Hepatic myofibroblasts: a heterogenous population of multifunctional cells in liver fibrogenesis

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Abstract

Hepatic myofibroblasts constitute a heterogenous population of highly proliferative, pro-fibrogenic, pro-inflammatory, pro-angiogenic and contractile cells that sustain liver fibrogenesis and then fibrotic progression of chronic liver diseases of different aetiology to the common advanced-stage of cirrhosis. These α-smooth muscle actin-positive myofibroblast-like cells, according to current literature, mainly originate by a process of activation and trans-differentiation that involves either hepatic stellate cells or fibroblasts of portal areas. Hepatic myofibroblasts can also originate from bone marrow-derived cells, including mesenchymal stem cells or circulating fibrocytes able to engraft chronically injured liver, as well as, in certain conditions, by a process of epithelial to mesenchymal transition involving hepatocytes and cholangiocytes. Hepatic myofibroblasts may have also additional crucial roles in modulating immune response and in the cross talk with hepatic progenitor (stem) cells as well as with malignant cells of either primary hepatocellular carcinomas or of metastatic cancers.

1. Introduction

Hepatic myofibroblasts (MFs) represent a heterogenous population of α-smooth muscle actin (αSMA)-positive pro-fibrogenic cells, mainly found in chronically injured livers (i.e., fibrotic and or cirrhotic), expressing a peculiar repertoire of antigens (Cassiman et al., 2002). Clinical and experimental evidence suggest that different populations of MFs exist that may be recognized by means of tissue localization and/or antigen profile (Fig. 1): (a) portal/septal MFs (PS/MFs), displaying an overlapping antigen repertoire, commonly found in the expanded connective tissue around portal tracts (portal MFs) or in the inner part of fibrotic septa (septal MFs), (b) interface MFs (IF/MFs), found at the edge between fibrotic septa and the surrounding parenchyma (i.e., where active fibrogenesis occurs), (c) activated, myofibroblast-like, hepatic stellate cells (HSC/MFs), αSMA-positive cells found primarily in or around capillarised sinusoids of fibrotic/cirrhotic livers.

Origin of hepatic MFs and processes leading to MF like phenotype (following liver injury)

Hepatic Stellate Cells (HSC)

GFs, chemokines, ROS, adipokines, cannabinoids, apoptotic bodies, etc.

Portal Fibroblasts (PS/MFs)

Activation and transdifferentiation
- initiation
- perpetuation

MF

Hepatocytes

Epithelial to mesenchymal transition

Activation & Transdifferation ?

Colangiocytes

Bone Marrow — MSC

Recruitment/engraftment into injured liver

Circulating Fibrocytes

Antigen repertoire of subpopulations of MFs in fibrotic/cirrhotic human livers

	HSC/MFs	IF/MFs	PS/MFs
α-SMA	+++	+	+
N-CAM	+++	±	-
SYN	+/++	-	-
NGF	+/++	±	-
NT3	++	-	-
NT4	+	±	-
Trk B Trk C p75	+	-	-
α, β CRYS	+	+	±

HSC/MFs — MFs from HSC
IF/MFs — Interface MFs
PS/MFs — Portal/Septal MFs

Roles of MFs in human liver pathologies (ascertained or suggested)

Pro-Fibrogenic
- proliferation (all MFs)
- migration (all MFs)
- ECM synthesis (all MFs)
- contractility (HSC/MFs)
- proinflammatory (HSC/MFs)

