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Cellular Sources of Extracellular Matrix in Hepatic Fibrosis

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Synopsis

The deposition of increased and abnormal extracellular matrix is the hallmark of liver fibrosis. Hepatic stellate cells are well known as the major source of the fibrillar collagens and other components of the liver scar, but it is now appreciated that they are only one of many potentially fibrogenic cell populations in the diseased liver. Portal fibroblasts as well as circulating mesenchymal cells derived from the bone marrow are also important sources of matrix proteins in fibrosis. Recent data suggest that hepatocytes and biliary epithelial cells undergo an epithelial to mesenchymal transition, similarly assuming a fibrogenic phenotype. Sinusoidal endothelial cells and hepatocytes both produce specific matrix proteins important in liver health and disease. The challenge of the future will be to define more explicitly the roles of these different fibrogenic cell populations in fibrosis in a disease-specific way.

Keywords

Collagen; hepatic stellate cells; portal fibroblasts; sinusoidal endothelial cells; liver fibrosis; extracellular matrix; epithelial to mesenchymal transition

I. Introduction

The extracellular matrix (ECM) is a complex and dynamic component of the liver which has multiple functions. ECM proteins have architectural and mechanical roles, providing tensile strength and resilience, modulating diffusion and vascular flow, and regulating cell movement. ECM mechanics are increasingly recognized as key determinants of normal and pathologic cell behavior.[1] ECM proteins regulate signaling, serving as ligands, storage depots, and receptors. Importantly, the interaction between the cells and the matrix of the liver is bidirectional. Most cells both produce matrix and respond phenotypically to that matrix. Defining the cellular sources of ECM in the normal and diseased liver is thus critical to understanding the pathophysiology of liver fibrosis.

The quantity of ECM in the fibrotic liver can be up to eight-fold higher than that of the normal liver. Perhaps even more dramatic are changes in the quality of the matrix expressed. There are significant increases specifically in the ECM proteins that make up the fibrotic scar, including the fibrillar collagens, which provide rigidity, and fibronectin splice variants and

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proteoglycans.[2] Basement membrane proteins are similarly increased, reflecting the capillarization of the sinusoids. Less well understood are changes in matrix protein modifications including glycosylation, intermolecular cross-linking, and glycosaminoglycan side chain sulfation.[3]

Understanding these changes in ECM proteins and the cells that synthesize them is critical to understanding fibrosis and, ultimately, to identifying new therapies. Although increasing evidence points to significant limitations in the "final common pathway" model of fibrosis, our knowledge of cell- and disease-specific matrix synthesis is rudimentary. This review summarizes the current literature on the cellular source of ECM proteins in fibrosis, highlighting recent advances that broaden the concept of the fibrogenic cell and outlining areas of ongoing and future research.

II. Stellate cells and other myofibroblast precursors

Myofibroblasts are the workhorses of fibrosis. Wound healing in general and pathologic tissue fibrosis in particular result primarily from myofibroblast-mediated ECM synthesis and deposition, and the state of the myofibroblast population in a diseased tissue reflects the direction of fibrosis. Myofibroblasts are contractile and secretory cells that express de novo the microfilament protein α-smooth muscle actin (α-SMA), which is used in practice as a myofibroblast marker.[4] There are three conditions required for myofibroblastic differentiation: high levels of the growth factor TGF-β, the presence of the fibronectin splice variant EDA, and increased local mechanical tension.[5] These factors act on a variety of potential myofibroblast precursors in the body, including fibroblasts and smooth muscle cells. An appreciation for the heterogeneity of the myofibroblast precursor population in the fibrotic liver is one of the most dramatic results of the research of the last decade.

a. Hepatic stellate cells

Hepatic stellate cells (HSC), the stellate-shaped, vitamin A-storing cells located in the Space of Disse, are without question the most studied fibrogenic population in the liver. HSC have important functions in liver development, metabolism, the immune response, and angiogenesis, but are best known for their key role in fibrosis.[6] Several seminal papers established that HSC-derived myofibroblasts produce the majority of the abnormal ECM – including fibrillar collagens and basement membrane proteins – in the fibrotic liver, particularly from non-biliary disease.[7–9] Although many studies over the last decade failed to use appropriately HSC-specific markers, the overwhelming consensus of the extensive HSC literature, summarized in several excellent recent reviews, is that HSC are highly fibrogenic participants in liver fibrosis.[10–12]

