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REVIEW ARTICLE

Hepatic sinusoidal cells in health and disease: update from the 14th International Symposium

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Abstract

This review aims to give an update of the field of the hepatic sinusoid, supported by references to presentations given at the 14th International Symposium on Cells of the Hepatic Sinusoid (ISCHS2008), which was held in Tromsø, Norway, August 31–September 4, 2008. The subtitle of the symposium, 'Integrating basic and clinical hepatology', signified the inclusion of both basal and applied clinical results of importance in the field of liver sinusoidal physiology and pathophysiology. Of nearly 50 oral presentations, nine were invited tutorial lectures. The authors of the review have avoided writing a 'flat summary' of the presentations given at ISCHS2008, and instead focused on important novel information. The tutorial presentations have served as a particularly important basis in the preparation of this update. In this review, we have also included references to recent literature that may not have been covered by the ISCHS2008 programme. The sections of this review reflect the scientific programme of the symposium (http://www.ub.uit.no/munin/bitstream/10037/1654/1/book.pdf):
1. Liver sinusoidal endothelial cells.
2. Kupffer cells.
3. Hepatic stellate cells.
4. Immunology.
5. Tumor/metastasis.
Symposium abstracts are refered to by a number preceded by the letter A.

Liver sinusoidal endothelial cells

Fenestrations

In his key note lecture (A1), Eddie Wisse underlined the fact that electron microscopy is still the only method of observing fenestrations in the liver sinusoidal endothelial cells (LSEC) and that sinusoidal cells of different mammalian species are amazingly similar in fine structure. He further provided information that pore size varies among species and strains and between periportal and perivenous areas of the sinusoid. After isolation, the porosity of LSECs decreases from approximately 10% at 6 h to 1% at 48 h (1) (A9). Porosity is influenced by the isolation techniques (2), the culture conditions and the presence of vascular endothelial growth factors (VEGFs) (3). Potential LSEC lines such as SKHep1 (4) might overcome some of these issues. Fenestrations are supported by actin cytoskeletal filaments, and disruption of the cytoskeleton is associated with a dramatic increase in porosity. Cellular signals involved in the regulation of actin, such as cortactin and transforming growth factor (TGF)-β1 (A15), and the Rho-like GTPases (5) influence porosity. VEGF produced by hepatocytes is probably the key cytokine involved in the regulation of LSEC fenestration mediated by actions on VEGF-R2 expressed on LSEC (6). The role of fenestrations in the bi-directional transfer of substrates between hepatocytes and sinusoidal blood is now well established (7) (A1, 13). Defenestration impairs the hepatic disposition of lipoproteins, albumin-bound drugs and other particulate substrates (7, 8) (A11, A13) and potentially impacts on T-cell interactions with hepatocytes (9). In studies using adenoviral delivery of transgene DNA, uptake of the transgene in hepatocytes correlated strongly with the LSEC pore size. The size of the adenovirus particle is 93 nm, with protruding fibres of 30 nm. Thus, the use of adenoviral-mediated gene therapy in humans may be difficult owing to the small LSEC pore size (103–107 nm) (10).

Scavenger function

Endocytic rate and capacity of LSECs are probably the highest known of any cell type in the human body. Peter McCourt presented an update on endocytosis receptors in these cells (A2). The cells carry three major types of endocytosis receptors to keep the blood clean.

The liver sinusoidal endothelial cell mannose receptor

It is known that mannose receptor (MR) clearance of several blood-borne soluble macromolecules carrying mannose in the ultimate position is carried out mainly in the LSECs, but not in the Kupffer cells (KCs) (11). Studies including MR-deficient knockout (KO) mice showed that the clearance of denatured collagen is MR mediated (12). Using the same KO mice showed that LSEC MR mediates the import of blood-borne lysosomal enzymes for re-use in the endo/lysosomal apparatus (13).

The liver sinusoidal endothelial cell scavenger receptor

Previous studies established that blood-borne negatively charged soluble macromolecular scavenger receptor (SR) ligands are cleared mainly by endocytosis in LSECs (14, 15). Hyaluronan and chondroitin sulphate, which are negatively charged connective tissue polysaccharides believed to be cleared by a highly specific hyaluronan receptor, are taken up by the same SRs that take up aminoterminal propeptides of type I and III procollagen (16). Studies employing KO mice lacking SR-A showed that the LSEC SR is distinct from SR-A (17). Recent evidence indicates that the major LSEC SR is represented by the two closely related receptors, stabilin-1 and -2 (18).

The liver sinusoidal endothelial cell Fc-γ receptor IIb2 (IIb2)

Of the known Fc-γ receptors, only IIb2 is able to mediate endocytosis of immune complexes (ICs) and only IIb2 is expressed in LSECs. Clark Anderson noted that the presence of this receptor in LSECs has, astonishingly, been ignored by immunologists (A4). Trond Berg (A5) reported that ICs endocytosed via IIb2 are degraded at a lower rate than antigens endocytosed via LSEC SR (19). Moreover, the ICs are associated with lipid rafts after cross-linking before internalization via clathrin-coated pits, and a large proportion of the internalized ICs is recycled back to the plasma membrane. Both these events delay receptor–ligand transport to later endocytic compartments. Cross-linking of LSEC IIb2 does not lead to tyrosine phosphorylation. It was suggested that the LSEC IIb2, similar to its role in dendritic cells, is able to present antigens to B-cells (A4). IIb2 in LSECs and placenta endothelium may share a similar role in local vascular immunity (20).

Comparative aspects of scavenger function

Liver sinusoidal endothelial cells represent the mammalian counterpart of vertebrate scavenger endothelial cells (SEC) (21). Using highly efficient clathrin-mediated endocytosis, these cells clear an array of colloids and soluble macromolecules from the circulation, whereas macrophages use phagocytosis to remove particles of size > 200 nm. Martin–Armas (A7) presented a study on SR-mediated endocytosis of immune-stimulating bacterial oligonucleotides, CpG (22) in Atlantic cod SEC. Preincubation of cultured cod SECs with CpG or poly I:C selectively downregulated SR-mediated endocytosis, but only marginally affected MR-mediated endocytosis. In his tutorial presentation (A6), Clive Crossley gave an update of the invertebrate scavenger cell system, the nephrocyte, which is functionally strikingly similar to the vertebrate SEC system. He focused on insect nephrocytes that display an extensively well-developed clathrin-mediated endocytosis (23). These cells endocytose ligands that are also avidly endocytosed by the mammalian LSEC SR. At present, no information is available on the structure of these receptors. Nephrocytes produce large amounts of lactate, just as the mammalian LSEC and fish SEC do. It is assumed that this lactate is used as a high-energy fuel by neighbouring energy-demanding cells.

