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REVIEW ARTICLE

15th International Symposium on Cells of the Hepatic Sinusoid, 2010

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Abstract

This is a meeting report of the presentations given at the 15th International Symposium on Cells of the Hepatic Sinusoid, held in 2010. The areas covered include the contributions of the various liver cell populations to liver disease, molecular and cellular targets involved in steatohepatitis, hepatic fibrosis and cancer and regenerative medicine. In addition to a review of the science presented at the meeting, this report provides references to recent literature on the topics covered at the meeting.

The 15th International Symposium on Cells of the Hepatic Sinusoid (ISCHS) took place in Pasadena, California on 29 August–1 September and was attended by 205 scientists from 21 nations. It marked an inaugural meeting for the newly formed International Society for Hepatic Sinusoidal Research, an organizational backbone for the historical series of ISCHS, which dates back to 1977. The ISCHS is an unique and probably the sole, meeting in which scientists from multidisciplinary backgrounds come together to discuss the anatomy, evolutional biology and functions in health and disease of all different cell types in the liver, in addition to crosstalk among them.

The 15th ISCHS showcased five state-of-the-art lectures: cluster of differentiation (CD)8 T cell migration by Michael Dustin, Wnt signalling in development and disease by Randall Moon, hepatocyte–endothelial cell interactions in the developing zebrafish liver by Didier Stainier, the VHL tumour suppressor in health and disease by William Kaelin, and inflammation and metabolism in liver tumourigenesis by Michael Karin. The symposium provided a platform for 57 oral and 98 poster presentations in seven thematic sessions: steatohepatitis; hepatic stellate cell biology; innovative therapeutic targets of liver fibrosis; progenitor cells, liver development and regeneration; liver sinusoidal endothelial cells (LSECs); cancer biology and comparative anatomy and evolutional biology. Notable at this meeting were presentations of many molecular and cellular studies aimed at disclosing potential therapeutic targets of steatohepatitis, liver fibrosis and cancer, as well as cell lineage or fate mapping studies of liver progenitor cells, especially for those of liver mesenchymal cells. In addition, a translation of findings in embryo to adult parenchymal and non-parenchymal liver cell biology was evident in many studies. At the same time, a picture is still worth a thousand words: elegant images of electron microscopy powerfully captured our imagination by showing morphological evidence of cell movement and fate. The meeting reconfirmed the importance of cell-type-specific studies and cellular crosstalk in understanding normal liver biology and the pathogenesis of liver diseases. As we begin to identify new molecular mechanisms of cellular interactions, we are also humbled by the fact that our fundamental knowledge of each cell type of the liver is far from complete. The symposium presented a superb

opportunity to deepen mechanistic insights into the biology of a liver cell type of interest in a particular discipline and to broaden the perspective to other cell types and different disciplines. For just this reason, the scientific and social activities at the meeting spontaneously stimulated interactions among the participants and allowed collaborative arrangements to be discussed and conceptualized. The symposium also honoured two of our colleagues who passed away in the past 2 years, the late Professors Albert Geerts of Belgium and Hiromasa Ishii of Japan, with tributes during tutorial lectures by Scott Friedman and Makoto Suematsu respectively.

This historical symposium was made possible by grants from National Institute of Alcohol Abuse and Alcoholism (NIAAA) and Office of Rare Diseases Research of NIH, American Association for the Study of Liver Diseases and Japan Science and Technology Agency's Exploratory Research for Advanced Technology and by industrial support. This communication summarizes the highlights of the oral presentations made at the meeting.

Steatohepatitis

The tutorial in the steatohepatitis session presented by Anna Mae Diehl (Duke University) addressed the critical question why non-alcoholic steatohepatitis (NASH) more likely progresses to cirrhosis and hepatocellular carcinoma (HCC) than steatosis alone. Because the main difference between NASH and steatosis is cell injury in NASH, her focus was on identifying factors released by injured hepatocytes that promote fibrogenesis. She demonstrated that Sonic and Indian hedgehog ligands (Shh, Ihh) are not present in healthy human or mouse hepatocytes, but are produced by stressed/injured cells. Shh/Ihh are mediators that can promote inflammation, stellate cell differentiation and expansion of liver progenitor cells. The number of Shh^+ hepatocytes correlated with progenitor cell accumulation and fibrosis stage, suggesting that injury to hepatocytes promotes formation and release of soluble Hh ligands, which induce fibrosis in chronically damaged livers.

Andrew Miller (NIAAA) investigated the association of steatosis with inflammation in alcoholic and non-alcoholic liver injury using various cytokine-deficient mice. He reported that there is dissociation between steatosis and hepatic inflammation in both alcoholic (Lieber DeCarli diet) and non-alcoholic (high fat diet) liver injury. In these models, interleukin (IL)-10 is a key negative regulator of inflammation, which does not cause either liver injury or steatosis. In contrast, IL-6 and signal transducers and activators of transcription (STAT)3 in hepatocytes regulate steatosis and liver injury, but not inflammation, in alcoholic and non-alcoholic liver disease (1).

The topic of hepatic inflammation in the pathogenesis of alcoholic liver injury was expanded by Ekihiro Seki (UC San Diego), who presented his work on the role of toll-like receptor (TLR)4 expressed on Kupffer cells (KCs) and hepatic stellate cells (HSCs). Using TLR4 chimeric mice, he showed that wild type (WT) mice treated with WT bone marrow cells developed hepatic steatosis, inflammation, cell necrosis and moderate fibrosis during 4 weeks of chronic intragastric alcoholic feeding. TLR4-deficient mice with TLR4 $-/-$ bone marrow were completely protected. Interestingly, TLR4 $-/-$ mice with WT bone marrow (TLR4-deficient stellate cells) and WT mice with TLR4 $-/-$ bone marrow (TLR4-deficient KCs) showed a partial reduction of all parameters, suggesting that TLR4 on both KCs and HSCs are important for inflammation, steatosis and fibrosis after chronic alcohol exposure (2).

The role of KC activation in alcoholic liver disease was also the topic of the next talk by Fatima Teixeira-Clerc (INSERM, France). She investigated the potential of the cannabinoid receptor 2 (CB2) in facilitating the transition of KCs from a pro-inflammatory (M1) to an anti-inflammatory (M2) phenotype, which may protect against alcohol-induced steatosis. Feeding WT animals and CB2 $-/-$ mice an ethanol-containing Lieber DeCarli diet resulted in increased steatosis and a pro-inflammatory state of KCs, which was exaggerated in the CB2 $-/-$ mice. However, a CB2 agonist could attenuate these effects in WT animals and promote the transition to an anti-inflammatory phenotype in KCs. These findings suggest that the CB2 receptor could be a promising target to reduce steatosis and inflammation in alcoholic liver disease (3).

The pro-inflammatory activation status of KCs was also the focus of the presentation by Laura Nagy (Cleveland Clinic). She investigated the role of macrophage migration inhibitory factor (MIF), a cytokine that can activate macrophages to produce pro-inflammatory cytokines in alcohol-induced liver injury. WT mice fed a Lieber DeCarli diet for 28 days developed steatosis, mild liver injury and showed enhanced tumour necrosis factor (TNF)-α gene expression. All effects were significantly reduced in MIF $-/-$ mice suggesting that MIF may be a contributor to ethanol-induced liver injury.

