Pflugers Arch - Eur J Physiol (2013) 465:775–778
DOI 10.1007/s00424-012-1209-5

INVITED REVIEW

Hepatic stellate cells—the pericytes in the liver

Claus Hellerbrand

Received: 3 December 2012 / Revised: 12 December 2012 / Accepted: 14 December 2012 / Published online: 5 January 2013

Abstract Hepatic stellate cells (HSCs) are pericytes of liver in the space between parenchymal cells and sinusoidal endothelial cells of the hepatic lobule. HSCs comprise specialized functions such as vitamin A storage, hemodynamic functions, support of liver regeneration, and immunoregulation. In pathological conditions, HSCs transform to an activated myofibroblasts-like phenotype, start to proliferate, and de novo express several proinflammatory and profibrogenic genes. These processes are particularly important in the development of cirrhosis, portal hypertension, and hepatocellular cancer. This review highlights recent findings in understanding the biology of HSCs and discusses the physiological functions of HSCs and the role of activated HSCs in pathophysiology and disease.

Keywords Hepatic stellate cell · Myofibroblast · Pericyte · Fibrosis · Immunoregulation · Vasoregulation · Hepatocellular cancer

Abbreviations

α-SMA	Alpha-smooth muscle actin
ECM	Extracellular matrix
HSCs	Hepatic stellate cells
HCC	Hepatocellular carcinoma
MMPs	Metalloproteinases

Hepatic stellate cells (HSCs) are liver-specific pericytes within the vasculature of the hepatic sinusoid. Stellate cells ("Sternzellen") were first described by the German anatomist Carl von Kupffer in the nineteenth century. In 1952, the Japanese anatomist Toshio Ito defined HSCs as fat-storing cells. HSCs have been given various names as perisinusoidal cell, pericyte, lipocytes, interstitial cells, fat-storing cells, vitamin A-storing cells, or Ito cells [12, 14]. Standardization of the nomenclature as "hepatic stellate cells" took place in 1996 [1].

HSCs are the major nonparenchymal element and constitute approximately 10–15 % the total number of resident cells in normal liver including hepatocytes [12]. HSCs are located in the perisinusoidal space of Disse between the fenestrated liver endothelium and epithelial hepatocytes. HSCs display a dendritic morphology and embrace with thornlike microprojections the endothelial cell layer of the sinusoids providing physical contact not only to sinusoidal endothelial cells but also with the cell body to the hepatocytes (Fig. 1).

The origin of HSCs is still a matter of debate. HSCs express both mesenchymal cell markers such as desmin, type I collagen, and vimentin, as well as neuronal or glial cell markers such as neural cell adhesion molecule, synaptophysin, glial fibrillary acidic protein, nestin, and p75Ntr [10]. This expression pattern suggested the neural crest as the origin of HSCs but lineage-tracing studies performed by Cassiman et al. failed to detect neural crest-derived HSCs in developing liver [6]. Another hypothesis suggests multipotent mesenchymal progenitor cells as the origin of HSCs, particularly because these cells also give rise to neural cells as well as mesenchymal lineages for smooth muscle cells or adipocytes whose markers are also expressed by HSCs [10]. Consistent with this hypothesis, Asahina et al. demonstrated that HSCs are derived from mesoderm and at least in part via septum transversum and mesothelium [3]. Still, one limitation of this elegant study was that the lineage-tracing analyses were carried out in fetal liver.

C. Hellerbrand (✉)
Department of Internal Medicine I, University Hospital Regensburg, 93053 Regensburg, Germany
e-mail: claus.hellerbrand@ukr.de