Molecular biology

Sergij Goerdt, in his tutorial (A3), looked for LSEC-specific features in his own work and in the literature, and found that (a) stabilin-2 is lost from non-sinusoidal hepatic endothelium late in hepatic vascular differentiation (24); (b) activation of the G-protein-coupled bile acid receptor (Gpbar1/TGR5) by bile salts leads to overexpression/activation of eNOS and enhanced NO production mediating sinusoidal relaxation and hepatic stellate cell (HSC) quiescence. In contrast, endothelin (ET)-1 induces LSEC constriction and defenestration (25–28); (c) insulin is an important LSEC growth factor cross-activating the VEGF pathway (29). Moreover, endocytosis/intracellular trafficking was recently shown to be distinct in LSECs compared with other cells (30). The LSEC-specific features are as follows: (a) a remarkable net-like distribution of clathrin heavy chain, fully associated with microtubules, but not with actin; (b) clathrin-coated vesicles only partially colocalized with early endosome antigen 1 and adaptor protein 2; (c) Wnt2, an autocrine growth and differentiation factor specific for LSECs that synergizes with the VEGF signalling pathway to exert its effects (31). As a strategy to study the specialized differentiation parameters of LSECs, highly purified LSECs and lung microvascular endothelial cells (LMECs) were compared with respect to gene expression. It was found that 319 genes are overexpressed (> 4-fold) in LSECs. Interestingly, the expression of stabilin-1 and -2 were about 25 and 1000 times higher in LSECs, whereas the von Willebrand factor was 100 times higher in LMECs.

Ageing

Old age is associated with substantial thickening and defenestration of the LSEC, sporadic deposition of collagen and basal lamina in the extracellular space of Disse and increased numbers of fat-engorged, non-activated HSCs (32, 33) (A10–13). Defenestration is also apparent in isolated LSECs (A10). There is perisinusoidal upregulation of the von Willebrand factor, VEGFR-2, collagen I and IV and intercellular adhesion molecule (ICAM)-1, and reduced expression of caveolin-1 and F-actin (32). There is a 35% reduction in sinusoidal perfusion and five-fold increase in leucocyte adhesion (33) (A12). Unlike most liver diseases, there is no change in the expression of α-smooth muscle actin (α-SMA), desmin or VEGF, reduced expression of caveolin-1 and HSCs are not activated (32) (A11). These age-related changes have been termed pseudocapillarization. Pseudocapillarization is reversed by caloric restriction and resveratrol (A48). Defenestration leads to impaired transfer of lipoproteins and provides a novel mechanism and therapeutic

target for age-related dyslipidaemia (8) (A11, 13). Old age is associated with impaired endocytic capacity by the LSEC (33) (A10, 12).

Microcirculation

LSECs originate between the 4th and the 6th gestational week from the vitelline veins and/or the septum transversum. Initially, LSECs are non-fenestrated and continuous with a basal lamina; they then differentiate into fenestrated LSECs between 10 and 17 gestational weeks (34). Transgenic mice with selective hepatic impairment of VEGF transduction have severely disrupted sinusoidal endothelium, indicating that VEGF is a pivotal cytokine in sinusoidal development (6). Cultures of fetal rat LSECs confirmed the role of VEGF and also of TGF-β1 in fetal LSEC development (A16). After development, the liver architecture is best described by hepatic microvascular subunits, the group of sinusoids supplied by a single inlet venule (35).

The responses of the microcirculation to many toxicants are similar. Initially, LSECs become swollen, develop large gaps that can lead to extravasation of erythrocytes into the space of Disse, and in severe toxicity, LSECs disintegrate and become detached debris (35–37). NO donors or metalloproteinase inhibitors ameliorate damage (36). Changes in the microcirculation occur in many chronic liver diseases. In non-alcoholic fatty liver disease, there is disruption of sinusoidal blood flow (38). Initially, this is generated by hepatocytes swollen by lipid droplets (LDs) narrowing the sinusoidal lumen. Microvascular impairment and trapping of leucocytes might contribute to HSC activation. Steatohepatitis leads to defenestration and capillarization of the LSEC with further impairment of blood flow (38). In primary biliary cirrhosis, there is aberrant expression of aquaporin-1 on the LSEC membrane, suggesting that this water channel may have a role in the development of capillarization (A14).

Role of oxygen tension

Tissue hypoxaemia is common under several pathological conditions, and LSECs are the primary targets of ischaemia-reperfusion injury following liver preservation. Hypoxia induces profound changes in the cellular gene expression profile. A major transcription factor family activated by hypoxia, hypoxia-inducible factor (HIF), contributes to the molecular regulation of the hypoxic response. High blood alcohol levels are accompanied by hypoxia and activation of HIF-1α in the liver. Ethanol increases the mRNA expression of chemokine genes (MCP-1, RANTES and MIP-2), vasoconstrictor molecules (ET-1) and HIF-1α, and activates ET-1 via HIF-1α, independent of hypoxia. The ethanol-mediated release of ET-1 may activate HSCs and exaggerate vasoconstriction and hepatic blood flow, and inflammation in the liver (A8).

The normal oxygen tension in the hepatic sinusoids is considerably lower than the atmospheric oxygen tension. Cultivation of LSECs under 5% (normoxic) oxygen tension, as opposed to hyperoxia (20%), which is used in most incubators, improved the survival of LSECs and SR-mediated endocytosis, reduced the production of interleukin (IL)-6 and increased the production of IL-10. Under normoxia, generation of H_2O_2 was reduced drastically. Thus, the viability, structure and many of the essential functional characteristics of isolated LSECs are clearly better preserved when the cultures are maintained under more physiological oxygen levels (1) (A9).

Development

Embryonic development of the liver is closely associated with vascular organization. Co-expression of SE-1 (24, 39, 40) and stabilin-2 is an adequate marker for differentiated LSECs, and both molecules are co-expressed in LSECs at the late stage of liver development (E15.5–17.5). After culturing E13.5 fetal liver cells for 7 days in the presence of VEGF, the proliferated endothelial sheets expressed neither SE-1 nor stabilin-2. In the presence of both VEGF and SB-431542 (an inhibitor of TGF-β1 receptor kinase; ALK-5), the endothelial sheets started to express stabilin-2 and contained some SE-1 co-expressing cells. These findings suggest that VEGF plays a role in the endothelial sheet formation, and blocking of TGF-β1 signalling may be involved in the differentiation of LSECs (24) (A16).

