang, Hao Liu, Lanfen Chen, Cai-Hong Yun, Xianming Deng, Dawang Zhou [20']
Presenter affiliation: Xiamen University, Xiamen, China.
Differential roles of CIDE proteins in promoting lipid droplet fusion and growth in subpopulations of hepatocytes
Wenyi Xu, Lizhen Wu, Peng Li, Linkang Zhou [10']
Presenter affiliation: Tsinghua University, Beijing, China. 45

Coffee / Tea Break

Identification of novel targets to treat NASH and its transition to HCC
Elena Kotsiliti, Dominik Pfister, Mohsen Malehmir, Valentina Leone, Kristian Unger, Achim Weber, Mathias Heikenwalder [20']
Presenter affiliation: Technische Universität München (TUM), Helmholtz Zentrum München (HMGU), Munich, Germany; German Cancer Research Center (DKFZ), Heidelberg, Germany. 46

Endogenous molecular-cellular network theory and its applications in hepatocellular carcinoma (HCC)
Gaowei Wang, Xiaomei Zhu, Ping Ao [10']
Presenter affiliation: Shanghai Jiao Tong University, Shanghai, China. 47

Assessing liver fibrosis in patients with chronic hepatitis B—Comparisons of T1 mapping on Gd-EOB-DTPA-enhanced 1.5T MRI with aspartate aminotransferase-to-platelet ratio index and fibrosis-4
Li Yang, Ying Ding, Shengxiang Rao, Mengsu Zeng, Heqing Wang, Ruofan Sheng [10']
Presenter affiliation: Shanghai Institute of Medical Imaging, Zhongshan Hospital, Fudan University, Shanghai, China. 48

Demethyleneberberine attenuates non-alcoholic fatty liver disease with activation of AMPK and inhibition of oxidative stress
Xiaoyan Qiang, Lulu Xu, Miao Zhang, Yubin Zhang [10']
Presenter affiliation: State Key Laboratory of Natural Medicines, Nanjing, China. 49

Free Open Bar
Sponsored by BioBAY
SESSION 7  CANCER GENOMICS AND EVOLUTION

Chairpersons:  
Eithan Galun, Hadassah Hebrew University Hospital, Jerusalem, Israel  
Xin W. Wang, National Cancer Institute, NIH, Bethesda, Maryland, USA

Integrated omics investigation of tumor heterogeneity and drivers in liver cancer  
Xin W. Wang  [20']  
Presenter affiliation: National Cancer Institute, Bethesda, Maryland.  

Programmed cell death pathways in liver injury and hepatocarcinogenesis  
Tom Lüdde  [20']  
Presenter affiliation: University Hospital RWTH Aachen, Aachen, Germany.

The potential of novel tetrahydroxylated bile acids (THBAs) to prevent cholangitis and liver cancer in Abcb4−/− mice  
Renxue Wang, Jonathan Sheps, Lin Liu, Ian Welch, Victor Ling  [10']  
Presenter affiliation: British Columbia Cancer Agency, Vancouver, Canada.

Coffee / Tea Break

Mir 122 is a hepatic-hormone with systemic effects  
Eithan Galun  [20']  
Presenter affiliation: Hadassah Hebrew University Hospital, Jerusalem, Israel.

Evolution of cancer cell populations  
Xuemei Lu, Chung-I Wu  [20']  
Presenter affiliation: Beijing Institute of Genomics, Beijing, China.

Exploiting the pentose phosphate pathway as therapeutic target in hepatocellular carcinoma treatment  
Iris Ming-Jing Xu, Robin Kit-Ho Lai, Shu-Hai Lin, Aki Pui-Wah Tse, David Kung-Chun Chiu, Hui-Yu Koh, Cheuk-Ting Law, Chun-Ming Wong, Zongwei Cai, Carmen Chak-Lui Wong, Irene Oi-Lin Ng  [10']  
Presenter affiliation: The University of Hong Kong, Hong Kong, Hong Kong.
Role of methionine adenosyltransferase 1A in liver cancer
Shelly C. Lu [20’]
Presenter affiliation: Cedars-Sinai Medical Center, Los Angeles, California.

Reduced growth ability and increased nuclear abnormality in HBV-infected human hepatocytes of humanized chimeric mouse liver
Chise Tateno, Yasumi Yoshizane, Chihiro Yamasaki, Ami Yanagi, Yuko Ogawa, Yuji Ishida [10’]
Presenter affiliation: PhoenixBio Co., Ltd., Higashihiroshima, Japan; Hiroshima University, Hiroshima, Japan.

Role for the ER stress sensor IRE1α in promotion of hepatocellular carcinoma
Ying Wu, Bo Shan, Yong Liu [10’]
Presenter affiliation: Institute for Nutritional Sciences, Shanghai, China.

Genomics analysis reveals a novel regulatory role of hepatocyte orosomucoid on their proliferation during liver regeneration
Xian-Yang Qin, Mitsuko Hara, Erik Amer, Harukazu Suzuki, Piero Carninci, Alistair Forrest, Soichi Kojima [10’]
Presenter affiliation: RIKEN, Wako and Yokohama, Japan.

Coffee / Tea Break

Direct reprogramming of human fibroblasts to functional hepatocyte-like cells
Lijian Hu [20’]
Presenter affiliation: Chinese Academy of Sciences, Shanghai, China.
The regulation of metabolic reprogramming in hepatocellular carcinoma cells
Ping Gao [20']
Presenter affiliation: University of Science and Technology, Hefei, China. 61

β-catenin-dependent erythropoiesis in adult mice deficient in hepatic ARID1A chromatin remodeler
Rozenn Riou, Cécile Godard, Angélique Gougelet, Frédérique Verdier, Meriem Ladli, Franck Lager, Zhong Wang, Christine Perret, Sabine Colnot [10']
Presenter affiliation: INSERM, Paris, France; CNRS, Paris, France; Université Paris Descartes, Paris, France. 62

Mapping the tissue microenvironment—BioImaging informatics and the tissue section
Christopher Johnson [20']
Presenter affiliation: PerkinElmer Inc, Melbourne, Australia. 63

Liver-directed gene therapy for transplantation tolerance induction
Alexandra Sharland [10']
Presenter affiliation: University of Sydney, Sydney, Australia. 64

THURSDAY, April 28—6:00 PM

COCKTAILS and BANQUET

Poster Awards given by
Geng-Sheng Feng
Hongyang Wang
Jessica Zucman-Rossi
SESSION 9  ANIMAL MODELS FOR LIVER DISEASES AND CANCER

Chairpersons:  Young-Joon Surh, Seoul National University, Seoul, South Korea
Lars Zender, University Hospital Tübingen, Tübingen, Germany.

Direct in vivo shRNA screening for functional target discovery in hepatocellular carcinoma
Lars Zender  [20']
Presenter affiliation: University Hospital Tuebingen, Tuebingen, Germany.  65

CRL4(DCAF8) ubiquitin ligase targets histone H3 and controls mouse liver homeostasis
Gaofeng Li, Tong Ji, Jiang Chen, Xiujun Cai, Yong Cang  [20']
Presenter affiliation: Zhejiang University, Hangzhou, China.  66

Kupffer-cell derived TNF triggers cholangiocellular neoplasia via JNK in the context of hepatic mitochondrial dysfunction and oxidative stress
Presenter affiliation: Technische Universität München and Helmholtz Zentrum München, Munich, Germany; German Cancer Research Center (DKFZ), Heidelberg, Germany.  67

Coffee / Tea Break

Mutation and subsequent activation of Nrf2 in diethylnitrosamine-treated mouse liver accelerates progression of hepatocellular carcinoma through metabolic alterations
Hoang Kieu Chi Ngo, Do-Hee Kim, Young-Nam Cha, Young-Joon Surh  [20']
Presenter affiliation: Seoul National University, Seoul, South Korea.  68
Hepatocellular carcinoma repression by TNFα-mediated synergistic lethal effect of mitosis defect-induced senescence and cell death sensitization
Dan Li, Jing Fu, Min Du, Haibin Zhang, Lu Li, Jin Cen, Weiyun Li, Xiaotao Chen, Yunfei Lin, Edward Conway, Eli Pikarsky, Hongyan Wang, Yuan Ji, Hong-Yang Wang, Guoyu Pan, Lijian Hui [10']
Presenter affiliation: Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences, Shanghai, China.

Deregulation of epigenetic regulator SETDB1 contributed to hepatocellular carcinoma progression and cancer metastasis
Chun-Ming Wong, Lai Wei, Cheuk-Ting Law, Daniel W. Ho, Felice H. Tsang, Sandy L. Au, Karen M. Sze, Joyce M. Lee, Carmen C. Wong, Irene O. Ng [10']
Presenter affiliation: The University of Hong Kong, Hong Kong, China.
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Cancer arises through an evolutionary process whereby normal cells acquire mutations that erode growth controls, leading to the expansion of aberrantly proliferating cells. Such mutations activate oncogenes or inactivate tumor suppressors, each bestowing new capabilities to emerging tumors. Still, cancer is not an inevitable consequence of mutation but is instead kept in check by intrinsic tumor-suppressor programs activated in damaged cells. Accordingly, our laboratory studies such mechanism in order to reveal key regulatory nodes controlling basic cellular processes and to identify the strategies nature uses to combat cancer. More recently, our interests have expanded to explore the action of tumor maintenance genes – those genes needed to sustain the proliferation and survival of malignant cancer cells – with the goal of identifying cancer vulnerabilities and therapeutic targets. Our approach combines powerful mouse models, genetics, and genomics in a coordinated manner that allows us to study tumor suppressor and tumor maintenance networks in a comprehensive way. Recent efforts to apply these tools towards the identification and characterization of tumor maintenance genes in hepatocellular carcinoma will be discussed.
REVISITING NOSOLOGY OF LIVER TUMORS THROUGH THE SCOPE OF GENOMICS

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Hepatocellular carcinoma (HCC) is one of the leading causes of death by cancer worldwide. It is mainly developed on cirrhosis due to chronic hepatitis B and C, metabolic and alcoholic liver diseases in western countries. In contrast, hepatocellular adenomas are rare benign liver tumors frequently developed in women after oral contraception. Recent advances in molecular classification and dissection of genetic and epigenetic drivers have increased our knowledge of the molecular diversity of benign and malignant liver tumors. Using genomic approaches, we identified several new oncogenes and tumor suppressor genes and we described a molecular classification of hepatocellular adenomas that is used in clinical routine. Recently, using sequencing, we identified TERT promoter mutations activating telomerase as the most important mechanism of malignant transformation of both adenoma in carcinoma and of cirrhotic nodules in carcinoma. We also found new etiological factors predisposing to liver tumor development with the finding of recurrent AAV2 insertions in cancer driver genes but also mutational signatures as the result of exposure to specific genotoxic agents. Finally, next generation sequencing was particularly fruitful to identify new drug targets in hepatocellular carcinoma and these finding open new avenues to develop genome based clinical trials.
GENETICS OF LIVER FIBROSIS.

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Following chronic liver injury of any etiology, there is progressive fibrosis. To date, removing the causative agent is the only effective therapy to stop or even reverse liver fibrosis. Induction of liver fibrosis in mice such as with chronic carbon tetrachloride administration, bile duct ligation, chronic ethanol administration, or diet induced steatohepatitis have provided models to study the genetics of liver fibrosis. Furthermore, the identification of activated hepatic stellate cells (HSCs) as the major fibrogenic cell type in the injured liver, as well as the recognition of key cytokines involved in this process, have provided new insights into the pathogenesis of liver fibrosis. Early insight into the genetics of fibrosis was provided by the observation that different strains of mice have different sensitivities to the above models of liver fibrosis. Subsequent backcrossing studies have revealed quantitative trait loci (QTLs) that account for strain differences, including compliment factor 5. Most studies though have used the sensitivity of knockout mice to demonstrate sensitivity or resistance compared to wild-type mice. Analysis of these studies have revealed detailed pathways leading to liver fibrosis that can be characterized genes by modifying: (1) the response to hepatic injury, (2) the interaction with bacterial products, (3) the generation of oxidant stress, (4) the resultant inflammation, (5) the activation and proliferation of hepatic stellate cells, (6) abnormal angiogenesis and (7) the development of the fibrous scar.

A genetic component for human liver fibrosis has been demonstrated by twin studies. The level of liver fibrosis correlated between monozygotic twins ($r^2 = 0.48; P < .002$) but not between dizygotic twins ($r^2 = 0.12; P = .7$). In multivariable models adjusted for age, sex, and ethnicity, the heritability of hepatic fibrosis (based on liver stiffness) was 0.5 (95% confidence interval, 0.28-0.72; $P < 6.1 \times 10^{-11}$). Candidate gene studies based on the genetics of the mouse studies have tried to identify genetic risk factors for human liver fibrosis using single nucleotide polymorphisms in genes that were previously identified in the mouse models of liver fibrosis. However, these studies have been difficult to reproduce, perhaps reflecting insufficient statistical significance. Genome wide association studies (GWAS) have identified PNPLA3 (which regulates hepatic triglyceride content), TM6SF2 (which regulates hepatic lipid secretion), TLR4 (the receptor for LPS, and identified in mouse liver fibrosis), and aquaporin (a water channel, and identified in mouse liver fibrosis). Association studies using whole genome sequencing are underway.
Sustained hepatic inflammation resulting from parenchymal liver injury is a major driving force of both fibrosis progression and fibrosis resolution. Convergent data demonstrate that the hepatic innate and adaptive immune system play a key role in the initiation, perpetuation and maintenance of the inflammatory response, with major deleterious impact on hepatocyte lesions and fibrosis. Recent studies have also highlighted the contribution of innate lymphoid cells in the fibrogenic process. We will summarize our recent findings on the identification of anti-inflammatory targets and pathways that control macrophage phenotype in experimental models of fibrosis, in particular components of the endocannabinoid system and the autophagy pathway. We will also present some of our new data regarding the role of non-conventional T cells such as MAIT cells in the control of fibrosis progression.
Background and Aim  Liver fibrosis is a common pathological process resulting from chronic liver damage, and oxidative stress is involved in this process. The intracellular oxidative stress sensor Keap1 (Kelch-like ECH-associated protein 1) negatively regulates Nrf2 (Nuclear factor-erythroid2 p45-related factor 2) which activates cellular antioxidant response against oxidative stress. In this article, we use the small hairpin RNA (shRNA) to silence oxidative stress sensor with the hope to explore a novel therapeutic target for liver fibrosis.