Pro-angiogenic
- documented for HSC/MFs and IF/MFs

Immunomodulatory
- documented for HSC/MFs

In liver regeneration
- proposed for HSC/MFs

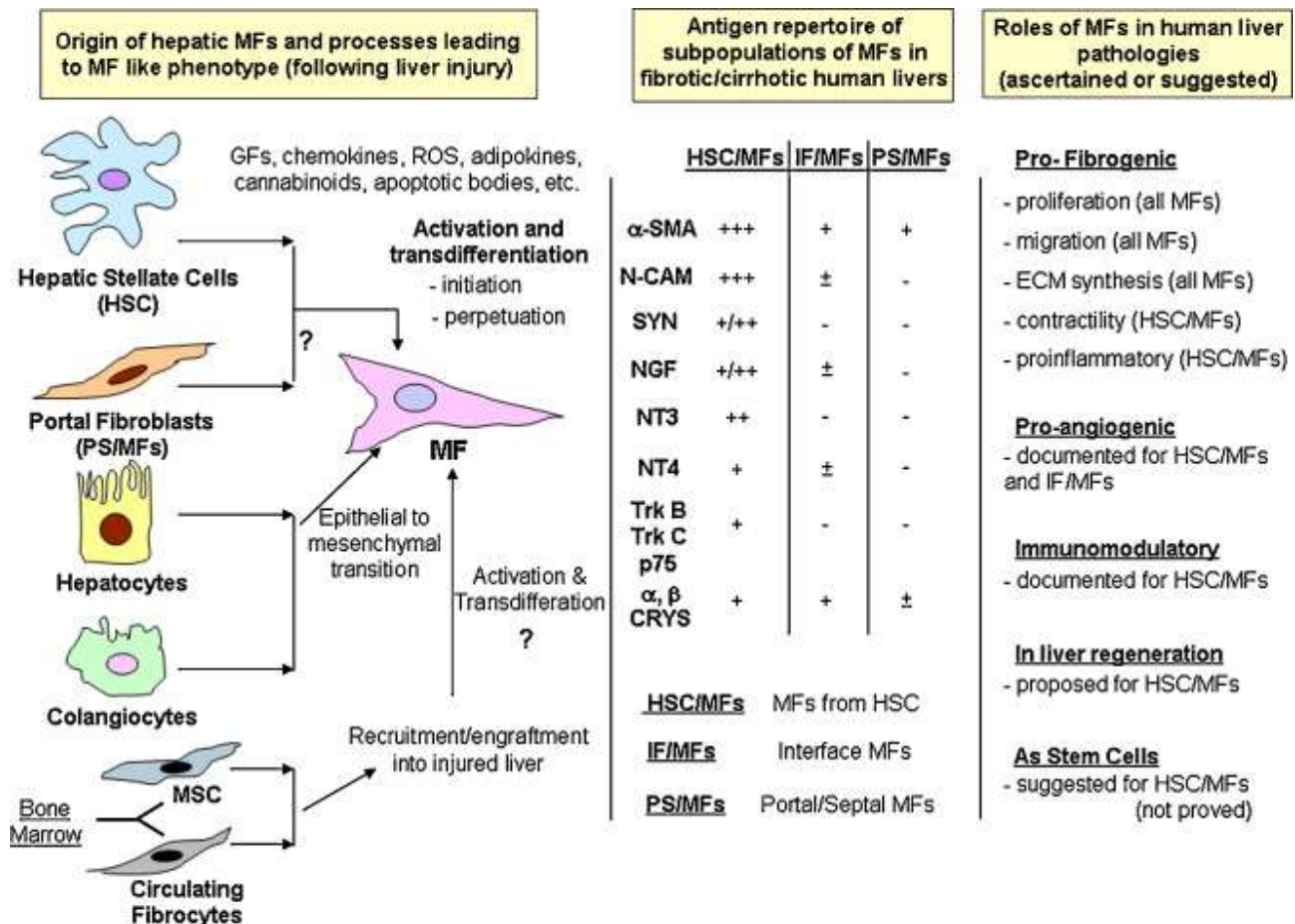**As Stem Cells**
- suggested for HSC/MFs (not proved)

Fig. 1.

Subpopulations of MFs, that may originate from different cell sources, can be recognized by means of tissue localization (portal/septal MFs or PS/MFs; interface MFs or IF/MFs; activated myofibroblast-like hepatic stellate cells or HSC/MFs) as well as by their specific antigen repertoire (this part adapted from Cassiman et al., 2002). In the figure are also summarized processes/mechanisms leading to acquisition of MFs-like phenotype and ascertained and proposed roles in human liver pathologies. The reader should note the following: (1) because of MFs heterogeneity, at present available methods to isolate MFs from chronically injured livers suffer of a significant lack of specificity; in particular, the so called "outgrowth" technique is likely to result in the isolation of a mixed population of MFs. (2) At present, it is difficult to propose a single and unequivocal marker for MFs originating from bone marrow: unequivocal identification of cells coming from bone marrow has been obtained only using sex-mismatch transplant in murine models (see Forbes et al., 2004 and Russo et al., 2006) or by identifying transplanted human bone marrow cells engrafting the injured liver of immunopermissive mice (Valfrè di Bonzo et al., 2008). (3) Identification of MFs originating after EMT is also difficult, although several studies (see Zeisberg et al., 2007 and Friedman, 2008a and ref. therein) have proposed the use of FSP1 (fibroblast-specific protein 1, also known as S100A4) for this purpose.