HSC are easily isolated from normal liver and undergo spontaneous myofibroblastic differentiation when cultured under standard conditions. This model system has resulted in the detailed characterization of the ECM deposited by HSC myofibroblasts, although HSC populations are recognized to be heterogeneous. HSC produce most of the major and minor matrix proteins of the fibrotic liver including fibrillar and non-fibrillar collagens, components of the organized sinusoidal basement membrane (collagen IV, laminin, and perlecan), cellular fibronectin, tenascin-C, the elastic fibril component fibrillin, and many of the small proteoglycans (see [2], [10–12] and references therein).

b. Portal fibroblasts and other resident liver fibroblast populations

One of the notable controversies in the hepatology literature over the last decade has been whether or not non-HSC-derived myofibroblasts contribute to fibrosis. Several authors have now convincingly and elegantly demonstrated in rodent model systems that there are

fibrogenic, α-SMA-positive myofibroblast populations that are distinct from HSC by marker analysis.[13, 14] These cells, many of which are derived from portal fibroblasts, appear to be particularly abundant in biliary fibrosis.[14–17] In one important recent study, the livers of rats with biliary fibrosis of two different etiologies (bile duct ligation and arterial ischemia) were immunostained for α-SMA, as a marker for myofibroblasts, and desmin, as a marker for HSC. The majority of myofibroblasts in fibrotic regions were found to be desmin negative, derived from portal mesenchymal cells rather than HSC.[14] The matrix expression profile of portal fibroblasts and myofibroblasts has not been investigated with the exception of one study that demonstrated upregulation of the α1 subunits of collagens I, III, and IV when primary portal fibroblasts underwent myofibroblastic differentiation in vitro.[18] Portal myofibroblasts are likely, however, to secrete a broad variety of ECM proteins. Many of the studies examining HSC matrix expression in vivo used α-SMA as a marker of fibrogenic cells, and thus the conclusions are applicable to portal myofibroblasts as well as HSC.

Resident non-portal fibroblast populations in the liver that may activate to myofibroblasts and contribute to fibrogenesis include the fibroblasts of Glisson's capsule, smooth muscle cells of the vasculature, and so-called second layer cells around central veins.[19] In some forms of fibrosis, there is also a significant population of α-SMA-negative activated fibroblasts.[20, 21] The matrix secreting profile of these cells has not been studied, and their relative contributions to liver diseases of different etiologies is not known.

c. Bone marrow derived myofibroblast precursors

Although fibrogenic cells in the liver have been assumed to arise from resident populations, recent evidence suggests that circulating cells derived from the bone marrow contribute, possibly significantly, to fibrogenesis. One group demonstrated the presence of green fluorescent protein (GFP)-positive HSC in the livers of mice that had received bone marrow transplants with GFP-expressing cells. These HSC could undergo myofibroblastic differentiation in culture, and appeared to deposit collagen.[22] In human liver specimens from patients who received gender mismatched bone marrow or liver transplants, there also appeared to be a significant population of marrow-derived myofibroblasts.[23] An elegant study using mice which had undergone bone marrow transplants followed by CCl_4- or thioacetamide-induced liver injury demonstrated that close to 70% of HSC and myofibroblasts in the fibrotic liver were derived from the bone marrow, and that these cells produced type I collagen.[24] Other groups, however, have been unable to identify HSC derived from the bone marrow and instead have observed a population of fibrocytes (bone marrow-derived circulating mesenchymal cell precursors) that appear to undergo myofibroblastic differentiation and deposit collagen after lodging in the liver.[25] The role of circulating cells in the fibrotic response to damage is thus yet to be definitively determined.