Methods  Four pairs of Keap1-shRNA (named as Plvx/GFP/puro-Keap1-mus-1781,1894,1934,2097) and a pair of negative control shRNA were designed, then were respectively transfected into a mouse liver cell(AML12) in order to choose the target sequence whose expression was lower than others by qRT-PCR. Keap1-shRNA transfected cells were exposed to 1% hypoxia for various time points up to 48 hours. Subsequently, the expression of Keap1, Nrf2, collagen I(COL1A1), collagenIII(COL1A3), transforming growth factor -β(TGF-β1), and vascular endothelial growth factor -A(VEGF-A), insulin-like growth factor 1 (IGF-1) were analyzed either by Western blot or qRT-PCR. Next, the Enzyme-linked immunosorbent assay (ELISA) was employed to determine the COL1A1, COL1A3 levels in supernatant of transfected cells. The analysis of apoptosis were assessed using flow cytometry and caspase 8 protein expression, the Lipid Peroxidation and antioxidant Enzymes activity were carried out by MDA and GSH assay and the lactate dehydrogenase (LDH) assays were performed to evaluate the potential cellular activity.

Results  The target pair Keap1-mus-1781 was selected and the rate of this gene/GAPDH expression was 0.34±0.003, which is the lowest expression compared with the others (P<0.05). The silencing of Keap1 gene increased Nrf2 protein and mRNA expression under hypoxic conditions, western blot indicated that COL1A1, COL1A3, TGF-β1, VEGF-A, IGF-1 expression decreased as well(P<0.05). ELISA also revealed the decreased COL1A1, COL1A3 expression in transfected cells compared with the negative control, which is consistent with western blot results. In addition, transfection of the Keap1-shRNA displayed higher cellular activity, antioxidant effect and reduced hypoxia-induced cell apoptosis.

Conclusion  Taken together, our results indicate that silencing of Keap1 significantly attenuated liver fibrosis progression through the decreased hepatocyte oxidative stress, offering a new treatment perspectives for liver fibrosis.
NEW INSIGHTS INTO INFLAMMATION REGULATION AND LIVER CANCER

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Inflammation is an essential immune response that enables survival during infection or injury and maintains tissue homeostasis under a variety of noxious conditions. Inflammation contributes significantly to the pathogenesis of many diseases, including tumor. Several experimental and epidemiological evidence indicate that, inflammation is associated with the most of, if not all, tumors and supports their progression. Inflammatory mediators and cells are involved in the proliferation, migration, invasion and metastasis of malignant cells. Inflammatory cytokines such as TNF, IL-1, IL-6 and IFN, and chemokines receptors and ligands play important role in tumor initiation and progression.

Recently, we demonstrated that DEN-induced precancerous lesions and hepatocellular carcinoma were dramatically impaired in miR-484−/− mice. Mechanistically, ectopic expression of miR-484 initiates tumourigenesis and cell malignant transformation through synergistic activation of the transforming growth factor-β/Gli and nuclear factor-κB/type I IFN pathways. Specific acetylation of H3K27 is indispensable for basal IFN-induced continuous transcription of miR-484 and cell transformation. Moreover, we also found that Inc-DILC expression was preferentially down-regulated in liver cancer stem cells (LCSCs). Depletion of Inc-DILC markedly enhanced LCSC expansion and facilitated HCC initiation and progression. Inc-DILC inhibited the autocrine IL-6/STAT3 signaling, and mediated the crosstalk between TNF-α/NF-κB signaling and IL-6/STAT3 cascade. Clinical investigation showed decreased Inc-DILC expression in HCCs predicts early recurrence and short survival of patients, highlighting its prognostic value. These findings connect hepatic inflammation with precancerous lesions and liver cancer stem cells, providing a potential therapeutic strategy to manipulate or reverse liver precancerous lesions and tumorigenesis.
A BIFUNCTIONAL ROLE FOR C-JUN IN REGULATING LIVER HOMEOSTASIS DURING FIBROSIS

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c-Jun, a major component of the AP-1 transcription factor complex, has been implicated in a wide range of physiological and pathological processes. In the liver, c-Jun has been shown to be required for regeneration following partial hepatectomy, and is a promoter of hepatocellular carcinoma formation induced by chemical carcinogens. In the present study, we have evaluated the role of c-Jun in hepatic fibrosis, using conditional knockout mice to inactivate c-jun in adult liver (1) hepatocytes and (2) hepatic stellate cells (HSCs), using a variety of transgenic mouse strains expressing the cre-recombinase under various promoters. Fibrosis was induced by chronic injections of carbon tetrachloride and livers were analyzed for HSC activation, degree of fibrosis and many other parameters. Our results suggest that deletion of c-Jun in different cell-types result in opposite effects on liver fibrosis, suggesting a bifunctional role for c-Jun in regulating liver homeostasis during the fibrotic process. Detailed results will be presented.
TARGETING NUCLEAR RECEPTOR CAR IN HCC

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Human hepatocellular carcinoma (HCC) is one of the most common cancers worldwide. In contrast to the great progress made against many other cancers, the study of HCC treatment has been poor and therapeutic options are extremely limited. We use genomic, genetic and biochemical approaches to elucidate function and molecular mechanism of HCC.

Aberrant β-catenin activation contributes to a third or more of (HCC), but β-catenin activation alone is not sufficient to induce liver cancer in mice. Nuclear receptor CAR has been implicated in HCC promotion for a long time. Interestingly, β-catenin is activated in nearly all of the CAR-dependent tumors generated by the tumor promoter phenobarbital. Here, we show that full activation of β-catenin in the liver induces senescence and growth arrest, which is overcome by combined CAR activation, resulting in uncontrolled hepatocyte proliferation, hepatomegaly and rapid lethality despite maintenance of normal liver function. Combining CAR activation with limited β-catenin activation induces tumorigenesis, and the tumors share a conserved gene expression signature with β-catenin-positive human HCC. These results reveal an unexpected route for hepatocyte proliferation and define a murine model of hepatocarcinogenesis with direct relevance to human HCC. In addition, we found significant suppression of tumor incidence and growth by using CAR antagonist Androstanol in HBV-related liver cancer mouse model where β-catenin is activated. Nuclear receptors are natural targets for therapeutic intervention, thus our study has the potential to provide novel approaches for the treatment and prevention of HCC.
COMPENSATORY ROLES OF CD8\(^+\) T CELLS AND DENDRITIC CELLS IN IMMUNE REGULATION IN THE GUT WITH NON-FUNCTIONAL CD4\(^+\) TREGS

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CD4\(^+\) Tregs need to migrate from the mucosal periphery into the draining lymph node via CCR7 to exert their suppressive effects. In this study, we investigated whether CCR7 deficiency resulted in failure of immune suppression in 2% dextran sulfate sodium-induced colitis. Unexpectedly, intestinal inflammation was not exacerbated in the absence of CCR7. Expression of IL-10, a representative suppressive cytokine, was enhanced in CCR7KO CD8\(^+\) T cells. Colon CCR7KO CD8\(^+\) T cells reduced the activation of CD4\(^+\) T cells. Depletion of CD8\(^+\) T cells using anti-CD8 antibody exacerbated colitis in CCR7KO mice. Plasmacytoid dendritic cell numbers were also slightly increased during intestinal inflammation in the absence of CCR7, and the depletion of those cells exacerbated DSS-induced colitis in CCR7KO mice. These results suggest that CD8\(^+\) T cells and plasmacytoid dendritic cells have compensatory roles in immune regulation in the gut for impaired function of CD4\(^+\) Tregs.
Non-alcoholic fatty liver disease (NAFLD) is up to 30% in developed
countries and nearly 10% in developing nations but its precise mechanisms
are still unclear. Succinylation is a new identified post-translational
modifications (PTMs) and plays important regulatory role in cellular
process. Here we performed the comparative succinylome study in the liver
of NASH model through highly sensitive immune-affinity purification,
high-resolution LC–MS/MS and bioinformatics analysis. Overall, we
identified 815 succinylation sites in 407 proteins and 243 succinylation
acetylation sites corresponding to 178 proteins were quantified.
Bioinformatic analysis indicated the different expressed proteins were
involved in a variety of cellular functions such as carbon metabolism,
amino acid metabolism, fat acid metabolism, binding and catalyzing, anti-
oxidation and xenobiotics metabolis. Subcellular location analysis showed
they were mainly localized to cytoplasm and mitochondria. Motif analysis
obtained 8 conserved succinylation site motifs. These different succinylated
proteins may change many normal metabolism pathways and promoted
NASH development. This is the first quantitative succinylome study in the
liver tissues of NASH rat model, which may confer a new concept for
NASH genesis and development as well as exploring new therapy avenues
to cure NASH.
GENE NETWORKS ACTIVATED IN CULTIVATED PRIMARY HUMAN AND MOUSE HEPATOCYTES REPRESENT IN VIVO DISEASE STATES

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It is well-known that isolation and cultivation of primary hepatocytes causes major gene expression alterations. In the present genome-wide, time resolved study of cultivated human and mouse hepatocytes, we made the observation that expression changes in culture strongly resemble alterations in liver diseases. Hepatocytes of both species were cultivated in collagen sandwich and in monolayer conditions. Genome-wide data were also obtained from human NAFLD, cirrhosis, HCC, and hepatitis B virus infected tissue as well as mouse livers after partial hepatectomy, CCl4 intoxication, obesity, HCC and LPS. A strong similarity between cultivation and disease induced expression alterations was observed. For example, expression changes in hepatocytes induced by one day cultivation and one day CCl4 exposure in vivo correlated with R=0.615 (P<0.001). Interspecies comparison identified predominantly similar responses in human and mouse hepatocytes but also a set of genes that responded differently. Unsupervised clustering of altered genes identified three main clusters: (1) downregulated genes corresponding to mature liver functions, (2) upregulation of an inflammation/RNA-processing cluster, (3) upregulated migration/cell-cycle associated genes. Gene regulatory network analysis highlights overrepresented and deregulated HNF4 and CAR (cluster 1), Krüppel-like factors MafF and ELK1 (cluster 2), as well as ETF (cluster 3) among the interspecies conserved key regulators of expression changes. Interventions ameliorating but not abrogating cultivation induced responses include removal of non-parenchymal cells, generation of the hepatocytes’ own matrix in spheroids, supplementation with bile salts and siRNA mediated suppression of key transcription factors.

In conclusion, this study shows that gene regulatory network alterations of cultivated hepatocytes resemble those of inflammatory liver diseases and should therefore be considered and exploited as disease models.
HEPATOCYTE-SPECIFIC SMAD7 DELETION ACCELERATES DEN INDUCED HCC VIA ACTIVATION OF STAT3 SIGNALING IN MICE

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TGF-β signaling plays critical roles in different liver cell types, in multiple liver diseases, including hepatocellular carcinoma (HCC), with Smad7 as one of the most important endogenous negative regulators. We previously found that high Smad7 levels correlate with better clinical outcome in HCC patients. However, the underlying tumor suppressive molecular mechanism is still not clear. In this study, conditional (TTR-Cre) hepatocyte specific (Alb-promotor) Smad7 knockout (ko) mice developed more tumors than wildtype (Wt) and corresponding Smad7 transgenic (Tg) mice, 9 months after DEN challenge, verifying Smad7 as a tumor suppressor in progressed liver disease. In line with our findings in patients, Smad7 levels showed a significant inverse correlation with tumor numbers in both, tumor as well as surrounding tissue. Smad7 Ko mice exhibited increased p-Smad2/3 levels and reduced apoptosis in the tumor tissue. Higher tumor incidence was accompanied low p21 and high c-MYC expression in the tumors, and activation of STAT3 signaling was found in Smad7 deficient tumors. Together, our results provide new mechanistic insights into the tumor suppressive functions of Smad7 in hepatocarcinogenesis, linking Smad7 deficiency with enhanced Stat3 signaling.
METABOLIC ALTERATIONS OF CLINICAL SIGNIFICANCE IN LIVER CANCER

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Introduction: Liver cancer, predominantly hepatocellular carcinoma (HCC), is one cancer for which there is currently no effective therapy, especially at the advanced stage. A detailed understanding of metabolic alterations or regulators in HCC could increase the chances of discovering novel anti-HCC therapies. In this study, we aimed to identify clinically significant metabolic genes and pathways, and also to investigate the molecular consequences of targeting metabolism in HCC.

Materials and methods: We queried the expression of >2000 metabolic genes across published clinical HCC datasets. We correlated consistently altered genes with invasive/epithelial-to-mesenchymal (EMT) markers and subsequently performed survival analyses using statistical approaches. Furthermore, we applied bioinformatics techniques to delineate and construct the metabolic portrait of HCC. In vitro, we modulated the glutaminolytic pathway in HCC cells of well and poor differentiation stages followed by mass spectrometry-based metabolomics, gene expression analysis, immunoblotting and phenotypic assays.

Results and discussion: We identified over 400 consistently altered metabolic targets, whose expression strongly correlates with invasive/EMT markers. Of these, expression of about 150 genes predicted patient survival outcome. Overall, the genomic landscape of HCC revealed downregulation of genes involved in traditional hepatocyte metabolic functions such as xenobiotics/drug metabolism, urea cycle, gluconeogenesis, and fatty acid metabolism. On the other hand, we found that HCC upregulates glycolysis, nucleotide biosynthesis, proton transport, and other pathways including glutaminolysis. Interference with glutamine metabolism in poorly differentiated HCC cells, either by deprivation or inhibitors, led to profound proliferation and cell cycle arrest, suppressed levels of ATP and TCA intermediates, decreased oxygen consumption, impaired clonogenicity and deregulation of several targets, including GLS, FASN and MYC.