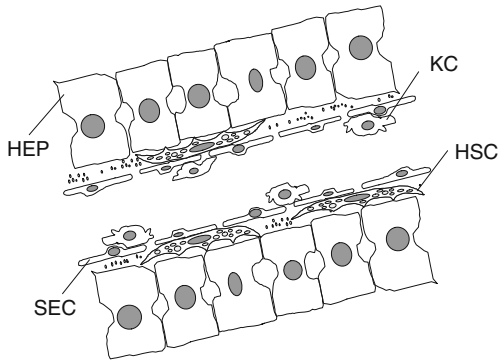

Fig. 1 Structure of hepatic lobule. The liver lobule is formed by parenchymal cells, i.e., hepatocytes and nonparenchymal cells. Parenchymal hepatocytes form the cords of the lobule while nonparenchymal liver cells are localized in the sinusoidal compartment of the tissue. Sinusoidal endothelial cells (SECs) form the lining of the sinusoids. Kupffer cells (KCs), which are liver-resident macrophages, are mainly located within the sinusoids. Hepatic stellate cells (HSCs) are located in the space of Disse between hepatocytes and SECs. Further nonparenchymal liver cells not depicted in this cartoon include dendritic cells, which are located in the portal triad, and intrahepatic lymphocytes, including pit cells, i.e., liver-specific natural killer cells

Physiological functions of hepatic stellate cells

The most characteristic feature of HSCs in normal liver is their role in vitamin A (retinol) transport and storage. HSCs store 80 % of total body retinol as retinyl esters in the lipid droplets in the cytoplasm and regulate both transport and storage of vitamin A [5]. Vitamin A autofluorescence allows imaging of HSCs along the hepatic sinusoids (Fig. 2).

HSCs also contribute to the three-dimensional architecture of the normal liver. They regulate the extracellular matrix (ECM) turnover in the space of Disse by secreting correct amounts of ECM molecules accompanied by secretion of degrading enzymes called MMPs and their (tissue) inhibitors (TIMPs) [22].

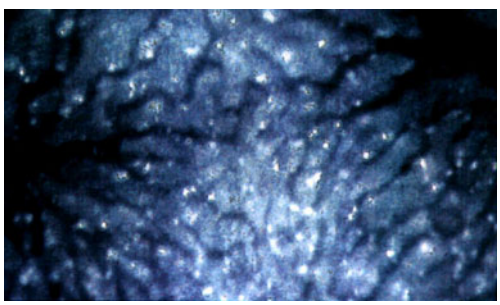

Fig. 2 Fluorescence microscopy for detection of vitamin A carrying lipid droplets in hepatic stellate cells. Vitamin A exhibits a *blue-green* autofluorescence when excited with the light of approximately 328-nm wavelength. Thus, fluorescence microscopy of murine liver tissue allows specific demonstration of autofluorescence of vitamin A emitted from the lipid droplets of HSCs and demonstrates localization of HSCs lining the hepatic sinusoid (magnification×200)

Furthermore, HSCs are critical regulators of liver regeneration as it occurs after partial hepatectomy. The liver has a remarkable growth capacity, and following resection of two-third of the liver, the hepatic mass is almost completely restored after 2 weeks. A recent study demonstrated that HSCs act as a positive regulator at the early phase and a negative regulator at the terminal phase of liver regeneration through cell–cell interaction and cytokine networks [8].

Moreover, HSCs are implicated in the regulation of sinusoidal tone and are now regarded as the principal cells involved in sinusoidal blood flow regulation [19]. In line with their potential role in vasoregulation, HSCs express a large number of receptors and intracellular mediators that modulate cellular contraction [20]. Further, nerve endings in the intralobular spaces are localized mainly in the Disse spaces and are oriented and especially closely related to HSCs [24]. The exact meaning of this remains to be elucidated, but it can be speculated that HSCs could be involved in neurotransmission.

Furthermore, HSCs display immunological properties such as modulation of hepatic tolerance. The liver is considered to be an immune-privileged organ. For example, recipients of human liver allografts require less immunosuppression than is necessary with other organs. Ichikawa et al. demonstrated that HSCs function as regulatory bystanders, capable of enhancing differentiation and accumulation of regulatory T cells [13], which contribute significantly to the tolerogenic nature of the liver. Further, HSCs may play an immunoregulatory role in chronic viral hepatitis recipients to minimize the effect of immunosuppressants without affecting rejection [21].

