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Review

Liver fibrosis: a dynamic and potentially reversible process

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Summary. In any chronic liver disease (CLDs), whatever the aetiology, reiteration of liver injury results in persisting inflammation and progressive fibrogenesis, with chronic activation of the wound healing response in CLDs, representing a major driving force for progressive accumulation of ECM components, eventually leading to liver cirrhosis. Cirrhosis is characterized by fibrous septa dividing the hepatic parenchyma into regenerative pseudo-lobules, as well as by extensive changes in vascular architecture, the development of portal hypertension and related complications. Liver fibrogenesis (i.e., the dynamic process leading to increased deposition of ECM and much more) can lead to different patterns of fibrosis and is sustained by myofibroblast-like cells (MFs) of different origin, with activated hepatic stellate cells (HSC/MFs) being the major cell type involved. Major pro-fibrogenic mechanisms also include oxidative stress, as well as derangement of epithelial-mesenchymal interactions and, as recently suggested, the process of epithelial to mesenchymal transition (EMT).

Liver fibrosis has been considered traditionally as an irreversible process but experimental and clinical literature data published in the last decade have suggested that both the removal of the aetiological agent or condition, as well as an effective therapy, can result in significant regression of liver fibrosis. This is usually associated, particularly in animal models, with induction of apoptosis in MFs but, unfortunately, human HSC/MFs are much more resistant to apoptosis than murine MFs. However, clinical studies provided no unequivocal evidence for a complete reversal of cirrhosis or a significant reversal of vascular changes in conditions of established cirrhosis.

Key words: Liver fibrosis, Hepatic stellate cells, Hepatic myofibroblasts, Liver cirrhosis, Myofibroblast apoptosis

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Progressive liver fibrogenesis: the process leading to excess deposition of extracellular matrix and eventually to liver cirrhosis

Introductory remarks

Chronic liver diseases (CLDs) are typically characterized by reiteration of hepatocyte injury due to either chronic viral infection by hepatotropic viruses (mainly hepatitis B and C viruses) or to autoimmune injury, as well as to metabolic and toxic/drug – induced causes, with chronic alcohol consumption being predominant in western countries (Fig. 1). This results in a chronic activation of the wound healing response that, together with other mechanisms like oxidative stress, derangement of epithelial-mesenchymal interactions and epithelial to mesenchymal transition, sustains persistent liver fibrogenesis (i.e., the process) and represents a major driving force for liver fibrosis (i.e., the result) (Parola and Robino, 2001; Friedman, 2003, 2004; Bataller and Brenner, 2005; Friedman 2008b; Novo and Parola, 2008; Parola et al., 2008).

Liver fibrogenesis can be envisaged as a dynamic and highly integrated molecular, tissue and cellular process that leads to the progressive accumulation of extracellular matrix (ECM) components in an attempt to limit hepatic damage in a CLD irrespective of the aetiology. This eventually leads to liver cirrhosis and

hepatic failure, with cirrhosis (Fig. 2) being currently defined as an advanced stage of fibrosis, 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 related complications (variceal bleeding, hepatic encephalopathy, ascites and hepatorenal syndrome) (Friedman, 2003, 2004, 2008b; Pinzani and Rombouts, 2004; Bataller and Brenner, 2005).

The clinical relevance of fibrogenic progression in CLDs

Progressive liver fibrogenesis has a tremendous clinical impact which is best described by the following scenario:

a) on the basis of current reports, it can be estimated that millions of patients worldwide are affected by a form of CLD, with HCV chronic infection becoming predominant in western countries, followed by and/or associated with chronic alcohol consumption, and HBV and HCV chronic infections being predominant in Asia and Africa;

b) 25-30% of patients affected by CLDs are expected to develop significant fibrosis and cirrhosis;

c) liver cirrhosis is currently the most common non-neoplastic cause of death in Europe and USA and overall represents the 7th most common cause of death in western countries;

d) mortality rate is further increased by primary liver cancer (i.e., hepatocellular carcinoma or HCC) which is strictly associated with liver cirrhosis;

e) a peak for advanced CLD (mainly associated with HCV chronic infection) has been predicted for Europe in the next decade which is likely to face a significant reduction of organ donation for liver transplants, which at present represent the only effective treatment option;

f) a number of clinical features have been identified that may serve as major predictors for the development of significant fibrosis and cirrhosis:

- male gender (age <50 years);
- age at infection (particularly for HCV);
- daily alcohol intake;
- hepatic iron content;
- obesity and diabetes mellitus;
- individual factors, including mainly differences in immune responses vs infectious agents and related auto-antigens as well as individual differences in drug metabolism.

In fact, fibrotic progression towards cirrhosis has been estimated to last for at least 10 – 15 years; however, progression towards cirrhosis may be extremely rapid and completed within 2-3 years in children affected by biliary atresia or familiar progressive intrahepatic cholestasis, as well as in a subset of patients following OLT for HBV- or HCV – related cirrhosis (Poynard et al., 1997; Pinzani et al., 2001; Parola et al., 2008).

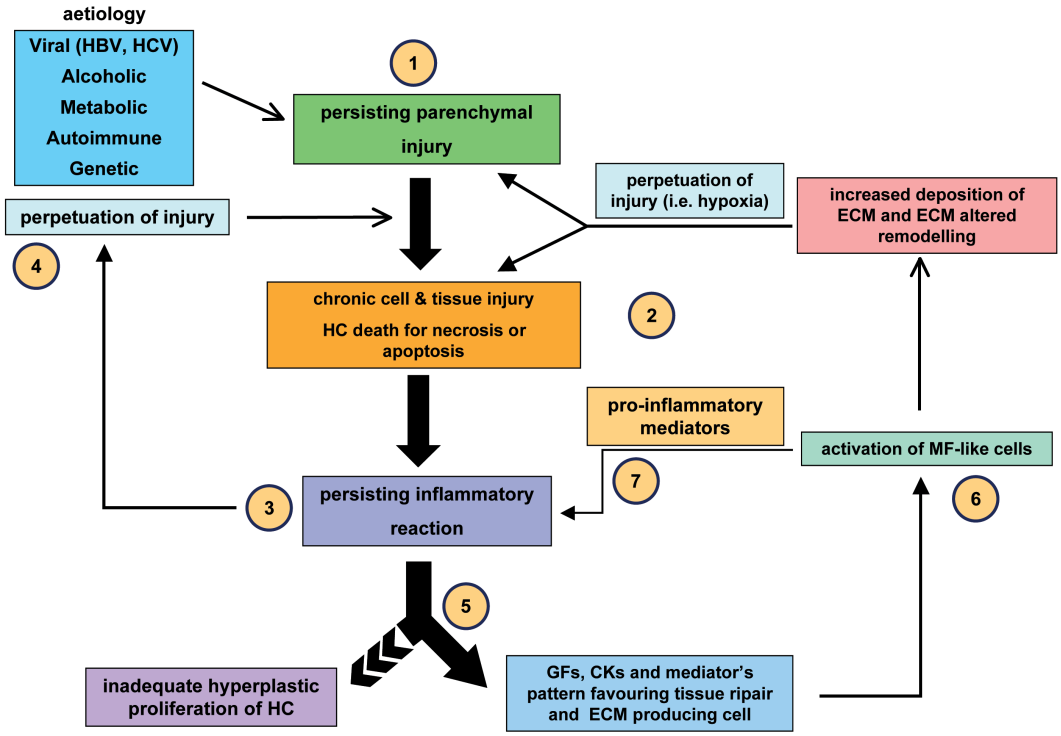

Fig. 1. Events involved in fibrosclerotic development of CLDs. CLDs may involve different aetiological agents or conditions able to cause persisting parenchymal liver injury (1) and then hepatocyte (HC) necrotic or apoptotic cell death (2). As a result, a persistent inflammatory reaction can occur (3), which may significantly affect the progression of the disease by either contributing to the perpetuation of injury (4) or by 'creating' a growth factor, cytokine and mediator pattern favoring tissue repair and the activation of ECM-producing cells (5). This chronic scenario, with time will lead to activation of myofibroblast-like cells that, in turn, will contribute either to perpetuation of inflammation by releasing pro-inflammatory mediators (7) or to the wound healing response by excess and progressive accumulation of fibrillar ECM components. If the aetiological agent or causal condition persists, the CLD can undergo a fibrosclerotic progression to cirrhosis and liver failure.

