

# The Hepatic Sinusoid in Aging and Disease: Update and Advances From the 20th Liver Sinusoid Meeting

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This is a meeting report of the 2019 Liver Sinusoid Meeting, 20th International Symposium on Cells of the Hepatic Sinusoid, held in Sydney, Australia, in September 2019. The meeting, which was organized by the International Society for Hepatic Sinusoidal Research, provided an update on the recent advances in the field of hepatic sinusoid cells in relation to cell biology, aging, and liver disease, with particular focus on the molecular and cellular targets involved in hepatic fibrosis, nonalcoholic hepatic steatohepatitis, alcoholic liver disease, hepatocellular carcinoma, and cirrhosis. In addition, the meeting highlighted the recent advances in regenerative medicine, targeted nanotechnologies, therapeutics, and novel methodologies. (*Hepatology Communications* 2020;4:1087-1098).

The theme of the 2019 Liver Sinusoid Meeting (formerly known as the International Symposium on Cells of the Hepatic Sinusoid) was the role of the sinusoidal cells in disease and aging. This meeting discussed the advances in our understanding of the basic biology and pathobiology of sinusoidal cells: the liver sinusoidal endothelial cells (LSECs), hepatic stellate cells (HSCs), and Kupffer cells (KCs). Here, we report recent advances in their cell biology and how they contribute to the development of multiple liver diseases, including hepatic fibrosis, nonalcoholic steatohepatitis (NASH), alcoholic liver disease (ALD), hepatocellular carcinoma

(HCC), and cirrhosis. One of the key messages from this conference is that these conditions may be targeted with novel therapeutic agents. The advances in liver regeneration and aging along with the development of innovative nanomedicines to target the liver cell types were also covered.

The symposium also honored the passing of one of our esteemed colleagues, Professor Soichi Kojima, from the RIKEN Center for Integrative Medical Sciences in Wako, Japan. His significant contributions to the study of the liver were remembered in a commemorative address by Professor Norifumi Kawada. The 20th Liver Sinusoid Meeting was cosponsored

*Abbreviations: ALD, alcoholic liver disease; BCAA, branched-chain amino acid; CD, cluster of differentiation; DAMP, damage-associated molecular pattern; DMNA, dimethylnitrosamine; FFA, free fatty acid; GLMP, glycosylated lysosomal membrane protein; HCC, hepatocellular carcinoma; Hh, Hedgehog; HMGB-1, high-mobility group box 1; HSC, hepatic stellate cell; IFC-305, adenosine-derivative compound; IL, interleukin; ISHSR, International Society for Hepatic Sinusoidal Research; IVM, intravital fluorescence microscopy; KC, Kupffer cell; LSEC, liver sinusoidal endothelial cell; MoDM, monocyte-derived macrophage; NASH, nonalcoholic steatohepatitis; NOSTRIN, nitric oxide synthase trafficking inducer; NOX, nicotinamide adenine dinucleotide phosphate oxidase; NR4A1, nuclear receptor subfamily 4, group A, member 1; OGFRL1, opioid growth factor receptor like-1; p52Shc, 52 kDa isoform Src homology 2 domain containing; pHx, partial hepatectomy; PS-ASO, phosphorothioate antisense oligonucleotide; QD, quantum dot; RUNX1, runt-related transcription factor 1; SMAD, mothers against decapentaplegic homolog; Sox9, sex determining region Y-box 9; TAA, thioacetamide; TAZ, transcriptional coactivator with PDZ-binding motif; TGF- $\beta$ , transforming growth factor  $\beta$ ; VEGF, vascular endothelial growth factor.*

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## LSECs

LSECs are unique among other endothelial cells in the body because they are characterized by transcellular pores known as *fenestrae* and the absence of a basement membrane.<sup>(1,2)</sup> These pores allow bidirectional transfer of material between blood, HSCs, and hepatocytes. Functionally, the LSECs are antigen-presenting cells with roles in immune recognition, scavenging of waste-borne molecules by endocytosis, and regulation of endothelial homeostasis by secretory materials, such as NO.<sup>(1)</sup> Stabilin-1 and stabilin-2 play a major role as waste scavenger receptors.<sup>(3,4)</sup> Edward Harris (University of Nebraska, Lincoln, NE) revealed a new role of stabilins as phosphorothioate antisense oligonucleotide (PS-ASO) scavengers.<sup>(5)</sup> He demonstrated that injection of radiolabeled PS-ASOs in mice led to their accumulation in the liver, particularly within LSECs, through clathrin-dependent endocytosis.

It has been shown that the mannose receptor scavenging capacity is independent of the base macromolecule but requires a glycosylated side chain.<sup>(1)</sup> Karen Sørensen (Arctic University of Tromsø, Tromsø, Norway) demonstrated that LSECs have a significant reduction in scavenging of fluorescein isothiocyanate (FITC)-bovine serum albumin in a glycosylated lysosomal membrane protein (GLMP) knockout

transgenic mouse model. This reduced scavenging was associated with increased liver collagen deposition. However, Glmp<sup>gt/gt</sup>(goldenticket) mice maintained the fenestrated cell surface, suggesting that maintenance of scavenger mechanisms and regulation of fenestrations are independently controlled.

Further examination of the mechanisms involved in endocytosis and fenestration formation were evaluated by multiple groups in response to treatments with branched-chain amino acids (BCAAs), free fatty acids (FFAs), glucocorticoids, and acetaminophen, using *in vitro* and *in vivo* models. BCAA dietary imbalance leads to obesity and reduced life expectancy in mice.<sup>(6)</sup> Sun Woo Kang (University of Sydney, Sydney, Australia) demonstrated that a 2-fold BCAA dietary imbalance reduced the frequency of fenestrations but not their porosity or diameter. This effect was inhibited by caloric restriction. Work by Karolina Szafranska (Arctic University of Tromsø) supported the detrimental effect of unbalanced BCAAs on LSEC morphology, showing reduced fenestration porosity and increased scavenging capacity. This group also presented on the ability of sildenafil and its analogues to increase porosity (confirming previous data<sup>(7,8)</sup>), without any alteration in endocytic capacity. Building on previous work demonstrating the toxic effects of palmitic acid (a FFA) on hepatic toxicity,<sup>(9)</sup> Yana Geng (University of Groningen, Groningen, the Netherlands) presented data on the toxicity of oleate and palmitate on *in vitro* rat LSECs. Each treatment alone induced necrosis; however, combination treatment did not induce cell death and led to increased lipid droplet formation.

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It was hypothesized that lipid droplet formation is a protective mechanism that guards against FFA-induced toxicity. Sabin Bhandari (Arctic University of Tromsø) shared some data on the *in vitro* effects of glucocorticoids in LSECs. Glucocorticoids are known to promote endothelial dysfunction, induce hepatic stenosis, and lead to insulin resistance in rats.<sup>(10,11)</sup> Proteomic analyses of LSECs after glucocorticoid treatment demonstrated down-regulation of major energy metabolism pathways and increased expression of proinflammatory cytokines and components of redox systems 24 hours after treatment. Finally, Ingelin Kyrrestad (Arctic University of Tromsø) followed up published work on the negative impacts of high-dose acetaminophen<sup>(12)</sup> on *in vitro*-isolated LSECs. It was reported that high-dose acetaminophen treatment induces rapid dose-dependent impairment of LSEC redox potential, loss of endocytic capacity, and defenestration.