Activation of hepatic stellate cells

In the normal liver under physiological conditions, HSCs reside in a quiescent stage. Following liver injury, HSCs undergo an activation process to a highly proliferative, myofibroblast-like cell type. HSC activation in diseased liver is characterized morphologically by enlargement of rough-surfaced ER, loss of vitamin A droplets, ruffled nuclear membrane, and appearance of contractile filaments. The expression of the cytoskeletal protein alpha-smooth muscle actin (α-SMA) is increased, which confers increased contractile potential, and α-SMA is recognized as a marker of activated HSCs [4, 22].

Activated HSCs synthesize and secrete a large amount of ECM components such as collagen, proteoglycan, glycosaminoglycan, and glycoprotein. Deposition of ECM is further enhanced by the production of TIMPs, which prevent the degradation of ECM, leading to a net accumulation of ECM with a gradual disruption of normal liver architecture [16].

During the activation of HSCs, there also occur dramatically altered and enhanced expression and secretion of numerous pro- and anti-inflammatory cytokines and growth factors [4, 22]. The most potent inducer of the expression of collagen I and other ECM constituents by HSCs is transforming growth factor-β. Platelet-derived growth factor is a potent inducer of HSC proliferation. Both factors are de novo expressed during HSC activation as well as their corresponding receptors [4, 10, 22]. Generally, autocrine signaling is a critical process during HSC activation, underlining the importance of tightly regulated local control of cytokine and growth factor action within the pericellular milieu.

Establishment of cell isolation and culture methods of HSCs from rodent and human livers promoted the development of HSC research in the early 1980s [10]. Thus, the activation process of HSCs can be simulated by culturing freshly isolated HSCs on uncoated plastic cell culture dishes. Within 1–2 weeks, also in vitro HSCs undergo an activation process towards a myofibroblast-like phenotype. Numerous studies have shown that gene expression pattern and cellular functions of in vitro activated HSCs closely resemble pathological characteristics of activated HSCs in diseased livers [4, 10, 22].

Role of activated hepatic stellate cells in pathophysiology and disease

The activation of HSCs is the key event of hepatic fibrogenesis. Fibrosis during chronic disease can be considered as deregulated wound healing. Persistent hepatocellular injury leads to perpetuation and acceleration of HSC activation with increased ECM synthesis and impaired ECM degradation (fibrolysis). Increased net ECM deposition leads to a gradual disruption of normal liver architecture and ultimately liver cirrhosis [4, 16].

The best studied ECM component of hepatic fibrosis is collagen type I, the expression of which is regulated both transcriptionally and posttranscriptionally in HSCs. Together with other HSC-produced ECM constituents such as sulfated proteoglycans and adhesive glycoproteins, collagen I forms the fibrotic bands surrounding nodules in cirrhotic livers. However, already early deposition of ECM in the subendothelial space of Disse—so-called “capillarization” of the sinusoid—causes diminished liver function and also contributed to the perpetuation of HSC activation [16, 22].

Activated HSCs also are significant mediators of hepatic immunoregulation. They secrete numerous different chemokines and cytokines such as interleukin-6, IL-8, monocyte chemotactic peptide-1, and regulated on activation normal T cell expressed and secreted [4, 15]. Thus, HSCs initiate and amplify hepatic inflammation by inducing infiltration of mono- and polymorphonuclear leukocytes. Further, activated HSCs promote differentiation of liver macrophages with proinflammatory as well as profibrotic functions [7]. In addition, they produce complement protein C4 [9], which contributes to the hepatic inflammatory response. Activated HSCs also function as antigen-presenting cells that can stimulate lymphocyte proliferation or apoptosis [24]. Furthermore, activated HSCs modulate the hepatic immune response by their expression of the costimulatory molecules as B7-H1 (PDL-1 or programmed death ligand-1) [17], which plays a crucial role in regulating T cell apoptosis. Moreover, activated HSCs express Toll-like receptors 4 and 9 [11, 18], which indicates their capacity to interact with bacterial products.