Kupffer cells

Role of Kupffer cells in alcoholic and non-alcoholic liver disease

Alcoholic liver disease (ALD) and non-alcoholic steatohepatitis (NASH) are common forms of liver disease that are histologically indistinguishable (41). Approximately 20% of alcoholics will develop ALD, while the prevalence of NASH in obese adults has been estimated at 40–100% (41). The progression of ALD and NASH is a complex process involving both parenchymal and non-parenchymal cells in the liver. There is growing appreciation for the role of the KC in both ALD and NASH.

Activation of Kupffer cells in alcoholic liver disease and non-alcoholic steatohepatitis

Chronic ethanol consumption, in both animal models and humans, increases circulating endotoxins (41, 42). Endotoxin also increases in both high-fat diet and methylcholine-deficient diet (MCD) models of NASH (1). Activation of Toll-like receptor (TLR)-4 signalling by endotoxin increases the production of inflammatory cytokines and reactive oxygen species (ROS). Mice lacking TLR-4 or CD14 are protected from both ethanol-induced and high-fat-diet-induced liver injury (41, 43).

Toll-like receptor-4 signalling is mediated by MyD88-dependent and -independent pathways. Interestingly, while TLR-4$^{-/-}$ mice are protected from ethanol-induced liver injury, MyD88$^{-/-}$ mice develop hepatic steatosis and increased alanine aminotransferase (44). In contrast, TIR-domain-containing adapter-inducing interferon (TRIF)$^{-/-}$ mice are protected from ethanol-induced liver injury (45). No studies have yet tested the differential roles of MyD88 and TRIF in models of NASH, but such studies would probably provide insights into the comparative pathophysiology of ALD and NASH.

Pro- and anti-inflammatory mediators regulating Kupffer cell activity: NADPH oxidase

Chronic ethanol feeding also sensitizes KCs to activation by lipopolysaccharides (LPS) (43). Increased production of ROS via NADPH oxidase contributes to increased LPS-stimulated ERK1/2 and p38 activation, as well as tumour necrosis factor (TNF)-α expression, in KCs from ethanol-fed rats (46) (A18). These data are consistent with the protection of p47$^{phox-/-}$ mice from ethanol-induced liver injury (43). In contrast, NADPH oxidase may not be as important in NASH as it is in ALD.

Liver International (2009)

For example, mice lacking gp91, one of the two membrane-bound proteins comprising NADPH oxidase cytochrome b_{558}, are susceptible to NASH in the MCD model (47).

Adiponectin

Adiponectin, an adipokine secreted by adipocytes, has insulin-sensitizing actions, as well as potent anti-inflammatory effects. Circulating adiponectin concentrations are decreased in animal models of NASH and ALD (46). Treatment of mice with exogenous adiponectin during ethanol feeding or in the *ob/ob* mouse model of NASH prevents liver injury (46). This protection may be due, at least in part, to the anti-inflammatory effects of adiponectin on KCs in the liver, in that treatment of KCs isolated from ethanol-fed rats with adiponectin normalizes LPS-stimulated TNF-α expression (46) (A18).

Cyclic AMP

Cyclic AMP (cAMP) is an important anti-inflammatory signal in KCs; abnormal regulation of cAMP production during NASH or ALD may contribute to increased inflammatory cytokines in the liver. For example, LPS can decrease the expression of adenylyl cyclase (48) (A24) and chronic ethanol decreases Gs (43) and increases phosphodiesterase 4 (49) in KCs. These combined effects suppress agonist-stimulated cAMP production in KCs, probably contributing to the increased inflammatory cytokine production in both NASH and ALD.

Interactions of Kupffer cells with hepatocytes

KCs, because of their proximity, influence hepatocyte function. KC-derived TNF-α has cytotoxic effects on hepatocytes (42). KC-derived mediators, including ROS and cytokines, are likely critical contributors to hepatic insulin resistance (50) (A20), a characteristic of both ALD and NASH. Finally, KCs influence lipid metabolism in hepatocytes. KC-derived endocannabinoids, interacting with the CB-1 receptor on hepatocytes, contribute to liver injury in response to both ethanol and high-fat diets, at least in part via the regulation of fatty acid synthesis and oxidation (51, 52). Further, arachidonic acid-derived lipid mediators produced by KCs also contribute to hepatic steatosis in the *ob/ob* model of NASH (53) (A21).

Role of Kupffer cells and infiltrating leucocytes in drug-induced hepatotoxicity

Acetaminophen (APAP) is a safe analgesic at therapeutic levels but overdoses cause liver injury and even liver failure. Studies on the mechanisms of cell death mainly focus on intracellular signalling events (54), but recently, the pathophysiological role of the innate immune response has received more attention (55) (A23).

Role of tissue macrophages, natural killer cells and neutrophils in acetaminophen hepatotoxicity

Based on the beneficial effects of compounds such as gadolinium chloride, which are thought to inactivate KCs, it was hypothesized that tissue macrophages contribute to APAP hepatotoxicity (reviewed in (55)). However, several lines of evidence argue against an involvement of KCs in the injury process. Firstly, the centrilobular area of necrosis is inconsistent with the predominant periportal localization of the most active KCs. Secondly, animals deficient in a functional NADPH oxidase, the main enzyme of phagocytes that produces ROS, show the same APAP-induced oxidant stress and liver damage as wild-type animals (56). Furthermore, elimination of KCs with liposomal clodronate aggravated APAP-induced liver injury presumably due to the lack of anti-inflammatory mediator production (57). These data indicate that KCs are actually beneficial during APAP-induced liver injury because of the prevention of an excessive inflammatory response.

Based on experiments with elimination of natural killer (NK) and natural killer T (NKT) cells, it was concluded that these resident lymphocytes contribute to APAP hepatotoxicity (58). However, a recent study indicated that the involvement of NK and NKT cells is dependent on the use of the solvent dimethyl sulfoxide, which can activate these lymphocytes (59). These findings suggest that NK and NKT cells do not contribute to APAP-induced liver injury unless these cells are activated through independent stimuli before APAP administration. This may have some implications for the susceptibility of individuals to APAP overdose but it appears to be of limited relevance for the general toxicity of APAP.