Conclusion: Our study has identified consistently altered metabolic targets that are prognostic indicators, biomarkers and potential therapeutic targets, and show that glutaminolysis is selectively druggable in exogenous glutamine-reliant HCC.
VESSELS THAT ENCAPSULATED TUMOR CLUSTERS:
IMPLICATION IN HEPATOCELLULAR CARCINOMA METASTASIS
AND ITS REGULATION MECHANISM

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Early metastasis is responsible for frequent relapse and high mortality of hepatocellular carcinoma (HCC), but its underlying mechanisms remain unclear. Based on histological examination on serial HCC sections and three-dimensional reconstruction, we found a novel and prevalent vascular pattern, vessels that encapsulated tumor clusters (VETC) and formed cobweb-like networks. The presence of VETC (VETC+) predicted higher metastasis and recurrence rates of HCC. Interestingly, although Epithelial-mesenchymal transition (EMT) has been considered as a key event in metastasis, abrogation of EMT by knockdown of Snail or Slug significantly diminished in vivo metastasis of VETC− xenografts but did not affect that of VETC+ ones, while silencing of Snail or Slug substantially reduced the in vitro migration of both VETC− and VETC+ HCC cells. Further analysis revealed that VETC provided an efficient metastasis mode by facilitating the release of whole tumor cluster into the bloodstream. Mechanism study showed that HCC cell-derived Ang2 was a prerequisite for VETC formation. Knockdown of Ang2 abolished VETC formation and consequently attenuated in vivo tumor metastasis. Further more, based on both in vitro and in vivo evidence, we uncovered that miR-125b and miR-100 were able to negatively regulate Ang-2 expression. Restoration of miR-125b or miR-100 dramatically abolished VETC formation in tumor xenografts by reducing Ang2 expression, and consequently inhibited in vivo metastasis. Collectively, our study identify a novel metastasis mechanism that relies on VETC pattern but is independent of EMT, characterized miR-125b and miR-100 as novel VETC suppressors by suppressing Ang-2 expression, and thus provides novel targets for anti-metastasis therapy of HCC.

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DIFFERENTIAL ROLE OF HEPATIC INSULIN RECEPTOR/PTEN AND IGF-1 RECEPTOR/PTEN SIGNALING IN LIVER METABOLISM AND INTER-ORGAN COMMUNICATION.

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Background: The insulin receptor (IR) and IGF-1 receptor (IGF1R) trigger through the central PI3K/PTEN signalling node, specific events regulating metabolic and mitogenic processes in hepatocytes. We previously reported that PTEN deficiency in hepatocytes triggers hepatomegaly, steatosis and impaired gluconeogenesis in the liver, but in contrast it improves muscle insulin sensitivity and decreases adiposity through poorly characterized crosstalk mechanisms. Herein, we investigated the specific contributions of IR and IGF1R signalling in association with PTEN to regulate lipid and glucose metabolism in the liver, as well as homeostasis of other metabolically active peripheral organs.

Methods: Mice with hepatocyte-specific deletions of IR, IGF1R, or both, in addition to deletion of PTEN were generated and metabolically phenotyped. Histological and molecular analyses were performed on explanted tissues.

Results: IR, but not IGF1R, signalling was required for the development of hepatomegaly in mice bearing a PTEN deletion in hepatocytes. However, signalling by both receptors contributed through different mechanisms to steatosis development, and deletion of both receptors was required to prevent abnormal lipid accumulation in the absence of PTEN. Unexpectedly, impaired hepatic gluconeogenesis and improved systemic glucose tolerance in the absence of PTEN were abolished by preventing IGF1R signalling, whereas adiposity and browning of white adipose tissues were dependent of hepatic IR signaling. Finally, abrogation of hepatic IR/PTEN and IGF1R/PTEN signalling differentially modulate the morphology/function of brown adipose tissue.

Conclusions: Hepatic IR and IGF1R signalling in conjunction with PTEN regulate distinct hepatic metabolic processes and differentially affect homeostasis of peripheral organs such as muscles, white and brown adipose tissues.
Hepatocellular carcinoma (HCC) represents the second most common cause of cancer-related death. 80% of HCC occurs in patients with cirrhosis. The intriguing tumor-stroma and tumor-extracellular matrix (ECM) interactions, as well as the intricate interplay between biochemical and mechanical cues make HCC microenvironment an extremely interesting but complicated system to study. Here we focused on the biomechanics of HCC microenvironment. Using atomic force microscopy (AFM) and ultrasound elastography, we found increased stiffness in the paracancerous tissue from HCC patients and syngenic mouse models. We further identified the changes of cytoskeleton and cell-cell adhesion proteins correlated with the upregulated stiffness in HCC paracancerous tissue. These results suggest a unique mechanical microenvironment in HCC with liver cirrhosis. Studying the underlying mechanisms may contribute to the understanding of HCC progression and invasion, as well as to the understanding of targeted drug resistance.
HCCDB: HEPATOCELLULAR CARCINOMA GENE EXPRESSION ATLAS

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Hepatocellular carcinoma (HCC) is one of the most prevalent cancer types worldwide. The heterogeneity of HCC is a big challenge for disease diagnosis and treatment. The accumulation of genome-wide gene expression data provide the opportunity to classify HCCs into different subgroups based on gene expression signatures. We carefully curated more than ten public large-scale gene expression datasets of more than 2,000 clinical HCC samples. Then, a web-based database HCCDB (http://bioinfo.au.tsinghua.edu.cn/database/hccdb) was developed to visualize basic data analysis results, including differential analysis and survival analysis. Based on the curated data, we developed a signature-network based meta-analysis method to identify candidate HCC subtypes. A few consistent subtypes and molecular signatures were successfully identified. We also identified several epi-drivers by integrating gene expression data with DNA methylations.
NON-CELL-AUTONOMOUS FUNCTION OF YAP IN INITIATING LIVER TUMORS BY RECRUITING TUMOR ASSOCIATED MACROPHAGES

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Tumor-associated macrophages (TAMs) have been found widely present in established human tumors with a generally tumor-promoting role. However, in most cancers it is unknown whether TAMs are recruited by the single tumor-initiating cell and play a role in the survival and expansion of this single cell toward a tumor. Hepatocellular carcinomas (HCCs) often develop after viral infection, liver damage, and the following regeneration, which more or less involves increasing number of hepatic progenitor cells (HPCs), which are often associated with macrophages. However, the origin of true tumor-initiating cells of HCC is still under debating, and the role of macrophages is unclear. Interestingly, it was recently found that activation of the Hippo pathway effector YAP leads to dedifferentiation of mature hepatocytes into cells resemble HPCs. Using a hydrodynamic injection approach we were able to confirm that expression of active YAP in a single hepatocyte drives dedifferentiation. To our surprise, YAP expression also immediately induces massive recruitment of macrophages. In a couple of months, these YAP-expressing livers develop massive tumors. This indicates that macrophages could be recruited by single tumor-initiating cells and allows us to study the function of these macrophages. We found that YAP expression or inhibition of Hippo pathway kinases Lats1/2 or Mst1/2 recruits macrophages through direct induction of chemokine CCL2 and also CSF1. These macrophages express markers of TAM and block of macrophage recruitment by CCL2/CSF1 knockdown leads to clearance of YAP positive HPCs through a p53-dependent process. Strikingly, loss of macrophage recruitment completely eliminates tumors induced by active YAP. These findings identify a non-cell-autonomous function of YAP indispensable for tumorigenesis and demonstrate that macrophages recruited by single tumor-initiating cells promote tumorigenesis by preventing these cells from clearance.
INHIBITORY ROLE OF WW45/MST1 ON PI3K PATHWAY IN LIVER DISEASE

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The coordination of “PI3K as a growth stimulatory pathway” and “Hippo as a growth inhibitory pathway” has an important role in controlling organ size. However, the physiological meaning of bridging PI3K pathway and Hippo pathway remained unclear, especially in the liver.

Our preliminary results revealed that liver-specific WW45-/-;Pten-/- mice have a synergistic effect on development of NAFLD-NASH-Cirrhosis-HCC via increased pAKT(active AKT). This suggests that “not only PTEN, but also WW45 can suppress the PI3K pathway.” In vitro system showed that MST1 also play a role in this suppressive mechanism.

Moreover, the Pten-/-;MST1tg mice restored the fatty liver phenotype of Pten-/- by decreasing pAKT.

All of these findings indicate that WW45/MST have a suppressive role on PI3K pathway in fatty liver development.

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Hepatic steatosis is the accumulation triglyceride in the liver. Recently, hepatic steatosis has become more important because it occurs in the patients with obesity, type 2 diabetes, and hyperlipidemia and is associated with endoplasmic reticulum (ER) stress and insulin resistance. CCR2 inhibitor has been reported to improve inflammation and insulin resistance in adipose tissue, but its mechanisms remained unknown in hepatic steatosis.

We examined whether CCR2 inhibitor improves ER stress-induced hepatic steatosis in type 2 diabetic mice. In this study, db/db and db/m (n = 9) mice were fed CCR2 inhibitor (2 mg/kg/day) for 9 weeks. CCR2 inhibitor decreased plasma and hepatic triglycerides levels and improved insulin sensitivity in diabetic mice. Moreover, CCR2 inhibitor treated db/db mice decreased ER stress markers (e.g., BiP, ATF4, CHOP, and XBP-1) and inflammatory cytokines (e.g., TNFα, IL-6, and MCP-1) while increasing markers of mitochondrial biogenesis (e.g., PGC-1α, Tfam, and COX1) in the liver. We suggest that CCR2 inhibitor may ameliorate hepatic steatosis by reducing ER stress and inflammation in type 2 diabetes mellitus.
DJ-1 PROMOTES EXPANSION OF LIVER PROGENITOR CELLS IN HBV PATIENTS AND IN A DDC-INDUCED MURINE MODEL OF CHRONIC LIVER INJURY

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Objective: Following chronic hepatic injury or once hepatocytes regeneration ability is severely impaired, liver progenitor cells (LPCs) appear in the liver and can mediate the liver regeneration. The inflammatory microenviroment of LPCs play important role in LPCs proliferation. We found DJ-1(Park7) is expressed in injured liver both in mice and human, our previous studies have suggested that DJ-1 is involved in ROS generation and inflammatory regulation. Whether DJ-1 correlates with LPC proliferation by inflammatory regulation remains unclear.

Design: We examined the role of DJ-1 in LPCs proliferation. DJ-1 expression were examined in patients with chronic hepatitis B virus (HBV) and a murine model induced by 0.1% 3, 5-diethoxycarbonyl-1, 4-dihydrocollidine (DDC) containing diet. LPCs proliferation was studied in WT and DJ-1 KO mice.

Results: Expression levels of DJ-1 were upregulated in the liver of HBV patients and DDC fed mice. Additionally, DJ-1 expression positively correlated with LPCs proliferation. Ductular reaction and LPCs numbers were significantly reduced in DJ-1 KO mice after DDC-feeding. Along with reduced hepatic stellate cells (HSCs) activation and collagen deposition in DJ-1 KO mice. Furthermore, infiltrated CD11b+Gr-1low macrophages and proinflammatory factors (IL-6, TNF-α) were attenuated in DJ-1 KO mice. In vitro, DJ-1 KO LPCs and DJ-1 knockdown in epithelial progenitor cell (LEPC) line showed similar proliferation rates indicating that DJ-1 does not stimulate liver progenitor cells proliferation directly. Mechanistically we found DJ-1 KO impaired the secretion of macrophage-mobilizing chemokines in HSCs leading the reduced inflammatory microenviroment and proliferation of LPCs.

Conclusion: We conclude that DJ-1 is required for the inflammatory microenviroment and activation of LPCs in injured liver.
LOSS OF ARID1A EXPRESSION POTENTIATES ANGIOGENESIS OF LIVER CANCER BY UP-REGULATING ANG2

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Arid1a, a component of SWI/SNF complexes, is one of the most mutated genes in cancers, including hepatocellular carcinoma and ovarian cancers. However, how Arid1a deficiency promotes tumor development remains largely unknown. Here we demonstrate that loss of Arid1a expression in hepatocellular carcinoma (HCC) is featured with increased vessel density. Mechanistically, loss of Arid1a expression induces ectopic angiopoietin-2 (Ang2) expression, which promotes tumor angiogenesis and tumor progression. Ang2 blockade or Sorafenib treatment reduced vessel density in HCC of Arid1aΔli mice. Furthermore, we demonstrated that Sorafenib profoundly inhibits Arid1aΔli HCC progression, suggesting that Sorafenib treatment could be the first-line therapy for the HCC patient with Arid1a somatic deficiency.
DIFFERENTIAL ROLES OF YAP ACTIVITY IN REGULATION OF
HEPATOXYTE DEDIFFERENTIATION

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YAP (Yes-associated protein) is a transcriptional co-activator whose
activity is widely known to be regulated by the canonical Hippo pathway.
Thus far, regulating the function of YAP has been focused on directing
cytoplasmic to nuclear translocation via phosphorylation by LATS1/2.
However, recent evidences suggest that the activities of YAP can be further
altered to varying degrees. However, the altered functions exerted by YAP
with different activity levels have not been carefully investigated.
Here, we show that YAP with varying activity levels performs different
roles in the liver. In order to deliver and thereby overexpress YAP in a
hepatocyte-specific manner, we utilized the Adeno-associated virus (AAV)
system. As already known, overexpression of active YAP indeed induced
liver enlargement. However, only the highly active mutant form of YAP
(YAP-5SA) was able to induce hepatocytes to undergo cell fate transition to
become immature biliary epithelial cells (BECs). Not only did the
overexpression of YAP-5SA upregulate BEC-related genes, but it also
repressed those that direct hepatocyte fates. This suggests that YAP can
maintain the differentiation status of hepatocytes depending on the degree
of its activity.
HIGH LEVELS OF PEROXIREDOXIN 1 IN LIVER CANCERPREDICT POOR PROGNOSIS

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Introduction and Objectives: Peroxiredoxin 1 (PRDX1) is an antioxidant enzyme in mammalian cells. However, recent various evidences revealed that PRDX1 can act in a manner independent of its peroxide detoxifying function. Here, we investigate the expression characteristics of peroxiredoxin 1 (PRDX1) mRNA and protein in liver cancer cell lines and tissues.

Methods: The expression and clinical characteristics analysis of PRDX1 mRNA was evaluated in the RNA sequencing data of liver cancer which were downloaded from The Cancer Genome Atlas (TCGA). The Kaplan-Meier and Cox regression survival analysis was performed to determine the relationship between PRDX1 levels and patient survival. Western blotting was used to demonstrate the expression of PRDX1 protein in 6 liver cancer cell lines and 29 paired fresh tissue specimens.

Results and Discussion: The mRNA of PRDX1 gene was up-regulated approximately 1.3-fold in tumor tissue compared with the adjacent non-tumor control. Its abundance was significantly higher in men. High levels of PRDX1 mRNA were associated with a shorter overall survival time, but not with recurrence-free survival. The Cox regression analysis demonstrated that patients with high PRDX1 mRNA showed ~1.55-fold increase of risk for death. In liver cancer cells, PRDX1 protein was strongly expressed with multiple different bands. The theoretical PRDX1 band was increased, whereas the high molecular weight form was down-regulated in tumor tissues.

