Liver fibrosis occurs as a result of the deposition of excess matrix proteins. These include collagen types I and III which are synthesized by hepatic stellate cells which, after liver injury, become activated to a myofibroblastic phenotype. These activated hepatic stellate cells are also able to synthesize enzymes called metalloproteinases, which have the ability to degrade matrix proteins. The extracellular activity of the metalloproteinases is carefully regulated by controlling their activation from proenzymes to the catalytic form and by a family of specific inhibitors, called tissue inhibitors of metalloproteinases (TIMPs). Recent evidence indicates that hepatic stellate cells play a key role in regulating matrix degradation in liver; (i) they express several metalloproteinases, (ii) they express cell surface associated metallo- and serine proteinases that can cleave prometalloproteinases to the active form and (iii) they express TIMPs which inhibit extracellular degradative activity. The complex interplay of these different factors plays an important role in the pathogenesis of liver fibrosis. The purpose of the lecture is to review this data and to set it in the context of matrix biology in the liver.

Liver Fibrosis: Cellular events and matrix biology

In normal liver, hepatic stellate cells (HSC) are situated in the Space of Disse and have an important role in retinoid storage and metabolism. After liver injury these cells proliferate and become activated to a myofibroblastic phenotype. In their normal quiescent phenotype, HSC synthesize small quantities of type IV collagen, but when activated they synthesize large quantities of collagen types I and III, which are the principal collagens found in fibrotic or cirrhotic liver.

This central role for activated HSC in liver fibrosis has led to many studies investigating the factors that regulate their proliferation and phenotypic alteration. HSC proliferate in response to growth factors released by damaged hepatocytes and activated Kupffer cells including platelet derived growth factor (PDGF), fibroblast growth factor (FGF) and insulin-like growth factor-1 (IGF-1). The precise mechanisms which regulate alteration of proliferating HSC to the activated phenotype are poorly understood, but early features include autocrine expression of transforming growth factor beta-1 (TGF-β1) and expression of TGF-β1 receptors.

Matrix Degradation by Hepatic Stellate Cells

The family of enzymes involved in degradation of matrix proteins in the extracellular space are the matrix metalloproteinases (or MMPs). There has been a dramatic increase in our knowledge of this family of enzymes with at least 20 members now cloned and sequenced and many of their biochemical properties described. This family of enzymes can be subdivided into four broad categories:

i) The collagenases, which degrade fibrillar interstitial collagens (types I, II and III)
ii) The gelatinases, which degrade denatured collagens (gelatins) but which can also degrade basement membrane (type IV) collagen.
iii) The stromelysins which degrade proteoglycans and laminin in addition to a wide variety of other proteins.
iv) The newly described membrane-type matrix metalloproteinases which are involved in cell surface proteolytic events.

To date, HSC activated by primary culture on plastic have been described to synthesize several members of the matrix metalloproteinase family. In early primary culture (days 1-3) there is transient expression of interstitial collagenase (MMP-1 in humans, MMP-13 in rats) and stromelysin-1 (MMP-3) which are then down-regulated (days 3-5) and disappear by the time HSC are fully activated (days 7-21). In contrast, gelatinase A (MMP-2) expression does not occur in early culture but increases from days 3-5 and is a continuous prominent feature of fully activated HSC. Recent evidence indicates that this is accompanied by a similar pattern of expression of the progelatinase...
A activator, recently identified as membrane-type 1-MMP (MT1-MMP), and this is associated with the appearance of active gelatinase A in the cell culture media. These data indicate that HSC are clearly capable of degrading matrix proteins, particularly during the early phase of HSC activation.

These data obtained in cell culture have been complemented by molecular studies in whole liver. Expression of gelatinase A mRNA (MMP-2) is low in normal liver but increases dramatically in liver disease or after experimental liver injury. There is a progressive increase in detection of the active form of gelatinase A (which is also a type IV collagenase) as determined by zymography as liver fibrosis progresses, which appears paradoxical as there is also an increase in deposition of type IV collagen. Recent data from our laboratory suggest that gelatinase A promotes proliferation of hepatic stellate cells by a mechanism which has yet to be defined. This may involve disruption of cell-matrix interaction.

Inhibition of matrix degradation in progressive liver fibrosis

TIMPs are low molecular weight glycoproteins that are specific inhibitors of the metalloproteinase family of matrix-degrading enzymes. Four distinct members of this gene family have now been described to include TIMP-1, TIMP-2, TIMP-3 and TIMP-4.

