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Invited Review

Liver fibrosis, the hepatic stellate cell and tissue inhibitors of metalloproteinases

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Summary. Liver fibrosis occurs as a consequence of net accumulation of matrix proteins (especially collagen types I and III) in response to liver injury. The pathogenesis of liver fibrosis is underpinned by the activation of hepatic stellate cells (HSC) to a myofibroblast like phenotype with a consequent increase in their synthesis of matrix proteins such as interstitial collagens that characterise fibrosis. In addition to this there is increasing evidence that liver fibrosis is a dynamic pathologic process in which altered matrix degradation may also play a major role. Extracellular degradation of matrix proteins is regulated by matrix metalloproteinases (MMPS) – produced by HSC - which in turn are regulated by several mechanisms which include regulation at the level of the gene (transcription and proenzyme synthesis), cleavage of the proenzyme to an active form and specific inhibition of activated forms by tissue inhibitors of metalloproteinases (TIMPS). Insights gained into the molecular regulation of HSC activation will lead to therapeutic approaches in treatment of hepatic fibrosis in the future, and could lead to reduced morbidity and mortality in patients with chronic liver injury

Key words: Hepatic Stellate Cell (HSC), Matrix Metalloproteinase (MMP), Tissue Inhibitor of Metalloproteinase types 1 to 4 (TIMPs 1, 2, 3 & 4), Liver fibrosis, Collagenase

Introduction: Liver Fibrosis

Fibrosis is the liver's wound healing response to a variety of chronic insults including autoimmune damage (primary biliary cirrhosis, chronic active hepatitis), infection (particularly hepatitis B and C), parasitic infestation (schistosomiasis) and toxic damage (principally alcohol) (Friedman, 1997). The high prevalence of these illnesses make liver fibrosis a worthy cause of study: $250x10^6$ people worldwide are infected with Hepatitis B infection alone. To date, progressive fibrosis and cirrhosis have been viewed as a static irreversible response to chronic injury. Evidence is accumulating that liver fibrosis can now be considered as a dynamic and potentially reversible process in which changes in matrix degradation occur in addition to matrix synthesis: moreover it mirrors parenchymal wound healing in other tissues.

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Pathogenesis

Hepatic fibrosis is characterised by an accumulation of extracellular matrix (ECM) in response to chronic liver injury and is usually distinguished from cirrhosis (advanced fibrosis) which is considered irreversible and in which thick bands of matrix fully encircle the parenchyma forming abnormal nodules. By definition cirrhosis results in the disruption of the normal liver architecture. However this definition is potentially misleading; fibrosis and cirrhosis should be considered part of a disease process continuum in which cirrhosis represents the most advanced stage. Similarities of the wound healing response in liver, kidney, lung and arteries have increased our knowledge of how tissues respond to ongoing injury. Central to the processes in all of these tissues is the recruitment of inflammatory cells, and the activation of mesenchymal myofibroblast like cells (Stellate cells in liver) onto which cell signals converge. The activated myofibroblast cells proliferate and secrete interstitial or fibrillar collagens (collagen I and III). Ultimately the activated myofibroblast-like stellate cells express cytokines in an autocrine manner. Central too, is the release of matrix degrading proteases, matrix metalloproteinases (MMPs) and their regulation by the specific inhibitors - the so called tissue inhibitors of metalloproteinases (TIMPs) and other protease inhibitors. Experimental liver injury and human cirrhosis are characterised by an increased content of extracellular matrix (ECM) constituents predominantly the interstitial or fibrillar collagen: collagen types 1 and III (Rojkind et al., 1979; Siebold et al., 1988). Indeed quantitative as

well as qualitative changes in other matrix components occur including sulphated proteoglycans (Arenson et al., 1988), and matrix glycoproteins including laminin (Maher et al., 1988), cellular fibronectin (Martinez-Hernandez, 1985) and tenascin (Ramadori et al., 1991). The changes in matrix composition are similar whatever the liver insult: this underscores the importance of understanding the central regulatory elements of the fibrotic process which in turn may identify novel therapeutic strategies that may intervene or reverse the fibrosis response.