## HSCs and Inflammation

HSCs are liver-residing pericytes involved in vitamin A storage and the regulation of extracellular matrix metabolism.<sup>(7)</sup> Following insult, stimulation, or activation, HSCs are the main fibrogenic cells of the liver and contribute extensively to hepatic fibrosis.<sup>(13,14)</sup>

Grant Ramm (Queensland Institute of Medical Research, Berghofer Institute, Brisbane, Australia) gave a keynote address describing the recently identified role of heavy-chain ferritin as a novel damage-associated molecular pattern (DAMP) that activates nucleotide-binding oligomerization domain-like receptor family pyrin domain-containing 3 (NLRP3) inflammasome by intercellular adhesion molecule 1, further driving HSC-derived interleukin (IL)-1 $\beta$  secretion.<sup>(15)</sup> Moreover, biomarker studies designed for the early detection of liver disease and staging of hepatic fibrosis revealed microRNA 25 (miRNA-25) as an antifibrotic “brake” on HSC collagen synthesis through the inhibition of regulators of Notch signaling/span and suppression of transforming growth factor beta (TGF- $\beta$ )-induced collagen synthesis.<sup>(16)</sup>

Novel data on the initiation step of HSC activation following loss of adherent junctions between HSCs and hepatocytes were presented by Hayato Urushima (Osaka City University, Osaka, Japan). Using immune-electron microscopy of an acute liver injury model (single CCl<sub>4</sub>

injection), this group demonstrated that E-cadherin is a key adhesion molecule between HSCs and hepatocytes. Loss of these adherent junctions in the early stages of inflammation was followed by HSC activation and enhancement of the Yes-associated protein/transcriptional coactivator with PDZ-binding motif (TAZ) expression. *In vitro* overexpression of TAZ in human HSCs up-regulated activation, whereas reconstruction of E-cadherin-mediated adherent junctions led to TAZ suppression and HSC quiescence. This work adds to published work by this group in understanding the role of senescence HSCs in liver disease.<sup>(17)</sup>

In-depth characterization of the HSC activation pathway TGF- $\beta$ 1/mothers against decapentaplegic homolog (SMAD) was performed by Misako Sato-Matsubara (Osaka City University). Following previous studies on the role of cytoglobin in attenuating liver fibrosis in thioacetamide (TAA) mice,<sup>(18)</sup> Dr. Sato-Matsubara described the role of TGF- $\beta$  in reactive oxygen species-induced DNA damage and demonstrated that TGF- $\beta$ 1 reduced cytoglobin, a free radical scavenger expressed uniquely in HSCs within the liver.<sup>(19)</sup> Further work showed that TGF- $\beta$ 1/SMAD2 reduced cytoglobin in HSCs from patients with NASH through recruitment of Sp3 transcription factor (SP3) and increasing alpha smooth muscle actin ( $\alpha$ SMA) expression through SMAD3. Following from this, Le Thi Thanh Thuy (Osaka City University) highlighted the effects of overexpression and knockout of cytoglobin. Within models of bile duct ligation, TAA-induced fibrosis, and diethylnitrosamine-induced liver cancer, it was shown that cytoglobin regulates HSC activation and suppresses inflammation in liver fibrosis and cancer.<sup>(18)</sup>

The key role that inflammatory pathways play in the development of liver diseases was highlighted by Frank Tacke (Charité Medical University, Berlin, Germany). In a recent review,<sup>(20)</sup> this group demonstrated the importance of KCs and monocyte-derived macrophages (MoDMs) in health and disease. Analysis of single-cell RNA sequencing showed that the old dogma of M1 and M2 macrophages should be reconsidered in favor of a spectrum of activation states/functions.<sup>(20)</sup> This approach identified MoDMs as interesting targets for novel therapeutic approaches to immunomodulate the progression of liver disease.<sup>(20)</sup> In addition, Dr. Tacke's group has developed expertise in intravital fluorescence microscopy (IVFM) for tracking, observing, and understanding

the role of macrophages in liver disease.<sup>(21)</sup> Finally, he demonstrated that within progressively worsening liver disease, KCs and MoDMs will respond to different metabolic cues than in nondiseased states and may be critical targets for attenuating the progression of nonalcoholic fatty liver disease (NAFLD) and reducing cardiovascular risk.<sup>(16)</sup>

## Liver Diseases

### FIBROSIS

Stiffening of the liver was originally understood to occur as the result of HSC activation, extracellular matrix deposition, and modulation of the liver angi-architecture during fibrosis progression. This topic was a focus of research for several groups attending the meeting, which led to in-depth characterization and discussion of the effect of matrix stiffness on the modulation of sinusoidal cell phenotypes during the initial stages of liver disease.<sup>(22)</sup> Sergi Guixé-Muntet (Inselspital, Bern, Switzerland) reported the effect of stiffness on healthy primary cells by using collagen-coated tuned polyacrylamide gels with physiologically relevant stiffness (0.5 kPa as healthy, 13.5 kPa as fibrotic, and 30 kPa as cirrhotic). High stiffness induced cell dysfunction in all cell types. Hepatocytes demonstrated reduced albumin and urea synthesis, HSCs become activated with increased  $\alpha$ SMA and collagen deposition, and LSECs were capillarized (loss of fenestrated surface) with reduced NO production. Martí Ortega-Ribera (Institut d'Investigacions Biomèdiques August Pi i Sunyer [IDIBAPS], Barcelona, Spain) highlighted the role of stiffness on primary cells isolated from cirrhotic livers and showed that culturing cells on soft substrates ameliorated the stiff-cell phenotype. Moreover, he showed that the antifibrotic drug liraglutide was effective in deactivating HSCs when cultured on stiff compared to softer matrices. As a molecular mechanism, high stiffness was associated with increased nuclear deformation. Alleviating the nuclear-cytoskeleton tension was proposed as a strategy to recover nuclear sphericity and cellular phenotype. Finally, Srivatsan Kidambi (University of Nebraska) recreated a fibrotic-like microenvironment using innovative, biomimetic, polymer film-coated polydimethylsiloxane gels. This work indicated that LSECs cultured on these gels exhibited

loss of fenestrations, impaired hyaluronic acid endocytosis, and increased expression of cell adhesion molecules. Overall, these findings suggest a leading role of matrix stiffness in deregulating sinusoidal cells during liver disease progression.

### NASH

Jean-François Dufour's presentation (Inselspital) described the contribution of hepatic endothelial dysfunction to poor health outcomes in patients with NASH. Professor Dufour has previously outlined the therapeutic approaches for the treatment of NASH,<sup>(23)</sup> and in this lecture he updated the delegates on the current understanding of the role of sinusoidal cells in NASH.<sup>(24,25)</sup> Sinusoidal endothelial dysfunction precedes inflammatory and fibrotic progression in liver disease.<sup>(24)</sup> Of note, the loss of NO release from the endothelium contributes to increased intrahepatic resistance and activation of HSCs.<sup>(24)</sup> Targeting of cells expressing endothelial dysfunction markers, such as vascular adhesion-factor 1, with antifibrotic and inflammatory drugs may reduce the migration of immune cells to the liver and limit liver disease progression.<sup>(25)</sup> Other presented therapeutic approaches focused on targeting LSECs to resolve NASH. Miren Bravo (Vall d'Hebron Institut de Recerca [VHIR], Barcelona, Spain) presented data on the role of statins in restoring the LSEC phenotype and reducing portal pressure in a rat model of NASH without fibrosis.<sup>(26)</sup> NASH animals exhibited increased portal pressure, mainly due to endothelial dysfunction (decreased phosphorylated protein kinase B and phosphorylated endothelial nitric oxide synthase [NOS], increased endothelin 1 [ET-1] levels, and a higher predominance of dedifferentiated cluster of differentiation [CD]32b<sup>-</sup> LSECs). HSCs from these animals had reduced vitamin A storage and displayed activation of ET-1 downstream signaling. Treatment with statins resulted in a reduction in portal pressure levels, NASH reversion, and significant recovery of LSEC morphology, leading to a more quiescent phenotype of HSCs.<sup>(26)</sup>

Finally, the involvement of the role of runt-related transcription factor 1 (RUNX1) in NASH by clinical, *in vitro*, and *in vivo* studies was presented by Savneet Kaur (Institute of Liver and Biliary Sciences [ILBS], New Delhi, India).<sup>(27)</sup> This group found that messenger RNA and protein expression of RUNX1



was enhanced in patients with NASH (correlating with disease severity) and colocalized with vascular endothelial growth factor receptor 3 (VEGFR3)<sup>+</sup> LSECs and CD45<sup>+</sup> liver-infiltrating macrophages. Knockdown approaches using nanolipocarriers containing RUNX1 small interfering RNA (siRNA) in steatotic LSECs (>50%) resulted in substantial down-regulation of angiogenic, inflammatory, and adhesion gene expression with decreased infiltration of dendritic and T cells in the liver. *In vivo*, silencing of RUNX1 markedly reduced CD45<sup>+</sup>CD3<sup>+</sup> liver-infiltrating lymphocytes and CD11c<sup>+</sup> dendritic cells in both infiltrating and circulating lymphocytes.<sup>(27)</sup>

