

# Gut

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## Leading article

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### Is liver fibrosis reversible?

Liver fibrosis and cirrhosis result from the majority of chronic liver insults and represent a common and difficult clinical challenge of worldwide importance. At present, the only curative treatment for end stage cirrhosis is transplantation, but even in the developed world, the number of donor organs available and the clinical condition of the potential recipient limit the applicability of this technique. The alternative clinical course is one familiar to gastroenterologists—that of a progressive damage limitation exercise in which the complications of fibrosis and cirrhosis are treated with greater or lesser success. The development of fibrosis, and particularly cirrhosis, is associated with a significant morbidity and mortality. Thus, there is a considerable imperative to develop antifibrotic strategies that are applicable to liver fibrosis. Such an approach is attractive precisely because it is aimed at the final common pathological pathway of chronic liver disease, regardless of aetiology. However, because fibrotic liver disease may not present clinically until an advanced or cirrhotic stage, the possibility of reversing the fibrosis is an essential issue for developing therapeutic approaches.

Liver fibrosis represents the wound healing response of the liver, as such it demonstrates generic aspects that characterise tissue healing elsewhere in the body — a wound healing response that is dynamic and has the potential to resolve without persistent scarring. This may seem at odds with the clinical impression that advanced fibrosis and cirrhosis are at best irreversible and at worst progressive. However, recent developments in our understanding of the process of hepatic fibrogenesis confirm that the process is dynamic with respect to both cell and extracellular matrix (ECM) turnover and suggest that a capacity for recovery from advanced cirrhosis and fibrosis is possible. Moreover, with the advent of effective antiviral therapies, biopsy documented examples of improvements in fibrosis and in some examples resolution, including that of cirrhotic change, are accumulating in the literature.<sup>1–4</sup> To utilise these observations and establish the attributes required of an effective antifibrotic therapy, we need to understand the nature and origin of the fibrotic ECM, the methods by which the ECM is degraded and the essential processes which occur when fibrosis undergoes recovery with restoration of the normal liver architecture.

#### Nature and origin of fibrosis

Development of liver fibrosis entails major alterations in the both quantity and quality of hepatic ECM and there is overwhelming evidence that activated hepatic stellate cells (HSC, Ito, fat storing cell, or lipocyte) are the major producers of the fibrotic neomatrix.<sup>5–6</sup> Hepatic stellate cells reside in the space of Disse and in normal liver are the

major storage sites of vitamin A, stored in the cytoplasm as retinyl esters. Following chronic liver injury, HSC proliferate, lose their vitamin A and undergo a major phenotypical transformation to smooth muscle  $\alpha$ -actin positive myofibroblasts (activated HSC) which produce a wide variety of collagenous and non-collagenous ECM proteins. Cirrhotic liver contains approximately six times more ECM overall than normal liver, and in the space of Disse collagen types III and V and fibronectin accumulate in early injury.<sup>7</sup> In chronic injury there is increasing deposition of collagen types I and IV, undulin, elastin, and laminin.<sup>8</sup> Hyaluronan, normally a minor component of the space of Disse, is increased more than eightfold<sup>9</sup> and dermatan and chondroitin sulphate and heparan sulphate proteoglycans also increase. Although collagen types I, III, and IV are all increased, type I increases most and its ratio to types III and IV therefore increases.<sup>7–10–12</sup> Culture studies have suggested that the neomatrix laid down in the space of Disse may itself contribute to the disease associated alterations in the phenotype of HSC, sinusoidal endothelial cells, and hepatocytes.<sup>13–16</sup> With progressive injury ECM spurs link the vascular structures, ultimately resulting in the architecturally abnormal nodules that characterise cirrhosis.

Complete recovery from liver fibrosis would involve remodeling and breakdown of these multiple ECM components, with degradation of the predominant component, collagen I, being particularly important for recovery of normal liver histology. At present, the identities of the enzyme(s) that degrade the fibrillar collagens (collagens I and III) in the liver are unclear. The matrix metalloproteinases (MMP), a family of zinc dependent endoproteinases, have the capability to degrade these various ECM components and are expressed particularly by HSCs and Kupffer cells.<sup>17</sup> The first discovered and best characterised interstitial collagenase in humans is MMP-1, which is widely expressed in human tissues including liver, but other human interstitial collagenases with a more limited cell expression include neutrophil collagenase (MMP-8) and collagenase 3 (MMP-13). The enzymes MMP-2 and MMP-14 have also recently been ascribed interstitial collagenolytic activity.<sup>18–19</sup> However, studies in animal models and human liver fibrosis indicate that interstitial collagenolytic activity decreases in liver extracts in advanced fibrosis,<sup>20–24</sup> which would promote net collagen deposition. There is increasing evidence that collagenase

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**Abbreviations used in this article:** ECM, extracellular matrix; HSC, hepatic stellate cell; MMP, metalloproteinase; PAI, plasminogen activator inhibitor; TIMP, tissue inhibitor of metalloproteinases.

