HEPATIC FIBROSIS: NEW CONCEPTS AND CONTROVERSIES

SEPTEMBER 14–15, 2018
DFW AIRPORT, TEXAS

Program Chairs
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#STC18
Schedule-at-a-Glance and Meeting Locations
Wi-Fi Network: AASLD
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Thursday, September 13
5 PM – 7 PM  Registration  Enterprise Foyer

Friday, September 14
6:30 AM – 6 PM  Registration  Enterprise Foyer
7 AM – 8 AM  Breakfast  Aviators B
8 AM – 6 PM  General Session  Enterprise 1 & 2
9:40 AM – 10:10 AM  Break  Enterprise Foyer
11:45 AM – 1:15 PM  Lunch and Poster Viewing  Aviators B
6 PM – 7 PM  Networking Reception  Aviators B

Saturday, September 15
6:30 AM – Noon  Registration  Grand Ballroom Foyer
7 AM – 8 AM  Breakfast  Aviators B
8 AM – Noon  General Session  Enterprise 1 & 2
10:10 AM – 10:30 AM  Break  Enterprise Foyer

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Continuing Education Information
Upon completion of this activity, participants will be able to:
- Understand basic concepts underlying tissue fibrosis.
- Grasp new principles surrounding the biology of the microbiome in the pathogenesis of fibrosis.
- Gain insights into how basic science discoveries may be translated to new therapeutics.

This activity was planned in the context of the following ACGME/IOM/IPEC competencies: Patient-centered Care, Work in Interdisciplinary Teams, Evidence-based Practice, Quality Improvement Utilize Informatics, Medical Knowledge, Teams and Teamwork and Translating Data From the Bench to Medical Therapy.

Accreditation and Designation Statements
Continuing Medical Education (CME)
The American Association for the Study of Liver Diseases (AASLD) is accredited by the Accreditation Council for Continuing Medical Education (ACCME) to provide continuing medical education for physicians. AASLD designates this live activity for a maximum of 11.00 AMA PRA Category 1 Credits™. Physicians should claim only the credit commensurate with the extent of their participation in the activity.

Claiming CME Credits
Physicians and other health care professionals seeking AMA PRA Category 1 Credits™ for this live continuing medical education activity must complete an evaluation by Monday, October 15, 2018. A link to the CME and MOC evaluation will be emailed to attendees after the conference.

Certificates will only be issued to those who complete an evaluation by the deadline. CME certificates will be emailed upon successful completion of the evaluation.

Disclosures
This live educational activity has been planned in accordance with AASLD and ACCME Standards of Commercial Support by members of the Single Topic Conference faculty and the Basic Research Committee.

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Statement on off-label and investigational use: Speakers are asked to make a reasonable effort to identify during their presentation any discussion of off-label or investigational use or application of a product or device.

Financial disclosures will appear at the beginning of each session and are provided below.

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## Conference Agenda

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SPEAKER SUMMARIES
Hepatic Fibrosis: New Concepts and Controversies
Welcome and Overview

The fourth AASLD-sponsored Single Topic Conference (STC) on hepatic fibrosis will be held in Dallas on Sept 14th and 15th, 2018 in Dallas, Texas. This meeting will serve as an update on progress made in the field of hepatic fibrosis with a focus on the multiple new discoveries that have occurred since the last fibrosis STC, held in northern Virginia in 2006. The conference will encompass a diverse range of topics and expertise, from basic molecular and cellular biology of nonparenchymal cells to clinical trial design for emerging antifibrotic therapies.

The history of AASLD sponsored STCs on hepatic fibrosis is perhaps one of the most remarkable in the field of hepatology. The first conference, held nearly 30 years ago in 1989, focused on the biochemistry of extracellular matrix and its role in determining cellular behavior 1. At this meeting, there was great focus on the structure of the extracellular matrix, the cellular source of extracellular matrix, particularly in the space of Disse (where the question of hepatocytes vs. stellate cells as a source of matrix was actively debated). The role of extracellular matrix function, particularly the role of the basement membrane, in liver function was also addressed. Regulation of matrix synthesis was addressed and so was what would soon become a fundamentally central topic - matrix degradation, and the idea that matrix is remodeled. There was brief mention of experimental models of liver injury and fibrosis, but there was no discussion of the translational relevance of evolving basic science discoveries in the field.

During the second AASLD fibrosis STC in 2000, with the recognition that hepatic stellate cells were key components of the wound healing process, there was extensive focus on stellate cell biology, including signaling, cytokine and nuclear receptor pathways of gene regulation and multiple aspects of stellate cell behavior 2. Translational aspects of fibrosis, including emerging antifibrotic therapies were advanced. Further, clinical aspects important in fibrosis, including the concept of assessing fibrosis progression was introduced.

The third AASLD fibrosis STC, held in 2006, was marked by a veritable explosion of new data, with substantial novel mechanistic insight 3. Basic science advances were presented in multiple areas, including in NFkB signaling, cytokine and vascular mediator biology, oxidant stress, the role of immune cells in fibrosis, and many other areas. Clinical and translational aspects of fibrosis were notable, and focused on the recognition that genetic determinants were important in fibrosis. There was substantial discussion about an expanded view of fibrogenic cells, including circulating and bone marrow-derived fibrogenic cells. Clinical aspects of fibrosis gained considerable attention, including the importance of assessment of fibrosis progression where the potential of serum markers and imaging was recognized. Finally, novel antifibrotic therapies garnered great attention, with discussion of clinical trial endpoints, emerging targets, and pathways relevant for design of antifibrotic treatment.

The fourth STC on liver fibrosis will offer tremendous breadth, covering many timely topics. The meeting includes 6 specific sessions including the following: 1) an update on the biology of organ fibrosis - with not only an overview of what’s new in the liver, but also expanding to mechanisms of lung and skin fibrosis; 2) the role of the microbiome, gut immunity, and the gut-liver axis; 3) non-alcoholic fatty liver disease (NAFLD), fibrosis progression, and hepatocellular...
cancer (HCC); 4) a review of nonparenchymal cells beyond hepatic stellate cells and their role in fibrosis; 5) a special session specifically dedicated to hot topics and emerging concepts in liver fibrosis; and 6) translational aspects of fibrosis and cirrhosis, including translation of findings into humans as well as discoveries that may lead to novel therapies. The conference will include not only 29 posters (including 4 oral presentations by young investigators), but also ample time for networking among participants - a critical and important goal of the conference.

It is notable that at the last fibrosis STC in 2006, participants unanimously agreed that 6 years (the time between the 2nd and 3rd STCs) was too long to wait for the next conference. Well, we didn’t make that timeline, but here we are 12 years later, with even more to talk about!

References
Liver Fibrosis from 2006-2018: Where We’ve Been, Where We’re Going

The 12 years since the last Fibrosis STC have been a time of remarkable progress in understanding the basic science of fibrosis and translating it to therapy. Reviewing the program for the 2006 conference makes it clear just how far the field has come. In 2006, genetic studies of fibrosis risk (in chronic hepatitis C) were just beginning, and the “omics” of the day were proteomics and gene arrays. NASH was barely mentioned. Concepts that are well accepted today – whether or not fibrosis can regress in humans1 and whether apoptosis is linked to fibrosis2 – were the subject of talks by senior members of the field. Hepatocyte EMT was a hot topic. The development and use of non-invasive imaging and biomarkers was (and continues to be) the subject of much research, although transient elastography is no longer a novelty and techniques and equipment have become more sophisticated. Similarly, the role of the immune system and inflammation in fibrosis was and is at the fore, although our approaches are more sophisticated. Perhaps most notable from the 2006 program, though, is Session 4, entitled “The Era of Antifibrotic Therapy” – a session that included many exciting ideas but was frustratingly far from realistic translation into the clinic.

The STC of 2018 reflects many of the key areas of progress over the last decade. First is an appreciation for the commonalities in fibrosis across organs and disease processes. The silos of the past are breaking down: several pan-fibrosis conferences have occurred in the last few years, NIH funding opportunities have targeted shared mechanisms, and this conference includes two fibrosis researchers outside of hepatology. The concept of a "wound healing triad" linking mechanisms underlying normal wound healing, pathological fibrosis, and cancer-associated matrix deposition is now well accepted and instructive at basic and translational levels.3

And yet – there is increasing appreciation for the heterogeneity of liver fibrosis resulting from different etiologies: for heterogeneity in mechanisms, cell-cell communication, inflammatory and immune responses, and fibrogenic cell phenotype and behavior. The idea that fibrosis is a “final common pathway” resulting from a uniform population of hepatic stellate cells is now viewed as a rough approximation. Research into unique mechanisms of fibrosis in NAFLD in particular has taken off, although heterogeneity is not limited to this disease.

The gut microbiome is the subject of headlines for its relevance to many diseases, liver fibrosis among them.4 In 2006, there was not a single mention of the microbiome in the STC program; in 2018, there is justifiably an entire session devoted to the gut-liver axis. The microbiome, its relationship to immunity, bile acids, and the rest of the body, and innovative new microbiome-based therapies represent an almost entirely new area of research over the last decade.

Genetics and epigenetics, too, are relatively new areas of research in liver fibrosis.5 From the first report that there were genetic determinants of disease severity in HCV, to new details about susceptibility to NASH, data collection and availability has surged, often proving to have direct clinical applicability. Epigenetics barely existed as a field 12 years ago; now there is extraordinarily exciting ongoing work on the role of epigenetic changes in many facets of fibrosis.
Emerging technologies were hot in 2006 and remain so in 2018, although what’s new has, not surprisingly, changed significantly. From early proteomics, the field has moved to glycomics and metabolomics. Imaging and single cell analyses have become ever more sophisticated.

Other hot topics in the last decade include cell-cell communication via exosomes, the role of cells other than stellate cells (including hepatocytes, sinusoidal endothelial cells, and novel immune cells) in fibrogenesis, angiogenesis in fibrosis, mechanical drivers of fibrosis, micro RNAs, and cell death and repair in many forms (necroptosis, autophagy, senescence).

Perhaps the biggest advance since the last STC is that antifibrotic therapies are close to becoming a clinical reality. Although there was great hope in 2006 that practical antifibrotic therapies were around the corner, the number of ongoing clinical trials today suggest that the corner is finally being turned. The approaches in many cases are novel, tapping into stem cells, gene therapy, novel “omics” data, and the microbiome.

Where are we going? The 2018 STC program offers hints about the course of the next decade of research. Technology will certainly continue to improve and with it our ability to understand, diagnose, and monitor fibrosis in humans. Translational research, including although not limited to new methods for stellate cell targeting, will be the focus of many labs, often in collaboration with industry, and increasingly leading to clinical trials. The next AASLD fibrosis meeting may be an Emerging Trends rather than a Single Topic Conference!

It’s worth emphasizing, however, that there will still be an important place for basic research. Studies of the microbiome, genetics and epigenetics, stem cells, fibrosis immunology and the tumor microenvironment, in particular as related to NASH, still have much to teach us. So, too, do research topics common in the older literature, and featured at the 2006 STC – soluble factors, matrix proteases, and basic liver cell biology. Merging existing knowledge with new fields and techniques will be an exciting challenge in the next decade.

References
Fibrosis of the lung involves pathophysiological mechanisms that may be similar across diverse organs (1). However, there are important inter-organ differences, both in regenerative capacity and pathobiology of fibrosis. Idiopathic pulmonary fibrosis (IPF) is the most common and recalcitrant of the fibrotic lung diseases. The approval of two drugs, Pirfenidone and Nintedanib, by the United States Food and Drug Administration (FDA) in 2014 has heralded a renewed interest in drug discovery and development for IPF and other fibrotic diseases.

Our understanding of the pathogenesis of IPF has evolved over the past three decades, and the role of aging in this disease process is gaining greater recognition (2, 3). The diagnosis of IPF is typically made beyond the fifth decade of life, and there is an increase in both the incidence and prevalence of the disease with advancing age. Biological hallmarks of aging (4) which include genomic instability, telomere attrition, epigenetic alterations, loss of proteostasis, deregulated nutrient sensing, mitochondrial dysfunction, cellular senescence, stem cell exhaustion, and altered intercellular communication, are linked to key pathobiological processes in fibrosis (5, 6).

Both clinicians and scientists intuitively approach fibrosis as a pathological process; however, it can be argued that fibrosis serves an adaptive host response function (7). Accordingly, fibrosis may be viewed as a physiological response conserved through evolution to survive tissue injury, even at the cost of a loss in organ structure/function. This "trade-off" would be predicted to select fibrotic repair over "perfect" organ regeneration in environments of limited bioenergetic resources. Progressive fibrosis may occur when the normal bidirectional signaling between the epithelium and mesenchyme that coordinates repair becomes aberrant in the context of chronic injury and aging. This aberrant signaling may result from several factors such as elevated oxidative stress, impaired fibrinolysis, and alterations in cytokines, chemokines, growth factors, and eicosanoids. Ultimately, the causes of pathological fibrosis likely involve impaired ability to clear antigens, autoimmunity, impaired regeneration, and the aberrant recapitulation of developmental or wound healing genes (7). In the context of aging, senescence of both the epithelium and mesenchyme may be predicted to give rise to cell phenotypes and fates that favors non-resolution/progression of fibrosis (8-10). In this session, I will discuss opportunities to target molecular pathways that determine the capacity for fibrosis resolution. Such strategies, if successful, can be leverages to not only halt disease progression, but to reverse fibrotic remodeling to achieve normal organ structure and function.

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References


Lessons Learned from Skin Fibrosis

Description:
Organ fibrosis is an irreversible endpoint of several diseases, leading to organ failure. Systemic sclerosis (SSc) is a prototypic fibrotic disease with fibrosis affecting multiple organs including the skin. The basic mechanisms responsible for the initiation and perpetuation of fibrosis in SSc are not completely understood, rendering early diagnosis unlikely and adding critical importance to the identification of an effective therapy. We characterized dermal fibrosis, a hallmark of SSc in twins discordant for the disease. We also compared the molecular fingerprint of SSc skin with that of an organ culture model utilizing human skin. We further expanded our studies to lung tissues of patients with SSc to identify disease- and organ-specific mechanisms. We identified insulin-like growth factor binding protein (IGFBP)-5 as a gene over-expressed in skin and lung tissues of patients with SSc. Comparison with lung tissues from patients with a related disease, idiopathic pulmonary fibrosis (IPF), suggested that increased IGFBP-5 levels is a feature of fibrosis in different organs and diseases. During these investigations, we noted increased endostatin levels in fibrotic tissues. Endostatin is a cleavage product of Collagen XVIII. To determine whether endostatin mediates tissue fibrosis, we tested its effects in vitro in primary fibroblasts, in vivo in murine models of fibrosis, and ex vivo in human tissues maintained in organ culture. Endostatin exerted anti-fibrotic effects in all these models. Furthermore, we identified a peptide sequence within endostatin that mediated its anti-fibrotic effects. Current work is underway to delineate the mechanism(s) by which endostatin and its active domain abrogate fibrosis.

References

The Microbiome and the Liver

The gut microbiota is the nidus for a panoply of pathophysiological processes in the host. The homeostasis of the intestinal microflora is regulated by several factors including environmental, genetic and mucosal barriers. Anatomic sequestration of commensal flora relies on intact barriers to ensure that microbes serve their respective functions in discrete locations. Recent evidences have linked disruption of intestinal microflora to a variety of metabolic disorders including obesity (Ley et al., 2006), type 2 diabetes (Qin et al., 2012), inflammatory bowel disease (IBD) (Frank et al., 2007), neurological disorders (Hsiao et al., 2013), cancers (Yoshimoto et al., 2013) and chronic liver diseases (Brenner et al., 2015). However, little is known about how a genetic disease with physiological consequences can alter the landscape of the microbiota, nor the subsequent effects of such changes.

Primary sclerosing cholangitis (PSC) is a complex liver disease with etiologies that involves genetic, environmental, immunological, and other potential factors i.e. gut dysbiosis. An association between PSC and ulcerative colitis in an estimated 75% of Western PSC patients implicates an etiological role for gut dysbiosis in this process (Loftus et al., 2005). An over-presentation of gut commensals Lactobacillus, Enterococcus, and Fusobacterium has been recently reported in PSC patients (Sabino et al., 2016). It is very likely that alterations in the intrahepatic as well as extrahepatic biliary ducts, and biliary epithelial cells (i.e. cholangiocytes) during cholestasis may promote microbial translocation to liver.

The liver is an anatomic site that is highly enriched in unconventional T cells including γδ T cells, which are capable of modulating liver injuries through IL-17 production. Mounting evidence demonstrate IL-17+ γδ T cells expand in response to inflammation, particularly important for TCR-mediated recognition of bacterial pathogens invading host tissues. In acute injury setting, such as Concanavalin (Con-A)-induced hepatitis and experimental hepatectomy regeneration, this hepatoprotective population is largely restricted to Vγ4 usage (Hammerich et al., 2014). However, in chronic models of liver injury, such as high-fat diet (Mehta et al., 2015) and biliary atresia (Klemann et al., 2016), γδ T cells-derived IL-17 is implicated in perpetuating disease pathogenesis; Vγ-chain usage has yet to be elucidated in this context. Interestingly, IL-17 has also been demonstrated to hypersensitize hepatic stellate cells (HSCs), a sentinel cell types in hepatic fibrosis, to TGF-β; addition of IL-17 to HSC cultures in vitro permits a robust response to sub-optimal concentrations of TGF-β. While this is advantageous in acute liver wound healing, perhaps prolonged hypersensitivity to profibrotic mediators encourages pathology during chronic liver disease. Therefore, we hypothesize that IL-17+ γδ T cells could potentially expand in respond to inappropriately localized commensal bacteria during cholestasis injury. However, the contributions of these mechanisms in the pathogenic progression of cholestatic liver disease remain largely unknown.

Using mice with an inborn error of metabolism, multidrug resistance gene 2 (Mdr2/-) modeling human PSC, we found that the physiological disturbances resulting in cholestasis selectively enriched for Lactobacillus gasseri within the intestinal microflora. Concomitantly, L. gasseri translocated to the liver where it triggered liver-specific expansion of an invariant population of...
IL-17A+ Vγ6Jγ γδ T cells. Disruption of this pathological process via antibody-mediated blockade of γδ TCR or IL-17A attenuated liver pathology and ameliorated disease, highlighting novel immunotherapeutic avenues for PSC. These results suggest that the etiology of a genetic disease can differentially reshape the microbiota with distant immunological and pathological consequences.

References
The human body and the enteric microbiome have an intimate and symbiotic relationship. A dysbalance of this delicate homeostasis between host and microbiome can lead to disease. It has been recognized for a long time that liver disease is associated with changes in the enteric microbiota and the gut barrier. Alterations in the microbiota can either occur as intestinal bacterial overgrowth and/or as changes in the bacterial composition, collectively named dysbiosis. Intestinal dysbiosis is a common feature in patients with liver cirrhosis, but it also occurs in early stages of liver injury and fibrosis. Qualitative changes of the human gut microbiome have now been characterized by deep sequencing, and several studies described the microbial taxonomy in patients with early and end-stage liver disease. Dietary factors including alcohol or a Western diet appear to be stronger determinants in changes of the microbiome than liver disease itself. The contribution of dysbiosis to chronic liver disease goes beyond disruption of the mucosal barrier and bacterial translocation. Microbial metabolites are equally important for the progression of liver disease. We review how the microbiota changes in chronic liver disease and fibrosis, and how dysbiosis contributes to bacterial translocation and liver disease progression.