1.1. Cell origin and plasticity

Profibrogenic hepatic MFs can originate from different cellular sources (Fig. 1), with a relative contribution that may vary depending on the specific aetiology of the chronic liver disease (CLD) and/or the prevailing fibrogenic mechanism (Friedman, 2008b and Parola et al., 2008).

1.1.1. Hepatic stellate cells

Hepatic stellate cells in normal liver are perisinusoidal cells of still uncertain embryological origin, responsible for the synthesis of basal membrane like-ECM components of the subendothelial space of Disse and for storage and metabolism of vitamin A and retinoids (Geerts, 2001). HSC have been also proposed to act as "liver specific pericytes" and to significantly contribute to hepatic development and regeneration (Friedman, 2008a). HSC have been the first cell source of pro-fibrogenic MF-like cells to be identified and HSC/MFs still remain the most investigated cell population for which the process of activation and pro-fibrogenic mechanisms are best characterized (Friedman, 2008a). HSC/MFs are involved in most of clinical conditions of CLDs, with a prevailing involvement in the pattern of fibrosis progression defined as "perisinusoidal/pericellular fibrosis", recognising a metabolic or alcoholic aetiology. HSC may contribute also to the origin of interface MFs and contribute to the pattern of "bridging fibrosis" found in patients affected by chronic viral hepatitis (Parola et al., 2008).

1.1.2. Portal fibroblasts

Portal fibroblasts are located in the connective tissue of portal areas and their activation into MFs is relevant in ischemic conditions and in obstructive cholestatic diseases (pattern of "biliary fibrosis", Parola et al., 2008). Because of overlapping antigen repertoire, portal fibroblasts are considered to give origin also to septal MFs.

1.1.3. Bone marrow-derived cells

Under conditions of chronic liver injury, pro-fibrogenic MFs (mainly IF/MFs and some portal MFs) have been described also to originate from progressive recruitment of bone marrow-derived cells (Forbes et al., 2004) like mesenchymal stem cells or MSC (Russo et al., 2006 and Valfrè di Bonzo et al., 2008) and circulating fibrocytes (Kisseleva et al., 2006).

1.1.4. Cholangiocytes and hepatocytes

Profibrogenic cells in CLDs may also originate from cells of epithelial origin like cholangiocytes and hepatocytes through the process of epithelial to mesenchymal transition or EMT (Zeisberg et al., 2007 and Friedman, 2008b), a process originally described in embryologic and fetal development and actually involved in cancer cell invasiveness and progression as well as in the development of chronic inflammatory and fibrogenic diseases of different organ and tissues (Lee et al., 2006). The real relevance of EMT in CLDs is actually a matter of intense debate.