inhibition may arise from increased expression in fibrotic liver of endogenous MMP inhibitors, the tissue inhibitors of metalloproteinases (TIMPs). Expression of both TIMP-1 and -2 is increased in human and rat model fibrotic liver<sup>25-31</sup> and in human liver the degree of TIMP-1 expression correlates with extent of fibrosis<sup>25</sup> assessed by hydroxyproline content. Studies by our group and others<sup>25 27 31-33</sup> indicate that activated HSC may be an important source of these TIMPs in injured liver. In rat models of liver fibrosis, TIMP-1 is expressed early in fibrogenesis before apparent collagen deposition.<sup>26</sup> In contrast to the TIMPs, mRNA for interstitial collagenase (MMP-1 in humans, MMP-13 in rats) remains unaltered in human and rat liver as fibrosis develops.<sup>25 26 34</sup> The resulting increase in TIMP:MMP ratio in liver may promote fibrosis by protecting deposited ECM from degradation by MMPs. However, other MMP inhibitory mechanisms might contribute to fibrosis. MMPs are released as inactive pro-enzymes, and an important regulatory step involves cleavage of the inhibitory N-terminal peptide to confer enzymatic activity.<sup>35</sup> The means of proenzyme activation varies between different MMPs, but the protease plasmin is required for efficient activation of proMMP-1.<sup>36</sup> Activated HSC may however inhibit plasmin synthesis in fibrotic liver through synthesis of plasminogen activator inhibitor-1 (PAI-1).<sup>37 38</sup> Plasmin may have an important antifibrotic role, as studies of fibrosis in lung and kidney utilising PAI-1 and urokinase plasminogen activator knockout mice suggest that an increased PAI-1:urokinase ratio in tissues promotes fibrogenesis.<sup>39</sup> In summary, activated HSC might produce a fibrogenic environment within the liver through a combination of ECM overproduction, diminished MMP activation and inhibition of active MMPs by TIMPs. The removal or inactivation of activated HSC from the liver is therefore likely to be a key process before recovery from fibrosis can occur.

### Resolution of fibrosis

In clinical circumstances where an effective treatment for the underlying insult is available, remodeling of the scar tissue can occur and a return towards architectural normality has been documented even in advanced fibrosis and cirrhosis. This has been most clearly documented in autoimmune disease, but is paralleled by observations of haemochromatotic patients after venesection and patients with hepatitis B and C after successful interferon therapy.<sup>1-4</sup> These observations are highly encouraging and suggest that the liver has a capacity to remodel scar tissue which, if harnessed and manipulated, would offer a novel therapeutic approach to the treatment of liver fibrosis. It is difficult, if not impossible to follow the cellular mechanisms mediating recovery in humans, as ethical considerations prevent serial biopsy samples from being taken from patients with liver disease and fibrosis which seems to be resolving clinically. However, recovery from fibrosis has been studied in rat models, which permit frequent sampling and control over the chronology and extent of the fibrotic lesion. Abdel-Aziz and colleagues<sup>40</sup> examined reversibility of fibrosis in experimentally induced cholestasis in rats. Following bile duct ligation for three weeks, the typical features of bile duct proliferation and periportal fibrosis developed with a notable increase in hepatic mRNA for collagens I and IV. However, three weeks after relief of bile duct ligation (by reanastomosis of the bile duct to a jejunal loop), there was resorption of periportal fibrosis and the liver ECM returned virtually to normal, except for a persistence of collagen IV in sinusoids. Moreover mRNAs for collagen I and IV became virtually undetectable. We have recently examined spontaneous recovery from liver fibrosis in carbon tetrachloride treated rats.<sup>41</sup>

Rats treated for four weeks with intraperitoneal carbon tetrachloride developed established liver fibrosis with extensive intervascular bridging with collagen fibres. Carbon tetrachloride dosing then stopped and livers were examined at various times up to four weeks of recovery. After this time, histological analysis showed a noticeable dissolution of the collagenous fibrotic matrix and a return of liver structure to virtual normality. The hepatic mRNA content of TIMP-1 and -2 and procollagen I all dropped greatly in livers the first week of recovery which coincided with the most rapid phase of collagen degradation, as assessed by hydroxyproline content. A key finding was that interstitial collagenase activity increased in the liver homogenates during this time. The data support the hypothesis that TIMPs play a predominant role in regulating fibrosis by protecting fibrotic ECM from degradation by collagenase and possibly other MMPs. Another important observation was that there was prominent apoptosis of activated HSC during recovery, particularly in the first three days concomitant with the largest drop in hepatic TIMP and procollagen I mRNA. Apoptosis therefore effectively removed the activated HSC, which were overproducing ECM and TIMPs. This mechanism may also effect removal of "professional" ECM producing cells in other organs during wound healing and resolution of fibrosis. For example, Baker and colleagues<sup>42</sup> showed that apoptosis removed surplus mesangial cells from glomeruli during resolution of mesangial proliferative nephritis and apoptosis also removes myofibroblasts during skin wound healing.<sup>43 44</sup> Our more recent studies suggest that during progressive fibrotic liver injury both HSC mitosis and apoptosis increase—that is, turnover of these cells is increased, although proliferation predominates such that there is net increase in HSC numbers. During recovery, apoptosis becomes the overriding process with resulting net HSC loss from the liver.

