Hepatic myofibroblasts and fibrogenic progression of chronic liver diseases

HEPATIC MYOFIBROBLASTS AND FIBROGENIC PROGRESSION OF CHRONIC LIVER DISEASES.

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Summary

Liver fibrogenesis is a dynamic and highly integrated molecular, tissue and cellular process that during the course of a chronic liver disease (CLD) leads progressively to an excess deposition of extracellular matrix (ECM) components in an attempt to limit the consequences of chronic parenchymal injury. Irrespective of etiology, liver fibrogenesis is sustained and modulated by an intense cross talk occurring between different hepatic cell populations that involves the synthesis and release of several mediators including growth factors, cytokines, chemokines, reactive oxygen species, adipokines, vasoactive agents and plasma proteins. In this scenario a major pro-fibrogenic role is played by a heterogeneous population of α-smooth muscle actin (α-SMA) positive cells defined as hepatic myofibroblasts (MFs). Hepatic MFs are highly proliferative and contractile cells, primarily responsible for excess deposition of ECM components and involved in ECM altered remodeling observed in CLDs. MFs also represents a unique and critical cellular crossroad able to integrate incoming paracrine or autocrine signals, released from all hepatic cell populations involved or available in the microenvironment, as well as to synthetize and release mediators which sustain and perpetuate fibrogenesis, chronic inflammatory response and neo-angiogenesis. This review has been designed to offer critical knowledge on hepatic MFs including terminology, essential definitions and characterization of MFs, with a focus on the origin of these cells (mainly from hepatic stellate cells and portal fibroblasts or, to a less extent, bone marrow-derived cells), the process of activation and the functional responses that these cells can afford in the fibrogenic progression of CLDs.

1. Liver fibrogenesis in the scenario of chronic liver diseases

1.1 Introductory remarks and definitions

Chronic liver diseases (CLD) are characterized by persisting parenchymal (i.e., hepatocyte) injury that is induced by a limited and well defined number of etiological agents or conditions. On a worldwide basis, according to the respective clinical relevance, chronic liver injury can be elicited by: i) chronic infection by hepatitis B and C viruses, ii) chronic exposure to altered metabolic conditions (non-alcoholic fatty liver disease or NAFLD), iii) chronic exposure to drug or toxins (with excess alcohol consumption being predominant in western countries), iv) autoimmune-mediated injury (mainly primary biliary cirrhosis or PBC and primary sclerosing cholangitis or PSC) or v) inherited defects (hereditary hemochromatosis, Wilson's disease). Persisting liver parenchymal injury then results in chronic activation of inflammatory and wound healing response that, together with other mechanisms (oxidative stress, derangement of epithelial-mesenchymal interactions and, possibly, epithelial to mesenchymal transition or EMT, reviewed in Novo et al., 2014), can sustain the process of liver fibrogenesis, now recognized as the major driving force for excess deposition of extracellular matrix (ECM) components and fibrotic scar formation (i.e., liver fibrosis, the net tissue result) (Friedman, 2008a,2008b; Parola et al., 2008; Dranoff and Wells, 2010; Povero et al., 2010; Forbes and Parola, 2011; Zhang and Friedman, 2012; Mallat and Lotersztajn, 2013; Novo et al., 2014; Marra and Tacke, 2014).

Liver fibrogenesis as a process should be then defined as a dynamic and highly integrated molecular, tissue and cellular process that during the course of a CLD leads to a progressive excess accumulation of ECM components in an attempt to limit the consequences of chronic parenchymal injury (Friedman, 2008; Parola et al., 2008; Parola and Forbes, 2012; Novo et al. 2014). Irrespective of the etiology, liver fibrogenesis is then critical for the progression of any form of CLD eventually leading to liver cirrhosis and hepatic failure (Friedman, 2008; Parola et al., 2008; Parola and Forbes, 2012; Rosselli et al., 2013; Novo et al. 2014). According to this definition of liver fibrogenesis, cirrhosis can be then defined as an advanced stage of CLD, characterized by the formation of regenerative nodules of parenchyma surrounded and separated by fibrotic septa, and associated with significant changes in organ vascular architecture, development of portal

hypertension and major related complications such as variceal bleeding, hepatic encephalopathy, ascites and hepatorenal syndrome as well as an increased risk of developing hepatocellular carcinoma (HCC) (Friedman, 2008; Parola et al., 2008; Parola and Forbes, 2012; Rosselli et al., 2013; Novo et al. 2014).

1.2 Liver fibrogenesis in the progression of chronic liver diseases

According to introductory remarks and basic definitions one can rationally envisage liver fibrogenesis as a major pathophysiological event driving the progression of CLDs, irrespective of etiology. Indeed, as suggested in Figure 1, liver fibrogenesis and of course chronic liver injury and persistent inflammatory response, are intrinsically correlated and span throughout the entire natural history of any CLDs, being fundamental for the other pathophysiological events to occur and for the effective progression of the disease (Friedman, 2008; Parola et al., 2008; Forbes and Parola, 2012; Rosselli et al., 2013; Novo et al. 2014). Along these lines, chronic activation of inflammation and the recruitment and activation of cells of either innate or acquired immunity is seen to progressively result in a pro-fibrogenic environment characterized by the significant synthesis and release of several mediators, including growth factors, cytokines, chemokines, adipokines, ROS and others. This environment is critical for the impairment of liver regeneration/hyperplasia observed in the progression of CLDs and is believed to favor persistent activation of MF-like cells, chronic activation of wound healing as well as excess deposition of ECM components, the latter event being usually paralleled by altered or inefficient ECM remodeling. The transition from the early stage of chronic injury and significant fibrosis towards a more advanced condition of CLD is then believed to involve hypoxia and angiogenesis. Indeed these two events are suggested to drive both fibrogenesis and the progressive development of vascular changes that will accompany CLD development through the conditions of pre-cirrhosis (Figure 1). Although liver fibrosis in the early stage and likely in the pre-cirrhosis one is potentially reversible (i.e., following the removal of chronic exposure to the specific etiology or to effective therapy), with the time deposition of ECM components becomes more and more significant. Fibrotic septa and strictly related vascular changes then start to significantly modify the overall structure of liver parenchyma leading to the classic histopathological features of cirrhosis and the related ensue of portal hypertension and related pathophysiological events. As recently suggested, from a clinical point of view cirrhosis does not simply represent the end-point of a defined CLD and at least two distinct stages of cirrhosis should be distinguished (Rosselli et al., 2013): i) compensated cirrhosis,

characterized by the lack of overt clinical manifestations and by a hepatic vein pressure gradient (HVPG) within the 5–10 mmHg range; ii) decompensated cirrhosis, this time characterized by overt clinical manifestations and HVPG values > 10–12 mmHg and, along with the risk to develop HCC, to eventually enter in the stage of liver failure and the disease becoming then systemic. Epidemiological data that have unequivocally outlined the highly significant worldwide clinical impact of CLDs and HCC (recently summarized in Novo et al., 2014) can then only emphasize the relevance of persistent liver fibrogenesis in the overall scenario of progressive CLDs.

1.3 Cell populations in liver fibrogenesis

Liver fibrogenesis, irrespective of etiology, can be considered as a process which is sustained and modulated by an intense cross talk occurring between different hepatic cell populations that involved the synthesis and release of several mediators, including growth factors, cytokines, chemokines, adipokines, ROS and vasoactive agents and plasma proteins. Any resident liver cell population, that may also respond to plasma proteins and to conditions of tissue hypoxia, has been described to significantly contribute to liver fibrogenesis during chronic liver injury and CLD progression (Friedman, 2008a, 2008b; Parola et al., 2008; Dranoff and Wells, 2010; Forbes and Parola, 2011; Parola and Marra, 2011; Zhang and Friedman, 2012; Iwaisako et al., 2012; Mallat and Lotersztain, 2013; Novo et al., 2014). In such a scenario of pro-fibrogenic environment (see Figure 2) injured hepatocytes represent during CLD progression a major source of ROS and of other redox-related mediators or reactive intermediates as well as the most relevant source (in quantitative terms) of vascular endothelial growth factor (VEGF), particularly in the presence of hypoxic areas. Parenchymal cell damage and death (either necrotic or apoptotic) will of course sustain the chronic activation of inflammatory response which mainly involves macrophages obtained from resident Kupffer cells as well as from monocytes recruited from peripheral blood and of bone marrow origin. Activated macrophages sustain liver fibrogenesis by synthetizing and releasing a huge number of pro-fibrogenic and pro-inflammatory mediators. As shown in Figure 2, the list of macrophage-derived mediators includes at least ROS, transforming growth factor β1 (TGFβ1), platelet-derived growth factor (PDGF), basic fibroblast growth factor (bFGF), the pro-inflammatory primary cytokines interleuchin-1 (IL-1) and tumor necrosis factor α (TNFα) as well as several chemokines with monocyte chemoattractant protein-1 (MCP-1 or CCL2) playing a major role in modulating the response by several liver cell populations (Marra and Tacke, 2014). Although not cited in Figure 2, the reader should note that other cells of the innate immunity like

natural killer T (NKT)-cells can modulate liver fibrogenesis (Novo et al., 2014). Liver sinusoidal endothelial cells, when damaged and /or activated during chronic liver injury, can contribute to the overall pro-fibrogenic scenario by synthetizing and releasing specific mediators like nitric oxide (NO), endothelins (ETs), insulin-like growth factor 1 (IGF1) and prostaglandins (PGs) in addition to other already cited like ROS, PDGF, TGFβ1, IL-1 and bFGF. Even platelets have been reported to contribute to this scenario of cell-cell interactions by releasing PDGF, TGFβ1, IGF1 as well as epidermal growth factor (EGF), transforming growth factor α (TGFα) and tromboxanes.

If one refer to the ability of release mediators (Figure 2), liver fibrogenesis is of course significantly sustained by hepatic myofibroblasts or MFs, to which this review is specifically dedicated. Details concerning origin, process of activation and role in fibrogenesis will be described starting from next section. Here it is sufficient to remark that these MFs are both target for mediators generated and/or released by other liver cells as well as active source of mediators acting in a paracrine/autocrine way.

2. Hepatic Myofibroblasts

2.1 Hepatic Myofibroblasts: what in a definition

For a liver - dedicated pathologist the term “hepatic myofibroblast” is a very familial one that apply to an apparently heterogeneous population of cells that share a mesenchymal-like ultrastructural phenotype, express α-smooth muscle actin (α-SMA, the most reliable marker for these cells) and/or other mesenchymal-like markers and are easily identified by immunohistochemistry in fibrotic and cirrhotic human liver specimens (Cassiman et al., 2001; Friedman, 2008a; Parola et al., 2008; Dranoff and Wells, 2010). Although activated hepatic MFs are likely to represent a rather heterogeneous population of cells in relation to their origin (see later), current literature emphasizes their major role in sustaining fibrogenic progression of any CLD, irrespective of their origin and of specific etiology (Friedman, 2008b; Parola et al., 2008; Forbes and Parola, 2011; Rosselli et al., 2013; Novo et al. 2014). Indeed, hepatic MFs are currently envisaged as highly proliferative and contractile cells able to contribute to liver fibrogenesis and CLD progression by displaying a number of phenotypic responses. Hepatic MFs are primarily responsible for excess deposition of ECM components but, in their activated state, also play a critical role in the altered remodeling of ECM which typically characterizes any progressive CLDs.