References
Role of T Cells in NASH Associated Mucosal Inflammation: Integrity Matters

Non-alcoholic steatohepatitis (NASH), an advanced progressive form of non-alcoholic fatty liver disease (NAFLD), afflicts ~16 million people in the US (1). An estimated one in six NASH patients progress to cirrhosis, and NASH-related cirrhosis is the 2nd leading indication of liver transplantation in the US. NASH is also a risk factor for the development of hepatocellular carcinoma (2, 3). Consequently, there is an urgent need to devise novel therapeutic interventions to halt progression of NASH, as no therapies currently exist.

Hepatic inflammation and fibrosis are the key features of NASH, with fibrosis emerging as the single most important indicator of disease progression and mortality (4). Fibrosis is a process of normal wound healing, following injury and inflammation, necessary for maintaining tissue architecture and function (5). Dysregulation of this process results in the excess deposition of extracellular matrix resulting in progressive fibrosis. While it is well established that inflammation is central to the initiation and progression of fibrosis in NASH (5), the mechanisms inciting the inflammatory process are still not fully understood. We recently demonstrated gut microbial antigens as among the primary drivers of hepatic inflammation in NASH (6). Our studies in a mouse model of compromised intestinal epithelial barrier substantiated clinical evidence linking intestinal epithelial permeability and NASH progression (6). Specifically, a Western diet (WD) of high fat, high fructose and high cholesterol significantly increased gut permeability resulting in severe NASH in mice with a compromised intestinal epithelial barrier. These pathological changes in the intestinal epithelial barrier were triggered by gut dysbiosis and mucosal inflammation, facilitating translocation of gut bacterial endotoxin, a potent inducer of hepatic inflammation (7). A similar increase in intestinal epithelial permeability was observed in WT mice fed WD for 32 weeks, and increased gut permeability in these mice correlated with histological, biochemical and metabolic parameters of NASH. We also demonstrated that humans with nonalcoholic fatty liver disease (NAFLD) but with no known inflammatory bowel disease (IBD) have increased colonic inflammation and defects in intestinal epithelial barrier (6). Together, these studies underscore the importance of diet-induced gut dysbiosis, mucosal inflammation, and subsequent disruption of intestinal epithelial barrier in NASH.

In this talk, I will cover our recent efforts to build upon these findings to uncover the cellular basis of mucosal inflammation in NASH. We will discuss the role of CD4 T cells, which play a critical role in maintaining intestinal mucosal homeostasis (8-10). Our recent data show that WD promotes recruitment of inflammatory CD4 T cells to the intestine, and that blocking the recruitment of CD4 T cells to the intestinal mucosa attenuates mucosal inflammation and improves intestinal epithelial barrier integrity. Strikingly, this intervention resulted in attenuated hepatic inflammation and fibrosis, and improved indices of the metabolic syndrome. These findings provide novel mechanistic insights into the involvement of inflammatory CD4 T cells in diet-induced disruption of intestinal epithelial barrier in NASH, and provide a framework for targeted therapy to block CD4 T cell trafficking to treat NASH.
References
Mechanisms of NASH Fibrosis

The natural history of nonalcoholic steatohepatitis (NASH) is highly variable. The disease can regress to simple steatosis or trigger progressive fibrogenesis that ultimately culminates in cirrhosis. Because fibrosis severity is the only histologic parameter that independently predicts the risk for liver-related morbidity and mortality in NASH it is critical to clarify tractable mechanisms that drive NASH fibrosis. The risk for progressive fibrosis is much greater in NASH than in simple steatosis. A key finding that distinguishes NASH from simple steatosis is hepatocyte ballooning. Fibrosis severity in NASH tightly parallels numbers of ballooned hepatocytes, suggesting that ballooned hepatocytes release fibrogenic factor(s). Ballooned hepatocytes have been shown to release Sonic (Shh) and Indian hedgehog (Ihh) ligands. These factors act as morphogens by activating an evolutionarily conserved signaling pathway that regulates cell viability, proliferation and differentiation.

Hedgehog ligands activate the canonical Hedgehog (Hh) signaling pathway by binding to their receptor, Patch, a transmembrane receptor expressed on the surface of Hedgehog-responsive cells. Binding of Hh ligands to Patch relieves Patch-dependent inhibition of Smoothened (Smo), a G protein coupled receptor that controls the stability of Gli family transcription factors. When Smo is inactive, Gli is sequentially phosphorylated and targeted for proteolytic cleavage that either degrades it or generates a truncated protein that functions as a transcriptional repressor. This constrains Hedgehog signaling activity until Hh ligands become available to interact with Patch. Hh-Patch interaction de-represses Smo and activated Smo blocks Gli phosphorylation, permitting full length Gli to accumulate, traffic to the nucleus, and engage Gli-target genes. The mammalian Hh pathway is quite complicated. In addition to 3 Gli proteins (which differ in their susceptibility to proteolytic cleavage), it includes a number of co-receptors that positively and negatively modulate Hh-Patch interaction, as well as intracellular proteins that can suppress Smo. Lipids (e.g., fatty acids, oxysterols and cholesterol) also directly modify the activities of both Hh ligand and Smo. In addition, core components of the Hedgehog pathway (e.g., Patch, Smo, and Gli proteins) can be involved in signaling that does not require Hh ligands. This flexibility permits exquisite context-dependent modulation Hh pathway activity and explains how it is able to play multiple roles in tissue development and regeneration.

Various types of adult liver cells respond to Hh ligands and/or other factors, such as TGFβ, that can activate the Hedgehog pathway downstream of Patch. Pathway activity has been shown to regulate fate decisions in all of the liver cell types that are critically involved in liver repair, including hepatic stellate cells, liver sinusoidal endothelial cells, immune cells, and cholangiocytes. Not surprisingly, the specific consequences of Hh signaling vary somewhat according to the type of target cell engaged but pathway activity generally promotes cell viability, motility, and proliferation. For example, Hedgehog signaling is necessary for hepatic stellate cells to become and remain myofibroblastic. Local activation of Hedgehog signaling in stellate cells adjacent to ballooned hepatocytes triggers a chicken wire pattern of fibrosis that is typical of early NASH fibrosis. Hedgehog signaling also stimulates loss of fenestrae (capillarization) in liver sinusoidal endothelial cells and promotes angiogenesis. Activating Hh signaling in ductal cells causes the cells to undergo partial epithelial-to-mesenchymal transition and induces their production of fibrogenic factors and chemokines. Hedgehog signaling also
promotes immune cell viability and typically triggers a switch from Th1/M1 polarization to Th2/M2 polarization. The net effect of all of these actions is scarring, i.e., accumulation of fibrous matrix and associated inflammatory cells, myofibroblasts, reactive endothelia, and immature liver epithelial cells (i.e., the ductular reaction). The intensity of the ductular reaction strongly correlates with fibrosis severity in NASH. In rodent models of NASH, Hh pathway inhibitors improve liver inflammation and fibrosis. In humans, therapeutic interventions that improve NASH reduce hepatic Hh pathway activity.

There is growing evidence that hepatocytes are also capable of Hedgehog signaling, although the role of Hh ligands in triggering this process remains debated because adult hepatocytes are thought to lack primary cilia (and the latter are classically involved in the canonical Hedgehog pathway). Gebhardt and colleagues have reported that Hedgehog is a critical regulator of lipid metabolism in hepatocytes. As in adipocytes, pathway activation appears to inhibit lipogenesis and promote lipid elimination. Humans with Smo mutations that inhibit Hedgehog signaling develop hepatic steatosis but may be relatively protected from advanced NAFLD because mice with haploinsufficiency of Gli2 are susceptible to hepatic steatosis, but protected from diet-induced NASH and related fibrosis. A chromosomal deletion that results in constitutive activation of Gli1 in hepatocytes was recently discovered in a subtype of human hepatic adenomas that have a high risk of clinically-significant hemorrhage. This phenotype is consistent with evidence that Hedgehog signaling generally promotes cell growth and stimulates vasculogenesis. Conversely, treating mice with Hedgehog inhibitors or deleting Smo via genetic approaches blocks hepatocyte regeneration after partial hepatectomy.

The aggregate data suggest that Hedgehog pathway activity must be maintained within an appropriate range for liver health. Too little Hedgehog activity promotes hepatic steatosis and inhibits hepatocyte regeneration, while excessive Hedgehog activity induces progressive fibrogenesis and generates a liver microenvironment that is conducive for hepatic neoplasia. Further, the Hedgehog pathway is commonly activated (typically by epigenetic mechanisms) in various types of primary liver cancer and the level of pathway activity correlates with poor tumor-free and overall survival. Given all of this, therapeutic intervention to “normalize” Hedgehog pathway activity would seem to be ideal to both prevent NAFLD and to inhibit NASH and its lethal complications, including cirrhosis and liver cancer. However, the complexity of pathway regulation, coupled with its broad spectrum activity in extrahepatic tissues are major challenges and thus, hedgehog inhibitors have not yet been tested as treatments for human NASH.

Lessons learned by studying NASH-related activation of Hedgehog signaling are helping to inform ongoing efforts to control fibrogenic repair. It is evident that both progression and regression of liver injury is a community affair, involving collaboration among multiple types of cells that modulate the activities of multiple interacting signaling pathways that must be appropriately coordinated to regenerate healthy hepatic parenchyma. Attention is focusing on identification of mechanisms that critically regulate fate decisions in cell types that are the key drivers of that process in the hopes of minimizing off-target effects on other cell types whose functions are essential for health. Progress will be enhanced by improved understanding of genetic and epigenetic mechanisms that establish cellular susceptibility to injury-related reprogramming.

References
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The use of animal models of liver fibrosis is crucial to determine novel molecular mechanisms and to identify effective molecular targets for treatment of liver fibrosis. Mouse models of liver fibrosis include toxin-induced (e.g. carbon tetrachloride, thioacetamide), cholestasis-induced (e.g. bile duct ligation, DDC diet), non-alcoholic steatohepatitis-induced (e.g. choline-deficient high-fat diet), infection-based (e.g. Schistosoma mansoni), and genetic models of (e.g. Mdr2−/−, Nemo−/−, Tak1−/−) liver fibrosis. None of these animal models can recapitulate full clinical spectrum of human liver fibrosis and cirrhosis. This may limit to determine effective molecular targets for human diseases. However, in addition to in vitro cell culture experiments using hepatic stellate cells, these animal models are still the gold standard to screen drugs from millions of candidates. Based on previous animal studies, a number of clinical trials have been performed and several (not many) drugs have been confirmed effectiveness in patients with fibrosis. This still indicates the importance of preclinical studies using animal models. On the other hand, many clinicians and researchers also recognize that so many pharmaceutical agents confirmed effectiveness in animal models have not shown such efficacy in patients with liver fibrosis through clinical trials. The discrepancies between animal models and human diseases may be explained by various factors, for examples, genetic heterogeneity in human vs inbred strains of mice, length of disease progression (10-30 years in humans vs weeks to months in mice), degree of fibrosis (established fibrosis in humans vs developing fibrosis in mice), etiologies (viral hepatitis, alcoholic cirrhosis in humans that cannot be recapitulated in mice), gut microbiome (Gram-negative dominant in humans vs Gram-positive in mice), different drug metabolism. In addition, diet-induced NAFLD animal models cannot reproduce full spectrum of human NAFLD well, for example, the progression from simple steatosis to NASH. In this presentation, limitations of currently available animal models for liver fibrosis studies and potential of new models for fibrosis studies will be discussed. Novel fibrosis models may be useful to identify novel molecular targets and developing novel effective medicine for liver fibrosis.

References
Angiocrine Signaling in Fibrosis and Cancer

Data sets will be presented from one or more of the following projects:

1. **CXCL1 drives portal hypertension by interfacing mechanosensing and angiocrine signals to generate sinusoidal microthrombi**
   The role of mechanical forces in portal hypertension (PHTN) and fibrogenesis is increasingly recognized. However, mechanisms by which forces are transduced by liver sinusoidal endothelial cells (LSECs) into pressure and matrix changes are incompletely understood and were a focus of this study. For *in vitro* studies, LSECs were subjected to mechanical stretch with a Flexcell device to recapitulate the *in vivo* pulsatile forces induced by congestion. For *in vivo* studies, partial ligation of the suprahepatic inferior vena cava (pIVCL) was used to simulate congestive hepatopathy-induced portal hypertension. Microarray analysis of primary LSECs exposed to mechanical stretch showed upregulation of the neutrophil chemoattractant CXCL1. Intravital imaging directly revealed sinusoidal neutrophil-platelet complexes and formation of neutrophil extracellular traps (NETs) after pIVCL. Mice deficient in neutrophil elastase (NE-/-), a key enzyme for neutrophil function, evidenced lower fibrin formation and portal pressure after pIVCL and bile duct ligation (BDL), suggesting that neutrophil recruitment into the sinusoidal lumen increases portal pressure by promoting sinusoidal microthrombi. RNA-sequencing (RNA-seq) from LSECs identified mechanosensitive pathways impacted by mechanical stretch that included integrins, Notch, and calcium signaling. *In vitro* studies showed that mechanical stretch upregulates CXCL1 through integrin dependent activation of transcription factors downstream from Notch and its interaction with the mechanosensitive Piezo calcium channel. Mechanosensitive angiocrine signals released by LSECs drive PHTN through sinusoidal neutrophil recruitment, NETs, and microthrombi formation. These results augment the molecular knowledge and spectrum of potential therapeutic targets for PHTN.

2. **Mechanotransduction-induced glycolysis mediates a CXCL1-dominant angiocrine signaling program**
   Integrins within focal adhesions (FA) sense increased extracellular stiffness to promote fibrosis. Angiocrine signaling with the release of chemokines from liver sinusoidal endothelial cells (LSEC) balance liver regeneration and fibrosis. The precise mechanisms by which mechanosensing occurs and angiocrine signaling is regulated remain elusive. Here, we investigated novel FA proteins involved in LSEC mechanotransduction, stiffness-induced angiocrine signaling, mechanisms of LSEC mechanosensing, and more specifically the role of FA proteins in liver fibrosis. Mass spectrometry revealed that glycolytic enzymes, particularly Phosphofructokinase 1 isoform P (PFKP), are enriched in isolated FA from LSEC on collagen-I coated gels with incremental stiffness. Increased stiffness resulted in PFKP co-localization with Vinculin in FA, which paralleled an increase in glycolysis. Co-immunoprecipitation experiments
revealed association of PFKP and Integrin beta 1. Mechanistically, stiffness-induced glycolysis was associated with expansion of actin dynamics and attenuated by inhibition of Integrin beta 1 signaling. Using RNA sequencing, we identified CXCL1-dominant neutrophil migration as one of the top mechanotransduction-induced pathways. Inhibition of glycolysis and glycolysis-mediated actin assembly attenuated CXCL1 expression. We demonstrate that CXC1 expression is mediated through a glycolysis-induced increase in nuclear pore size, which is associated with an increased nuclear translocation of the transcriptional regulator YAP. Congruently, inhibition of glycolysis was associated with a decrease in activating histone marks at the CXCL1 promoter region. Finally, endothelial cell specific knockdown of hexokinase 2, a rate limiting glycolytic enzyme, decreased CXCL1 expression and attenuated portal hypertension in vivo. Glycolytic enzymes represent druggable targets with the potential to alter disease progression.

3. P300 Acetyltransferase Mediates Stiffness-Induced Activation of Hepatic Stellate Cells Into Tumor-Promoting Myofibroblasts

Hepatic stellate cells (HSCs) contribute to desmoplasia and stiffness of liver metastases by differentiating into matrix-producing myofibroblasts. We investigated whether stiffness due to the presence of tumors increases activation of HSCs into myofibroblasts and their tumor-promoting effects, as well as the role of E1A binding protein p300, a histone acetyltransferase that regulates transcription, in these processes. HSCs were isolated from liver tissues of patients, mice in which the p300 gene was flanked by 2 loxP sites (p300F/F mice), and p300+/+ mice (controls). The HSCs were placed on polyacrylamide gels with precisely defined stiffness, and their activation (differentiation into myofibroblasts) was assessed by immunofluorescence and immunoblot analyses for alpha-smooth muscle actin. In HSCs from mice, the p300 gene was disrupted by cre recombinase. In human HSCs, levels of p300 were knocked down with small hairpin RNAs or a mutant form of p300 that is not phosphorylated by AKT (p300S1834A) was overexpressed. Human HSCs were also cultured with inhibitors of p300 (C646), PI3K signaling to AKT (LY294002), or RHOA (C3 transferase) and effects on stiffness-induced activation were measured. RNA sequencing and chromatin immunoprecipitation-quantitative polymerase chain reaction were used to identify HSC genes that changed expression levels in response to stiffness. We measured effects of HSC-conditioned media on proliferation of HT29 colon cancer cells and growth of tumors following subcutaneous injection of these cells into mice. MC38 colon cancer cells were injected into portal veins of p300F/Fcre and control mice, and liver metastases were measured. p300F/Fcre and control mice were given intraperitoneal injections of CC14 to induce liver fibrosis. Liver tissues were collected and analyzed by immunofluorescence, immunoblot, and histology. Substrate stiffness was sufficient to activate HSCs, leading to nuclear accumulation of p300. Disrupting p300 level or activity blocked stiffness-induced activation of HSCs. In HSCs, substrate stiffness activated AKT signaling via RHOA to induce phosphorylation of p300 at serine 1834; this caused p300 to translocate to the nucleus, where it up-regulated transcription of genes that increase activation of HSCs and metastasis, including CXCL12. MC38 cells, injected into portal veins, formed fewer metastases in livers of p300F/Fcre mice than control mice. Expression of p300 was increased in livers of mice following injection of CC14; HSC activation and collagen deposition were reduced in livers of p300F/Fcre mice compared with control mice. In studies of mice, we found liver stiffness to activate HSC differentiation into myofibroblasts, which required nuclear accumulation of p300. p300 increases HSC expression of genes that promote metastasis.
Despite continued efforts and research, incidence of HCC continues to increase. This is due to the rising incidence of chronic liver diseases which eventually lead to advanced fibrosis and cirrhosis over years of hepatobiliary insult (1). Most frequent causes of chronic liver diseases include viral hepatitis, non-alcoholic fatty liver disease, alcoholic liver disease and other diseases like hemochromatosis, PBC and PSC. These diseases are characterized by repeated cycles of hepatobiliary injury albeit due to distinct mechanisms, which lead to release of DAMPs while creating not only pro-regenerative environment but also pro-inflammatory and pro-fibrogenic milieu. Furthermore, hepatocytes and cholangiocytes can serve as facultative stem cells for each other. The process of epithelial cell proliferation and transdifferentiation may make chromatin susceptible to DNA aberrations including mutations especially within the adverse microenvironment composed of inflammatory cells, activated stellate cells and endothelial cells, all of which can be source of ROS and other DNA damaging cytokines, microbial products etc. Accumulating aberrations in the form of loss of tumor suppressor genes and activating mutations in oncogenes can suddenly provide a growth and survival advantage to a cell leading to its clonal outgrowth, dysplasia and frank HCC. Thus, the cellular basis of all HCC is complex and heterogeneous but includes cycles of cell death, proliferation, inflammation, myofibroblast activation and endothelial cell dysfunction over decades (2). While the cellular basis of hepatobiliary injury may be diverse and etiology-specific, the overall downstream molecular effects are rather conserved. Multiple high throughput studies (WGS, WES) have, for example, suggested common mutations across all HCC (3, 4). The most frequent mutation affected the promoter region of TERT promoter in around 60% of all HCCs that led to increased telomerase expression. Next common mutations were those affecting CTNNB1 and TP53 each of which were evident in around 35% and 25% of HCC cases. Several additional mutations occurred consistently albeit at lower frequencies including those affecting ARID1A, ALB, AXIN1, APOB, CDKN2A, ARID2, RPS6SKA3 and others (4). Rarely, there is predilection for specific pathways by specific innocuous agents, for example, aflatoxins, which are metabolized by cytochrome p450 to form an active metabolite, which leads to DNA adduct formation often leads to loss of function mutations in the tumor suppressor gene p53. Another exception is HBV, which is a DNA integrating virus and has been shown to integrate into TERT, MLL4 and CCNE1 and alter their function. Thus, molecular mechanisms that are the basis of HCC remain relatively conserved over multiple etiologies.