There are relatively few studies of how apoptosis of HSC is controlled in the liver. HSC activated in culture undergo spontaneous apoptosis *in vitro*, which can be greatly increased by serum deprivation and fas ligand.<sup>41 45 46</sup> Our recent studies show that a further cytokine present in injured liver, nerve growth factor, induces HSC apoptosis in culture. Mast cells, which become more abundant in fibrotic liver, are a rich source of nerve growth factor.<sup>47</sup> The proapoptotic receptor fas and its ligand are also expressed by activated HSC.<sup>45</sup> It is possible that persistence of HSC in fibrotic liver might therefore require undefined survival factors to offset the effects of these apoptotic stimuli, and removal of survival factors when liver injury ceases would then allow relatively rapid removal of HSC. Apoptotic signals in the liver might not be confined to soluble factors and the fibrotic neomatrix itself might render activated HSC susceptible to apoptosis. The role of cell-matrix interactions in regulating cell survival has most extensively been studied in epithelial cells in which absolute deprivation of contact with the ECM is a potent proapoptotic mechanism, a process that has been termed anoikis.<sup>48</sup> A recent study has shown that blocking HSC attachment to plastic induces apoptosis,<sup>49</sup> whereas data from our laboratory show that HSC cultured on plastic or collagen I are more susceptible to apoptosis induced by serum deprivation than HSC cultured on Matrigel, a basement membrane-like matrix which reduces HSC proliferation and activation. These final data raise the interesting idea that ECM degradation may result in HSC apoptosis rather than HSC apoptosis facilitating ECM degradation.

Although liver fibrosis in rats is reversible, the implications for recovery from cirrhosis in humans remain to be clarified. In our studies<sup>41</sup> and those of Abdel-Aziz and coworkers,<sup>40</sup> liver cirrhosis had not been achieved before

recovery was initiated. Clearly a key question which can be tackled using rat models is: does liver fibrosis reach a point where it becomes irreversible, and if so what are the qualitative and quantitative differences in the liver structure compared with recoverable fibrosis? Several factors might dictate whether liver fibrosis can recover. Firstly, it is clear that recovery requires degradation of the existing fibrotic matrix, but this matrix may be modified to resist degradation as fibrosis progresses. Newly secreted collagen fibrils can be cross-linked by both tissue transglutaminase and lysyl oxidase pathways; the activity of both pathways is increased during liver fibrogenesis.<sup>50-52</sup> Such cross-linking during maturation of collagen might reduce its susceptibility to collagenase.<sup>53</sup> A recent report also suggests that tissue transglutaminase can be released onto ECM from apoptotic hepatocytes which are found in increased numbers in fibrotic liver.<sup>54</sup> Mature ECM is also relatively rich in elastin; to date there are very limited data on the turnover of this important matrix protein in fibrosis. Secondly, recovery is unlikely if collagenolytic enzymes remain inactive following cessation of liver injury. The full range of enzymes having interstitial collagenase activities in liver still require identification. However, interstitial collagenase mRNA expression (MMP-1 in humans, MMP-13 in rats) is similar in normal compared with cirrhotic livers, and does not change during recovery in the rat model, even in the face of overt ECM degradation.<sup>25 26 41</sup> Previous studies suggest that collagenase activity becomes deficient during evolution of liver fibrosis in animal models and in humans,<sup>20-24</sup> and the studies described earlier suggest that this may be caused by TIMP overexpression. Continued inhibition of ECM degradation by TIMPs may block the ability to recover from fibrosis, even after removal of the injury. As activated hepatic stellate cells are an important source of both ECM and TIMPs, recovery from fibrosis might require either removal of the activated HSC population, as shown in rat models, or possibly the phenotypical reversal of stellate cell activation, a process yet to be observed *in vivo*. In non-recovering liver fibrosis activated HSC might persist as a result of a "memory" effect, possibly mediated by collagenous and non-collagenous components of the deposited fibrotic neomatrix, which either promote HSC activation or protect them from apoptotic stimuli.<sup>16 48 49 55</sup>

In summary, accumulating evidence suggests that liver fibrosis is reversible and that recovery from cirrhosis may be possible. Moreover, the application of cell and molecular techniques to models of reversible fibrosis are helping to establish the events and processes that are critical to recovery. It is anticipated that ultimately these approaches will lead to the development of effective antifibrotics, which harness or mimic the liver's capacity for reversal of fibrosis with resolution to a normal architecture.

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