2. Functions

Hepatic MFs may originate from different cell sources through a process of activation/transdifferentiation (Fig. 2) that may involve common mediators, mechanism and signalling pathways, but our present knowledge mostly derives from "in vivo" and "in vitro" studies performed on activated human or rodent HSC (Friedman, 2008a). Accordingly, here we will mainly refer to activation of HSC to HSC/MFs and to main responses operated by HSC/MFs.
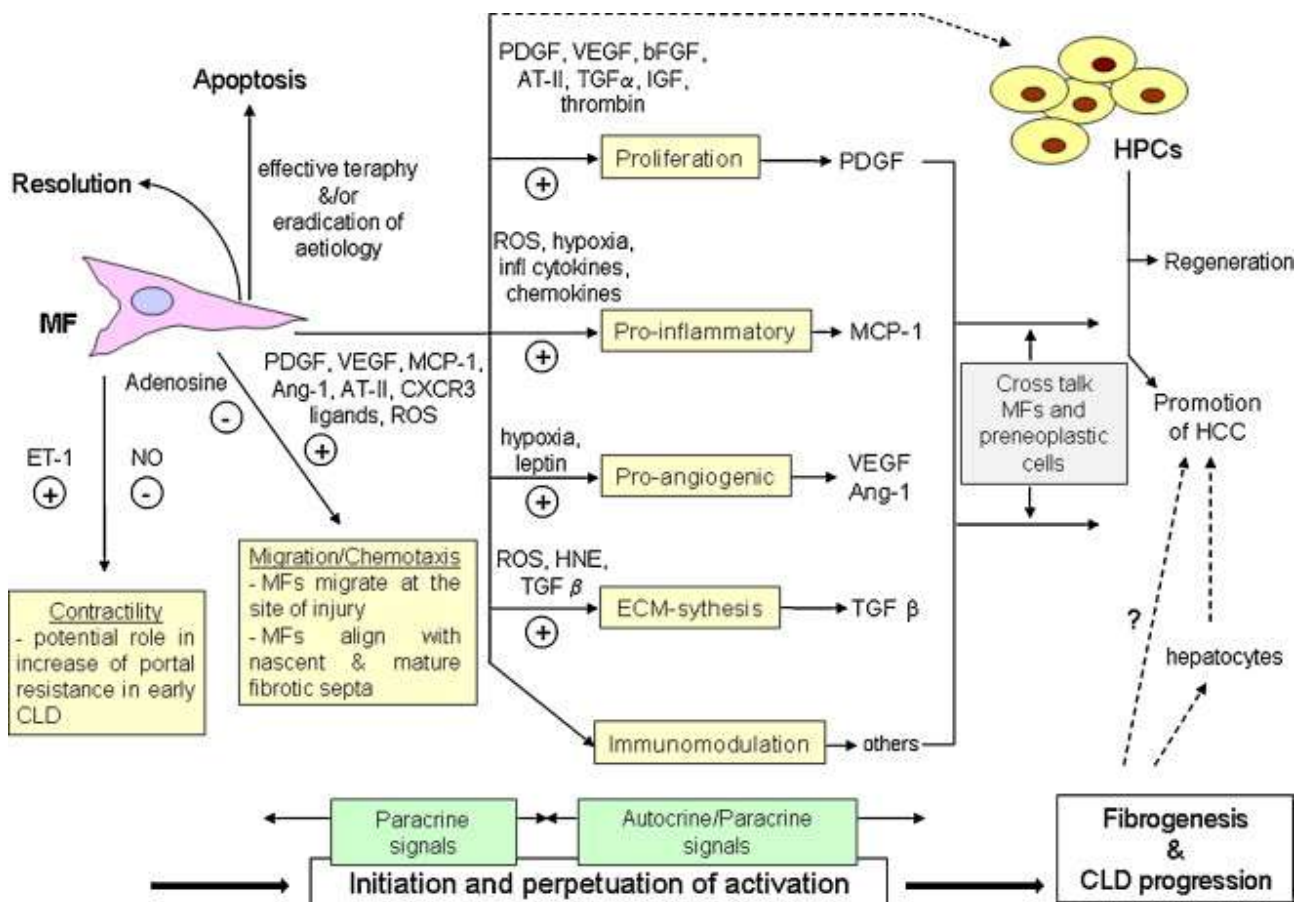
Fig. 2.

Phenotypic responses of fully activated MF-like cells include proliferation, migration/chemotaxis, contractility, excess deposition, altered remodelling of ECM. Perpetuation of activation of MFs is the main driving force for progressive fibrogenesis; moreover, cross-talk of HSC/MF and, likely, other MFs with either hepatic progenitor cells (HPCs) and/or pre-neoplastic cells may contribute to hepatocellular carcinoma (HCC).