The different patterns of fibrosis

Fibrotic progression of CLDs has been described to proceed through at least four distinct patterns of fibrosis that seem strictly related to the underlying cause of CLD. The main fibrotic patterns (as detailed in the next paragraphs), are also related to the "topographic site" of tissue injury, to the involvement of different populations of MFs and the predominant pro-fibrogenic mechanism (Cassiman and Roskams, 2002; Pinzani and Rombouts, 2004; Parola et al., 2008).

Bridging fibrosis

This is a pattern of ECM deposition and septa formation that is typically described mainly in the liver of patients carrying HBV- or HCV-related chronic hepatitis. In this pattern one can appreciate, as a result of portal-central bridging necrosis, the development of fibrotic septa that apparently connect portal areas with the area of central vein (i.e, portal-central septa) or different portal areas (portal-portal septa), as well as of blind septa in the parenchyma. Within this pattern are included the classic images of fibrotic septa leading to the obliteration of central veins and of early changes in vascular architecture and connections with the portal system, which eventually favor the development of portal hypertension. It is believed that chronic activation of wound healing is likely to represent the major pathogenic mechanism driving this pattern of fibrosis progression; moreover, hepatic pro-fibrogenic MFs in these settings can be either derived from HSCs, portal fibroblasts, or even from bone marrow stem cells (see later). However, oxidative stress and reactive oxygen species (ROS) have also been described to offer a significant pathogenic contribution.

Perisinusoidal/pericellular fibrosis

This is a pattern that has been described in CLDs due to excess alcoholic consumption (ASH or alcoholic steatohepatitis) or to metabolic derangement and then progressing from non-alcoholic fatty liver disease (NAFLD) to non-alcoholic steatohepatitis (NASH). In these clinical settings excess deposition of ECM components is seen first in the space of Disse and leads to the peculiar "chicken-wire" pattern. MFs derived from activation of hepatic stellate cells (HSC/MFs) and ROS and oxidative stress have a predominant pro-fibrogenic and pathogenic role, respectively.

Biliary fibrosis

This definition is used in those conditions affecting the biliary tree that are characterized by the rather peculiar scenario of concomitant proliferation of reactive bile ductules and periductular MFs (here mainly derived from periportal fibroblasts or, see later, by EMT transition of cholangiocytes). This scenario is dominated by the consequent formation of portal-portal septa that for a long time do not significantly affect vascular connections with the portal system. For this pattern, either significant alterations in the interactions between cholangiocytes and mesenchymal cells or cholangiocyte transition into MF-like phenotype, as well as oxidative stress, have been proposed as major mechanisms.

Centrilobular fibrosis

This is a pattern that has to be included in this classification but is really independent of CLDs

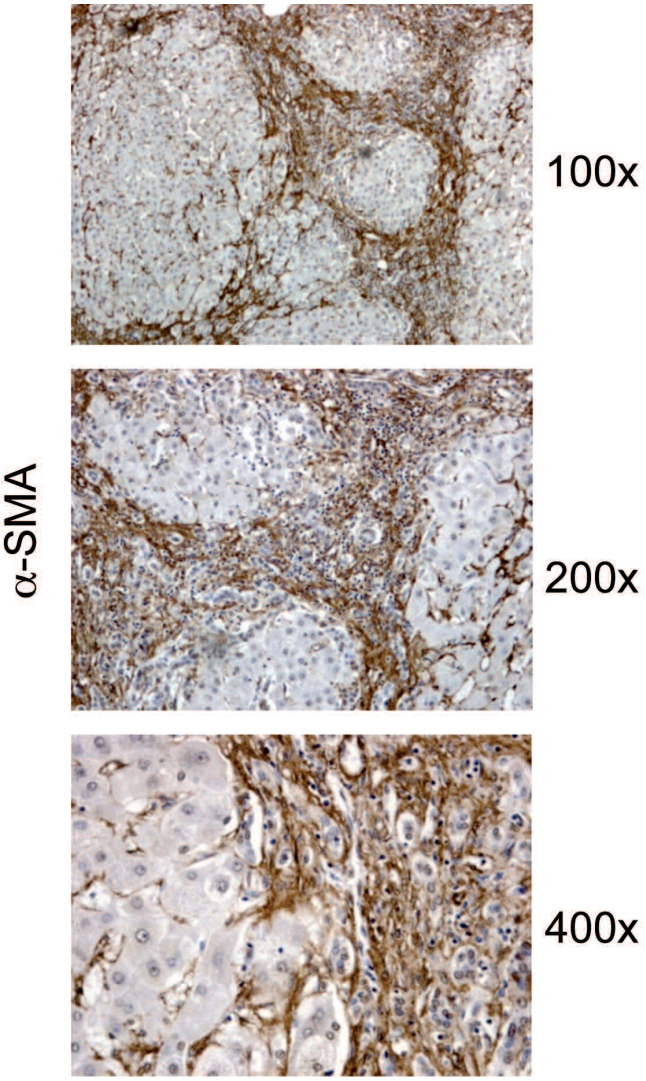

Fig. 2. Immunohistochemical analysis performed on paraffin liver sections from patients with hepatitis C virus (HCV) related liver cirrhosis (METAVIR F4). Sections (2 μm thick) were incubated with specific antibodies raised against α-SMA that positively stain myofibroblast – like cells. Primary antibodies were labeled by using EnVision, HRP-labelled System (DAKO) antibodies and visualized by 3'-diaminobenzidine substrate.

progression. Indeed, centrilobular fibrosis is typically described in patients affected by chronic heart failure in which a significant alteration of venous outflow is realized. In these patients fibrotic septa develop between central vein areas (central-central septa) and lead to the unique scenario often defined as "reversed lobulation".

Hepatic myofibroblasts.

Hepatic myofibroblasts (MFs) is a collective definition for a heterogenous population of pro-fibrogenic cells, mostly positive for α-smooth muscle actin (αSMA), which can be easily identified in chronically injured livers (i.e., fibrotic or cirrhotic) in either clinical or experimental conditions (Cassiman et al., 2002; Parola et al., 2008; Novo et al., 2009). On the basis of their antigen profile and/or tissue localization, different populations of MFs have been described: a) portal/septal MFs (PS/MFs), which express an overlapping antigen repertoire and are commonly found in the expanded connective tissue around portal tracts (portal MFs) as well as in the inner part of fibrotic septa (septal MFs); b) interface MFs (IF/MFs), which are primarily found where active fibrogenesis occurs, which is at the edge between fibrotic septa and the surrounding parenchyma; c) activated, myofibroblast-like, hepatic stellate cells (HSC-MFs), αSMA-positive cells found primarily in or around capillarised sinusoids of fibrotic/cirrhotic livers.

In the last decade, literature data have revealed that pro-fibrogenic hepatic MFs can originate from different cellular sources (Fig. 3), with a relative contribution that may vary depending on the aetiology of the chronic liver disease (CLD) and/or the prevailing fibrogenic mechanism (Friedman, 2008b; Parola et al., 2008). The following cell sources or mechanisms have been described:

Hepatic stellate cells

Hepatic stellate cells are perisinusoidal cells of still uncertain embryological origin, responsible for the synthesis of basal membrane like – ECM components of the sub-endothelial space of Disse and for storage and metabolism of vitamin A and retinoids. HSC have been also proposed to act as "liver specific pericytes" and to significantly contribute to hepatic development and regeneration (Friedman, 2008a). HSC are still considered as the most relevant cell source for pro-fibrogenic MF-like cells for which the process of activation and pro-fibrogenic mechanisms are best characterized (Friedman, 2008a). HSC-MFs are involved in most, if not all, clinical conditions of CLDs, with an involvement which is prevalent in the pattern of