In keeping with the topic of inflammatory mediators in alcoholic liver disease, Ramón Bataller (IDIBAPS, Barcelona) reported his results on the role of osteopontin in pathogenesis. Osteopontin, which can act as a neutrophil chemoattractant, was detected in high levels in livers of alcoholic hepatitis patients, but not in normal livers and the osteopontin content in these livers correlated with disease severity. Furthermore, osteopontin-deficient mice had less inflammation and liver injury compared with WT animals after chronic alcohol feeding. Thus, osteopontin may be a new potential target to treat patients with alcoholic hepatitis.

The next speaker, Cheng Ji (University of Southern California), focused on intracellular signalling mechanisms of cell death, especially endoplasmic reticulum (ER) stress, in alcohol-induced liver injury. Using a liver-specific glucose-regulated protein (GRP)78-deficient

mouse, he found enhanced liver injury after chronic ethanol feeding, but also after treatment with various hepatotoxic drugs. GRP78 is a master regulator of ER homeostasis. However, liver-specific GRP78-deficient mice suffer from chronic ER stress with significant apoptotic and necrotic cell death, inflammation and modulation of numerous genes. The presenter concluded that the aggravated liver injury after ethanol and other stressors in the liver-specific GRP78 −/− mice indicates the importance of ER stress in the pathophysiology.

In the first talk on NASH, Joan Claria (IDIBAPS, Barcelona) addressed risk factors involved in the transition of steatosis to steatohepatitis. He identified in apolipoprotein E-deficient (ApoE −/−) mice, which are prone to develop spontaneously steatohepatitis, the upregulation of pro-inflammatory 5- and 12/15-lipoxygenase genes. Comparing ApoE −/− mice with double deficient mice (ApoE −/−; 5-lipooxygenase (LO) −/− and ApoE −/−; 12/15-LO −/−), he found that the spontaneous macrophage infiltration, cytokine formation and liver injury observed in ApoE −/− mice was substantially reduced in the double-deficient animals. Although ApoE −/−; 5-LO −/− mice did not show reduced steatosis, there was an insulin-sensitizing effect in the adipose tissue. In contrast, the ApoE −/−; 12/15-LO −/− mice had reduced steatosis and insulin sensitization in the liver. These data suggest that 5-LO and 12/15-LO gene products are involved in promoting insulin resistance and hepatic inflammation in metabolic liver disease (4).

In his talk on the role of KCs in promoting insulin resistance in the liver, Nicolas Lanthier (Université Catholique de Louvain, Brussels) reported that 3 days of feeding a high fat diet caused KC activation and insulin resistance in the liver. In contrast, 4–16 weeks of high fat feeding caused hepatic and peripheral insulin resistance and macrophage infiltration into the adipose tissue. Depletion of KCs before high fat feeding prevented the initial hepatic insulin resistance and prolonged depletion prevented obesity, adipose tissue inflammation and insulin resistance. Thus, activation of KCs by high fat diet initially causes hepatic insulin resistance and promotes adipose tissue inflammation and peripheral insulin resistance during prolonged high fat feeding. Specific mediators of the effect remain to be identified.

Bruce Cronstein (New York University) reported that fructose feeding promotes adenosine triphosphate (ATP) hydrolysis to adenosine mainly by ecto-5'nucleotidase. Using various adenosine receptor deficient mice and receptor antagonists, he showed that high levels of fructose ingestion promote steatosis and non-alcoholic fatty liver disease through A1 and A2B receptors.

The last two presentations in the steatohepatitis session focused on xenobiotic-induced inflammation and fibrosis. Tomonori Aoyama (University of California, San Diego) reported on studies to identify chemokines and their receptors that are involved in macrophage and HSC migration during development of fibrosis. After chronic carbon tetrachloride (CCl_4) treatment, the CX3CR1 receptor was predominantly expressed on macrophages/KCs and the ligand, CX3CL1, was expressed by HSC. Interestingly, liver inflammation and fibrosis were enhanced in CX3CR1-deficient mice (5). Based on the observation that recombinant CX3CL1 can induce IL-10 in KCs, the presenter concluded that HSC-derived CX3CL1 can attenuate inflammation and fibrosis by inducing anti-inflammatory mediators in macrophages.

Kenichi Ikejima (Juntendo University, Tokyo) investigated the role of natural killer T (NKT) cells in thioacetamide-induced inflammation and fibrosis. Treatment with thioacetamide for 9 weeks resulted in necro-inflammation and extensive fibrosis in WT mice. However, CD1d knockout animals, which lack mature NKT cells, showed strongly attenuated inflammation, fibrosis and mortality suggesting that NKT cells contribute to thioacetamide-induced hepatic inflammatory injury and fibrogenesis (6).

In the poster session, a number of papers were presented that provided new insight into various aspects of the pathogenesis of alcoholic steatohepatitis, NASH and drug-induced liver injury. A focus of investigators was the innate immunity. Qifa Xie (University of Louisville) and Hisafumi Yamagata (Juntendo University) presented evidence for impaired innate immunity caused by NKT cell depletion in various models of NASH. Oygi Park (NIAAA) reported that IL-22, a newly identified cytokine of the IL-10 family, is down-regulated during alcoholic steatohepatitis and treatment with recombinant IL-22 ameliorates alcohol-induced steatosis, liver injury and oxidant stress. Furthermore, invariant NKT cells contribute to CCl_4-induced acute injury and inflammation, but appear to play a limited role during the later fibrosis because of depletion of iNKT cells. Palash Mandal (Cleveland Clinic) showed that full-length adiponectin suppressed the lipopolysaccharide (LPS)-induced activation of ethanol-primed KCs by interaction with the Adipo2 receptor and by inducing haemeoxygenase-1 expression, but also by shifting the macrophages to an anti-inflammatory phenotype. Ekihiro Seki (University of California, San Diego) demonstrated that steatohepatitis and fibrosis in mice on a choline-deficient diet were in part dependent on TLR9-mediated IL-1β formation (7). A similar mechanism involving TLR9 and inflammasome-dependent IL-1β formation was observed during acute acetaminophen hepatotoxicity, but this had no relevant effect on liver injury or the sterile inflammatory response (David Williams, University of Kansas) (8).

Hepatic stellate cell biology

Scott Friedman (Mount Sinai, NY) opened the HSC biology session by giving a tutorial overview of the field with a historical perspective on how the cells were identified and defined since the original discovery in 1876 until now (9). He pointed to several areas, which are yet to be explored including functional roles of HSCs in

liver and organ homeostasis, which may be determined by selective deletion of the cells in normal or injured liver; biological roles of retinoids stored in the cells and depleted in 'activated' cells; plasticity and heterogeneity of HSCs; relationship to stem cells and the stem cell niche; lineage of HSCs; the half life of HSCs in health and disease; immunological roles of HSCs; and interactions with other cell types, particularly LSECs and KCs. Subsequent speakers addressed some of these questions.

Tatiana Kisseleva (University of California, San Diego) reported that using genetic labelling techniques with the Rosa26EYFP/EYFP mice crossed to K19CreERT, or FSPCre, GFAPCre mice, there is no evidence of mesenchymal-to-epithelial transition to hepatocytes, cholangiocytes or oval cells, or of epithelial-to-mesenchymal transition to HSCs in liver fibrosis induced by CCl4 or bile duct ligation (BDL) (10).