Moreover, liver MFs operate also through the critical synthesis and paracrine/autocrine release of several growth factors and mediators that are able to sustain and perpetuate not only fibrogenesis but also chronic inflammatory responses and neo-angiogenesis. As recently pointed out (see Figure 2), the central role of MFs as effectors of fibrogenesis, is emphasized by their intrinsic ability to act as unique and critical cellular crossroad that, in the overall scenario of a progressive CLD, can integrate incoming paracrine or autocrine signals (including growth factors, pro-inflammatory cytokines, chemokines, proangiogenic mediators, adipokines, ROS and others) released from all hepatic cell populations involved and/or available in the microenvironment (Novo et al., 2014).

According to current literature, liver fibrogenesis in progressive CLDs has been proposed to be sustained by at least four main pro-fibrogenic mechanisms: i) chronic activation of the wound healing response, which is likely the most relevant mechanism from a general point of view for CLDs and that is believed to predominate in CLDs by hepatotropic viruses (HBV and HCV) as well as in CLD with autoimmune etiology; ii) oxidative stress, again a general mechanism intimately related to chronic liver injury that has been reported as predominant mechanism in CLDs by chronic alcohol consumption or altered metabolism (NAFLD/NASH - related CLD); iii) a derangement of epithelial-mesenchymal interactions, which is usually detected in the frame of chronic cholangiopathies; iv) epithelial to mesenchymal transition (EMT) of either hepatocytes or cholangiocytes, although this mechanism is still controversial and highly debated (Pinzani and Rombouts, 2004; Parola et al., 2008; Forbes and Parola, 2011; Novo et al., 2014). In addition to these major mechanisms, other mechanisms have emerged in the last decade that may significantly affect fibrogenic CLD progression and include the involvement of: i) hypoxia, hypoxia-inducible factors (HIFs) and angiogenesis, as well as ii) of inflammasomes as pro-inflammatory and pro-fibrogenic determinants; iii) adipokines which have a relevant role particularly in CLD associated to metabolic disturbances (metabolic syndrome and/or obesity and type 2 diabetes); iv) the process of autophagy and v) the role of natural killer and natural killer-T cells (the reader interested in pro-fibrogenic mechanisms can refer to a recent review by Novo et al., 2014).

More than a decade ago Cassiman et al. (2002) have proposed the possibility to recognize, irrespective of aetiology and according to their antigen profile and tissue localization, at least four different subpopulations of MFs in pathological human specimens obtained from patients affected by CLDs: i) portal MFs, which are MFs detected in the expanded connective tissue around portal

tracts; ii) septal MFs, which are detected in the inner part of fibrotic septa; it should be noted that portal MFs and septal MFs share a common strong positivity for α-SMA as well as a more variable positivity to other antigens like glial fibrillary acidic protein (GFAP), brain-derived nerve growth factor (BDNF) and α-B-crystallin (ABCRYS); iii) interface MFs that are, by definition, α-SMA positive cells detected at the edge between fibrotic septa and the surrounding parenchyma (i.e., where active fibrogenesis usually occurs); interface MFs express more intense stain for GFAP and ABCRYS as well a variable degree of positivity for other antigens like, in addition to BDNF, also neuronal cell adhesion molecule (N-CAM), neuronal growth factor (NGF) and neurotrophin 4 (NT-4); iv) activated, myofibroblast - like, hepatic stellate cells (HSC/MFs), which are α-SMA-positive that are easily recognized first by their localization in or around capillarised sinusoids of fibrotic/cirrhotic livers and as cells within pseudolobules; these cells, originating mainly from activation/transdifferentiation of hepatic stellate cells (HSC), are strongly positive for α-SMA ad N-CAM, very positive for GFAP, NGF, BDNF, NT-3 and synaptophysin (SYN) as well as significantly positive for other antigens like NT-4, ABCRYS, p75 and the NT receptors tyrosine kinase (Trk) A and B. On the basis of localization and antigen repertoire Cassiman et al. (2002) at that time proposed that, apart from HSC/MFs likely deriving mainly from HSC, portal MFs (and possibly septal MFs) may originate from portal fibroblasts whereas the origin of interface MFs was supposed to be more heterogeneous. Although this overall morphological interpretation has progressively changed with the advent of more powerful technologies for fate tracing and the availability of specific transgenic mice, terms like portal MFs, interface MFs and HSC/MFs are still widely used and easy to understand for any pathologist, basic scientist or clinician. On the other hand, one should recall that within the antigen repertoire displayed by HSC/MFs and by other types of MFs (Geerts, 2001; Cassiman et al., 2002) one can find several proteins usually associated to cells of neural origin, including GFAP, P75 and nestin. This led to the hypothesis that HSC may originate from neural crest during embryogenesis, rapidly abandoned following one of the pioneer attempt made to trace the origin of this peculiar cell type by employing Wnt1Cre and Rosa26 reporter mice (Cassiman et al., 2006), opening the way to the search for mesenchymal origin of HSC and of other cells reported to trans-differentiate into MFs.

2.2. The embryonal origin of mesenchymal cells able to give rise to liver MFs

Extensive literature data indicate that although HSCs are widely recognized as the most relevant source of MFs, indeed α-SMA positive profibrogenic MF-like cells have been shown to

originate from different cell sources during the progression of CLDs (Friedman, 2008a,2008b; Parola et al., 2008; Dranoff and Wells, 2010; Povero et al., 2010; Forbes and Parola, 2011; Asahina, 2012; Zhang and Friedman, 2012; ; Iwaisako et al., 2012; Mallat and Lotersztajn, 2013; Novo et al., 2014; Wells, 2014). The heterogenous origin of MFs is, in turn, somewhat intrinsically related to the complexity of the scenario concerning the origin of different mesenchymal cell types in the developing liver. According to a series of nice experimental studies by Asahina and coworkers (Asahina et al., 2009,2011,2012) as well as by other laboratories (Hoppo et al., 2004; Kubota et al., 2007; Suzuki et al., 2008) we have now a consistent knowledge of the different liver mesenchymal cell types in developing murine liver and of how these cells can differentiate in those reported to be able to be activated to a MF-like phenotype. As summarized in Figure 3, the origin of the cells having the potential to be activated into MFs is in the developing murine liver represented by the septum transversum mesenchyme (STM). As it is well known, in mice liver bud forms from the foregut endoderm at approximately embryonic day (E) 9. At that time the STM which surrounds both the foregut endoderm and cardiac mesoderm starts to secrete fibroblast growth factors (FGFs) and bone morphogenetic proteins (BMPs) as a relevant step to induce differentiation from endoderm of hepatoblasts, the bi-potent progenitor cells able to differentiate into both hepatocytes and biliary epithelial cells (Zaret, 2002). The liver bud in mice embryos is formed at E9.5 to E10.5 from hepatoblasts budding that leaving the foregut endoderm invade the surrounding STM (Enzan et al., 1997). However, elegant studies that employed MesP1^{Cre} and Rosa26lacZ reporter mice in order to follow cells of mesoderm origin have shown that, before the formation of liver bud, the STM is formed by MesP1 positive mesoderm (Asahina et al., 2011). Still before formation of liver bud the STM also express specifically Wilm's tumor 1 (Wt1), a protein that later in the development is expressed only by mesothelial and sub-mesothelial cells. By using Wt1^{Cre}ERT2 and Rosa26 reporter mice, Asahina and coworkers (2009, 2011) were able to label STM cells and to trace this cell lineage. Data indicate that these STM cells gives rise to both mesothelial cells (positive for activated leukocyte cell adhesion molecule or ALCAM, WT1 and podoplanin) and submesothelial cells, which express ALCAM and WT1 but also desmin and p75NTR. These cells are the most likely source of either HSCs, which are positive for WT1, desmin and p75NTR, as well as for the so-defined perivascular mesenchymal cells (PMCs). PMCs, which are positive for desmin, Jagged 1, p75NTR and α-SMA, are the cells proposed to give rise to portal fibroblasts, smooth muscle cells and the few fibroblasts detected around the central vein. This has been reported to happen in mouse embryos at E 10-13. As also shown in Figure 3, HSCs and

PMCs may also derive directly from WT1 positive STM cells but the most critical point here is that two of the most relevant mesenchymal cell types described to undergo activation/trans-differentiation into MF-like cells (HSCs and portal fibroblasts) have then a common origin from MesP1 positive mesoderm.

2.3 The adult cells that can give rise to hepatic MFs

As previously recalled, hepatic MFs represent a rather the heterogeneous population of α-SMA positive cells. As we will see in detail in the next sections, literature data indicate that hepatic MFs originate mainly from HSCs or portal fibroblasts through a process defined as of activation/trans-differentiation. Such a process leads to the appearance of a highly proliferative, migratory and contractile MF-like phenotype able to synthetize excess ECM components as well as and to remodel the extracellular matrix and to sustain angiogenesis and/or inflammatory response, also offering a contribute in modulating immune response (Friedman, 2008a, 2008b; Parola et al., 2008; Dranoff and Wells, 2010; Forbes and Parola, 2011; Zhang and Friedman, 2012; ; Iwaisako et al., 2012; Mallat and Lotersztain, 2013; Novo et al., 2014; Wells, 2014). Although most of present knowledge on liver MFs has been originated from studies performed on HSC/MFs it is generally accepted that major phenotypic responses of liver MFs may be shared also by MFs originating from portal MFs (with some exception for these cells) as well as, possibly, from other sources such as mainly cells recruited into the chronically injured liver from bone marrow like fibrocytes and mesenchymal stem cells or MSCs (Forbes and Parola, 2011). The next sections then offer an overview of major informations and concepts on the cells that can give rise to liver MFs. According to literature data, a section will be also dedicated to the controversial and highly debated hypothesis that at least some of the pro-fibrogenic cells in CLD progression may originate from either hepatocytes or cholangiocytes following a process of epithelial to mesenchymal transition (EMT).

3. Hepatic stellate cells and the process of activation/transdifferentiation into liver MFs

3.1 HSCs in normal liver or quiescent HSCs

Hepatic stellate cells (HSCs) are perisinusoidal cells that in the normal liver reside in the subendothelial space of Disse and are characterized by the presence of cytoplasmic processes defined as intersinusoidal (or interparenchymal) and subendothelial. These cytoplasmic processes allow these cells to establish contacts with a relevant number of hepatocytes as well as of liver sinusoidal endothelial cells (LSECs) but also with adjacent HSCs and nerve endings (Wake, 1980; Geerts, 2001). Under physiological conditions (or quiescent state) HSCs have been reported to play at least three major roles that are either lost or deregulated in CLD (Geerts, 2001; Friedman, 2008b): i) HSCs are responsible for intrahepatic uptake, storage and release of vitamin A and retinoids which are typically retained within lipid droplets in the perinuclear cytoplasm; this function is progressively lost during CLD when the cells acquire the MF-like phenotype; ii) HSCs have a role, together with LSEC and hepatocytes, in the synthesis and deposition in the space of Disse of basal membrane like – ECM components (mainly collagen type III, collagen type IV and laminin) as well as in the remodeling of such extracellular matrix on the basis of their ability to produce several metalloproteinases (mainly MMP1, MMP2 and, to a less extent, MMP3, MMP10, MMP13 and MMP14) and related tissue inhibitors (TIMP1 and TIMP2); under conditions of CLD this function in HSC/MFs is dramatically deregulated to favor excess deposition of ECM components versus remodeling; iii) HSCs have been proposed to act as 'liver specific pericytes' able to regulate sinusoidal blood flow by responding to vasoactive peptides (mainly ET-1 and NO) and neurotransmitters of the autonomous nervous system (ANS); this function, which is favored by the intimate contact established by HSCs with LSECs and by the axons of ANS with HSCs, is somewhat exacerbated during the progression of CLD.