## ALD

In ALD, the innate immune system contributes significantly to liver damage.<sup>(28)</sup> KCs and peripheral blood mononucleocytes are critical to this process because they become sensitized to bacterial lipopolysaccharides and elevate proinflammatory cytokines. Adam Kim (Cleveland Clinic, Cleveland, OH) presented next-generation sequencing data to understand why innate immune cells become hypersensitive to lipopolysaccharide. In response to chronic alcohol exposure, KCs are sensitized to activation by pathogen-associated molecular patterns and DAMPs, and this leads to a state of chronic inflammation in the liver through expression of tumor necrosis factor alpha, IL-6, IL-8, and IL-18; this chronic inflammation contributes to the progression of ALD through reactive oxygen species imbalance.<sup>(29)</sup>

From a different perspective, Kenichi Ikejima (Juntendo University, Tokyo, Japan) presented recent data on the alterations in small intestine microbiota following chronic ethanol feeding in obese KK-Ay mice and the actions of rifaximin, an antibiotic used to treat irritable bowel syndrome and hepatic encephalopathy, on ethanol-induced liver injury. This group found that ethanol administration led to an *Erysipelotrichales* abundance of microbiota, with rifaximin reversing this abundance and preventing hepatic steatosis in this obese mice model.<sup>(30)</sup>

## HCC

Advances in biomarkers and therapeutics targeting the Wnt/ $\beta$ -catenin pathway in HCC were reported at the meeting. This pathway is involved

in the epigenetic development of HCC and progression to metastasis.<sup>(31,32)</sup> The identification of a potential surrogate biomarker of sex determining region Y-box 9 (Sox9) in HCC was presented by Kristy Chan (University of Hong Kong, Hong Kong, China). Sox9 confers “stemness” properties in HCC through the Wnt/ $\beta$ -catenin pathway.<sup>(33)</sup> This group showed the correlation between Sox9<sup>+</sup> HCC and the serum marker clusterin. The involvement of the Wnt/ $\beta$ -catenin pathway in HCC was also examined by Nuria Guerrero-Celis (Universidad Autónoma de México, Mexico City, Mexico) who investigated the use of an adenosine-derivative compound (IFC-305) as an HCC therapeutic. This group reported that IFC-305 reduced cell survival in HCC cell lines but did not impair survival of a human hepatic progenitor cell line.<sup>(34)</sup>

## CIRRHOSIS

The etiology, association with vascular disease, and treatment of cirrhosis was one of the key areas of interest at this meeting. In his lecture on vascular changes in liver disease, Jaime Bosch (Inselspital) discussed the similarities between the pathobiology of cirrhosis and the changes seen in the endothelial wall in atherosclerosis, postulating that cirrhosis should be considered a vascular disease of the liver. Cirrhotic liver shows marked vascular distortion, stiffness, and irregular capillarized sinusoids.<sup>(35)</sup> The endothelium of cirrhotic livers is profoundly dysfunctional,<sup>(14)</sup> becoming proangiogenic, proinflammatory, prothrombotic, and provasoconstrictive, with a striking resemblance to the changes seen in atherosclerosis, such as increased perfusion pressure and sensitivity to vasoconstrictors, together with markedly impaired vasorelaxation mechanisms.<sup>(36,37)</sup> Overall, this suggests that these factors contribute to the development of portal hypertension through increased resistance to blood flow and vascular tone.<sup>(35,38)</sup>

The individual cells of the sinusoid undergo phenotypic changes during liver disease progression to cirrhosis. LSECs become capillarized, lose their protective NO regulation, and undergo immune cell infiltration<sup>(39)</sup>; HSCs become activated, proliferative, and fibrogenic<sup>(40)</sup>; and KCs become activated and proinflammatory with engorgement, similarly to systemic macrophages.<sup>(20)</sup> A recent advance in the transcriptome

of LSECs during the progression of cirrhosis was presented by Anabel Fernández-Iglesias (IDIBAPS). The progression of capillarization was observed in LSECs isolated from rats after undergoing 2, 6, or 14 weeks of CCl<sub>4</sub> treatment. She found that 7,349 genes were dysregulated in LSECs isolated from the acute liver injury model (2 weeks of CCl<sub>4</sub>). These genes were related to inflammation and cell-to-cell interaction pathways. Fibrotic LSECs (6 weeks of CCl<sub>4</sub>) had 6,989 dysregulated genes, which were primarily involved in cell proliferation and development, including angiogenesis and vascular proliferation. Finally, cirrhotic LSECs (14 weeks of CCl<sub>4</sub>) exhibited 4,069 dysregulated genes implicated in cell transformation and invasion and tumor development. Complex transcriptomic analysis highlighted similarities between capillarization of rat LSECs and primary human cirrhotic LSECs.

In addition, advancements in our understanding of posttranslational modifications in CCl<sub>4</sub> animal models and patients with liver fibrosis were presented by Natalia Nieto (University of Chicago, Chicago, IL). Dr. Nieto's group has identified a posttranslational signature of the oxidant stress sensate high-mobility group box 1 (HMGB-1) isoforms that may be measured in animal models and patients.<sup>(41)</sup> Interestingly, their mass spectrometry-based method could potentially be used to stage clinical liver fibrosis progression and measure therapeutic responses. HMGB-1 is a DAMP that is increased in expression by hepatocytes and KCs following CCl<sub>4</sub> treatment. HMGB-1 binds to the receptor for advanced glycation end products on HSCs, leading to its activation and increased collagen deposition.<sup>(41)</sup> Recently, this group demonstrated that ablation of HMGB-1 in intestinal epithelial cells reduced diet-induced NASH in mice.<sup>(42)</sup>

The role of LSECs in phagocytic and adaptive T-cell responses following CCl<sub>4</sub>-induced cirrhosis was discussed by Rubén Francés (Miguel Hernández University, Alicante, Spain). The innate immune response is directed in part by the activity of LSECs,<sup>(43)</sup> suggesting an increased phagocytic role for LSECs in cirrhosis. *Escherichia coli* challenge (intraperitoneally delivered, 24 hours before) activated toll-like receptors, integrins, members of the complement cascade, and regulatory molecules, such as liver and lymph node sinusoidal endothelial cell C-type lectin, in both KCs and LSECs, suggesting increased LSEC phagocytic activity.<sup>(44)</sup>

Multiple presented studies highlighted the potential of LSECs and HSCs as therapeutic targets in cirrhosis, using animal models. The therapeutic regression of cirrhosis by using vasoactive compounds, such as statins,<sup>(36)</sup> cyclic guanosine monophosphate activators/enhancers,<sup>(45)</sup> and strong antioxidants,<sup>(46,47)</sup> is all mediated through changes in LSECs and HSCs.<sup>(48)</sup> Balasubramaniyan Vairappan (Jawaharlal Institute of Postgraduate Medical Education and Research, Pondicherry, India) demonstrated in CCl<sub>4</sub>-induced animal models that the severity of portal hypertension was associated with increased expression of NOS trafficking inducer (NOSTRIN), with candesartan cilexetil treatment (angiotensin II type 1 receptor antagonist) reducing NOSTRIN and increasing NO. Similarly, Denise van der Graaff (University of Antwerp, Antwerp, Belgium) presented her work on intrahepatic vascular resistance in rats fed a choline-deficient diet. This study demonstrated that angiotensin II and cyclooxygenase 2 receptor antagonists mitigated the increased pressure gradient in this animal model. Chang-Peng Zhu (Second Military University, Shanghai, China) presented work using TAA and CCl<sub>4</sub> animal models to examine the role of serotonin and its receptor 1<sub>A</sub> in portal hypertension. Serotonin receptor 1<sub>A</sub> demonstrated a critical role in hepatic hemodynamics, with agonist treatment promoting increased portal pressure and antagonism reducing portal pressure by increased NO. Finally, Zhi-Ren Liu (Georgia State University, Atlanta, GA) presented work on ProAgio treatment, which induced apoptosis in integrin  $\alpha\beta$ 3-expressing cells. This promoted depletion in activated HSCs and capillarized LSECs, with significant improvements in hepatic fibrosis.<sup>(49)</sup> These integrin receptors are heavily expressed in the endothelium, macrophages, and activated HSCs in experimental animal models.<sup>(50)</sup>