The hepatic stellate cell

Central to the changes observed in matrix production (fibrogenesis) and degradation (fibrolysis) is the Hepatic Stellate Cell (HSC) which has now been identified as the major source of matrix in chronic liver injury (Friedman, 1997; Kawada, 1997). The HSC is a mesenchymal cell, which lies in the Space of Disse between the specialised hepatic sinusoidal epithelium and the palisades of hepatocytes (Wake, 1980). In normal liver HSC are distinguished by prominent intracellular lipid droplets that contain vitamin A stored as retinyl esters (Hendriks et al., 1988). In health HSC are the principal storage site for retinoids and the endogenous ultraviolet fluorescence of vitamin A which in these cells provides a convenient cellular marker for these cells and imparts a buoyancy which assists in their purification (Friedman et al., 1985).

HSCs lie in close proximity to a matrix consisting primarily of type IV collagen, laminin and heparan sulphate proteoglycans (Burt et al., 1990). In chronic injury the HSCs become activated - undergoing a phenotypic change to a myofibroblast type cell that expresses a smooth muscle actin (Rockey et al., 1992), proliferate and have been shown to secrete matrix proteins: specifically collagen I and III (Milani et al., 1989, 1990a,b). The primary location of fibrotic change exists according to the site of injury (eg perivenular in CCl4 intoxication and periductular following bile duct ligation in rats) but with progressive injury will spread to become panlobular. HSC can be demonstrated to undergo a transitional cell stage before becoming more fully activated in the early stages of fibrotic models (McGee and Patrick, 1972; Minato et al., 1983; Senoo et al., 1984; Maher et al., 1988; Takahara et al., 1988; Maher and McGuire, 1990). Studies of HSC biology have been enhanced by the observation that when primary HSC cultures are plated onto uncoated plastic they demonstrate the phenotypic changes which mirror activation. This observation has greatly facilitated the study of HSC activation and the culture model of activation is widely used and accepted in studies of hepatic fibrogenesis.

Table 1. Products and components of hepatic stellate cells.

1	*Vitamin A related compounds*	
	Retinoids	
	Nuclear retinoid receptors	
2	*Cytoskeletal markers*	
	desmin, alpha-smooth muscle actin	
3	*Extracellular Matrix*	
	Collagens	Types I, III, IV, V, VI, XIV
	Proteoglycans	eg Heparan sulphate
	Glycoproteins	eg laminin, tenascin
4	*Proteases and Inhibitors*	
	Matrix proteases:	eg Interstitial Collagenase/MMP-1, Gelatinase A/MMP-2 Stomelysin/MMP 3 Membrane-type metalloproteinase 1(MT-1 or MT-MMP)/MMP 14
	Protease Inhibitors:	eg TIMPs 1-4 and PA-1
5	*Cytokines, growth factors and inflammatory mediators*	
	Prostaglandins	
	Acute Phase components	
	Mitogens	
	Vasoactive Mediators	eg Endothelin 1 (ET-1), nitric oxide (NO)
	Fibrogenic compounds	eg TGF-beta 1-3
6	*Receptors*	
	Cytokine receptors	
7	*Signaling molecules and transcription factors*	
	Signaling components	eg MAP kinase
	Transcription factors	eg SP-1, NFKB, c-myb

Evidence for HSC as the main source of matrix in liver injury

In injury, HSC express collagen types I, III, IV (Takahara et al., 1988; Nakatsukasa et al., 1990) and laminin (Milani et al., 1990b) but little or no messenger RNA is localised to parenchymal cells (see Table 1). HSCs are therefore now considered to be the major source of fibrotic matrix in liver injury (Maher and McGuire 1990; Pietrangelo et al., 1992).