In addition to their critical role in hepatic inflammation and fibrosis, activated HSCs play a role in the pathogenesis of portal hypertension. According to their myofibroblastic phenotype, activated HSCs respond by contraction to vasoactive substances such as endothelin 1 and angiotensin II [19]. The acquisition of a contractile phenotype during HSC activation has been documented in culture and in vivo. Both the anatomical location of HSCs and the capacity to contract or relax in response to various vasoactive mediators indicate activated HSCs as major determinant of early and late increases in portal resistance during liver fibrosis.

Activated HSCs also infiltrate the stroma of liver tumors and localize around tumor sinusoids, fibrous septa, and capsules (Fig. 3). The interaction between tumor cells and activated HSCs has been recognized to fundamentally affect cancer development and progression of hepatocellular carcinoma (HCC). Conditioned media collected from activated HSCs induced proliferation and migration of HCC cells cultured in monolayers [2]. Moreover, in a three-dimensional spheroid coculture system, HSCs promoted HCC growth and diminish the extent of central necrosis through the activation of critical cancer-related signaling

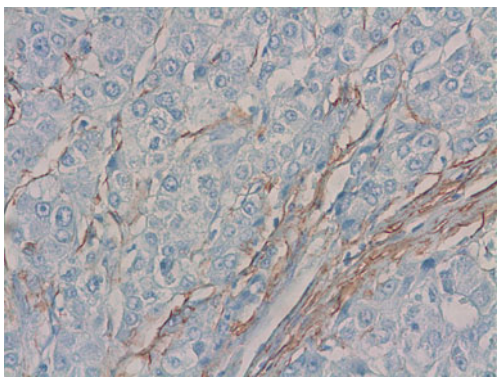

Fig. 3 Activated hepatic stellate cells in hepatocellular carcinoma. Immunohistochemical staining of human hepatocellular carcinoma tissue for alpha-smooth muscle actin, a marker for activated HSCs. Activated HSCs localize around fibrous septa and transduce the HCC stroma (magnification × 200, tissues was counterstained with hematoxylin)

pathways [2]. Consistent with these findings, simultaneous in vivo implantation of HSCs and HCC cells into nude mice promoted tumor growth and invasiveness as compared to tumors derived from HCC cells implanted without activated HSCs [2]. Further studies revealed function of activated HSCs in the process of tumor angiogenesis and sinusoidal remodeling [23]. Thus, disruption of the normal liver matrix is also a requirement for tumor invasion and desmoplasia.

In summary, HSCs not only share many anatomic and phenotypic similarities with pericytes in other organs but also exhibit remarkable and distinct competence in physiological as well as pathologic conditions. Since the discovery of these liver pericytes more than 100 years recognized within the vasculature of the sinusoid, we got several insights into the biology and function of these fascinating cells. Still, there are quite a few key issues which have to be addressed. Thus, it appears promising to further elucidate the role of HSCs in the remarkably high immunotolerance of liver. Further, the assessment of the complex cross talk of HSCs with other liver cells and infiltrating inflammatory cells is of importance. It further remains unclear whether the process of HSC activation can be reverted in vivo, which would provide further evidence of fascinating plasticity of these cells.