HSCs targeting pathways were demonstrated by Dinesh Mani Tripathi (ILBS). Dr. Tripathi highlighted orphan nuclear receptor subfamily 4, group A, member 1 (NR4A1) as a novel modulator of the HSC pathogenic phenotype. Increased NR4A1 expression is observed in patients with cirrhosis, CCl<sub>4</sub>- and TAA-treated rats, and *in vitro*-activated HSCs. Other therapeutic pathways discussed were farnesoid X receptor (FXR) agonists, peroxisome proliferator-activated receptor agonists, and caspase inhibitors, all related to regression of cirrhosis through actions on hepatocytes.<sup>(47,51-53)</sup> Clinically, statins, FXR agonists, and

obeticholic acid have demonstrated positive results in patients with cirrhosis.<sup>(54,55)</sup>

## Liver Regeneration

One of the most remarkable aspects of the liver is its ability to regenerate following acute injury, partial hepatectomy (pHx), and administration of damaging chemicals.<sup>(56)</sup> Following acute injury and pHx, the liver regenerates to “hepatostat,” the ideal size ratio to maintain body homeostasis. This process is heavily dependent on progenitor cells and cellular plasticity. Anna Mae Diehl (Duke University, Durham, NC) described the unique resilience that enables the liver to regenerate by the Hedgehog (Hh) signaling pathway after pHx. Cellular plasticity in the liver uses similar pathways between organogenesis and regeneration centralized around up-regulation of Hh signaling ligands.<sup>(57)</sup> In development, Hh signaling controls progenitor fate and tissue construction, whereas in adults, it regulates progenitor responses, matrix remodeling, and proliferation of hepatocytes and ductular cells.<sup>(57)</sup> This demonstrates that the mechanisms of liver organogenesis are retained with age and promote reconstruction following injury as long as there is a stimulus.<sup>(57)</sup> The Hh signaling pathway is a complex regulated transducer that may be a potential therapeutic target to regulate the liver’s response to injury, particularly because it is involved in liver regeneration, chronic liver disease, and HCC.<sup>(58)</sup> Activation of these pathways drives progressive loss of hepatocytes, leading to accumulation of fibrogenic cells and culminating in fibrosis and cirrhosis; targeting these pathways is a therapeutic area worth further investigation.<sup>(57,59,60)</sup>

Understanding gene expression and signaling pathways following pHx was discussed by Nur Raman (Universiti Sains Islam Malaysia, Kuala Lumpur, Malaysia). Using germ-free mice, this group demonstrated that intestinal microbiota drive liver regeneration, with a delayed response seen in germ-free animals. Superb three-dimensional image-based reconstruction of the liver microarchitecture following pHx was presented by Rajanikanth Vadigepalli (Thomas Jefferson University, Philadelphia, PA). This work advanced the group’s previous studies examining miRNA expression dynamics<sup>(61)</sup> and demonstrated the chronology of cellular vascular and biliary growth

in liver regeneration. Remodeling involved the formation of a temporary dense bile canalization network that regressed following regeneration. In contrast, the sinusoidal networks maintained a branched structure in full contact with hepatocytes throughout the two distinct stages of regeneration.

Takayo Yanagawa (Tokai University, Tokyo, Japan) demonstrated that crosstalk between blood cells and hepatocytes during liver regeneration is mediated by opioid growth factor receptor like-1 (OGFRL1), suggesting that OGFRL1-expressing cells may be an important potential regenerative therapy. Finally, an additional regenerative therapy was presented by Shunhei Yamashina (Juntendo University) showing that liver regeneration following pHx occurs by a cathepsin L inhibitor in CCl<sub>4</sub>-induced fibrotic livers. The group observed that their inhibitor Z-Phe-Tyr-aldehyde accelerated liver regeneration when given intraperitoneally for 3 days.<sup>(62,63)</sup>

## The Aging Liver

Building on the work delivered at the previous International Society for Hepatic Sinusoidal Research (ISHSR) conferences,<sup>(64)</sup> Professors Le Couteur, McCourt, and Török presented on the advancements made in the understanding of the aging liver, methods of viewing this process, and age-related components of accelerated fibrosis.

The aging liver undergoes the seven hallmarks of aging, leading to defenestration of LSECs, accumulation of lipid droplets and collagen deposition in HSCs, low-grade activation and inflammation by KCs, and cellular senescence and reactive oxygen species imbalance in hepatocytes.<sup>(65-67)</sup> These changes contribute to poor liver function with advancing age in a clinical setting.<sup>(67,68)</sup> Mashani Mohamad (Universiti Teknologi Majlis Amanah Rakyat, Selangor, Malaysia) built on her published data<sup>(69)</sup> and further demonstrated how age-related and diabetes-induced defenestration of LSECs contributed to changes in circulating lipoproteins and hepatic insulin sensitivity. Therapeutic targets for age-related changes in LSECs have been shown with multiple agents that target either the VEGF/NO pathway or the nutrient-sensing pathways through adenosine monophosphate-activated protein kinase/nicotinamide adenine dinucleotide.<sup>(70,71)</sup> The limitation of using these agents is the broad

off-target effects; David Le Couteur (University of Sydney) presented novel data on targeting individual cell types in the liver by using orally administrated nanomedicines.<sup>(72)</sup>

Visualization of age-related defenestration has to date been limited to electron microscopy and fixed-cell super-resolution microscopy techniques.<sup>(73)</sup> However, impressive data derived from direct stochastic optical reconstruction, structured illumination, atomic force, and stimulated emission depletion microscopy presented by Peter McCourt (Arctic University of Tromsø) demonstrated that it is possible to stain and visualize the LSECs as they move between defenestrated, fenestrated, and ultrafenestrated states.<sup>(8,74)</sup> These techniques allow for the effects of therapeutic agents on the ultrastructure of LSECs to be viewed in real time.<sup>(8,67)</sup>

With age, there is an accelerated development of fibrosis in the liver.<sup>(66,75)</sup> Natalie Török (Stanford University, Sacramento, CA) presented data on the age-related changes in 52 kDa isoform Src homology 2 domain containing (p52Shc) in the liver and its contribution to accelerated fibrosis in aging. p52Shc is the human homolog of the adaptor protein SHC-1 that has been shown to modulate lifespan and the stress response by targeting insulin/insulin-like growth factor 1 and c-Jun N-terminal kinases signaling pathways in *Caenorhabditis elegans*.<sup>(76)</sup> Professor Török demonstrated that p52Shc-mediated activation of phagocyte oxidase (p47phox)/nicotinamide adenine dinucleotide phosphate oxidase 2 (NOX2) leads to heightened redox stress, exacerbates inflammatory responses, and accelerates fibrotic progression in animal models. This finding is particularly important because dysregulation of redox pathways occurs within aging hepatocytes<sup>(65,67)</sup> and directly contributes to liver fibrosis.<sup>(77)</sup> Therapeutic targeting of this pathway is applicable to both age-related and liver disease progression given that the NOX pathway promotes cellular injury, impairs hepatic microcirculation, and contributes to NAFLD progression.<sup>(78)</sup>