CONCLUSION: PRDX1 was overexpressed in the tumor tissues of liver cancer and serves as a poor prognostic factor for overall survival.
ROLE OF C-JUN IN HEPATIC FIBROSIS

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c-Jun, a major component of AP-1 transcription factor complex, has been implicated in a wide range of physiological and pathological processes. To identify novel biological processes regulated by c-Jun, we analyzed differentially expressed genes between wildtype (c-jun+/+) and c-jun knockout (c-jun−/−) mouse embryonic fibroblasts (MEFs) by Ingenuity Pathway Analysis. The Hepatic fibrosis/Hepatic stellate cell (HSC) activation pathway was the top one affected, leading us to assess activated HSC status in c-jun+/+ and c-jun−/− embryos. c-jun−/− embryos showed dramatically high levels of activated HSCs compared to c-jun+/+ embryos. Hence we hypothesized that c-Jun may play a role in hepatic fibrosis.

Thus to investigate the effect of c-Jun in hepatic fibrosis, we used c-jun conditional knockout mice to inactivate c-jun in various cell types of adult liver. Fibrosis was induced by chronic injections of carbon tetrachloride and livers were analyzed for HSC activation and degree of fibrosis. Surprisingly, we found that deletion of c-jun in hepatocytes and HSCs resulted in differential effects on fibrosis, suggesting that c-Jun acts as a dual regulator in hepatic fibrosis. Detailed data will be presented.
Identification of metabolic alterations in hepatitis B virus core protein transfected hepatocellular carcinoma cell by integrative multi-omics analysis

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Chronic infection caused by the hepatitis B virus (HBV), is strongly associated with hepatitis, fatty liver and hepatocellular carcinoma. Hepatitis B virus core protein (HBc), encoded by the HBV genome, may play the center role of HBV life cycle. However, the function of HBc in this process still remains unclear. To investigate the underlying mechanisms, we characterize the features of hepatocellular carcinoma cell transfected with HBc using multi-omics analysis. The results show that HBc transfection promote the expression of metabolism-related proteins and the secretion of metabolites in hepatocellular carcinoma cell, especially up-regulates the glycolysis and glycine metabolism pathway when combined proteomics with metabolomics analysis. In addition, this change of metabolic pathways are mainly contributed in two ways. First, HBc can directly bind to the enzymes of the glycine metabolic pathway. Then, it can regulate the glycolysis pathway by increasing the expression of Max-like protein X (MLX). This study provides further insights into the function of HBc in the pathogenesis of HBV-induced diseases. Besides, metabolic reprogramming appears to be a hallmark of HBc transfection and provides an interesting therapeutic target.
ESSENTIAL ROLES OF MYC IN MOUSE HEPATOCARCINOGENESIS INDUCED BY THE ACTIVATION OF AKT AND RAS PATHWAYS

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The significance of MYC activation in liver tumors has remained obscure. Here, we examined the effect of Myc in mouse liver tumor models induced by hepatocytic expression of myristoylated AKT (AKT) and/or mutant HRASV12 (HRAS) via transposon-mediated gene integration. Although AKT or HRAS alone induced multiple liver tumors following incubation periods of approximately 5 months, their combination facilitated the process with the appearance of hepatocellular carcinoma within 8 weeks. Introduction of AKT/HRAS induced lipid-laden preneoplastic cells that grew into nodules composed of tumor cells with or without intracytoplasmic lipid, with the latter being more proliferative and associated with spontaneous Myc expression. The induction of MadMyc, a competitive Myc inhibitor, in preneoplastic cells blocked the progression of AKT/HRAS-induced tumors, and the co-introduction of MadMyc with AKT and HRAS almost completely abolished tumorigenesis. Although transposon-mediated Myc overexpression itself was insufficient to induce tumors, it facilitated tumorigenesis by AKT or HRAS, and when it was cointroduced with AKT and HRAS, diffusely infiltrating tumors devoid of lipid accumulation developed as early as two weeks. Myc overexpression suppressed the mRNA expression levels of genes involved in fatty acid synthesis in the tumors, and when it was combined with HRAS, it also suppressed the mRNA expression levels of the genes involved in β-oxidation. Finally, examining the dose-responses of Myc in the enhancement of AKT/HRAS-induced tumorigenesis, we found that a reduction to one-third retained enhancing effect but three-times greater introduction damped the process with increased apoptosis. Conclusion: Our results demonstrate that in hepatocarcinogenesis induced by activated AKT and HRAS, activation of endogenous Myc is a prerequisite and adequate levels of Myc deregulation facilitate the process with alterations in cellular metabolism.
DIRECT REVERSION OF HCC CELL TO FUNCTIONAL LIVER-LIKE CELL WITH THE LOSS OF TUMORIGENECITY POTENTIAL BY SMALL-MOLECULE COMPOUNDS

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Hepatocellular carcinoma (HCC) is the second leading cause of cancer-related mortality worldwide. Till today, surgical resection remains the primary and most effective treatment method for early-stage HCC patient. Other treatments, including traditional chemotherapy as well as newly emerged immunotherapy and target therapy (precision medicine), have yet achieve significant efficacy in HCC treatment due to the disease’s complex molecular mechanisms, tumor heterogeneity and high tendency of drug-resistance. Cell reprogramming, which leads to cell fate switch, may have the potential to effectively address these issues. To reprogram cell, the key is to re-constitute target cell-specific biological platform while the initial source cell, regardless of its original characteristics, to overcome the signaling and epigenetic barriers in the process. Based on these, we hereby developed an effective approach to revert HCC cells into normal functioning liver-like cells using a small molecule combination (SMC). Our results showed that SMC treatment led to HCC cells losing their tumorigenicity potential both in vitro and in vivo, and in the meantime the treated cell highly expressing liver cell specific markers such as ALB, HNF4a, AAT, Asgpr1 and CYP3A as well as obtaining normal liver cell functions such as glycogen storage, ALB and urea production and CYP activity. The reversal was accompanied with cell apoptosis induction. Results from HCC cell lines were confirmed by experiments on HCC patient-derived cells and xenograft (PDX) animal models. Mechanically, the strong suppression of tumor-related signaling such as Akt, Erk, Stat3 and Smad2/3 by SMC might partially contribute to this process. In summary, our research data suggest that reprogramming strategy can be applied to tumor treatment research as demonstrated in our HCC cell reversion experiments which lead to effective HCC depletion. Our results may also shed a light on other solid tumor controlling or abrogation research.
BIOMARKERS OF COLORECTAL CARCINOMA LIVER METASTASIS IN URINE

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Introduction and Objectives: Colorectal carcinoma (CRC) is one of the most common gastrointestinal carcinomas and more than one third of CRC patients accompanied by liver metastasis. The routine diagnostic methods of CRC liver metastasis are ultrasonography and CT scan. But these methods are high-cost and need good medical conditions. The recent clinical serum biomarker of CRC liver metastasis is CEA, but it is lack of sensitivity and specificity. So it’s important to find a better way to detect CRC liver metastasis. Urine can be obtained non-invasively and continually, and the proteins in urine are mainly from plasma proteins, so urine proteins can reflect the changes of physiopathologic conditions directly and serve as good resource of biomarkers.

Methods: We used a new filter device to gather the urine proteins onto the NC membrane, then dissolved the membrane in acetone to extract the urine proteins and the proteins can be used for Western Blotting experiments. The arbitrary absorbance units of the Western blot bands were normalized to the urinary creatinine excretion.

Results and Discussion: We have collected urine samples from CRC liver metastasis patients. The preliminary results showed a high quality of urine proteins, so it seems possible to find something useful in urine. Then we have tried to detect Cathepsin D, which had been reported as a potential biomarker of renal cell carcinoma. The result shows that there is a significant difference between CRC patient and healthy urine samples.

Conclusions: The enrichment approach of urine proteins provides a new way of biomarker discovery in CRC liver metastasis and urinary Cathepsin D maybe a potential marker of CRC liver metastasis.
REMODELING OF THE BILIARY TREE DURING LIVER REGENERATION

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The liver epithelium comprises the parenchymal cells (hepatocytes) and the biliary epithelial cells (BECs), also known as cholangiocytes. In the adult liver, these cells are maintained in a quiescent state to form stable epithelial sheets and tubules, while they enter dynamic regeneration processes once the organ is suffering from tissue loss or various types of injury. In particular, the regeneration process against chronic injury proceeds through drastic changes in the morphology and phenotype of liver epithelial tissues, and in many cases, accompanies a dynamic remodeling of the intrahepatic biliary epithelial tissue, known as ductular reaction, in both human pathologies and animal models. However, the cellular basis for this phenomenon remains unclear. We took an unbiased approach based on an in vivo clonal labeling and tracking of the biliary epithelial cells in the three-dimensional landscape, in combination with mathematical simulation, to understand their mode of proliferation in a mouse model where the nascent biliary structure formed in a tissue-intrinsic manner. An apparent heterogeneity among the biliary epithelial cells was observed: whereas most of the cells that entered the cell cycle upon liver injury exhibited a limited and tapering growth potential, a selected population continued to proliferate, making a major contribution to the biliary expansion. Our study has highlighted a unique mode of epithelial tissue dynamics, which depends not on a hierarchical system driven by fixated stem cells but rather on a stochastically maintained progenitor population with persistent proliferative activity.
HEPATOCYTE PLASTICITY IN LIVER REGENERATION AND CANCER

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The liver is known for its regenerative capacity. Hepatocytes regenerate largely by self-duplication. However, when hepatocyte proliferation is impaired, e.g., in response to chronic hepatocyte injury, cells within bile ducts are thought to contribute to hepatocyte regeneration by giving rise to bipotential liver progenitor cells. In analogy, hepatocytes have been shown to undergo biliary differentiation under conditions of biliary injury, although it remains to be determined whether hepatocytes can transdifferentiate into fully functional cholangiocytes and regenerate the biliary system. Biliary differentiation of hepatocytes has also been suggested as a mechanism by which hepatocytes escape hepatocyte-specific injury, i.e., a form of metaplasia. This plasticity of hepatocytes may predispose to malignant transformation as suggested by findings that hepatocytes can give rise to cholangiocarcinomas in mice. Collectively, these findings raise the questions of whether plasticity of hepatocytes is effective in bile duct regeneration and/or contributes to the formation of liver cancer.
CRISPR/CAS9-MEDIATED KNOCK-IN OF LARGE DNA IN HUMAN CELLS

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CRISPR/Cas9-induced site-specific DNA double-strand breaks (DSBs) can be repaired by homology-directed repair (HDR) or non-homologous end joining (NHEJ) pathways. Extensive efforts have been made to knock-in exogenous DNA to a selected genomic locus in human cells; which, however, has focused on HDR-based strategies and was proven inefficient. Here, we report that NHEJ pathway mediates efficient rejoining of genome and plasmids following CRISPR/Cas9-induced DNA DSBs, and promotes high-efficiency DNA integration in various human cell types. With this homology-independent knock-in strategy, integration of a 4.6 kb promoterless ires-eGFP fragment into the GAPDH locus yielded up to 20% GFP+ cells in somatic LO2 cells, and 1.70% GFP+ cells in human embryonic stem cells (ESCs). Quantitative comparison further demonstrated that the NHEJ-based knock-in is more efficient than HDR-mediated gene targeting in all human cell types examined. These data support that CRISPR/Cas9-induced NHEJ provides a valuable new path for efficient genome editing in human ESCs and somatic cells.
ACYL-COA THIOESTERASE-MEDIATED METABOLIC
REGULATION

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Acyl-CoA thioesterases (Acots) are enzymes that catalyze the hydrolysis of acyl-CoA molecules, including fatty acyl-CoAs. Although their reactions are relatively well defined, the cellular and biological consequences of Acot activity are incompletely understood. Hypotheses include control of the intracellular balance between fatty acyl-CoAs and free fatty acids, the intracellular and intra-organelle concentrations of CoASH and the availability of fatty acids for the biosynthesis of inflammatory mediators.

Acots have been identified in a broad array of organisms from prokaryotes to mammals. In mammals, convergent evolution has resulted in two structurally distinct types of Acot enzymes. Type I enzymes (Acots 1-6) contain an α/β-hydrolase domain near the C-terminus, which comprises all amino acid residues required for catalysis, and typically an N-terminal regulatory acyl-CoA thioester hydrolase domain that is not required for enzymatic activity. Members of the α/β-hydrolase fold enzyme superfamily include enzymes such as carboxyl-esterases and lipases.

Type II enzymes (Acots 7-15) are related by a common hotdog fold structural motif. The ‘hotdog’ domain proteins contain highly divergent sequences that fold to create very well conserved structural elements in which an antiparallel β-sheet is the ‘bun’, which wraps around an α-helical ‘hotdog’. With the exception of Acots 13-15, which consist of a single hotdog fold domains, the other established type II Acots (i.e. Acots 7-12) comprise tandem hotdog fold domains. The significance of tandem hotdog fold thioesterase domains is presumably related to the requirement that these structures oligomerize to create enzyme active sites. Uniquely, Acots 11 and 12 each contain a C-terminal lipid-binding steroidogenic acute regulatory transfer-related (START) domain.

Our research has focused on the metabolic functions of Acot11 (common synonym: Thioesterase superfamily member 1, Them1) and Acot13 (common synonym: Thioesterase superfamily member 2, Them2). Them1 is highly expressed in brown adipose tissue, whereas Them2 is broadly distributed and enriched in oxidative tissues. Studies using purified recombinant proteins have revealed that preferred substrates of these Acots are long-chain fatty acyl-CoAs. Utilizing mice with targeted disruption of each gene, we have demonstrated key roles in the regulation of glucose and lipid metabolism, as well as energy homeostasis. Both Them1 and Them2 function to limit energy expenditure under conditions of metabolic stress. In the setting of overnutrition, these genes contribute to obesity and features of the metabolic syndrome.
LIVER POLYPLOIDY: DR JEKYLL OR MR HIDE?