Neutrophils accumulate in the liver in response to APAP-induced necrosis (60). A number of therapeutic interventions directed against neutrophil functions and recruitment had no effect on the oxidant stress and liver injury during the first 24 h after APAP overdose (56, 60, 61). The only exception to this rule appeared to be pretreatment with a neutropaenia-inducing antibody (62). However, this beneficial effect may be independent of the inhibition of neutrophil cytotoxicity, as the removal of the neutrophils activates KCs and preconditions hepatocytes to the APAP-induced stress (63). In support of this conclusion, the neutropaenia-inducing antibody is not effective if administered after APAP (61).

Role of macrophages and neutrophils in regeneration after acetaminophen-induced liver injury

The main purpose of inflammatory cell recruitment into the liver after extensive cell necrosis is to remove dead cells. Necrotic hepatocytes are replaced by dividing hepatocytes closest to the area of necrosis (64). In addition to promoting cell division in healthy hepatocytes, the removal of necrotic cells is critical for the regeneration to be successful. Thus, neutrophils and monocyte-derived macrophages migrate into the necrotic areas and dissolve it. The recruitment of macrophages into the liver is triggered mainly by the formation of monocyte chemoattractant protein 1 (MCP-1), which is generated by macrophages and hepatocytes in the area of injury (65). The receptor for MCP-1 is expressed on infiltrating macrophages (65). Mice deficient in MCP-1 or its receptor have the same initial injury after APAP overdose but show a delayed regenerative response (65). These data suggest that newly recruited macrophages are important for regeneration. In contrast to the critical role of oxidant stress in phagocyte cytotoxicity, the process of necrotic cell removal does not require ROS as animals deficient in a functional NADPH oxidase show a similar regenerative response as wild-type animals (A23). Future studies are needed to elucidate whether these phagocytes are also involved in regulating cell cycle activation and the division of healthy hepatocytes around the area of necrosis.

Hepatic stellate cells

Storage of vitamin A as a function of stellate cells

Hepatic stellate cells store about 80% of the body's total vitamin A as retinyl esters (RE) in their lipid droplets (LDs) and play pivotal roles in the regulation of vitamin A homeostasis. HSCs take up retinol from blood by receptor-mediated endocytosis and store vitamin A mainly as retinyl palmitate in LDs in their cytoplasm, and secrete retinol–retinol-binding protein complex into the blood. Unlike adipocytes, HSCs are not involved in energy storage, but they represent a particular cell population specialized in maintaining the concentration of vitamin A in the bloodstream within the physiological range. Under pathological conditions such as liver fibrosis, HSCs lose their LDs and RE (66).

It has been reported that LDs in adipocytes are surrounded by PAT proteins, which were named after perilipin, adipocyte differentiation-related protein (ADRP)/adipophilin and TIP47. In this symposium, Yoshikawa *et al.* (A32) reported the expression of ADRP, and TIP47 around LDs of HSCs. ADRP localized around LDs emitting vitamin A-autofluorescence in quiescent HSCs and the culture-activated HSCs administered with retinol, while TIP47 did not localize around the LDs but diffusely localized in the cytosol in quiescent HSCs, although the colocalization of TIP47 and LDs was observed in activated HSCs. These data suggested that the different palmitoyl acyl transferase (PAT) proteins play specific roles during the formation and maturation of LDs in HSCs. Recently, Straub *et al.* (67) demonstrated that, in the normal liver, PAT proteins were colocalized with the vitamin A-autofluorescence of LDs of HSCs, while in the steatotic liver, ADRP and TIP47 were expressed in LDs of HSCs and additionally in LDs of steatotic hepatocytes. Taken together, the dynamic changes of PAT proteins in HSCs will provide us with considerable knowledge to help in our understanding of the mechanisms leading to the formation and loss of LDs containing vitamin A.

Lecithin: retinol acyltransferase (LRAT) is a retinol esterification enzyme, and it is markedly activated especially in HSCs. Cellular retinol-binding protein-1 (CRBP-1) also mediates retinoid metabolism, and retinol-bound CRBP-1 is a substrate of LRAT. Nagatsuma *et al.* (68) demonstrated that LRAT may be an excellent alternative marker to identify quiescent HSCs as well as CRBP-1 in the normal liver (A34). In the fibrotic/cirrhotic liver, the different patterns of expression for LRAT and α-smooth muscle actin (SMA) facilitated the differentiation between various subsets of fibroblast-like cells involved in fibrogenesis. They also revealed that LRAT was mainly distributed in the rough endoplasmic reticulum and multivesicular bodies of HSCs. The upstream regulatory mechanisms of the expressions of LRAT and CRBP-1, which are retinoic acid-responsive genes, were demonstrated by Mezaki *et al.* (69) in this symposium (A31). Nagatsuma *et al.* reported the co-expression of LRAT and CRBP-1 in the polar bear liver, which was compared with that in human liver. The interaction between LRAT and CRBP-1 could play an important role in the unique vitamin A storage function of HSCs. The cells expressing both LRAT and CRBP-1 were recognized as the functional quiescent HSCs concerned with vitamin A metabolism.

Comparative biology and stellate cells

To demonstrate the origin of hepatic and extrahepatic SCs in phylogeny, vitamin A and vitamin A-storing cells were investigated in arrowtooth halibut (*Atheresthes evermanni*) (70), lamprey (*Lampetra japonica*) (71) and ascidian (*Halocynthia roretzi*) (72). In the arrowtooth halibut, the highest concentration of stored vitamin A was present in SCs in the pyloric cecum, a teleost-specific organ protruding from the intestine adjacent to the pylorus. Considerable amounts of vitamin A were also stored in SCs in the intestine and liver. In the lamprey, vitamin A was stored in SCs in the intestine, liver, kidney, gill and heart. In the ascidian, retinal is the essential form of vitamin A for storage, and no SCs were observed. Thus, the distribution of SCs with vitamin A-storing capacity differs between mammalian and non-mammalian vertebrates, suggesting that the SCs appeared in the lamprey, and the vitamin A-storing site has shifted during vertebrate evolution.

The bile ducts of larval lamprey degenerate and disappear during metamorphosis, so that no bile duct is observed in the adult liver, which offers a valuable model for studying the liver pathogenesis of human biliary atresia. Miura *et al.* (A42) reported the microstructural analyses of the bile duct degeneration of the lamprey in larval and spawning stage. In larval lamprey, bile canaliculi, intra- and extrahepatic bile ducts, and gall bladder were clearly observed. Apoptotic cells were detected in the epithelium of extrahepatic bile ducts in the latter larval stage of larva. Convoluted bile ducts were surrounded by fibrous deposits of extracellular matrix (ECM) components, where sinusoids were abundant. The HSCs in the perisinusoidal space stored LDs, and several liver parenchymal cells constructed bile canaliculi. In the adult lamprey, the entire biliary system and thick periductal fibrosis disappeared. HSCs containing large quantities of vitamin A and hepatic parenchymal cells with large amount of LDs were observed. However, neither was accompanied by hepatic fibrosis or cirrhosis. These results strongly suggest that the degeneration and disappearance of bile ducts in the lamprey were caused by apoptosis of the bile duct epithelium during metamorphosis when the larvae transformed into the adults. The HSCs were probably responsible for the fibrosis that accompanies the degeneration of bile ducts.