## Nanomedicines

Current approaches for ameliorating liver diseases are focused on deactivating HSCs. However, interaction between LSECs and HSCs provides a unique cellular target for therapies. The development of new

techniques to target drug/material delivery to LSECs has become a research focus. In the first approach, Nicholas Hunt (University of Sydney) described silver sulfide quantum dots (QDs; 8 nm in diameter) for *in vivo*-targeted delivery to LSECs.<sup>(70,72)</sup> These orally administrated QDs are rapidly transferred across the small intestine and taken up by the liver (75% of ingested material within 30 minutes). They do not enter systemic circulation, are cleared by hepatobiliary excretion within 24 hours, and do not demonstrate hepatic toxicity or immunorecognition. When coated with a formaldehyde-treated bovine serum albumin biopolymer, improved liver targeting and enhanced LSEC localization occur. Coupling of metformin to these QDs increased the bioavailability of this drug 5-fold in the liver and 50-fold in LSECs.<sup>(72)</sup>

A second targeting approach was presented by Diana Hide (VHIR) who provided data on polymeric micelles (22.4 nm in diameter) that were rapidly internalized by healthy LSECs and HSCs *in vitro*. Although LSECs isolated from cirrhotic rats maintained their internalization capacity, this was dramatically reduced in HSCs. Validation experiments were performed with encapsulation of simvastatin (entrapment efficiency >96%), showing fewer toxic effects than the nonencapsulated drug and an improved phenotype of LSECs and HSCs isolated from bile duct ligation animals (Krüppel-like factor 2 up-regulation and increased fenestrae and porosity). Polymeric micelles administered to animals intravenously were found in the liver (40% accumulation) in both healthy and cirrhotic animals.

Finally, Bård Smedsrød (Arctic University of Tromsø) demonstrated the uptake of nanocrystals in liver cells. Two different formulations (1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[amino(polyethylene glycol)-200 [DSPE-PEG200] and Pluronic F-127) were investigated with or without polyvinylpyrrolidone K30/aerosol OT and administered *in vitro* or by intravenous injection in mice. *In vivo*, the particles distributed primarily into hepatocytes, while *in vitro* they were mainly found in LSECs or KCs. DSPE-PEG200 outperformed Pluronic F-127.

## Methods

Methodological advances in hepatic sinusoidal research range from novel animal models to

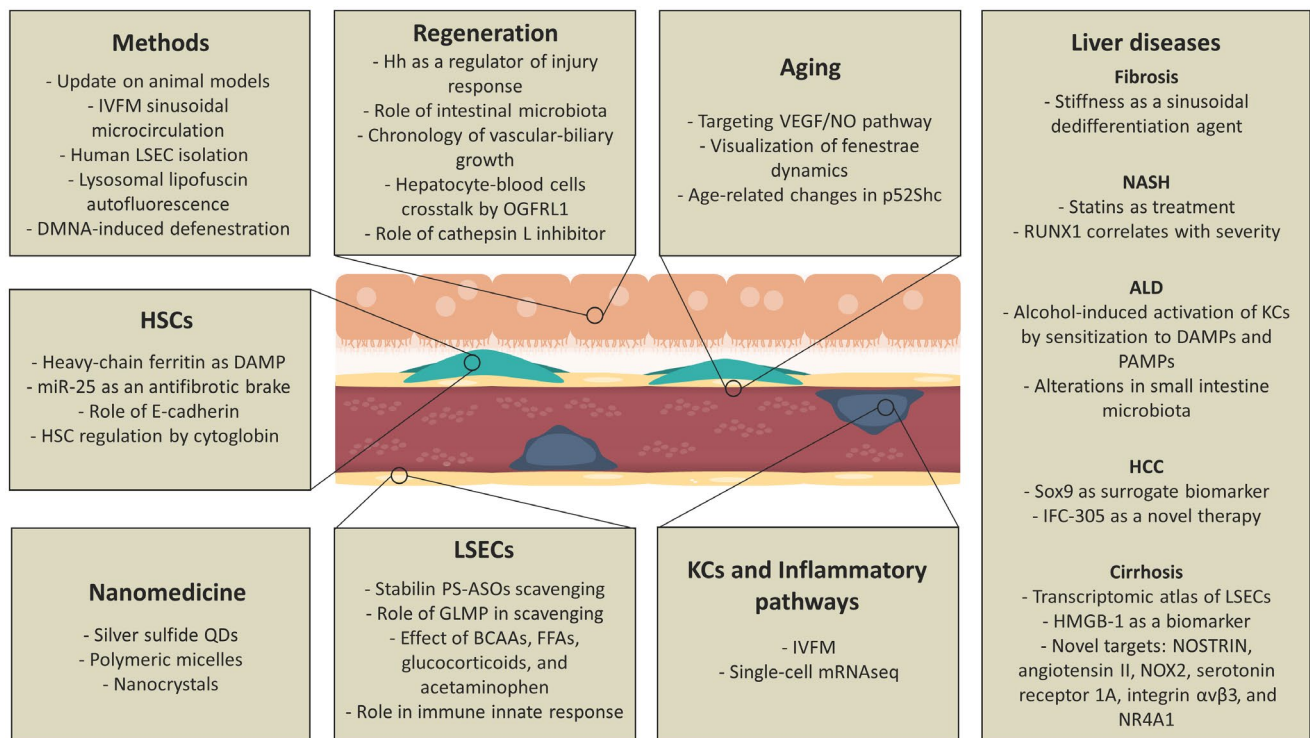


cellular isolations and microscopy techniques. The use of animal models to study liver diseases was widely addressed by Francisco Javier Cubero (Complutense University, Madrid, Spain). Although most animal models of liver diseases are very useful for elucidating pathophysiology, some only partially represent the characteristics of human liver diseases.<sup>(79,80)</sup> Therefore, special caution needs to be taken when selecting the most suitable animal model for a given purpose.

Advances in hepatic sinusoidal hemodynamics and IVFM techniques were presented by Antony Wheatley (National University of Ireland, Galway, Ireland). This group has shown that changes that occur in the hepatic microcirculation under physiological circumstances (e.g., homeostasis or liver regeneration)<sup>(81)</sup> following acute liver injury (e.g., ischemia-reperfusion injury)<sup>(82)</sup> or chronic liver disease are important to the understanding of the whole pathophysiological process. With widefield IVFM, this process can be visualized using FITC-labeled dextran or erythrocytes to

assess intrahepatic blood flow, rhodamine-6G to visualize rolling and adhering of white blood cells, nuclear stain Hoechst 33362 to identify normal or apoptotic hepatocytes, and vitamin A-related autofluorescence to identify HSCs.

A novel human LSEC isolation and characterization method was presented by Pantelitsa Papakyriacou (University of Birmingham, Birmingham, United Kingdom). After finely slicing livers, Dr. Papakyriacou adjusted enzymatic digestion according to the texture of the tissue (increased time for cirrhotic livers). Cell isolation was enhanced by gradient centrifugation, followed by immunomagnetic selection. Isolated LSECs were cultured in endothelial cell media with human serum, VEGF, and hepatocyte growth factor. Immunohistochemistry analysis from healthy livers showed high type II Fc gamma receptor (FcγRII2) and low CD31<sup>+</sup> expression, whereas cirrhotic livers exhibited low FcγRII2 and high CD31<sup>+</sup> staining, confirming pseudocapillarization during cirrhosis.



**FIG. 1.** Summary overview of the recent discoveries presented at the 20th Liver Sinusoid Meeting. The diagram summarizes the main topics discussed, including the physiology of LSECs, HSCs, and KCs and their contribution to the development of multiple liver diseases (fibrosis, NASH, ALD, HCC, and cirrhosis). In addition, advances in liver regeneration and aging along with the development of novel methods and sinusoidal-targeted nanomedicines were covered at this meeting. Abbreviations: miR, micro RNA; mRNA-Seq, messenger RNA sequencing; PAMP, pathogen-associated molecular pattern.

Anett Larsen (Arctic University of Tromsø) studied the autofluorescence of adult human LSECs. This work showed lysosomal lipofuscin autofluorescence may impede fluorescent-based techniques and the use of TrueBlack as a lipofuscin autofluorescence quencher. *In vitro* cultures revealed a time-dependent reduction of autofluorescence with TrueBlack in the ultraviolet spectrum with traces of autofluorescence remaining in the red and far-red channels.