Lina Lu (Cleveland Clinic) presented data on intriguing immunosuppressive activity of HSCs using an islet allograft transplantation model. Cotransplantation of islets with HSCs under the renal capsule caused accumulation of $CD11b^+CD11c^-$ cells and prevented rejection. Isolated $CD11b^+CD11c^-$ cells exhibited the phenotype of myeloid-derived suppressor cells (MDSC). Furthermore, HSC-conditioned dendritic cells showed the MDSC phenotype, inhibited T cell response via induction of activated T cell apoptosis and augmentation of Treg cell expansion, and attenuated ovalbumin-specific T cell induced liver damage *in vivo*.

Hydrophobic bile acids activate HSCs and this may underlie biliary liver fibrosis. This activation mechanism was described by Roland Reinehr (University of Düsseldorf) to entail NADPH oxidase complex (NOX)-derived reactive oxygen species (ROS) generation leading to Yes-dependent epidermal growth factor receptor (EGFR) phospho-activation and consequent ERK1/2 activation and cell proliferation (11). With additional stress with cycloheximide or hydrogen peroxide, Jun N-terminal kinase (JNK) was also activated, resulting in association of activated EGFR with CD95, CD95 tyrosine-phosphorylation, DISC formation and apoptosis, highlighting the role of JNK in switching between bile acid-induced HSC proliferation and apoptosis.

Heterogeneity of human HSCs was demonstrated by the expression of CD45, a haematopoietic marker. Chuansheng Wang (Mount Sinai, NY) reported that the majority of human HSCs sorted by fluorescence excited by UV 355 nm light was $CD45^+$. These cells are rich in lipid droplets, express glial fibrillary acidic protein (GFAP), alpha smooth muscle actin (α-SMA) and type I collagen, but also human leucocyte antigen (HLA)-DR and CD86, suggestive of antigen presentation function. On the other hand, $CD45^-$ HSCs are positive for GFAP and express α-SMA and type I collagen after culture activation but do not express high levels of HLA-DR or CD86. Multiple passages selectively expand the $CD45^+$ HSCs and sorted $CD45^+$ HSCs do not survive beyond 2–3 weeks in culture.

Leptin is a profibrogenic adipokine and activates HSCs, but detailed information on signalling remains lacking. Fabio Marra (University of Florence) discussed their finding on leptin-induced vascular endothelial growth factor (VEGF) upregulation and cell migration in a manner dependent on mammalian target of rapamycin (mTOR) (12). Leptin also induces hypoxia-inducible factor (HIF)-1α, an effect not mediated via mTOR, but by ROS generated from NOX. These results demonstrate leptin's angiogenic and chemotactic effects via mTOR and oxidant stress.

The final speaker for the HSC session was Norifumi Kawada (Osaka City University) who provided an update on cytoglobin (CYGB), which he isolated from activated rat HSC by proteomic and mass-spectrometry analyses in 2001 (13). CYGB has peroxidase activity towards hydrogen peroxide and fatty acid peroxides. It may possess tumour suppressor function as it is increased in cancer, and mice haplo-insufficient in this protein are more susceptible to carcinogen-induced liver tumourigenesis. However, α-SMA expression is increased in CYGB haploinsufficient mice subjected to experimental liver fibrosis, suggesting its possible antifibrogenic role. More functional and mechanistic analyses will clarify the significance of this protein with regards to HSC.

There were also excellent poster presentations on HSCs, including the genetic basis for susceptibility to liver fibrosis by Colleen Croniger (Case Western Reserve University), who suggested that the small region of mouse chromosome 17 contains susceptibility genes; Stephen Hill (Newcastle University) demonstrated that a soluble factor released from hepatic myofibroblasts activates nuclear factor κB (NF-κB) in monocytes *in vitro*; osteopontin-mediated profibrogenic signalling was described by Ariz Lopategi (Mount Sinai); and reduced expression of glutathione synthetic enzymes was shown in HSCs activated *in vitro* and *in vivo* by Komal Ramani (University of Southern California).

Innovative therapeutic targets of liver fibrosis

One of the key issues for identifying new targets for the treatment of liver fibrosis is the identification of the cell producing the fibrous scar. Although clinical and experimental research clearly identifies that myofibroblasts are the source of the extracellular matrix proteins, the origin of these myofibroblasts is still unclear. David Brenner (University of California, San Diego) reported that using novel transgenic mice to mark the cell fate of hepatic cells, progressively more studies support the concept that quiescent HSCs become activated during fibrogenesis to become the major source of the myofibroblasts responsible for the fibrous scar (10). Therefore, understanding the activation process of the HSC as well as developing techniques to target drug delivery to HSCs has become the focus of current research.

Derek Mann (Newcastle University) described that several signalling molecules and transcription factors

have been identified as critical in determining the quiescent vs activated phenotype of HSCs. NF-κB is the best-studied transcription factor that is required for HSC activation. The activity of NF-κB is continuously elevated in activated HSCs, and NF-κB activity is required for the survival of activated HSCs. Profibrogenic angiotensin II leads to phosphorylation of the p65 subunit of NF-κB at its Ser536 residue (14). General inhibitors of NF-κB including gliotoxin and sulphasalazine are antifibrotic in rodent models of hepatic fibrosis. Furthermore, a specific peptide mimetic that competitively inhibits Ser536 phosphorylation inhibits NF-κB activity and fibrosis.

Hide Tsukamoto (University of Southern California) discussed that peroxisome proliferator-activated receptor (PPAR)-γ is a critical transcription factor required to maintain HSCs in a quiescent state or to convert cultured activated HSCs back to a quiescent phenotype. Three different signalling pathways, Necdin, Wnt and Shh, all act on the PPARγ gene to induce epigenetic repression via MeCP2 recruitment and H3K27di/tri-methylation (15). Therefore, blocking the necdin, Wnt or Shh pathways results in increased expression of PPARγ and reversion to a quiescent phenotype.

Steven Dooley (University of Heidelberg) reported that the Notch pathway is highly induced in experimental and clinical liver fibrosis. Furthermore, a Notch inhibitory molecule, gamma secretase inhibitors, blocks the activation by transforming growth factor (TGF)-β of HSCs in culture. Notch signalling is closely associated with angiogenesis, and the relationship of increased Notch expression to the pathogenesis of hepatic fibrosis needs to be further studied.

Massimo Pinzani (University of Florence) discussed that many fibrogenic agonists such as platelet-derived growth factor (PDGF), leptin and angiotensin II, mediate their profibrogenic affects on HSCs by activating NADPH oxidase to produce reactive oxygen species. Therefore, many studies have used antioxidants to block rodent models of liver fibrosis. However, perhaps a more effective drug might be pirfenidone (16). This drug has both anti-oxidant and anti-inflammatory affects. Pirfenidone blocks many of the profibrogenic activities of activated HSCs. As this drug has already been approved for treatment in patients, it appears to be ready for a clinical trial in the therapy of liver fibrosis.

Krista Rombouts (University of Florence) discussed that in addition to secreting extracellular matrix proteins, chemokines and cytokines, a characteristic of activated HSCs is their increased rate of proliferation. Therefore, a new potential target for blocking liver fibrosis would be to inhibit HSC mitosis. Both myristoylated alanine-rich protein kinase c-substrate and aurora b kinase are required for the mitotic cycle of cultured HSCs, because use of siRNAs against either of these proteins resulted in decreased proliferation. Converting these observations into potential drugs would probably require the development of small molecules with similar activities.