To examine the characteristics of ECM components supporting the sinusoidal wall (scaffolding function) of the liver, the livers of two frozen baby mammoths that died about 40 000 years ago and were buried in permafrost in Siberia, were analysed by Senoo *et al.* (A41). The livers were preserved at gross anatomical and histological levels. The ultrastructure of ECM components, namely the fibrillar structure showing a characteristic pattern of cross striation and basement membrane structure, were clearly demonstrated by transmission and scanning electron microscopy. Type I and type IV collagens were shown in ECM components by immunofluorescence. These findings suggested that the three-dimensional structure of ECM was important for maintaining the gross and histological morphology of the sinusoidal wall in the liver. Thus, comparative biology and phylogeny of HSCs and the liver are indicative and useful for the research of the hepatic sinusoid.

Regulation of hepatic stellate cell activation

Several recent reports have indicated that hepatic fibrosis and even cirrhosis may regress (73, 74). These observations have toppled the established theory that cirrhosis is an incurable liver disease, particularly from a pathological point of view, and has increased the enthusiasm for developing antifibrogenic therapies. In experimentally induced liver fibrosis in rodents, the cessation of further liver injury by stopping hepatotoxin administration results in fibrosis regression, usually mediated by the reduction of tissue inhibitor of matrix metalloproteinase-1

Liver International (2009)

and apoptosis of HSCs. In humans, the spontaneous resolution of liver fibrosis can occur after successful treatment of the underlying disease. In particular, chronic hepatitis C virus infection has been studied most extensively, and interferon (IFN) therapy with viral eradication results in fibrosis improvement. Among IFNs, IFN-γ is the strongest inhibitor of HSC activation, as revealed by its inhibitory effect on collagen synthesis and α-SMA expression (75). In this symposium, Maubach *et al.* (A27) (76) reported that IFN-γ induces the class II transactivator, the invariant chain (CD74), the major histocompatibility complex (MHC) class II molecules and cathepsin S in activated rat HSCs, indicating that IFN-γ is an important regulator in antigen presentation in HSCs. Other recent studies have indicated that HSCs in culture undergo apoptosis via pentapeptide GRGDS (Gly-Arg-Gly-Asp-Ser), nerve growth factor (NGF), a high dose of sphingosine-1-phosphate, gliotoxin, and so on. The regulation of HSC activation by C-reactive protein, an acute-phase reactant that participates in inflammatory responses and is produced by hepatocytes, has shed a new light on the local cell–cell interactions (A39).

Tsukamato *et al.* (A28) showed that transcriptional regulation essential for adipocyte differentiation is required for the maintenance of HSC quiescence. Quiescent HSCs express peroxisome proliferators-activated receptor (PPAR-γ), CCAAT/enhancer-binding protein (C/EBP)-α, -β, -δ, liver X receptor α (LXR-α) and sterol-regulatory element-binding protein-1c (SREBP-1c), which are adipogenic transcription factors and are downregulated by the HSC activation process (77). Activated HSCs show phenotypic reversal by the forced expression of PPAR-γ or SREBP-1c, or by the treatment of cells with the adipocyte differentiation cocktail MDI (methylxanthin, dexamethasone and insulin). They further reported the involvement of a new class of antiadipogenic factors: the Wnt family of proteins. That is, canonical (Wnt3a and 10b) and non-canonical (Wnt4 and 5) Wnts, their receptors (Frizzeled-1 and -2) and coreceptors [lipoprotein receptor-related protein (LRP) 6 and Ryk] are induced in activated rat HSC *in vitro* and *in vivo*. Most interestingly, Wnt antagonism using the LRP coreceptor antagonist Dkk-1 restores both the expression of the adipogenic transcription factors listed above and the HSC quiescence (78).

Another interesting transcription factor is early growth response 1 (Egr-1). Egr-1 is an immediate early gene that is both rapidly and transiently induced in response to a variety of stress factors. It also regulates the expression of genes involved in the fibrotic process including basic fibroblast growth factor (bFGF), VEGF, TGF-β and platelet-derived growth factor (PDGF). Pritchard *et al.* (A35) demonstrated the development of enhanced fibrosis and augmented α-SMA expression after carbon tetrachloride injection in Egr-$1^{-/-}$ mice compared with wild-type mice. These data suggest that Egr-1 plays a protective role in fibrosis.

In addition to vitamin A, vitamin E proved to be absorbed and accumulated in HSCs. Vitamin E is composed of eight different forms: α-, β-, γ- and δ-tocopherols and -tocotrienols. Since the discovery of vitamin E in 1922, studies on tocopherols and tocotrienols have focused mainly on their antioxidant properties. Recently, the non-antioxidant functions of vitamin E were verified, and it was shown to deactivate protein kinase C in smooth muscle cells, lower cholesterol level, inhibit platelet adhesion and exhibited anticancer property (79). Furthermore, tocopherols reportedly promote the transciption of Bcl2, α-tocopherol transfer protein, cytochrome P450, tropomyosin and PPAR-γ, and inhibit that of CD36, SR-BI, collagen α1, matrix metalloproteinase-1 (MMP-1), MMP-19, E-selectin, ICAM-1, integrins and cyclins D1 and E (80). In this context, Yamaguchi *et al.* showed the inhibitory effects of the four tocopherols and tocol on HSC proliferation and the induction of apoptosis (A36). The addition of δ-tocopherol and tocol to culture-activated HSCs resulted in marked morphological changes leading to detachment from a substratum. According to these observations, the authors suggested that vitamin E would be a promising candidate for the treatment of hepatic fibrosis and liver cirrhosis.