Activation consists of early (initiation) and late (perpetuation) phases (Friedman, 1997). Early activation may be provoked by rapid deposition of cellular fibronectin and release of soluble stimuli by Kupffer cells (hepatic macrophages) (Friedman and Arthur, 1989). The late phases of activation are characterised by proliferation (Wong et al., 1994), fibrogenesis, contractility, release of cytokines (Maher et al., 1988) and certain MMPs. The most effective stimulus for proliferation described to date is platelet derived growth factor (PDGF) - a cytokine which also plays a role in smooth muscle cell proliferation during atherosclerosis. The expression of PDGF receptors is a feature of HSC activation (Wong et al., 1994). Other factors that may be important include epidermal growth factor (Marra et al., 1996), fibroblast growth factor (Pinzani et al., 1989), endothelin (Housset et al., 1994), insulin-like growth factor (Pinzani et al., 1990), thrombin (Marra et al., 1995) and transforming growth factor alpha (TGFα).

In fibrogenesis, alongside the increased ECM accumulation through HSC proliferation, matrix production per cell is also increased. The most effective stimulus to collagen-1 synthesis yet described is transforming growth factor beta 1 (TGF $ß_1$). TGF $ß_1$ can

be derived from HSC (autocrine) (Bachem et al., 1992; Houglum et al., 1994) and from Kupffer cells (paracrine), (Friedman and Arthur, 1989). TGF $ß_1$ binding to HSC increases with HSC activation (Houglum et al., 1994), and activation of latent TGF $ß_1$ itself may also contribute to a net increase in its activity. Activation of HSC may also be associated with an increase in contractile activity, a quality common to myofibroblast-like cells in other tissues. HSC contractility is regulated by endothelin (Housset et al., 1994), nitric oxide (Rockey and Chung, 1995) and potentially by eicosanoids and modifiers of cyclic AMP (Pinzani et al., 1990).

HSC may regulate the inflammatory response to liver injury by releasing cytokines chemotactic for leukocytes. Colony stimulating factor (Pinzani et al., 1992) and monocyte chemotactic peptide (Marra et al., 1993), both secreted by HSC, are upregulated during HSC activation. These findings may contribute to the marked infiltration of mono-nuclear leukocytes which accompanies most forms of experimental and human liver injury. Most recently HSC have been demonstrated to release Il-10, a cytokine with modulatory effects on macrophages suggesting that rather than being a passive recipient of fibrogenic signals the HSC may influence the inflammatory response by down regulating Kupffer cell activity (Thompson et al., 1998a,b).

Matrix degradation in liver fibrosis

The resorption of extracellular matrix is a normal event in the remodelling seen in a variety of tissues eg. in embryogenesis, trophoblastic implantation, angiogenesis, tissue morphogenesis and growth. In pathological processes the accelerated breakdown occurring in for example arthritides, periodontal disease, tumour invasion and metastases may in part be due to a breakdown in the tight control of degradative processes (Murphy et al., 1991).

In fibrotic liver injury there is evidence to suggest that: 1) Degradation of the normal basement membrane-like liver matrix (in the Space of Disse) occurs. This may disturb hepatocyte function and promote deposition of a fibrillar liver matrix. 2) In progressive fibrosis there is a failure to degrade excess fibrillar collagens (Arthur, 1994a). The major class of enzyme expressed by mesenchymal cells to mediate this matrix remodelling are the Matrix Metalloproteinases (MMPs) (see Table 2).

The MMPs are a family of zinc and calcium dependent endopeptidases secreted by connective tissue cells that have activity against the major constituents of matrix including fibrillar (interstitial and banded) and non-fibrillar collagens. MMPs are important in ECM turnover (Murphy et al., 1991), and there is strong evidence for their expression by HSC (Emonard et al., 1990; Herbst et al., 1991; Milani et al., 1992) and Kupffer cells (Winwood et al., 1995). The MMPs can be grouped according their enzymatic substrate (see Table 2). The first group are the collagenases: these MMPs are central to the process of remodelling/repair of fibrotic tissue as they cleave the helix of native fibrillar collagens I, II and III to render the collagen susceptible to degradation by other MMPs to which they were previously resistant (Goldberg et al., 1986). Thus activation of Interstitial Collagenase (MMP-1) or an alternative analogue is crucial to the process of remodelling of fibrotic matrix - the expression of Interstitial Collagenase would be necessary to initiate degradation of the most abundant fibrotic collagen (types I and III) (Friedman, 1997). A recent report that Gelatinase A (MMP-2) may have degradative activity against collagen I (Franklin, 1995) would, if confirmed, have implications for the process of matrix remodelling

Table 2. The matrix metalloproteinases family (MMPs).