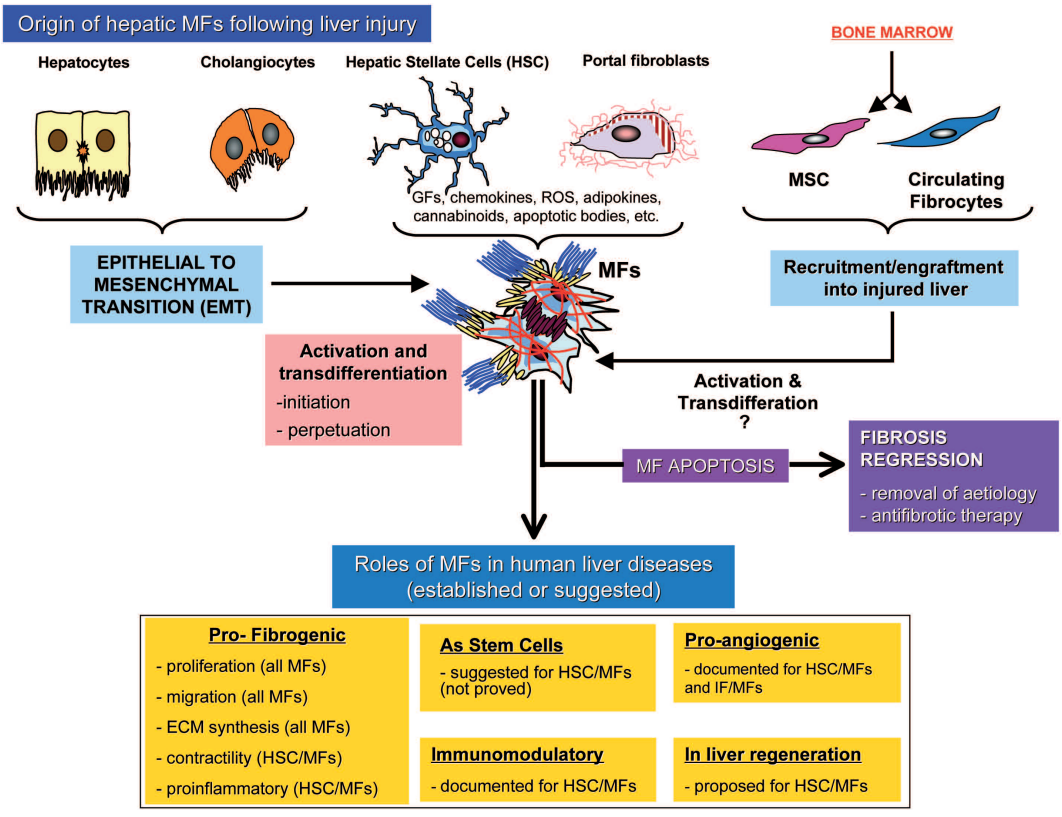

Fig. 3. Hepatic MFs have been proposed to originate following a common process of activation/trans-differentiation from hepatic stellate cells and portal fibroblasts, or by recruitment and differentiation of bone marrow-derived cells like mesenchymal stem cells (MSC) or circulating fibrocytes, as well as by a process of epithelial to mesenchymal transition involving hepatocytes or cholangiocytes. In the figure are also indicated the established or suggested role for human MFs.

"perisinusoidal/pericellular fibrosis" that is typically found in the early stages of both NASH and ASH. Moreover, HSC may significantly also contribute to the origin of interface MFs and then to the pattern of "bridging fibrosis" which predominates in the fibrotic/cirrhotic liver of HCV chronic patients (Parola et al., 2008).

Where the process of activation of HSC to HSC/MFs is concerned, this process has been investigated by following changes in morphology and phenotypical responses observed in HSC, obtained from either human or rat liver, when cultured on plastic substrate. HSC in these conditions undergo a trans-differentiation from the original "storing phenotype" to the one of activated MFs, classically including the following relevant features (Friedman, 2000, 2003, 2004; Pinzani and Marra, 2001; Pinzani and Rombouts, 2004; Bataller and Brenner, 2005):
- high proliferative attitude;
- increased synthesis of ECM components, particularly fibrillar collagens, as well as of factors involved in ECM remodelling;
- ability to migrate (chemotactic response);
- increased synthesis of growth factors (autocrine loops) and pro-inflammatory cytokines;
- contractility in response to vaso-active compounds;
- resistance to apoptosis induction (see later).

Although the scenario proposed on the basis of "in vitro" studies may represent an over-simplification, it is still considered as reasonably similar to the one that is likely to occur "in vivo". It is likely that at least portal fibroblasts as well as bone marrow – derived cells may undergo a similar process of activation in conditions of CLD.

Portal fibroblasts

These cells are located in the connective tissue of portal areas and their activation into MFs is believed to be relevant in ischemic conditions, as well as 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.

Bone marrow-derived cells

Recent studies have provided evidence indicating that under conditions of chronic liver injury, pro-fibrogenic MFs (mainly IF/MFs and some portal MFs) can originate from progressive recruitment of bone marrow derived cells (Forbes et al., 2004). At least two different populations of bone marrow have been shown to engraft chronically injured livers and differentiate into α-SMA positive MFs, including mesenchymal stem cells or MSC (Russo et al., 2006; Valfrè di Bonzo et al., 2008; Li et al., 2009) and circulating fibrocytes (Kisseleva et al., 2006). The effective contribution of MSCs has been found to vary depending on the source (human or murine), as well as the experimental protocol for cell transplant, with a recent report even suggesting that collagen deposition by bone marrow-derived cells may be negligible (Higashiyama et al., 2009), although the conclusions of the latter study are at present a matter of debate (Kallis and Forbes, 2009).

Cholangiocytes and hepatocytes

Recent reports have suggested that pro-fibrogenic cells may also originate from either cholangiocytes or hepatocytes through a process of epithelial to mesenchymal transition. Such a process has been described originally in embryologic and fetal development, and then involved in cancer cell invasiveness and progression, as well as in organ fibrogenesis. Concerning liver fibrogenesis, only two reports have suggested that α-SMA negative but FSP-1 (fibroblast specific protein type 1) positive MFs may originate from hepatocytes that underwent full EMT program (Zeisberg et al., 2007) and that fibrogenesis may be prevented by treatment with BMP-7 (bone morphogenic protein – 7), a molecule that has been reported to significantly affect EMT process (Dooley et al., 2008). More data have been published that suggest a putative contribution by EMT of cholangiocytes in human and experimental conditions of biliary fibrosis and these studies will be recalled in the following section dedicated to the pro-fibrogenic mechanisms. At present, the real contribution of EMT to the fibrogenic progression of CLDs is a matter of intense debate.

Major pro-fibrogenic mechanisms in liver fibrogenesis

A limited number of mechanisms have been proposed to sustain liver fibrogenesis, including chronic activation of the wound healing response, oxidative stress and altered modulation of epithelial-mesenchymal interactions, as well as the already cited mechanism of EMT (Pinzani and Marra, 2001; Pinzani and Rombouts, 2004; Bataller and Brenner, 2005; Elsharkawy et al., 2005; Iredale, 2007; Kallis et al., 2007; Friedman, 2008a,b; Novo and Parola, 2008; Parola et al., 2008).

Chronic activation of the wound healing reaction

This is believed to represent the most common and relevant mechanism in hepatic fibrogenesis. The hallmarks of the involvement of chronic activation of the wound healing reaction are the following (Parola et al., 2008):
- the persistence of hepatocellular and cholangiocellular damage with variable degree of necrosis and apoptosis;
- the presence of an inflammatory infiltrate composed of mononuclear cells and lymphocytes;
- the involvement of different pro-fibrogenic and ECM – producing cells that, as just mentioned, are also characterized by intense proliferative attitude, migration and contractility;

- quantitative (excess deposition) and qualitative alteration in the composition of the ECM, being associated with a limited ability to remodel and remove the typical fibrillar-like collagens and a persistent attempt at liver regeneration.

Chronic activation of wound healing reaction, as well as of hepatic myofibroblasts is, in turn, sustained by several growth factors and cytokines. Several experimental and clinical studies have dissected those intracellular signalling pathways relevant for the pro-fibrogenic effect of these soluble factors and their receptors, as a basis for future development of selective therapeutic strategies. These factors include platelet-derived growth factor (PDGF), transforming growth factor (TGF)ß, connective tissue growth factor (CTGF), endothelin-1 (ET-1), monocyte chemotactic protein (MCP)-1, and tumour necrosis factor (TNF), to name just a few (Bataller and Brenner, 2005; Friedman 2008a,b; Parola et al., 2008). More recently, the list of polypeptide mediators has been implemented by the addition of other soluble factors, including adipokines and pro-angiogenic cytokines.

Where adipokines are concerned, they have been proposed as pro-fibrogenic mediators in conditions of non alcoholic fatty liver disease (NAFLD) and non alcoholic steatohepatitis (NASH). Adipokines are cytokines exclusively or predominantly secreted by the adipocytes and the best known representative adipokine, leptin, has been found to be increased in obese patients with NASH as well as in other patients with a CLD of different aetiology.