III. The fibrogenic hepatocyte controversy

Hepatocytes are the major cell population in the liver and a significant potential source of ECM proteins. The role of hepatocytes in fibrogenesis was the source of intense debate in the literature through the mid 1990s. Although the controversy was initially resolved in favor of myofibroblasts as the primary matrix producing cells of the liver, recent reports suggest that hepatocytes can undergo an epithelial to mesenchymal transition (EMT) and may contribute to fibrogenesis in the form of fibroblasts and myofibroblasts.

Throughout the 1980s and early 1990s, different groups arrived at different conclusions about the role of hepatocytes in the deposition of fibrillar collagens (collagen I and III) and basement membrane proteins (collagen IV, laminin, and perlecan) in fibrosis (for review, see refs. [8, 26]). Definitive identification of the cells responsible for depositing specific ECM proteins proved difficult using tissue sections because secreted matrix proteins can adhere to adjacent

cells, while cells in culture have a propensity to adopt new and potentially non-physiologic phenotypes. Several studies employing different techniques to overcome these limitations were particularly influential in demonstrating ultimately that mature hepatocytes have little if any role in the synthesis of fibrillar collagens and basement membrane proteins. In situ hybridization, alone or in combination with immunostaining to mark specific cell populations, yielded no evidence that hepatocytes as opposed to non-parenchymal cells expressed collagen I, III, or IV in CCl_4-induced fibrosis in the rat or in human livers affected by variable degrees of fibrosis from a variety of causes (including alcoholic, biliary, and viral).[9, 26, 27] Similarly, mRNA expression analyses of freshly isolated, non-cultured hepatocytes, sinusoidal endothelial cells, and HSC demonstrated minimal synthesis of laminin or collagens I, III, or IV by hepatocytes from either normal, bile duct ligated, or CCl_4-treated livers.[8]

New data have again raised the specter of the fibrogenic hepatocyte. This work is based on studies from the renal fibrosis field demonstrating that tubular epithelial cells undergo EMT, and that such cells comprise a large percentage of fibrogenic cells in the diseased kidney. Initial studies on fibrotic kidneys showed that renal tubular epithelial cells expressed fibroblast-specific protein-1 (FSP1, also known as S100 A4), believed to be both a mediator and a marker of EMT.[28] Tubular epithelial cells also expressed HSP47, a collagen-specific chaperone indicative of ongoing collagen synthesis, and the myofibroblast marker α-SMA.[28, 29] Lineage tracing studies, in which transgenic reporter mice were used to identify all epithelial cell descendents, provided definitive evidence in support of the contribution of EMT to kidney fibrosis: in one model, 36% of FSP1-positive fibroblasts in the fibrotic kidney were derived from epithelial cells.[30]

Both neonatal and adult hepatocytes can undergo EMT in culture, losing epithelial characteristics and becoming α-SMA-expressing myofibroblasts.[31–35] Hepatocyte EMT in vivo is less well established. Human explant livers from patients with a variety of biliary and non-biliary diseases exhibited no colocalization between hepatocyte markers and the EMT markers FSP1, HSP47, and α-SMA.[36] Lineage tracing studies using transgenic mice in which GFP was expressed under the alpha-fetoprotein promoter failed to demonstrate evidence of α-SMA expression in labeled cells two weeks after bile duct ligation (unpublished results). A recently published lineage tracing study using transgenic mice expressing β-galactosidase under the control of the albumin promoter did show, however, that hepatocytes undergo EMT in CCl_4-induced fibrosis.[37] The authors of this work suggested that there is a significant population of hepatocyte-derived, FSP-1-positive, α-SMA-negative fibroblasts in the fibrotic liver. Further work will be required to determine the magnitude and timing of the contribution of these cells to ECM synthesis in fibrosis.

Hepatocytes are the major source in the body of plasma fibronectin, one of the two major products (along with cellular fibronectin) of the fibronectin gene.[38] In the normal liver, plasma fibronectin is the most abundant matrix protein in the Space of Disse, coating hepatocyte membranes and collagen fibrils.[39, 40] In the setting of fibrosis, fibronectin increases dramatically and is one of the first matrix proteins to do so; however, almost all of this increase is in cellular fibronectin.[40] While cellular fibronectin plays an important role in myofibroblastic differentiation, the function of plasma fibronectin in either the normal liver or in the context of the altered milieu of the injured liver is not well understood.