NAME	NUMBER	SUBSTRATE
The Collagenases		
Interstitial Collagenase	MMP-1	Collagen I, II, III, VII, VIII, X, gelatins, aggrecans, tenascin
Neutrophil Collagenase	MMP-8	Collagen I, II, III, VII, X, aggrecan
Collagenase 3	MMP-13	Collagen I, II, III, VII, X, aggrecans, gelatins
The Gelatinases		
Gelatinase A	MMP-2	Gelatin, collagen types IV, V, VII, X (may also have Interstitial Collagenase activity against collagen 1), fibronectin, elastin, laminin, aggrecan, vitronectin
Gelatinase B	MMP-9	Gelatin, collagen IV, V, VII, X, XI, vitronectin, elastin, aggrecan
The Stromelysins		
Stromelysin 1	MMP-3	Aggrecan, link protein, fibronectin, laminin, elastin, transin, gelatins
Stromelysin 2	MMP-10	Gelatins I, III, IV, V; collagens III, IV, V, VIII, IX, activates procollagenase, fibronectin, laminin, elastin, aggrecan
Stromelysin 3	MMP-11	N-terminal domain cleaves casein
Matrilysin	MMP-7	Gelatins, elastin, aggrecans, fibronectin, link protein, activates procollagenase, vitronectin, tenascin C, entactin, laminin
Membrane-type MMPs		
MT-1	MMP-14	Activates progelatinase A and possibly other MMPs, collagen I, II, III; dermatan sulphate, laminin B chain, fibronectin, gelatin, vitronectin

- in the liver and other organ/tissue systems. This second group of MMPs, the Gelatinases, have activity against denatured collagen (gelatin), some collagens, elastin and laminin. The gelatinases are significant for their important role in degrading normal type IV collagen in the basement membrane in the Space of Disse during the early stages of the fibrotic process (Murphy et al., 1991). The third group, the Stromelysins, have activity against a variety of collagen: II, IV, IX, X, XI, denatured collagen (or gelatin), laminin and fibronectin. Stromelysins are also important in that they activate procollagenase (Murphy et al., 1991; Matrisian, 1992). A fourth group, the membrane type MMPs (MT-MMP), comprises of three members and serve to activate gelatinase and by virtue of a unique structure localize this activity to the cell surface (Sato et al., 1994; Takino et al., 1995; Will and Hinzmann 1995).

Regulation of MMP expression and activity

The extracellular activity of MMPs is regulated at various stages: 1) by transcriptional activation at the level of the gene; 2) by activation of the latent proenzyme when the propiece is cleaved; and 3) by extracellular inhibition by the specific Tissue Inhibitors of Metalloproteinases (TIMPs) or more general protease inhibition such as α-2 macroglobulin. A series of growth factors including Il-1, TNF-α, PDGF, ß-FGF, and EGF regulate MMPs at the level of the gene (Matrisian, 1990; Murphy et al., 1990, 1991; Murphy and Hembry 1992). An important cytokine in the context of liver fibrosis is TGF-$ß_1$ which differentially regulates certain MMPs and is expressed by Kupffer cells (Matsuoka and Tsukamoto, 1990) and activated HSCs (Bachem et al., 1992). Activated HSCs express TGF-$ß_1$ receptors (De Bleser et al., 1995), TGF-$ß_1$ mRNA and also secrete this cytokine in an autocrine manner. In fibroblasts TGF-$ß_1$ down regulates interstitial collagenase (MMP-1): important in the degradation of collagen I (Edwards et al., 1987), whilst upregulating gelatinase A (MMP-2) (Overall et al., 1989) TIMP-1 and collagen I (Matsuoka and Tsukamoto, 1990; Wahl et al., 1993). Hence TGF-$ß_1$ is a profibrogenic cytokine and is likely to play an important role in the fibrotic process.