Studies linking leptin to fibrogenesis range from experimental animal models (Ikejima et al., 2001) to "in vitro" studies showing that leptin can indeed exert a number of biological actions on HSC, including up-regulation of collagen, stimulation of cell proliferation, prevention of apoptosis and up-regulation of pro-inflammatory and pro-angiogenic cytokines (Aleffi et al., 2005; Marra and Bertolani, 2009). This scenario is likely to be counteracted by adiponectin, another adipokine which is known to be decreased in obese and/or diabetic (Type II) patients. Adiponectin has been shown to ameliorate metabolic derangements and liver damage in mouse models of NASH (Xu at al., 2003), to exert a direct antifibrogenic role and to induce HSC apoptosis (Ding at al., 2005).

Concerning angiogenesis and pro-angiogenic cytokines, formation of new vessels is known to occur in several organs and to be critical for both growth and repair of tissues in several pathophysiological conditions (Carmeliet, 2003). As will be emphasized in a following section, the current feeling is that angiogenesis, as triggered by conditions of hypoxia and by directly involving hepatic MFs, can proceed in association with and favour progressive liver fibrogenesis.

Oxidative stress

Involvement of oxidative stress, reactive oxygen species (ROS) and other reactive intermediates has been unequivocally documented in all human major clinical conditions of CLDs, as well as in most experimental models of liver fibrogenesis, with a predominant role in sustaining fibrogenesis in NASH and ASH patients. An extensive analysis of the role of oxidative stress in liver fibrogenesis is out of the scope of the present paper and the interested reader can find more details in specialized reviews (Parola and Robino, 2001; Novo and Parola, 2008). Here only major concepts will be briefly recalled.

1. Oxidative stress in CLDs results from either increased generation of ROS and other reactive intermediates, as well as by decreased efficiency of antioxidant defences, a typical feature of CLDs. It should be noted that oxidative stress in CLDs can be either responsible for potentially toxic consequences or actively contributes to excessive tissue remodelling and fibrogenesis. ROS and other reactive mediators such as the aldehydic end-product of lipid peroxidation 4-hydroxy-2,3-nonenal (HNE) can be released either by activated inflammatory cells or from hepatocytes directly or indirectly damaged by the specific aetiological agent or conditions. As a relevant example, generation of ROS within hepatocytes may represent a consequence of an altered metabolic state (like in NAFLD and NASH) or of ethanol metabolism (ASH), with ROS being then mainly generated by mitochondrial electron transport chain or through the involvement of selected cytochrome P450 isoforms like CYP2E1 (Angulo, 2002; Albano, 2006; Tilg and Hotamisligil, 2006; Zamara et al., 2006). Severe oxidative stress, presumably by favouring mitochondrial permeability transition as well as by affecting the integrity of biological membranes, cytoskeleton as well as transcription and protein synthesis, is indeed able to promote hepatocyte death (necrotic and/or apoptotic).

2. ROS, HNE and other oxidative stress – related mediators released by damaged hepatocytes or activated inflammatory cells can directly affect the behaviour of human HSC/MFs and likely of other MF-like cells (Fig. 4). As a matter of fact, ROS and HNE have been reported to up-regulate expression of critical pro-fibrogenic genes, including procollagen type I, TIMP-1 and the pro-inflammatory chemokine MCP-1, possibly through activation of a number of critical signal transduction pathways and transcription factors, including activation of JNKs, AP-1 and, only for ROS, NF-κB (Parola et al., 1998; Parola and Robino, 2001; Zamara et al., 2004; Friedman, 2004; Bataller and Brenner, 2005; Novo et al., 2006a; Novo and Parola, 2008).

ROS may also contribute to positively modulate proliferation of rat HSC/MFs but this does not apply to human MFs exposed to HNE, which rather is able to inhibit basal and PDGF-stimulated DNA synthesis or even induce cell death (Robino et al., 2000; Zamara et al., 2004; Novo et al., 2006a). Low levels of extracellularly generated superoxide anion, but not H_2O_2 or HNE, are able to stimulate migration of human

HSC/MFs through activation of Ras/Erk signalling (Novo et al., 2006a) and also of isoforms 1 and 2 of c-Jun-NH_2-kinases (JNK1/2) (Novo et al., 2009; submitted).

3. A redox sensitive role should also be recognized to intracellular and NADPH-oxidase-dependent ROS generation within human and rat HSC/MFs that has been reported to occur in response to several known pro-fibrogenic mediators, including PDGF-BB, angiotensin II, and the adipokine leptin (De Minicis et al., 2006). ROS generated within HSC/MFs can act as positive modulators or pro-fibrogenic signalling pathways and, indeed, selective inhibition of NADPH oxidase can effectively reduce phenotypic responses of HSC/MFs (reviewed by De Minicis et al., 2006). Moreover, genetically manipulated mice lacking p47phox (i.e., a crucial subunit of NADPH oxidase) have been shown to be protected from development of experimental fibrosis (Bataller et al., 2003). Intracellular and NADPH oxidase-related generation of ROS has also been reported to follow "in vivo" and "in vitro" phagocytosis of apoptotic bodies from damaged hepatocytes by HSC/MFs and to result in procollagen type I increased expression (Zhan et al., 2006).

4. Oxidative stress may contribute to CLDs progression also by affecting immune response. Experimental studies (alcohol fed rodents) and clinical data (patients affected by ALD, chronic HCV infection or NAFLD) indicate that oxidative stress is associated with the development of circulating IgG antibodies directed against epitopes derived from protein modified by lipid peroxidation products or against oxidized cardiolipin. Titre of these antibodies correlate with disease severity and, as recently proposed for NAFLD patients, may serve as a prognostic predictor of progression of NAFLD to advanced fibrosis (Albano et al., 2007 and reference therein). Along these lines, a T cell mediated response towards lipid peroxidation derived antigens has been described in patients with advanced ALD (Stewart et al., 2004). Immune response triggered by oxidative stress may then play a significant role in the progression of ALD, in the worsening of chronic hepatitis C by alcohol intake and, possibly, even in the progression of NAFLD.

Derangement of epithelial – mesenchymal interactions in cholangiopathies and/or epithelial to mesenchymal transition?

Clinical conditions known as cholangiopathies, a group of progressive disorders representing a major cause of chronic cholestasis in adult and pediatric patients, share a common scenario characterized by cholestasis, necrotic or apoptotic loss of cholangiocytes, cholangiocyte proliferation and portal/periportal inflammation and fibrosis. In these settings the so called