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Polyploidy, the addition of one or more complete sets of chromosomes, is the most dramatic change known to occur in the genome. The liver is a polyploid organ. During post-natal growth, the liver undergoes dramatic changes characterized by gradual polyploidization. We previously unveiled that binucleation process has a pivotal role in establishing physiological liver polyploidy. In rodent’s liver, binucleation takes place around weaning, hepatocytes accomplish mitosis without performing cytokinesis. Insulin signaling through the PI3K/AKT pathway has a critical role in the generation of binucleate polyploid hepatocytes. The close connection between liver physiology and insulin signaling prompted us to investigate whether polyploidy is modified during metabolic disorders of the liver, such as nonalcoholic fatty liver disease (NAFLD). Interestingly, using murine NAFLD models and cohort of patients, we demonstrated the conversion of a physiological polyploidy (binucleate polyploid hepatocytes, DNA integrity) into a pathological polyploidy (mononucleate polyploid, DNA instability). We demonstrated that this polyploid contingent is generated under a "DNA damage signal" (ATR/p53/p21) that precludes the activation of mitotic kinase (endoreplication cycle). Importantly, oxidative stress was evidenced as a key player. Indeed, antioxidant treatment (NAC) was sufficient to: dampen ROS level in damaged livers, inhibit ATR activation, reduced pathological polyploidization. Collectively, these findings demonstrate for the first time within damaged liver, the genesis of pathological polyploid hepatocytes exhibiting a strong potential of genomic instability. Futures work will unravel how this pathological polyploidy behaves in a damaged livers and whether it can influence disease progression.
STRESS-ACTIVATED MIR-21/MIR-21* IN HEPATOCYTES PROMOTES LIPID AND GLUCOSE METABOLIC DISORDERS ASSOCIATED WITH HIGH-FAT DIET CONSUMPTION.

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**Objective:** miR-21 is an oncomir highly upregulated in hepatocellular carcinoma, but also in early stages of liver diseases characterized by the presence of steatosis. Whether upregulation of miR-21 contributes to hepatic metabolic disorders and their progression towards cancer is unknown. This study aims at investigating the role of miR-21/miR-21* in early stages of metabolic liver disorders associated with diet-induced obesity (DIO).

**Design:** Constitutive miR-21/miR-21* knockout (miR21KO) and liver-specific miR-21/miR-21* knockout (LimiR21KO) mice were generated. Mice were then fed with high-fat diet (HFD) and alterations of the lipid and glucose metabolism were investigated. Serum and ex-vivo explanted liver tissue were analyzed.

**Results:** Under normal breeding conditions and standard diet, miR-21/miR-21* deletion in mice was not associated with any detectable phenotypic alterations. However, when mice were challenged with an obesogenic diet, glucose intolerance, steatosis and adiposity were improved in mice lacking miR-21/miR-21*. Deletion of miR-21/miR-21* specifically in hepatocytes led to similar improvements in mice fed a HFD, indicating a crucial role for hepatic miR-21/miR-21* in metabolic disorders associated with DIO. Further molecular analyses demonstrated that miR-21/miR-21* deletion in hepatocytes increases insulin sensitivity and modulates the expression of multiple key metabolic transcription factors thereby reducing fatty acid uptake, de novo lipogenesis, gluconeogenesis and glucose output.

**Conclusion:** Hepatic miR-21/miR-21* deficiency prevents glucose intolerance and steatosis in mice fed an obesogenic diet by altering the expression of several master metabolic regulators. This study point out miR-21/miR-21* as a potential therapeutic target for NAFLD and the metabolic syndrome.
Fibrolamellar carcinoma (FLC) is a unique type of liver cancer that primarily affects teenagers and young adults. FLC differs from other types of liver cancers in terms of histology, known biomarkers, and etiology. We recently established the first-ever disease model of FLC, a patient-derived xenograft (PDX), and demonstrated a striking enrichment in cancer stem cells (CSCs). Further RNA-sequencing (RNA-seq) studies of FLC and several maturational lineage stages of the liver revealed that the gene expression profile of FLCs most closely resembles that of normal biliary tree stem cells (BTSCs). The molecular changes that drive FLC are unknown. The only recurrent mutation identified so far in FLC is a ~400 kb deletion on chromosome 19 that leads to the fusion gene DNAJB1-PRKACA, which couples a heat shock protein (DNAJB1) with a cAMP-dependent kinase (PKA). Bioinformatic analysis of RNA-seq data from ~10,000 tumors across 29 different cancer types in The Cancer Genome Atlas (TCGA) revealed that expression of DNAJB1-PRKACA is specific to FLCs. We found that 16 other protein-coding genes are dramatically up-regulated in FLCs relative to other liver cancers, and 8 of these are uniquely elevated in FLCs compared to 28 other tumor types. Preliminary analysis also revealed at least 5 long, non-coding RNAs (lncRNAs) that are significantly altered in FLC, including one that has been associated previously with cAMP-mediated transcriptional regulation. Finally, we showed that many of these FLC markers are also dysregulated in our PDX disease model of FLC relative to BTSCs, the presumptive FLC cell type of origin. We are currently using the PDX disease model to evaluate the oncogenic potential of these FLC marker genes and lncRNAs.
CONTRIBUTION OF HEPATIC STELLATE CELLS TO HEPATOCARCINOGENESIS

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80% of hepatocellular carcinomas (HCC) develop in fibrotic or cirrhotic livers. However, the contribution of fibrosis to the development or progression of HCC remains elusive, and it is not clear whether fibrosis and hepatocarcinogenesis represent two parallel but functionally independent biological processes, or whether fibrosis promotes hepatocarcinogenesis. Of note, recent data in pancreatic cancer suggest that cancer-associated fibroblasts (CAF) may also restrict tumor growth. Hence, functions of CAF and fibrosis may be organ- or cancer-specific. Here, I will discuss the cellular source and the functional contribution of myofibroblasts to hepatocarcinogenesis. Our preliminary data suggest that hepatic stellate cells (HSC) not only represent the primary source of myofibroblasts in the precancerous liver but also the main source of cancer-associated fibroblasts (CAF) within HCC lesions. Moreover, experiments in which HSC activation status was genetically manipulated suggest that HSC promote hepatocarcinogenesis in diethylnitrosamine-induced HCC.
MicroRNAs (miRNAs) belong to a class of short non-coding RNAs that regulate the expression of protein-coding genes. Aberrant expression of miRNAs is a common event in different types of cancers. We have identified a set of miRNAs that are down-regulated in hepatocellular carcinomas (HCCs). Further investigations on these miRNAs, based on clinical samples and on both cell and animal models, have revealed that: (1) HCC-associated miRNAs, together with proteins, constitute the regulatory networks that provide elaborate control on gene expression and cellular activity. (2) HCC-associated miRNAs are critical regulators of cell proliferation, apoptosis, tumor angiogenesis and metastasis. A single miRNA can regulate different proteins within the same or distinct signaling pathways, therefore its deregulation confers cells with different malignant phenotypes. (3) Abnormal transcription regulation contributes to miRNA deregulation in HCCs. (4) A serum-microRNA classifier is able to identify small-size, early-stage, AFP-negative and preclinical HCCs.
Despite the recent success of sorafenib in hepatocellular carcinoma (HCC), prognosis remains poor for advanced disease patients. The mammalian Target of Rapamycin (mTOR) was an attractive target for HCC since it is hyperactivated in at least 50% of cases. However, the FDA-approved mTOR-allosteric inhibitor RAD001 has failed in two clinical trials as a single agent in HCC, most likely due to incomplete mTOR Complex 1 (mTORC1) inhibition. Likewise, we showed that RAD001 had minimal effects on HCC cell proliferation or tumor regression, but when combined with BEZ235, a PI3K/mTOR ATP binding-site competitive inhibitor, synergistically blocked the growth of HCC cells and caused tumor regression in a mouse model approximating human HCC with bad prognosis. We also found that RAD001/BEZ235 induced the upregulation of autophagy, a tumor suppressor event in liver, and particularly stimulates mitophagy. Despite this observation, it is argued that a small population of human cancer stem cells (CSC) or tumor initiating cells (TICs) is protected by mTOR inhibitors, potentially leading to the emergence of resistance. In contrast, the biguanides, phenformin and metformin, also potent inhibitors of mTORC1, are reported to selectively suppress the proliferation of CSCs. These effects are argued to be through inhibition of OXPHOS, and consequent blockage of ATP production, AMPK activation, and the induction of autophagy. Given the ability of biguanides to inhibit mTORC1 and selectively inhibit the proliferation of CSCs, we set out to determine their effect as single agent or in combination with RAD001/BEZ235 on tumor progression, maintenance of CSCs and whether we could recapitulate these findings in a 3D-culture tumor model (organoids). We have found that the triple combination (phenformin together with RAD001/BEZ235) is more potent than either phenformin or RAD001/BEZ235 alone in inhibiting both tumor progression and the maintenance of CSCs. As expected, phenformin efficacy is strikingly dependent on glucose concentration and induces mitochondrial damage, but does not promote autophagy. We have been able to recapitulate the effects of the drugs on CSCs in 3D-cultures, which we are now generating from human patients. Our immediate goal is to determine whether we can use the human organoids from patients to guide clinical treatment in real time.
Delineating molecular signaling cascades has guided the design of many therapeutic chemicals that target specific signaling molecules for treatment of various types of cancer. However, the crosstalk between signaling pathways may confound patients’ responses to pharmaceuticals designed to disrupt a specific pathway. Pten, as a tumor suppressor, acts to counteract the PI3K/Akt signaling, and Shp2 promotes signaling through the Ras-Erk pathway. We have found that additional deletion of Shp2 indeed suppressed the myeloproliferative effect of Pten loss, indicating directly opposing functions between pathways regulated by these two enzymes. Surprisingly, the Shp2 and Pten double knockout mice suffered lethal anemia, a phenotype that reveals previously unrecognized cooperative roles of Pten and Shp2 in erythropoiesis. Consistently, treatment of Pten-deficient mice with a specific Shp2 inhibitor or the Mek inhibitor (Trametinib) suppressed myeloproliferative neoplasm (MPN) while causing anemia. These results identify concerted actions of Pten and Shp2 in promoting erythropoiesis, while acting antagonistically in MPN development.

Although Shp2/Ptpn11 was identified as the first proto-oncogene encoding a tyrosine phosphatase in leukemia, our recent data indicate surprisingly a tumor suppressor role of Shp2 in the liver. Interestingly, dual deficiency of Shp2 and Pten dramatically accelerates and enhances HCC initiation and progression. Additional removal of Shp2 aggravates the development of NAFLD, fibrosis and multiple hepatic injuries induced by Pten loss. Furthermore, combined Shp2 and Pten loss promoted genesis of liver tumor-initiating cells (TICs) in the pre-cancer stage. In sum, these data indicate complexity in signal cross-talk and may explain the frequently seen adverse effects in cancer patients that received drugs designed to target the Ras-Erk pathway.
Human hepatocellular carcinomas are the most prevalent primitive liver cancers, with a still poor prognosis. Thirty per cent of them present activating mutations in the gene encoding beta-catenin. In the lab, we produced transgenic mice with hepatospecific gain- or loss-of-function of beta-catenin signaling, deciphering how it is at work in the liver. Here, I will show that beta-catenin is not only a hepatic oncogene, but also patterns cell specification in the embryonic and the adult liver. These unexpected roles of hepatic beta-catenin make this pathway being a master metabolic integrator in the liver. Similar properties are found in beta-catenin-mutated liver cancers, and have a future as targets for new therapeutic strategies.
Oxidative stress plays an important role in the pathogenesis of many liver
diseases. Mitochondria are the main source of reactive oxygen species (ROS) within hepatocytes. SIRT3, a sirtuin family member of NAD+-dependent deacetylases, is the major mitochondrial protein deacetylase. SIRT3 deacetylates many mitochondrial enzymes to augment anti-oxidant action, including SOD2 and isocitrate dehydrogenase 2. However the role of SIRT3 in protecting hepatocytes oxidative stress remains unknown. We firstly generated acute and chronic CCl4 treated mice models and found SIRT3 expression level in liver decreased in acute liver injury mice, but unchanged in chronic liver injury mice. Next we established a SIRT3 overexpressed murine hepatocyte AML12 cell line. SIRT3 overexpress protected AML12 cells from t-BHP induced oxidative injury through reducing ROS accumulation. Further studies revealed SIRT3 overexpress reduced SOD2 acetylation level and increased catalase expression level, resulting in increased anti-oxidant capacity. t-BHP treatment caused mitochondrial DNA damage, mitochondrial membrane potential reduction and mitochondrial fusion, which resulted in mitochondrial dysfunction. Interestingly, overexpression of SIRT3 totally reversed these adverse effects through upregulating PGC-1α level to promote mitochondria biogenesis, and NRF2 level to maintain mitochondrial dynamics. Furthermore, t-BHP treatment resulted in nuclear DNA damage, while SIRT3 overexpress increased DNA repair through upregulation of OGG1/2. In summary, these results suggest that SIRT3 improves nuclear DNA repair, maintains mitochondrial homeostasis and increases anti-oxidant capacity under oxidative stress status, to enhance hepatocytes surviving, which could be a potential therapy target for liver diseases.
INFLAMMATION-DEPENDENT IL-18 SIGNALING RESTRICTS
HEPATOCELLULAR CARCINOMA GROWTH BY SYSTEMATIC
MODULATION OF LYMPHOCYTE ACTIVITY

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Hepatocellular carcinoma (HCC) is intimately associated with chronically diseased liver tissue. The inflammatory cytokine Interleukin-18 (IL-18) is increased in the circulation of patients with HCC and correlated with poor prognosis, a feature often associated with tumor promoters. However, conflicting results have been reported for IL-18 in HCC development and progression. In this study, we interrogated IL-18’s expression profile in tissue specimens from HCC patients, and used clinically relevant mouse models to explore the functional role of this cytokine. Our results indicate that IL-18 exerts a potent inflammation-dependent tumor-suppressive effect, mediated largely by tumor-infiltrating lymphocytes. We demonstrate that comparisons of IL-18 expression between tumor and matched non-tumor tissue provide more reliable predictions of patient prognosis than overall expression. Taken together, our findings resolve a long-standing contradiction regarding a tumor-suppressive role for IL-18 in established HCC and provide a mechanistic explanation for the complex relationship between its expression pattern and HCC prognosis.
Tissue repair and regenerative medicine address the important medical needs to replace damaged tissue with functional tissue. Kinases Mst1 and Mst2 (Mst1/2), the mammalian Hippo orthologs, are dominant determinants of tissue growth and regeneration. Here, we report the discovery of a reversible and selective Mst1/2 inhibitor, XMU-MP-1, using a high-throughput assay. The co-crystal structure and the structure-activity relationship confirmed that XMU-MP-1 is ‘on-target’ to Mst1/2. XMU-MP-1 blocks Mst1/2 activity, thereby activating downstream effector Yap and promoting cell growth. XMU-MP-1 displays excellent in vivo pharmacokinetics and efficacy to augment mouse liver repair as well as intestinal repair and regeneration. Importantly, XMU-MP-1 treatment exhibits substantially greater repopulation efficacy regarding the Fah-deficient mouse liver with human hepatocytes than a vehicle control. Thus, the pharmacological modulation of Mst1/2 kinase activities might provide a novel approach to potentiate tissue repair and regeneration. Moreover, XMU-MP-1 might serve as a lead compound for developing regenerative-targeted therapeutics.
Liver plays a major regulatory role in whole-body lipid metabolism. Disruption of the hepatic lipid metabolism could lead to the initiation and progression of several metabolic disorders. The accumulation of fat in the form of lipid droplets (LDs) is an early pathophysiological feature of altered liver metabolism that is linked to insulin resistance and the potential progression of severe liver diseases, such as liver steatosis, liver cirrhosis and hepatocellular carcinoma.