Transforming growth factor-β as a key regulator of hepatic stellate cell activation

Transforming growth factor (TGF)-β is a key regulatory molecule for ECM metabolism, and it functions as an autocrine and a paracrine mediator (66). Cellular sources of TGF-β1 are diverse, including HSCs, KCs, hepatocytes, LSECs and platelets. Proteolytic cleavage of latent TGF-β-binding protein is a prerequisite for the release and generation of bioactive (mature) TGF-β, which is induced by urokinase plasminogen activator or tissue plasminogen activator. The impact of TGF-β1 on liver fibrosis has been well documented via the marked attenuation of liver fibrosis development using the soluble type II TGF-β receptor and in a model of adenoviral delivery of the dominant-negative TGF-β receptor. The role of the Smad cascade in TGF-β signalling has been characterized in HSCs (68). Furthermore, bone morphogenic protein-7 antagonizes TGF-β signalling through Smad1/5/7 and Id-2, and thereby suppresses collagen gene expression (81).

In this symposium, Kojima S *et al.* (A40) showed that TGF-β is activated by proteases such as plasmin (PLN) and plasma kallikrein (PLK) on the surface of HSCs during pathogenesis of liver fibrosis and that blockage of these activation reactions with a protease inhibitor, camostat mesilate, prevented the disease development. They further showed that PLN and PLK cleaved between K^{56}–L^{57} and R^{58}–L^{59}, respectively, within the latency-associated protein portion of human latent TGF-β1; this finding indicates a novel detection system of TGF-β activation *in vivo* using the antibodies recognizing PLK cut ends. They further demonstrated that peptides containing protease cleavage sites as well as their decoy peptide effectively suppressed the TGF-β activation reaction and prevented the activation of HSCs in culture.

The hepatic stellate cell as a principal player in liver fibrosis

Hepatic stellate cells, which reside in Disse's space in close contact with both LSECs and hepatocytes, play multiple roles in hepatic pathophysiology (82) Quiescent HSCs represent a vitamin A-storing phenotype and metabolize a small amount of basement membrane-forming laminin and type IV collagen. When hepatitis is induced by iron overload, alcohol consumption, infection with hepatitis viruses B or C, NASH, autoimmune hepatitis and bile duct obstruction, local inflammation and damaged hepatocytes activate HSCs. This process is triggered by oxidative stress due to lipid hydroperoxide and reactive aldehyde generated in and released from damaged or apoptotic hepatocytes and KCs, via the paracrine stimulation of PDGF-BB, insulin-like growth factor-1 and TGF-β derived from sinusoidal cells, platelets and infiltrating leucocytes, and by the production of a splice variant of cellular fibronectin (EIIIA isoform) (83–86). Activated HSCs change their phenotype to 'myofibroblast'-like cells that produce increased

amounts of types I and III collagens, show augmented contractility accompanied by the expression of α-SMA and the production of ET-1, secrete TGF-β and MCP-1, lose retinoid and exhibit active apoptosis. Transcriptional activation by Kruppel-like factor 6, activator protein 1 and C/EBP enhances gene expression regulating ECM accumulation (87).

Involvement of iron in hepatic stellate cell function and liver fibrosis

The role of iron in the hepatic pathophysiology has long been studied in fields of haemochromatosis and alcoholic liver injury (88). Recently, an unusual accumulation of iron in the liver has also been observed in chronic hepatitis C and NASH. The activation process of HSCs is triggered by oxygen free radicals, including hydrogen peroxide (H_2O_2), which can be produced by the Fenton reaction of $Fe^2+H_2O_2 \rightarrow Fe^3+HO^-+HO^{\cdot}$. Free iron induces the production of TNF-α and TGF-β1 and nuclear factor-κB (NF-κB) activation in hepatic macrophages (89). The free radicals generated induce lipid peroxidation, DNA breakage and 8-hydroxy-2′-deoxyguanosine formation, resulting in tissue damage and DNA mutagenesis (90). Thus, iron is also a key molecule for liver fibrogenesis.

Ferritin is an iron-binding protein that is composed of 24 individual proteins of either heavy (H) or light (L) subtypes and is important for iron homeostasis. Although it is well known that serum ferritin level increases in the course of liver inflammation, the exact reason for this elevation is unclear. Ruddel *et al.* (A33) identified the role of T-cell immunoglobulin and mucin domain-2 (Tim-2), which is a receptor for H ferritin endocytosis, in HSC activation. Tim-2 mRNA and proteins were present in HSCs and were weakly induced in the process of activation. The incubation of HSCs with tissue ferritin augmented the phosphorylation of the PI3-kinase target motif YXXM, protein kinase Cz (PKCz), p42/p44 mitogen-activated protein kinase and IKK, leading to the activation of NF-κB. These data suggest a novel bioactive function of ferritin independent of its binding to iron.

Kawada *et al.* (91) discovered a new iron-binding protein, cytoglobin, the fourth globin in mammals by proteomics analysis of HSC activation. Cytoglobin shows amino acid sequence homology with vertebrate myoglobin, haemoglobin and neuroglobin. Cytoglobin is uniquely localized in fibroblast-like cells in splanchnic organs, namely vitamin A-storing cell lineages, including pancreatic SCs, reticular cells in the spleen and mesangial cells in the kidney (92). The oxygen- and carbon monoxide-binding equilibrium and kinetic properties are nearly identical between cytoglobin and myoglobin, indicating that cytoglobin may convey oxygen to the mitochondria of actin-rich non-muscle cells to facilitate cell contraction (A25). On the other hand, a recent report by Xu *et al.* (93) demonstrated the anti-oxidative and cytoprotective action of cytoglobin by over-expressing this protein using adenovirus-associated gene transfer in the rat liver injured by carbon tetrachloride injection.

Are hepatic stellate cells a pure single population?

As stated above, the contribution of HSCs to the hepatic fibrotic process is well recognized. However, recently, several lines of evidence have pointed out the heterogeneity of hepatic 'myofibroblasts'. In other words, it is now questionable whether activated HSCs are identical to myofibroblasts (94). Both activated HSCs and myofibroblasts express α-SMA and collagens α1(I) and α1(III). However, desmin, P100 and α2-macroglobulin are expressed in activated HSCs, but not in myofibroblasts. Additonally, fibulin-2, gremlin, cardiac troponin T and lumican are present in myofibroblasts, but absent in activated HSCs. A recent study using transgenic mice that express the red fluorescent protein and enhanced green fluorescent protein reporter genes under the direction of the mouse α-SMA and collagen α1(I) promoter/enhancer, respectively, demonstrated that there are at least three myofibroblastic populations: α-SMA-only expressing cells, collagen-only expressing cells and dual-positive cells (95). Activated and myofibroblast-like HSCs are derived from vitamin A-storing quiescent HSCs. In contrast, the origin of myofibroblasts remains controversial: they may be derived from portal fibroblasts, vitamin A-free HSCs or hepatic stem cells. Fascin has been proposed as a novel marker that distinguishes human HSCs from portal fibroblasts (A57). The theory of epithelial–-mesenchymal transition has brought about further confusion in this field.

