Fibrogenesis
II. Metalloproteinases and their inhibitors in liver fibrosis

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Arthur, Michael J. P. Fibrogenesis. II. Metalloproteinases and their inhibitors in liver fibrosis. Am J Physiol Gastrointest Liver Physiol 279: G245–G249, 2000.—Liver fibrosis is characterized by activation of hepatic stellate cells, which are then involved in synthesis of matrix proteins and in regulating matrix degradation. In the acute phases of liver injury and as liver fibrosis progresses, there is increased expression of matrix metalloproteinases (MMPs) and their tissue inhibitors (TIMPs). Among the changes described, striking features include increased expression of gelatinase A (MMP-2) and membrane type 1-MMP (MT1-MMP; MMP-14) as well as TIMP-1 and TIMP-2. These molecules and other family members are involved in regulating degradation of both normal and fibrotic liver matrix. This article outlines recent progress in this field and discusses the mechanisms by which MMPs and TIMPs may contribute to the progression and regression of liver fibrosis. Recently described properties of MMPs and TIMPs of relevance to the pathogenesis of liver fibrosis are outlined. The proposal that regression of liver fibrosis is mediated by decreased expression of TIMPs and involves degradation of fibrillar collagens by a combination of MT1-MMP and gelatinase A, in addition to interstitial collagenase, is explored.

LIVER FIBROSIS is traditionally viewed as a progressive pathological process involving multiple cellular and molecular events that lead ultimately to deposition of excess matrix proteins in the extracellular space. When this process is combined with ineffective regeneration and repair, there is increasing distortion of the normal liver architecture, and the end result is cirrhosis. Studies of the role of hepatic stellate cells (HSCs) in this process and a more detailed knowledge of their cell biology have led to important questions about the validity of this “progression-only” model. Current evidence indicates that liver fibrosis is dynamic and can be bidirectional (involving phases of progression and regression) and that in addition to increased matrix synthesis, this pathological process involves major changes in the regulation of matrix degradation.

In the extracellular space, matrix degradation occurs predominantly as a consequence of the action of a family of enzymes called the matrix metalloproteinases (MMPs). These are secreted from cells into the extracellular space as proenzymes, which are then activated by a number of specific, usually cell surface-associated, cleavage mechanisms. The active enzymes are in turn inhibited by a family of tissue inhibitors of metalloproteinases (TIMPs). By this combination of mechanisms, extracellular matrix degradation is closely regulated, which prevents inadvertent tissue damage. This themes article will examine the current state of our knowledge of the role of these pathways of matrix degradation and their regulation in progression and regression of liver fibrosis. Although there has been significant progress, there are important new questions and hypotheses emerging in this field.

DEGRADATION OF NORMAL LIVER MATRIX

In normal liver, HSCs in the space of Disse are maintained in a quiescent, nonfibrogenic phenotype, in part because they are in contact with a complex extracellular matrix comprised of type IV collagen, laminin, and proteoglycans. When removed from this environment, they “activate” to a profibrogenic myofibroblastic phenotype, typical of that seen in fibrotic liver. These observations suggest that degradation of normal liver matrix may contribute to the pathogenesis of liver fibrosis, particularly in the early stages of response to liver injury. There is some evidence to suggest that this may occur, but to date this hypothesis is based largely on the observation that MMPs capable of degrading normal liver matrix are expressed in liver injury and fibrosis. The three most relevant MMPs are gelatinase A (MMP-2), gelatinase B (MMP-9), and stromelysin (MMP-3), all of which have been studied in liver.

Progelatinase A. Progelatinase A is secreted by HSCs in culture as they become activated to a myofibroblastic phenotype (1). This proenzyme is activated at the surface of cells in a trimolecular interaction, which
involves formation of a complex with membrane type 1-MMP (MT1-MMP, also known as MMP-14) and TIMP-2. Recent studies have shown that culture-activated HSCs express all three components of this system and that formation of active gelatinase A is regulated by MT1-MMP expression and activity (2, 20). Because MT1-MMP is a transmembrane molecule, the implication is that active gelatinase A is formed at the cell surface of HSCs, where it would be well placed to disturb normal cell-matrix interactions. An interesting recent observation is that HSC-mediated activation of progelatinase A is significantly induced in the presence of collagen type I (the principal matrix protein found in fibrotic liver) (20). This could contribute to further degradation of the normal liver matrix, leading to increased activation of HSCs and increased synthesis of type I collagen. This positive feedback loop would theoretically promote progression of liver fibrosis (but see section on regression of liver fibrosis below).