IV. Biliary epithelial cells

Defining the role of biliary epithelial cells (BEC) in fibrosis and, more specifically, their direct contributions to fibrogenesis is an area of intense and evolving research. Whereas the major contribution of BEC to matrix synthesis (in both the normal and diseased liver) was once thought to be limited to the production of basement membrane, new research suggests that

BEC may play a direct role in synthesis of the fibrotic scar, particularly in conditions where there is a ductular reaction.

BEC, in contrast to hepatocytes, rest on a fully organized basement membrane. In vivo and in vitro data indicate that this basement membrane is synthesized by both portal mesenchymal cells on one side and by BEC on the other.[26, 41] In culture, human BEC isolated from normal cystic ducts demonstrated intense immunoreactivity for two major components of the basement membrane, collagen IV and laminin [42]. BEC in normal and fibrotic rat and human liver tissue (from patients with a variety of liver diseases including viral, alcoholic, and biliary) expressed mRNA for the B1 chain of laminin, the α1 chain of collagen IV, and the basement membrane proteoglycan perlecan.[26, 43] BEC, along with hepatocytes, also synthesize collagen XVIII, a newly appreciated collagenous component of the basement membrane which is a precursor of the angiogenesis inhibitor endostatin.[44] Synthesis of basement membrane components by BEC was observed in normal as well as fibrotic liver, with slight increases seen in fibrosis[26, 41].

Studies have consistently shown that BEC do not directly synthesize significant amounts of the fibrillar collagens or fibronectin [26, 41]. In the last several years, however, data on the role of EMT in renal fibrosis (see section III above) has raised the possibility that EMT also contributes to liver fibrosis. It has been known for at least 20 years that, in the setting of biliary injury, newly formed BEC (especially cells within the ductular reaction) express the intermediate filament vimentin and it has been postulated that this reflects “cellular reorganization” of BEC.[45] Recent data suggest that this reorganization reflects EMT, with BEC in the damaged liver undergoing a process analogous to that of renal tubular epithelial cells in the damaged kidney. Preliminary experiments with BEC in vitro and in vivo are suggestive of EMT, although it is important to note that definitive lineage tracing studies have not been reported.

EMT is driven largely by TGF-β, and multiple studies have now demonstrated that TGF-β-treated BEC in culture undergo EMT and exhibit changes including the acquisition of a myofibroblast-like morphology and de novo expression of α-SMA and collagen I.[46, 47] As is the case for hepatocytes, in vivo studies are less definitive. In the mouse, CK19-positive BEC 12 weeks after bile duct ligation demonstrated synthesis of collagen I as well as morphological changes and basement membrane disruption consistent with EMT.[47] Similarly, in human liver tissue taken from patients with a variety of different diseases, BEC were shown to co-express epithelial cytokeratin markers and FSP-1, HSP47, and vimentin, with associated cytoplasmic redistribution of E-cadherin and nuclear localization of one or both of the TGF-β downstream signaling molecules Smad2 and Smad3.[36, 46, 48] The co-localization was particularly marked in small ducts and cells of the ductular reaction, and in diseases like primary biliary cirrhosis and biliary atresia in which the ductular reaction is prominent.[36, 46, 48] Few cells in any of these in vivo analyses co-expressed epithelial markers and α-SMA, and evidence for direct fibrogenesis is largely circumstantial (for example, expression of the collagen chaperone HSP47).[49] It is not yet clear whether expression of all mesenchymal markers is required to demonstrate the presence of fully fibrogenic cells. Thus the data so far are consistent with mesenchymal changes, but not yet full EMT. Lineage tracing studies are required before concluding definitively that biliary EMT occurs. Additionally, such studies are needed to determine the relative contribution of EMT to early and late stages of fibrosis.