LDs are dynamic subcellular organelles whose growth is closely linked to obesity and hepatic steatosis. CIDE proteins including Cidea, Cideb and Cidec (also called Fsp27) play important roles in lipid metabolism. Cidea and Cidec are LD-associated proteins that promote atypical LD fusion in adipocytes. Here, we find that CIDE proteins are all localized to LD-LD contact sites (LDCSs) and promote lipid transfer, LD fusion and growth in hepatocytes. We have identified two types of hepatocytes, one with small LDs (small LD-containing hepatocytes, SLHs) and one with large LDs (large LD-containing hepatocytes, LLHs) in the liver. Cideb is localized to LDCSs and promotes lipid exchange and LD fusion in both SLHs and LLHs, whereas Cidea and Cidec are specifically localized to the LDCSs and promote lipid exchange and LD fusion in LLHs. Cideb-deficient SLHs have reduced LD sizes and lower lipid exchange activities. Fasting dramatically induces the expression of Cidea/Cidec and increases the percentage of LLHs in the liver. The majority of the hepatocytes from the liver of obese mice are Cidea/Cidec-positive LLHs. Knocking down Cidea or Cidec significantly reduced lipid storage in the livers of obese animals. Our data reveal that CIDE proteins play differential roles in promoting LD fusion and lipid storage; Cideb promotes lipid storage under normal diet conditions, while Cidea and Cidec are responsible for liver steatosis under fasting and obese conditions.
IDENTIFICATION OF NOVEL TARGETS TO TREAT NASH AND ITS TRANSITION TO HCC

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Due to the consumption of high caloric food combined with an increased sedentary lifestyle, the incidence of overweight and obesity has grown rapidly in Western cultures, like the USA and Europe but notably also in developing countries (e.g. India, China). Although chronic viral infections with Hepatitis B or C are still the leading etiology causing hepatocellular carcinoma (HCC), it has become more and more clear that non-alcoholic fatty liver disease (NAFLD) and subsequent non-alcoholic steatohepatitis (NASH) are increasingly important etiologies for HCC development. Dietary etiology greatly contributes to the fact that HCC currently is the fastest rising cancer in the USA, with a similar trend in Europe. In the recent past, we and others have generated several pre-clinical mouse models that enabled to study the cellular and molecular mechanisms of NASH development and NASH to HCC transition in the context of a chronic metabolic syndrome. Remarkably, these models recapitulated several pathophysiological hallmarks of NASH on the basis of a metabolic syndrome in humans and develop HCC. Here, I will report on the characterization and identification of novel targets that could be used to treat NASH and subsequent liver cancer development.
Emerging of systems and quantitative biology has provided us a new and powerful framework to explore the genesis and progression of cancer. A quantitative cancer endogenous molecular-cellular network theory has been proposed [1, 2] and implemented in Hepatocellular Carcinoma (HCC) [3]. In light of this theory and working model, we found that the normal hepatocyte and cancerous hepatocyte can be represented by robust stable states of one single endogenous network, and the genesis and progression of cancer can be viewed as transitions from normal tissue state and cancer state. Accordingly, here we addressed the issues of the mutation regularity of HCC [4] and the potential strategies to cure or relieve HCC [3]. First, normal and cancer states predicted a set of most probable genetic mutations in HCC. Subsequent inspection of experimental data demonstrated agreement with this prediction. Other testable predictions, such as mutation regularity in normal liver tissue and similar mutation regularity in other cancers, were also obtained. Second, In the light of this theory, we demonstrate that positive feedback loops must be existed as a general molecular basis for the maintenance of normal liver and HCC. Regulating the positive feedback loops directly or indirectly provides potential strategies to cure or relieve HCC. Specifically, inhibiting proliferation and inflammation related positive feedback loops, and simultaneously inducing liver-specific positive feedback loop were predicated as the potential strategy to cure or relieve HCC. Other classical issues in cancer research such as drug resistance, cancer diagnosis, cancer epidemiology can also be discussed in the present theory.

References
ASSESSING LIVER FIBROSIS IN PATIENTS WITH CHRONIC HEPATITIS B: COMPARISONS OF T1 MAPPING ON GD-EOB-DTPA-ENHANCED 1.5T MRI WITH ASPARTATE AMINOTRANSFERASE-TO-PLATELET RATIO INDEX AND FIBROSIS-4

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Objectives: To assess accuracy of T1 mapping on Gd-EOB-DTPA-enhanced 1.5T magnetic resonance imaging (MRI) for staging liver fibrosis in chronic hepatitis B (CHB) and to compare it with aspartate aminotransferase-to-platelet ratio index (APRI) and FIB-4.

Methods: This retrospective study included 100 patients with CHB (mean age: 54 years old; 85 men and 15 women) who underwent gadoxetic acid-enhanced MRI including T1 mapping on a 1.5T scanner (MAGNETOM Aera, Siemens Healthcare, Erlangen, Germany). T1 mapping using 2 flip angle method was performed before and 20-min hepatobiliary phase (HBP) after injection of gadoxetic acid (Primovist, Bayer-Schering) with following parameters: repetition time/echo time = 4.38 ms/1.93 ms, flip angle = 2°and 12°, FOV = 380–400mm×300–324mm, matrix = 216×288, slab thickness = 200 cm resulting in an interpolated section thickness of 5 mm. Liver fibrosis stage was histologically determined according to the Scheuer scoring system: S0 (n=21), S1 (n=12), S2 (n=14), S3 (n=18) and S4 (n=35). Region of interests were drawn on the homogenous area of the right liver lobe on the T1 maps, avoiding major vessels and bile ducts. Mean T1 relaxation times were measured and the reduction rate of the T1 relaxation time (ΔT1) was calculated. Liver function tests and platelet counts were performed, the APRI and FIB-4 index were calculated using the corresponding formulas. Diagnostic performance of T1 relaxation times, APRI and FIB-4 for staging significant fibrosis (≥S2), advanced fibrosis (≥S3) and cirrhosis (S4) was compared.

Results: Precontrast T1 (r=0.34), HBP T1 (r=0.69), ΔT1 relaxation time (r=0.50), APRI (r=0.59) and FIB-4 (r=0.55) correlated significantly with fibrosis stages (all P<0.001). Areas under the curves (AUCs) of HBP T1 relaxation time for significant fibrosis, advanced fibrosis and cirrhosis were 0.81, 0.87 and 0.90, with sensitivities of 67.16%, 81.13%, 91.43% and specificities of 84.85%, 85.11% and 83.08%, respectively, and the AUCs were greater than that of APRI (0.79, 0.84, 0.81) and FIB-4 (0.75, 0.80, 0.80), although the differences were not significant (P>0.05). However, APRI and FIB-4 exhibited limited sensitivity (<72.00%) for cirrhosis compared with HBP T1 value.

Conclusion: Gd-EOB-DTPA-enhanced T1 relaxometry is more accurate than APRI and FIB-4 for staging liver fibrosis in CHB.
DEMETHYLENEBERBERINE ATTENUATES NON-ALCOHOLIC FATTY LIVER DISEASE WITH ACTIVATION OF AMPK AND INHIBITION OF OXIDATIVE STRESS

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Non-alcoholic fatty liver disease (NAFLD) has reached an epidemic level globally, which is recognized to form non-alcoholic steatohepatitis (NASH) by the “two-hit” model, including oxidative stress and inflammation. As approximately 20-30% of the general population suffers from NAFLD, the growing prevalence pushes it into a social problem in the Western countries, which is a much more common rather than alcoholic fatty liver disease (ALD).

Demethyleneberberine (DMB) is a novel cationic antioxidant which comes from Chinese herb Cortex Phellodendri chinensis (CPC) that has a long history of traditional Chinese medicine use. Theoretically, DMB could be guided into mitochondrion by the high negative potential inside mitochondrion. Recent studies revealed that CPC has many pharmacological activities such as anti-microbial, anti-inflammatory, anti-diarrhoea and anti-cancer, but the particular biological properties of DMB are still elusive.

AMP-activated protein kinase (AMPK) has long been regarded as a key regulator of energy metabolism, which is recognized as a critical target for NAFLD treatment. Here we introduce ameliorated NAFLD by activating AMPK pathways. Our study showed that the intraperitoneal injection of DMB (20 or 40 mg/kg body weight) decreased hepatic lipid accumulation in methionine and choline deficient (MCD) high-fat diet feeding mice and db/db mice. The further investigation demonstrated that DMB activated AMPK by increasing its phosphorylation in vitro and in vivo. Accompanied with AMPK activation, the expression of lipogenic genes were significantly reduced while genes responsible for the fatty acid β-oxidation were restored in DMB-treated NAFLD mice. In addition, the remarkable oxidative damage and inflammation induced by NAFLD were both attenuated by DMB treatment, which is reflected by decreased lipid oxidative product, malonaldehyde (MDA) and inflammatory factors, tumor necrosis factor α (TNFα) and interleukin 1β (IL-1β). Based on all above, DMB could serve as a novel AMPK activator for treating NAFLD and preventing the pathologic progression from NAFLD to NASH by inhibiting the oxidative stress and inflammation.

Key words: Demethyeneberberine; Non-alcoholic liver disease; Hepatosteatosis; AMPK; Oxidative stress; Inflammation

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Liver cancer is extremely heterogeneous in its tumor biology and clinical presentation, which poses a significant challenge to cancer management. Tumor heterogeneity may emanate from the presence of cancer stem cells or selection by clonal evolution. To overcome this problem, molecular-based technologies including genomic, transcriptomic and metabolomic profiling, have been used to distinguish tumor subgroups, which allow for stratification of patients with greater homogeneity and can assist in molecular re-staging. These various genome-based signatures also delineate critical gatekeepers of cancer initiation and progression which can be further honed by integrative genomics to identify key driver genes and functionally linked networks capable of determining patient prognosis or therapeutic outcome. Examples of biologically relevant molecular signatures and drivers include those linked to metastasis, tumor recurrence, cancer stem cells, tumor metabolism, tumor stroma and gender disparity. Furthermore, comparative genomics has revealed that although signatures may share a common prognostic space, each carries unique molecular changes linked to different sets of cancer hallmarks, which collectively occupy different tumor biological space. Integrative genomic approaches allow us to tease apart these differences, rooted in tumor heterogeneity, to identify critical biomarkers for cancer diagnosis and clinically relevant therapeutic targets that represent convergent cancer driving molecular nodes.
Hepatocellular death is present in almost all types of human liver disease and is used as a sensitive parameter for the detection of acute and chronic liver disease of viral, toxic, metabolic, or autoimmune origin. Clinical data and animal models suggest that hepatocyte death is the key trigger of liver disease progression, manifested by the subsequent development of inflammation, fibrosis, cirrhosis, and hepatocellular carcinoma. For years, the term apoptosis was used synonymously for programmed cell death. Apoptosis is triggered by ligation of death receptors like tumor necrosis factor (TNF) receptor by their cognate ligands and represents a highly synchronized procedure depending on activation of aspartate-specific proteases known as Caspases. Apoptotic death of hepatocytes is a common feature of viral hepatitis, acute liver failure, alcoholic and nonalcoholic steatohepatitis and is associated with fibrosis. However, a growing number of recent studies showed that there are distinct programmed cell death modes other than apoptosis. As such, necroptosis – relying on the kinases Receptor-Interacting Kinase 3 (RIPK3) and RIPK1 – represents a novel form of programmed cell death in development, tissue homeostasis and inflammation. In our work, we have focused on evaluating the functions of RIP Kinase-dependent signaling pathways in liver physiology and pathology. More specifically, data on the roles of RIPK1 and RIPK3 in acute liver injury, NASH and HCC development as well as their interaction with inflammatory signaling pathways will be presented and discussed.
THE POTENTIAL OF NOVEL TETRAHYDROXYLATED BILE ACIDS (THBAS) TO PREVENT CHOLANGITIS AND LIVER CANCER IN ABCB4⁻/⁻ MICE

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THBA was discovered in the bile of Abcb11⁻/⁻ mice, a genetic model for Progressive Familial Intrahepatic Cholestasis 2 (PFIC2) [1]. Unlike PFIC2 patients, Abcb11⁻/⁻ mice do not exhibit progressive cholestasis. Our hypothesis is that production of THBA in the bile of Abcb11⁻/⁻ mice, has dual benefits. First, THBA a highly hydrophilic bile salt reduce bile acid pool hydrophobicity and toxicity. Second, THBA can use another canalicular transporter, Abcb1 (Mdr1), to promote bile flow and reduce cholestasis. Progressive Sclerosing Cholangitis (PSC) is a chronic cholestatic disease. PSC patients have a greatly increased risk of liver and GI cancers. Abcb4⁻/⁻ mice are a genetic model for PSC. They develop cholangitis at 2 weeks, gallstones and progressive fibrosis after 8 weeks, and extensive liver damage leading to hepatocellular carcinoma after 12 months.

The goal of this study is to determine if THBA can prevent cholangitis and liver cancer in the Abcb4⁻/⁻ mice. We constructed a double knockout (DKO) (Abcb11⁻/⁻ and Abcb4⁻/⁻) mouse to determine if THBA produced by the Abcb11⁻/⁻ mice can protect the DKO mice from developing cholangitis. We observed that the liver in DKO mice over the course of up to 13.5 months is similar to wild-type mice. However, the non-DKO Abcb4⁻/⁻ littermates develop firm livers with diffuse, chronic cholangitis and intra-hepatic bile duct sclerosing. In addition they develop nodules often of hepatocellular carcinoma origin. To determine if THBA alone was able to alleviate progressive cholangitis, we synthesized different isoforms of THBA and showed they are efficient at stimulating bile flow. Feeding synthesized THBA to Abcb4⁻/⁻ mice greatly reduced gallstone formation, and liver chemistry and histology showed that THBA-fed Abcb4⁻/⁻ mice had less cholangitis and fibrosis.