We have investigated the role of TIMPs in liver fibrosis, concentrating mainly on TIMP-1 and -2 and have examined their expression in hepatic stellate cell (HSC) culture, in human liver disease and in animal models of liver fibrosis. In early primary culture (day 3) HSC transiently express interstitial collagenase in the absence of TIMP-1. As the HSC become activated by culture on plastic and this becomes fully established (as defined by expression of α-smooth muscle actin and procollagen I) the early pattern is reversed; MMP-1 expression is not detectable and there is a striking increase in TIMP-1 expression, synthesis and extracellular release. When TIMPs were removed from the culture medium of activated HSC’s the measurable total metalloproteinase activity increased by more than 20-fold. These studies have led to the hypothesis that alterations in matrix degradation, mediated by increased localised expression of TIMP-1 by activated HSC (relative to interstitial collagenase) play a significant role in the pathogenesis of liver fibrosis.

This hypothesis was examined further in studies of fibrotic human liver, which demonstrated a 5-fold increase in mRNA levels for TIMP-1 with no significant change in interstitial collagenase (MMP-1) expression. These single time point studies were complemented by temporal analysis after rat liver injury induced by CCl₄ or bile duct ligation (BDL). In both these models, TIMP-1 mRNA was upregulated within 6 hours, peaked at 72 hours post injury and remained elevated throughout the time course of the study. There was no parallel change in rat interstitial collagenase (MMP-13) gene expression.

We have recently extended our analysis of the role of TIMP-1 in liver fibrosis by studying a variant of the CCl₄ model, in which fibrotic liver (after 4 weeks of repeated injury) is allowed to recover. Early in the recovery period (day 3-10) both TIMP-1 and procollagen I mRNA levels return to control values with no change in rat interstitial collagenase (MMP-13). These observations imply that the changes in TIMP-1 relative to rat interstitial collagenase is the mechanism by which the observed degradation of fibrillar liver matrix occurs in this model.

As changes in TIMP-1 are mediated largely via alterations in gene transcription we have studied regulation of this gene in HSC’s using a strategy of transient transfection of primary cultures with a series of TIMP-1 promoter-CAT constructs. These studies have revealed interesting results which indicate that TIMP-1 regulation in HSC differs from that observed in skin fibroblasts. Truncation analysis revealed a minimal promoter that contained AP-1, PEA 3 and Sp1 transcription factor binding sites. Use of mutated constructs indicated that TIMP-1 promoter activity was classically AP-1 dependent and mediated by c-fos and c-jun in fibroblasts but not in activated HSC. Moreover, electrophoretic mobility shift assays indicated that AP-1 binding due to c-fos and c-jun was a feature of the early phase of HSC activation (20hrs) at a time when TIMP-1 was not expressed. Conversely in activated HSC, TIMP-1 expression was increased in the absence of the classic c-fos and c-jun mediated AP-1 binding activity. We have recently shown that this prolonged late expression of
TIMP-1 in activated HSC is mediated by Fra-2 and Jun-D transcription factors.

TIMP-2 expression by activated HSC and in models of liver injury parallels that observed for TIMP-1. In early primary HSC culture, TIMP-2 is not detectable, but low levels of TIMP-2 mRNA and TIMP-2 activity are evident in activated HSC. TIMP-2 mRNA expression is also increased in human liver disease as determined by RNA'ase protection analysis of total liver RNA obtained from liver explants at the time of transplantation. Similarly TIMP-2 mRNA increases in rat models of progressive liver fibrosis and like TIMP-1 is rapidly down regulated in the CCl₄ recovery model described earlier.

Summary

The lecture will describe recent progress in understanding the cell and molecular pathogenesis of liver fibrosis. There is now a substantial body of evidence indicating that alterations in matrix degradation are important in pathogenesis. In the early phase, gelatinase A appears to be involved in liver fibrosis by promoting proliferation of HSC. In contrast, in more advanced disease matrix degradation is inhibited by TIMPs -1 and -2 derived from activated hepatic stellate cells. As liver fibrosis resolves naturally our data show that decreased TIMP-1 expression relative to interstitial collagenase may mediate matrix remodelling in the liver. Finally, TIMP-1 gene transcription in activated HSC differs from that of skin fibroblasts and is mediated by a novel mechanism that involves the transcription factors Fra-2 and Jun-D. Understanding the role of matrix degradation and specifically TIMPs in liver fibrosis is now informing the design of strategies for the development of antifibrotic therapies.