If activated HSCs are the key cell synthesizing the fibrous scar, then developing drug delivery systems to target these cells specifically would be a key breakthrough in antifibrotic therapy. Klaas Poelstra (University of Groningen) reported that three drug delivery systems have been developed to target HSCs in rodent models of hepatic fibrosis. To target the mannose-6-phosphate/insulin-like growth factor 2 receptor (M6P/IGF-2R), drugs are coupled to mannose-6-phosphate human serum albumin (M6PHSA), which is taken up specifically in activated HSCs through this receptor. Recently, a ρ-kinase inhibitor and an angiotensin receptor blocker have been conjugated to M6PHSA and were used successfully in rodent models to attenuate hepatic fibrosis (17). The PDGF receptor is also upregulated on activated HSCs, leading to receptor expression levels that are much higher than on other cell types. Interferon-γ was conjugated to a peptide that binds the PDGF-β receptor. This drug inhibited liver fibrosis in a rodent model.

Yoshiro Niitsu (Sapporo Medical University) described that vitamin A-coupled liposomes have been used to carry siRNA to block a fibrogenic gene (18). The concept is that activated HSCs will take up the vitamin A liposome containing the siRNA by receptor-mediated uptake bound to retinol-binding protein. This technique has been used in culture and *in vivo* to direct the siRNA for the collagen chaperone p47 into activated HSCs, resulting in decreased hepatic fibrosis.

Isao Sakaida (Yamaguchi University, Ube, Japan) described follow-up studies to their earlier study that showed that autologous bone marrow infusion improved liver function in cirrhosis. In the studies presented, the effect of splenectomy was examined in experimental cirrhosis and in patients. In mice, splenectomy increased engraftment of bone marrow, decreased liver fibrosis and increased serum albumin. In patients, splenectomy improved serum albumin and prothrombin time and decreased type III procollagen-*N*-peptide 24 weeks after infusion of autologous bone marrow.

Fibromodulin is an extracellular matrix protein that plays a role in collagen fibril formation and matrix assembly. Elisabetta Mormone (Mt Sinai, New York) reported on their studies to determine whether fibromodulin modulates HSC production of collagen I in liver disease. These investigators found that not only collagen I but also fibromodulin expression is upregulated in cirrhotic and alcoholic patients. Fibromodulin increases in hepatocytes and sinusoids from mice with liver fibrosis induced by either drugs or BDL. Increased fibromodulin protein was observed in HepG2 cells overexpressing cytochrome P450 2E1 compared with control HepG2 cells suggesting that fibromodulin is induced by oxidative stress Finally, $Fmod^{-/-}$ mice showed reduced fibrosis compared with WT mice in the BDL model. These studies indicate that fibromodulin effects on collagen I deposition could contribute to liver fibrosis.

The posters in this section analysed several strong candidates for antifibrotic therapy, including matrix

metalloprotease (MMP)-13 by Yutaka Inagaki (Tokai University), heparin binding-epidermal growth factor by David R. Brigstock (Ohio State University), PPARδ agonists by Bernd Schnabl (University of California, San Deigo), the herbal drug Yan-Gin-Wan by Hide Tsukamoto (University of Southern California) and the EZH2 inhibitor 3-deazaneplanocin A (DZNep) by Jelena Mann (Newcastle University). Furthermore, Soichi Kojima (RIKEN) demonstrated that the TGFβ propeptide LAP is a potential new serum biomarker for ongoing liver fibrosis.

Progenitor cells, liver development and regeneration

Randy Moon (University of Washington) gave a state-of-the-art lecture on the role of Wnt signalling in regeneration and regenerative medicine. He described Wnt signalling as tightly regulated, both spatially and temporally, and noted that deviation from homeostatic ranges has been linked to diseases (19). Responses of cells and tissues are context dependent, so that the same pathway can develop very different structures in early development. Elevated β-catenin signalling can enhance liver development, but too much signalling has adverse consequences. The Wnt-β-catenin pathway is activated during liver regeneration and activity correlates with elevated cell proliferation and accelerated liver regeneration. β-catenin signalling is an evolutionarily conserved response to acute injury and likely involved in reestablishing tissue homeostasis.

Atsushi Miyajima (University of Tokyo) reported on the role of non-parenchymal cells in liver development and in liver regeneration in the adult mouse (20). In liver development, mesothelial cells support proliferation of fetal hepatocytes, whereas in the adult mouse periportal mesenchymal cells provide a niche for oval cells.

Cell lineage tracing studies performed by Kinji Asahina (University of Southern California) demonstrate that in liver development, HSCs and perivascular mesenchymal cells are derived from mesothelial cells and submesothelial cells on the liver surface (21). In contrast, LSECs, KCs and hepatoblasts do not derive from mesothelial or submesothelial cells.

Dieter Häussinger (Heinrich Heine University of Düsseldorf) described the expression of multiple stem/progenitor cell markers and pluripotency genes in HSC, data on their *in vitro* and *in vivo* differentiation capacity, suggestive for a role of HSC as stem/progenitor cells. The stem cell niche for the HSC stem cell is the space of Disse. Niche factors include Wnt7a/b, Wnt10b and Jag1 from hepatocytes, SDF1 from LSECs, collagen IV and laminin, and sympathetic innervation.

Yuji Yokouchi (Kumamoto University) discussed the reciprocal relationship between hepatoblasts and blood vessels during chick embryogenesis. Neurturin from the endothelium of the ductus venosus and Wnt9a in the walls of the hepatic sinusoid co-operatively control formation of hepatoblast cords (22, 23). Conversely forced expression of Noggin, a bone morphogenic proteins (BMP) antagonist, decreases endothelial number beneath the hepatic mesothelium.

Lola Reid (University of North Carolina) described the presence of multipotent stem cells in the liver and the extrahepatic biliary tree. These cells express endodermal transcription factors, stem/progenitor cell markers and, weakly and variably, adult liver, bile duct and pancreatic genes. In three dimensional (3D) culture and depending on the culture conditions, these cells can form bile ducts, hepatocytes and islet-like structures (24).

Shi Yin (NIAAA) presented their studies that demonstrates that activated type I natural killer T cells accumulate in the liver after partial hepatectomy and inhibit liver regeneration through production of interferon-γ and STAT1 signalling.

Kenjiro Wake (Tsurumi University and Minophagen Pharmaceutical Co.) presented electron microscopy evidence that mesenchymal cells provide the initial lining of the primitive sinusoids and hepatic vessels in the embryo with subsequent adherence of endothelial cells formed by circulating angioblasts.

Lin Wang (University of Southern California) reported that bone marrow progenitor cells of LSECs respond to partial hepatectomy with increased proliferation within the bone marrow, increased mobilization to the circulation, and increased engraftment within the liver. Influx of these LSEC progenitor cells into the liver promotes hepatocyte proliferation and is necessary for normal liver regeneration.

José Pérez-Pomares (University of Malaga) discussed that coelomic epithelial (mesothelial) cells, particularly from the septum transversum, are critical to the development of the liver cytoarchitecture (25). These mesothelial cells undergo epithelial-to-mesenchymal transition, giving rise to highly invasive, migratory cells, including HSCs. These mesothelial cells express the Wilms tumour gene (Wt1), a mesodermal gene required for the development of a variety of organs. In the absence of Wt1, retinoid signalling is disrupted, sinusoid development is abnormal, and the liver becomes reduced in size, also displaying an anomalous lobulation.