We conclude that progression of cholangitis in Abcb4⁻/⁻ mice is a result of chronic exposure to toxic, hydrophobic bile acids and reducing the hydrophobicity of the bile acid pool by feeding Abcb4⁻/⁻ mice with THBA, or creating a DKO with Abcb11⁻/⁻ results in reducing cholangitis and liver cancer. Therefore THBAs can potentially be used to treat liver diseases where a therapeutic agent with high choleretic efficiency and low toxicity may be advantageous.

MIR 122 IS A HEPATIC-HORMONE WITH SYSTEMIC EFFECTS

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Anemia is commonly associated with acute and chronic inflammation however; the underlying mechanism is not fully resolved. Our aim was to determine whether miR-122, which is generated in the liver and is also secreted to the blood, participates in the development of anemia associated with inflammation. We characterized the primary transcript of the human liver-specific miR-122, which spans 5 kb consisting of three exons, the third of which gives rise to miR-122. Within the miR-122 promoter region we identified an NF-kB binding site and demonstrate that RelA, as well as the NF-κB stimulators, Tumor Necrosis Factor Alpha (TNFα) and lipopolysaccharide (LPS), mediators of inflammation-induced anemia, all enhance miR-122 promoter activity. LPS, potentially derived from gram-negative gastrointestinal bacterial flora, traveling from the gut microbiome to the liver, also induces miR-122 secretion into the blood in a TNFα-dependent manner. We established that erythropoietin (EPO) is a miR-122 target and an increase in miR-122 in the blood results in reduced Epo expression level. In accordance, repression of miR-122 by antago-miR-122, results in increased EPO, reticulocyte and hemoglobin levels. An inverse miR-122/EPO correlation was found in the blood of mice with acute pancreatitis and steatohepatitis and in human patients with acute inflammations. Conclusion: We propose that by targeting EPO, miR-122 contributes to inflammation-induced anemia and accordingly, inhibition of miR-122 may offer a new therapeutic approach for this malady.
Adaptive evolution can happen at various levels, population, organism, or cellular. Cancer has been recognized as an evolutionary process driven by mutation, drift, migration and natural selection. Cells from within a tumor and from the adjacent normal tissues may be treated as human individuals sampled from all over the world. Recent large-scale genomic sequencing studies have revealed extensive genetic heterogeneity within a tumor which has been believed to be driven by positive selection, thus retaining high frequency in the tumor. However, it lacks a set of empirical data with a precise estimation of low frequency mutations and a null model to test the force of selection.

To provide the statistical power, multiregional samples in hepatocellular carcinoma (HCC) tumors were sampled and sequenced using either whole-exome sequencing (WES) or genotyping. Population genetic theories to model the mutation process during tumor growth were applied with aims to measure genetic heterogeneity driven by neutral forces and tested the action of selection both within and between tumors by comparing the observations with the null model. Those studies indicate that neutral process inevitably results in rapid and extreme genetic diversification of tumor cells within tumors. The non-Darwinian process may not accurately characterize the evolution between tumor and non-tumor tissues, or between different tumors in the same individual. Rigorous testing of predictions against observations is necessary. The genetic diversity under a Darwinian model would generally be orders of magnitude smaller. Because the level of genetic diversity will have implications on therapeutic resistance, non-Darwinian evolution should be heeded in cancer treatments even for microscopic tumors.
EXPLOITING THE PENTOSE PHOSPHATE PATHWAY AS THERAPEUTIC TARGET IN HEPATOCELLULAR CARCINOMA TREATMENT

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Hepatocellular carcinoma (HCC), the most common type of primary liver cancer, is the fifth most prevalent and the third most lethal cancer worldwide. Only a minority of HCC patients are suitable for surgical treatments. HCC has a high recurrent rate and is resistant to conventional chemotherapies. So far, there is only one FDA-approved targeted therapy, Sorafenib, for advanced HCC patients. Knowledge regarding the metabolic regulation in HCC is warranted for the identification of novel therapeutic targets. HCC cells experience an elevation of oxidative stress due to aberrant signaling pathways and activated metabolic machinery. To combat oxidative stress, we found that HCC cells had increased reliance on the pentose phosphate pathway (PPP), a major antioxidant producing pathway. PPP is composed of the oxidative and the non-oxidative arms. We showed that all enzymes in the PPP were over-expressed in human HCC. Transketolase (TKT), a reversible enzyme in the non-oxidative arm which controls the direction of the metabolic flux in the PPP, was the most up-regulated enzyme and its expression was governed by the NRF2/KEAP1/BACH1 oxidative stress sensor pathway in HCC. Metabolomic and metabolic flux analyses suggested that knockdown of TKT truncated the PPP, forcing metabolites to enter the oxidative arm. This led to an accumulation of ribulose-5-phosphate (Ru5P) and ribose-5-phosphate (R5P) which in turn inhibited the activity of glucose-6-phosphate dehydrogenase (G6PD), the NADPH-producing enzyme in the PPP. As a result, knockdown of TKT drastically increased reactive oxygen species (ROS) accumulation and caused ROS-associated cell cycle delay. Knockdown of TKT markedly reduced NADPH production and repressed HCC growth in vitro and in vivo. Therapeutically, disturbing the redox homeostasis of HCC cells by stable knockdown or pharmacologic inhibition of TKT sensitized cancer cells to Sorafenib treatment in vitro and in vivo. Here, using HCC as a cancer model, we highlighted the pro-tumorigenic roles of antioxidants. We also demonstrated that the PPP can be exploited as a therapeutic target for cancer treatment.
ROLE OF METHIONINE ADENOSYLTRANSFERASE 1A IN LIVER CANCER

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Methionine adenosyltransferase (MAT) catalyzes the biosynthesis of S-adenosylmethionine (SAMe), the principal biological methyl donor. MAT1A encodes mammalian MAT in normal hepatocytes and MAT2A in all non-hepatocytes. Patients with chronic liver disease have reduced hepatic MAT activity and SAMe levels. Mat1a knockout mice have low hepatic SAMe levels, develop steatohepatitis and hepatocellular carcinoma (HCC). In these mice, several oncogenic signaling pathways are induced. SAMe protects against carcinogen-induced HCC in rodents in part by preventing hepatic SAMe depletion. SAMe also prevents HCC establishment--but not growth of already established HCC--in an orthotopic HCC model due to hepatic compensation that prevented SAMe accumulation. A more effective strategy is to enhance MAT1A expression in HCC, which raised steady state SAMe level, promoter methylation and silencing of the oncogene LIN28B. Besides HCC we have uncovered another liver cancer where MAT1A plays a key role. We found MAT1A expression is reduced in two mouse models of chronic cholestatic liver injury and in a mouse model of cholestasis-associated cholangiocarcinoma (CCA). Importantly we found MAT1A-encoded protein (MATα1) is highly expressed in normal bile duct epithelial cells and hepatocytes, but reduced in both chronic cholestatic liver injury and mouse and human CCA. We used anti-MATα1 antibody followed by proteomics MS to identify proteins that interact with MATα1 and found that MATα1 interacts with Mnt, Max and c-Myc. In normal liver, MATα1 mainly interacts with Mnt and Max, but this switches to c-Myc and Max in cholestatic livers and CCA. Interestingly promoter regions of MAT1A and c-Myc have E-box sequences that are bound by MATα1, Mnt and Max in normal liver that switched to c-Myc and Max in cholestatic livers and CCA. MATα1 binds to the E-box as a complex with c-Myc and Max, but not by itself or with c-Myc. While E-box positively regulates c-Myc, it negatively regulates MAT1A. Furthermore MATα1 binding represses E-box whereas c-Myc binding activates E-box. This results in reciprocal regulation between MAT1A and c-Myc. Overexpressing MAT1A lowered c-Myc expression even after inhibiting DNA methylation or H3K27 trimethylation. Knocking down MAT1A was as effective as overexpressing c-Myc in enhancing CCA growth and invasion in vivo. The same reciprocal regulation between MAT1A and c-Myc also occurs in HCC cells. Taken together, MAT1A is a tumor suppressor gene in both HCC and CCA. Lower SAMe level activates many oncogenic pathways as well as turning on oncogenes via epigenetics. In addition we have uncovered a reciprocal regulation between MAT1A and c-Myc by a mechanism that appears independent of epigenetics. Therapies targeted at raising MAT1A expression may benefit both HCC and CCA.
REDUCED GROWTH ABILITY AND INCREASED NUCLEAR ABNORMALITY IN HBV-INFECTED HUMAN HEPATOCYTES OF HUMANIZED CHIMERIC MOUSE LIVER

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Several studies performed in established cell lines and transgenic mice have reported that expression of hepatitis B virus (HBV) proteins, such as surface and X proteins, affect hepatocyte proliferation and induce growth of hepatocellular carcinoma. However, it has not been elucidated how HBV infection affects phenotypes of normal human hepatocytes (HHs), which are the natural host for HBV. In the present study, we examined the effects of HBV infection on the morphology and proliferation of HHs by using cDNA-uPA/SCID chimeric mice with humanized livers.1) Seven-week-old chimeric mice were inoculated with HBV genotype C. At 12 and 20 weeks after infection, HHs were collected by collagenase perfusion. Compared with naïve HHs, HBV infection induced robust HH hypertrophy, and increased the number of binuclear HHs at 12 weeks after infection. At 20 weeks after infection, the HHs were more enlarged and many atypical nuclei were observed. Flow cytometric analysis and microscopic observation revealed that ploidy increased depending on the duration of infection, and pegylated interferon alpha-2a treatment for 8 weeks reduced the cell size and inhibited the ploidy increase caused by HBV infection. To examine the effects of HBV infection on HH proliferation, HHs were isolated from naïve and HBV-infected chimeric mice and transplanted into 3-week-old cDNA-uPA/SCID mice. At 16 weeks after transplantation, blood human albumin levels reached 9 mg/mL. On the other hand, maximum human albumin level was approximately 1 mg/mL in chimeric mice transplanted with HBV-infected human hepatocytes. Histological examination indicated that the repopulation ratio of chimeric mouse liver transplanted with naïve HHs was more than 80%, but that of chimeric mouse liver transplanted with HBV-infected HHs was less than 10%. These results suggest that HBV infection not only induced hepatocyte hypertrophy and ploidy increase but also inhibited hepatocyte proliferation in vivo.

ROLE FOR THE ER STRESS SENSOR IRE1α IN PROMOTION OF HEPATOCELLULAR CARCINOMA

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In eukaryotes, accumulation of unfolded/misfolded proteins in the endoplasmic reticulum (ER) lumen triggers the unfolded protein response (UPR), which reduces ER stress by enhancing the ER’s capacity to manage the overload of protein folding. A growing number of studies have indicated the implication of ER stress in various cancers, but the mechanistic role of individual UPR pathways in tumorigenesis and/or tumor progression are poorly understood. The mammalian inositol-requiring enzyme 1 α (IRE1α), an ER-localized Ser/Thr kinase/endonuclease, mediates a critical signaling branch of the UPR. IRE1α is activated through trans-autophosphorylation and cleaves Xbp1 mRNA to produce a functionally active spliced form of this transcription factor upon ER stress. We previously demonstrated in mice that IRE1α promotes hepatocyte proliferation and liver reparative regeneration through regulating STAT3, a critical molecule in the development of hepatocellular carcinoma (HCC). Here, we report a crucial role for IRE1α in diethylnitrosamine (DEN)-induced HCC in mice. We found that abrogation of hepatic IRE1α protected mice from DEN-initiated tumorigenesis, exhibiting fewer and smaller tumors in the liver. Furthermore, high-fat diet (HFD)-induced obesity, which accelerated HCC development in DEN-treated mice, did not affect the protective effect of hepatocyte IRE1α deficiency. Further studies showed that loss of IRE1α resulted in attenuated hepatosteatosis in HFD-fed mice, and enhanced hepatocyte apoptosis in mice regardless of feeding a normal chow diet or HFD. Together, these results suggest that IRE1α can promote or even drive HCC development through suppressing cell death in hepatocytes. Thus, targeting IRE1α may serve as an intriguing strategy for HCC therapeutics.
Liver regeneration requires the integration of multiple signals and interaction among different hepatic cell populations that orchestrally stimulate quiescent hepatocytes (HPC) to reenter cell cycle and proliferate. We aimed to investigate the regulatory signaling networks underlying transcriptional interaction between liver sinusoidal endothelial cells (LSEC) and HPC during liver regeneration. Genome-wide transcriptional profiling of mouse LSEC and HPC, isolated 2, 30, 48, and 168 h after partial hepatectomy (PH) and 2 h after sham operation, was measured using the Cap Analysis of Gene Expression technology. The results of Ingenuity Pathway Analysis showed enrichment in LSEC for genes encoding molecules regulating wound-healing pathways as early as 2 h after PH, while in HPC pathways related with cell cycle control were abundantly enriched at 30 h and 48 h after PH. To investigate interaction between HPC and LSEC, correlation analysis on gene expression was performed and important genes implicated in liver regeneration were extracted from various importance parameters (VIPS) found in partial least squares-discriminant analysis (PLS-DA) modeling. As a result, a key regulator, orosomucoid (Orm1), known to be involved in lipid homeostasis and energy balance, was identified and further characterized by Bayesian network analysis. Expression of Orm1 significantly increased in HPC at 30 h and peaked at 48 h after PH. Gene expression analysis of a panel of human liver cell lines showed that Orm1 was restrictedly expressed by HPC. Immunohistochemical (IHC) staining of liver tissues of hepatectomized mice showed a strong expression of Orm1 at 48 h after PH in portal vein (PV) area, compared to central vein (CV) areas with strict localized positivity only in pericentral HPC adjacent to CV. In vivo knockdown of Orm1 with its siRNA administered to mice using Invivofectamine 3.0 by a single injection from their tail-vein resulted in the suppressed expression of a proliferating marker Ki-67 in regenerating liver at 48 h after PH. Furthermore, fold change in serum levels of Orm1 before and 48 h after PH was significantly correlated with the postoperative liver-to-body weight ratio of mice, suggesting a proper response of Orm1 is required for liver regeneration. In human HPC cell line FLC4, Orm1 siRNA inhibited cell proliferation suppressing the expression of cell cycle-associated genes such as cyclin B. These findings suggested a potential role of the lipid homeostasis and cell cycle regulator, Orm1, in the regulation of HPC proliferation during liver regeneration.
DIRECT REPROGRAMMING OF HUMAN FIBROBLASTS TO FUNCTIONAL HEPATOCYTE-LIKE CELLS

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The generation of functional hepatocytes is key for cell-based therapy for liver diseases. We have previously converted non-hepatic mouse and human cells into mature hepatocyte-like cells with potential for biomedical and pharmaceutical applications (named as iHep and hiHep, respectively). Lately, we have characterized whether hiHeps could be used in a bio-artificial liver support system (hiHep-BAL) and demonstrated that hiHep-BAL could treat large animals with D-gal-induced acute liver failure. In generation of iHep and hiHep cells, we found that lineage conversion by expression of lineage-specific transcription factors is a process with low efficiency. We analyzed the mechanism by which a cell resists hepatic lineage conversion. Our recent data shed light on cellular responses to hepatic conversion by revealing a function of the ATM-p53 pathway in sensing chromatin opening.
One of the emerging hallmarks of cancer has been the deregulated cellular metabolism, which is well beyond the Warburg effect, or aerobic glycolysis, as Otto Warburg described some 90 years ago. In this presentation, I will discuss our recent evidence regarding alterations as well as the underlying mechanisms of glucose, lipid and amino acid in cancer cells, especially in hepatocellular carcinoma cells.
β-CATENIN-DEPENDENT ERYTHROPOIESIS IN ADULT MICE DEFICIENT IN HEPATIC ARID1A CHROMATIN REMODELER

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Genetic and epigenetic alterations play key roles in cancer, as illustrated by the identification of preferential mutations in SWI/SNF chromatin remodeling components in Hepatocellular Carcinoma (HCC), the most common primitive liver cancer and the second most frequent cause of cancer-related deaths worldwide. One third of HCCs depend on activating mutations in CTNNB1 gene encoding β-catenin. ARID1A, a SWI/SNF component, is the chromatin modifier the most frequently mutated in more than 13% of HCCs, initially found preferentially in CTNNB1-mutated ones (Guichard, Nat Genet, 2012). ARID1A would rather be tumor suppressive, as its mutations are mainly inactivating. We hypothesized that HCC emergence and/or progression could be linked to the loss of ARID1A in a β-catenin-dependent context.