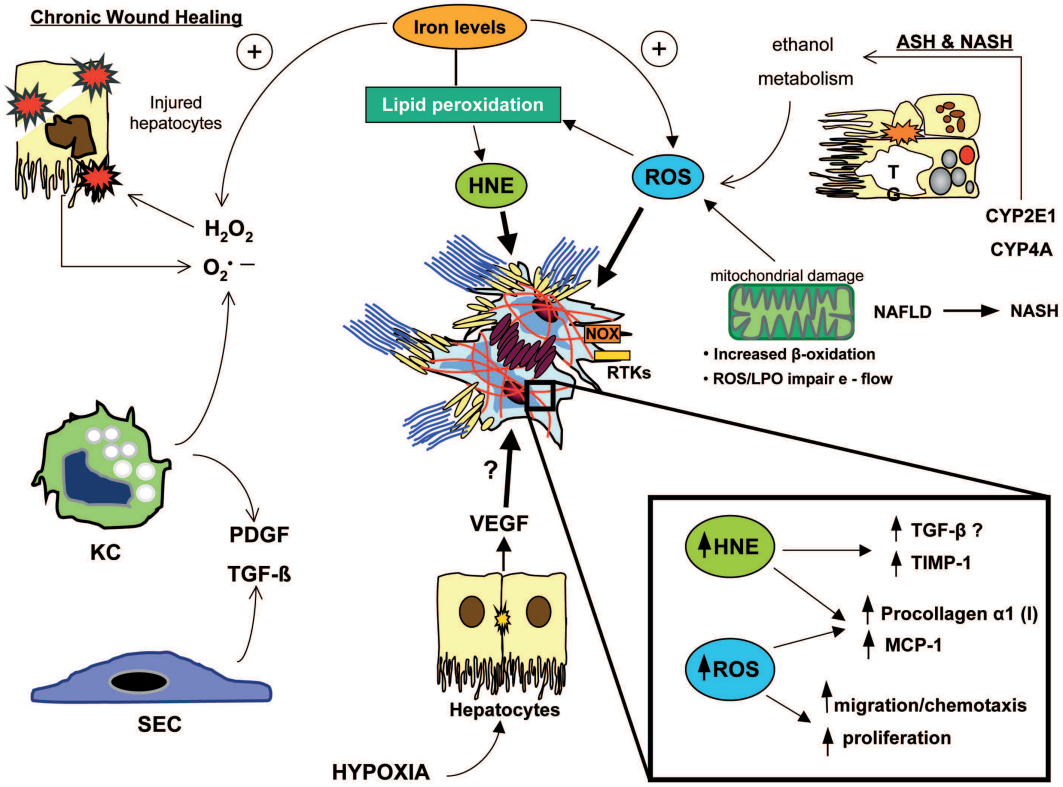

Fig. 4. ROS and related mediators (HNE, 4-hydroxy-2,3-nonenal) as generated by injured hepatocytes (including hepatocytes in ASH and NASH), activated Kupffer cells or sinusoidal endothelial cells as well as by damaged mitochondria can operate as pro-fibrogenic stimuli directly on activated, MF-like, HSC (HSC/MFs): a 'stellate centric' view. MFs react by up-regulating the expression of pro-collagen type 1, MCP-1, TIMP-1, TGFß1 as well as by migrating and/or proliferating. See text for more details.

"ductular reaction" (i.e., proliferation of bile ductular cells or cholangiocytes) has been reported to be relevant and to behave as the "pace maker of portal fibrosis" (Roskams and Desmet, 1998). Ductular reaction is characterized by intense proliferation of cholangiocytes that is associated with significant changes in the surrounding mesenchymal cells, first portal fibroblasts in the portal connective tissue and then HSC when the reaction starts to invade the surrounding parenchyma, as well as with significant changes in the ECM. It is still unclear whether the first event is represented by phenotypic changes in proliferating cholangiocytes or by changes in ECM, that in turn can lead to cholangiocyte proliferation. However, several studies of the pre-EMT era have reported evidence for an intense cross-talk between cholangiocytes and surrounding mesenchymal cells, possibly resulting in the release of cytokines and pro-inflammatory mediators that are likely to be responsible for the overall scenario described for cholangiopathies. In fact, cholangiocytes are considered active "cellular actors" in pathological conditions for their ability to secrete several chemokines (IL-6α TNFß, IL-8, MCP-1) and pro-fibrogenic polypeptide mediators (PDGF-BB, ET-1, CTGF, TGFß2). This putative pro-fibrogenic scenario is also sustained by the fact that almost the same chemokines and pro-fibrogenic polypeptides can also be produced by infiltrating immune, inflammatory or mesenchymal cells (reviewed in Pinzani and Rombouts, 2004).

The scenario just described (i.e., alteration of epithelial-mesenchymal interactions) has received a relevant contribution by studies of the last 3-4 years that have proposed a major pro-fibrogenic role for cholangiocyte which in chronic conditions of injury may undergo EMT to transform themselves into pro-fibrogenic MF-like cells.

As a necessary premise, it should be noted that EMT is a fundamental process, paradigmatic of the concept of cell plasticity, which has been originally described in embryonic development, where cell migration and tissue remodeling play a primary role in regulating morphogenesis in multi-cellular organisms. As for its original definition, EMT was focused on the formation of mesenchymal cells from epithelial cells in different areas of embryos. This process is characterized by the loss of epithelial cell polarization as a result of disappearance of specialized junctional structures, cytoskeleton reorganization and organelle redistribution and gradual acquisition of typical EMT – related mesenchymal features and behavior (Lee et al., 2006; Thiery and Sleeman, 2006; Moustakas and Heldin, 2007; Baum et al., 2008; Cannito et al., 2009). The EMT process in embryo development has a natural counterpart, since embryonic mesenchymal cells can eventually undergo a reverse transition process, known as mesenchymal to epithelial transition or MET (Chaffer et al., 2007), leading them to regain a fully differentiated epithelial phenotype.

In the last decade the EMT process (and then possibly MET) has been identified in at least two other well defined pathophysiological conditions, including organ fibrosis and cancer progression and metastasis, leading to the recent suggestion that EMTs may even be classified into three corresponding different subtypes (Acloque et al., 2009; Kalluri and Weinberg, 2009; Zeisberg and Neilson, 2009), defined as: Type 1 EMT, involved in embryonic development; Type 2 EMT, associated to tissue damage, regeneration and organ fibrosis; Type 3 EMT, involved in cancer progression and metastasis.

However, the EMT programs and related major morphological and functional events that have been detected in either physiological or patho-physiological conditions, are stimulated and regulated by a common set, although very complex, of inducing stimuli, signal transduction pathways, transcription factors and post transcriptional mechanisms.

Coming back to cholangiopaties, an involvement of EMT has been first proposed for the animal model of biliary fibrosis induced by bile duct ligation in rats (Xia et al., 2006), in which cholangiocytes were found to co-express α-SMA and cytokeratin 19 (CK-19, a BDEC and HPCs marker), an EMT scenario fully reproduced by treating *in vitro* primary cultures of cholangiocytes with TGFß1 and prevented, both *in vivo* and *in vitro*, by pre-treatment with HGF. The same scenario in BDL was confirmed by other studies (Omenetti et al., 2008a, 2008b) where a clear cause-effect relationship between EMT of cholangiocytes, appearance of portal MFs and biliary fibrosis, as well as the closely related major involvement of Hedgehog signalling pathway was first described.

Most importantly, an identical scenario was also described in specimens from human patients affected by primary biliary cirrhosis (Jung et al., 2007; Robertson et al., 2007; Omenetti, 2008b), primary sclerosing cholangitis (Kirby et al., 2008) and biliary atresia (Diaz et al., 2008), again with a major pathogenic role for Hedgehog and TGFß1-Smad2/3 signaling.

At present, two reports from different laboratories (Jung et al., 2008; Rygiel et al., 2008) have also provided the first evidence for EMT in cholangiocytes and/or hepatic progenitor cells (HPCs) in biopsies from patients affected by alcoholic liver disease, again with Hedgehog and TGFß1-Smad2/3 signalling having a major role. These latter two studies may suggest that, although the predominant role of HSC/MFs still remains as a dogma (particularly in the early phases and during progressive fibrogenesis), in a rather advanced phase of ALD this process may potentially contribute to increased ECM deposition in alcoholic patients.

The emerging major role of angiogenesis in CLDs

Angiogenesis is currently defined as a dynamic, hypoxia - stimulated and growth factor - dependent process, eventually leading to the formation of new vessels from pre-existing blood vessels. In the last

decade experimental and clinical studies have described the occurrence of hepatic angiogenesis in a number of different patho-physiological conditions, including those involving inflammatory, fibrotic and ischemic features. In particular, literature evidence is now suggesting that hepatic angiogenesis is strictly associated with, and may even favour fibrogenic progression of chronic inflammatory liver diseases of different aetiology. Indeed, in the context of chronic activation of the wound healing response, typical of fibrogenic conditions, an excess of aberrant neo-angiogenesis can occur. Mainly for anatomical reasons, including the existence of two different types of micro-vascular structures in the liver (i.e. large vessels lined by a continuous endothelium vs. sinusoids lined by a fenestrated endothelium), the formation of new vessels assumes very distinct features in chronic liver diseases (CLDs) and, ultimately, represent a key factor in determining disease progression to cirrhosis.

This hypothesis relies first on the established concept that angiogenesis is a major process involved in wound healing, being integrated with the inflammatory process (Carmeliet, 2003). Moreover, human cirrhotic livers, irrespective of the aetiology, are characterized by enhanced vascular remodelling. Where angiogenesis in CLDs is concerned, one also has to remember that formation of fibrotic septa, as well as capillarization of sinusoids (due to deposition of fibrillar ECM in the space of Disse) can result in an increased resistance to blood flow and oxygen delivery. These are the premises for the development of hypoxia, which is the main and most obvious stimulus for angiogenesis in any organ or tissue, with transcription of hypoxia - sensitive pro-angiogenic genes being modulated usually through hypoxia inducible factors (HIFs).