Chronic Notch signalling is necessary to maintain an intact intrahepatic bile duct network in mice. Stacey Huppert (Vanderbilt University) discussed three possible reasons for this: developmental lack of bile duct formation, post-natal lack of branching, or inability to maintain formed ducts and described studies to examine these possibilities (26). Using resin casting and microcomputed tomography studies of a conditional Notch loss mouse model, their studies suggest that there is a structural disconnect in the intrahepatic bile ducts that can only be visualized in a 3D but not in a two dimensional system and that this is possibly because of obstruction. Thus, these findings suggest that chronic loss of Notch signalling is necessary to maintain communicating, formed intrahepatic ducts.

Toru Nakamura (Korume University) described experiments in which human $CD34^+$ cells were injected

into the spleen of immunodeficient rats halfway through a course of CCl_4 to induce hepatic fibrosis (27). The transplanted human cells differentiated into vascular endothelial cells, LSECs and vascular smooth muscle cells. The transplanted cells increased HGF, EGF, TGFβ and VEGF in liver tissue, increased hepatocyte proliferation, and prevented liver fibrosis in this model of CCl_4-induced fibrosis.

Andrew Cox (Harvard Medical School) discussed the role of nitric oxide in liver development of zebrafish. Two thousand six hundred and forty compounds were screened to examine the effect on liver development. Nitrous oxide (NO) donors increased liver size, whereas an NO antagonist decreased liver size. The effect of the NO antagonist was confirmed using a morpholino knockdown of nitric oxide synthase (NOS)1, which also decreased liver size, whereas knockdown of NOS2 had no effect.

Didier Stainier (UCSF) gave a state-of-the-art lecture on cell-cell interactions in the developing zebrafish liver. He described single-cell-lineage tracing to analyse hepatic vs pancreatic fate decision. In the early embryo, endodermal cells located at least two cells away from the midline can give rise to both liver and pancreas, whereas cells closer to the midline give rise to pancreas and intestine (28). Bmp2b regulates liver fate. It is expressed highly in the lateral plate and this induces endodermal cells to become liver. Overexpression of Bmp2b in the medially located endodermal cells drives cells fated to become pancreas to become liver.

In the poster session, Kristen Alexa (Harvard Medical School) described the role of vitamin D3 in early liver formation in the zebrafish. Andrew Axon (Newcastle University) reported that oestrogen increased proliferation of progenitor cells isolated from the rat biliary tree. Steven Dooley (University of Heidelberg) described a decrease in cytokeratin-19 positive cells (i.e. putative hepatic progenitor cells) in hepatitis B patients treated with interferon (IFN)-γ and an increase in cytokeratin-19 positive cells in IFN-γ and IFN regulator factor-1 knockout mice treated with 3,5-diethoxycarbonyl-1,4-dihydrocollidine, suggesting that IFN-γ inhibits hepatic progenitor cell activation in chronic liver damage. Steven Dooley also reported that TGFβ signalling correlated with cytokeratin-19 expression in hepatitis B patients, but not schistosomiasis-associated liver damage, suggesting that TGFβ signalling in progenitor cell activation is aetiology dependent. Ange-Clarisse Dusabineza (Catholic University of Louvain) reported that liver progenitor cells are activated in a rat model of chronic liver disease, but are not fully able to restore liver mass after partial hepatectomy or to maintain liver function. Regina Espanol (Catholic University of Louvain) presented data in a liver disease model that myofibroblasts ($FSP1^+$ cells) associated with progenitor cells do not show evidence of epithelial–mesenchymal transition from cytokeratin-19^+ liver progenitor cells. Emma Fairhall (Newcastle University) reported that a hepatic progenitor cell line (B-13) showed significantly enhanced transdifferentiaton to a hepatocyte phenotype in the presence of myofibroblasts. Kouichi Hasegawa (University of Southern California) reported that the monoclonal antibody, GCTM-5, which reacts with fetal human hepatoblasts also recognizes human hepatic progenitors/stem cells. Yoshiya Ito (Kitasato University) described impaired liver regeneration in VEGF receptor-1 knockout mice given toxic doses of acetaminophen (29). Tomokazu Matsuura (Jikei University) reported that implantable artificial liver tissue showed superior function when implanted in the omentum or kidney (30). Mayako Morii (Akita University) described the use of a Japanese lamprey as a model to study human biliary atresia (31). Michele Pritchard (Cleveland Clinic) reported in an Egr-1 knockout mouse model that hepatocyte proliferation after CCl_4 exposure was delayed and injury was greater (32). Chise Tateno (Hiroshima University) compared hepatocyte sensitivity to TNF-α in severe combined immunodeficiency (SCID) mice vs SCID mice transplanted with human hepatocytes and found that human hepatocytes are resistant to TNF-α. Noemi van Hul (Catholic University of Leuven) reported that in a liver injury model KCs promote liver progenitor cell migration into the parenchyma. Karen Wallace (Newcastle University) presented their data that the glucocorticoid-dependent transdifferentiation of the B-13 rat pancreatic acinar cell line into hepatocytes is dependent on transient suppression of Wnt signalling (33).

Liver sinusoidal endothelial cells

Liver sinusoidal endothelial cells have a distinct morphological phenotype among endothelial cells with open fenestrae and lack of an organized basement membrane. These cells are among the most active scavenger cells in the body, and their unique functional and structural features as specialized endocytes are important for homeostasis of the blood. Under pathological conditions, such as alcohol injury or cirrhosis, the number and size of fenestrae have been shown to decrease with concomitant appearance of a basal lamina. However, relatively less is understood at the molecular level about the mechanism of defenestration of LSEC, the role of LSECs in the clearance of virus and lipoproteins, and recruitment of white cells into inflamed liver.

In this symposium, Guanhua Xie (University of Southern California) showed that NO-mediated regulation of the LSEC phenotype occurs through the cyclic guanosine monophosphate, soluble guanylate cyclase (sGC), protein kinase G pathway. In a thioacetamide model of cirrhosis, administration of an activator of sGC (Bay 60-2770) completely reversed capillarization without directly altering HSC activation. Once capillarization was reversed, HSC activation reversed and cirrhosis regressed, suggesting that the normalized LSEC phenotype attenuates cirrhosis. Administration of the sGC activator also prevented progression of cirrhosis in the face of ongoing thioacetamide treatment.

Cyril Géraud (University of Heidelberg) identified liver endothelial differentiation-associated protein-1 (Leda-1), a single-pass transmembrane protein with a molecular mass 26 kDa that is homologous to Ajap-1/Shrew1 in polarized cells (34). Leda-1 is preferentially localized to the abluminal surface of LSECs, sorts basolaterally to E-cadherin-positive adherens junctions when overexpressed in Madin Darby canine kidney cells, and may regulate cell–cell and/or cell–matrix interactions in LSECs.

Previous studies have shown that stabilin-1 and stabilin-2 scavenger receptors are expressed on LSECs. Ruomei Li (University of Tromsø) reported that the mildly oxidized form of low-density lipoprotein (LDL) was endocytosed by LSECs, and not by KCs, preferentially utilizing stabilin-1 (35). These results suggest the importance of stabilins on LSECs in the uptake of oxidized LDL, and the implications of these receptors in the development of atherosclerosis. Poster presentations from the Tromsø group also showed that LSECs express LDL-receptor-related protein (LRP-1), which is also expressed in liver parenchymal cells. The identification of LRP-1 on LSECs provides a new mechanism for the uptake of pro-atherogenic ligands, such as APOE-rich remnant lipoproteins. Additional studies from the Tromsø group showed that nidogen, a component of basement membrane, was cleared by LSECs from the blood by endocytosis through stabilins.