Based on the commonalities in cellular and molecular basis of HCC development, our lab has focused on genomic aberration events occurring downstream to chronic injury, inflammation and fibrosis that non-preferentially lead to mutations in the same genes like TERT promoter, CTNNB1 and others. We posit that generating animal models of human HCC using an inside-out or a reductionist approach by expressing clinically relevant genes without the need to develop chronic liver injury or use of chemical carcinogens, will yield highly relevant models to allow study of hepatocarcinogenesis. This can be achieved by use of sleeping beauty transposon/transposase (SB) or CRISPR/Cas9 constructs delivered to the liver via hydrodynamic tail vein injection (HTVI) (5). If the mutants singly or in combination are truly
oncogenic, the models can be established in weeks and gene expression changes can be computationally compared to relevant human HCC subsets for validation and further studies. Also, these tumors should be able to create their own microenvironment allowing them to thrive optimally within the livers.

One pathway of interest is the Wnt/β-catenin signaling known to be active in 20-40% of all HCCs (6). Missense mutations mostly affecting CTNNB1 are observed in majority of these cases. Mutations have also been reported in other components of the degradation complex of β-catenin including AXIN1 in around 3-16% and AXIN2 in around 3% of all HCC cases. In HCC patients, exome sequencing has also revealed cooperating mutations of CTNNB1 with mutations in ARID2, NFE2L2, TERT, APOB and MLL2 (4). This is particularly relevant since expression of mutant β-catenin alone is insufficient to lead to HCC development in mice. Likewise, certain mutations were also always mutually exclusive from CTNNB1 and demonstrated lack of cooperation in development of HCC. These included TP53 and AXIN1 (4).

Based on the fact that β-catenin mutations alone are not able to cause HCC in mice and coexistence of CTNNB1 mutations with others in notable subsets of HCC, we have generated several animal models and even tested them for clinical relevance. For example, examining human HCC landscape, we identified around 9-12.5% of all HCCs to demonstrate Met activation or overexpression along with CTNNB1 mutations (7). When mutant β-catenin and Met were co-expressed using SB-HTVI, it led to rapid development of HCC which molecularly resembled with high concordance a subset of human HCC displaying CTNNB1 mutations and Met activation. Once validated, we have used this model to test biology and shown activation of Ras signaling downstream of Met and expression of Glutamine synthetase downstream of β-catenin to be critical for HCC development ((7) and unpublished). Likewise, we have shown the importance of β-catenin inhibition in this model for therapeutic benefit. We have also generated relevant models of CTNNB1 mutants with TERT and NFE2L2.

Thus, based on highly relevant clinical studies evaluating genomic aberrations in HCC cases, and using CRISPR/Cas9 or SB along with HTVI to express these aberrations in the liver will allow generation of relevant animal models of human HCC downstream of complex cellular mechanisms that may be distinct and heterogeneous based on etiology.

References
Myofibroblasts and Tumor Microenvironment in Liver Cancer

The majority of liver cancers arises in fibrotic livers. Whereas fibrosis precedes the development of hepatocellular carcinoma (HCC), it often develops as desmoplastic response in patients with cholangiocarcinoma (CCA). Hence myofibroblast accumulation and fibrosis in the “premalignant” environment (PME) may affect hepatocarcinogenesis, and myofibroblasts fibrosis in the tumor microenvironment (TME) may alter tumor progression. Here, we will present data demonstrating the origin of myofibroblasts by multiple methods, as well as their functions in the development of HCC and CCA, using tools to functionally manipulate or genetically deplete hepatic stellate cell-derived myofibroblasts. We will discuss mediators and pathways through which myofibroblasts contribute to cancer development, including extracellular matrix proteins, growth factors and inflammatory mediators as well as mechanosensitive signaling pathways.
The Role of Macrophages in Resolving Inflammation

Inflammation is a defensive response to injury and infection, but it must be resolved in a timely fashion, as unresolved inflammation lead to tissue damage and a range of chronic diseases. The initial phase of inflammation is characterized by the rapid infiltration of neutrophils, which constitute the first line of defense by neutralizing invading microorganisms or noxious materials by phagocytosis and netosis. The resolution phase is characterized by neutrophil apoptosis and subsequent clearance by macrophages, which plays a critical role in resolving inflammation and restoring tissue structure and function.

In the liver, resident Kupffer cells are the predominant population of macrophages in naïve state. Upon acute or chronic hepatic injury in mice and humans, circulation Ly6C\textsuperscript{hi} monocytes are massively recruited into the liver and initially differentiate into Ly6C\textsuperscript{hi} macrophages that have pro-inflammatory and pro-fibrogenic actions (1-4). As wound healing occurs, the Ly6C\textsuperscript{hi} macrophages differentiate into Ly6C\textsuperscript{low} macrophages (5), which promote tissue repair and injury resolution (6,7). The switch from pro-inflammatory to reparative macrophages is regulated by tissue micro-environmental cues, one of which is sensing and uptake of apoptotic cells. As an example, in a murine model of alcoholic liver disease (ALD), we have shown that phagocytosis of dead cells (efferocytosis) triggers macrophage switching from Ly6C\textsuperscript{hi} to Ly6C\textsuperscript{low} phenotypes. Our data also show that macrophage efferocytosis is defective in NOX2\textsuperscript{-/} mice. As result, we observe increased hepatic neutrophil accumulation, exacerbated inflammatory phenotype of macrophages, and worsened liver injury after chronic ethanol feeding in NOX2\textsuperscript{-/} mice compared to wild-type controls.

In summary, macrophage reprogramming through efferocytosis and other mechanisms plays a critical role in resolving inflammation and limiting tissue injury. When the molecular pathways regulating this process are impaired, which is more likely in severe chronic injury conditions and in genetically predisposed individuals, macrophages could exacerbate inflammation and promote fibrosis. Thus, modulating macrophage phenotypes may be a promising approach in both promoting tissue repair/regeneration in acute injury, and also slow down the progression of chronic diseases.

References


Liver sinusoidal endothelial cells (LSECs) in healthy liver promote quiescence of hepatic stellate cells (HSC), both by preventing HSC activation and promoting reversion to quiescence (1, 2). Prior to fibrosis LSECs undergo “capillarization” and capillarized LSECs lose the ability to promote hepatic stellate cell quiescence. This raises four questions. What is capillarization? Why are LSECs capillarized? How do LSECs maintain HSC quiescence? Why is this function lost in capillarized LSECs?

What is capillarization? We have now shown, both in the thioacetamide model of cirrhosis and in a high fat, high fructose (HFHF) model of NAFLD, that capillarization is not a de-differentiation process, but is due to repair of injured LSECs by liver sinusoidal endothelial cell progenitor cells (sprocs) from the bone marrow (BM) that engraft but fail to fully mature (3). Around two-thirds of LSECs in the thioacetamide model and 30% of LSECs in the HFHF diet model are derived from BM sprocs. The resident LSECs in these two models of chronic liver disease are fully fenestrated and the BM-derived LSECs are capillarized.

Why don't BM-derived LSECs fully mature in fibrosis? Two pathways are upregulated in the immature LSECs that suppress the nitric oxide pathway: Thrombospondin-1 (TSP-1) and ADAMTS13 are both upregulated in immature LSECs (3). TSP-1 signals through CD47 and CD36 to inhibit soluble guanylate cyclase, NOS activation, and cGMP-dependent protein kinase I (cGKI) to inhibit the NO pathway (4). ADAMTS13, which has several TSP-1 repeat domains, also inhibits the NO pathway by binding to CD36 (3). Upregulation of TSP-1 in cirrhotic LSECs is due to a difference in TGF receptors in normal and immature LSECs: normal LSECs express the ALK1 kinase receptor, but this is downregulated in immature LSECs. TGF signaling through the ALK1 receptor downregulates TSP-1 expression, whereas TGF signaling through the ALK5 receptor increases TSP-1 expression (3). When cirrhotic LSECs are incubated with VEGF plus an ALK5 receptor antagonist, cGMP increases and LSECs develop normal fenestration overnight (3).

How do LSECs promote HSC quiescence? HB-EGF prevents HSC activation and promotes reversion to quiescence (5). LSECs isolated from normal liver release HB-EGF extracellularly, but LSECs from cirrhotic liver do not (3). We have now have shown that HB-EGF is necessary for the ability of normal LSECs to maintain HSC quiescence. Given that HB-EGF is both necessary and sufficient (5) to maintain HSC quiescence, logic dictates that HB-EGF release alone can fully account for the LSEC effect on HSC quiescence.

Why don't immature LSECs maintain HSC quiescence? HB-EGF is synthesized as pre-pro-HB-EGF and is expressed across the cytoplasmic membrane as pro-HB-EGF. Release of HB-EGF from the cytoplasmic membrane requires cleavage by metalloproteinases termed sheddases. Normal LSECs express 9 metalloproteinases that have been identified as HB-EGF sheddases and 6 of these 9 sheddases are downregulated in immature LSECs (3). Thus the inability of immature LSECs to release HB-EGF extracellularly and thereby maintain HSC quiescence is due to downregulation of sheddases needed to release HB-EGF ectodomain from the cytoplasmic membrane.
References
Vascular Control of Liver Function During Fibrosis and HCC Progression

Blood vessels form one of the body's largest surfaces serving as critical interface between the circulation and the different organ environments. They thereby don't just support rheological functions, but act through paracrine (angiocrine) cytokines as gatekeeper of tissue homeostasis and adaptation to pathologic challenge. The liver with its two vascular beds - the arterial and the sinusoidal vasculature - has emerged as a prototypic organ to study the mechanisms of angiocrine signaling.\(^1\) During development, endothelial programs involving GATA4 and other transcription factors orchestrate liver organogenesis.\(^2\)

Endothelial cell (EC)-derived Wnt signals act angiocrine and provide positional cues controlling liver zonation.\(^3,4\) Central vein-derived Wnt signals have been shown to control the proliferation of pericentral hepatocyte progenitor cells during liver regeneration in an angiocrine manner.\(^3\) Subsequent work has demonstrated that central vein EC produce the Wnt signaling enhancer Rspondin3, which controls liver zonation in an angiocrine manner.\(^4\) In the adult, EC-derived cytokines, such as Angiopoietin-2, control hepatocyte proliferation during liver regeneration.\(^5\) EC thereby act as gatekeeper of their microenvironment controlling development, homeostatic organ function, and regeneration. But angiocrine regulation of organ function is not just restricted to EC. Pericytes increasingly gain attention as angiocrine regulators. Pericytes are organotypically differentiated microvascular murals cells.\(^6\) They are in intimate contact with lumen-lining EC and thereby control microvascular plasticity and function. In the liver, hepatic stellate cells (HSC) comprise a specialized population of pericytes.\(^6\) Activated HSC express the C-type lectin receptor Endosialin which controls liver function contextually in an angiocrine manner, including the progression of fibrosis and hepatocellular carcinoma.\(^8\) This presentation will review the state of the art of vascular control of liver function focusing on tissue regeneration, fibrosis and HCC progression. It will also show in press data on the source of EC during liver regeneration.\(^9\)

References


Proteostasis And Fibrosis Resolution

The proteostasis network is a highly conserved molecular response to disruptions in the cellular proteome. Efficient functioning of the proteome is fundamental to all cellular processes and crucial in maintaining homeostasis (1). The stability and function of all proteins is achieved through interactions of the components of the proteostasis network. This intricate sensing network includes molecular chaperones that influence protein folding, conformation and stability, ubiquitin-dependent proteasomes, and autophagic activities to clear damaged proteins during chronic cellular stress. In various human chronic diseases including liver disease, exposure to stress results in activation of the proteostasis network to prevent damage and protect from stress signals. Imbalance in the functioning of this network can contribute to loss of protein homeostasis and development of disease.

Liver fibrosis is a significant health problem, accounting for 1.5 million deaths worldwide and its mortality is attributed to cirrhosis and liver cancer (2). Fibrosis results from dysregulation of the normal wound healing process and leads to excessive accumulation of extracellular matrix (ECM) proteins and scar, primarily due to unresolved chronic inflammation in the liver. Variety of etiologies such as chronic HBV and HCV infection, biliary disease, alcoholic steatohepatitis (ASH), and non-alcoholic steatohepatitis (NASH) have been linked to fibrosis and cirrhosis (3, 4). To date there is no FDA-approved anti-fibrotic drug, hence there is a pressing need to understand pathogenic mechanisms to guide development of effective therapies. Recent reports on success of antivirals for HBV and HCV (5) and models of fibrosis resolution (6) suggest the likelihood of reversion of hepatic fibrosis. It becomes pertinent to understand not only the mechanisms of initiation and progression but also of reversion of fibrosis to identify effective anti-fibrotic therapies.

Oxidative stress and inflammation play a major role in stellate cell activation and fibrosis (7, 8). In response to oxidative stress and tissue injury, components of the proteostasis network, such as heat shock proteins (HSPs) that serve as molecular chaperones, play an important role to restore homeostasis (9). Heat shock factor 1 (HSF1) is a master transcriptional regulator of proteostasis machinery and is activated during oxidative stress and inflammation. While the importance of HSF1 in restoring cellular homeostasis is well-established, its potential role in liver fibrosis remains poorly understood. We hypothesize that HSF1 is induced during liver fibrosis and plays a protective role by decreasing hepatic stellate cell (HSC) activation. Our results reveal that HSF1 is induced in human fibrotic livers and in livers of murine models of inflammation and fibrosis. Mice lacking HSF1 exhibit augmented liver fibrosis despite limited pro-inflammatory cytokine response. HSF1-deficient mice demonstrate delayed reversal in the model of liver fibrosis resolution. In addition, HSCs lacking HSF1 demonstrate elevated profibrogenic gene expression. Our findings identify a novel role for HSF1 in liver fibrosis. Thus, modulating HSF1 activation may be an attractive therapeutic target for ameliorating liver fibrosis.
References


Epigenetic Regulation of Stellate Cell Activation

My work has revealed that epigenetic processes are crucial for control of fibrogenesis and fibrosis (1,2). In my talk, I aim to describe how hepatic stellate cell activation, a pivotal event in fibrogenesis, is controlled by DNA methylation, which is one of the key transmitters of information from genome to phenotype and now known to be responsive to cues from cellular microenvironment. DNA methylation in HSCs is interpreted via MeCP2, a DNA methyl binding protein, which has been recently discovered to exert broad influence over the coding and non-coding transcriptional landscapes of the activated HSCs and is required for the expression of transcripts that control DNA integrity and replication (3,4). We have also identified a site-specific phosphorylation event on the MeCP2 protein that is required for its stimulation of HSC proliferation and which upon mutation reduces the level of CCl4-induced liver fibrosis when compared with wild type.

Irrespective of the cause of chronic liver disease, only a minority of patients will develop severe fibrosis; the basis for this variance is not known and we currently have no methodologies for predicting prognosis. I will discuss the idea that propensity to develop fibrosis can be epigenetically inherited, lasting at least two successive generations (5). The epigenetic mechanisms involved in this inheritance will be discussed as well as the presence of such mechanisms in patients that show highly variable speed of fibrosis progression in liver disease.

The possible use of epigenetic marks as biomarkers for patient stratification will also be mentioned and data discussed that shows differential DNA methylation can be detected within the pool of cell-free genomic DNA found in human plasma, which can be used to stratify liver fibrosis severity in patients with a variety of human liver diseases. These signatures can be traced back to the fibrotic liver tissue, providing a biomarker of the underlying pathological process that may be able to track severity of fibrosis using a blood test (6, 7).

References


Fibrosis and the Aging Liver

Non-alcoholic steatohepatitis (NASH) is a leading cause of liver-related mortality in the U.S. and worldwide. The incidence of cirrhotic NASH rises with age, contributing significantly to elderly mortality. Older patients are excluded from liver transplantation; therefore, an increase in NASH-related mortality is expected since NASH has no approved medical treatment. Addressing the factors that elicit progressive fibrosis would allow earlier interventions, reducing disease burden. Most data in NASH/fibrosis research is derived from experiments using young animals; in clinical trials, however, the target population is usually middle-aged or elderly. To overcome this contradiction and facilitate translation, animal experiments addressing fibrosis should also be performed in older animals.

Activation of the Src homology 2 domain containing (Shc) proteins and enhanced production of ROS has been recognized in aging. p46, p52, and p66Shc are encoded by the ShcA locus in mammals and are involved in regulating metabolic and oxidative pathways. P52Shc is a membrane-associated protein whereas p46 and 66 are mitochondrial. While the role of the mitochondria as a source of reactive oxidative species (ROS) in aging has been recognized, targeting mitochondrial pathways of redox stress has been difficult. We previously showed that NADPH oxidases (NOXs) are important sources of ROS. NOXs catalyze the reduction of molecular oxygen to superoxide, using NADPH as an electron donor. In the liver hepatocytes, hepatic stellate cells (HSC) and macrophages express the phagocytic NOX2. During activation of NOX2, the regulatory subunit p47phox coordinates membrane translocation of the other subunits, assembling the active oxidase, and catalyzing the formation of superoxide. The mechanism by which NOX2 gets activated in non-phagocytic hepatocytes and its link to fibrogenic responses in aging has not been addressed. We show that Shc, especially p46 and p52, are induced in older patients, and liver fibrosis, Shc expression, and p52Shc activation (pY317) are more enhanced in aged compared to young mice on fast food diet (FFD). NOX2 was induced in a Shc-dependent manner during FFD, and inflammatory and fibrogenic transcripts were blunted, and less fibrosis was seen in total and hepShcKO mice. These mice exhibited attenuated oxidative stress, lipid peroxidation, and triglyceride content. Direct binding of p52Shc to p47phox was a key to NOX2 activation in older hepatocytes. Therefore, enhanced p52Shc/NOX2 expression/activation and redox stress during aging contribute to an exacerbated inflammatory and fibrogenic response in NASH.

References
Extracellular Vesicles as a Driver of Liver Fibrosis

A newly-recognized component of cell-cell communication in the liver is intercellular trafficking of molecules that are contained within nano-sized extracellular vesicles (EVs) such as exosomes or microvesicles. Microvesicles are 200-1000nm in diameter and are formed by budding of the plasma membrane while exosomes are typically 50-200nm in diameter and arise by inward budding of multivesicular bodies which then fuse with the plasma membrane and cause exosomes to be released from the cell. Despite their distinct pathways of biogenesis, both exosomes and microvesicles mediate cell-cell transfer of their respective molecular payloads that can potentially effect reprogramming events in recipient cells. EV cargo molecules includes a broad spectrum of microRNAs, mRNAs and proteins that are protected from extracellular degradation by the presence of the outer vesicular membrane which also functions to present docking proteins and receptors to recipient cells, allowing for EV uptake either by fusion with the plasma membrane or by endocytosis.