The participation of cells in the blood in the hepatic fibrotic process has additionally been proposed. These cells are generated from bone marrow-derived mesenchymal cells or circulating fibrocytes and may serve as a substantial fraction of the fibrogenic cell population in the liver during chronic injury. However, there also exists some controversy; bone marrow-derived cells may provide fibrogenic cells, while bone marrow-derived endothelial cell progenitor cells can be antifibrogenic. Hepatocyte growth factor (HGF) gene transfer accelerated the recruitment of bone marrow-derived mesenchymal cells into the liver, increasing the gelatinase activity in the fibrotic area (Iimuro *et al.*, A61). On the other hand, Witters *et al.* (A37) described an impairment of blood platelet function in cholestatic liver disease. Because platelets are one of the major sources of growth factors, such as PDGF, HGF, prostaglandins and platelet-activating factors, involved in inflammatory and fibrotic processes, analysis of the function of platelets in liver fibrosis should be considered further. Ogawa *et al.* (A38) emphasized the involvement of senescent erythrocytes in the pathogenesis of a rabbit model of steatohepatitis.

Immunology (A44)

The cells of the hepatic sinusoid have a strategic position to interact with immune cells passing with the blood stream through the hepatic sinusoids. Interaction is further facilitated by the narrow sinusoidal diameter, slow and irregular blood flow as well as low perfusion pressure. The liver is known as an immune regulatory organ, which contributes to the elimination of pathogens from the circulation but at the same time favoring the induction of immune tolerance rather than adaptive immunity (96, 97). At the ISCHS-meeting in Tromsø, various groups presented the involvement of KCs in innate immune reactions that are critical for the development of drug-induced liver disease (please see symposium session V). KCs have also been shown to contribute to bystander-hepatitis during extra-hepatic influenza infection (98) and are known to engage in a cross-talk with hepatic NK cells upon contact with TLR ligands (99). These interactions often lead to an increased expression of cytokines with effector function such as TNF-α, and thereby promote innate immunity against infectious microorganisms or induce liver damage. Other cell populations such as NK cells, NKT cells and γδ-T cells represent significant populations in the liver but their contribution to local immune regulation is currently not well defined, although their role in antiviral defence has been demonstrated recently (100). These cell

populations either recognize pathogens or altered cells through genetically conserved surface receptors or express a skewed repertoire of T-cell receptors recognizing their antigen in the context of the evolutionaryly conserved CD1 molecule. Nevertheless, *in vivo* imaging revealed that NKT cells continuously patrol the hepatic sinusoids and are arrested upon specific recognition of a cognate ligand, α-galactosyl-ceramide, which is presented in a CD1-restricted fashion (101). Certainly, these innate immune cells play a key role in local pathogen defense in the liver but the exact cellular and molecular mechanisms involved remain to be identified.

An important link between innate and adaptive immune responses is the expression of chemokines, which recruit lymphocytes with effector functions. The Adams group in Birmingham has recently revealed that chemokine CCL25 recruits pathogenic $CCR9^+$ CD8 T cells into the liver in patients with primary sclerosing cholangitis (102). These findings further demonstrate an important connection between the gut and the liver, as hepatic recirculation of T cells initially primed in the intestinal tract is involved in the manifestation of hepatic autoimmunity (103). Also, expression of chemokines by LSECs is important for the transendothelial migration of T cells and subsequent development of local effector function (104, 105).

Induction of antigen-specific tolerance in CD8 T cells has been attributed to hepatic cell populations that bear the capacity to function as antigen-presenting cells. Besides hepatocytes, which represent the most prominent hepatic cell population and induce deletional tolerance in CD8 T cells (106, 107), KCs (108), HSCs (109) and LSECs have been implicated in mediating T-cell tolerance (110). KCs bear the capacity to present antigen on MHC class I and MHC class II molecules to CD8 and CD4 T cells respectively. Using a model of murine liver transplantation, Klein *et al.* (79) reported that KCs can be divided in a bone marrow derived and an organ-resident cell population with distinct functional and phenotypic characteristics. The group of Yamamoto *et al.* further expanded our knowledge on organ-resident KCs by reporting that this cell population constitutes a fixed proportion of the entire population of KCs and therefore presumably depends on local signals for survival and growth (A19). Further research is required in order to clearly assign particular functional properties to this organ-resident KC population.

It is accepted that the initial antigen-specific stimulation of naïve CD8 T cells in the liver determines their subsequent functional capacity, i.e. tolerance, whereas extrahepatic priming of T cells in the secondary lymphatic tissue leads to the development of T-cell immunity that upon antigen-recognition in the liver can develop into autoimmunity (107). Bertolino *et al.* (111) reported that ubiquitous antigen-presentation on MHC class I molecules leads to rapid hepatic recruitment of circulating naïve CD8 T cells. Knolle *et al.* (A44) presented data that LSECs cross-present circulating antigens to naïve CD8 T cells, leading to a rapid and liver-specific recruitment of antigen-specific T cells to the liver. The consequence of antigen-specific retention is an initial stimulation of naïve CD8 T cells but ultimately the development of CD8 T-cell tolerance. T-cell tolerance induced by antigen-presenting LSEC is characterized by mutual upregulation of co-inhibitory molecules on LSEC (B7H1) and the interacting T cells (PD1). The balance of co-inhibitory and costimulatory signals determines whether LSEC induce T-cell tolerance. $B7H1^{-/-}$ LSEC that fail to trigger PD1 stimulation also fail to induce T-cell tolerance, whereas additional costimulation through CD28 overrides tolerogenic signals promoting effector cell generation (112). Tolerance induction by LSEC has been shown to play a role in oral tolerance and development of tumour-specific tolerance following systemic tumour cell distribution (113, 114). Collectively, the early steps in recruiting naïve T cells to the liver and the functional outcome of these physical interactions influence subsequent systemic immune responses. Furthermore, cross-talk between liver sinusoidal cells and tumour cells may enhance the hepatic metastasis of circulating tumour cells, as was reported by Vidal-Vanaclocha *et al.* at this meeting (A45).

Taken together, an understanding of the cellular and molecular mechanisms determining the local regulation of immune responses in the liver will not only further our knowledge on the pathophysiological principles underlying persistent viral infection of the liver but will also allow us to develop therapeutic principles to deliberately increase tolerance during autoimmunity or to increase immunity in persistent viral infection or cancer.