A fourth additional role has been suggested more recently for HSCs that are now envisaged as cells able also to contribute significantly to hepatic development and regeneration (reviewed in Yin et al., 2013). Along these lines, HSCs are believed to contribute to vessel formation and integrity through their intimate cross-talk with LSECs based on the ability of HSCs to secrete the chemokine SDF1 and to express PDGF β receptor whereas LSECs produce PDGFβ and the SDF1 receptor CXCR4, with SDF1 reported to also facilitate the recruitment of hematopoietic stem cells in the liver. HSCs have been reported to also stimulate the proliferation of hepatoblast progenitor cells and hepatocytes through the release of hepatocyte growth factor (HGF), Wnt, fibroblast growth factor (FGF), and retinoic acid (Yin et al., 2013). HSCs may also modulate the differentiation of hepatoblast into hepatocytes or cholangiocytes through the control of ECM composition within the liver as well as, at least in CLDs, to drive the differentiation of hepatic progenitor cells into

cholangiocytes through the release of the Notch ligand jagged 1 (Jag1) (Boulter et al., 2012).

It should be noted that HSCs have been also proposed as putative progenitors of epithelial cells in the liver (Yang et al., 2008; Michelotti et al., 2013) but this hypothesis was not confirmed by a successive fate-tracing experimental study (Mederacke et al., 2013).

3.2 The process of activation / transdifferentiation of HSCs into MF-like cells (HSC/MFs)

HSC/MFs (i.e., activated myofibroblast-like cells originated from HSCs) have been historically the first pro-fibrogenic cells identified in either experimental and clinical conditions. These cells have been extensively investigated in the past two decades and represent the cell population for which the process of activation and profibrogenic mechanisms are best characterised (Friedman, 2008b; Forbes and Parola, 2012; Mallat and Lotersztajn, 2013; Novo et al., 2014). HSC/MFs are still believed to be the most relevant pro-fibrogenic cells by most laboratories involved in liver fibrogenesis and this view has been recently reinforced by an elegant fate tracing experimental study that, by employing a novel Cre-transgenic mouse able to label 99% of HSCs, showed that as much as 82-96% of liver MFs originated directly from HSCs (Mederacke et al., 2013) in murine models of toxic, cholestatic and fatty liver disease.

Extensive literature data support the notion that quiescent HSCs, in conditions of chronic liver injury, undergo a peculiar process of activation/trans-differentiation which involves significant changes in their morphology and the induction of pro-fibrogenic phenotypic responses that are indeed very close to those observed in human or rodent HSCs when cultured on plastic substrate (Friedman, 2008b; Parola et al., 2008; Forbes and Parola, 2012; Mallat and Lotersztajn, 2013; Novo et al., 2014). This process of activation has been described to proceed in sequential stages of initiation and perpetuation (Friedman 2008a,2008b)(Figure 4). The step of initiation represents an early response which is stimulated by several paracrine signals that leads HSCs to evolve in a transient, potentially reversible, contractile and pro-fibrogenic cellular phenotype. This transient phenotype is characterised by a rapid induction of PDGFβ receptor expression and is potentially primed to respond, in conditions of chronic liver injury, to all those growth factors and mediators (see specifically section 3.3 as well as Figure 2 and Figure 4) that are critical in sustaining the phenotypic responses operated by fully activated MF-like phenotype (i.e.,

perpetuation) (Friedman, 2008b; Parola et al., 2008; Forbes and Parola, 2012; Mallat and Lotersztajn, 2013; Novo et al., 2014). Of course, if liver injury is acute, resolution of tissue injury that follows activation of inflammatory response and conventional wound healing would result in the progressive disappearance of mediators and the recovery of normal microenvironment conditions, leading eventually to either apoptotic cell death of transiently activated cells or to their reversion to a quiescent phenotype. As we will see later, induction of apoptosis of transiently activated cells may require the intervention of restorative macrophages as well as of NKT cells.

3.3 The functional responses of HSC/MFs: a paradigm for the multiple role of hepatic MFs

Once fully activated by pro-fibrogenic mediators and by conditions related to the chronically injured tissue microenvironment, HSC/MFs have been described to operate a number of phenotypical responses or functions that may serve as a paradigm for the entire population of hepatic MF-like cells (summarized in Figure 4). Indeed, one should take in mind that most of present knowledge on liver MFs comes directly from studies investigating properties of human as well as rodent (murine and rat) HSC/MFs (Friedman, 2008b; Parola et al., 2008; Forbes and Parola, 2012; Mallat and Lotersztajn, 2013; Novo et al., 2014). Accordingly, one should also takes into account the widely acknowledged issue that HSC/MFs can operate during CLD progression so-defined paracrine-autocrine loops (i.e. synthetizing and releasing mediators for whom these cells also express cognate receptors). This has been characterized mainly for HSC/MFs and for mediators like TGFβ1, PDGF-BB, MCP-1 or CCL2, ET-1 and VEGF. Phenotypic responses or pro-fibrogenic properties of HSC/MFs will be synthetically described in the following sub-sections by focusing the attention to the mediators and related receptors involved.

3.3.1 HSC/MFs as rapidly proliferating cells. HSC/MFs are by definition a cell population characterized by an impressive proliferative attitude. These cells proliferate, display typically enhanced survival and then migrate and accumulate in response to paracrine/autocrine effects of several growth factors, cytokines, lipid mediators, or adipokines produced by the injured liver or profibrogenic environment. Proliferation of HSC/MFs, in particular, is elicited by a number of mitogens, including PDGF, basic fibroblast growth factor (bFGF), Angiotensin II, VEGF and thrombin (Pinzani and Marra, 2001; Friedman, 2008a; Parola et al., 2008; Forbes and Parola, 2012; Mallat and Lotersztajn, 2013), with PDGF isoforms (particularly PDGF-BB) considered as the most potent and effective mitogenic stimuli. PDGF-BB has been shown to be released by activated

kupffer cells, sinusoidal endothelial cells and platelets but a very significant contribution (i.e., during active fibrogenesis) in the synthesis and release of this mediator is by activated HSC/MFs themselves in an autocrine/paracrine pathway.

3.3.2 HSC/MFs as cells able to migrate. A critical property of HSC/MFs and, likely, of all hepatic MFs is the ability to migrate. This is an essential issue since it allows these MF-like cells to reach the site of injury and, as a part of the wound healing and pro-fibrogenic response, to align with nascent as well as already established fibrotic septa. Migration/chemotaxis of human HSC/MFs has been shown to be stimulated by a variety of mediators including polypeptide chemoattractant like PDGF-BB, monocyte chemoattractant protein 1 (MCP-1 or CCL2), angiotensin II, the two pro-angiogenic cytokines VEGF and angiopoietin-1 as well as CXCR3 ligands (Pinzani and Marra, 2001; Novo et al., 2007; Parola et al., 2008; Mallat and Lotersztajn, 2013; Novo et al., 2014). Most of these peptides (in particular PDGF-BB, CCL2 and VEGF) operate by stimulating a Ras/ERK and JNK1/2 signalling in a redox- and NADPH oxidase - dependent manner (Novo et al., 2011). Interestingly, a raise in the intracellular levels of reactive oxygen species (as induced by defined compounds like menadione or 2,3-dimethoxy-1,4-naphtoquinone, or by exposure of cells to hypoxic conditions) has been shown to be sufficient to stimulate oriented migration of either HSC/MFs or even of MF-like cells derived from mesenchymal stem cells by eliciting the same Ras/ERK and JNK1/2 signaling pathway (Novo et al., 2006a; Novo et al., 2011; Busletta et al., 2012; Novo et al., 2012).

3.3.3 HSC/MFs as cells involved in ECM synthesis and remodeling. According to current literature, fibrogenesis progression is mainly characterised by the replacement of the typical low-density basement membrane of the space of Disse with fibrillar matrix. In particular, this is believed to result primarily from an unbalance between excess deposition of fibrillar collagens (mainly collagen type I and III) as well as other ECM components and integrin ligands, and a reduced/altered degradation and remodeling of ECM itself (Pinzani and Marra, 2001; Friedman, 2008a; Parola et al., 2008; Forbes and Parola, 2012; Mallat and Lotersztajn, 2013). HSC/MFs, as major profibrogenic cells, have a prominent role in ECM deposition during CLDs in response mainly to the master profibrogenic cytokine TGFβ1 that, similar to what described for PDGF-BB, can be released by activated macrophages, activated/damaged LSECs or platelets as well as from fully activated HSC/MFs, once again as an autocrine/paracrine loop. In addition to TGFβ1, several other mediators of ECM excess deposition have been identified and the list include ROS and the major

aldehydic end-product of lipid peroxidation 4-hydroxynonenal (HNE) (Parola et al., 2008; Novo and Parola, 2008) as well as connective tissue growth factor (CTGF) and cannabinoids (Mallat and Lotersztajn, 2013). In relation to ECM remodeling, it has been shown in the past that HSC/MFs their ability to produce ECM-degrading enzymes according to the activation state; in their quiescent state HSCs produce metalloproteinases (MMPs), MMP activators able to cleave pro-MMPs into their active form as well as specific tissue inhibitors of the metalloproteinases (TIMPs). In the early stage of activation HSCs can still produce MMPs and their activators but do not significantly express TIMPs. Of relevance, fully activated HSC/MFs behave as a phenotype expressing low levels of MMP-1 (interstitial collagenase) but, at the same time, high levels of MMP-2, MMP-9, MMP3 or Stromelysin, which are not really so efficient in degrading fibrillary matrix, as well as high levels of TIMP-1.