Recent evidence suggests that EV production and action in the liver likely represents a central regulatory system that ensures its many functions are correctly orchestrated or, alternatively, that can drive or dampen pathophysiological pathways during times of organ damage, infection, or disease. EVs from hepatic cancer cells mediate delivery of RNAs or proteins and subsequent regulation of cancer cell proliferation, chemo sensitivity or migration, intercellular transmission of the hepatitis C virus, or acquisition of a pro-angiogenic phenotype by endothelial cells. However, a more expansive and highly complex role for exosomes in the liver is now emerging with the recognition that hepatocytes, macrophages, cholangiocytes and hepatic stellate cells (HSC) produce exosomes that can influence a broad spectrum of cellular processes involved in liver repair after hepatectomy or pathogenesis associated with fibrosis, alcoholic liver disease, non-alcoholic steatohepatitis, or non-alcoholic fatty liver disease (see (1-8) for reviews).

We have begun to examine the regulation of HSC function and liver fibrosis by EVs from other HSC, hepatocytes or the systemic circulation. As assessed by expression of collagen, αSMA, or CTGF/CCN2, EVs from activated HSC can drive pathways of activation and fibrogenesis in quiescent HSC whereas EVs from quiescent HSC can suppress activated HSC. Regarding the latter observation, we found all components of a Twist-1-miR-214/-199a axis to be transferred via EVs to recipient HSC in which CTGF/CCN2 was directly targeted thereby suppressing its expression and that of its downstream fibrogenic mediators (9-11). We found that i.p. administration of fluorescently-labeled HSC-derived EVs resulted in their preferential localization to HSC in the liver while in vitro binding studies revealed that cell-surface heparan sulfate proteoglycans (HSPG) and integrins mediate binding of HSC-derived EVs to other HSC (12). Proteomic analysis reveals striking differences in protein content between EVs from quiescent HSC versus activated HSC.

HSC also respond to EVs produced by hepatocytes. When such hepatocytes are subjected to stress or damage, the EVs produced by these cells can drive fibrogenic pathways in HSC (13, 14). On the other hand, we find that EVs from normal hepatocytes have suppressive actions on activated HSC in vitro (15) and are anti-fibrotic in vivo, where they localize mainly to HSC or hepatocytes via cell surface HSPG or integrins. Similar actions are exhibited by EVs from the
serum of healthy donors which cause attenuated HSC activation and fibrogenesis, hepatocyte recovery, reduced hepatic inflammation, and modulation of circulating inflammatory mediators (16). The direct therapeutic properties of serum EVs on HSC or hepatocytes appear to be due, at least in part, to the action of EV miRs that are suppressed in serum EVs during fibrosis (16).

The emerging picture is that liver fibrosis is influenced by multiple sub-populations of EVs, at least in part through their direct interactions with HSC. The challenge ahead is to fully discriminate the origin, targets, and roles of various EV populations in the liver at different stages of injury or disease and to identify key components in their respective molecular payloads that are responsible for mediating either pathogenic or therapeutic outcomes in fibrosis.

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References
NAFLD is a spectrum of disease that includes hepatic steatosis, NASH, fibrosis, cirrhosis and hepatocellular carcinoma (reviewed in (1)). Not all patients that develop steatosis will invariably go on to get more advanced liver disease. Since very few patients are followed over time with serial liver biopsies the pathophysiology of liver disease progression is debated. Since the prevalence of steatosis > NASH > fibrosis > cirrhosis > HCC some have proposed that just having steatosis is not enough to cause more advanced liver disease and a “second hit” is needed to allow for progression similar to what is seen for the adenoma to carcinoma transition in colon cancer (reviewed in (2)). However, more recent observations that about 22-49% individuals can develop hepatocellular carcinoma even in the absence of cirrhosis (reviewed in (1)) calls into question the linear progression model of liver disease. Furthermore, the presence of hepatic steatosis correlates to the presence of metabolic syndrome traits and forebodes the development of cardiovascular disease which is the number one cause of death in NAFLD and thus suggests that even just hepatic steatosis is a marker of future morbidity and mortality. Human genetic studies allow us to identify genetic variants that associate with disease. Because genetic changes predated any pathophysiology these changes are unquestionably causally related to disease development and allow us to 1. Define the biological underpinnings of disease development 2. Better define personal risk of disease and 3. Identify targets for therapeutic intervention to alter disease outcomes. We and others have shown that common NAFLD and common related metabolic diseases and traits such as obesity, diabetes, dyslipidemia, and hypertension are heritable (genetically influenced) and through the use of Genome Wide Association Studies (GWAS) we have identified many genetic changes that associate with these common diseases (3-10). Further we and others have shown a difference in prevalence of NAFLD across ancestries and more than 70% of this difference is likely genetic (9, 11-13).

We and others have carried out the largest studies to date on NAFLD and obesity related disease. We find that genetic variants that associate with hepatic steatosis also associate with NASH/fibrosis. The exact mechanism by which these variants affect genes to cause disease is still being worked out but from what has so far been reported these variants can cause problems with lipid and glucose metabolism in liver (reviewed in (14)). These data support a model where primary defects in lipid metabolism causally contribute to NASH fibrosis and suggest that reversing the primary lipid abnormality can reverse not only steatosis but also NASH/fibrosis. We have also found variants that predispose to liver inflammation and fibrosis more than they contribute to hepatic steatosis suggesting that modifiers of these processes are identifiable and can also contribute to liver inflammation and fibrosis more than to steatosis (15-18). Finally, NAFLD predisposing variants do not always predispose to NAFLD correlated metabolic diseases and indeed sometimes protect against epidemiologically correlated diseases suggesting both a common and unique genetic predisposition to metabolic disease that can be used to define disease subclasses, tailor therapeutics based on subtype and forms the basis for precision medicine ((3) and reviewed in (14)).
References


Currently Available and Emerging Antifibrotic Therapies in Human Liver Fibrosis

The response to recurrent injury, in liver and in other organs is one of wound healing. Many different types of injury (i.e., chronic hepatitis, ethanol, biliary tract disease, excess iron or copper, etc.) lead to hepatocellular injury and ultimately to hepatic fibrosis and cirrhosis. A key pathogenic event in this process is transition of resident perisinusoidal cells known as hepatic stellate cells (Ito or stellate cells) from a quiescent to an "activated" state. This process is characterized by production of increased amounts of extracellular matrix and de novo expression of smooth muscle $\alpha$ actin, the latter characteristic consistent with their transformation to myofibroblasts. One of the most remarkable aspects of this response (as in all tissues) is enhanced extracellular matrix production or fibrogenesis. Fibrogenesis after injury to the liver is characterized by a multi-fold increase in collagen (type I>III>IV) as well as an increase in other extracellular matrix constituents such as proteoglycans (dermatan sulfate>chondroitin sulfate > heparan sulfate. The wounding process is complex and integrated and involves aspects of matrix synthesis and deposition as well as degradation.

In multiple different liver (and other parenchymal organ) diseases, evidence suggests that the wounding process is a dynamic one with not only elements of extracellular matrix deposition, but also degradation. This realization is supported by evidence that fibrosis and even cirrhosis is reversible (see 1,2 for review). For the purposes of this discussion, the terms “reversible”, “reversion”, “reversal”, and “regression” are used interchangeably to indicate that fibrosis progression has been interrupted; these terms are not meant to insinuate or imply that the liver has returned to a normal histopathological state.

Multiple pathogenic mechanisms underlie fibrosis regression. These are largely centered around modulation of stellate cell activation, stimulation of matrix degradation or stimulation of stellate cell death. Indeed, a critical mechanism of fibrosis regression is tied to inhibition or reversal of stellate cell activation. This phenotypic reversal occurs both in cell culture models as well as in vivo, although “deactivated” stellate cells appear to exhibit greater responsiveness to recurring fibrogenic stimulation.

The most effective approach to reverse fibrosis is to remove the primary injury. Evidence now exists in many liver diseases to support the concept that treatment of the underlying process leads to fibrosis regression, including hepatitis B, delta hepatitis, hemochromatosis, removal of alcohol in alcoholic liver disease, decompression of biliary obstruction in chronic pancreatitis, immunosuppressive treatment of autoimmune liver disease, and treatment of schistosomiasis, and others. Growing evidence also suggests that fibrosis is reversible after eradication of hepatitis C, and it is likely that with the widespread introduction of direct acting antiviral therapy that many patients will experience substantial reversion of fibrosis.

In terms of primary antifibrotic therapy (i.e. directed at a fibrogenic pathway, rather than the underlying disease), a number of approaches have been proposed. To date, several studies in humans have examined primary anti-fibrotic treatment in humans (including with colchicine, silymarin, and polyenylphosphatidylcholine, and others); these studies have largely found
the proposed treatments to be ineffective. However, given the newer understanding of the pathogenesis of fibrosis, novel therapies continue to evolve. Preclinical studies have highlighted many potentially novel therapies that may abrogate fibrogenesis - focused on many different pathways - some of which have been highlighted at this STC. Unfortunately, a number of approaches based on excellent preclinical mechanistic data have failed, including interferon gamma 20, 21, PPAR gamma 22, 23, lysl oxidase-like 2 24, and others. Yet, multiple studies of potentially antifibrotic therapies are currently underway, including large well-conceived and well powered studies. It is likely only a matter of time before an effective agent (or agents) is (are) identified.

References
Fibrosis is an intrinsic response to chronic injury, maintaining organ integrity when extensive necrosis or apoptosis occurs. With protracted damage, fibrosis can progress toward excessive scarring and organ failure. To date, antifibrotic treatment represents an unconquered area for drug development, with both enormous potential and challenges. While mild fibrosis remains largely asymptomatic, its progression to cirrhosis is the major cause of liver-related morbidity and mortality. Highly effective antiviral regiments for viral hepatitis are decreasing the burden of viral-related cirrhosis and HCC; however, progressive hepatic fibrosis from NAFLD has taken center-stage as the leading cause of chronic liver disease globally. Those with the progressive variant of NAFLD, non-alcoholic steatohepatitis (NASH), are at risk for fibrosis progression, the primary determinant of liver-related morbidity and mortality (1). However, there are currently no FDA approved pharmacologic therapies for NASH. Antifibrotic therapies that prevent progression toward cirrhosis or induce regression of advanced fibrosis and cirrhosis are urgently needed. However, multiple challenges in clinical trial development in liver fibrosis exist.

The underlying pathophysiologic mechanisms that contribute to the development and progression of NAFLD and NASH are complex and reflected by the myriad of therapies, with different targets, currently under investigation. In broad strokes, drug development has focused on modulation of metabolic pathways, inflammatory cascades, and/or pathogenic mechanisms underlying the development and progression of liver fibrosis. Although much progress has been made in enhancing our understanding of NAFLD pathogenesis, development of pharmacologic treatments has been hindered by challenges in clinical trial enrollment and complexities in clinical trial design (2).

Determining optimal yet feasible endpoints for clinical trials in liver fibrosis is hindered by the chronic nature of the disease, with typically slow progression (over decades of time) to clinically significant outcomes (3). Thus, relevant and acceptable surrogate endpoints that accurately depict disease biology and activity need to be broadly validated. In addition, the interconnected and dynamic nature of metabolic, inflammatory and fibrotic aspects of the disease, in response to an intervention, add to the difficulty in identifying clear and precise endpoints. Although the presence of NASH has been clearly linked with fibrosis development, an individual treatment can have different impacts on these two endpoints (i.e. treatment with drug X can improve NASH but worsen fibrosis and vice versa). Moreover, during disease progression fibrosis may worsen, while features of steatohepatitis resolve or “burnout”. As a result, NASH and fibrosis need to be evaluated independently to ensure a beneficial impact on one parameter does not simultaneously result in a negative impact on another endpoint of interest, particularly given that individual treatments tend to focus on one primary mechanism of action (i.e. metabolic or NASH disease modifier vs. antifibrotic).

Another challenge for clinical development in liver fibrosis is the histologic parameter used to stage this outcome. The inherent limitations of liver biopsies (interobserver reliability and sampling variability) presents a unique set of barriers and limitations for early phase trials investigating whether relevant information can be derived from small sample size and short-term endpoints to inform future trials. The challenge remains with long-term registration trials which...
are required to demonstrate clinically meaningful hard outcomes which are in line with FDA and EMA requirements. While there is mounting interest in identifying study endpoints that utilize non-invasive serologic or radiologic biomarkers of fibrotic changes in response to therapeutic intervention, these biomarkers will need further data and regulatory qualification before they can replace a liver biopsy, both in clinical trials and subsequently in clinical practice (4).

The indolent and dynamic bidirectional nature of disease progression poses challenges in the assessment of these outcome types among individuals with early stage disease. In the setting of cirrhosis, relevant endpoints include changes in degree of portal hypertension or the development of hepatic decompensation. In clinical trials of cirrhosis, all-cause and liver-related mortality are also important and tenable outcomes. Changes in portal hypertension, which is tightly linked to clinical outcomes, can be assessed directly through measurement of the hepatic venous pressure gradient (HVPG) or determined by the impact of the intervention on liver-related events. To evaluate changes in hepatic synthetic function and the development of hepatic decompensation, serial monitoring of Child-Turcotte-Pugh (CTP) score, model for end-stage liver disease (MELD) score, and new onset or worsening of liver-related events such as ascites, variceal bleeding, hepatic encephalopathy and HCC are needed (5). Other non-invasive serial monitoring tests (i.e. methacetin breath test and HepQuant) to assess changes in liver function are being investigated. With an increasing number of patients developing NASH-related end-stage liver disease and pharmacological treatments on the horizon, there is a pressing need to develop biomarkers for prognostication, selection of patients for treatment and monitoring (6).

Optimal selection and stratification of patients also poses a challenge in clinical trials design. Subjects should ideally be matched according to etiology of disease, age, gender, signs of the metabolic syndrome, and other confounders of fibrosis progression (i.e. alcohol or tobacco consumption). Studies may enrich recruitment with patient intermediate fibrosis stage disease (stage 2-3), where dynamic changes of fibrosis are best detectable. Such inclusion criteria lend higher screen fail rates, increased enrollment time-lines, and associated increase in study costs. Another variable for consideration is stratification according to genetic risk to progression to cirrhosis (7). Optimal selection and stratification of patients on the basis of potential confounders poses additional challenges in clinical trial development.

We have gained remarkable insight into the cellular/molecular mechanisms of liver fibrosis and reversal. Even reversal of cirrhosis appears feasible in preclinical models. As the field has progressed toward clinical translations, knowledge of foreseeable challenges in clinical trials development in liver fibrosis, lends opportunities for strategies by which to optimize study designs while working to develop and validate novel noninvasive markers and techniques to quantify liver fibrosis and especially fibrogenesis.

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POSTER PRESENTATIONS
TGFB INDUCES LIVER FIBROSIS VIA MIRNA-181A-MEDIATED DOWN REGULATION OF AUGMENTER OF LIVER REGENERATION (ALR) IN HEPATIC STELLATE CELLS

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Background: Liver damage caused by fibrosis pushes the liver to recover itself by regenerating itself. Liver stellate cells are the major cells involved in fibrosis. ALR is a crucial protein in liver regeneration as loss of ALR impairs liver regeneration. Thus, we hypothesized that ALR may help liver to overcome TGFβ induced fibrosis in human liver stellate cell line, LX2, via miRNA-181a.

Methods: LX2 cells were treated with 20 ng/ml TGFβ for 24 hr. Transfections were performed using siPORT NeoFx reagent following manufacturer’s protocol for miRNA-181a, ALR plasmid or empty vector. Cells were harvested after 48 hr or 72 hr of transfection for protein or RNA analysis. Western blotting was performed for ALR, Col1A1, α-SMA, E-cadherin and β-actin. qRT-PCR was conducted for ALR, GAPDH, miRNA-181a or 5S rRNA.

Results: TGF-β is a known inducer of cell fibrosis and it was found to up-regulate miRNA-181a expression by 3-fold and caused down-regulation of ALR to half. Co-transfection of ALR and TGF-β also showed 1.6-fold decreased expression of ALR compared to ALR transfected cells. miRNA-181a also down-regulated expression of ALR by 3.33-fold and up-regulated expression of fibrosis marker α-SMA by 10-fold. On the other hand, ALR over-expression in LX2 cells caused the reversal of these effects by down-regulating the expression of Col1A1 by 2-folds and α-SMA by 5-folds compared to control. This reversal of fibrosis seems to occur because of reversal of EMT as protein from ALR over-expressing cells showed a 6.5-fold increase in epithelial marker, E-cadherin, expression.

Conclusion: TGF-β induces fibrosis via miRNA-181a which inhibits ALR expression. ALR can overcome fibrosis by reversing EMT and increasing expression of E-cadherin, causing down-regulation of fibrosis markers, Col1A1 and α-SMA.

Disclosure: Nothing to disclose.
HYALURONAN SYNTHASE 2 OVEREXPRESSION IN HEPATIC STELLATE CELLS CONTRIBUTES TO LIVER FIBROSIS THROUGH NOTCH1 ACTIVATION

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Background: Liver cirrhosis as the advanced stage of liver fibrosis, is the 12th leading cause of death in the US. Hyaluronic acid, a major extracellular matrix (ECM), has been used as a biomarker of liver cirrhosis. However, the regulatory mechanisms of and the mechanistic role of hyaluronan (HA) in liver fibrosis is unknown.

Methods: Liver biopsy samples were obtained from 65 patients with chronic hepatitis B-mediated liver fibrosis and 28 patients with NAFLD or NASH. Hepatic stellate cell (HSC)-specific Has2 (Has2ΔHSC) or Notch1 (Notch1ΔHSC) knockout mice were generated by crossing Has2fl/fl or Notch1fl/fl mice with Lrat-Cre transgenic (Tg) mice, respectively. αSMA promoter-driven HAS2 Tg mice were also used.

Results: Liver HA contents and HA synthase 2 (HAS2) levels were increased in both human and mouse liver fibrosis. Has2ΔHSC mice had reduced liver fibrosis, whereas mice with HAS2 overexpression showed enhanced liver fibrosis. HAS2 promoted fibrogenic, proliferative, and invasive properties of HSCs through HA receptors, CD44 and TLR4. This study demonstrated that a transcription factor WT1 transcriptionally regulates HAS2 expression, and an oncogene Notch1 is a new downstream target of HAS2 and hyaluronic acids. Furthermore, we showed that a hyaluronic acid biosynthesis inhibitor, 4-methylumbelliferone, has the anti-fibrotic effect for liver fibrosis.

Conclusion: In conclusion, we suggested that HA is actively synthesized by HAS2 during HSC activation and promotes liver fibrosis through Notch1 activation. Targeted HA synthesis inhibition may be an effective strategy to treat or prevent liver fibrosis.