A second level of control resides where the secreted form of all MMPs (pro-MMP) is converted to the active MMP by cleavage of the propiece (Bachem et al., 1989; Friedman et al., 1989; McDonald, 1989). This process may be mediated by plasmin (Matrisian, 1992; Murphy et al., 1992). Active stromelysin cleaves activated interstitial collagenase (MMP-1) (Murphy et al., 1987; Matrisian, 1990) whilst the fourth group of MMPs: MT-MMP activates gelatinase A (MMP-2) at the cell membrane.

The TIMPs

Tissue Inhibitors of Metalloproteinases (TIMPs) function at two levels: certain TIMPs stabilise specific pro-MMP species and all TIMPs inhibit all active MMP enzymes: thus acting as an important regulatory brake on metalloproteinase activity (see Table 3) (Docherty et al., 1985; Boone et al., 1990). Four TIMPs have been identified to date (TIMP 1, 2, 3 and 4) - each is a separate gene product (Pavloff et al., 1992; Denhardt et al., 1993; Apte et al., 1994; Wu and Moses, 1998) (see Table 3). Structurally, there are similar features running through the TIMP family. TIMP 1 and 2 share 40% amino acid homology and both have three looped structures stabilised by six disulphide bonds (Boone et al., 1990; Murphy and Docherty, 1992; Denhardt et al., 1993). TIMPs bind to MMPs in a stoichiometric manner which is irreversible under normal physiological conditions. The MMP is rendered inactive when the TIMP binds to its active site (Cawston et al., 1983; Bo and Denhardt, 1992; Murphy and Docherty, 1992). Binding is non-covalent so under certain *in vitro* conditions TIMPs can be separated from the MMP species with the former retaining activity against MMPs (Murphy et al., 1989). TIMPs 1 and 2 inhibit the active form of all MMPs (Denhardt et al., 1993) and TIMPs 3 and 4 probably have the same spectrum of activity. Two functional domains exist on the TIMP molecule: the N terminal is vital for activity against MMPs - truncation

Table 3. Properties of tissue inhibitors of metalloproteinases (TIMPs).

	TIMP-1	TIMP-2	TIMP-3	TIMP-4
MMP inhibition	All	All	All?	All?
Mature protein size (kDa)	28.5	21	21	23
Glycosylation	Yes	No	Yes	No
Localization	Diffusible	Diffusible	ECM bound	Unknown
Expression	Inducible	Constitutive Possibly Inducible in Hepatic Stellate Cells	Inducible	Unknown
Major tissue sites	Liver, Bone, Ovary	Liver, Lung, brain ovaries, testis, heart, placenta	Kidney, brain, lung, heart, ovary	Kidney, colon, placenta, testis, brain, heart, ovary, skeletal muscle
Binding to pro-MMP	MMP-9	MMP-2	Unknown	Unknown
Binding to all active MMPs	Yes	Yes	Probably	Probably

ECM: extracellular matrix

of the TIMP molecule which spares the N-terminal allows continued inhibitory activity against MMPs. The C terminal facilitates interaction with the prometalloproteinases (Howard and Banda, 1991; Howard et al., 1991; Ward et al., 1991; Fridman et al., 1992). TIMPs 1 and 2 bind to pro-Gelatinase species (Gelatinase B/MMP 9, Gelatinase A/MMP 2 respectively) thus preventing activation (De Clerck et al., 1991; Goldberg et al., 1992). TIMPs are regulated at the level of transcription by cytokines and growth factors that also govern MMP expression and that are also important both in HSC activation and HSC synthetic function. For example TGF-ß$_1$ upregulates TIMP-1 and gelatinase A whilst down regulating TIMP-2, interstitial collagenase (MMP-1) and stromelysin (Edwards et al., 1987; Overall et al., 1989, 1991). TNF-alpha upregulates TIMP-1 and interstitial collagenase (MMP -1) (Marshall et al., 1993).