In addition, one should emphasize the well known relationships in CLDs between the inflammatory process and angiogenesis, with inflammatory response gaining the role of a dynamic state relevant fibrogenesis progression (Friedman, 2004, 2008b). Indeed, several mediators of the inflammatory response are known to stimulate other surrounding cells to express VEGF and other pro-angiogenic factors (Carmeliet, 2004). Moreover, other cytokines and mediators, known to be over-expressed during the condition of chronic inflammatory liver injury have been suggested to sustain angiogenesis, including HGF, NO and PDGF (Medina et al., 2004). Moreover, one should consider that: a) neo-vessels are likely to significantly contribute to perpetuation of the inflammatory response by expressing chemokines and adhesion molecules promoting recruitment of inflammatory cells; b) angiogenesis, early in the course of a CLD, may even contribute to the transition from acute to chronic inflammation (Jackson et al., 1997).

In this section, a brief analysis of major findings and concepts supporting the emerging pathogenic role of angiogenesis in CLDs is offered, with a specific emphasis on the crucial role of hypoxic conditions and hepatic stellate cells (HSCs), particularly when activated to the myofibroblast-like pro-fibrogenic phenotype. The interested reader can refer to recent more comprehensive reviews for more details (Medina et al., 2004; Fernandez et al., 2009; Valfrè di Bonzo et al., 2009). The following major concepts and findings should be outlined.

1. Angiogenesis and up-regulation of VEGF expression has been documented in different models of acute and chronic liver injury, including fibrosis induced by chronic CCl_4 treatment, bile duct ligation, administration of diethylnitrosamine and methionine and choline deficient diet, which is able to induce NAFLD and NASH (Ankoma Sey et al., 1998; Rosmorduc et al., 1999; Corpechot et al. 2002; Yoshiji et al., 2003; Medina et al., 2004; Kitade et al., 2006; Novo et al., 2007; Tugues et al., 2007; Taura et al., 2008; Moon et al., 2009). The same scenario was also documented in specimens from human fibrotic/cirrhotic liver and hepatocellular carcinoma (Medina et al., 2003-2005, Novo et al., 2007; Fernandez et al., 2009). Human conditions of CLDs include chronic infection by HBV and HCV, as well as autoimmune diseases such as primary biliary cirrhosis (PBC) and primary sclerosing cholangitis (PSC).

2. In both experimental and clinical conditions angiogenesis and fibrogenesis develop in parallel, as unequivocally indicated by morphological images showing a very high number of endothelial cells and microvascular structures in portal tracts and, more generally, within established fibrotic septa. Moreover, the strict relationships between hypoxia, angiogenesis and VEGF expression, as well as fibrogenesis in liver specimens from fibrotic/cirrhotic rodent and human livers, have been documented by several studies (Rosmorduc et al., 1999; Corpechot et al., 2002; Tugues et al., 2007) and unequivocally confirmed by a recent report in which liver conditional HIF-1α - deficient mice were subjected to BDL (Moon et al., 2009): in these mice a very significant decrease in collagen type I and α-SMA transcripts and related protein levels, as well as of transcripts for PDGF and plasminogen activator inhibitor-1 (PAI-1) was detected as compared to wild type mice in which the typical scenario of biliary type fibrosis and cirrhosis was associated to early and sustained up-regulation of HIF-1α.

3. VEGF expression, as detected in either experimental and clinical conditions, is mostly limited to hepatocytes and to HSC/MFs and, possibly, other hepatic myofibroblasts (Medina et al., 2004; Fernandez et al., 2009; Valfrè di Bonzo et al., 2009). In normal liver, VEGF expression is mostly limited to a few hepatocytes, showing nuclear positivity for HIF1α, constituting the first row around the centrilobular vein (Bozova and Elpek, 2007; Tugues et al., 2007).

4. Other polypeptides have been involved in hepatic angiogenesis associated with the fibrogenic progression process in CLDs, including, in particular, leptin. This adipokine, which has been suggested to exert a pro-fibrogenic effect by promoting the development from

non alcoholic fatty liver disease (NAFLD) to non alcoholic steatohepatitis (NASH), can up-regulate the expression of VEGF and Ang-1 expression, as well as of the pro-inflammatory chemokine monocyte chemoattractant protein 1 or MCP-1 (Aleffi et al., 2005). The pro-angiogenic role of leptin has been confirmed by a study performed on Zucker rats, animals that naturally develop leptin receptor mutations (Kitade et al., 2006), receiving the steatogenic choline-deficient and aminoacid defined (CDAA) diet. To the list of pro-angiogenic mediators that may have a role in vascular remodelling in cirrhosis, one should now include also Hedgehog (Hh) ligands that, released from HSC/MFs, cholangiocytes and possibly hepatic progenitor cells, may promote changes in sinusoidal endothelial cell gene expression resulting in capillarization of sinusoids and in the release of vasoactive factors such as nitric oxide (Witek et al., 2009).

5. Hepatic MFs, which are involved in fibrogenesis, have also been reported to play a significant pro-angiogenic role. This is particularly true for hepatic stellate cells even in physiological angiogenesis because of their role as liver specific pericytes, connected to their strategic location in the space of Disse and intimate contact with sinusoidal ECs (Friedman 2008a). As for recent literature data, HSC/MFs should be considered as a hypoxia-sensitive, cyto- and chemokine-modulated cellular crossroad between necro-inflammation, pathological angiogenesis and fibrogenesis on the basis of the following studies that outlined the following major concepts:

- HSC and HSC/MFs react to conditions of hypoxia and to leptin by up-regulating transcription and synthesis of VEGF, Angiopoietin 1, as well as of their related receptors VEGFR-2 and Tie2 (Ankoma-Sey et al., 2000; Wang et al., 2004; Aleffi et al., 2005; Novo et al., 2007)

- HSC/MFs respond to the action of VEGF and Angiopoietin 1 in terms of proliferation (Ankoma Sey et al., 1998; Yoshiji et al., 2003; Olaso et al., 2003), increased deposition of ECM components (Corpechot et al., 2002; Olaso et al., 2003; Yoshiji et al., 2003), and increased migration and chemotaxis (Novo et al., 2007).

This is a scenario that is likely to be relevant for the *in vivo* progression of a CLD. In either human and rat fibrotic/cirrhotic livers (Novo et al., 2007) α-SMA-positive MFs able to express concomitantly VEGF, Ang-1 or the related receptors VEGFR-2 and Tie-2, are found at the leading edge of tiny and incomplete developing septa, but not in larger bridging septa. This distribution may reflect the existence of an early phase of CLD, occurring in developing septa, in which fibrogenesis and angiogenesis may be driven/modulated by HSC/MFs, and of a later phase occurring in larger and more mature fibrotic septa where the chronic wound healing is less active and fibrogenic transformation more established. In the late setting pro-angiogenic factors are expressed only by endothelial cells, a scenario that is likely to favour the stabilization of the newly formed vessels.

6. Angiogenesis may represent a putative therapeutic target in the treatment of CLDs. The bulk of available experimental data indeed indicate that antiangiogenic therapy is effective in preventing progressive fibrogenesis, as shown by administering old anti-angiogenic drugs (Wang et al., 2000), antibodies able to neutralize either VEGFR-1 (Flt-1) and/or VEGFR-2 (Flk-1) (Ankoma Sey et al., 1998; Morales-Ruiz and Jimenez 2005; Morales-Ruiz et al., 2005), adenovirus expressing soluble Tie-2 (AdsTie-2, where Tie-2 is the receptor for Ang-1) (Taura et al., 2008), the multi-targeted tyrosine kinase receptor inhibitors Sunitinib (Tugues et al., 2007) or Sorafenib (Mejias et al., 2009). Moreover, most of these treatments were also able to significantly inhibit the development of portal hypertension, porto-systemic collateral vessels and hyperdynamic splanchnic circulation (Morales-Ruiz and Jimenez 2005; Morales-Ruiz et al., 2005 ; Tugues et al., 2007; Mejias et al., 2009). All these data suggest that anti-angiogenic drugs may then represent an attractive alternative therapeutic tool to prevent or significantly slow down fibrosis progression towards cirrhosis, bearing also in mind that this may also affect the development of portal hypertension, including its complications, and of liver cancer. This putative new therapeutic strategy is at present debated and requires properly designed clinical trials. A note of caution is based on a very recent negative report in which the use of the anti-angiogenic drug Celingitide (a specific inhibitor of integrin αvß3) in two different animal models of fibrosis surprisingly resulted in an overall worsening of liver fibrosis (Patsenker et al., 2009).