Sarah Mitchell (University of Sydney) described studies in the isolated perfused liver that showed that age-related loss of LSEC fenestration reduced the apparent volume of distribution and the transfer of acetaminophen across the sinusoidal endothelium. This is consistent with impaired access of acetaminophen to the space of Disse, which has implications for pharmacokinetics and toxicity of acetaminophen in old age.

Studies presented by Clark Anderson (Ohio State University) demonstrated that LSECs play a much greater role in the endocytosis and clearance of blood-borne adenovirus than KCs.

Utilizing metabolomic analysis Makoto Suematsu (Keio University) showed that carbon monoxide (CO), a gaseous product of heme oxygenase, altered microvascular responses in the circulation, and identified cystathionine β-synthase as a novel CO-responsive enzyme (36). Paul Kubes (University of Calgary) presented data that recruitment of white cells into the inflamed liver differs from other organs, as sinusoids recruit the majority of these cells. In liver sinusoids, selectins do not play a role in the recruitment of white cells, while integrins contribute to cell recruitment under some conditions. Spinning disc confocal intravital microscopy was used to assess the function of integrins in the adhesion of neutrophils in sinusoids. Studies revealed that Mac-1 was necessary for neutrophil adhesion and crawling under local inflammatory stimuli in sinusoids (37). However, during endotoxaemia adhesion of neutrophils in sinusoids was shown to be CD-44 dependent and integrin independent. These studies provide a new mechanism for intravascular trapping of neutrophils in the sinusoids.

Vijay Kalra (University of Southern California) reported on the innate immunity of LSECs in response to ethanol feeding and *in vitro*. Their studies showed that ethanol induced the mRNA expression of monocyte chemotactic protein-1, MIP-1β, regulated upon activation, normal T cell expressed and secreted (RANTES), endothelin (ET)-1 and HIF-1α in LSECs. The upregulation of ET-1 and RANTES by ethanol in endothelial cells was shown to involve HIF-1α, and HIF-1α was negatively regulated by miR-199, which target the 3'UTR of HIF-1α mRNA (38).

In the poster session, Robin Fraser (University of Otago, New Zealand) presented a novel method for delivery of siRNAs to hepatocytes *in vivo*, utilizing lipid nanoparticles of sizes (< 100 nm), which could pass through the fenestrae. David Le Couteur (University of Sydney) presented a novel 3D structural illumination light microscopy tool to investigate functional information about fenestration with great clarity and spatial resolution (39). Aisling McMohan's (University of Sydney) data showed that anti-oxidant therapy could reverse phenotypic changes in fenestrae induced by oxidative stress in diabetes. Sugiru Pak (University of Tsukuba) showed that during liver ischaemia reperfusion injury platelets adhere to LSECs (40). Studies of Leela Paris (Indiana University) showed that LSEC function to transcytose platelets to hepatocytes during xenotransplantation induced thrombocytopenia (41).

Cancer biology

Inflammation is linked to tissue repair and tumourigenesis in the liver (42). In this session, four speakers presented molecular mechanisms of liver injury, tumourigenesis and cancer metastasis mediated by alcohol, hepatitis virus and cell–cell interactions.

Alcohol intake is known to synergistically increase the risk of HCC in hepatitis C virus (HCV)-infected patients (43). However, a molecular link between alcohol and HCV remained to be determined. Keigo Machida (University of Southern California) found that HCV NS5A protein induces TLR4, which is activated by LPS associated with alcohol intake. TLR4 signalling induces Nanog, a stem cell marker, in $CD133^{+}$ $CD49f^{+}$ cancer stem cells from alcohol-fed NS5A transgenic mice (44). Nanog-dependent expression of Yap1 and Igf2bp3 suppresses TGFβ/Smad3 signalling in the cancer stem cells. Both TLR4 signalling and suppression of TGFβ signalling appear to be linked to HCC cancer stem cells.

TGFβ-activated kinase 1 (TAK1) is a member of the mitogen-activated protein kinase (MAP3K) family and a component in the signalling of TLRs and receptors for IL-1, TNF-α and TGFβ. TAK1 activates both NF-κB and JNK, which have opposite effects on cell survival and death. Sayaka Inokuchi (University of California San

Diego) deleted TAK1 in hepatocytes (TAK1ΔHep) and examined its role in mouse livers. The TAK1ΔHep mice, which lacked activation of both NF-κB and JNK pathways, showed increased hepatocyte death, inflammation and fibrosis, which ultimately resulted in tumourigenesis (45). Hepatocyte death stimulates KCs to produce TNF-α, which causes further hepatocyte death in the TAK1Δ-Hep mice. Indeed, deletion of TNFR1 reduced the effects of TAK1ΔHep. Deletion of TLR4 also reduced inflammation and fibrosis. TNFR and TLR4 might be therapeutic targets for preventing liver fibrosis and carcinogenesis.

During metastasis, cancer cells interact with hepatic sinusoids and modulate the immune response. Fernando Vidal-Vanaclocha (CEU-San Pablo University) showed that colon carcinoma cells induce mannose receptor on LSECs. Blocking the mannose receptor on LSECs increased cytotoxicity of lymphocytes to the carcinoma cells (46). In addition to LSECs, cancer cells also activate HSCs. As reviewed by the presenter, discoidin domain receptor 2 (DDR2) is a tyrosine kinase receptor expressed in activated HSCs. DDR2-null mice had increased metastasis of colon carcinoma cells. Conditioned medium from the carcinoma cells increased expression of mRNAs associated with immune tolerance, HSC activation and cell adhesion in HSCs lacking DDR2 gene. These data suggest that tumour-activated LSECs and HSCs provide a prometastatic microenvironment in the liver.

Metastatic carcinoma cells elicit an inflammatory response that promotes expression of cell adhesion molecules on LSECs and metastasis (47). Pnina Brodt (McGill University) analysed the role of TNFR2 in promoting tumour-LSEC interactions. TNFR2-null mice had reduced recruitment of neutrophils and reduction of metastasis. Although the inhibitory effect was only seen in females, a tumour-LSEC interaction via TNFR2 seems to be a rate-limiting step for liver metastasis. Manipulation of liver-specific antitumour immunity might prevent cancer metastasis in the liver.

There were eight posters in the Cancer Biology session. Nigel Bird (University of Sheffield) reported that expression of uPA, uPAR and MMP-2 are not required for invasion of colon cancer liver metastasis when the cancer cells are not associated with a desmoplastic growth pattern (48). Matt Cave (University of Louisville) presented evidence that haemangiosarcoma develops after a long period of occupational vinyl chloride exposure (49). Jian-Chang Liu et al. (University of Southern California) isolated liver cancer stem cells from their mouse model (44) and showed that HSCs might provide a niche for the stem cells. Excessive body weight is associated with an increased risk for HCC. Douglas Feldman (University of Southern California) found that leptin stimulates the expression of pluripotency factors in liver cancer stem cells. MAID is a helix-loop-helix transcription factor and controls proliferation and tumourigenesis (50). Koichi Fujisawa (Yamaguchi University) showed increased expression of MAID in the hepatic neoplasms induced by diethylnitrosamine treatment in Zebrafish. Hua Wang (NIAAA) reported that myeloid-specific STAT3 knockout mice enhance antitumour immunity and decrease in carcinogenesis (51). Shuping Zhong's (University of Southern California) data showed that alcohol increases transcription activity of RNA polymerase III via JNK1 that may be required for oncogenic transformation (52).