Studies mapping the gene promoters of TIMPs and MMPs show some common regulatory motifs that differ in individual TIMPs/MMPs in terms of their frequency and position in relation to the transcription start site. For example the murine TIMP-1 and interstitial collagenase (MMP-1) promoters both have AP-1 and PEA-3 binding sites but in different configurations (Edwards et al., 1992; Schorpp et al., 1995). An AP-1 site is noted in the promoters of stromelysin and gelatinase B but not in gelatinase A (MMP-2). The human TIMP-2 gene on chromosome 17 is flanked by 5' AP-1 and AP-2 consensus sequences and several SP-1 sites in association with a TATA box (De Clerck et al., 1994). The AP-1 consensus site in the TIMP 2 promoter is further upstream from the transcription start site than that found in the TIMP-1 promoter and is not associated with a PEA-3 motif. As in TIMP 1 and 2, the TIMP 3 promoter has multiple SP-1 sites which confer a high basal expression in growing cells (Wick et al., 1995). The spatial distribution and differing frequencies of transcription binding sites in the promoters of TIMPs 1 and 2 may explain in part the differential expression observed in response to cytokines such as TGF ß$_1$ and TNF-α. The promoters also provide a mechanism whereby TIMPs can be coregulated and independently regulated to inhibit MMP activity in a wide variety of physiological (eg growth and development) and pathological processes (eg arthritides and liver fibrosis). Moreover, from what has been discussed it can be proposed that through relatively small changes in the ratio of TIMP: MMP concentrations alterations in matrix degradation can be effected and regulated.

There are large regions of the TIMP promoter regions still undescribed. Recently for example a novel transcription factor binding site "Upstream TIMP element 1" has been described along with its associated binding proteins (Trim et al., 2000).

Interaction of TIMPs with MMPs in the fibrotic process and evidence for MMP Inhibition during liver fibrosis

Stellate cells express a variety of MMPs: both human and rat HSCs express gelatinase A (MMP-2) and rat HSC express Stromelysin (MMP-3) (Arthur et al., 1989, 1992; Vyas et al., 1995). Gelatinase A expression is upregulated with HSC activation whilst Stromelysin is transiently expressed with HSC activation over a period of 72 hours. Both MMPs can be immunolocalized to HSC and MMP activity can be detected in cell culture supernatants. Both MMPs are also expressed in acute liver injury in perisinusoidal cells (Herbst et al., 1991; Iredale et al., 1993). Collagenase can be localised to the HSC cytoplasm during activation by culture on uncoated plastic (Arthur, 1994b).

Messanger RNA (mRNA) for collagenase cannot be detected in activated rat HSC but is detected in freshly isolated cells (Iredale et al., 1996). However TNF-α and IL-1 can induce interstitial collagenase expression in activated human HSCs (Emonard et al., 1990; Iredale et al., 1995). Cultured HSC also release interstitial collagenase in response to polyunsaturated lecithin (Li et al., 1992). HSCs therefore possess the ability to remodel matrix during activation and specifically to mediate remodelling of interstitial collagens by expressing interstitial collagenase (MMP-1). Other cells may play a role in matrix degradation such as Kupffer cells which express Gelatinase B (Winwood et al., 1995) and sinusoidal endothelial cells which express stromelysin (Herbst et al., 1991).

TIMP expression in progressive fibrosis

To address the concept that TIMP expression may promote fibrosis by reducing collagenase activity in progressive liver fibrosis, the gene expression of TIMPs 1 and 2 and MMPs have been studied in HSC activation both in tissue culture and in vivo. When HSC are cultured in uncoated tissue culture plastic, a process which recapitulates many of the features of activation including α Smooth Muscle Actin (α SMA) and ProCollagen 1 (PC-1) expression, there is an increase in the transcription of TIMP-1 mRNA in activated cells compared to quiescent (freshly isolated) cells (Iredale et al., 1992). TIMP-1 can be immunolocalized to HSC and also detected extracellularly in HSC cell culture supernatants by ELISA. When HSC conditioned media is subjected to gelatin sepharose chromatography, TIMP-1 bound to Gelatinase A (MMP-2) is separated: removal of TIMP-1 is associated with a twenty-fold increase in gelatinase activity. Returning TIMP-1 to the media results in re-inhibition of Gelatinase A and a reduction in its activity (Iredale et al., 1992). Both TIMP 1 and 2 are found in HSC conditioned media and TIMP 2 mRNA is observed in northern analysis of activated HSC total RNA (Iredale et al., 1992; Benyon et al., 1996). It is of interest that HSC activation appears to be associated with an upregulation of TIMP-2 expression: a feature not observed in other cell lines studied to date. HSC may not be the sole source of TIMPs in the liver: TIMPs 1 and 2 are detected in Hep G2 hepatoma cell lines (Kordula et al., 1992; Roeb et al., 1993; Benyon et al., 1996). In these studies TIMP 1 expression was found to increase