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Disclosure: Nothing to disclose.
LACTOBACILLUS RHAMNOSUS GG PREVENTS LIVER FIBROSIS THROUGH FXR-MEDIATED INHIBITION OF BILE ACID SYNTHESIS AND MICROBIOTA-REGULATED BILE ACID DE-CONJUGATION AND EXCRETION IN MICE

Authors: Y. Liu¹; C. McClain¹; W. Feng¹

Institution(s): University of Louisville¹

Background: Gut microbiota play an critical role in liver disease. The purpose of this study is to examine the effect of probiotics Lactobacillus rhamnosusGG (LGG) on the prevention of liver injury and to understand the underlying mechanisms in a mouse model of bile-duct ligation (BDL)-induced liver fibrosis.

Methods: Liver fibrosis was induced in mice by BDL for 11 days. One group of BDL mice received LGG at a dose of 10⁹ CFU/day. Bile acid compositions were determined. Liver fibrosis and injury were assessed by histology liver fibrotic gene expression and serum AST, ALT and bilirubin analysis. Intestinal microbiota were determined by pyrosequencing and gut lumen bile salt hydrolase (BSH) activity was determined. FXR luciferase reporter activity was analyzed.

Results: LGG treatment significantly decreased hepatic total bile acid concentration, liver injury and fibrosis in BDL mice. Metabolomics profiling showed a distinct bile acid composition in serum, liver and feces among Sham, BDL and BDL-LGG groups. Hepatic concentration of T-βMCA, a FXR antagonist, was markedly elevated, and CDCA, a FXR agonist, was reduced in BDL mice, which all were normalized in LGG treated mice. LGG significantly increased the expression of ileum FGF15, which is a FXR target and suppressor of hepatic bile acid synthesis through gut-liver axis. As a result, hepatic expression of key enzymes in bile acid synthesis, CYP7A1 and CYP27A1, was significantly reduced by LGG. Furthermore, LGG supernatant attenuated T-βMCA-mediated suppression of FXR activities in a reporter system and in HepG2 cells. SHP expression was increased and CYP7A1 expression was deceased by LGG supernatant in HepG2 cells. Fecal BSH activity was significantly increased by LGG, along with an increased gut BSH-producing bacteria population and de-conjugated bile acid concentration in feces. Bile acid transporters, MRP2 and MRP4, were significantly increased in kidney, indicating a likely increased bile acid urinal excretion.

Conclusion: LGG treatment prevented BDL-induced liver fibrosis by inhibition of bile acid synthesis and increase of fecal and urinal bile acid excretion. LGG may inhibit the suppression of FXR by T-βMCA and increase SHP expression in the liver and FGF15 expression in the intestine, leading to the reduction of CYP7A1 expression. In addition, LGG increased gut bacterial BSH activity and bile acid de-conjugation leading to an increased bile acid excretion.

Disclosure: Nothing to disclose.
POTENTIAL PATHOGENIC ROLE OF INCREASED CAMP-SPECIFIC PDE4 IN THE DEVELOPMENT OF HEPATIC FIBROSIS

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Institutions: University of Louisville Medical Center¹

Background: Several studies have demonstrated that activation of cAMP signaling decreases hepatic stellate cell (HSC) differentiation and proliferation in response to various stimuli. Our earlier work showed that HSC activation and development of hepatic fibrosis is accompanied by a significant increase in cAMP-degrading PDE4 expression. We also showed that inhibition of PDE4 activity prevents trans-differentiation of HSCs and development of fibrosis in a rat model of cholestatic liver injury. In this study, we examined normal and fibrotic human liver tissues to evaluate the role of cAMP/PDE4 pathway in the development of fibrosis in humans. We also evaluated the efficacy of PDE4 inhibition in alleviating carbon tetrachloride (CCl₄) induced fibrosis in mice.

Methods: Fibrotic livers were examined from patients with alcoholic hepatitis (AH) (n=9) and cancer patients with various primary cancer sites undergoing liver resection (n=8). Livers from healthy donors were also examined (n=5). C57Bl/6 mice were subjected to repeated CCl₄ injection for 4 weeks to establish hepatic fibrosis. One group of mice received a PDE4 inhibitor, Rolipram (targeted to the liver), twice a week. Expression levels of fibrotic genes and cAMP-specific PDE4 sub-family of enzymes (PDE4A, B and D) were quantified by RT qPCR and Western blot. Differences were analyzed by ANOVA. To examine if increased expression of PDE4 enzymes was associated with increased fibrotic markers in the liver, correlation analysis was performed.

Results: PDE4A, B, D and fibrotic markers were significantly higher in fibrotic human livers compared to normal livers. PDE4A, B and D were positively correlated with fibrotic genes (TGFB1, ACTA2, ACTB, and COL1A1) across all groups of human livers. Importantly, hepatic cAMP levels were significantly lower in patients with AH, indicating that increased PDE4 expression likely resulted in accelerated degradation of this critical second messenger. Further, Pde4a, b and d expression was also higher in mouse livers from the CCl₄ model. CCl₄ injection for 4 weeks led to the development of fibrosis as confirmed by Sirius red staining and increased hydroxyproline (HYP) content. Genes involved in HSC activation were significantly increased, demonstrating the active fibrogenesis. Importantly, inhibition of PDE4 activity by Rolipram prevented HYP accumulation and attenuated expression of genes involved in collagen synthesis (Hsp47) as well as crosslinking (Lox). Additionally, Mmp2 and Timp2 increases were markedly attenuated and anti-fibrotic Il-10 was significantly increased by Rolipram.

Conclusion: These results clearly indicate that PDE4 enzymes are involved in the development of liver fibrosis in both humans and mice. These data also suggest that PDE4 isoforms are...
potential therapeutic targets in the prevention/treatment of liver fibrosis.

**Disclosure:** Nothing to disclose.
FBLN5 COULD BE A NOVEL TREATMENT TARGET FOR ELASTIC FIBROSIS IN THE LIVER

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Institutions: Osaka City Juso Hospital; Osaka City University; Musashino Red Cross Hospital; Takatsuki General Hospital

Background: Although viral hepatitis has been successfully controlled, numbers of patients with HCC are still increasing, particularly due to the rise in patients with nonalcoholic steatohepatitis and the age of patients with viral hepatitis. Previously, we reported an increased accumulation of elastin fiber in advanced hepatic fibrosis, correlated with higher risk of developing HCC. However, synthesis and degradation of elastic fiber remain unclarified. In this study, we aimed to clarify the mechanism of fibrosis especially focusing on elastic fiber in advanced stage fibrosis.

Methods: First, we analyzed human liver biopsy specimens from 18 chronic hepatitis C (CH-C) patients obtained both before and after achieving sustained virological response (SVR) by direct acting antivirals (DAAs) treatment. Using Azan and Orcein stained sections we quantified amounts of collagen and elastic fiber, respectively, and evaluated the pathological fibrosis staging according to METVIR scoring system. Second, to reveal elastic fiber synthesis we conducted an in vitro study using two kinds of human hepatic stellate cell lines; i.e. HHSteC (ScienCell Research Laboratories, San Diego, CA) and LX-2 (American Type Culture Collection, Manassas, VA). Third, to clarify the relationship of elastic fiber and aging in mouse NASH model, we evaluated mRNA expression in the liver of young (18 weeks-old) and aged (56 weeks-old) mice with/without western diet (WD).

Results: (1) Serial biopsy samples from CH-C patients during a median of 2.3 years revealed that elastic fiber amounts had slightly increased after DAAs treatment (p = 0.08) whereas collagen fiber amounts had unchanged (p = 0.74). The activity grade had significantly improved during the same period. When patients whose initial diagnosis was ≥ F2 stage were analyzed, elastic fiber amount had remained unchanged (p = 0.22) while collagen fiber amount showed tendency of decrease (p = 0.053), implying that elastic fibers are not rapidly degraded even after the removal of initial causes of fibrosis. (2) In vitro study using stellate cell lines revealed that among five elastic fiber-related genes, i.e., elastin, galactosidase beta 1 (also known as elastin-binding protein), fibrillin 1, fibrulin 5 (FBLN5; necessary to organize mature elastic fibers), and microfibrillar-associated protein 4, only FBLN5 was significantly overexpressed upon TGF-b stimulation in both HHSteC (3.1 ± 0.1 fold change, p < 0.001) and LX-2 (6.0 ± 0.5 fold change, p < 0.001). (3) Finally, in mouse NASH model as expected, collagen 1A1 mRNA expression was elevated by WD in both young and aged mice (3.4 ± 1.7, 5.6 ± 1.1 fold change, respectively). In contrast, no alteration of FBLN5 expression was observed in young mice between WD and normal diet (ND), whereas aged mice with WD
showed increased FBLN5 expression compared to those with ND (1.5 ± 0.2 fold change).

**Conclusion:** Elastic fiber was undegraded shortly after SVR in CH-C patients. Activated hepatic stellate cells produced FBLN5, the component of elastic fiber, of which expression increased only in aged mice in response to western diet in vivo. These preliminary results indicate that FBLN5 could be a novel target for the treatment of advanced stage fibrosis, especially in aged patients.

**Disclosure:** Nothing to disclose.
 ROLE OF CYTOGLOBIN IN BILE DUCT LIGATION-INDUCED BILIARY INFLAMMATION AND FIBROSIS IN MICE

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Institution(s): Osaka City University1

Background: We assessed the role of Cytoglobin (CYGB), a gas-binding protein uniquely presented in hepatic stellate cell (HSCs), on bile duct ligation (BDL)-induced biliary inflammation and fibrosis.

Methods: Cygb transgenic (TG) and Cygb knockout (KO) mice as well as wild-type (WT) mice were employed and sacrificed at 24, 48, and 72 hours (acute phase) and 1, 2, and 3 weeks (chronic phase) after performing BDL. Histological and molecular biological analyses were performed by using obtained blood samples and tissues. CYGB expression was assessed in liver sections from patients with primary biliary cholangitis (PBC) or primary sclerosing cholangitis (PSC).

Results: In vivo: Recent our series of experiments imply protective role of CYGB in liver injuries and fibrosis development. In BDL model, Cygb expression increased time-dependently in WT mice, peaked 4.5-folds at the mRNA and 18.7-folds at protein levels, possibly for the defense against tissue damage. During the acute phase, KO mice showed severe liver damage compared to WT as indicated by a large number of huge bile infarcts (25.2 ± 13.6 vs 5.1 ± 2.5, p<0.05), increased serum level of AST (3930 ± 1611 vs 1243 ± 434 IU/l, p<0.05), ALT (788 ± 179 vs 489 ± 119 IU/l, p<0.05), bile acid (2068 ± 791 vs 1035 ± 345 nmol/g, p<0.01), and total bilirubin (554 ± 215 vs 186 ±37 mmol/l, p<0.05). In contrast, TG mice showed minimum liver injury. Under BDL for 1-3 weeks, compared to WT, fibrosis was robustly developed in KO mice as indicated by increased expression of α-smooth muscle actin (α-SMA, 5.7-fold, p<0.01), collagen 1α1 (3-fold, p<0.001), and Sirius-red positive area (2.8-fold, p<0.01). In contrast, TG mice showed suppression of α-SMA, Coll1α1, Sirius red-positive area up to 55% compared to WT mice. The infiltration of neutrophils observed in KO livers (4-fold higher than WT mice, p<0.001) were rarely seen in TG livers. Myeloperoxidase expression was 2.6-fold higher in KO mice, but 0.5-fold lower in TG mice compared to WT. Nitric oxide (NO) metabolites including NO2, NO3, and cGMP, and the reactive oxygen species (ROS) markers 4-hydroxynonenal and malondialdehyde were markedly increased in KO mice. Treatment with an NO inhibitor subdued these phenotypes in BDL-KO mice, while administration of an NO donor aggravated liver damage in BDL-WT mice to the same extent as in BDL-KO mice. In culture: Isolated HSCs from KO mice (HSCsCygb-null) became enlarged with a developed αSMA network after 7 days in culture, and these cells lost cellular vitamin A-lipid droplets more rapidly than HSCs from WT (HSCsCygb-wild). Moreover, HSCsCygb-null demonstrated a pre-activated phenotype with increased production of O2- and marked expression of interleukin 6 (II-6), tumour necrosis factor (Tnf) α, Il-1β, chemokine
(C-X-C motif) ligand 1 (Cxcl-1), Cxcl-2, and Chemokine (C-C motif) ligand 2 (Ccl-2), -3, and -4. Similar results were produced when primary HSCsCygb-wild were treated with siCygb. In contrast, primary HSCs isolated from TG mice (HSCCygb-TG) exhibited significantly decreased mRNA levels of αSMA, collagen 1α1, and transforming growth factor β-3 at 4 days in culture compared with HSCsCygb-wild. Interestingly, when both HSCsCygb-wild and HSCCygb-TG were challenged with different doses of H2O2, we found that H2O2 dose-dependently induced αSMA expression in HSCsCygb-wild, which was significantly attenuated in HSCsCygb-TG. In human liver: PBC and PSC patients had higher levels of Cygb-positive cells along sinusoids and bile ducts compared to normal livers.

**Conclusion:** CYGB in HSCs is thought to be involved in the regulation of BDL-induced liver injury and fibrosis development possibly via nitrosative and oxidative stress.

**Disclosure:** Nothing to disclose.
IL13-STAT6 MEDIATED ACTIVATION OF HEDGEHOG SIGNALING REGULATES SCHISTOSOMIASIS FIBROSIS

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Institution(s): Stanford University Medical Center1; Newcastle Fibrosis Research Group2; Federal University of Minas Gerais3; Faculdade de Medicina4; Cincinnati Children’s Hospital5; UNC Medical Center6; National Institute of Allergy and Infectious Diseases7

Background: IL13 and Hedgehog signaling pathways have both been implicated in the pathogenesis of fibrosis. In this study, we investigated if there is cross-talk between IL13 and Hh pathways in an experimental model of IL-13 induced liver fibrosis and in mouse and human schistosomiasis.

Methods: Hedgehog/IL13 signaling were investigated by qRT-PCR, immunohistochemistry and ELISA in uninfected healthy transplant donors (n=22), infected hepatointestinal schistosomiasis patients (liver granulomas, low fibrosis, n=17), infected hepatosplenic patients (advanced fibrosis and portal hypertension n=72); in Schistosoma mansoni infected mice (wild-type, IL13Rα1-/- and TKO (IL-10-/- IL12p40-/-IL13Rα2-/-) treated with anti-IL13 antibody, Hh inhibitors (Vismodegib or AsO3) or vehicle; in mice overexpressing IL13 (plasmid) and in human liver cells stimulated with rIL13 and treated with STAT6 siRNA or Vismodegib.

Results: Hedgehog signaling is upregulated in human schistosomiasis and correlates with IL13, fibrosis stage and severity of portal hypertension. Overexpression of IL13 (plasmid, infected TKO mice, rIL13) induced Hedgehog production/activation; lack of IL13 signaling (IL13Rα1-/- infected mice, anti-IL13 Ab, STAT6 siRNA) implicated in reduced Hedgehog pathway, indicating that Hedgehog signaling is dependent on IL13. STAT6 ChIP assay further demonstrated that STAT6 directly regulate the transcription of Hedgehog ligands and transcription factors. Smoothened antagonist Vismodegib effectively blocked fibrosis during acute schistosomiasis but failed to inhibit Hh pathway/fibrogenesis when treatment was initiated in chronic phase due to Smoothened-independent IL13-mediated Gli activation. Gli inhibition with AsO3 in the chronic phase impaired Hedgehog signaling and fibrogenesis.

Conclusion: Activation of the Hedgehog pathway in schistosomiasis is highly dependent on IL13-mediated signaling. Targeting Hedgehog pathway with Gli antagonists may be a novel therapeutic strategy to treat schistosomiasis fibrosis and related portal hypertension.

Disclosure: Nothing to disclose.
REGULATION OF THE MESENCHYMAL TRANSITION OF DUCTULAR CELLS BY AUTOPHAGY: A CRITICAL ROLE IN LIVER CIRRHOSIS

Authors: T. Hung

Institution(s): National Taiwan University Hospital

Background: We previously found that autophagy is the link between ductular reaction (DR) and liver cirrhosis. However, the subsequent fibrogenic response, which is regulated by increased autophagy in DR, remains unclear. Here, we examined the mechanism by focusing on the regulation of the mesenchymal transition.

Methods: Primary rat ductular cells were purified from an experimental rat cirrhosis model induced by 2-acetylaminofluorene (AAF) and carbon tetrachloride (CCL4). Western blot analysis, dual immunofluorescence staining and transmission electron microscopy were used to evaluate autophagy in ductular cells. The effect of autophagy on the mesenchymal transition was assessed in both primary rat ductular cells and human cirrhotic livers.

Results: Significantly increased autophagic flux was observed in ductular cells isolated from AAF/CCL4 livers, compared to normal livers. Treatment of ductular cells with the autophagy inhibitor bafilomycin A1 significantly reduced mesenchymal marker levels in ductular cells. In human cirrhotic livers, ductular cells positive for the autophagy marker LC3B also showed increased expression of the profibrogenic cytokine transforming growth factor-β (TGF-β) and the mesenchymal marker fibroblast-specific protein-1 (FSP-1).

Conclusion: Increased autophagy may contribute to the mesenchymal transition of cells during DR, a critical mechanism responsible for the pathogenesis of cirrhosis.

References

Disclosure: Nothing to disclose.
PERETINOIN, AN ACYCLIC RETINOID, INHIBITS HEPATOCARCINOGENESIS THROUGH SUPPRESSION OF SPHINGOSINE KINASE 1 EXPRESSION IN VITRO AND IN VIVO

Authors: M. Funaki

Institution(s): Kanazawa University

Background: Sphingosine-1-phosphate is a potent bioactive lipid metabolite that regulates cancer progression. Because sphingosine kinase 1 and sphingosine kinase 2 (SPHK 1/2) are both essential for sphingosine-1-phosphate production, they could be a therapeutic target in various cancers. Peretinoin, an acyclic retinoid, inhibits post-therapeutic recurrence of hepatocellular carcinoma via unclear mechanisms. Peretinoin (NIK-333), an acyclic retinoid, was reported to inhibit the post therapeutic recurrence of hepatocellular carcinoma (HCC) in patients with chronic hepatitis C. However, the mechanisms of its inhibitory effects against recurrent HCC remains unclear. We hypothesized that peretinoin could prevent hepatocarcinogenesis by modifying a SPHK-S1P axis. In the present study, we assessed the effect of peretinoin on SPHK activation and development of liver cancer in vivo and in vitro.

Methods: We examined effects of peretinoin on expression, enzymatic and promoter activity of SPHK1 in a human hepatoma cell line, Huh-7. We also investigated effects of SPHK1 on hepatocarcinogenesis induced by diethylnitrosamine (DEN) using SPHK1 knockout mice and transgenic mice.

Results: [In vitro] The treatment with peretinoin (10 to 40 μM) reduced the mRNA and protein expression of SPHK1 in Huh-7 cells in a time- and dose-dependent manner. However, peretinoin did not change the expression of SPHK2 in Huh-7 cells. Furthermore, peretinoin markedly reduced the enzymatic activity of SPHK1 assessed by in vitro 32P labeled SPHK activity assay. Next, we performed reporter gene assays by using constructs containing the SPHK1 promoter region. Peretinoin reduced the promoter activity of SPHK1 and overexpression of SP1 restored the promoter activity. In addition, three deletion constructs with and without SP1 binding sites were created. The deletion constructs without SP1 binding sites abolished the promoter activity. Interestingly, we previously reported that peretinoin suppresses the expression of SP1. Collectively, peretinoin reduced the expression of SPHK1 mRNA via SP1.