3.3.4 HSC/MFs and their ability to contract. HSC/MFs, when activated, have been proposed to contribute to increased portal resistance during CLD fibrogenic progression. This contribution is believed to be reversible until the major cirrhosis related changes are developed, including the formation of thickened fibrotic septa and intrahepatic shunts as well as lobular distortion, and the increase in portal pressure is fixed (Friedman, 2008a). As already mentioned in a previous section, contractility in response to vasoactive mediators or and neurotransmitters of the ANS is a feature of quiescent HSCs (i.e., liver specific pericytes) which is emphasized even in earlier stages of fibrosis when activated HSCs start to express contractile filaments including α-SMA and myosin (Rockey et al., 1992a; Saab et al., 2002) which, in turn, can generate those calcium-dependent and calcium-independent contractile forces contributing to cellular contractility (Bataller et al., 2001; Yee, 2001; Laleman et al., 2007). Similarly to what described for HSCs during regeneration (i.e., following partial hepatectomy or acute injury), HSC/MFs during chronic liver injury take contact with LSECs and contribute to angiogenesis, an essential event that is associated to fibrogenesis and CLD progression. In advanced cirrhosis HSC/MFs, which accumulate in large numbers, have been proposed to progressively impede portal blood flow, possibly with responses to vasoactive mediators and signaling pathways related to interaction with ECM that result either in the constriction of individual sinusoids or in the contraction of the cirrhotic liver (Pinzani et al., 1992; Melton et al., 2006). Contractility of HSC/MFs, which increase their density and coverage of sinusoidal lumen, is controlled mainly the opposite action of NO and ET-1 as well as by a number of additional mediators like angiotensin II, eicosanoids, carbon monoxide, somatostatin and atrial

natriuretic factor (Reynaert et al., 2002; Rockey, 2003; Friedman, 2008a). Of interest, HSC/MFs are likely to contribute (by their pro-angiogenic response, see later) to the progressive development of intrahepatic shunts (Friedman, 2008a; Novo et al., 2014).

Extensive literature data indicate that conditions of chronic liver injury can lead to vascular disorder in which ET-1 is overproduced by HSCs whereas NO release by LSECs is reduced (Kawada et al., 1993; Geerts, 2001; Rockey, 2001; Friedman 2008; Iwakiri et al., 2014). Although ET-1 was originally identified as vasoconstrictor produced mainly by endothelial cells [Yanagisawa et al., 1988), HSCs represent both a a major source as well as a target for ET-1 during liver injury (Kawada et al., 1995; Mallat et al., 1996; Pinzani et al., 1996; Rockey and Weisiger, 1996). ET-1 has a prominent contractile effect on HSCs and MFs, which has been proposed to contribute to portal hypertension in the cirrhotic liver (Rockey et al., 1992b; Kawada et al., 1993; Kawada et al., 1995; Mallat et al., 1996; Pinzani et al., 1996; Rockey and Weisiger, 1996; Geerts, 2001; Friedman 2008a; Iwakiri et al., 2014). The scenario of interactions between LSECs and HSC/MFs is likely to be more complex in regulating intrahepatic vascular pathophysiology. Indeed, the LSEC phenotype is presumably the result of the action of several polypeptides, including VEGF angiopoietins, ephrins, and fibroblast growth factors (FGFs) but is also sensitive to mechanical forces like those due to shear stress: both pro-angiogenic polypeptides and shear stress can modulate endothelial NO synthase (eNOS) activity in LSECs, thereby regulating flow and vascular tone in the sinusoids [Shah et al., 1997]. LSECs can also affect the early response of HSCs by means of factors released in a paracrine way such as the cellular isoform of fibronectin that, in turn, can contribute to early HSC activation and, particularly, their synthesis of ET-1 (Jarnagin et al., 1994). However, ET-1 (that can stimulate proliferation of early-cultured HSCs) has been reported to inhibits fully activated HSC/MFs (Rockey et al., 1998). This is relevant in relation to the change of phenotype (i.e., capillarization) the LSEC undergo during chronic liver injury which is associated with a reduction in eNOS activity and NO synthesis after injury, possibly because of post-translational de-regulation of eNOS (Iwakiri et al., 1914). Since NO is believed to maintain HSCs in a quiescent state, NO reduction can facilitate HSCs activation and contribute to switch on fibrogenic progression of CLDs (Langer et al., 2008; Deleve et al., 2008). A final degree of complexity relies on the fact that during injury also HSCs have been reported to produce NO, likely following iNOS up-regulation in response to pro-inflammatory cytokines or endotoxemia (reviewed in Iwakiri et al., 2014). An overall interpretation of this complex scenario is that all these interactions are reasonably able to

facilitate remodeling and constriction of the sinusoidal vasculature, resulting in an increase of hepatic vascular resistance as an early feature of intrahepatic portal hypertension.

3.4 HSC/MFs as cells involved in liver angiogenesis.

Fibrogenic progression of CLDs is associated to significant vascular remodeling affecting mainly liver sinusoids as well as to changes in the phenotype of LSECs and of their close interactions with activated HSC/MFs. As it is well known, the LSECs lose their fenestrae and the space of Disse becomes the site of abnormal deposition of basement membrane matrix, a change overall defined as "capillarization" of sinusoids. These changes, according to what already mentioned in the previous section, are accompanied by alterations in the reciprocal synthesis of NO and ET-1 between LSECs and HSC/MFs. Vascular remodeling also involves additional and pro-angiogenic cross-talk between LSECs and HSC/MFs that are critical for CLD progression with several research groups envisaging liver pathological angiogenesis as an event facilitating fibrogenesis (Medina et al., 2004; Fernández et al., 2009; Novo et al., 2014; Iwakiri et al., 2014). LSECs in conditions of chronic injury can release PDGF, the most potent mitogenic and chemotactic stimulus for HSC/MFs and even offer a contribute to the synthesis of TGF-β1 (with activated macrophages and HSC/MFs being the major contributors) (Friedman, 2008a, 2008b; Parola et al., 2008; Mallat and Lotersztajn, 2013; Novo et al., 2014; Pellicoro et al., 2014; Iwakiri et al., 2014). The phenotype of LSECs is highly affected mainly by VEGF that under physiological conditions has been reported to modulate the size and number of LSECs fenestrae by operating through the receptor VEGFR1 (Shah et al., 1999; Funyu et al., 2001; Yokomori et al., 2003; May et al., 2011). This is relevant because introduces the essential role of hypoxia and hypoxia-inducible factors (HIFs) in sustaining liver pathological angiogenesis associated to the fibrogenic progression of CLD (Medina et al., 2004; Fernández et al., 2009; Novo et al., 2014; Iwakiri et al., 2014). Indeed, the presence of hypoxic areas within chronically damaged liver parenchyma is very common and progressive during CLD development, with HIFs primarily involved in mediating the switch of pro-angiogenic response (Rockey et al., 1998; Langer et al., 2008). Liver angiogenesis in CLD progression is closely related to chronic activation of wound healing and histopathological changes occurring in liver parenchyma. Increased deposition of ECMs and formation of fibrotic septa, which are paralleled by vascular changes, are by themselves events favoring an impairment

of oxygen diffusion (Fernández et al., 2009; Novo et al., 2014; Iwakiri et al., 2014). During CLD both sprouting and intussusceptive angiogenesis are involved and have a role in the genesis of portal hypertension in both intra- and extrahepatic circulation (Iwakiri et al., 1914). Along these lines, elegant studies performed in conditional Notch1 knockout mice have outlined the critical role of Notch1 in LSECs for maintaining fenestration of LSECs, suggesting that the loss of Notch1 can result in pathological angiogenesis, the development of nodular regenerative hyperplasia and portal hypertension in intrahepatic circulation (Radaeva et al., 2007). Histopathological analysis of cirrhotic livers by different laboratories has indeed indicated the significantly increased number of new vessels in fibrotic septa as well as around regenerative nodules (Medina et al., 2004).

As previously mentioned, hypoxia, angiogenesis and liver fibrogenesis are believed to be intrinsically correlated with both clinical studies on human patients and experimental rodent model of fibrosis indicating that angiogenesis and fibrogenesis develop in parallel. Moreover, data from experimental studies employing antiangiogenic therapeutic strategies unequivocally indicate that these strategies are extremely effective in reducing fibrogenic progression, inflammatory infiltrate, the number of α-SMA positive MFs as well as the increase in portal pressure (Medina et al., 2004; Novo et al., 2007; Fernández et al., 2009; Moon et al., 2009; Valfrè di Bonzo et al., 2009; Novo et al., 2014; Novo et al., 2012; Iwakiri et al., 2014; Cannito et al., 2014). Along these lines, VEGF expression has been detected in hypoxic areas of chronically injured livers being limited to LSECs, hepatocytes and activated HSC/MFs (Medina et al., 2004; Novo et al., 2007; Fernández et al., 2009; Valfrè di Bonzo et al., 2009; Novo et al., 2014; Iwakiri et al., 2014; Cannito et al., 2014). This means of course that HSC/MFs and likely all MF-like cells can be modulated in their behavior by proangiogenic cytokines released by hypoxic hepatocytes LSECs and, in an additional autocrine/paracrine loop, also by profibrogenic cells themselves. Indeed human and rodent HSC/MFs have been shown to respond to hypoxia by expressing VEGF-A and angiopoietin 1 as well as their related receptors VEGFR2 and Tie-2, in CLDs (Novo et al., 2007; Taura et al., 2008; Choi et al., 2010). At the same time, one should consider that HSC/MFs are also critical cellular target for the action of VEGF and angiopoietin I. VEGF has been shown to stimulate in HSC/MFs proliferation and increased deposition of extracellular matrix as well as increased migration and chemotaxis (Novo et al., 2007,2012). In addition, HSC/MFs oriented migration has been reported to just require the exposure to hypoxic conditions (Novo et al., 2012). Our group has outlined that hypoxia- or VEGF stimulated oriented migration of human HSC/MFs relies on a biphasic

mechanism that requires: i) an early phase that is switched on by ROS released either by mitochondria under hypoxic conditions or through ligand-receptor related activation of NADPHoxidase (following ligand receptor interaction), resulting in redox-dependent activation of Ras/ERK and JNKs; ii) a late and delayed phase of oriented migration depending on HIF-1α-mediated, ROS-stabilized, upregulation of VEGF expression which, in turn, can operate as autocrine/paracrine chemoattractant when released extracellularly (Novo et al., 2011,2012). These data offer a rational explanation for images collected by immunohistochemical analysis designed to investigate HIFs and major hypoxia-sensitive gene targets like VEGF, VEGFR2, angiopoietin I and Tie2 and performed on human and rodent fibrotic/cirrhotic liver. These data led to the hypothesis that hypoxia, through HIF-mediated pathways, may affect the migration of MF-like cells and their proangiogenic behavior, leading these cells to align with developing septa and then drive both fibrogenesis and angiogenic response (Novo et al., 2007,2012,2014; Fernández et al., 2009).

3.5 HSC/MFs interact with cells of innate immunity: pro-fibrogenic and pro-resolution issues

According to the definition of cells at the crossroad of relevant pathophysiological events, HSC/MFs are involved during CLD in a critical cross-talk with inflammatory cells, particularly cells of innate immunity like Kupffer cells and macrophages of extrahepatic origin as well as natural killer (NK) and natural killer T (NKT) cells. These interactions are of extreme interest because they can either sustain CLD fibrogenic progression as well as to facilitate resolution of fibrosis.