in the presence of IL-6, an acute phase cytokine, suggesting that TIMP 1 may be released by hepatocytes in acute liver injury.

A number of other studies have indicated that a reduction in matrix remodelling activity occurs as fibrosis progresses manifested by a fall in interstitial collagenase (MMP-1) activity (essential in the degradation of collagens I and III). This is noted in models of alcoholic liver injury in humans and primates and carbon tetrachloride injury in rats (Okazaki and Maruyama, 1974; Maruyama et al., 1981, 1982; Perez-Tamayo et al., 1987). This suggests that inhibition of matrix degradation is a feature of fibrogenesis. Further evidence to support this concept is provided by models of recovery from hepatic fibrosis. During carbon tetrachloride induced liver injury in the rat progressive fibrosis can be documented and becomes established after 4 weeks of treatment. At this stage prompt withdrawal of the toxin results in resolution of the fibrotic changes over a period of 28 days (Iredale et al., 1998). Analysis of the TIMP/Collagenase relationship in this model indicates that during progressive fibrosis interstitial collagenase continues to be expressed but its activity falls with a concurrent upregulation in TIMP 1 and 2 expression (Mallat et al., 1995). In the recovery phase expression of TIMPs 1 and 2 decreases with a concurrent rise in collagenase activity accompanied by histological evidence of matrix remodelling (Iredale et al., 1998). The expression of Collagenase mRNA remains relatively constant.

In a model of fibrotic liver from murine schistosomiasis expression of interstitial collagenase, detected immunologically, remains relatively constant (Takahashi et al., 1980; Takahashi and Simpser 1981; Truden and Boros, 1988) whilst collagenase activity decreases emphasizing the importance of expression of collagenase inhibitors during fibrogenesis. Further evidence for the important roles of TIMPs in fibrogenesis come from the analysis of serum in patients with hepatic inflammation and established cirrhosis reveals an increase in TIMP 1 levels by ELISA (Murawaki et al., 1993, 1994; Muzzillo et al., 1993). In addition, when TIMPs 1 and 2, interstitial collagenase and Gelatinase A mRNA expression in fibrotic liver compared to normal were studied by Ribonuclease Protection Analysis (Iredale et al., 1995; Benyon et al., 1996), TIMP 1 and 2 transcripts were increased in fibrotic liver, as are Gelatinase A transcripts (Benyon et al., 1996). In contrast Interstitial collagenase transcripts were only marginally increased in primary sclerosing cholangitis and primary biliary cirrhosis (Benyon et al., 1996). TIMP 1 was also immunolocalized to peri-sinusoidal cells in fibrotic liver in 75% of biopsies positive for interstitial collagenase suggesting that coexpression of TIMP 1 with interstitial collagenase occurred (Benyon et al., 1996). This data provides powerful evidence that progressive fibrosis is associated with changes in the pattern of matrix degradation in addition to matrix synthesis. Moreover, current evidence suggests that such changes may be mediated by the powerful MMP inhibitors the TIMPs.

Conclusion

In summary, the Hepatic Stellate Cell has been examined in the context of progressive fibrotic liver injury. There is considerable evidence that HSC are the principle effector cells for matrix remodeling in liver injury and that during HSC activation TIMPs 1 and 2 mRNA are expressed. Understanding the dynamic changes in TIMP expression in HSC activation will enhance our knowledge of fibrogenesis and may lead to the development of novel therapeutic strategies based on promoting matrix degradation. It is anticipated that further insights into the relative roles of the TIMPs and MMPs will become possible as experimental tools such as the development of TIMP gene knockout mice become available to facilitate definitive mechanistic studies.

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