Comparative anatomy and evolutional biology

The function of the hepatic reticuloendothelial system has been attributed to the mononuclear phagocyte system (MPS) mainly governed by KCs. However, LSECs remove colloid waste and soluble macromolecules from the blood by non-phagocytic, receptor-mediated endocytosis, while KCs clear larger particles (53). Bård Smedsrød (University of Tromsø) reported that this dual cell principle of waste clearance is central in all animals (scavenger endothelial cells+MPS in vertebrates, and haemocytes+nephrocytes in invertebrates).

Polar bears (Ursus maritimus) store a large amount of vitamin A in HSCs (54). Haruki Senoo (Akita University) reported that a polar bear kept in a zoo under a standard animal diet low in vitamin A lost storage of vitamin A lipids in HSCs compared with polar bears in the wild. The liver from the bear in the zoo developed fibrosis, implying a protective role of vitamin A in the liver.

Conclusion

The symposium demonstrated that research on cells of the hepatic sinusoid is flourishing. Studies examined the role of non-parenchymal cells, inflammatory cells, progenitor cells and parenchymal cells in liver disease, signalling pathways potentially amenable to therapeutic intervention, cell origin in liver development, stem and progenitor cells in the adult liver, novel cell functions, and crosstalk between non-parenchymal cells and between non-parenchymal and parenchymal cells in liver development and liver disease.

The diversity and sophistication of research presented at the 15th ISCHS symposium reflects the continuing strength of Hepatology research in general. The small meeting format of this bi-annual meeting has created a community over the years of investigators of non-parenchymal cell biology and pathophysiology. This most recent symposium included investigators across a wider spectrum of Hepatology and the resulting cross-fertilization and future collaborations will undoubtedly enrich the field.

To avoid other small format liver meetings held bi-annually in the fall, the timing of the next meeting has been moved up by one year. The 16th ISCHS meeting will be held in Florence, Italy on 22–24 September 2011.

References

1. Miller AM, Horiguchi N, Jeong WI, Radaeva S, Gao B. Molecular mechanisms of alcoholic liver disease: innate immunity and cytokines. *Alcohol Clin Exp Res* 2011; **35**: 787–93.

2. Inokuchi S, Tsukamoto H, Liu Z, Brenner DA, Seki E. Toll-like receptor 4 induces alcoholic liver disease through bone marrow-derived and endogenous liver cells. *Alcohol Clin Exp Stud* 2010; **107**: 844–9.
3. Teixeira-Clerc F, Belot MP, Manin S, *et al.* Beneficial paracrine effects of cannabinoid receptor 2 on liver injury and regeneration. *Hepatology* 2010; **52**: 1046–59.
4. Martinez-Clemente M, Ferre N, Titos E, *et al.* Disruption of the 12/15-lipoxygenase gene (Alox15) protects hyperlipidemic mice from nonalcoholic fatty liver disease. *Hepatology* 2010; **52**: 1980–91.
5. Aoyama T, Inokuchi S, Brenner DA, Seki E. CX3CL1-CX3CR1 interaction prevents carbon tetrachloride-induced liver inflammation and fibrosis in mice. *Hepatology* **52**: 1390–400.
6. Ishikawa S, Ikejima K, Yamagata H, *et al.* CD1d-restricted natural killer T cells contribute to hepatic inflammation and fibrogenesis in mice. *J Hepatol* 2011; doi:10.1016/j.jhep.2010.08.022 [Epub ahead of print].
7. Miura K, Kodama Y, Inokuchi S, *et al.* Toll-like receptor 9 promotes steatohepatitis by induction of interleukin-1beta in mice. *Gastroenterology* 2010; **139**: 323–34, e7.
8. Williams CD, Farhood A, Jaeschke H. Role of caspase-1 and interleukin-1beta in acetaminophen-induced hepatic inflammation and liver injury. *Toxicol Appl Pharmacol* 2010; **247**: 169–78.
9. Friedman SL. Hepatic stellate cells: protean, multifunctional, and enigmatic cells of the liver. *Physiol Rev* 2008; **88**: 125–72.
10. Scholten D, Osterreicher CH, Scholten A, *et al.* Genetic labeling does not detect epithelial-to-mesenchymal transition of cholangiocytes in liver fibrosis in mice. *Gastroenterology* 2010; **139**: 987–98.
11. Sommerfeld A, Reinehr R, Haussinger D. Bile acid-induced epidermal growth factor receptor activation in quiescent rat hepatic stellate cells can trigger both proliferation and apoptosis. *J Biol Chem* 2009; **284**: 22173–83.
12. Aleffi S, Navari N, Delogu W, *et al.* The Mammalian target of rapamycin mediates the angiogenic effects of leptin in human hepatic stellate cells. *Am J Physiol Gastrointest Liver Physiol* 2011; doi:10.?1152/?ajpgi.?00047.?2010 [Epub ahead of print].
13. Kawada N, Kristensen DB, Asahina K, *et al.* Characterization of a stellate cell activation-associated protein (STAP) with peroxidase activity found in rat hepatic stellate cells. *J Biol Chem* 2001; **276**: 25318–23.
14. Oakley F, Teoh V, Ching ASG, *et al.* Angiotensin II activates I kappaB kinase phosphorylation of RelA at Ser 536 to promote myofibroblast survival and liver fibrosis. *Gastroenterology* 2009; **136**: 2334–44, e1.
15. Hattori K, Dias S, Heissig B, *et al.* Vascular endothelial growth factor and angiopoietin-1 stimulate postnatal hematopoiesis by recruitment of vasculogenic and hematopoietic stem cells. *J Exp Med* 2001; **193**: 1005–14.
16. Grizzi F. Pirfenidone: a potential therapeutic option in the treatment of liver fibrosis. *Clin Exp Pharmacol Physiol* 2009; **36**: 961–2.
17. Colina F, Lopez-Carreira M, Moreno E, *et al.* A clinopathologic review of 8 liver graft primary nonfunctions. *Hepatogastroenterology* 1995; **42**: 212–21.
18. Sato Y, Murase K, Kato J, *et al.* Resolution of liver cirrhosis using vitamin A-coupled liposomes to deliver siRNA against a collagen-specific chaperone. *Nat Biotechnol* 2008; **26**: 431–42.
19. Goessling W, North TE, Lord AM, *et al.* APC mutant zebrafish uncover a changing temporal requirement for wnt signaling in liver development. *Dev Biol* 2008; **320**: 161–74.
20. Onitsuka I, Tanaka M, Miyajima A. Characterization and functional analyses of hepatic mesothelial cells in mouse liver development. *Gastroenterology* 2010; **138**: 1525–35, e1–6.
21. Asahina K, Zhou B, Pu WT, Tsukamoto H. Septum transversum-derived mesothelium gives rise to hepatic stellate cells and perivascular mesenchymal cells in developing mouse liver. *Hepatology* 2010; **53**: 983–95.
22. Matsumoto K, Miki R, Nakayama M, Tatsumi N, Yokouchi Y. Wnt9a secreted from the walls of hepatic sinusoids is essential for morphogenesis, proliferation, and glycogen accumulation of chick hepatic epithelium. *Dev Biol* 2008; **319**: 234–47.
23. Tatsumi N, Miki R, Katsu K, Yokouchi Y. Neurturin-GFRalpha2 signaling controls liver bud migration along the ductus venosus in the chick embryo. *Dev Biol* 2007; **307**: 14–28.
24. Wang Y, Cui CB, Yamauchi M, *et al.* Lineage restriction of human hepatic stem cells to mature fates is made efficient by tissue-specific biomatrix scaffolds. *Hepatology* 2011; **53**: 293–305.
25. Ijpenberg A, Perez-Pomares JM, Guadix JA, *et al.* Wt1 and retinoic acid signaling are essential for stellate cell development and liver morphogenesis. *Dev Biol* 2007; **312**: 157–70.
26. Sparks EE, Perrien DS, Huppert KA, Peterson T, Huppert SS. Defects in hepatic Notch signaling result in disruption of the communicating intrahepatic bile duct network in mice. *Dis Model Mech* 2011; **4**: 359–67.
27. Nakamura T, Torimura T, Sakamoto M, *et al.* Significance and therapeutic potential of endothelial progenitor cell transplantation in a cirrhotic liver rat model. *Gastroenterology* 2007; **133**: 91–107, e1.
28. Chung WS, Shin CH, Stainier D. Bmp2 signaling regulates the hepatic versus pancreatic fate decision. *Dev Cell* 2008; **15**: 738–48.
29. Kato T, Ito Y, Hosono K, *et al.* Vascular endothelial growth factor receptor-1 signaling promotes liver repair through restoration of liver microvasculature after acetaminophen hepatotoxicity. *Toxicol Sci* 2010; **120**: 218–29.
30. Saito R, Ishii Y, Ito R, *et al.* Transplantation of liver organoids in the omentum and kidney. *Artif Organs* 2011; **35**: 80–3.
31. Morii M, Mezaki Y, Yamaguchi N, *et al.* Onset of apoptosis in the cystic duct during metamorphosis of a Japanese lamprey, Lethenteron reissneri. *Anat Rec (Hoboken)* 2010; **293**: 1155–66.