Activated Kupffer cells indeed can release a complex panel of mediators which includes pro-inflammatory cytokines like tumor necrosis factor-α (TNF), interleukin 1 (IL-1), IL-6, IL-10, and IL-18 as well as several chemokines such as macrophage chemotactic protein-1 (MCP-1, CCL2), macrophage inflammatory protein 2 (MIP-2, CXCL2), RANTES (CCL5 or regulated on activation, normal T cell expressed and secreted), macrophage inflammatory protein (MIP)-1α (CCL3) and -1β (CCL4) (Friedman, 2008a; Smith, 2013; Tacke and Zimmermann, 2014; Marra and Tacke, 2014). Activated Kupffer cells also release other polypeptides like osteopontin, PDGF and TGFβ1 but also reactive oxygen species (ROS). Of course in the chronic liver injury scenario most of these mediators are mainly intended to sustain and/or modulate the inflammatory response by sustaining the infiltration of inflammatory cells into the liver and modulating wound healing response. However, apart from what already described for PDGF, TGFβ1 and ROS, HSC/MFs

respond also to CCL2 (oriented-migration) which is indeed a prominent chemokine during the progression of CLDs being also actively synthetized and released by fully activated HSC/MFs in another relevant autocrine/paracrine loop (Pinzani and Marra, 2001; Parola et al., 2008; Marra and Tacke, 2014). Literature indicated that HSC/MFs upon activation by TLR4 ligands or other stimuli, represent a very significant additional source of CCL2 and critically sustain monocyte recruitment in chronically injured livers. This is relevant since the number of Kupffer cells seems to decrease during inflammation and fibrogenesis whereas the number of monocyte-derived pro-fibrogenic macrophages (designated as $CD11b^{+}F4/80^{+}$) has been found to increase in inflamed liver (Duffield et al., 2005). Indeed, CCL2 seems to play a major role during CLD fibrogenic progression by significantly contributing to the recruitment into injured liver of inflammatory monocytes leading to the so-defined population of pro-inflammatory and pro-fibrogenic macrophages which, in a study employing tracking of the glycoprotein Ly6C (marker of circulating monocytes) have been identified as $Gri^{+}Ly6C^{hi}$ macrophages (Karlmark et al., 2009). These $Gri^{+}Ly6^{hi}$ macrophages, in addition to the conventional role in sustaining inflammatory response and further hepatocyte injury, are in turn those involved in regulating the classic phenotypic responses of HSC/MFs by favoring the process of HSC activation/transdifferentiation (Tacke and Zimmermann, 2014). These pro-inflammatory and pro-fibrogenic macrophages are different from those defined as pro-resolution macrophages ($Ly6C^{low}$) that originate in response of mediators from hepatic MFs or other hepatic cells such as CX3CL1. $Ly6^{low}$ macrophages, which are typically involved in the removal of cell debris and pro-fibrogenic signals, express and release TRAIL and MMP9, that can promote apoptosis of MFs, as well as matrix metalloproteases such as MMP12 and MMP13 that can efficiently degrade/remodel ECM (Fallowfield et al., 2007; Ramachandran et al., 2012). Of relevance, $Ly6C^{low}$ macrophages are apparently derived from a phenotypic transition of the profibrogenic $Ly6C^{hi}$ macrophages and characterized by evidence of prior phagocytosis of dying cells (Ramachandran et al., 2012). Interestingly, removal of etiology or efficient therapy can then either favor apoptosis of HSC/MFs and/or result in a prominent role of pro-resolution macrophages, potentially leading to fibrosis reversal (Schuppan and Pinzani, 2012; Mallat and Lotersztajn, 2013; Schuppan and Kim, 2013; Novo et al., 2014). Indeed, regression of liver fibrosis (evident in experimental rodent models of fibrosis but also described, at least in pre-cirrhotic stage, in human livers) involves four major aspects that are the regeneration of hepatocytes, the reversal of HSC/MFs to the vitamin A-storing quiescent phenotype, the removal of MFs by apoptosis and, finally, the lysis of ECMs.

Along these lines, and in addition to the role of pro-resolution macrophages, one should also take in mind that liver fibrosis regression requires the removal of HSC/MFs and, more generally, of hepatic MFs from fibrotic septa. This can happen either following reversal of HSC/MFs towards a more quiescent phenotype, as shown in a murine model of liver injury (Troeger et al., 2012), or through the induction of HSC/MFs apoptosis which is facilitated by the fact that these cells can express CD95, TNF receptor 1, p75 and TRAIL receptors (Iredale et al., 1998; Wright et al., 2001; Kendall et al., 2009). It should be stressed once again that both these events (phenotype reversal and apoptosis) can occur when the etiological condition has been discontinued (typical in murine models of fibrosis) or therapy is efficient (in both murine models and human conditions) since HSC/MFs during CLD progression are typically set to a resistant, anti-apoptotic and NF-kB – related phenotype (Novo et al., 2006b; Oakley et al., 2009; Iredale et al., 2013). A third possible mechanism potentially able to favor removal of HSC/MFs relies on their senescence. Indeed, an elegant study has shown that hepatic MFs can undergo senescence, with senescent MFs being characterized by a block of proliferation, down-regulation of the expression of ECM proteins and up-regulation of the expression of matrix degrading enzymes (Krizhanovsky et al., 2008). In the same study, senescent MFs have been shown to be then cleared from the site of injury by the intervention of liver specific natural killer (NK) cells, usually located in the sinusoids at close proximity to liver non-parenchymal cells. These NK cells (abundant and having a rapid turn-over being substituted by bone marrow-derived cells), which increase enormously in relation to chronic viral infection and/or chronic inflammation, are able to selectively kill early activated HSC but not quiescent or fully activated and MF-like cells (Gao and Radaeva, 2013). This because during the process of activation/transdifferentiation early activated HSCs produce and release retinoic acid which, in turn, can upregulate on transient HSCs the expression of NK cell activating ligand retinoic acid inducible gene 1 (RAE1). Since transiently activated HSCs overexpress on their surface TRAIL receptors (Taimr et al., 2003), RAE1 by binding to NKG2D on NK cells can lead to NK cell activation resulting in the death of HSCs through TRAIL- and NKG2D dependent mechanisms (Radaeva et al., 2006). Fully activated HSC/MFs have lost retinol stores and can not produce any more retinoic acid or RAE1 and for this reason are then resistant to the action of NK cells. NK-mediated killing of human HSCs can also depend on the expression by HSCs of the NK cell activating receptor NKp46 (Gur et al., 2012) as well as by the fact that activated HSCs apparently have lost the ability to express MHC-1 antigen (i.e., able to suppress NK cell function) (Melhem et al., 2006; Muhanna et al., 2011). A number of elegant studies has also provided evidence that

activated NK cells, through the release of interferon-γ (IFN-γ), can induce HSCs cell cycle arrest ad apoptosis (Rockey et al., 1992b; Melhem et al., 2006) as well as to enhance their intrinsic ability to kill activated HSCs (Radaeva et al., 2006).

3.6 HSC/MFs and interactions with cells of the adaptive immunity.

T lymphocytes are believed to contribute to the modulation of liver fibrogenesis by means of their interactions with either pro-fibrogenic cells and/or other cells of innate and adaptive immunity. Th1 and Th2 lymphocytes have been proposed to differently affect fibrogenic progression of CLDs as suggested by data from liver fibrosis studies performed on different strains of mice. By using the same murine model of liver fibrosis C57BL/6 mice, in which Th1 response is known to predominate, display a weaker fibrotic reaction than BALB/c mice, the latter model displaying the predominance of Th2 response (Shi et al., 1997). This can suggest that Th1 cells, through typical cytokines like IL-12 and IFN-γ may act by limiting or inhibiting liver fibrogenesis (Pellicoro et al., 2014; Muhanna et al., 2008; Wynn et al., 1995). IFN-γ can suppress collagen deposition by regulating the balance of MMPs and TIMPs expression and, together IL-12, can also negatively affect the expression and release of pro-fibrogenic cytokines by Th2 cells (Wynn, 2004). Concerning Th2 lymphocytes, the proposed predominant profibrogenic action, shown in the experimental models by Schistosoma spp. (Wynn et al., 1995), is believed to rely mainly on the ability of these lymphocytes to release IL-13 (Pellicoro et al., 2014; Wynn, 2004). IL-13 can up-regulate TGF-β1 and MMP9 expression as well as promote fibrogenesis by controlling on MFs the relative expression of IL-13 receptor α1 (IL-13Rα1) versus the related decoy receptor IL-13Rα2 (Wynn, 2004; Chiaramonte et al., 1999).

The role of the Th1/Th2 paradigm in regulating liver fibrogenesis has been clarified by studies investigating the role of Th17 and cells regulatory T (Treg) cells, the latter being a subset of CD4+ T helper cells expressing CD25 and able to release the anti-inflammatory and immunosuppressive cytokine IL-10. Treg cells have been shown to strongly increase in the liver of patients affected by chronic HCV infection, primary biliary cirrhosis and other autoimmune liver diseases (Pellicoro et al., 2014) and depletion of these cells has been reported to exacerbate fibrosis in the rat BDL model (Katz et al., 2011). Moreover, Treg cells have been proposed to depress fibrogenesis in the Schistosoma spp.-induced fibrosis through their ability to specifically suppress Th2 cells (Turner et al., 2011). In addition, in primary human HSCs, Tregs were found to

upregulate the expression of typical pro-fibrogenic genes like TGF-β1, α-SMA, procollagen type I, CCL2, TIMP1, and MMP2 in an IL-8-dependent manner (Langhans et al., 2013).

Whether Th17 cells are concerned, these cells are known to increase in number both in the serum and the liver of patients affected by several forms of either acute or chronic liver injury as well as to release, when activated, both IL-17 and IL-22. The putative pro-fibrogenic role of IL-17 relies on the knowledge that IL-17 receptor, in particular, is expressed by HSCs, monocytes, Kupffer cells as well as cholangiocytes. HSCs respond to IL-17 by upregulating collagen type I by involving activation of the STAT3 signaling pathway, but IL-17 is also known to upregulate expression of other mediators like IL-1β, IL-6, TNF-α and TGF-β in target cells (Meng et al., 2012).

4. Portal fibroblasts as a source of portal MFs

Portal fibroblasts are liver resident fibroblasts located in the portal tract mesenchyme that surrounds bile ducts that can be easily detected since, unlike HSCs, they express several specific markers like fibulin 2, elastin, IL-6, cofilin 1 and the ecto-ATPase nucleoside triphosphate diphosphohydrolase-2 (NTPD2) (Dranoff and Wells, 2010; Wells, 2014). As mentioned in a previous section, portal fibroblasts have been proposed to be able to give raise to α-SMA positive MFs defined as portal MFs, as also reproduced in vitro when portal fibroblasts are cultured on plastic or glass (Dranoff and Wells, 2010; Forbes and Parola, 2011; Iwasaiko et al., 2012; Mallat and Lotersztajn, 2013; Lemoinne et al., 2013; Wells, 2014). However, it should be noted that at least in theory portal MFs may also originate also from other sources, including vascular smooth muscle cells of the wall of hepatic artery or portal vein (Dranoff and Wells, 2010). Portal MFs have been suggested to primarily act as pro-fibrogenic cells in conditions of biliary fibrosis, likely together with activated HSC/MFs (Kinnmann and Housset, 2002; Dranoff and Wells, 2010; Wells, 2014). In particular, it has been proposed that portal fibroblasts and portal MFs are the first cells responding to bile duct ligation (BDL, an experimental model of secondary biliary cirrhosis), with HSCs and then HSC/MFs migrating later at the site of biliary injury (Kinnmann and Housset, 2002). The same group went further in suggesting that HSCs and portal fibroblasts may occupy different niches, with the HSCs niche being induced by hypoxia during injury and portal fibroblasts niche by the

ductular reaction (Lemoinne et al., 2013). According to this interpretation, HSC/MFs may mediate liver wound healing whereas portal MFs may regulate scar formation.