[In vivo] We analyzed SPHK1 transgenic and knockout mice under DEN-induced hepatocarcinogenesis conditions. Although all of the wild-type mice and 86% of the SPHK1 transgenic mice developed a liver tumor, only 53% of the SPHK1 knockout mice developed liver tumors. The frequency and numbers of liver tumors per knockout mouse were significantly lower than those of the wild-type and transgenic mice.
The liver tumor cells from wild-type and SPHK1 knockout mice had almost similar size of nucleus to that of non-tumorous liver cells frequently accompanied with Mallory body and moderate fatty change, and these liver tumors were diagnosed as well-differentiated HCC. On the other hand, the liver tumor cells from SPHK1 Tg mice showed nuclear atypia with higher tumor cell density and milder fatty change than wild-type and SPHK1 knockout mice, and these liver tumors were diagnosed as moderately differentiated HCC.

**Conclusion:** Our data showed crucial roles of SPHK1 in hepatocarcinogenesis and suggests that peretinoin prevents hepatocarcinogenesis by suppressing mRNA levels of SPHK1.

**Disclosure:** Nothing to disclose.
EFFECTS OF OBETICHOLIC ACID AND ELAFIBRANOR IN DIET-INDUCED OR CHEMICALLY-INDUCED ANIMAL MODELS OF NASH AND HEPATIC FIBROSIS

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Background: The FXR agonist obeticholic acid (OCA) and the PPARalpha/delta dual agonist elafibranor (GFT505), currently evaluated in clinical trials, are benchmarks in preclinical development of drugs targeting Non Alcoholic Steato-Hepatitis (NASH). Here we evaluated both drugs on several animal models of NASH and hepatic fibrosis, to select relevant models for drug efficacy studies.

Methods: Curative effects of OCA and GFT505 were evaluated in Diet-Induced NASH (DIN) obese and insulin resistant mouse or hamster fed a high fat/cholesterol/fructose rich (HFCF) diet for up to 25 weeks, as well as in chemically-induced mouse (carbon tetrachloride (CCl4) intoxication for 4 weeks) or rat (thioacetamide (TAA) intoxication for 13 weeks) models. In each model, plasma ALT/AST were measured and liver histology analysis (H&E and Sirius Red staining) and NAS scoring were performed.

Results: In DIN mice, 25 weeks of HFCF diet induced a strong liver steatosis, limited inflammation and perisinusoidal to periportal fibrosis. Compared with vehicle, both OCA and GFT505 significantly improved ALT/AST levels and NAS scoring in DIN mice, as compared with vehicle. However, OCA showed a better reduction in fibrosis score (61% lower, p<0.001 vs. vehicle) than GFT505 (26% lower, p<0.05 vs. vehicle).

In DIN hamsters, HFCF diet also induced a strong liver steatosis with evident inflammation and hepatocyte ballooning. The extent of hepatic fibrosis was also more pronounced than found in DIN mice, with peri-portal to bridging fibrosis. Compared with vehicle, GFT505 reduced plasma ALT by 52% (p<0.01 vs. vehicle) and significantly improved total NAS score in DIN hamsters, including fibrosis score (21% lower, p<0.01 vs. vehicle). In contrast, OCA showed limited benefits on NASH with concomitant dyslipidemic side effects, similar to those observed in humans (increased LDL-cholesterol and reduced HDL-cholesterol plasma levels).

Intoxication with TAA for 13 weeks resulted in bridging fibrosis in rats. OCA, but not GFT505, significantly reduced plasma ALT and AST by 57% and 43%, respectively. However, both OCA and GFT505 did not change either total NAS score, fibrosis score and % Sirius Red labelling in this rat model. Administration of CCl4 for 4 weeks also resulted in bridging fibrosis in mice. Both OCA and GFT505 significantly reduced % Sirius Red labelling in the liver, but GFT505 showed a more pronounced effect and was the only drug to reduce fibrosis score significantly (33% lower, p<0.001 vs. vehicle).
**Conclusion:** Our data indicate that depending on the preclinical model, OCA and GFT505 show variable effects. Overall, the 4-week CCl4-injected mouse represents a fast and convenient model to rapidly evaluate drugs targeting hepatic fibrosis. Given the similar cholesterol and bile acids metabolism and liver lesions as compared to humans, the DIN hamster also represents a useful diet-induced model to evaluate drugs targeting NASH and hepatic fibrosis.

**Disclosure:** Company Employee, Officer, Director with PHYSIOGENEX;
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MATRIX STIFFNESS REGULATE LIVER SINUSOIDAL ENDOTHELIAL CELLS (LSECS) FUNCTION: IMPORTANCE FOR LIVER FIBROSIS PROGRESSION

Authors: S. Kidambi

Institution(s): University of Nebraska-Lincoln

Background: Liver sinusoidal endothelial cells (LSECs) are a highly specialized endothelial cell that participates in numerous liver metabolic activities and collectively function as a scavenger system in the liver by removing waste macromolecules playing a vital role in the balance of lipids, cholesterol, and vitamins. Prior to hepatic fibrosis, independent of their etiology, LSECs become highly pro-inflammatory, capillarized (loss in fenestrations), and loss in specialized receptors (Stabilin-1, Stabilin-2, CD31 and SE-1). Liver fibrosis also leads to significant loss in the endocytosis function of LSECs. Thus understanding regulation of LSEC phenotype may be critical to understanding fibrosis. Extensive remodeling of the extracellular matrix occurs during fibrosis that leads to liver stiffening. The role of matrix stiffness as related to subtle but pivotal changes in LSECs physiology is under explored. The overall goal of our study is the development and implementation of a platform that enables the convergence of engineered cell microenvironment with the phenotypic and functional analysis of LSECs. Using our innovative biomimetic liver fibrosis model that allows modulation of substrate stiffness, we investigated the role of liver matrix stiffness in modulating LSECs function in fibrotic-like microenvironment.

Methods: Primary LSECs were cultured on our novel polymer film coated polydimethylsiloxane (PDMS) gels with 2 kPa, 9 kPa 25 kPa and 55 kPa elastic modulus mimicking healthy, early fibrotic, fibrotic and extremely fibrotic substrates. SEM was used to image to fenestrations of LSECs and HA endocytosis assay was performed to measure the LSECs function.

Results: LSECs cultured on stiffer environment had significant remodeling of cytoskeletal proteins and morphology indicated of stress fibers. Also we observed that LSECs on fibrotic substrates resulted in loss of fenestrations (capillarization). This is critical as capillarization has been show to precede hepatic fibrosis and “capillarized” LSECs lose the ability to promote hepatic stellate cell (HSC) quiescence. LSECs on stiffer environment also had higher expression of cell adhesion molecules, VCAM-1 and ICAM-1 indicating the loss of phenotype of the cells. Fibrotic stiffness also impeded the HA endocytosis in LSECs, one of the main functions of the cells. These data suggest a plausible mechanism that increased stiffness modulates hepatocyte and LSEC function causing liver functional failure. Similar effect was observed in LSECs isolated from Non-Alcoholic Fatty Liver Disease (NAFLD) rat models indicating correlation to physiological conditions.
Conclusion: Together, all these data demonstrates the plausible role of stiffness in regulating LSECs function and contribute to HSC activation and progression of liver fibrosis.

Disclosure: Nothing to disclose.
DEFICIENCY OF BOTH FARNESOID X RECEPTOR AND G PROTEIN-COUPLED BILE ACID RECEPTOR-1 EXACERBATED LIVER FIBROSIS IN MICE

Authors: J. Ferrell¹; J. Chiang¹

Institution(s): Northeast Ohio Medical University¹

Background: The bile acid-activated receptors farnesoid X receptor (FXR) and G protein-coupled bile acid receptor-1 (TGR5) regulate bile acid synthesis, liver metabolic homeostasis and energy metabolism. FXR is involved in feedback regulation of bile acid synthesis and protects against liver inflammation, and TGR5 increases insulin sensitivity and promotes adipocyte browning. Here, we determined the effects of double-deletion of these two bile acid receptors on liver metabolism, inflammation and fibrosis.

Methods: Heterozygote FXR and TGR5 deficient mice were mated to generate FXR/TGR5 double knockout mice (DKO). Liver transcriptomes were analyzed by RNA-sequencing and the metabolic phenotype was characterized. Cohorts of mice were fed standard chow diet, chow diet + 0.5% cholic acid, chow diet + 2% cholestyramine, or high fat, high cholesterol Western diet. Bile acid composition, pool size and key genes/enzymes in bile acid synthesis and regulation were measured. Liver inflammation and fibrosis markers were determined.

Results: Liver Cyp7a1 and Cyp8b1 were induced in chow-fed DKO mice while FXR target genes were suppressed in the ileum. Bile acid pool size was significantly increased in DKO mice, as was Cyp7a1 enzyme activity. Taurocholic acid was significantly induced and tauromuricholic acids were reduced in gallbladder bile. Cholestyramine induced Cyp7a1 expression further in DKO mice, as did cholic acid feeding, indicating dysregulation of bile acid homeostasis in these mice. Liver transcriptome analysis in chow-fed mice identified induction of bile acid synthesis, cholesterol synthesis and fibrosis gene expression, and the top regulated pathways in DKO mice were steroid/cholesterol synthesis, liver cirrhosis, and connective tissue disease. Western diet increased serum lipids and markers of oxidative stress and liver fibrosis in DKO mice, but these mice had less hepatic steatosis compared to control mice.

Conclusion: Fxr and Tgr5 double knockout mice have increased Cyp7a1 activity, cholic acid synthesis, and bile acid pool, and increased liver inflammation and fibrosis. DKO mice may serve as a novel model for liver fibrosis.

Disclosure: Nothing to disclose.
NAMODENOSON ANTI-FIBROGENIC EFFECT IS MEDIATED VIA DE-REGULATION OF THE WNT/β-CATENIN PATHWAY

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Institution(s): CanFite Biopharma Ltd¹; Hadassah Hebrew University Medical Center²

Background: The Wnt/β-catenin signaling pathway plays an important role in the pathogenesis of liver fibrosis (1-2). Namodenoson is an A3 adenosine receptor (A3AR) agonist, highly expressed in liver pathological cells but not in normal liver cells. Namodenoson was found to induce robust anti-inflammatory and anti-cancer effects, mediated via de-regulation of the Wnt/β-catenin signaling pathway (3-4). In this study we show the anti-fibrogenic effect of namodenoson in 3 experimental models, and demonstrate that the effect is conferred via the Wnt/β-catenin pathway.

Methods: The hepatic fibrosis model was induced in C57BL/6 mice by repetitive CCl4 injections, twice weekly i.p., for 6 weeks. Namodenoson 100µg/kg was administered IP 24 hours after CCl4 injections. At termination, liver enzyme levels were determined and liver tissue was subjected to pathological and blots analysis. NASH model was induced in C57BL/6 mice by injection of a single subcutaneous streptozotocin 200µg two days after birth followed by high fat diet feeding since age of 4 weeks. Namodenoson or vehicle oral treatments were administered along 6-9 weeks of age. At termination histologic analysis took place. The LX2 cells were incubated in vitro in the presence of namodenoson (10nM). Protein and mRNA analysis assays were performed.

Results: In the CCL4 model namodenoson significantly reversed the high liver enzyme levels to normal values (ALT: 8747±1785 vs. 203±66 IU/L; AST: 11,261±2205 vs. 152±93 respectively). Liver tissue extracts from the namodenoson treated animals showed significant reduction in fibrosis and inflammation compared to the vehicle demonstrated in Sirius-red and H&E staining respectively (p<0.01). Also, de-regulation of the Wnt/β-catenin pathway took place, manifested by an increase in GSK-3β and down regulation of β-catenin, Lef-1 and cyclin D1. In addition, a marked significant decrease in α-SMA expression level took place upon namodenoson treatment, supporting the anti-fibrogenic effect of the drug. In the NASH model, namodenoson significantly decreased fibrosis manifested by Sirius-red staining compared to the vehicle (fibrosis score % : 1.73±0.18 vs. 0.93±0.30, respectively; p<0.05). In the LX-2 hepato-stellate cells, namodenoson decreased the expression levels of A3AR, β-catenin, Lef-1 and cyclin D1 accompanied by an increase in GSK-3β and caspase-3 expression levels. The protein and mRNA α-SMA expression levels were markedly decreased upon namodenoson treatment.

Conclusion: Namodenoson, a small molecule orally bioavailable drug, induces an anti-fibrogenic effect mediated via the A3AR and the de-regulation of the Wnt/β-catenin pathway.
References

Disclosure: Company Employee, Officer, Director with CanFite biopharma Ltd;
THERAPEUTIC EFFECTS OF EXOSOMES DERIVED FROM HUMAN UMBILICAL CORD PLASMA IN MOUSE MODEL OF LIVER FIBROSIS

Authors: J. Huang¹

Institution(s): National Taiwan University Hospital¹

Background: Liver fibrosis is a passive and irreversible process characterized by excessive extracellular matrix accumulation leading to disrupted liver architecture and dysfunction. Currently, an effective therapy for liver fibrosis is lacking. Here, we hypothesized that umbilical cord blood plasma (UCBP)-derived exosomes have therapeutic potential for liver fibrosis.

Methods: We examined histology and collagen levels in a liver-fibrosis mouse model induced by carbon tetrachloride. Besides, in vitro experiments was focus on stellate cells to study whether exosomes could suppress cell activity and collagen production by western blotting, zymography, and ELISA.

Results: We observed that exosome treatment significantly decreased collagen deposition, improved liver function and affecting the balance of matrix metalloproteinase/tissue inhibitor of metalloproteinase.

Conclusion: These results demonstrated that exosomes available in UCBP exhibited anti-fibrosis effects in liver fibrosis mouse model and activated stellate cells, offering valuable insight into a novel cell-free anti-fibrosis strategy.

Disclosure: Nothing to disclose.
MULTIPARAMETRIC QUANTITATIVE LIVER MAGNETIC RESONANCE IMAGING.
CORRELATION WITH NON INVASIVE FIBROSIS SCORES

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Institution(s): CEDIMBA Clínica DIM; DIM Clinica Privada; Hospital Nacional; Hospital Universitario Austral

Background: Non alcoholic fatty liver disease (NAFLD) is now the most common chronic hepatic condition among adults. It is certainly important to distinguish those who are at higher risk of fibrosis progression. Non invasive imaging modalities are being used. Multiparametric quantitative magnetic resonance imaging (MRI) offers quantification of fat, iron and fibrosis. Non invasive serum fibrosis markers are being incorporated into the routine clinical care to evaluate which are eligibility to access the MRI studies. The aim is to evaluate the relationship between MRI elastography and serum liver fibrosis scores to aid to distinguish the high risk NAFLD patients.

Methods: Abdominal ultrasound with steatosis and exclusion of other causes of liver disease set the diagnosis of NAFLD. NAFLD patients (n=11, age 57.7±8.6, range=42 to 74, 7 females) have been evaluated with liver function tests, metabolic determinations (glycemia, serum insulin, HOMA index, lipids profile), platelet count, serum albumin and anthropometric measures. Non invasive scores were performed as published (APRI, AST/ALT, FIB4, BARD and NAFLD score). MRI studies were done with a Philips Ingenia 1.5 T scanner, version 5.3.1, which include magnetic resonance elastography (MRE) software to quantify fibrosis (liver stiffness, LS) and proton density fat fraction (PDFF) package to quantify fat fraction. The statistic analysis was performed using PASW Statistics 17.0. Pearson’s linear correlation coefficient (r) and two-tailed test of significance were used. P values <0.05 were considered statistically significant.

Results: LS was correlated with non-invasive liver fibrosis scores. There was a significant correlation with APRI (r=0.695; P=0.018) and FIB4 score (r=0.664; P=0.026), whereas non significant correlation was observed with NAFLD score (r=0.415; P=0.204), AST/ALT (r=-0.122; P=0.720) and BARD (r=-0.190; P=0.575). Additionally LS have positive correlation with HOMA index (r=0.816; P=0.002), serum insulin (r=0.725; P=0.012), AST (r=0.705; P=0.015), ALT (r=0.653; P=0.029) and glycemia (r=0.602; P=0.050). PDFF did not correlated with LS, neither than with fibrosis scores but it showed high correlation with glycosilated hemoglobin (r=0.749; P=0.008).
**Conclusion:** Among many of the non invasive serum fibrosis markers used in clinical care, FIB4 and APRI scores seems to be useful markers to stratify the risk of significant liver fibrosis development in NAFLD patients. These scores could allow to decide the time to evaluate hepatic stiffness with MRE during the course of care of NAFLD subjects. As expected, metabolic disorders correlate with fibrosis stage.

**References**

**Disclosure:** Nothing to disclose.
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TGFB1 REDUCES CYTOGLOBIN EXPRESSION VIA SMAD2-SP3 PATHWAY IN HUMAN, BUT NOT MOUSE, HEPATIC STELLATE CELLS

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Institution(s): Osaka City University Graduate School of Medicine; University College London

Background: Cytoglobin (CYGB), the fourth globin in mammals, is exclusively expressed in hepatic stellate cells (HSCs) and a key molecule for the maintenance of HSCs in quiescent state. CYGB also acts as a reactive oxygen species (ROS) scavenger protecting cells from oxidative stress. Although molecular function of CYGB has been determined by several settings, the regulation of CYGB gene expression in human HSCs has remained uncharacterized. This study aimed to reveal a regulatory mechanism of human CYGB gene promoter by TGF-β/Smads.

Methods: Human hepatic stellate cell line, HHSteCs, purchased from ScienCell and primary human HSC, isolated from intact human liver at University College London (UCL), were cultured in 2% FBS/SteCM-complete medium. Mouse HSCs were isolated and cultured in 10% FBS/DMEM. Protein and mRNA levels were determined using western blotting and quantitative PCR, respectively. The promoter activity was analyzed using luciferase assay. The chromatin immunoprecipitation (ChIP) assay was performed using anti-SP1 and SP3 antibodies at CYGB promoter region and the protein-protein interaction of Smad2 and SP3 was confirmed by immunoprecipitation (IP) assay. Human NASH specimens were obtained from Osaka City University Hospital and used for immunohistochemistry (IHC). 2',7’–dichlorofluorescin diacetate was used to measure intracellular ROS.

Results: Treatment of HHSteCs with 5 ng/mL TGF-β1 for 72 h significantly suppressed CYGB mRNA (< 61%) and promoted αSMA mRNA (> 57%) expressions compared with untreated ones, whereas TGF-β1 failed to change Cygb expression in primary mouse HSCs. TGF-β type I receptor inhibitors (SB431542) blocked the TGF-β1 (2 ng/ml) effect on CYGB and αSMA expressions in HHSteCs. siRNA knockdown system revealed that TGF-β1 reduced CYGB expression via Smad2 and increased αSMA expression via Smad3. Mithramycin, a SP1/3 DNA binding inhibitor, attenuated the TGF-β1-dependent reduction of CYGB expression. A putative SP1/3 response element is present in transcriptional initiation site of human CYGB promoter, but absent in that of mouse Cygb promoter. TGF-β1 suppressed luciferase activity of human CYGB promoter (-2133/+73), which was abolished by mutating the SP1/3 response element (+3/+10). ChIP and IP analyses revealed that TGF-β1/Smad2 reduced CYGB transcription by recruiting SP3, but not SP1, to CYGB promoter in HHSteCs. Finally, in human NASH specimens, majority of CYGB-positive cells were unstained with pSmad2 and αSMA antibodies and CYGB-positive cell number is negatively correlated with fibrosis stage. Overexpression of CYGB suppressed TGF-β1-induced intracellular ROS activity. In addition, staining for
4-hydroxynonenal and 8-hydroxy-2′-deoxyguanosine, markers of oxidative damage for lipid peroxidation and DNA damage, respectively, showed increased number of positive cells in advanced fibrosis.