In human liver disease and in animal models of liver fibrosis, there is evidence of increased expression of progelatinase A and increased formation of the active enzyme (18, 19). In patients with chronic hepatitis or cirrhosis, gelatinase A and MT1-MMP mRNA have been colocalized to activated HSCs (19), suggesting that the observations made in cultured HSCs are relevant in vivo in chronic liver disease. Further evidence for this is provided by temporal analysis of the CCl4 model of rat liver fibrosis, in which there is a clear relationship among progression of liver fibrosis, increased hepatic content of active gelatinase A, and an increased expression of progelatinase A, and an increased hepatic content of active gelatinase A, as determined by gelatin zymography (18).

Progelatinase B. Progelatinase B (MMP-9) is also found in liver, but the principal cellular source is the Kupffer cell (21). Progelatinase B expression is significantly increased with Kupffer cell activation, but the majority is secreted in the proenzyme form. Progelatinase B can be activated by plasmin andstromelysin. Because cultured HSCs express both urokinase-type plasminogen activator (uPA) and uPA receptor, which will, in the presence of plasminogen, generate plasmin (15), this combination could theoretically localize progelatinase B activation to the cell surface of HSCs. Increased gelatinase B activity has been reported in the livers of bile duct-ligated rats (14), suggesting that this enzyme may also play a role in liver fibrosis.

Prostromelysin 1. Prostromelysin 1 (MMP-3) is also secreted by HSCs in primary culture, but in contrast to gelatinase A, expression is transient, occurring principally in the first 48–72 h of culture. Prostromelysin expression is increased in the early phases of acute liver injury, with expression detected within 6 h and peaking at 48–72 h after injury (5). There have been no published data on expression of stromelysin in chronic liver injury and advanced liver fibrosis.

Although these enzymes have the ability to degrade normal liver matrix, there have never been conclusive data that demonstrate that degradation of this matrix actually does occur after liver injury or as liver fibrosis progresses. In fact, the relevant matrix proteins (type IV collagen, laminin, and proteoglycans) actually accumulate in fibrotic liver. There are several possible explanations for this apparent paradox. First, there may be matrix degradation in a pericellular environment with important consequences for HSC function, together with subsequent matrix deposition in a remote site. Second, the increased amounts of type IV collagen, laminin, and proteoglycans in fibrotic liver may not form a matrix that is capable of forming cell-matrix interactions with HSCs in the same way as normal liver matrix. Third, it is possible that the function of these enzymes may differ from that expected. For example, their target matrix substrate(s) in fibrotic liver may include different matrix proteins (see section on regression of liver fibrosis), or perhaps their proteolytic activity is directed against another class of substrate altogether. In other cell systems (keratinocytes and tumor cells) the description of an interaction between the cell surface molecule CD44 and active gelatinase B provides a recent example of this phenomenon (22). In this interaction, CD44 acts as a docking station on the cell surface for active gelatinase B, and this complex is then involved in activation of latent transforming growth factor (TGF)-β1, -2, and -3 to their profibrogenic forms. The same group described activation of latent TGF-β subtypes by active gelatinase A. Although it is not known whether such a system is in operation in the liver, this work suggests that there may be other potential profibrogenic mechanisms that are initiated by active metalloproteinases. Another example is provided by our recent observation (2) that active gelatinase A is involved in promoting HSC proliferation, through an unknown mechanism that was, however, dependent on metalloproteinase activity.

DEGRADATION OF FIBROTIC LIVER MATRIX

In fibrotic liver there is net deposition of fibrillar matrix that is comprised predominantly of the interstitial collagens, types I and III. These triple helical molecules are relatively resistant to protease activity but are cleaved at a specific Gly-Ile/Leu site in their α-chain by interstitial collagenases (MMP-1 in humans, MMP-13 in rats) and neutrophil collagenase (MMP-8). Other MMPs that can contribute to degradation of fibrillar collagens are discussed in more detail below (see section on regression of liver fibrosis). The active forms of these MMPs can be inhibited by all of the TIMPs, which are important regulatory molecules in tissue remodeling and repair.

Progression of liver fibrosis is associated with inhibition of matrix degradation. The hypothesis that progression of liver fibrosis is associated with inhibition of matrix degradation in liver is strongly suggested by studies of the relevant MMPs and TIMPs in cultured HSCs (9, 11, 12). In rat HSCs, MMP-13 was transiently expressed in the early stages of primary culture (days 1–4, in a similar pattern to stromelysin) but then decreased to become undetectable in late culture. In contrast, expression of TIMP-1 and TIMP-2 increases dramatically with duration of HSC culture. Similar
results were obtained in cultured human HSCs, except that MMP-1 expression was only observed if cells were exposed to tumor necrosis factor (TNF)‐α. Functional studies confirmed release of TIMP activity as demonstrated by reverse zymography, but, in addition, the importance of the TIMPs in inhibiting matrix degradation was demonstrated by experiments that separated TIMPs from MMPs in cell culture supernatants using affinity chromatography. In these studies, a 20-fold increase in detectable MMP activity was observed.