Reversal of liver fibrosis

As the reader should have realized from previous sections, liver fibrogenesis can be envisaged as a very dynamic process of wound healing, and possibly much more, which is in the end responsible for excess deposition of ECM (i.e., liver fibrosis) and all the other related morphological and functional changes found in a chronically injured liver. For many years liver fibrosis and cirrhosis have been considered as irreversible, with ortothopic liver transplantion being the only therapeutic resolutive option. Although the concept of fibrosis and cirrhosis reversal was first introduced by Popper and Underfriend almost forty years ago (Popper and Undenfriend, 1970) and later on by Perez-Tamayo (Perez-Tamayo, 1979), the "irreversibility" postulate has been really challenged only recently by data obtained first in animal models of fibrosis and then in human studies. Most authors involved have identified the issues of MF apoptosis and increased degradation of excess ECM as the crucial ones, providing that the aetiological agent or condition may be eliminated or efficiently counteracted by means of selective therapy (Iredale, 2001; Elsharkawy et al., 2005; Henderson and Iredale, 2007; Muddu et al., 2007). This has opened the way to the search for more selective therapeutic strategies designed to target more specifically hepatic MFs or

specific ligand-receptor interactions or pro-fibrogenic signalling pathways, in order to switch on or at least potentiate fibrosis reversal. In the next sections, major findings and concepts of this relatively new scenario will be summarized, with an attempt to focus attention on those critical issues that have emerged in the last few years.

Experimental evidence of liver fibrosis reversion

The first unequivocal evidence for reversal of liver fibrosis was provided in 1998 by a study from Iredale and co-workers (Iredale et al., 1998) performed in the rat by administering CCl_4 for 4 weeks, a procedure resulting in significant liver fibrosis. In this study it was described for the first time that cessation of administration of the hepatotoxic and pro-oxidant compound was followed by a reversion to virtually normal histology. A few years later homologous evidence was provided by the same group using the BDL rat model of secondary biliary fibrosis (Issa et al., 2001). In this study biliodigestive anastomosis was undertaken after 3 weeks of BDL in order to relieve the condition and once again, this was followed by an almost complete histological reversion at the end of a 42 day period of recovery. This was also the first study to report that fibrosis reversal was associated with a fivefold decrease in activated HSC (more likely of MFs, as we will say today), as determined by α-SMA staining. TUNEL staining indicated that loss of activated HSC (HSC/MFs) resulted from an increase in the rate of apoptosis during the first two days post biliodigestive anastomosis. This study (see also later) was accompanied by evidence for induction of apoptosis in cultured rat HSC/MFs by TGFß1 and the protein synthesis inhibitor cycloheximide, but prevented by IGF-1, then suggesting a more general concept: HSC apoptosis plays a critical role in the recovery from biliary fibrosis and survival and, apoptosis of HSC are likely to be regulated by growth factors expressed during fibrotic liver injury.

Issa and coworkers went further to show that reversion was obtained in CCl_4 chronic model, even when an advanced stage of micronodular cirrhosis was obtained after 12 weeks of administration of the hepatotoxin. After one year from cessation of CCl4 administration micronodular cirrhosis underwent significant remodeling to a macronodular cirrhosis; moreover, expression of collagen type 1 and TIMP1 messenger RNA (mRNA) decreased significantly and active MMPs were shown in livers during remodeling of fibrosis (Issa et al, 2004). This was paralleled by significant loss of more recently formed fibrils and a decrease in perisinusoidal fibrosis and a thinning of fibrotic septa. Once again, resolution was characterized by apoptosis of HSC/MFs, predominantly at the borders of fibrotic septa. Residual septa, not remodeled after one year, were characterized by trans-glutaminase-mediated cross-linking and relative hypo-cellularity. This experimental study was the first to show that only a certain degree of reversion, but not complete reversal, from an advanced stage of cirrhosis, is likely to be possible.

These seminal studies were relevant to start to envisage a scenario in which the rise and fall of activated HSC during liver injury (Fig. 5) is then modulated by a balance of several anti-apoptotic and pro-apoptotic

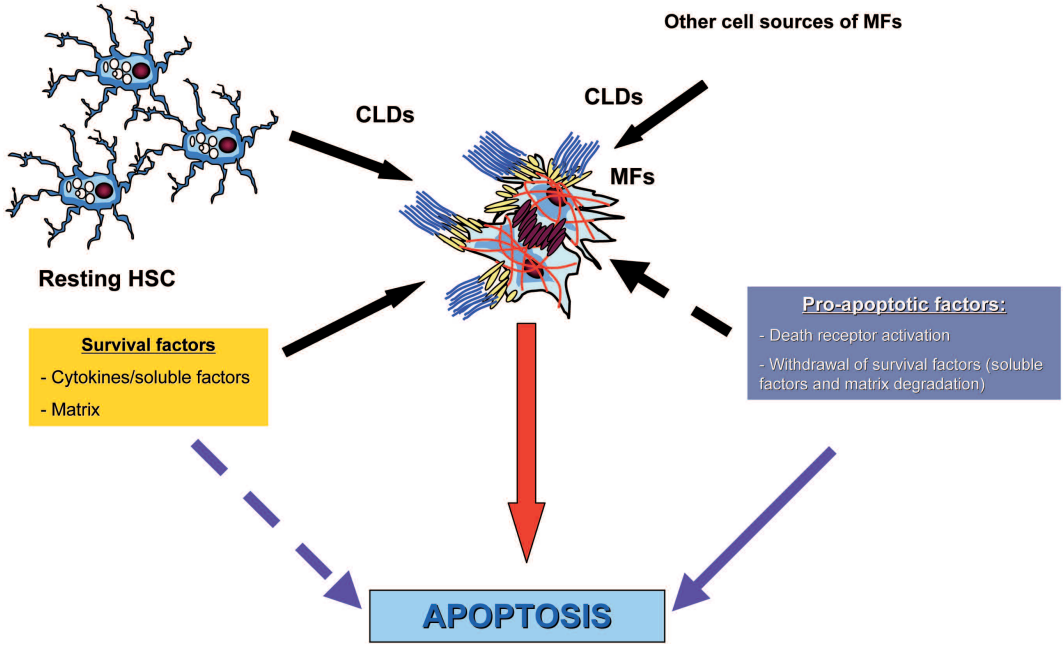

Fig. 5. The rise and fall of activated, MF-like, hepatic stellate cells as a balance between anti-apoptotic (i.e., pro-survival) and pro-apoptotic factors.

factors and signals coming from either the extracellular or intracellular environment (Elsharkawy et al. 2005). Moreover, apoptosis of HSC/MFs is of course followed by a decrease in collagen production and synthesis of tissue inhibitor of metalloproteases (TIMPs), two major features that favor accumulation of ECM in CLDs, as well as by an increase in activity of matrix metalloproteinases able to degrade fibrillar collagen.

These studies, showing that apoptosis of MFs is followed by reversion of experimental fibrosis, stimulated the search for drugs and therapeutic strategies designed to selectively stimulate apoptosis of pro-fibrogenic cells.

Clinical evidence of liver fibrosis reversion

A number of clinical studies have reported evidence suggesting that reversion or regression of fibrosis and cirrhosis may also occur in human patients affected by chronic viral infection (Shiratori et al., 2000; Kweon et al., 2001; Poynard et al., 2002; Dienstag et al., 2003; Farci et al., 2004; Serpaggi et al., 2006; Hui et al., 2007); autoimmune hepatitis (Dufour et al., 1997), alcoholic (Wakim-Fleming and Mullen, 2005) and non-alcoholic (Dixon et al., 2004; Kral et al., 2004) steatohepatitis following effective therapy and/or removal of aetiology. Along these lines, the most extensively investigated condition is represented by chronic HCV infection in patients receiving a standard therapy based on administration of IFN-α alone or in association with ribavirin (Shiratori et al., 2000; Poynard et al., 2002; Farci et al., 2004). Positive results in terms of fibrosis regression have been described for those responder patients in which viral clearance was obtained.