Indeed, from an historical point of view, a first evidence for the origin of portal MFs was obtained using the BDL model in which portal fibroblasts were shown to proliferate immediately after surgical intervention to give raise to a population of desmin-negative, α-SMA-positive cells adjacent to proliferating bile ducts and connective tissue stroma (Tuchweber et al., 1996; Beaussier et al., 1997). Similar data were obtained in another study in precision-cut liver slices where cells resembling portal fibroblasts, not HSCs, proliferated following exposure to bile acids (Clouzeau-Girard et al., 2006). Moreover, several in vivo and in vitro data (the latter obtained by employing primary culture of isolated portal fibroblasts, undergoing activation/differentiation to MF-like cells) support the hypothesis of transition of portal fibroblasts into portal MFs having the ability to produce and secrete fibrillary collagen (types I, III and IV) (reviewed in Dranoff and Wells, 2010; Wells, 2014).

The involvement of portal MFs, as originated by portal fibroblasts, in biliary fibrosis has been somewhat challenged, at least from a quantitative point of view, by a recent and already previously cited fate tracing study (Mederacke et al., 2013). These Authors developed a transgenic mice carrying a bacterial artificial chromosome with a Cre reporter driven by lecithin-retinol acyltransferase in which more than 99% of HSCs were reported to be specifically and efficiently labeled. In this study, overall suggesting HSCs as the major cell source of MF-like cells in CLDs irrespective of etiology, data related to the model of biliary fibrosis indicated in any case the involvement of an aliquot of MFs not derived from HSCs, likely then originated from portal fibroblasts. However (see Wells, 2014), although the study was nicely performed, the possibility exists that, due to technical limitations of the transgenic model employed, the real involvement of MFs derived from portal fibroblasts may have been underestimated. In addition, as also indirectly suggested by data from the fate tracing study, it has been proposed that portal MFs may have a role as contractile cells also in conditions in which predominate the pattern of bridging fibrosis. As suggested by Wells in a nice recent and detailed review on portal fibroblast and portal MFs (Wells, 2014) this may be relevant and plausible if one consider that bridging fibrosis connects fibroblast-rich regions such as the portal tract and the central vein.

If we come back to biliary fibrosis, the primary role of portal fibroblasts and portal MFs is supported by the knowledge that the injury to cholangiocytes is believed to represent a

prerequisite for the diffentiation of portal fibroblasts into portal MFs. The hypothesis (summarized in Figure 5) is here that once damaged, cholangiocytes acquire the ability to express and release TGFβ2 (Wells et al., 2004) as well as other relevant mediators like PDGF-BB, IL-6, CCL2 and connective tissue growth factor (CTGF). These mediators are likely responsible for the activation and differentiation of portal fibroblasts into portal MFs, also considering that portal fibroblasts are known to express the receptors for these polypeptide factors (Dranoff and Wells, 2010). This may be followed by an autocrine perpetuation by portal MFs not dissimilar from what reported for HSC/MFs. Accordingly, the strict interactions between portal MFs and activated/damaged cholangiocytes may also significantly contribute to CLD progression in other clinical setting, particularly those characterized by bridging fibrosis. Indeed, several laboratories have outlined the existence of a direct relationships between the intensity of the ductular reaction (a definition that applies to a peculiar form of hyperplastic response of cholangiocytes in pathological conditions) and the severity of ECM deposition in either animal models as well as human liver diseases of different etiologies, including chronic HCV and NAFLD/NASH (Clouston et al., 2005; Fabris et al., 2007; Richardson et al., 2007; Lorenzini et al., 2010). To this scenario one should ideally add concepts related to the possible involvement, particularly in pathophysiological conditions, of the so-called population of bi-potent hepatic progenitor cells (HPCs). As nicely reviewed by Wells, there are report from literature suggesting that portal fibroblasts may be actively involved in the peribiliary stem cell niche in either fetal development as well as during liver regeneration and repair in CLD (Wells, 2014), in an overall CLD scenario in which Wnt or Notch signaling modulate lineage differentiation of HPCs towards hepatocytes or cholangiocytes and Hedgehog signaling may be involved in the interactions between mesenchymal cells in the portal tract and epithelial cells (Omenetti et al., 2007; Boulter et al., 2012). As a matter of fact, HPCs have been reported to be surrounded by portal fibroblasts in injured livers (Greenbaum et al., 2011) and portal MFs have been proposed to secrete ECM required for progenitor cell expansion (Van Hul et al., 2009).

5. Bone marrow – derived MFs

The interest in bone marrow - derived cells as extrahepatic cells able to engraft injured liver initiated historically with studies designed in order to evaluate the possibility to employ these cells in terms of regenerative medicine (i.e., repopulation of liver parenchyma following an injury).

The original suggestion was that bone marrow - derived stem cells (BMdSCs) could transdifferentiate into either hepatocytes and cholangiocytes. Overall these specific attempts were unsatisfying and it was recognized that that in fact there is little if any contribution of the BM stem cell compartment to epithelial cells, with few positive results being possibly artefacts or even the result of cell fusion between BMdSCs and hepatocytes (reviewed in Newsome and Forbes, 2012). However, in one of these pioneer studies a laboratory noticed that MF-like cells in human livers were positive for markers that could only derive from bone marrow - derived cells recruited in liver parenchyma, as was the case of MFs carrying Y chromosome found in the liver of females that received previously a bone marrow transplant from male donors (Forbes et al., 2004). In a decade in which regenerative medicine was exploding, with several clinical studies trying to use bone marrow derived cells also in the field of hepatology (Houlihan and Newsome, 2008) the concept that these cells may give raise to fibrogenic cells was of course potentially a bad news. In the following years a number of studies have been performed to investigate this issue and the first one confirmed the existence of the problem since mice transplanted with traceable bone marrow cells (GFP positive) showed the presence of GFP positive stellate cells in their liver (Baba et al., 2004). Other studies employing the transplant of either murine or human BMdSCs showed a significant increase in the flux of bone marrow - derived MFs in the progression of chronic liver injury (Russo et al., 2006; Asawa et al., 2007; Valfrè di Bonzo, 2008; Fujimiya et al., 2009). Two of these studies proposed that bone marrow - derived mesenchymal stem cells were the most relevant population able to give raise to MFs (Russo et al., 2006; Valfrè di Bonzo et al., 2008). A further study reported the involvement of α-SMA negative bone marrow - derived cells defined as fibrocytes able to engraft injured livers and to contribute to liver fibrogenesis (Kisseleva et al., 2006). Studies performed on human liver specimens confirmed the hypothesis of MFs derived from bone marrow cells although the proportion of these cells was variable and usually representing a minority of the overall population of hepatic MFs (Forbes et al., 2004; Dalakas et al., 2010). At present this concept is the prevailing one: hepatic MFs from bone marrow cells can be found in either experimental or clinical conditions but their contribution is believed to be quite limited (Forbes and Parola, 2011; Newsome and Forbes, 2012). It is correct to mention that a study has questioned whether bone marrow cells may actually contribute to ECM deposition in any significant amount in the liver (Higashiyama et al., 2009). Properly designed experimental studies using modern lineage tracing experiments and reporter systems are required to finally clarify this issue.

6. MFs from hepatocytes and cholangiocytes ?

Literature from the last decade has offered several studies suggesting that pro-fibrogenic cells may originate, in addition to HSCs, portal fibroblasts and bone marrow - derived cells, also from either hepatocytes or cholangiocytes through a process of epithelial to mesenchymal transition or EMT (Choi et al., 2009; Cannito et al., 2010, Xie and Diehl, 2013; Novo et al., 2014). The term EMT conventionally refers to a critical biologic process, originally described in embryonic development, in which cells of epithelial origin undergo a phenotypic and functional transition towards the acquisition of a mesenchymal phenotype (i.e., by losing polarization and specialized junctional structures as well as by undergoing cytoskeletal reorganization) and related properties. In particular, the EMT process leads these cells to acquire the ability to migrate and to produce and secrete ECM components (Kalluri et al., 2009; Cannito et al., 2010). The involvement of EMT as a mechanism contributing to liver fibrosis was first proposed, following homologous studies in the field of renal and lung fibrosis, by a series of studies that initially showed the ability of either hepatocytes and cholangiocyte to undergo a transition to a fibroblastoid/mesenchymal-like morphology when exposed to TGFβ1 (reviewed in Kalluri et al., 2009; Cannito et al., 2010). In these experiments classic EMT changes were detected (E-cadherin down-regulation, acquisition of mesenchymal markers like vimentin, desmin, α-SMA and the protein S100A4, also known as fibroblast-specific protein 1 or FSP-1).

The first relevant study investigating the role of EMT of hepatocytes employed AlbCre.R26RstoplacZ double transgenic mice submitted to the chronic carbon tetrachloride (CCl_4) model (Zeisberg et al., 2007). The involvement of EMT in liver fibrogenesis was inferred by two order of data: first, when fibrosis was established approx. 15% of hepatic cells were found to be positive for FSP-1 expression, with 5% of cells co-expressing FSP-1 and albumin; second, inhibition of liver fibrosis and putative EMT-derived fibroblasts/MFs were significantly inhibited by treatment with bone morphogenetic protein-7 (BMP-7), a protein known to efficiently antagonize TGFβ1 signaling; these data, in particular, were homologous to those obtained in a parallel study using transgenic mouse over-expressing Smad7 in hepatocytes (Dooley et al., 2008). Evidence for some EMT-related changes were reported in the same study by analyses performed on liver specimens from patients affected by chronic HBV infection (Dooley et al., 2008).