In chronic liver disease and in animal models of liver fibrosis, significant increases in TIMP-1 and TIMP-2 expression have also been observed. Studies of human liver explants (obtained from patients undergoing liver transplantation) have found increased expression of TIMP-1, TIMP-2, or both in patients with sclerosing cholangitis, biliary atresia, primary biliary cirrhosis, and autoimmune chronic active hepatitis (3, 11). These changes in mRNA for TIMP-1 were accompanied by proportional changes in TIMP-1 protein, which was increased approximately fivefold in fibrotic compared with normal human liver, as determined by ELISA. By in situ hybridization, TIMP-1 and TIMP-2 transcripts have been localized predominantly to HSCs in fibrotic human liver and in animal models of liver fibrosis (6). Animal studies have also provided insights into the temporal sequence of molecular events following liver injury from either bile duct ligation or CCl4 (6, 9). These studies demonstrated that expression of TIMP-1 and TIMP-2 is rapidly and significantly increased within 3–6 h of injury and remains persistently increased in the chronic phase of both models.

These changes in TIMPs contrast with those observed for either MMP-1 or MMP-13 in fibrotic liver, because their expression remains unchanged from that observed in normal liver at any stage of the fibrotic process. This also contrasts markedly with the changes reported for gelatinase A and MT1-MMP (described above). The increased expression of TIMPs precedes increased expression of procollagen I mRNA, suggesting that fibrillar matrix proteins are laid down into an extracellular environment in which matrix degradation is already inhibited.

In addition to their role in inhibiting matrix degradation, TIMPs have recently been shown to play a significant role in regulating apoptosis of some cell types. TIMP-1 has been demonstrated to inhibit apoptosis of B lymphocytes and breast epithelial cells (4, 16). In both studies, this effect appears to be independent of the effect of TIMP-1 on inhibition of MMP activity and of any effect on cell-matrix interaction. If TIMP-1 has the same effect on activated HSCs, this raises another possible mechanism by which TIMPs may contribute to the pathogenesis of liver fibrosis.

The evidence for a role for TIMPs in progression of liver fibrosis still rests largely on the observed strong association between TIMP expression and progression of disease and the compelling data in cultured HSCs. To obtain conclusive evidence for an important role for TIMPs in liver fibrosis, more studies are required in which TIMP expression is manipulated in vivo using transgenic, gene deletion, or other technologies. These studies should help to dissect the relative roles of TIMP-1, TIMP-2, and TIMP-3 and should contribute to determining the overall importance, or otherwise, of the TIMP family in liver fibrosis. This may require the application of technologies such as conditional gene deletion, dual gene deletion, and cell- or organ-specific transgenic approaches to fully elucidate the role of each of the TIMPs.

Regression of liver fibrosis is associated with increased matrix degradation. There is increasing recognition that liver fibrosis may resolve spontaneously after successful treatment of chronic liver disease, as occurs, for example, after clearance of hepatitis C virus or after withdrawal of experimental liver injury in animal models. In rats treated for 4 wk with twice-weekly intraperitoneal CCl4, significant liver fibrosis develops, with formation of fibrous septae and early nodule formation. If CCl4 treatment is continued for a further 4 wk, the fibrosis progresses to cirrhosis. If, however, the liver injury is discontinued after 4 wk, the liver fibrosis resolves completely and normal liver histology is restored (10).

This has proven to be a valuable model because it facilitates study of the cellular and molecular events that underlie the natural resolution of liver fibrosis. Two key observations were made in this study. First, the activated myofibroblast-like HSCs undergo apoptosis. The rate of HSC apoptosis, relative to cell proliferation, is dramatically increased after withdrawal of injury, leading to a 50% reduction in numbers of activated HSCs in the liver within 72 h of withdrawal of the injury. Second, there is an increase in the detectable level of “collagenase” activity in the liver (as determined in whole liver homogenate), which increases approximately fivefold and coincides temporally with the observed degradation of the fibrotic matrix. Further analysis revealed that the observed increase in hepatic “collagenase” activity was associated with a rapid and significant decrease in the level of expression of TIMP-1 and TIMP-2. In contrast, the level of expression of MMP-13 did not change but remained at a constant level throughout the regression phase of the model. The fall in TIMP-1 expression may simply relate to the massive level of apoptosis of activated HSCs (which are the major cellular source of this inhibitor), but the converse is also possible in that decreased TIMP-1 expression may theoretically contribute to increased HSC apoptosis (see above). The constant level of expression of MMP-13 suggests that activated HSCs are not the main cellular source of this enzyme, unless there is a specific subset of activated HSCs that are resistant to apoptosis and continue MMP-13 expression. An alternative and more likely explanation is that MMP-13 is derived from other cells, such as Kupffer cells (7).