However, as pointed out by different authors (Desmet and Roskams, 2004; Henderson and Iredale, 2007; Ismail and Pinzani, 2009), no convincing or incontrovertible evidence has been reported for complete reversal of advanced cirrhosis. Indeed, when a careful analysis of these studies is performed, one can only prudently state that they describe a variable degree of fibrosis reversion in human patients. On the other hand, as shown also in animal models (Issa et al., 2004), in true cirrhosis a complete reversion of deranged vascular architecture is likely to be impossible.

As discussed by Ismail and Pinzani (2009), this may also be related to a substantial lack of a common language and of a worldwide consensus on definitions and terms, that is to be able to unequivocally distinguish conditions of pre-cirrhosis from true cirrhosis, the former being able to show some degree of reversal, sometimes even significant, the latter being unable to do so. Indeed, cirrhosis can appear in a very broad spectrum of variants, sometimes defined as early, fully developed, active and inactive, with a note of confusion added to the scenario by the too many histopathological scoring systems available. Moreover, as recently suggested, we definitively need a system (and the related technical tools) able to classify cirrhosis in different stages, including a clearcut distinction between compensated and de-compensated cirrhosis. The reader can refer for a more detailed analysis and discussion of this crucial point to the excellent recent review by Ismail and Pinzani (Ismail and Pinzani, 2009).

The issue of induction of MF apoptosis: relevant concepts and emerging therapeutic strategies

As mentioned in the previous sections, HSC/MFs and, likely, also MFs of different origin, can undergo apoptosis when the injurious stimulus responsible for chronic liver damage is withdrawn and remodeling of ECM is required. Indeed, HSC apoptosis is known to occur as a "normal" event after acute liver injury at the end of the healing reaction and then as a rather physiological and necessary "switch off" mechanism.

However, as pointed out by different laboratories (Elsharkawy et al., 2005; Novo et al., 2006b; Henderson and Iredale, 2007) progressive fibrogenesis in chronic liver injury, irrespective of aetiology, is characterized by an apparent failure or forestall of MFs apoptosis. This condition is believed to represent the consequence of either an altered balance between factors favouring survival and those favoring apoptosis (see Fig. 5) (Elsharkawy et al., 2005; Henderson and Iredale, 2007).

According to this view, it has been shown, for example, that apoptosis of HSC/MFs is significantly and directly inhibited by TIMP1 (Murphy et al., 2002), which is typically over-expressed in either experimental and clinical conditions of CLDs (Henderson and Iredale, 2007). Moreover, synthetic MMP inhibitors were also found in the same study to inhibit HSC apoptosis and this was confirmed by analyses showing that persistent expression of TIMP-1 mRNA during the course of experimental cirrhosis was correlated with persistence of activated HSC quantified by α-SMA staining, while in fibrosis, loss of activated HSC correlated with a reduction in TIMP-1 mRNA. The conclusion reached was that TIMP-1 was able to inhibit apoptosis of activated HSC via MMP inhibition.

Another obvious element in this puzzle is represented by the fact that several growth factors which are over-produced in the chronically injured liver can of course stimulate survival signals and pathways, with a central role identified for nuclear factor κB (NF-κB) activation deserving to be mentioned. Indeed, several studies have outlined that NF-κB activation, a well known event in activated HSC/MFs that may be related to the action of several growth factors and/or ROS (Bataller and Brenner, 2005; Elsharkawy et al., 2005; Friedman 2008a,b), is crucial for the survival of either rodent or human MFs (Oakley et al., 2005, 2009; Watson et al., 2008). This is the case, for example, for the recently identified "survival" role of angiotensin II which acts by activating IÎB kinase, leading to

phosphorylation of RelA at Ser536 (Oakley et al., 2009).

Where the equilibrium between survival and pro-apoptotic signals is concerned, another recent report has finally established a critical dual role of mature nerve growth factor (NGF) and of its pro-peptide (proNGF), acting as pro-apoptotic and survival signals, respectively. In the latter study (Kendall et al., 2009) authors were able to show that during resolution of fibrosis the cleavage of proNGF by MMP-7 altered the proNGF/mature NGF balance facilitating apoptosis of MFs (that co-express both p75 neurotrophin receptor and sortilin, the receptors for the two peptides).

The overall scenario has been implemented by a study in which activation of human HSC has also been reported to result in an anti-apoptotic gene reprogramming, leading human HSC/MFs to over-express Bcl-2 and to survive to pro-apoptotic stimuli found to be effective on rat HSC/MFs, including FasL, TNF and excess oxidative stress (Novo et al., 2006b). Immunohistochemical data provided in the same study clearly indicate that myofibroblast-like cells at the interface between septa and parenchyma significantly overexpress Bcl-2, confirming the possible *in vivo* relevance of *in vitro* data. Interestingly, this "survival" attitude related to Bcl2 overexpression, a finding also confirmed in another study (Watson et al., 2008), was lost during senescence of human HSC/MFs that became susceptibile to apoptosis induction. Indeed, a recent report has confirmed and elegantly further addressed the relevance of senescence for the balance between HSC/MFs survival and apoptosis (Krizhanovsky et al., 2008), These authors first detected the accumulation of senescent HSC-derived cells in murine fibrotic livers and then, by elegant experiments in which mice lacking key senescence regulators were used, were able to show that HSC/MFs continued to proliferate, leading to excessive fibrosis, then suggesting that senescence may represent a "natural" way to eliminate MFs. Indeed, senescent HSC were characterized by a gene expression profile consistent not only with cell cycle exit, but also with decreased synthesis of ECM components, increased release of ECM remodeling enzymes and enhanced immune surveillance. Of relevance, senescent HSC were found to release cytokines or receptors that are known to potentiate natural killer (NK) cell function, and indeed NK cells were found in close proximity to senescent cells in murine fibrotic livers and senescent cells were selectively targeted by NK cells, either in culture or *in vivo*, a scenario that becomes predominant under conditions compatible with fibrosis resolution (i.e., after cessation of administration of CCl_4) and is likely to contribute to eliminate activated HSC also under conditions of recovery from an acute liver injury.

The overall scenario just described, as has progressively emerged in the last decade, has stimulated the search for therapeutic strategies designed to selectively target HSC/MFs and, probably, to induce their apoptosis. Several drugs and agents have been tested and shown to be able to limit (to a variable extent) fibrosis in animal models, and the interested reader can refer to an excellent dedicated review by the group of Derek Mann (Muddu et al., 2007 and references therein). Within this list of drugs, several have been specifically found to induce MFs apoptosis, including the NF-κB inhibitor sulfasalazine, the mycotoxin gliotoxin, the proteasome inhibitor bortezomib, the agent curcumurin, as well as NGF or a soluble TGFß receptor. Other therapeutic options have been tested by attempting to enhance MMP activity, for example by employing antibodies against TIMPs, or by inhibiting HSC activation by treatment with hepatocyte growth factor (HGF).

Of relevance, a recent report (Oakley et al., 2009), in describing the pro-survival action of AT-II, has also provided convincing evidence for the potential antifibrotic efficacy of administration of captopril, an inhibitor of angiotensin-converting enzyme, as well as the necessary base of knowledge to understand why drugs able to affect the renin - angiotensin system have been shown to inhibit fibrosis in animal models (Bataller et al., 2007). Most important, this also explains the reported positive effects of candesartan, a drug designed to specifically block angiotensin receptor 1 (AT1), on both hepatic venous pressure and decreased serum levels of markers of fibrosis in compensated cirrhotic patients (Debernardi-Venon et al., 2007). The latter study is relevant, as recently discussed (Pinzani, 2009), because positive effects of candesartan have been obtained at a dosage not affecting mean arterial pressure in these patients.

Conclusions

Liver fibrogenesis is a dynamic and highly integrated molecular, tissue and cellular process that leads to the progressive accumulation of extracellular matrix (ECM) components in an attempt to limit hepatic damage in a CLD irrespective of the aetiology. Progressive fibrogenesis, sustained by a heterogeneous population of MFs that may originate from different cells sources, has a tremendous clinical impact because it can lead to the end-point of cirrhosis and related complications, including the development of hepatocellular carcinoma. Basic and clinical studies, by continuously unravelling molecular mechanisms involved in pro-fibrogenic phenotypic responses of MFs, including those involved in the critical survival/apoptosis equilibrium, are currently offering new "targets" for future anti-fibrotic strategies to test with properly designed clinical trials that may represent effective alternative options for a changing scenario in which fibrosis (but not advanced cirrhosis) can be reverted and liver transplantation (i.e., at present the only effective therapy for end-stage disease) possibly at least delayed.

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