**Conclusion:** Our study revealed for the first time that CYGB promoter activity is regulated by TGF-β1-Smad2-SP3 pathway in human HSCs, but not in mouse Cygb gene. Response to TGF-β1 is not always identical between human and mouse HSCs due to the unique promoter sequence of the specific gene like CYGB. TGF-β1 has a role in the activation of human HSCs to attenuate the protective function against oxidative stress by reducing CYGB expression.

**Disclosure:** Nothing to disclose.
THE LIVER MICROENVIRONMENT CONTROLS LYMPHATIC HETEROGENEITY TO MAINTAIN LIVER FUNCTION

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Institution(s): University of Colorado1

Background: Non-alcoholic fatty liver disease (NAFLD) is among the most common conditions afflicting American adults, with a prevalence of ~30%. Mounting evidence implicates the lymphatic vasculature as a key modulator of homeostasis in a number of different tissues. Lymphatic vessels support liver function by draining interstitial fluid, regulating cholesterol and lipid transport, and trafficking immune cells. While it is known that the frequency of lymphatic vessels is increased in patients with liver cirrhosis caused by viral infection (HCV), it is unclear whether expansion of the lymphatic vasculature is causative or consequential to disease progression. We show here that liver lymphatics support homeostasis, and that the liver microenvironment alters the expansion and functionality of lymphatic vessels. We further show that increased lymphatic vessel frequency is protective in a mouse model of chronic liver disease.

Methods: We sought to evaluate the role of pro-lymphangiogenic VEGFC/D-R3 signaling in the progression of liver fibrosis in C57BL/6 mice. Mice were fed a high fat/high cholesterol (HFHC) diet for 19 weeks to induce fibrosis. Subsequently, mice were maintained on the HFHC diet while supplementing with a blocking antibody to VEGF-R3 (αVEGF-R3), administered bi-weekly for 3 weeks. We then evaluated lymphatic vessel frequency, lymphatic endothelial cell (LEC) division, function and the extent of fibrogenesis in the presence or absence of the blocking VEGFR3 antibody.

Results: We found that HFHC-fed mice had significantly elevated lymphatic vessel frequency and LEC division. Interruption of lymphangiogenesis via administration of αVEGF-R3 significantly decreased LEC division, and αVEGF-R3 treatment coincided with increased inflammation and fibrosis in the liver. Furthermore, we found functional differences in LECs that could be attributed to the increased cholesterol.

Conclusion: These studies show that chronic liver inflammation, induced by an HFHC diet, is sufficient to promote LEC expansion and differentiation and increased lymphatic vessel density in the mouse liver. Furthermore, we show that inhibition of lymphangiogenesis results in accelerated liver fibrogenesis. These data identify the lymphatic vasculature as a key mediator of liver homeostasis, and suggest that promotion of lymphangiogenesis in the liver is a promising therapeutic target to improve liver functionality and regeneration.

References
Disclosure: Nothing to disclose.
MARKERS OF REGULATORY GENES FOLLOWING EXPOSURE TO A CCR2/CCR5 ANTAGONIST (CENICRIVIROC) IN HIV-INFECTED SUBJECTS

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Institution(s): University of Cincinnati1; University of Maryland2

Background: Chemokine receptors such as CCR2 and CCR5 are important in regulation of inflammation and immune responses to viral antigens. CCR2 is a receptor for monocyte chemoattractant protein-2 (CCL2), and involved in monocyte infiltration in inflammation. CCR5 is a key receptor for HIV-1 viral entry into T-cells and is expressed on subpopulations of Treg and Th1/Tc cells in humans. We examined the effect of cenicriviroc (CVC) CCR2/CCR5 antagonism in HIV infected persons not on antiretroviral therapy compared to patients treated with efavirenz (EFV), a non-nucleoside antiretroviral agent. All subjects received a tenofovir-emtricitibine backbone. Prior studies demonstrated a decrease in the ELF index in subjects treated with CVC compared to those treated with EFV, despite equivalent effects on HIV viral load reduction.

Methods: Five subjects in the CVC group and 5 in the EFV control group were evaluated. Expression of a group of 84 immunoregulatory/tolerance genes were assessed using a target chip approach (RT2 Profiler PCR Array, Qiagen). Fold-change (up and down regulation) were compared following 48 weeks of exposure to the study medications.

Results: The highest levels of downregulation were noted in ING4 and FOXP3 with greater than 3-fold change in those treated with CVC compared to EFV. Overall, more than 20 targeted genes were downregulated more than 2-fold.

Conclusion: In HIV-infected subjects, use of CVC preferentially downregulates a large number of immunoregulatory genes compared to efavirenz, despite equivalent suppression of HIV. This gene response may play an important role in regulating hepatic fibrosis.

Disclosure: Nothing to disclose.
OPIOID GROWTH FACTOR RECEPTOR-LIKE 1; AN EXTRACELLULAR VESICLE-MEDIATED CROSSTALK BETWEEN HEMATOPOIETIC CELLS AND HEPATOCYTES THAT ACCELERATES REGENERATION OF MURINE FIBROTIC LIVER

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Institution(s): Kitasato University Medical Center; Tokai University School of Medicine; Hokkaido University; Saitama University

Background: Interplay between hematopoietic cells and parenchymal hepatocytes has been implicated in the pathogenesis and treatment of liver fibrosis. We previously showed that granulocyte-colony stimulating factor (G-CSF) treatment of carbon tetrachloride (CCl4)-injected mice increased the number of bone marrow (BM)-derived cells migrating to the fibrotic liver and accelerated the regression of liver fibrosis. G-CSF administration was reported to stimulate the mobilization and proliferation of hepatic progenitor cells (HPC) in both rodents and humans suffering from advanced liver diseases. Here, we identified opioid growth factor receptor like-1 (OGFRL1) as a novel hematopoietic cell-derived accelerator of fibrotic liver regeneration through HPC mobilization in response to G-CSF treatment.

Methods: Acute liver injury and fibrosis were induced by single and repeated CCl4 administration, respectively. Human bone marrow mesenchymal stem cells were infected with recombinant OGFRL1-expressing lentiviruses and injected into the spleen of CCl4-treated nude mice. Total RNA was extracted from various organs/tissues of mice, and expression levels of Ogfrl1 gene were quantified using real time RT-PCR. Presence of OGFRL1 protein was detected by immunohistochemical staining, immunoelectron microscopy, or protein blot analysis. Fetal HPCs and hematopoietic cells were isolated from the E13.5 liver. After infection with recombinant retroviruses overexpressing OGFRL1, proliferation and differentiation of fetal HPCs were examined by colony forming assay and microarray analysis, respectively. EVs prepared from the sera were subjected to protein blot analysis.

Results: Endogenous Ogfrl1 was highly expressed in the hematopoietic organs such as the BM and spleen, while the liver contained a relatively small amount of Ogfrl1 mRNA. Twenty-four hours after a single CCl4 injection, OGFRL1 protein was observed as characteristic dots in the cytoplasm of zone 2 hepatocytes that surrounded the damaged hepatocytes in zone 3. OGFRL1 was transiently detected in the circulating blood as an EV protein upon acute liver injury. OGFRL1 was also detected in hepatocytes along the fibrous septa following repeated CCl4 injections. Administration of OGFRL1-expressing cells into mice with CCl4-induced liver fibrosis upregulated gene expression of α-fetoprotein (AFP) and cell cycle-related factors including cyclins. It also increased the number of AFP+ HPC and HNF4α+ parenchymal hepatocytes with active BrdU uptake after partial hepatectomy. In the E13.5 fetal liver, OGFRL1 was strongly expressed in both myeloid and erythroid cells, but not in Dlk-1+ HPC. When the fetal HPC were infected with OGFRL1-expressing retroviruses, it accelerated
the differentiation of fetal HPC as estimated by an increase in Hnf4a and Cyp2f2 expression. On the other hand, OGFRL1 overexpression had no effect on the colony-forming ability of HPC.

**Conclusion:** OGFRL1 secreted from the hematopoietic cells plays a critical role in the mobilization and differentiation of HPC in the fibrotic liver. Administration of OGFRL1-expressing cells or OGFRL1-containing EVs could be a potential regenerative therapy for advanced liver fibrosis.

**References**


**Disclosure:** Nothing to disclose.
NEUTROPHILS AND MAST CELLS ARE MAJOR PRODUCERS OF IL-17 IN HEPATOCELLULAR CARCINOMA

Authors: M. Flores Molina¹; T. Fabre¹; M. Abdelnabi²; G. Soucy¹; M. Bilodeau¹; S. Turcotte¹; N. Shoukry³

Institution(s): Centre de Recherche du Centre Hospitalier de l’Université de Montréal (CRCHUM)¹ ; Suez Canal University² ; Centre de recherche du CHUM³

Background: Hepatocellular Carcinoma (HCC) is the third cause of cancer-related death worldwide. IL-17 is upregulated in HCC and correlates with poor survival. Several studies link IL-17 to fibrosis progression and poor prognosis of HCC. However, the identity of the cellular sources of IL-17 and their target cells in the tumor tissue remain elusive. Furthermore, the activity of Th17 cells, the classical producers of IL-17, can be regulated by regulatory T cells (Tregs) that themselves favor an immunosuppressive pro-tumorigenic environment. We hypothesize that IL-17 from different cellular sources drives transition from fibrosis to HCC and HCC progression. Our primary objective is to define the cellular sources of IL-17 and their location within the tumor tissue, their target cells and their interaction with Tregs that may modulate their activity in HCC.

Methods: We developed a strategy combining multiplex immunofluorescence (IF), histochemistry (HC) and advanced image analysis to map IL-17+ cells and Tregs in situ in formalin fixed paraffin embedded (FFPE) HCC tumor tissue samples (tumor, peritumor and adjacent non tumoral tissue) obtained from the Hepatobiliary Biobank (HBP Biobank) of the University of Montreal Hospital (n=14). We have optimized a strategy for the analysis of the acquired images that combines the image analysis tools of Tissuealign™, Visiomorph™, and HISTOmap™ developed by Visiopharm. This strategy allows us to analyze how cell populations labelled in one image spatially relate to tissue compartments or cell populations in another image. We then count cell populations of interest in all the tissue and/or in specific tissue compartments and generate tissue heatmaps of these populations.

Results: Tissue heat maps demonstrate opposite compartmentalization of IL-17+ cells and Tregs, where IL-17+ cells are enriched in the peritumoral compartment and Tregs inside the tumor core (n=14). Density of Tregs is significantly higher in the tumor compared to non-tumoral adjacent tissue (p=0.0093), and the IL-17/Treg ratio is significantly reduced inside the tumor (p=0.0098). We have identified tumor associated neutrophils (TANs) and mast cells as major IL-17 producers accounting for up to 90 % of the total IL-17+ cells. IL-17+ TANs are located all over the tissue while mast cells are restricted to the fibrotic lesions.

Conclusion: Our data demonstrate opposite compartmentalization of IL-17+ cells and Tregs in the TME, and identify neutrophils and mast cells as major sources of IL-17 in HCC with distinct localization. Analysis of the clinical significance of these findings is ongoing.
Disclosure: Nothing to disclose.
THE KINASE ACTIVITY OF FOCAL ADHESION KINASE IS REQUIRED FOR PLASMA MEMBRANE TARGETING OF TGFβ RECEPTOR II AND TGFβ ACTIVATION OF HEPATIC STELLATE CELLS

Authors: Y. Chen; K. Tu; Y. Wang; N. Kang; C. Chen

Institution(s): University of Minnesota; Mayo Clinic Florida; Jiaotong University; Beth Israel Deaconess Medical Center/Harvard Medical School

Background: TGFβ induces hepatic stellate cells (HSCs) differentiate into tumor-promoting myofibroblasts. However, mechanisms underlying TGFβ-induced HSC activation remain incompletely understood. This study investigated how focal adhesion kinase (FAK) regulates TGFβ receptor trafficking and TGFβ-mediated signaling in HSCs.

Methods: Primary human HSCs cells were used. Myofibroblastic activation of HSCs was assessed by immunofluorescence (IF) and immunoblotting for alpha-smooth muscle actin. FAK was inactivated by a specific inhibitor, PF228, or a FAKY397F mutant. Protein subcellular localization was studied by IF. MTS Proliferation Assay and a HSC/tumor coimplantation mouse model were used to analyze the paracrine tumor-promoting effects of HSCs.

Results: Inhibition of FAK kinase activity by PF228 reduced TGFβ receptor II (TβRII) protein level of HSCs and TGFβ-induced activation of HSCs into myofibroblasts in vitro (P<0.05). Consistently, as compared to overexpression of wildtype FAK, overexpression of FAKY397F mutant by retroviral transduction of HSCs also reduced TβRII and the TGFβ-induced HSC activation process (P<0.05). Additionally, a lysosomal inhibitor, bafilomycin or chloroquine, abolished the suppressive effect of PF228 and FAKY397F on TβRII. IF demonstrated that TβRII was targeted to the peripheral plasma membrane by wildtype FAK but not by FAKY397F mutant and that TβRII colocalized with P-FAK at the peripheral plasma membrane. MTS Proliferation Assay revealed that conditioned medium of HSCs expressing FAKY397F mutant was less effective on promoting HT29 colorectal cancer cell proliferation in vitro, as compared to that of HSCs expressing wildtype FAK. Moreover, FAK knockdown HSCs were less effective than control HSCs on promoting HT29 growth in a HSC/HT29 coinjection mouse model.

Conclusion: The kinase activity of FAK stabilizes TβRII by targeting TβRII to the plasma membrane so as to facilitate TGFβ-induced activation of HSCs into tumor-promoting myofibroblasts. FAK represents a target for suppressing HSC activation and the prometastatic liver microenvironment.

Disclosure: Nothing to disclose.
MANNOSE PHOSPHATE ISOMERASE AND MANNOSE REGULATE HEPATIC STELLATE CELL ACTIVATION AND FIBROSIS IN ZEBRAFISH AND HUMANS

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Institution(s): Icahn School of Medicine at Mount Sinai1; Cleveland Clinic2

Background: The growing burden of liver fibrosis and lack of effective antifibrotic therapies highlight the need for identification of novel pathways and complementary systems to model the initiation and progression of hepatic fibrosis. Mutations in mannose phosphate isomerase (MPI), a mannose metabolizing enzyme, lead to a rare disorder in children (MPI-CDG) with characteristic early and progressive liver fibrosis. This association between mannose dysregulation and fibrosis led us to explore the broader involvement of MPI and mannose metabolism in liver development and adult liver diseases.

Methods: We performed in silico analysis on publicly available gene expression datasets from three liver disease cohorts to examine MPI gene expression in human samples of progressive liver fibrosis. Next, we characterized liver pathologies found in zebrafish models for Mpi deficiency that we had previously developed using morpholino oligonucleotides and TALEN-generated mutations. We used qPCR to analyze fibrotic gene expression in livers dissected from larvae with deficient Mpi, without or with mannose supplements. We extended the fibrotic gene expression analysis following MPI depletion and mannose supplementation in human hepatic stellate cells (HSC) in culture (LX-2 and TWNT-4) using qPCR, and identified potential pathway involvement through proteomics analysis using iTRAQ stable isotope tag labeling and LC-MS/MS separation and detection.

Results: Mutation of MPI leads to MPI-CDG and early liver fibrosis in children. In three adult human liver disease cohorts (including hepatitis B infection and non-alcoholic fatty liver disease), MPI gene expression was downregulated proportionate to fibrosis severity based on transcriptomic profiling and staging with Scheuer (HBV) or METAVIR (NAFLD) staging systems to score severity of fibrosis. Gene set enrichment analysis in samples with the lowest MPI expression revealed an increased expression of genes that cluster in focal- and cell-adhesion pathways. Separately, depletion of MPI amplified fibrotic gene expression in both zebrafish livers in vivo and cultured human HSC lines, indicating that loss of MPI promotes HSC activation. Importantly, mannose supplementation attenuated HSC activation and reduced fibrogenic gene expression in mpi morphant zebrafish, culture-activated HSCs, and in ethanol-activated HSCs. Proteomics analysis from mannose-treated HSC revealed decreased phosphoproteins that cluster in pathways involved with cell-cell adhesion.

Conclusion: These data introduce the novel prospect that modulation of mannose metabolism pathways could reduce HSC activation and improve hepatic fibrosis, and may be linked to cell-cell adhesion.
Disclosure: Nothing to disclose.
HEPATIC CD4+FOXp3+ TREGS ARE DYNAMICALLY RESPONSIVE TO CHRONIC CCl4-INDUCED LIVER FIBROSIS

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Institution(s): Cleveland Clinic1; Cleveland Clinic Foundation2

Background: Wound healing is a naturally occurring physiologic response; however, upon excessive exposure to toxicants (chronic alcohol abuse, CCl4) or persistent injury (viral infections, nonalcoholic steatohepatitis), fibrosis occurs. During fibrosis, hepatic stellate cells (HSCs) become activated and deposit extracellular matrix, hepatocytes are damaged, and hepatic nonparenchymal cells (NPCs) promote tissue injury. Both the progression and resolution of fibrosis depend on the interactions of NPCs, including HSCs, resident leukocytes, and infiltrating leukocytes. Regulatory T cells (Tregs) are key contributors to the resolution phase of inflammation and maintain immunological tolerance via secretion of a myriad of immunosuppressive and pro-resolving cytokines, including IL-10 and TGF-b. Here, we evaluated the dynamic regulation of inflammation and resolution in liver following chronic CCl4-induced liver fibrosis.

Methods: Wild-type C57BL/6J female mice were treated twice weekly with an injection of carbon tetrachloride (CCl4) for five weeks; control mice received olive oil (OO) (J Leuk Biology 2015 97(1):161-9). Final doses were timed so that mice were euthanized 18, 24, 48, or 72 h post-injection so that dynamic responses to the final challenge with CCl4 could be assessed.