Even more impressive were initial experimental and clinical reports in which EMT of cholangiocytes was suggested as putative pro-fibrogenic mechanism. These studies, mainly

related to conditions of biliary fibrosis, offered images of cholangiocytes apparently co-expressing α-SMA and cytokeratin 19 (CK19, a marker expressed by both adult cholangiocytes and HPCs) (Xia et al., 2006). The laboratory of Anna Mae Diehl produced several studies performed using the rat or murine BDL model of secondary biliary fibrosis (reviewed in Choi et al., 2009). These studies proposed a cause-effect relationships between EMT of cholangiocytes appearance of portal MFs and then biliary fibrosis as well as a major role for the Hedgehog signaling pathway. Of relevance, EMT-related changes for cholangiocytes as well as the relevance of Hedgehog signaling were also reported in human patients affected by primary biliary cirrhosis (PBC), by primary sclerosing cholangitis (PSC), or biliary atresia (Choi et al., 2009; Cannito et al., 2010). Additional mechanistic studies also proposed a critical role in EMT and liver fibrogenesis for Notch signaling on the basis of results from experiments based on the use of a specific c-secretase inhibitor or of neutralizing antibody against Jagged 1 (Chen et al., 2012; Liu et al., 2012; Xie et al., 2013). Because of the potential impact of these studies, different research laboratories became involved in investigating the role of EMT and starting from 2010 at least four elegant fate tracing studies, performed using properly designed triple transgenic mice, significantly challenged the real pro-fibrogenic relevance of EMT of either hepatocytes or cholangiocytes. A first study from David Brenner group employed a very complex transgenic model (triple transgenic mice expressing ROSA26 stop beta-galactosidase (beta-gal), albumin Cre, and collagen alpha1(I) green fluorescent protein or GFP) designed to trace hepatocyte-derived cells (labeled by beta-gal) and pro-fibrogenic cells (GFP labeled) (Taura et al., 2010). The conclusions from this study were straightforward: Authors could not find cells positive for both GFP and beta-gal, then apparently excluding origin of profibrogenic cells from hepatocytes. The same research group used the Cre/LoxP system in order to follow the cell fate of CK19 positive cells (i.e., cholangiocytes) in CK-19(YFP) or FSP-1(YFP) transgenic mice that were then subjected to BDL or chronic CCl_4 treatment (Scholten et al., 2010). Once again results were unequivocal in indicating that cholangiocytes were not expressing EMT markers and that cells positive for FSP-1(YFP) were negative for CK19. In a third study, the same group reported that FSP-1, a putative and widely used EMT marker, was not expressed by pro-fibrogenic and collagen type I producing cells. Moreover, FSP-1 positive cells in chronically injured livers were not co-expressing the typical markers of MFs like α-SMA and desmin but rather F4/80 and other markers of cells belonging to the myeloid-monocytic lineage, suggesting that FSP-1 cells may represent a subset of macrophages involved in CLD progression differing from Kupffer cells (Österreicher et al., 2011). A fourth study, this time from the group of Rebecca Wells, employed

the Cre/LoxP system to obtain transgenic mice designed to follow the fate of cells expressing alpha-fetoprotein (AFP), that is a way to label any hepatocyte and cholangiocyte since these cells derive all from AFP-positive precursors (Chu et al., 2011). These mice were then submitted to different experimental model of fibrosis and Authors reported that MFs were always AFP negative, then excluding an origin from either hepatocytes or cholangiocytes. These studies (Taura et al., 2010; Sholten et al., 2010; Österreicher et al., 2011; Chu et al., 2011) and others here not cited as well as the recent study from the Robert Schwabe laboratory (Mederacke et al., 2013) suggesting that HSCs are by far the major cell source of hepatic MFs, irrespective of etiology, strongly indicate that the involvement of EMT as pro-fibrogenic mechanism is highly controversial and, in case, of very minor relevance.

7. MFs and pro-fibrogenic mechanisms: implications for CLDs in terms of pattern of fibrosis and etiology

In this final section we will take advantage of the actual knowledge concerning the origin of MFs as well as of the established and emerging pro-fibrogenic mechanisms in order to offer in the end a synthetic view of how these concepts may impact the progression of CLD in terms of pattern of fibrosis with a reference to the specific etiology of the disease.

Indeed, an overall analysis of literature data clearly suggests that the specific etiology of a CLD is relevant in relation to CLD progression. The view we are going to offer is in line with the original proposal from Pinzani and Rombouts (Pinzani and Rombouts, 2004), as refined more recently (Parola et al., 2008; Novo et al., 2014). This view identifies four well defined and distinct patterns of fibrosis development that can be correlated to specific etiologies, prevailing pro-fibrogenic mechanisms and the type(s) of hepatic MFs involved. In this final section we will just mention the more relevant pro-fibrogenic mechanisms and the interested reader can refer to recent and exhaustive reviews for more details (Mallat and Lotersztajn, 2013; Novo et al., 2014).

7.1 Post-necrotic or bridging fibrosis

This is pattern that is typically observed in liver specimens from patients affected by chronic viral (HBV, HCV) infection or by an autoimmune disease. The pattern of bridging fibrosis is characterized by the predominant formation of fibrotic septa connecting portal areas with centrilobular vein (i.e., portal-central septa), which represent the consequence of portal-central bridging necrosis. This pattern is typically associated with the so-called interface hepatitis, as well as with the formation of blind septa or fibrotic septa connecting different portal areas in chronically damaged liver parenchyma. This is a pattern that leads to an early involvement of centrilobular vein, with formation of neo-vessels (i.e., angiogenesis) and of porto-central shunting. The prevalent fibrogenic mechanism proposed to be associated to this pattern and the related etiologies is represented by chronic activation of wound healing with a significant contribution of oxidative stress. According to current literature data fibrogenic progression is here provided mainly by HSC/MFs and portal MFs, with a possible minor contribution of MFs originated from bone marrow - derived cells.

7.2 Pericellular or perisinusoidal fibrosis

This peculiar pattern of fibrosis is also sometimes referred to as chicken-wire fibrosis and is mainly detected in liver specimens from patients with either alcoholic steatohepatitis (ASH) or suffering of metabolic derangements such as those affected by non-alcoholic fatty liver disease (NAFLD) progressing towards non-alcoholic steatohepatitis (NASH). A similar pattern has been detected also in the liver of patients with hemochromatosis. Excess deposition of ECM is found in the space of Disse and is usually proposed as the consequence of the activation of peri-sinusoidal HSCs to HSC/MFs. This pattern results in the previously mentioned capillarization of sinusoids that in the natural history of these diseases precedes the formation of fibrotic septa with a pattern of fibrosis development that progressively tends to connect cetrilobular vein areas to portal areas. The prevailing pro-fibrogenic mechanism in this pattern of fibrosis is represented by oxidative stress with a significant contribution, particularly in conditions of NASH, by lipotoxicity.

7.3 Biliary fibrosis

This pattern of fibrosis is observed in PBC and secondary biliary cirrhosis as well as in PSC and is characterized by the typical formation of fibrotic septa connecting portal areas and surrounding nodular areas of liver parenchyma. This is a rather peculiar pattern of fibrosis which develops by preserving rather normal connection between centrilobular vein and portal tracts and is typically associated to the so-called ductular reaction, that is an intense proliferation of reactive bile ductules and periductular MFs. In these conditions of chronic biliary damage, as previously mentioned, periductular MFs are believed to derive from portal fibroblasts as well as, in a later phase, from HSCs. The derangement of normal interactions between cholangiocytes, portal fibroblasts and, possibly, HPCs, is believed to predominate as pro-fibrogenic mechanism with an additional role played by oxidative stress.

7.3 Centrilobular fibrosis

This pattern of advanced fibrosis is unrelated to CLDs and is typically a secondary scenario found in conditions characterized by venous outflow obstruction, as is the case in patients affected by heart failure. In these clinical settings ECM deposition results in the formation of fibrotic septa connecting central vein areas (central-central septa) leading to the unique feature defined as reversed lobulation.

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Figure legends.

Figure 1. ***Major pathophysiological events involved in the fibrogenic progression of CLDs.*** The involvement of pathophysiological events is referred to the natural history of a typical disease (irrespective of etiology) starting from early injury and initial fibrosis, then leading to the development of vascular changes and tissue architecture derangement characteristic of liver cirrhosis and finally involving the development of portal hypertension and other complications, hepatic failure and hepatocellular carcinoma.

Figure 2. ***Cross-talk between hepatic cell populations in liver fibrogenesis.*** Liver fibrogenesis, irrespective of etiology, is a dynamic process sustained and modulated by an intense cross talk occurring between different hepatic cell populations, resident or recruited into chronically injured liver. These interactions involve the synthesis and release of several mediators, including growth factors, cytokines, chemokines, adipokines, ROS and vasoactive agents and plasma proteins, with functional responses of hepatic cells being also significantly modulated by conditions of hypoxia.

Figure 3. ***Liver Mesenchymal cells: from embryo development to liver diseases.*** The scheme illustrates actual knowledge on liver mesenchymal cells in normal and pathological conditions. Liver mesenchymal cells in the normal adult liver (middle panel) are proposed to originate from mesothelial cells deriving from the septum transversum mesenchyme (STM) during embryo development (left panel). HSCs originate from a population of sub-mesothelial cells (possibly also directly from STM) from which also originate perivascular mesenchymal cells (PMCs) that then give raise to portal fibroblasts, smooth muscle cells and fibroblasts around the centrilobular vein. Right panel offer the current literature view of hepatic myofibroblasts as a heterogeneous cell population originating from HSCs, portal fibroblasts and, to a less extent, bone marrow-derived cells. The origin of MFs throught epithelial-to-mesenchymal transition of hepatocytes or cholangiocytes is at present controversial.

Figure 4. ***The process of activation and trans-differentiation of HSCs into activated and MF-like cells (HSC/MFs).*** The process of activation and trans-differentiation of HSCs into HSC/MFs involves two major steps: i) the initiation step, potentially reversible, leading to a transiently activated phenotype, able to proliferate and critically involved in the resolution of parenchymal injury; if the injury is acute, these cells undergo either apoptosis or reversion to the quiescent phenotype; ii) the perpetuation step, in which the pro-fibrogenic environment and related mediators sustain a trans-differentiation of cells to a fully activated pro-fibrogenic phenotype that, with its functional responses, is critical in promoting the progression of the disease; removal of the etiology or efficient therapeutic approach can potentially lead to resolution of fibrosis associated to HSC/MFs senescence or apoptosis.

Figure 5. ***Activation of portal fibroblasts and bone marrow - derived cells.*** Portal fibroblasts, that can be recognized in vivo on the basis of their expression of a number of characteristic markers, are proposed to undergo a process of activation towards portal MFs that is essentially sustained by mediators expressed and released by damaged or activated cholangiocytes during the course of chronic injury affecting the biliary tree and leading to biliary-like fibrosis. Portal MFs are α-SMA positive cells but their positivity to other markers (like those of portal fibroblasts) may be not so

selective. In some pathophysiological conditions a relatively limited amount of activated MFs may originate from bone marrow - derived cells, like either mesenchymal stem cells (MSC) or fibrocytes, which are recruited into chronically injured liver by a restricted number of mediators (with a major role attributed to SDF-1) in a way which is also modulated by parenchymal hypoxia.

Figure 1.