References

1. Ahern M, Hall P, Halliday LC, Olynyk J, Ramm G, Denk H (1996) Hepatic stellate cell nomenclature (letter). Hepatology 1:193
2. Amann T, Bataille F, Spruss T, Muhlbauer M, Gabele E, Scholmerich J, Kiefer P, Bosserhoff AK, Hellerbrand C (2009) Activated hepatic stellate cells promote tumorigenicity of hepatocellular carcinoma. Cancer Sci 4:646–653
3. Asahina K, Tsai SY, Li P, Ishii M, Maxson RE Jr, Sucov HM, Tsukamoto H (2009) Mesenchymal origin of hepatic stellate cells, submesothelial cells, and perivascular mesenchymal cells during mouse liver development. Hepatology 3:998–1011
4. Bataller R, Brenner DA (2005) Liver fibrosis. J Clin Invest 2:209–218
5. Blomhoff R, Blomhoff HK (2006) Overview of retinoid metabolism and function. J Neurobiol 7:606–630
6. Cassiman D, Barlow A, Vander BS, Libbrecht L, Pachnis V (2006) Hepatic stellate cells do not derive from the neural crest. J Hepatol 6:1098–1104
7. Chang J, Hisamatsu T, Shimamura K, Yoneno K, Adachi M, Naruse H, Igarashi T, Higuchi H, Matsuoka K, Kitazume MT, Ando S, Kamada N, Kanai T, Hibi T (2012) Activated hepatic stellate cells mediate the differentiation of macrophages. Hepatol Res. doi:10.1111/j.1872-034X.2012.01111.x
8. Chen L, Zhang W, Zhou QD, Yang HQ, Liang HF, Zhang BX, Long X, Chen XP (2012) HSCs play a distinct role in different phases of oval cell-mediated liver regeneration. Cell Biochem Funct 7:588–596
9. Fimmel CJ, Brown KE, O'Neill R, Kladney RD (1996) Complement C4 protein expression by rat hepatic stellate cells. J Immunol 6:2601–2609
10. Friedman SL (2008) Hepatic stellate cells: protean, multifunctional, and enigmatic cells of the liver. Physiol Rev 1:125–172
11. Gabele E, Muhlbauer M, Dorn C, Weiss TS, Froh M, Schnabl B, Wiest R, Scholmerich J, Obermeier F, Hellerbrand C (2008) Role of TLR9 in hepatic stellate cells and experimental liver fibrosis. Biochem Biophys Res Commun 2:271–276
12. Geerts A (2001) History, heterogeneity, developmental biology, and functions of quiescent hepatic stellate cells. Semin Liver Dis 3:311–335
13. Ichikawa S, Mucida D, Tyznik AJ, Kronenberg M, Cheroutre H (2011) Hepatic stellate cells function as regulatory bystanders. J Immunol 10:5549–5555
14. Ito T, Nemoto M (1952) Kupfer's cells and fat storing cells in the capillary wall of human liver. Okajimas Folia Anat Jpn 4:243–258
15. Marra F (2002) Chemokines in liver inflammation and fibrosis. Front Biosci 7:1899–1914
16. Mormone E, George J, Nieto N (2011) Molecular pathogenesis of hepatic fibrosis and current therapeutic approaches. Chem Biol Interact 3:225–231
17. Muhlbauer M, Fleck M, Schutz C, Weiss T, Froh M, Blank C, Scholmerich J, Hellerbrand C (2006) PD-L1 is induced in hepatocytes by viral infection and by interferon-alpha and -gamma and mediates T cell apoptosis. J Hepatol 4:520–528
18. Paik YH, Schwabe RF, Bataller R, Russo MP, Jobin C, Brenner DA (2003) Toll-like receptor 4 mediates inflammatory signaling by bacterial lipopolysaccharide in human hepatic stellate cells. Hepatology 5:1043–1055
19. Reynaert H, Urbain D, Geerts A (2008) Regulation of sinusoidal perfusion in portal hypertension. Anat Red 6:693–698
20. Rocky DC (1995) Characterization of endothelin receptors mediating rat hepatic stellate cell contraction. Biochem Biophys Res Commun 2:725–731
21. Su YH, Shu KH, Hu C, Cheng CH, Wu MJ, Yu TM, Chuang YW, Huang ST, Chen CH (2012) Hepatic stellate cells attenuate the immune response in renal transplant recipients with chronic hepatitis. Transplant Proc 3:725–729
22. Tacke F, Weiskirchen R (2012) Update on hepatic stellate cells: pathogenic role in liver fibrosis and novel isolation techniques. Expert Rev Gastroenterol Hepatol 1:67–80
23. Thabut D, Shah V (2010) Intrahepatic angiogenesis and sinusoidal remodeling in chronic liver disease: new targets for the treatment of portal hypertension? J Hepatol 5:976–980
24. Ueno T, Bioulac-Sage P, Balabaud C, Rosenbaum J (2004) Innervation of the sinusoidal wall: regulation of the sinusoidal diameter. Anat Rec A Discov Mol Cell Evol Biol 1:868–873