Robin Fraser (University of Otago, Christchurch, New Zealand) presented on the health implications of the use of surfactants (dimethylnitrosamine [DMNA] and Poloxamer 407) in industry and agriculture. DMNA was banned; however, Poloxamer 407 is still widely used. These surfactants are used as defenestrating agents.<sup>(83)</sup> The development of liver diseases is highly prevalent in animal models and, given livestock exposure to these materials, it is suggested that liver function tests should be employed for animals.

## Conclusions

This meeting discussed the advances in our understanding of the basic biology and pathobiology of the cells of the sinusoid. We described the advancements in our understanding of LSEC endocytosis and fenestration regulation, HSC activation pathways, the contribution of stiffness to fibrotic cellular phenotypes, endothelial dysfunction in NASH and ALD, Wnt/ $\beta$ -catenin pathways in HCC, vascular dysfunction in cirrhosis and how this can be targeted with novel therapeutics, Hh signaling as a key regulator of liver plasticity, super-resolution microscopy of aging cells, nanomedicine development for targeting liver diseases, and finally, the recent progress in liver disease methodologies (Fig. 1). The symposium again demonstrated that research on cells of the hepatic sinusoid is flourishing. The 21st Liver Sinusoid Meeting will be held in Shanghai, China, in September 2021. Please check the ISHSR website ([www.ishsr.net](http://www.ishsr.net)) for further information. See you in Shanghai in 2021!

## REFERENCES

- 1) Sørensen KK, Smedsrød B. The liver sinusoidal endothelial cell: basic biology and pathobiology. In: Arias IM, Alter HJ, Boyer JL, Cohen DE, Shafritz DA, Thorgeirsson SS, et al., eds. *The Liver: Biology and Pathobiology*, 6th ed. Hoboken, NJ: Wiley-Blackwell; 2020:422-434.

- 2) Cogger VC, Hunt NJ, Le Couteur DG. Fenestrations in the liver sinusoidal endothelial cell. In: Arias IM, Alter HJ, Boyer JL, Cohen DE, Shafritz DA, Thorgeirsson SS, et al., eds. *The Liver: Biology and Pathobiology*, 6th ed. Hoboken, NJ: Wiley-Blackwell; 2020:435-443.
- 3) Politz O, Gratchev A, McCourt PA, Schledzewski K, Guillot P, Johansson S, et al. Stabilin-1 and -2 constitute a novel family of fasciclin-like hyaluronan receptor homologues. *Biochem J* 2002;362:155-164.
- 4) Hansen B, Longati P, Elvevold K, Nedredal GI, Schledzewski K, Olsen R, et al. Stabilin-1 and stabilin-2 are both directed into the early endocytic pathway in hepatic sinusoidal endothelium via interactions with clathrin/AP-2, independent of ligand binding. *Exp Cell Res* 2005;303:160-173.
- 5) Miller CM, Donner AJ, Blank EE, Egger AW, Kellar BM, Østergaard ME, et al. Stabilin-1 and stabilin-2 are specific receptors for the cellular internalization of phosphorothioate-modified antisense oligonucleotides (ASOs) in the liver. *Nucleic Acids Res* 2016;44:2782-2794.
- 6) Solon-Biet SM, Cogger VC, Pulpitel T, Wahl D, Clark X, Bagley E, et al. Branched-chain amino acids impact health and lifespan indirectly via amino acid balance and appetite control. *Nat Metab* 2019;1:532-545.
- 7) Hunt NJ, Lockwood GP, Warren A, Mao H, McCourt PA, Le Couteur DG, et al. Manipulating fenestrations in young and old liver sinusoidal endothelial cells. *Am J Physiol Gastrointest Liver Physiol* 2019;316:G144-G154.
- 8) Mao H, Diekmann R, Liang HP, Cogger VC, Le Couteur DG, Lockwood GP, et al. Cost-efficient nanoscopy reveals nanoscale architecture of liver cells and platelets. *Nanophotonics* 2019;8:1299-1313.
- 9) Geng Y, Hernández Villanueva A, Oun A, Buist-Homan M, Blokzijl H, Faber KN, et al. Protective effect of metformin against palmitate-induced hepatic cell death. *Biochim Biophys Acta Mol Basis Dis* 2020;1866:165621.
- 10) Kumar VH, Im NN, Huilgol SV, Yendigeri SM, K N, Ch R. Dose dependent hepatic and endothelial changes in rats treated with dexamethasone. *J Clin Diagn Res* 2015;9:FF08-FF10.
- 11) Nayak IMN, Narendar K, M PA, Jamadar MG, Kumar VH. Comparison of pioglitazone and metformin efficacy against glucocorticoid induced atherosclerosis and hepatic steatosis in insulin resistant rats. *J Clin Diagn Res* 2017;11:FC06-FC10.
- 12) Mitchell SJ, Huizer-Pajkos A, Cogger VC, McLachlan AJ, Le Couteur DG, Jones B, et al. Age-related pseudocapillarization of the liver sinusoidal endothelium impairs the hepatic clearance of acetaminophen in rats. *J Gerontol A Biol Sci Med Sci* 2011;66:400-408.
- 13) Youngmin AL, Friedman SL. Stellate cells and fibrosis. In: Arias IM, Alter HJ, Boyer JL, Cohen DE, Shafritz DA, Thorgeirsson SS, et al., eds. *The Liver: Biology and Pathobiology*, 6th ed. Hoboken, NJ: Wiley-Blackwell; 2020:444-454.
- 14) Fernández-Iglesias A, Gracia-Sancho J. How to face chronic liver disease: the sinusoidal perspective. *Front Med (Lausanne)* 2017;4:7.
- 15) Ruddell RG, Hoang-Le D, Barwood JM, Rutherford PS, Piva TJ, Watters DJ, et al. Ferritin functions as a proinflammatory cytokine via iron-independent protein kinase C zeta/nuclear factor kappaB-regulated signaling in rat hepatic stellate cells. *Hepatology* 2009;49:887-900.
- 16) Genz B, Coleman MA, Irvine KM, Kutasovic JR, Miranda M, Gratte FD, et al. Overexpression of miRNA-25-3p inhibits Notch1 signaling and TGF- $\beta$ -induced collagen expression in hepatic stellate cells. *Sci Rep* 2019;9:8541.
- 17) Odagiri N, Matsubara T, Higuchi M, Takada S, Urushima H, Sato-Matsubara M, et al. Involvement of ERK1/2 activation in the gene expression of senescence-associated secretory factors in human hepatic stellate cells. *Mol Cell Biochem* 2019;455:7-19.