Results: CCl4-treated mice displayed elevated indices of hepatocyte injury, including plasma alanine and aspartate aminotransferase (ALT, AST) activities; peak injury occurred 18 and 24hrs post-CCl4 injection, which almost resolved by 72hrs. Markers of liver fibrosis were elevated in CCl4-treated animals, with bridging deposition of collagen 1a1 (COL1A1) at 24hrs. Expression of pro-fibrotic genes alpha-smooth muscle actin (a-SMA) and COL1A1 were increased at early time points (18 and 24hrs), which was maintained at 72hrs. Similarly, CCl4 treatment increased the expression of pro-inflammatory mediators TNFa, MCP-1, and IL-6 in liver, with peak elevations at 18 and 24hrs. FACS analysis of liver NPCs revealed infiltration of CD11c+ dendritic cells, Ly6G+CD11b+ neutrophils and CD11b+Ly6Chi inflammatory scar-associated macrophages (SAM) at 24hrs following CCl4 treatment. Interestingly, CD11b+Ly6Clow tissue restorative SAMs persisted throughout the recovery period. The mRNA expression of pro-resolution mediators TGF-b and IL-10 robustly increased at 18 and 24hr, decreased at 48hr, and moderately increased at 72hrs. Liver CD4+FoxP3+Tregs displayed a biphasic response after CCl4 injection, with a modest increase at 18hrs, a decrease at 24hrs, a modest increase at 48hrs and a dramatic increase at 72hrs. The expression of FoxP3 mRNA in liver also followed this biphasic response. Interestingly, circulating CD4+FoxP3+Tregs increased at 18hrs, decreased at 24hrs, increased at 48hrs, and ultimately decreased again 72hrs post-CCl4.
**Conclusion:** These data demonstrate the dynamic response of the liver to the final challenge with CCl4 after chronic CCl4 exposure. Even after chronic injury, this additional insult led to leukocyte infiltration to the liver and induction of fibrogenic responses within 18-24hrs. By 48-72hrs after the final challenge, resolution of inflammation and extracellular matrix remodeling transpires. Here, we demonstrate that hepatic CD4+FoxP3+Tregs, major pro-resolution effector T cells, are also dynamically regulated, with increased recruitment to the liver during the wound healing/resolution phase of the response, likely serving to promote tissue repair following CCl4-induced liver fibrosis.

**Disclosure:** Nothing to disclose.
SINGLE-CENTRE, DOSE ESCALATION, OPEN-LABEL PHASE 1 TRIAL OF ANTI-FIBROTIC SMALL MOLECULE PRI-724 IN PATIENTS WITH HEPATITIS C VIRUS RELATED CIRRHOSIS

Authors: K. Kimura¹,²,³; K. Nishikawa¹; S. Shimoda²; T. Kanto³; M. Mizokami³

Institution(s): Tokyo Metropolitan Cancer and Infectious Diseases Center¹; Kyushu University²; National Center for Global Health and Medicine³

Background: Direct acting antivirals for hepatitis C virus (HCV) have proven very successful in many patients. However, a cure in advanced HCV cirrhosis is not adequate and anti-fibrotic drug therapy is not currently available. PRI-724 is a small molecule modulator of Wnt signaling that inhibits the cAMP response element binding protein (CREB) and β-catenin interaction. In pre-clinical models, PRI-724 inhibits activation of hepatic stellate cells and migrated CD11b+Ly-6C+ macrophages into the liver, thereby suppressing carbon tetrachloride and HCV transgenic mice mediated liver fibrosis.

Methods: In this single-centre, open-label, phase 1 trial, patients with HCV cirrhosis established by liver biopsy (Histology Activity Index [HAI] score: Grade IV-D) and Child-Pugh Class A or B at the time of informed consent were sequentially enrolled. PRI-724 was given as a continuous intravenous infusion 10, 40, 160 mg/m², 7 days with 7 days rest per one cycle. Patients were treated for 6 cycles. The primary safety endpoint was the frequency and severity of adverse events. The secondary endpoint was efficacy of PRI-724 for cirrhosis based on Child-Pugh score and liver biopsy (ClinicalTrials.gov, number NCT02195440)

Results: Between Sept 3, 2014, and May 24, 2016, 14 patients (Child-Pugh Class A/B; 6/8) were enrolled. median age: 62 (43-74) years; 9 (64%) males; 12 of 14 patients completed 6 cycles of treatment and one patient was withdrawn from the study due to other drug (cefaclor) induced liver injury (Grade 3), but was included in the intention-to-treat analysis. Safety: No patients discontinued treatment because of adverse events and grade 3 adverse events occurred with one patient that were related to study medication. Efficacy: Of the 7 Child-Pugh Class B enrolled patients 4 converted to Class A after conclusion of treatment. No Child-Pugh Class A patients worsened during treatment. PRI-724 treatment was associated with improved liver histology (HAI score) in 8 of the 12 patients in the first two treatment cohorts for whom biopsy results were available. Biopsy analysis showed that PRI-724 treatment reduced fibrosis area in the hepatic lobule in a dose dependent manner.

Conclusion: This is the report of PRI-724, a modulator of Wnt signaling for patients with HCV cirrhosis. Cyclic therapy with intravenous PRI-724 over 12 weeks was well tolerated by patients with HCV cirrhosis and resulted in improvement of liver histology and Child-Pugh score in a substantial proportion of patients.
Disclosure: Nothing to disclose.
MAST CELL REGULATION OF NAFLD/NASH INDUCED LIVER DAMAGE AND FIBROSIS

Authors: L. Kennedy¹; L. Hargrove¹; H. Francis¹

Institution(s): Texas A & M Health Science Center¹

Background: Consumption of a western diet causes obesity and results in non-alcoholic fatty liver disease. Mice fed a high fat diet (HFD) have increased biliary damage and fibrosis; however, these parameters are decreased in l-histidine decarboxylase knockout mice fed HFD. Mast cells (MCs) (i) infiltrate the liver and secrete histamine (HA) increasing biliary damage and liver fibrosis and (ii) are present in white adipose tissue of diabetic obese patients. Our aim was to evaluate the role of MCs on biliary damage, inflammation, steatosis and liver fibrosis during HFD feeding.

Methods: WT (c57) and Kit W-sh (MC-deficient) mice were fed a control or high fat, trans-fat diet (45% of calories from fat) coupled with a high fructose corn syrup equivalent for 16 wks. Animals were weighed weekly and food/water intake measured. Hepatocyte ballooning, liver damage and steatosis were determined by H&E and Oil Red O. Intrahepatic biliary mass (IBDM) and biliary proliferation were evaluated by immunohistochemistry for CK-19 and Ki67, respectively. Liver fibrosis was determined by qPCR for fibronectin-1, a-SMA and TGF-b1 and semiquantification of Sirius Red staining. MC infiltration was measured in liver by immunohistochemistry for mouse mast cell protease-1 and by qPCR for c-kit, chymase and tryptase. CCL3, CCL4 and CCL5 were evaluated by qPCR in isolated cholangiocytes. Serum HA and tryptase levels were measured by EIA. In vitro, cultured cholangiocytes were treated with MC conditioned medium and CCL4 and CCL5 measured by qPCR. Cholangiocytes were treated with oleate (OA), stearate (SA) or palmitate (PA) and MC migration was measured using Boyden chambers. Toluidine blue staining was performed to count migrating MCs.

Results: Liver weight was significantly reduced in Kit W-sh mice fed HFD compared to WT HFD. Steatosis and lipid droplet size/number increased in WT HFD mice and these parameters decreased in Kit W-sh mice fed HFD. IBDM and cholangiocyte proliferation increased in WT HFD mice, whereas HFD Kit W-sh mice had reduced IBDM and cholangiocyte proliferation. Kit W-sh HFD mice had reduced liver fibrosis compared to WT HFD mice. MC infiltration increased in WT HFD mice, whereas Kit W-sh mice fed HFD or control diet had no visible MCs. WT HFD mice had increased chemokine expression and liver inflammation, both of which were decreased in HFD Kit W-sh mice. Serum HA and tryptase levels were increased in WT HFD mice compared to control and reduced in both control and HFD Kit W-sh mice. In vitro, biliary CCL4 and CCL5 expression increased with MC treatment. MC migration was increased when cholangiocytes were treated with PA, SA or OA versus control.
Conclusion: Damaged cholangiocytes induce MCs migration into the liver during HFD feeding and contribute to liver inflammation, steatosis, biliary proliferation and liver fibrosis via upregulated chemokine signaling.

Disclosure: Nothing to disclose.
SEX-SPECIFIC ROLE OF PKLR IN NON-ALCOHOLIC FATTY LIVER DISEASE

Authors: K. Chella Krishnan¹; S. Sabir¹; D. Jayasekera¹; M. Shum²; R. Floyd¹; S. Nand¹; L. Stiles¹; A. Lusis¹

Institution(s): University of California, Los Angeles¹; Simon Fraser University²

Background: Non-alcoholic fatty liver disease (NAFLD) is increasingly recognized as the hepatic manifestation of the metabolic syndrome. NAFLD is not a single disease and ranges from simple steatosis to non-alcoholic steatohepatitis (NASH), fibrosis and cirrhosis. An important feature of NAFLD is their differential prevalence and disease phenotypes between males and females. Besides, NAFLD is associated with both obesity and insulin resistance and its prevalence has increased multifold over the past few decades. However, the etiology of the disease is not completely understood and there are no available prevention and/or treatments for NAFLD. To address this problem, we used multi-omics data, such as genotypes, transcriptomes and phenotypes, collected from an extensively phenotyped mouse reference population and identified several potential candidate genes driving NAFLD. We followed up on one of these candidates, namely pyruvate kinase, liver isoform (PKLR). Interestingly, our multi-omics analyses also demonstrated that PKLR might have sex-specific effects in developing NAFLD.

Methods: To further understand the role of PKLR in developing NAFLD, we used liver-specific loss-of-function and gain-of-function mouse models using recombinant replication-defective adeno-associated virus (AAV) under thyroxine binding globulin (Tbg) promoter. Following the treatment, animals were fed a high fat/high sucrose diet for 16-weeks. We measured phenotypes such as body composition, glucose-, insulin- and pyruvate/lactate- tolerance tests, plasma and liver lipid analyses, and fasting glucose and insulin measurements, whole-body energy homeostasis by indirect calorimetry. Liver tissues were used to isolate RNA for gene expression analyses and mitochondria for bioenergetics.

Results: We observed that knockdown of PKLR did not affect body weight composition. However, these animals had improved glucose tolerance, insulin sensitivity and pyruvate/lactate tolerance compared to controls. Further, these animals had reduced liver triglyceride accumulation and reduced fasting glucose and insulin levels as well as HOMA-IR measurements. Follow-up bioenergetics studies demonstrated that down regulation of PKLR lowered mitochondrial respiration. Our gain-of-function studies demonstrated the converse effect, i.e. augmented disease phenotypes. In contrast, when we overexpressed PKLR in female mice, we observed no changes. Doing pathway enrichment analysis using transcriptomic data from our mouse reference population for possible mechanism(s), we found that mitochondrial associated gene sets were highly enriched with liver Pklr expression between males and females.

Conclusion: We report that PKLR has a sex-specific role in developing NAFLD possibly through differentially affecting mitochondrial function.
References

Disclosure: Nothing to disclose.
IRE1Α ACTIVATION OF C/EBPB IS CRITICAL FOR HSC ACTIVATION AND IS ANTAGONIZED BY ADEFOVIR DIVIPOXIL

Authors: J. Maiers¹; Z. Liu¹; C. Li²; N. Kang¹; H. Malhi¹; V. Shah¹

Institution(s): Mayo Clinic Rochester¹; VCU School of Medicine²

Background: A key characteristic of Hepatic Stellate Cell (HSC) activation is increased expression of extracellular matrix (ECM) proteins and fibrogenesis. Increased ECM production and secretion is associated with ER stress and the Unfolded Protein Response (UPR). The mechanisms of UPR signaling, its role and regulation during HSC activation, and therapeutic implications, are unclear, and were the focus of this study.

Methods: For in vivo experiments, age and sex-matched C57Bl/6 mice received CCl₄ or vehicle twice a week for 6 weeks, along with daily IP injections of 10μM ADV or vehicle. Fibrosis was assessed by Sirius Red staining, immunoblotting, qPCR, and immunofluorescence of whole liver. In vitro experiments were conducted with LX-2 cells (immortalized HSCs) or primary rat HSCs, and HSC activation by TGFβ was assessed by immunoblotting and qPCR.

Results: Activation of LX-2 cells by TGFβ induced phosphorylation of the UPR sensor Inositol-requiring enzyme 1α (IRE1α) in a SMAD2/3 or procollagen I-dependent manner (p<0.01). Mutational analysis of IRE1α showed that IRE1α kinase activity was critical for HSC activation, through a signaling cascade involving Apoptosis Signaling Kinase 1 (ASK1) and c-Jun N-terminal Kinase (JNK) (p<0.05). IRE1α signaling through ASK1-JNK led to phosphorylation of the UPR-associated transcription factor CCAAT/enhancer binding protein β (c/EBPβ), a protein we identify as critical for TGFβ- or IRE1α-mediated HSC activation (p<0.05-0.01). Furthermore, pharmacological inhibition of c/EBPβ expression through the antiviral drug Adefovir Divipoxil inhibited TGFβ-stimulated HSC activation in vitro and fibrogenesis in vivo (p<0.05-0.01). Finally, we identified a critical relationship between c/EBPβ and the transcriptional regulator p300 during HSC activation. p300 knockdown disrupted TGFβ or UPR-induced HSC activation, and pharmacological inhibition of c/EBPβ binding to p300 decreased TGFβ-induced HSC activation (p<0.05-0.01).

Conclusion: These data indicate a novel mechanism whereby IRE1α signaling is critical for HSC activation in a c/EBPβ-p300 dependent mechanism, and identifies c/EBPβ as a novel pharmacological target for hepatic fibrosis.

Disclosure: Nothing to disclose.
SYSTEM GENETIC ANALYSIS OF HEPATIC FIBROSIS IN HYBRID MOUSE DIVERSITY PANEL IDENTIFIES MATRIX GLA PROTEIN AS A CAUSAL GENE FOR NON-ALCOHOLIC STEATOHEPATITIS

Authors:  S. Hui; D. Dirks; C. Pan; I. Tuominen; S. Beaven; K. Bostrom; A. Lusis

Institution(s): University of California at Los Angeles

Background: Non-alcoholic fatty liver disease (NAFLD) comprises of a spectrum of hepatic abnormalities ranging from simple steatosis and steatohepatitis (NASH) to fibrosis and cirrhosis. Despite significant research efforts, the etiology of this disease is poorly understood; in particular, factors governing the progression from bland steatosis to NASH and fibrosis are largely unknown. The goal of this study is to use a system genetics approach, integrating transcriptomics and phenotypic data in mice, to identify genetic factors contributing to the pathogenesis of NASH.

Methods: We have developed a novel mouse model for NASH and fibrosis using transgenic mice expressing human APOE*3-Leiden and human CETP (cholesteryl ester transfer protein). These mice exhibit a “humanized” lipoprotein profile and display the entire spectrum of NAFLD when fed a “Western diet” high in fats and cholesterol. We introduced the transgenes onto a panel of over 100 different inbred strains of mice known as the Hybrid Mouse Diversity Panel (HMDP). Each strain of mice was fed a “Western diet” for 16 weeks. Liver fibrosis were quantified by picrosirus red staining using an automated computer algorithm. Hepatic gene expression was determined by microarray. Correlation analysis on gene expression and hepatic fibrosis was performed to identify genes associated with hepatic fibrosis and related traits.

Results: We observed vast differences in hepatic fibrosis that was highly dependent on genetic background, indicating a strong genetic component. We found that the genetic variation predisposing to NASH and fibrosis differs markedly from that predisposing to simple steatosis. Among the most highly correlated genes with fibrosis, matrix gla protein (Mgp) was identified as a candidate gene for hepatic fibrosis based on additional evidence from expression quantitative trait loci (eQTL) analysis. We validated Mgp as a causal gene for hepatic inflammation and fibrosis by knockout mouse and cultured cell studies. When fed a low-methionine and choline-deficient high-fat diet, heterozygous whole body Mgp knockout mice displayed a dramatic reduction in hepatic inflammation and fibrotic gene expression, despite having a similar degree of steatosis and liver injury as wild-type mice. Single-cell sequencing identified Mgp had the highest expression level in stellate cells among all cell types in the liver, suggesting a functional role in stellate cells. Knockdown of Mgp by siRNA in cultured LX-2 stellate cells resulted in blunted TGF-β induced cell activation and fibrotic program, suggesting Mgp may play a role in stellate cell activation.
**Conclusion:** Our data suggested that Mgp plays a causal role in NASH pathogenesis and stellate cell activation which may represent a potential pharmacological target for intervening NASH.

**Disclosure:** Nothing to disclose.
ACTIVATION OF THE CELLULAR PROTEOSTASIS REGULATOR HEAT SHOCK FACTOR 1 ALLEVIATES HEPATIC STELLATE CELL ACTIVATION

Authors: A. Choudhury¹, A. Lim¹, R.M. Sebastian², C. L. Moore², M. Shoulders², P. Mandrekar¹

Institutions: Department of Medicine, University of Massachusetts Medical School¹; Department of Chemistry, Massachusetts Institute of Technology²

Background/Aims: Liver fibrosis is a wound healing response to hepatocellular injury and represents a significant morbidity and mortality. Various studies demonstrate that oxidative stress mediate the progression of fibrosis. Chronic exposure to cellular stressors increases accumulation of oxidized and misfolded proteins, activating the cellular proteostasis machinery. Heat shock factor 1 (HSF1) is a cellular sensor that gets activated in response to oxidative stress. Previously we demonstrated that absence of HSF1 exacerbates liver fibrosis. Here we hypothesized that activating the cellular stress response can effectively decrease the hepatic stellate cell (HSC) activation in response to pro-fibrogenic mediators.

Methods: Liver fibrosis was induced in mice by the intoxication of carbon tetrachloride (CCl₄) for 6 weeks. Activation of HSF1 was analyzed in human and murine fibrotic liver tissues by IHCs, EMSA, and mRNA expression of downstream genes regulated by HSF1, such as heat shock protein (HSP) 40, HSPA1A, and HSP90AA1. Activation of HSF1 was also evaluated in the primary HSC and LX-2 cells stimulated with the transforming growth factor beta (TGFβ). Activation of HSF1 was induced in the LX-2 cells either by heat shock, pharmacologically by celastrol, or through chemical genetic techniques by stably expressing a constitutively active form of HSF1 (cHSF1). These cells were then stimulated with TGFβ and the induction of pro-fibrogenic genes was evaluated.

Results: Human cirrhotic and mouse fibrotic livers demonstrated elevated activation of HSF1 as evaluated by the increased mRNA expression of HSF1, HSP40, HSPA1A, and HSP90AA1. Activation of HSF1 was further confirmed by the nuclear localization of HSF1 in the human cirrhotic livers. On the contrary, LX-2 cells stimulated with TGFβ failed to demonstrate nuclear binding activity of HSF1. In addition, primary HSCs and LX-2 cells stimulated with TGFβ did not demonstrate any induction of HSP40, HSPA1A, or HSP90AA1, confirming non-functional HSF1 in the activated HSCs. In an attempt to test whether activation of HSF1 could rescue the phenotype, we activated HSF1 either by heat shock or celastrol. Stimulation of these cells with TGFβ significantly decreased the expression of pro-fibrogenic genes αSMA and COL1A1. In order to surpass the pleiotropic effects beyond just HSF1 activation that may be mediated by heat shock and celastrol, we engineered LX-2 cells to stably express cHSF1 fused to a ligand-regulated, FKBP (FKBP.cHSF1.LX-2 cells). Addition of Shield-1, to these cells resulted in transcriptional upregulation of HSF1 target genes, confirming stress-independent, small molecule-regulated activation of HSF1. FKBP.cHSF.LX-2 cells stimulated with Shield-1 and TGFβ significantly attenuated the expression of pro-fibrogenic.
**Conclusion**: We conclude that the activation of HSF1 is elevated in fibrotic and cirrhotic livers, probably as an adaptive response to combat cellular stress mediated by pro-fibrogenic stimuli. However, this response is impaired in the stellate cells. Activation of HSF1 in LX-2 cells attenuates the response to TGFβ, suggesting its protective role in HSC activation.
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