There is a longstanding assumption that degradation of fibrillar matrix in liver (and the type I and III collagens that this contains) is mediated by the interstitial collagenases (MMP-1 in humans and MMP-13 in rats). Recent studies suggest that the true picture in
vivo may be significantly more complex and could involve other MMPs. Particularly noteworthy is the recent observation that MT$_1$-MMP (MMP-14) gene-deleted mice have a runted phenotype with skeletal dysplasia, arthritis, osteopenia, and generalized soft tissue disorders (8). These abnormalities were associated with evidence of increased fibrosis in the periosteal osteogenic layer of long bones, which occurred as a result of inadequate degradation of interstitial collagens. Skin fibroblasts prepared from MT$_1$-MMP-deficient mice and plated on a reconstituted type I collagen film were unable to degrade this matrix, unlike cells prepared from wild-type controls. Biochemical analyses have previously shown that MT$_1$-MMP can function as a “collagenase” capable of degrading collagen types I and III (17). In addition, gelatinase A has also been demonstrated to function as a “collagenase,” with cleavage of type I collagen occurring at the same Gly-Leu bond in the α-chains as described for MMP-1 and MMP-13 (13). Evidence of a role for gelatinase A in degradation of interstitial collagens in whole tissues is provided by studies of rabbit periosteal explants. In this model, type I collagen degradation correlated with gelatinase A (and not MMP-1) activity and was almost completely abolished (~80%) in the presence of specific gelatinase A inhibitors (13).

These studies raise the possibility that such alternative mechanisms may underlie degradation of fibrotic matrix in the liver. In contrast to MMP-1 and MMP-13, gelatinase A and MT$_1$-MMP are both expressed in culture-activated HSCs, and expression of both is increased in liver fibrosis (see section on degradation of normal liver matrix). These enzymes are both associated with the cell surface of HSCs in their active forms, situating them in an ideal location to play a significant role in pericellular degradation of fibrotic liver matrix. During regression of liver fibrosis, expression of both gelatinase A and MT$_1$-MMP gradually returns to baseline control values (unpublished observations) in a similar manner to that described for TIMP-1 and TIMP-2. If gelatinase A and MT$_1$-MMP are involved in degradation of fibrotic liver matrix, it is likely that the local balance of the active forms and the TIMPs in the pericellular environment will be critically important in determining the fate of fibrotic matrix. Further work is required to accurately dissect the relative roles of these different MMPs in degradation of the interstitial collagens in fibrotic liver, particularly during regression of liver fibrosis.

CONCLUSIONS

Through studies of cultured HSCs and molecular or biochemical analyses of fibrotic liver (human and animal models), there is now a significant body of evidence to indicate that liver fibrosis occurs as a consequence of alterations in matrix degradation, in addition to increased matrix synthesis. Much of the work over the past decade or more has described the key MMPs and TIMPs that are involved in this process. These studies have identified a key role for regulation of MMP activity in liver by TIMP-1 and TIMP-2 and have also identified several MMPs that are likely to be of major importance, including gelatinase A and B, stromelysin, MT$_2$-MMP, and the interstitial collagenses (MMP-1 and MMP-13). The prospective role of each of these MMPs and TIMPs in liver fibrosis is currently based mainly on a knowledge of their functions in cells and tissues other than liver, and there is little direct or conclusive evidence of their specific, detailed role in liver fibrosis. In addition to regulating degradation of extracellular matrix, newly described functions such as involvement in the activation of latent growth factors, inhibition of apoptosis, and effects on cell proliferation may all contribute to progression of liver fibrosis. Finally, there is increasing recognition that liver fibrosis is dynamic and involves phases of both progression and regression of this pathological process. There is good reason to suggest that degradation of fibrotic matrix in liver is complex and may possibly involve a combination of MT$_1$-MMP and gelatinase A in addition to the interstitial collagenses, with regulation mediated by local concentration of TIMPs. Further research is required to dissect the relative roles of each of these molecules in the pathogenesis of liver fibrosis in specific detail, because this will be important in the development of novel approaches to antifibrotic therapy.

REFERENCES