- 18) Thi Thanh Hai N, Thuy LTT, Shiota A, Kadono C, Daikoku A, Hoang DV, et al. Selective overexpression of cytoglobin in stellate cells attenuates thioacetamide-induced liver fibrosis in mice. *Sci Rep*2018;8:17860.
- 19) Motoyama H, Komiya T, Thuy le LT, Tamori A, Enomoto M, Morikawa H, et al. Cytoglobin is expressed in hepatic stellate cells, but not in myofibroblasts, in normal and fibrotic human liver. *Lab Invest*2014;94:192-207.
- 20) Guillot A, Tacke F. Liver macrophages: old dogmas and new insights. *Hepatology Commun*2019;3:730-743.
- 21) **Heymann F, Niemietz PM**, Peusquens J, Ergen C, Kohlhepp M, Mossanen JC, et al. Long term intravital multiphoton microscopy imaging of immune cells in healthy and diseased liver using CXCR6.Gfp reporter mice. *J Vis Exp*2015;97:e52607.
- 22) **Guixé-Muntet S, Ortega-Ribera M**, Wang C, Bosch J, Dufour JF, Berzigotti A, et al. Matrix stiffness modulates the phenotype of hepatic cells in cirrhosis and modifies the sinusoidal effects of statins [Abstract]. *J Hepatology*2019;70(Suppl.):e437.
- 23) Perazzo H, Dufour JF. The therapeutic landscape of non-alcoholic steatohepatitis. *Liver Int*2017;37:634-347.
- 24) Pesarín M, La Mura V, Gracia-Sancho J, García-Calderó H, Rodríguez-Vilarrupla A, García-Pagán JC, et al. Sinusoidal endothelial dysfunction precedes inflammation and fibrosis in a model of NAFLD. *PLoS One*2012;7:e32785.
- 25) **Francque S, Laleman W**, Verbeke L, Van Steenkiste C, Casteleyn C, Kwanten W, et al. Increased intrahepatic resistance in severe steatosis: endothelial dysfunction, vasoconstrictor overproduction and altered microvascular architecture. *Lab Invest*2012;92:1428-1439.
- 26) Bravo M, Raurell I, Hide D, Fernández-Iglesias A, Gil M, Barberá A, et al. Restoration of liver sinusoidal cell phenotypes by statins improves portal hypertension and histology in rats with NASH. *Sci Rep*2019;9:20183.
- 27) **Kaur S, Rawal P**, Siddiqui H, Rohilla S, Sharma S, Tripathi D, et al. Increased expression of RUNX1 in liver correlates with NASH activity score in patients with non-alcoholic steatohepatitis (NASH). *Cells*2019;8:pii: E1277.
- 28) **Gao B, Ahmad MF**, Nagy LE, Tsukamoto H. Inflammatory pathways in alcoholic steatohepatitis. *J Hepatology*2019;70:249-259.
- 29) **Kim A, McCullough RL**, Poulsen KL, Sanz-García C, Sheehan M, Stavitsky AB, et al. Hepatic immune system: adaptations to alcohol. *Handb Exp Pharmacol*2018;248:347-367.
- 30) Kitagawa R, Kon K, Uchiyama A, Arai K, Yamashina S, Kuwahara-Arai K, et al. Rifaximin prevents ethanol-induced liver injury in obese KK-Ay mice through modulation of small intestinal microbiota signature. *Am J Physiol Gastrointest Liver Physiol*2019;317:G707-G715.
- 31) Nishida N, Goel A. Genetic and epigenetic signatures in human hepatocellular carcinoma: a systematic review. *Curr Genomics*2011;12:130-137.
- 32) Hu Z, Wang P, Lin J, Zheng X, Yang F, Zhang G, et al. MicroRNA-197 promotes metastasis of hepatocellular carcinoma by activating wnt/ $\beta$ -catenin signaling. *Cell Physiol Biochem*2018;51:470-486.
- 33) Leung CO, Mak WN, Kai AK, Chan KS, Lee TK, Ng IO, et al. Sox9 confers stemness properties in hepatocellular carcinoma through Frizzled-7 mediated Wnt/ $\beta$ -catenin signaling. *Oncotarget*2016;7:29371-29386.
- 34) Rodríguez-Aguilera JR, Pérez-Cabeza de Vaca R, Guerrero-Celis N, Velasco-Loyden G, Domínguez-López M, Recillas-Targa F, et al. Molecular and cellular aspects of cirrhosis and how an adenosine derivative could revert fibrosis. In: Tsoulfas G, ed. *Liver Cirrhosis: Debates and Current Challenges*. London: Intech Open; 2019:433-467. <https://www.intechopen.com/books/liver-cirrhosis-debates-and-current-challenges/molecular-and-cellular-aspects-of-cirrhosis-and-how-an-adenosine-derivative-could-revert-fibrosis>.
- 35) Gracia-Sancho J, Marrone G, Fernández-Iglesias A. Hepatic microcirculation and mechanisms of portal hypertension. *Nat Rev Gastroenterol Hepatol*2019;16:221-234.
- 36) **Marrone G, Maeso-Díaz R**, García-Cardena G, Abalde JG, García-Pagán JC, Bosch J, et al. KLF2 exerts antifibrotic and vasoprotective effects in cirrhotic rat livers: behind the molecular mechanisms of statins. *Gut*2015;64:1434-1443.
- 37) Gracia-Sancho J, Laviña B, Rodríguez-Vilarrupla A, García-Calderó H, Bosch J, García-Pagán JC. Enhanced vasoconstrictor prostanoid production by sinusoidal endothelial cells increases portal perfusion pressure in cirrhotic rat livers. *J Hepatology*2007;47:220-227.
- 38) García-Pagán JC, Gracia-Sancho J, Bosch J. Functional aspects on the pathophysiology of portal hypertension in cirrhosis. *J Hepatology*2012;57:458-461.
- 39) Marrone G, Gracia-Sancho J. Hepatic microcirculation in chronic liver disease. In: Peralta C, Fontana L, eds. *Liver Failure: Etiologies, Neurological Complications and Emerging Therapies*. Hauppauge, NY: Nova Science Publishers; 2013:89-106.
- 40) Higashi T, Friedman SL, Hoshida Y. Hepatic stellate cells as key target in liver fibrosis. *Adv Drug Deliv Rev*2017;121:27-42.
- 41) Ge X, Arriazu E, Magdaleno F, Antoine DJ, delaCruz R, Theise N, et al. High mobility group box-1 drives fibrosis progression signaling via the receptor for advanced glycation end products in mice. *Hepatology*2018;68:2380-2404.
- 42) Gaskell H, Ge X, Desert R, Das S, Han H, Lantvit D, et al. Ablation of Hmgb1 in intestinal epithelial cells causes intestinal lipid accumulation and reduces NASH in mice. *Hepatology Commun*2019;4:92-108.
- 43) Shetty S, Lalor PF, Adams DH. Liver sinusoidal endothelial cells - gatekeepers of hepatic immunity. *Nat Rev Gastroenterol Hepatol*2018;15:555-567.
- 44) Caparrós E, Piñero P, Juanola O, Gómez-Hurtado I, Zapater P, González-Navajas JM, et al. LSEctin and CD206 participate in the regulation of the hepatic inflammatory response to E coli in a model of cirrhosis [Abstract]. *J Hepatology*2018;68(Suppl. 1):S472-S473.
- 45) Schwabl P, Brusilovskaya K, Supper P, Bauer D, Königshofer P, Riedl F, et al. The soluble guanylate cyclase stimulator riociguat reduces fibrogenesis and portal pressure in cirrhotic rats. *Sci Rep*2018;8:9372.
- 46) **Gracia-Sancho J, Laviña B**, Rodríguez-Vilarrupla A, García-Calderó H, Fernández M, Bosch J, et al. Increased oxidative stress in cirrhotic rat livers: a potential mechanism contributing to reduced nitric oxide bioavailability. *Hepatology*2008;47:1248-1256.
- 47) Boyer-Díaz Z, Domingo JC, De Gregorio E, Manicardi N, Aristu-Zabalza P, Cordobilla B, et al. A nutraceutical rich in docosahexaenoic acid improves portal hypertension in a preclinical model of advanced chronic liver disease. *Nutrients*2019;11:pii: E2358.
- 48) **Vilaseca M, Guixé-Muntet S**, Fernández-Iglesias A, Gracia-Sancho J. Advances in therapeutic options for portal hypertension. *Therap Adv Gastroenterol*2018;11:1756284818811294.
- 49) **Turaga RC, Yin L**, Yang JJ, Lee H, Ivanov I, Yan C, et al. Rational design of a protein that binds integrin  $\alpha\beta 3$  outside the ligand binding site. *Nat Commun*2016;7:11675.
- 50) Patsenker E, Popov Y, Stickel F, Schneider V, Ledermann M, Sägeser H, et al. Pharmacological inhibition of integrin  $\alpha\text{v}\beta 3$  aggravates experimental liver fibrosis and suppresses hepatic angiogenesis. *Hepatology*2009;50:1501-1511.
- 51) Úbeda M, Lario M, Muñoz L, Borrero MJ, Rodríguez-Serrano M, Sánchez-Díaz AM, et al. Obeticholic acid reduces bacterial translocation and inhibits intestinal inflammation in cirrhotic rats. *J Hepatology*2016;64:1049-1057.