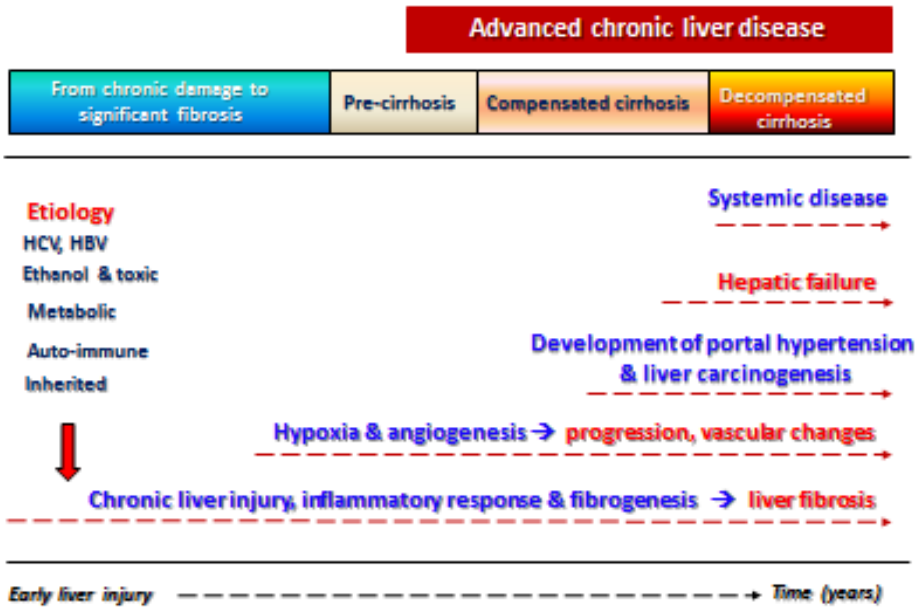


Figure 2.

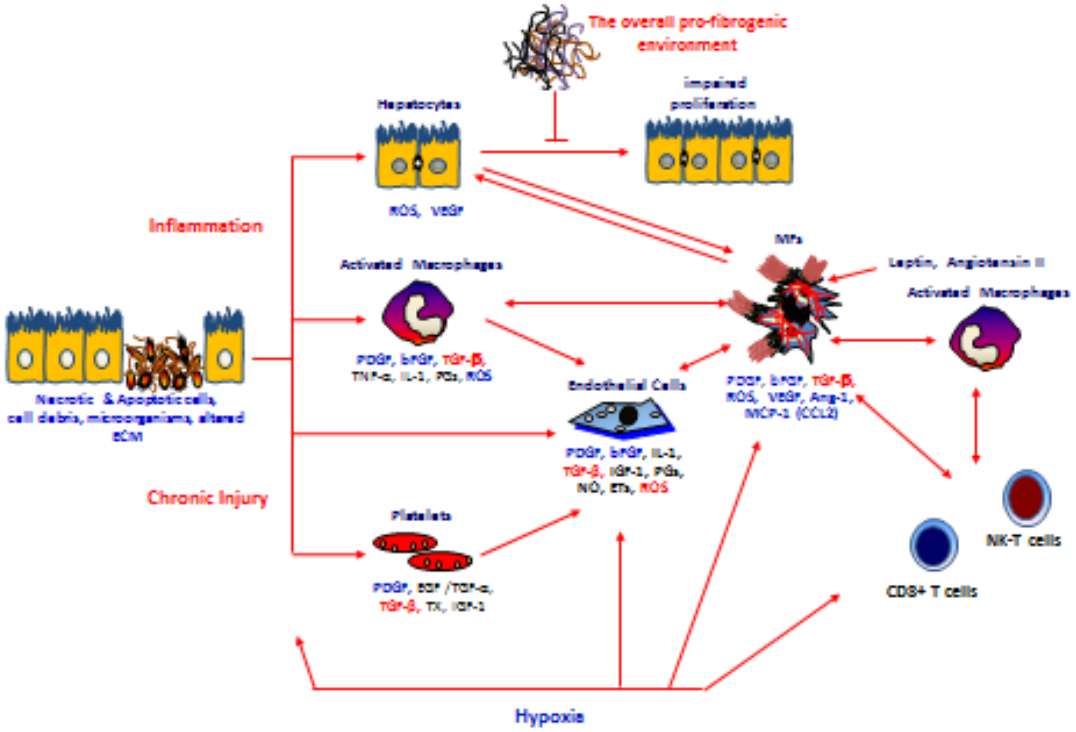


Figure 3.

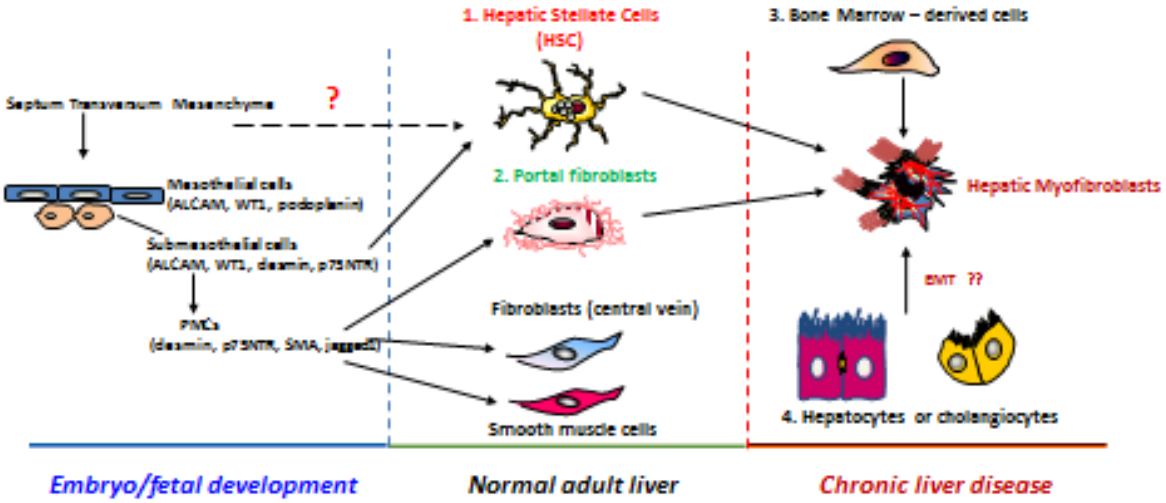


Figure 4.

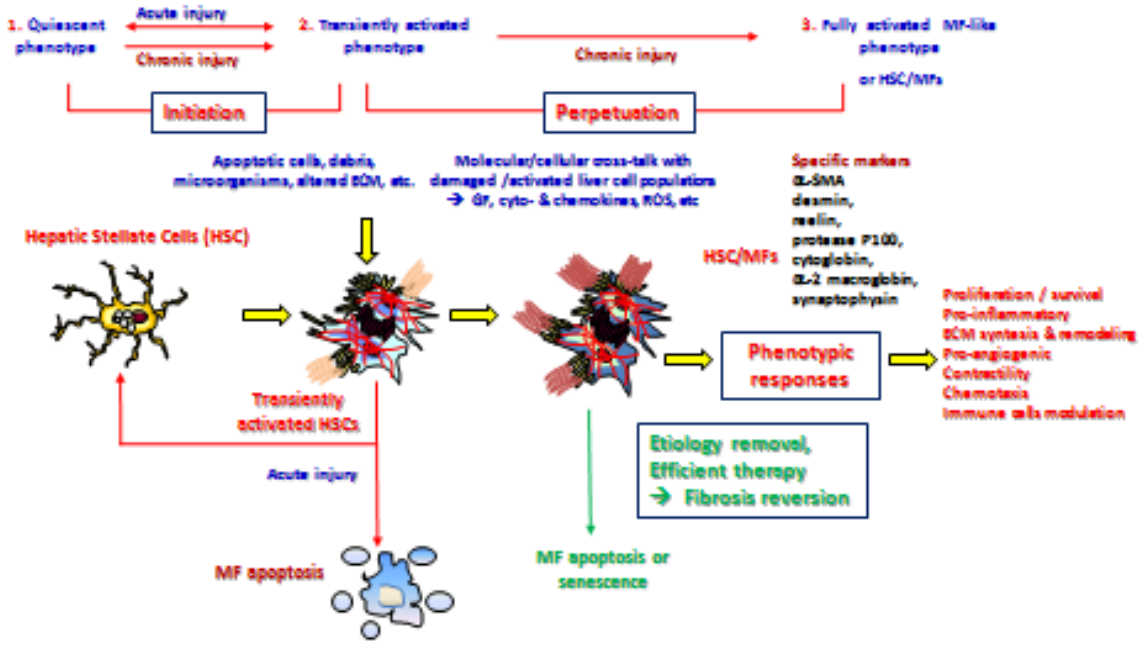
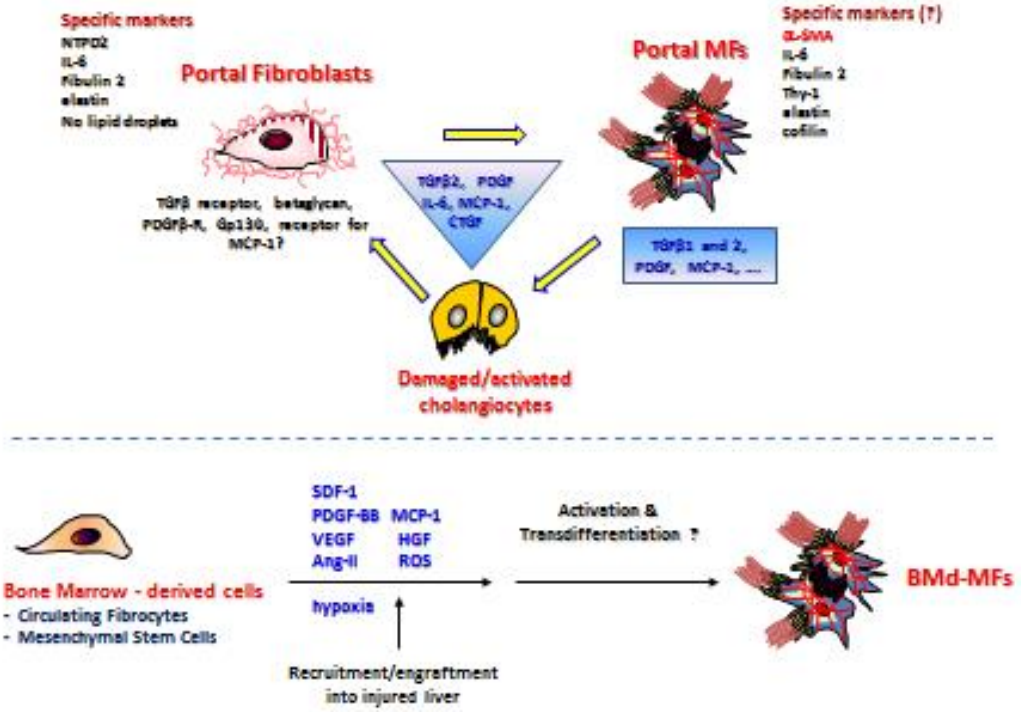


Figure 5.


Specific markers
NTPD2
IL-6
Fibulin 2
elastin
No lipid droplets
Portal Fibroblasts
TGFβ receptor, betaglycan, PDGFβ-R, Gp130, receptor for MCP-1?
TGFβ2, PDGF IL-6, MCP-1, CTGF
Portal MFs
Specific markers (?)
α-SMA
IL-6
Fibulin 2
Thy-1
elastin
cofilin
TGFβ1 and 2, PDGF, MCP-1, ...
Damaged/activated cholangiocytes
Bone Marrow - derived cells
- Circulating Fibrocytes
- Mesenchymal Stem Cells
SDF-1
PDGF-BB MCP-1
VEGF HGF
Ang-II ROS
hypoxia
Recruitment/engraftment into injured liver
Activation & Transdifferentiation ?
BMd-MFs