32. Pritchard MT, Cohen JI, Roychowdhury S, Pratt BT, Nagy LE. Early growth response-1 attenuates liver injury and promotes hepatoprotection after carbon tetrachloride exposure in mice. *J Hepatol* 2010; **53**: 655–62.
33. Wallace K, Marek CJ, Hoppler S, Wright MC. Glucocorticoid-dependent transdifferentiation of pancreatic progenitor cells into hepatocytes is dependent on transient suppression of WNT signalling. *J Cell Sci* 2010; **123**(Part 12): 2103–10.
34. Geraud C, Schledzewski K, Demory A, *et al*. Liver sinusoidal endothelium: a microenvironment-dependent differentiation program in rat including the novel junctional protein liver endothelial differentiation-associated protein-1. *Hepatology* 2010; **52**: 313–26.
35. Li R, Oteiza A, Sorensen KK, *et al*. Role of Liver Sinusoidal Endothelial Cells and Stabilins in Elimination of Oxidized Low-Density Lipoproteins. *Am J Physiol-Gastrointest Liver Physiology* 2010; **300**: G71–81.
36. Shintani T, Iwabuchi T, Soga T, *et al*. Cystathionine beta-synthase as a carbon monoxide-sensitive regulator of bile excretion. *Hepatology* 2009; **49**: 141–50.
37. Menezes GB, Lee WY, Zhou H, *et al*. Selective down-regulation of neutrophil Mac-1 in endotoxemic hepatic microcirculation via IL-10. *J Immunol* 2009; **183**: 7557–68.
38. Yeligar SM, Machida K, Tsukamoto H, Kalra VK. Ethanol augments RANTES/CCL5 expression in rat liver sinusoidal endothelial cells and human endothelial cells via activation of NF-kappa B, HIF-1 alpha, and AP-1. *J Immunol* 2009; **183**: 5964–76.
39. Cogger VC, Mcnerney GP, Nyunt T, *et al*. Three-dimensional structured illumination microscopy of liver sinusoidal endothelial cell fenestrations. *J Struct Biol* 2010; **171**: 382–8.
40. Pak S, Kondo T, Nakano Y, *et al*. Platelet adhesion in the sinusoid caused hepatic injury by neutrophils after hepatic ischemia reperfusion. *Platelets* 2010; **21**: 282–8.
41. Burlak C, Paris LL, Chihara RK, *et al*. The fate of human platelets perfused through the pig liver: implications for xenotransplantation. *Xenotransplantation* 2010; **17**: 350–61.
42. Grivennikov SI, Greten FR, Karin M. Immunity, inflammation, and cancer. *Cell* 2010; **140**: 883–99.
43. Schiff ER, Ozden N. Hepatitis C and alcohol. *Alcohol Res Health* 2003; **27**: 232–9.
44. Machida K, Tsukamoto H, Mkrtchyan H, *et al*. Toll-like receptor 4 mediates synergism between alcohol and HCV in hepatic oncogenesis involving stem cell marker Nanog. *Proc Natl Acad Sci USA* 2009; **106**: 1548–53.
45. Inokuchi S, Aoyama T, Miura K, *et al*. Disruption of TAK1 in hepatocytes causes hepatic injury, inflammation, fibrosis, and carcinogenesis. *Proc Natl Acad Sci USA* 2010; **107**: 844–9.
46. Arteta B, Lasuen N, Lopategi A, *et al*. Colon carcinoma cell interaction with liver sinusoidal endothelium inhibits organ-specific antitumor immunity through interleukin-1-induced mannose receptor in mice. *Hepatology* 2010; **51**: 2172–82.
47. Khatib AM, Auguste P, Fallavollita L, *et al*. Characterization of the host proinflammatory response to tumor cells during the initial stages of liver metastasis. *Am J Pathol* 2005; **167**: 749–59.
48. Illemann M, Bird N, Majeed A, *et al*. Two distinct expression patterns of urokinase, urokinase receptor and plasminogen activator inhibitor-1 in colon cancer liver metastases. *Int J Cancer* 2009; **124**: 1860–70.
49. Cave M, Falkner KC, Ray M, *et al*. Toxicant-associated steatohepatitis in vinyl chloride workers. *Hepatology* 2010; **51**: 474–81.
50. Takami T, Terai S, Yokoyama Y, *et al*. Human homologue of maid is a useful marker protein in hepatocarcinogenesis. *Gastroenterology* 2005; **128**: 1369–80.
51. Wang H, Park O, Lafdil F, *et al*. Interplay of hepatic and myeloid signal transducer and activator of transcription 3 in facilitating liver regeneration via tempering innate immunity. *Hepatology* 2010; **51**: 1354–62.
52. Zhong S, Machida K, Tsukamoto H, Johnson DL. Alcohol Induces RNA Polymerase III-dependent Transcription through c-Jun by Co-regulating TATA-binding Protein (TBP) and Brf1 Expression. *J Biol Chem* 2011; **286**: 2393–401.
53. Elvevold K, Smedsrød B, Martinez I. The liver sinusoidal endothelial cell: a cell type of controversial and confusing identity. *Am J Physiol Gastrointest Liver Physiol* 2007; **294**: 391–400.
54. Higashi N, Imai K, Sato M, *et al*. Intralobular distribution of vitamin a-storing lipid droplets in hepatic stellate cells with special reference to polar bear and arctic fox. *Comp Hepatol* 2004; **3**(Suppl. 1): S16.