- 52) Aristu P, Boyer-Diaz Z, Broqua P, Junien JL, Wettstein G, Bosch J, et al. The Pan-Ppar agonist lanifibranor improves portal hypertension and hepatic fibrosis in experimental advanced chronic liver disease [Abstract]. *Hepatology*2019;70:A63.
- 53) Gracia-Sancho J, Manicardi N, Ortega-Ribera M, Maeso-Díaz R, Guixé-Muntet S, Fernández-Iglesias A, et al. Emricasan ameliorates portal hypertension and liver fibrosis in cirrhotic rats through a hepatocyte-mediated paracrine mechanism. *Hepatology*2019;3:987-1000.
- 54) Hirschfield GM, Mason A, Luketic V, Lindor K, Gordon SC, Mayo M, et al. Efficacy of obeticholic acid in patients with primary biliary cirrhosis and inadequate response to ursodeoxycholic acid. *Gastroenterology*2015;148:751-761.e8.
- 55) Tsochatzis EA, Bosch J. Statins in cirrhosis: ready for prime time. *Hepatology*2017;66:697-699.
- 56) Michalopoulos GK. Hepatostat: liver regeneration and normal liver tissue maintenance. *Hepatology*2017;65:1384-1392.
- 57) Ochoa B, Syn WK, Delgado I, Karaca GF, Jung Y, Wang J, et al. Hedgehog signaling is critical for normal liver regeneration after partial hepatectomy in mice. *Hepatology*2010;51:1712-1723.
- 58) Machado MV, Diehl AM. Hedgehog signalling in liver pathophysiology. *J Hepatol*2018;68:550-562.
- 59) Yang L, Jung Y, Omenetti A, Witek RP, Choi S, Vandongen HM, et al. Fate-mapping evidence that hepatic stellate cells are epithelial progenitors in adult mouse livers. *Stem Cells*2008;26:2104-2113.
- 60) Du K, Hyun J, Premont RT, Choi SS, Michelotti GA, Swiderska-Syn M, et al. Hedgehog-YAP signaling pathway regulates glutaminolysis to control activation of hepatic stellate cells. *Gastroenterology*2018;154:1465-1479.e13.
- 61) Dippold RP, Vadigepalli R, Gonye GE, Patra B, Hoek JB. Chronic ethanol feeding alters miRNA expression dynamics during liver regeneration. *Alcohol Clin Exp Res*2013;37(Suppl. 1):E59-E69.
- 62) Manchanda M, Das P, Gahlot GPS, Singh R, Roeb E, Roderfeld M, et al. Cathepsin L and B as potential markers for liver fibrosis: insights from patients and experimental models. *Clin Transl Gastroenterol*2017;8:e99.
- 63) Sato T, Yamashina S, Izumi K, Ueno T, Koike M, Ikejima K, et al. Cathepsin L-deficiency enhances liver regeneration after partial hepatectomy. *Life Sci*2019;221:293-300.
- 64) Smedsrød B, Le Couteur D, Ikejima K, Jaeschke H, Kawada N, Naito M, et al. Hepatic sinusoidal cells in health and disease: update from the 14th International Symposium. *Liver Int*2009;29:490-501.
- 65) Maeso-Díaz R, Ortega-Ribera M, Fernandez-Iglesias A, Hide D, Muñoz L, Hessheimer AJ, et al. Effects of aging on liver microcirculatory function and sinusoidal phenotype. *Aging Cell*2018;17:e12829.
- 66) Maeso-Díaz R, Ortega-Ribera M, Lafoz E, Lozano JJ, Baiges A, Francés R, et al. Aging influences hepatic microvascular biology and liver fibrosis in advanced chronic liver disease. *Aging Dis*2019;10:684-698.
- 67) Hunt NJ, Kang SWS, Lockwood GP, Le Couteur DG, Cogger VC. Hallmarks of aging in the liver. *Comput Struct Biotechnol J*2019;17:1151-1161.
- 68) Hide D, Warren A, Fernández-Iglesias A, Maeso-Díaz R, Peralta C, Le Couteur DG, et al. Ischemia/reperfusion injury in the aged liver: the importance of the sinusoidal endothelium in developing therapeutic strategies for the elderly. *J Gerontol A Biol Sci Med Sci*2020;75:268-277.
- 69) Mohamad M, Mitchell SJ, Wu LE, White MY, Cordwell SJ, Mach J, et al. Ultrastructure of the liver microcirculation influences hepatic and systemic insulin activity and provides a mechanism for age-related insulin resistance. *Aging Cell*2016;15:706-715.
- 70) Hunt NJ, Lockwood GP, Kang SWS, Pulpitel T, Clark X, Mao H, et al. The effects of metformin on age-related changes in the liver sinusoidal endothelial cell. *J Gerontol A Biol Sci Med Sci*2020;75:278-285.
- 71) Hunt NJ, McCourt PAG, Le Couteur DG, Cogger VC. Novel targets for delaying aging: the importance of the liver and advances in drug delivery. *Adv Drug Deliv Rev*2018;135:39-49.
- 72) Hunt NJ, Lockwood GP, Le Couteur FH, McCourt PA, Singla N, Kang SWS, et al. Rapid intestinal uptake and targeted delivery to the liver endothelium using orally administered silver sulfide quantum dots. *ACS Nano*2020;14:1492-1507.
- 73) Øie CI, Mönkemöller V, Hübner W, Schüttpeiz M, Mao H, Ahluwalia BS, et al. New ways of looking at very small holes - using optical nanoscopy to visualize liver sinusoidal endothelial cell fenestrations. *Nanophotonics*2018;7: <https://doi.org/10.1515/nanoph-2017-0055>.
- 74) Zapotoczny B, Szafranska K, Owczarczyk K, Kus E, Chlopicki S, Szymonski M. Atomic force microscopy reveals the dynamic morphology of fenestrations in live liver sinusoidal endothelial cells. *Sci Rep*2017;7:7994.
- 75) Kim IH, Kisseleva T, Brenner DA. Aging and liver disease. *Curr Opin Gastroenterol*2015;31:184-191.
- 76) Neumann-Haefelin E, Qi W, Finkbeiner E, Walz G, Baumeister R, Hertweck M. SHC-1/p52Shc targets the insulin/IGF-1 and JNK signaling pathways to modulate life span and stress response in *C. elegans*. *Genes Dev*2008;22:2721-2735.
- 77) Török NJ. Dysregulation of redox pathways in liver fibrosis. *Am J Physiol Gastrointest Liver Physiol*2016;311:G667-G674.
- 78) **Matsumoto M, Zhang J**, Zhang X, Liu J, Jiang JX, Yamaguchi K, et al. The NOX1 isoform of NADPH oxidase is involved in dysfunction of liver sinusoids in nonalcoholic fatty liver disease. *Free Radic Biol Med*2018;115:412-420.
- 79) Lamas-Paz A, Hao F, Nelson LJ, Vázquez MT, Canals S, Gómez del Moral M, et al. Alcoholic liver disease: utility of animal models. *World J Gastroenterol*2018;24:5063-5075.
- 80) Fickert P, Wagner M. Animal models in liver disease. *Biochim Biophys Acta Mol Basis Dis*2019;1865:867-868.
- 81) Marlini M, Mabuchi A, Mallard BL, Hairulhisyam N, Akashi-Takamura S, Harper JL, et al. Delayed liver regeneration in C3H/HeJ mice: possible involvement of haemodynamic and structural changes in the hepatic microcirculation. *Exp Physiol*2016;101:1492-1505.
- 82) Mabuchi A, Wake K, Marlini M, Watanabe H, Wheatley AM. Protection by glycyrrhizin against warm ischemia-reperfusion-induced cellular injury and derangement of the microcirculatory blood flow in the rat liver. *Microcirculation*2009;16:364-376.
- 83) Warren A, Benseler V, Cogger VC, Bertolino P, leCouteur DG. The impact of Poloxamer 407 on the ultrastructure of the liver and evidence for clearance by extensive endothelial and Kupffer cell endocytosis. *Toxicol Pathol*2011;39:390-397.

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