Activation of HSC, following liver injury, progress in sequential stages of initiation and perpetuation (Friedman, 2008a and Friedman, 2008b). Initiation is an early response stimulated by a number of paracrine signals, leading to a transient and potentially reversible contractile and profibrogenic phenotype, characterized by rapid induction of platelet-derived growth factor (PDGF) β receptor and primed to respond to several growth factors and mediators that will be crucial in eliciting phenotypic responses operated by fully activated MF-like phenotype (perpetuation), including proliferation, migration/chemotaxis, contractility, excess deposition and altered remodelling of ECM and much more (see Fig. 1, Fig. 2 and Fig. 3).
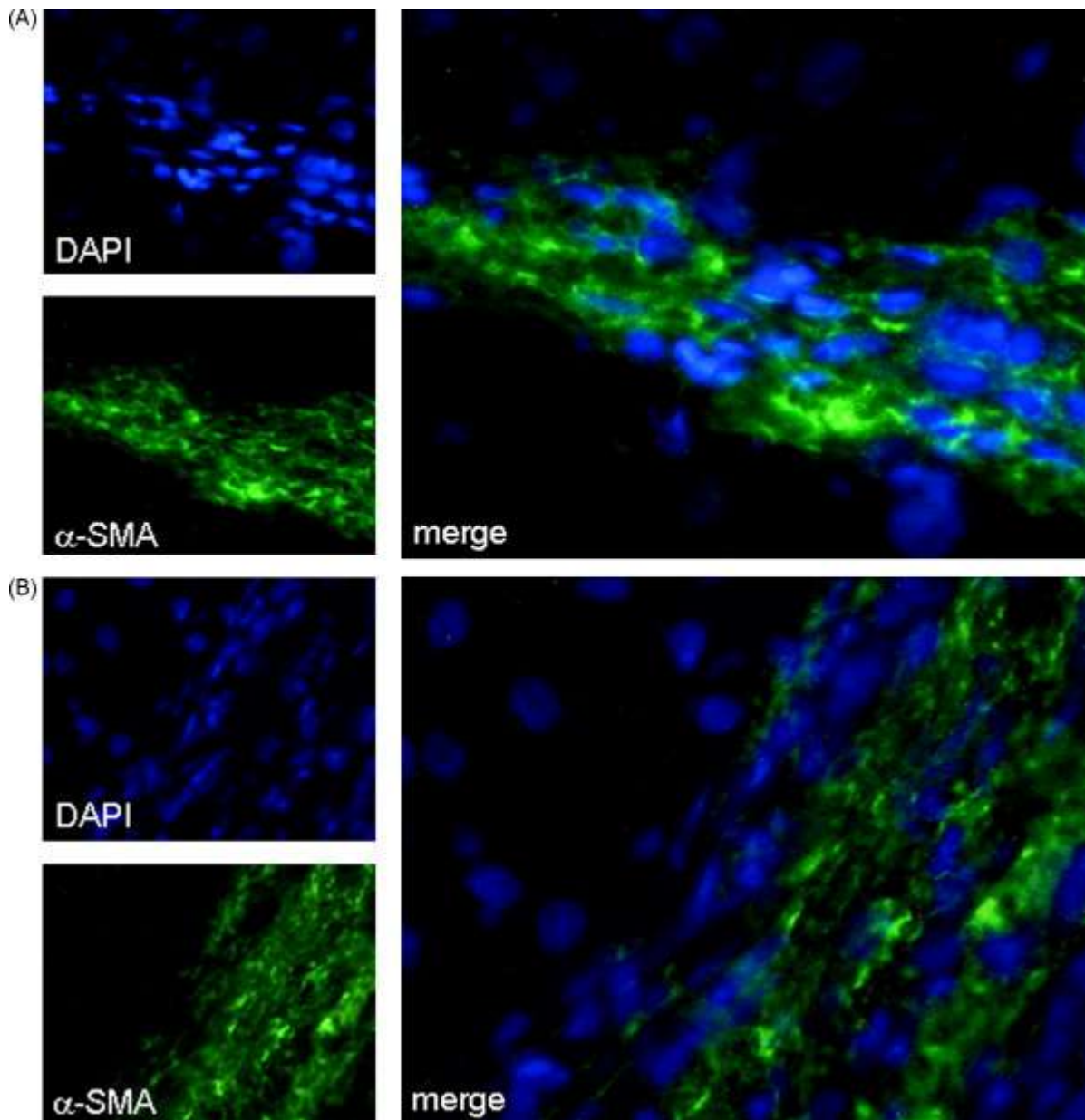
Fig.

Fig. 3.