V. Sinusoidal endothelial cells

Sinusoidal endothelial cells (SEC) line the sinusoids, in constant contact with sinusoidal blood flow and in close association with hepatocytes, HSC, and Kupffer cells. Changes in SEC can be detected significantly before fibrosis is visible by light microscopy,[36, 50, 51] leading some authors to suggest that SEC might drive or even initiate fibrosis.[43, 51, 52] The study of SEC

in fibrosis has been hindered, however, by controversies regarding their isolation, culture, and identification,[53] and correlating in vivo and in vitro findings has proven particularly difficult. Additionally, SEC have a high endocytic capacity and are located adjacent to highly fibrogenic HSC, making the interpretation of immunostains prone to error.

Whether or not SEC synthesize fibrillar collagens and other components of the fibrotic scar is controversial. Early studies showed that isolated SEC expressed mRNA for collagens I and III, and that SEC isolated from fibrotic liver had higher type I collagen expression than cells from normal liver.[8] More recent studies have questioned the contribution of SEC to the production of the fibrillar collagens, [26] and the literature as a whole suggests that in most cases, HSC and other myofibroblasts, not SEC, produce the bulk of the fibrotic ECM.

SEC are likely, however, to play two important roles in fibrosis, particularly in the early stages before HSC undergo myofibroblastic differentiation. First, SEC have an important role in capillarization of the sinusoids. This process, characterized by the loss of typical SEC fenestrations and the formation of an organized basement membrane in the Space of Disse, has been recognized as one of the hallmarks of liver fibrosis since it was first described in 1963.[54] The literature is unclear on the relative contributions of HSC and SEC to this process, but it is clear via a variety of techniques that SEC secrete significant amounts of the components required for an organized basement membrane including collagen IV, laminin, entactin, and perlecan.[8, 26, 43, 55–60] SEC appear to be the major perisinusoidal source of perlecan, a large and complex heparan sulfate proteoglycan that binds other matrix components including fibronectin.[43, 61] The formation of an organized basement membrane with both different organization and different type IV collagen composition results in hepatocyte dedifferentiation and dysfunction and may in this way facilitate hepatic injury and the perpetuation of fibrosis.[50, 62]

The second important role attributed to SEC is the production of fibronectin EDA, a splice variant of cellular fibronectin expressed primarily during development and in response to injury. In vitro studies suggest that fibronectin EDA, acting in concert with the potent profibrogenic mediator TGF-β, is necessary for the general process of fibroblast to myofibroblast differentiation.[5, 63] Fibronectin EDA is produced early in rodent models of fibrosis, preceding collagen deposition, and it persists at high levels in advanced fibrosis.[40, 64] Fibronectin EDA induces the myofibroblastic differentiation of HSC in culture, and it has been elegantly demonstrated that TGF-β acts on SEC to rapidly upregulate production of fibronectin EDA, thus linking TGF-β, SEC, and HSC activation.[64, 65] Treatment of SEC using albumin modified with malondialdehyde-acetaldehyde (an ethanol byproduct) resulted in increased fibronectin EDA expression, suggesting multiple potential inducers of SEC.[66] Although HSC themselves, and potentially hepatocytes as well, produce fibronectin EDA, SEC are the first responders and therefore may play a crucial role in the early stages of fibrosis.[67–69] Why fibronectin EDA, unlike plasma fibronectin or other forms of cellular fibronectin, is able to induce myofibroblast activation is unknown.

VI. Summary

The cellular basis of liver fibrosis appeared a decade ago to be a closed question. HSC had been shown to be the source of fibrillar collagens and basement membrane proteins, and the fibrosis field moved on to study HSC regulation. Today, HSC are still considered to be important myofibroblast precursors in the diseased liver; however, the recent literature has forced a reevaluation of the view that HSC are the dominant fibrogenic cells in all forms of fibrosis. Other mesenchymal cell populations, most notably portal fibroblasts and circulating cells from the bone marrow, are emerging as significant matrix producing cells. Hepatocytes and BEC, previously relegated to minor roles in fibrosis, are hypothesized to undergo EMT

and participate in fibrogenesis in the form of myofibroblasts. The challenges of the next decade are to define the role of different fibrogenic cells in different forms of fibrosis, to characterize more systematically the ECM produced by distinct fibroblast and myofibroblast populations, to define the source and function of newly appreciated and minor matrix proteins, and to identify the cell-based, soluble, and mechanical factors underlying fibrogenic cell differentiation.

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