Tumour/metastasis

Contribution of sinusoidal cells to hepatic metastasis

This section describes the contribution of sinusoidal cells to metastatic cancer cell regulation. Four phases of the metastasis process have been considered: (a) the microvascular phase of liver-infiltrating cancer cells, including mechanisms of intravascular arrest, death and survival of cancer cells within the inflammatory micro-environment of tumour-activated sinusoidal cells and immune escape mechanisms; (b) the intralobular micrometastasis phase, including growth activation of cancer cells and stromal cell recruitment into avascular micrometastases; (c) the angiogenic micrometastasis phase, including endothelial cell recruitment and blood vessel formation supported by proangiogenic myofibroblasts; and (d) the established hepatic metastasis phase, whose clinical significance is still affected by intratumoral stroma, blood vessel density, tumour-infiltrating lymphocytes and gene expression profile of cancer cells.

The microvascular phase of liver-infiltrating cancer cells

The hepatic metastasis process begins with the microvascular retention of circulating cancer cells. Mechanical stress suffered by cancer cells on entry and residence in the hepatic microvasculature contributes to cancer cell death. Infiltrating cancer cells can induce the obstruction of sinusoids, leading to transient micro-infarcts that damage hepatic cells. In turn, reoxygenation of ischaemic sinusoids induces the killing of cancer cells as a result of the release of NO and reactive oxygen intermediates from sinusoidal cells (115, 116). KCs can phagocytose cancer cells and modulate the antitumour immune response by releasing cytotoxic products and immune-stimulating factors activating hepatic NK cells (117, 118). In turn, these cells produce antitumour cytotoxicity via perforin/granzyme-containing granule secretion and death receptor-mediated mechanisms (119). However, some arrested cancer cells can resist and even deactivate antitumour defense mechanisms through several mechanisms: tumour-derived CEA (carcinoembryonic antigen) can prevent cancer cell death by inducing IL-10 to inhibit inducible NO synthase upregulation in sinusoidal cells (120). Expression of MHC class I on cancer cells can also promote immune escape via the negative regulation of hepatic NK cells. The high intracellular content of glutathione can also protect cancer cells from oxidative stress

(116). It was also reported at the symposium (A17) that IL-1-dependent MR upregulation in tumour-activated LSECs inhibits IFN-γ secretion and antitumour cytotoxicity of hepatic lymphocytes. The release of MR-stimulating factors was an immunosuppressant feature induced by ICAM-1-depedent COX-2 in liver-colonizing cancer cells expressing LFA-1. Because IL-1, COX-2 and ICAM-1 inhibitors show antimetastatic effects, it was suggested that MR-dependent hepatic immune suppression constitutes a common mediator for prometastatic effects induced by IL-1, COX-2 and ICAM-1. Liver-infiltrating cancer cells that survive in the microvasculature adhere to hepatic endothelial cells via vascular adhesion receptors regulated by proinflammatory cytokines and H_2O_2 (121–123). These microvascular events influence metastasis, and factors that neutralize inflammatory cytokines or adhesion receptors for cancer cells have therapeutic potential (124–126).

The intralobular micrometastasis phase

Intrasinusoidal cancer cell proliferation is activated by growth factors released from LSEC (122), while extravascular cancer cell proliferation is activated by factors released from tumour-activated perisinusoidal HSCs (127) and hepatocytes. These mechanisms are affected by both the phenotypic heterogeneity of hepatocytes and sinusoidal cells and the gradients of oxygen, hormones and ECM across the liver lobule.

A rich tumour growth-stimulating stroma is recruited into micrometastases before angiogenesis. The main sources of stromal cells are as follows: (a) HSCs (127), which support intralobular micrometastases and are transdifferentiated into myofibroblasts firstly at sites of cancer cell adhesion to LSEC before extravasation (A77) and secondly induced by paracrine factors from extravasated cancer cells. (b) Portal tract fibroblasts, which support perilobular micrometastases and are activated by cancer cells extravasated at terminal portal venules (128). (c) Perimetastatic hepatocytes, which sometimes suffer an epithelial to mesenchymal transition induced by cancer cells and tumour-activated HSCs, and express NGF as reported at the symposium (A46).

The angiogenic micrometastasis phase

Proangiogenic factors from hypoxic tumour-activated stromal cells and cancer cells contribute to this phase. Endothelial cell migration occurs only towards avascular micrometastases containing a high density of myofibroblasts and not towards those not containing myofibroblasts (127). Both myofibroblasts and endothelial cells colocalize, and their densities consistently correlate in well-vascularized metastases (127). Two predominant angiogenic patterns occur (129), which correlate with the site of metastatic cell implantation, the distinct stromal cell types, the invasion and growth patterns and the treatment resistance (130): (a) Sinusoidal-type angiogenesis occurring in metastases with replacement growth-pattern, where the liver architecture is preserved because cancer cells co-opt the existing network of sinusoids. Here, desmin- and glial fibrillary acidic protein-expressing myofibroblasts suggest their HSC origin (127). (b) Portal-type angiogenesis occurring in metastases with desmoplastic and pushing growth patterns. Here, the liver micro-architecture is not preserved and the growing metastatic tissue is delineated by desmoplastic stroma and compresses the surrounding parenchyma. Here, vimentin- and Thy-1 phenotype-expressing myofibroblasts suggest their portal tract fibroblast origin (128). Specific angiogenic factors produced by sinusoidal- and portal tract-derived myofibroblasts also contribute to angiogenic pattern differentiation (127, 128).

The established hepatic metastasis phase

Cancer cells can still be micro-environmentally modulated by stromal myofibroblasts and tumour-infiltrating hepatic lymphocytes, including CD4/CD25 regulatory T cells. The high intrahepatic concentration of proinflammatory and immunosuppressant cytokines, angiogenic and stromagenic factors, and soluble adhesion molecules also has regulatory effects on cancer cells, and prognostic implications. Consistent with this molecular micro-environment, remarkable gene expression alterations occur at hepatic metastases in this phase. Some originate at the primary tumours and may support hepatic metastasis, while others are differentially promoted by hepatocytes and hepatic myofibroblasts (A45). Consistent with the tumour proliferation-stimulating activity of sinusoidal cells, around 50% of hepatic metastasis genes regulated by factors from HSC-derived myofibroblasts belonged to the cell cycle-regulation class. Therefore, despite the occurrence of hepatic metastasis genes in the primary tumours, which may predict metastasis risk, tumour-activated hepatic cells may create a micro-environment contributing to the expression of genes operating at advanced phases of the hepatic metastasis process that may have therapeutic implications.

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