Our aim was to determine whether the loss of ARID1A increase the tumorigenic potential of β-catenin aberrant signaling in the liver. We took advantage of a transgenic murine model in which APC, the major brake of β-catenin signaling, is lost, leading within 9 months to HCC emergence (Colnot, PNAS 2004). We thus engineered new transgenic models using Cre-loxP strategy, allowing hepato-specific and inducible inactivation of Apc (leading to hepatic gain-of-function of β-catenin) and/or Arid1a in single hepatocytes.

Seven months after the loss of both APC and ARID1A in single hepatocytes, we unexpectedly observed intrahepatic macroscopic amounts of red blood cells, associated with a dramatically increased hematocrit and a de novo transcription of hepatic erythropoietin (EPO). This phenotype observed with complete penetrance in compound Apcko/Aridako mice, is not seen in either Apcko or Aridako mice. We confirmed this de novo transcription of Epo in compound Apcko/Aridako livers, as soon as 7 days after panlobular genic inactivations. These data show that chromatin remodeling induced by ARID1A loss can unmask new targets of β-catenin, such as Epo. Moreover, we found that this loss of both APC and ARID1A leads to a splenomegaly with a dramatic increase of erythroid progenitor cells suggesting the establishment of a stress erythropoiesis in response to the aberrant transcription of Epo by liver.

In this experimental setting, we did not find any effect of ARID1A loss on β-catenin-dependent tumor initiation, suggesting that this loss would rather have an effect on tumor progression. We currently investigate the mechanistics of this new relationship, as it could play a dual role. In liver cancers, transcriptional reprogramming through ARID1A loss could be a feature contributing to HCC progression. In liver physiology, chromatin remodeling through ARID1A could be a powerful epigenetic brake, contributing to the shift from an embryonic liver that produces EPO to an adult liver status.
The histological sections of liver like many tissues offer the potential of many layers of additional data over just a traditional morphological description. This presentation will look briefly at a newly developed range of histological based protocols and novel imaging techniques that are bringing a new importance to tissue imaging as a quantitative tool in understanding relationships in tissue microenvironments, particularly in the relation to tumorgenesis, inflammation and immunity.

This new development combines: single section multi-marker fluorescent labelling of up to 8 antigens using antibodies all of the same species; automated multispectral imaging (MSI) to remove the typically problematic FFPE tissue auto fluorescence and correct cross-talk between fluorescent channels; and an automated analysis that can quantitate the per-cell marker expression, determine the cellular phenotype, and elevates the power of fluorescence imaging from a cell based to tissue based analysis and bioinformatics.

This combined staining and detection strategy is ideal solution for delivering quantitative per-cell marker expression and phenotyping in any tissue and, analogous to that obtained from flow cytometry, but from within the intact tissue microenvironment, imaged in situ from standard FFPE tissue blocks.
LIVER-DIRECTED GENE THERAPY FOR TRANSPLANTATION TOLERANCE INDUCTION

Alexandra Sharland

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The liver is a tolerogenic site for the expression of foreign antigens. Liver allografts are spontaneously accepted in both small and large animal experimental models, and up to 20% of clinical liver transplant recipients can be weaned from all immunosuppression. Primary infections of hepatocytes are difficult for the recipient immune response to eradicate and often persist, whilst gene delivery to the liver can induce tolerance to both viral capsid antigens and to the transgene products.

Our group uses a gene therapy approach to express allogeneic MHC molecules in the recipient liver, for the induction of transplantation tolerance. High-level expression of donor MHC class I overcomes both naïve and memory responses against skin allografts. Tolerance induction is dependent upon the direct recognition of intact donor MHC, and upon absent or low-level expression of co-stimulatory molecules by transduced hepatocytes. Survival of fully-allogeneic heart grafts is significantly prolonged by the expression of multiple mismatched class I, while specialized MHC class II antigen processing and presentation pathways can be recapitulated in hepatocytes through the co-expression of allogeneic class II and class II transactivator. This work and its potential for clinical translation will be discussed.
DIRECT IN VIVO shRNA SCREENING FOR FUNCTIONAL TARGET DISCOVERY IN HEPATOCELLULAR CARCINOMA

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In contrast to some hematopoietic malignancies, for which molecular therapies can induce long-lasting tumor remissions, clinical experiences over the past couple of years have revealed that in most common types of solid tumors, acquired therapy resistance against molecular therapies is inevitable. Hepatocellular carcinoma (HCC) can be seen as a prototype of a therapy resistant tumor, and it represents a major health problem, causing more than 700,000 deaths annually worldwide. HCC shows intrinsic resistance to cytotoxics, and although the multikinase inhibitor sorafenib was recently approved as the first systemic treatment for patients with advanced HCC, the survival advantage conferred to these patients from sorafenib therapy averages only 2.8 months. In my talk I will give examples how innovative mosaic cancer mouse models can be combined with stable in vivo shRNA technology to identify new cancer genes and therapeutic targets in liver carcinomas. Taking advantage of a recently developed system for transposon-mediated in vivo delivery of miRNA-based short hairpin RNAs (shRNAs) (Rudalska et al., Nature Medicine 2014, Wuestefeld et al., Cell 2013, Kang et al., Nature 2011) we developed a platform that can be used to conduct negative-selection shRNA screens directly in mouse liver carcinomas in vivo. In my talk I will discuss novel therapeutic targets in HCC which were functionally identified by direct in vivo shRNA screening.
Postnatal liver maturation follows a programmed silencing of embryonic and cell cycle gene expression, which is often re-activated in hepatocellular carcinoma (HCC). The underlying mechanism for this highly coordinated program is largely unknown. Here we report that chromatin-bound histone H3 is increasingly poly-ubiquitinated during postnatal mouse liver development. An E3 ubiquitin ligase complex consisting of Cul4, DDB1 and DCAF8 adaptor protein, CRL4(DCAF8), binds and targets nucleosomal histone H3 for ubiquitination. Inducible deletion of DDB1 in adult mouse hepatocytes abolishes histone H3 ubiquitination, decreases methylated H3K9 on the promoters of fetal liver genes, reactivates their expression, and eventually causes cell senescence. Overexpression of a ubiquitination-defective H3 mutant or knockdown of DCAF8 results in reduced H3 ubiquitination and reactivation of fetal gene expression. Finally, some human hepatocellular carcinoma (HCC) samples shows a significant reduction of H3 ubiquitination. Our results suggest that histone H3 ubiquitination plays a critical role in postnatal liver maturation and its dysregulation might contribute to liver tumorigenesis.
The adult liver possesses the remarkable ability to fully regenerate upon tissue injury, disruption of which may lead to cancer development. How the liver micro-environment shapes liver cell plasticity upon liver damage remains elusive. We show that persistent hepatic mitochondrial dysfunction and oxidative stress trigger a niche favoring cholangiocellular overgrowth. Mechanistically, ROS and liver damage stimulate Kupffer-cell derived TNF production, activating JNK signaling in cholangiocytes, leading to cholangiocellular hyperproliferation and neoplasia. Anti-oxidant treatment, Kupffer-cell depletion, genetic TNFR1 deletion and pharmacological JNK inhibition reversed the phenotype. In different ICC mouse models and in patients with intrahepatic cholangiocellular carcinoma (ICC) strong TNF expression and cholangiocellular JNK activation can be detected within ICC, as well as ROS accumulation is found in surrounding hepatocytes. Our findings identify Kupffer-cell derived TNF as a pivotal regulator of cholangiocellular proliferation and subsequent neoplasia in liver damage. Targeting this ROS/TNF/JNK axis provides novel opportunities for liver injury and ICC therapy.
Nuclear factor E2-related factor 2 (Nrf2) is a transcription factor regulating the expression of a battery of genes encoding phase II carcinogen detoxifying enzymes and other cytoprotective proteins, hence being considered as an important target for cancer chemoprevention. However, recent studies have demonstrated the oncogenic potential of Nrf2. Here, we report that Nrf2 deficiency confers resistance to diethylnitrosamine (DEN)-induced hepatocarcinogenesis in mice. In the DEN-treated wild-type mouse liver, Nrf2 was overactivated. Mechanistically, the functionally activated Nrf2 was accompanied by overexpression of some metabolic enzymes involved in utilization of glutamine and channeling of glucose intermediates into the pentose phosphate pathway. This appeared to result in anabolic needs for rapid cell proliferation and growth of tumors. Notably, Nrf2 was mutated in amino-terminal motif DLG located in Keap1 binding domain, which may account for its enhanced activity in hepatocarcinomas formed in DEN-treated mice. Collectively, our results demonstrate an unexpected oncogenic potential of mutated Nrf2 in experimentally induced hepatocarcinogenesis through metabolic alterations.
HEPATOCELLULAR CARCINOMA REPRESSION BY TNFα-MEDIATED SYNERGISTIC LEthal EFFECT OF MITOSIS DEFECT-INDUCED SENESCENCE AND CELL DEATH SENSITIZATION

Dan Li¹, Jing Fu², Min Du ³, Haibin Zhang², Lu Li¹, Jin Cen¹, Weiyun Li¹, Xiaotao Chen¹, Yunfei Lin⁴, Edward Conway⁵, Eli Pikarsky⁶, Hongyan Wang¹, Yuan Ji³, Hong-Yang Wang⁷, Guoyu Pan⁴, Lijian Hui¹

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Hepatocellular carcinoma (HCC) is a cancer lacking effective therapies. Several measures have been proposed to treat HCCs, such as senescence induction, mitotic inhibition and cell death promotion. However, data from other cancers suggest that single use of these approaches may not be effective. Here, by genetic targeting Survivin, an inhibitor of apoptosis protein (IAP) that plays dual roles in mitosis and cell survival, we identified a TNFα-mediated synergistic lethal effect between senescence and apoptosis sensitization in malignant HCCs. Survivin deficiency results in mitosis defect-associated senescence in HCC cells, which triggers local inflammation and increased TNFα. Survivin inactivation also sensitizes HCC cells to TNFα-triggered cell death, which leads to marked HCC regression. Based on these findings, we designed a combination treatment using mitotic inhibitor and pro-apoptosis compounds. This treatment recapitulates the therapeutic effect of Survivin deletion and effectively eliminates HCCs, thus representing a potential strategy for HCC therapy. Conclusion: Our data demonstrate that Survivin ablation dramatically suppresses human and mouse HCCs by triggering senescence-associated TNFα and sensitizing HCC cells to TNFα-induced cell death. Combined use of mitotic inhibitor and SMAC mimetic can induce senescence-associated TNFα and enhance TNFα-induced cell death and synergistically eliminate HCC.
DEREGULATION OF EPIGENETIC REGULATOR SETDB1 CONTRIBUTED TO HEPATOCELLULAR CARCINOMA PROGRESSION AND CANCER METASTASIS

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Epigenetic deregulation plays an important role in liver carcinogenesis. Using transcriptome sequencing, we interrogated the expression of 591 epigenetic regulators in hepatitis B-associated human hepatocellular carcinoma (HCC). We found that aberrant expression of epigenetic regulators was a common event in HCC. We further identified SETDB1 (SET domain, bifurcated 1), a H3K9 specific histone methyltransferase, as the most significantly up-regulated epigenetic regulator in human HCCs. Up-regulation of SETDB1 was significantly associated with HCC disease progression, cancer aggressiveness and poorer prognosis of HCC patients. Functionally, we showed that knockdown of SETDB1 reduced HCC cell proliferation in vitro and suppressed orthotopic tumorigenicity in vivo. Inactivation of SETDB1 also impeded HCC cell migration and abolished lung metastasis in nude mice. Interestingly, SETDB1 protein was consistently up-regulated in all metastatic foci found in different organs, suggesting that SETDB1 was essential for HCC metastatic progression. Mechanistically, we showed that the frequent up-regulation of SETDB1 in human HCC was attributed to the recurrent SETDB1 gene copy gain at chromosome 1q21. In addition, hyperactivation of SP1 transcription factor in HCC enhanced SETDB1 expression at transcriptional level. Furthermore, we identified miR-29 as a negative regulator of SETDB1. Down-regulation of miR-29 expression in human HCC contributed to SETDB1 up-regulation by relieving its post-transcriptional regulation. Finally, transcriptome sequencing analyses showed that SETDB1 epigenetically silenced multiple downstream targets involved in cadherin mediated cell adhesion and Wnt signaling pathways and thereby contributed to liver carcinogenesis. In conclusion, we showed that SETDB1 is frequently up-regulated in human HCCs. The multiplicity of SETDB1 activating mechanisms at chromosomal, transcriptional, and post-transcriptional levels together facilitates SETDB1 up-regulation in human HCC. SETDB1 promotes HCC progression and metastasis through epigenetic silencing of multiple tumor suppressor genes.
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