Indirect immuno-fluorescence staining of α-SMA positive MFs in nascent (Panel A) or mature (Panel B) fibrotic septa, as detected on cryostat section of cirrhotic liver from HCV chronic patients, as previously described (Novo et al., 2007). Tiny images on the left always represent image acquisition of single fluorescence identifying nuclei (blue fluorescence, DAPI staining) or α-SMA immuno-positive cells (green fluorescence). The two large images represent the electronic merging of their respective tiny images. Original magnification: ×400.

2.1. Phenotypic responses of HSC/MFs, likely to be common to all hepatic MFs

Proliferation of activated MF-like cells is elicited by a number of mitogens but it is believed to be mainly sustained by homo- and heterodimeric PDGF isoforms, either released by activated Kupffer cells, sinusoidal endothelial cells, platelets or by activated MFs in an autocrine pathway, as well as by sustained expression of related receptors. PDGF-related down-stream signalling involves Ras/ERK pathway, phosphatidyl-inositol 3-kinase (PI-3K), ERK5 and others (Pinzani and Marra, 2001 and Friedman, 2008b). PDGF-BB also represent

the best characterized and most potent chemoattractant for HSC/MFs (Pinzani and Marra, 2001) and MF-like cells from human MSC (Valfrè di Bonzo et al., 2008), with migration/chemotaxis being an essential feature of MFs in order to reach the site of injury and to align with both nascent and established fibrotic septa. Migration/chemotaxis of MFs is also elicited by monocyte chemoattractant protein 1 (MCP-1), angiotensin II, vascular endothelial growth factor (VEGF), angiopoietin-1, reactive oxygen species (ROS), CXCR3 ligands, all involving primarily Ras/ERK signalling (Novo et al., 2007, Friedman, 2008a and Novo and Parola, 2008).

MFs are responsible for excess deposition of fibrillar matrix (mainly collagen type I and III), a hallmark of fibrotic and cirrhotic livers as a response to transforming growth factor (TGF) β1 released by Kupffer cells and by HSC/MFs (paracrine and autocrine sources), through downstream signalling involving Smads 2 and 3. Connective tissue growth factor (CTGF) and cannabinoids have been also identified as potent profibrogenic signals for HSC/MFs (Friedman, 2008b).

HSC/MFs show features of smooth muscle cells and contractility; by responding to opposing vasoactive mediators like endothelin-1 and nitric oxide, they contribute to increased portal resistance during early stages of fibrosis (late and fixed increase in portal pressure being due to distortion of angioarchitecture in the cirrhotic liver).

2.2. MFs in matrix degradation/remodelling and resolution of fibrosis

Progressive fibrogenesis is characterized by replacement of low-density basement membrane of the subendothelial space of Disse with fibril-forming matrix, a scenario that negatively affect differentiated cell functions (mainly of hepatocytes). This scenario essentially results from a disequilibrium between excess deposition of fibrillar collagens and reduced/altered degradation/remodelling of fibrotic ECM. Accordingly, HSC/MFs mainly express metallo-proteinases (MMPs) able to degrade basement membrane (MMP-2, MMP-9, MMP3 or stromelysin) that are less efficient to degrade fibrillar matrix, with a low expression of MMP-1 (interstitial collagenase). HSC/MFs and MFs also overexpress tissue inhibitor of metalloproteinase type I (TIMP-1) that inhibit interstitial collagenases and act as anti-apoptotic for HSC/MFs. Deposition of fibrillar matrix and formation of fibrotic septa is also favoured by the fact that HSC/MFs and, likely, all MFs, develop resistance to induction of apoptosis (El-Sharkawy et al., 2005, Novo et al., 2006 and Friedman, 2008b). Related to this concept are findings of the last decade suggesting that (Iredale, 2007) liver fibrosis and, possibly, initial stages of cirrhosis are potentially reversible in the presence of effective therapy and/or aetiology eradication. Regression of histopathology develops as a result of increased apoptosis of HSC/MFs and MFs and is paralled by increased expression of interstitial collagenases by hepatic macrophages.

2.3. HSC/MFs in inflammatory signalling and regulation of immune response

Persisting inflammatory response in a CLD is one of the "driving forces" sustaining fibrogenesis. HSC/MF and, likely, all MFs are target cells for inflammatory cytokines and other pro-inflammatory signals like (a) ROS and other oxidative stress-related mediators like 4-hydroxy-2,3-nonenal or HNE, generated as a consequence of hepatocyte injury and necrosis, (b) apoptotic bodies (engulfing and activating), and (c) bacterial endotoxin or other endogenous activators of Toll Like Receptor 4 (TLR4) of innate immunity displayed by HSC/MFs. On the other hand, HSC/MFs are also the cell source (even in an autocrine manner)

of a number of pro-inflammatory molecules, including TLR ligands, MCP-1 and other chemoattractants and chemokines (Bataller and Brenner, 2005 and Friedman, 2008b).

HSC/MFs can also play a crucial role in modulating hepatic immune responses (reviewed in Friedman, 2008a and Friedman, 2008b), and then fibrogenesis, by behaving as antigen presenting cells, by inducing locally immunotolerance throughout T cell suppression or by interacting directly with subsets of T lymphocytes (mainly CD8). Finally, natural killer cells (NK cells) seem to be able to selectively kill HSC/MFs (stimulated by interferon and inhibited by ethanol).

2.4. MFs in sustaining angiogenesis

HSC/MFs and at least IF/MFs have an additional dual role in pathological angiogenesis that accompanies progression of CLDs. Under conditions of hypoxia they respond by up-regulating pro-angiogenic cytokines like VEGF-A and angiopoietin-1 (also elicited by leptin) and related receptors like VEGFR type II and Tie-2, respectively. In biopsies from HCV-positive cirrhotic patients this is detected in tiny developing fibrotic septa. Pro-angiogenic cytokines can also sustain chemotaxis of these cells (VEGF and Ang-1) as well as stimulate ECM synthesis and proliferation (VEGF), suggesting a role for HSC/MFs and IF/MFs in the active interplay between angiogenesis and fibrogenesis, eventually favouring development of CLDs (Aleffi et al., 2005, Novo et al., 2007 and Lee et al., 2007).

3. Associated pathologies

3.1. CLDs and progressive fibrogenesis

Hepatic MFs play a major role in all conditions of CLDs characterized by a persisting scenario of necrosis/apoptosis, inflammatory response and progressive fibrogenesis, including those related to chronic infection by hepatotropic viral agent (mainly hepatitis B and C viruses) or to metabolic, autoimmune and toxic or drug-induced causes (with alcohol consumption being predominant). Although other liver cell populations will of course offer a significant contribution to fibrogenesis (injured hepatocytes, activated Kupffer and sinusoidal endothelial cells) in all CLDs hepatic MFs represent a unique and crucial cellular crossroad where incoming paracrine and autocrine signals (ROS from injured cells, growth factors, inflammatory and angiogenic signals, chemokines, adipokines, etc.) are integrated in order to "operate" all those phenotypic responses designed to sustain fibrogenesis and the progression of CLDs to the end-points of cirrhosis and hepatic failure.

Accordingly, MFs-dependent progressive fibrogenesis is sustained by at least three main pro-fibrogenic mechanisms (Parola et al., 2008): (a) chronic activation of the wound healing response (apply to any CLDs, predominates in chronic injury by viral agents or autoimmunity, (b) ROS and other oxidative stress-related reactive mediators (apply to all CLDs, mainly associated to metabolic or alcoholic aetiology), and (c) derangement of epithelial–mesenchymal interactions and EMT, detected in chronic cholangiopaties.

3.2. Liver regeneration and cancer

HSC/MFs, which are known to contribute to liver stem cell niche, also express the stem cell marker CD133 (Kordes et al., 2007) and it has proposed that they may then directly differentiate into stem or precursor cells. As discussed by Friedman (2008b), this is a fascinating hypothesis for liver regeneration but it may

also offer a possible explanation for the fact that fibrosis is a "near-absolute" requirement for the development of hepatocellular carcinoma (HCC). Related facts and hypothesis are the following: neoplastic cells may derive either from hepatic progenitor cells (HPCs) or adult and DNA-damaged hepatocytes sustained by paracrine or survival factors released by MFs or directly from HSC/MFs through a process of mesenchymal to epithelial transition into HPCs (speculative, but supported by the notion that in HSC/MFs operate hedgehog and Wnt signalling, commonly implicated in stem cell differentiation and cancer). This still speculative but fascinating scenario related to HCC may include the reduced tumour surveillance and decrease in NK cells number and function detected particularly in HCV patients.

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