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2.4.3 Function and metabolism of collagen and other extracellular matrix proteins

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Introduction

The extracellular matrix (ECM) occupies a small percentage of the volume of the normal liver, yet plays a disproportionately important role in liver function in health and disease. ECM proteins are both signalling molecules and architectural elements of the liver and are responsible for maintaining the differentiated state of normal hepatocytes and non-parenchymal cells. In liver fibrosis and cirrhosis, there are changes in the distribution, quantity and relative proportions of collagens and other ECM proteins (Fig. 1); these result in altered cell phenotypes, architectural distortion with abnormal blood flow, impaired diffusion and altered cell signalling.

ECM proteins in the normal liver

The normal liver encompasses physically separate regions with different matrix and cellular compositions. The matrix in the capsule and surrounding the bile ducts and central venous region is similar to that in other epithelial organs, with an organized basement membrane of collagen IV, laminin, entactin and perlecan. The interstitium of the portal space contains the fibrillar collagens I, III and V as well as collagen VI and fibronectin [1].

The space of Disse, which lies in between sinusoidal endothelial cells (SEC) and hepatocytes, has a matrix unique in the liver and the body. This narrow space, less than 1 μM wide, lacks the continuous laminin, perlecan and entactin that are found in most basement membranes [2]. Although collagen IV is present, it is in discrete discontinuous deposits not associated with laminin or perlecan. Fibronectin is abundant, applied closely to the microvilli of hepatocytes. Collagens III and VI are also found in the space of Disse, collagen III in discontinuous deposits and collagen VI arranged relatively homogeneously, increasing from the portal to the central region [3]. Structure is provided by

a continuous network of thick collagen I cables, which extend into the lobular areas from the adjacent portal tracts [1]. This unusual matrix is essential for maintaining the differentiation of hepatocytes and SEC as well as other non-parenchymal cells [4]. Gradients of matrix material are found in the sinusoids and may have functional relevance, resulting in phenotypical differences between cells in the periportal vs. central regions [1,5].

ECM proteins in the fibrotic liver

Fibrosis results in a nearly 10-fold increase in the expression of matrix proteins in the liver. The most impressive change is the capillarization of the sinusoids, in which the sparse, atypical matrix of the space of Disse is replaced by a complete and continuous basement membrane, with accompanying loss of fenestration of the sinusoidal endothelium. This process begins with an increase in cellular isoforms of fibronectin in the space of Disse, followed by increases in collagens I, III and IV and the appearance of laminin. As fibrosis progresses, portal to central gradients are lost, and the new matrix becomes continuous [6] (see also Chapters 4.1 and 6.1). This results in loss of the differentiated phenotype of the resident cells of the space of Disse, in particular SEC, hepatocytes and hepatic stellate cells (HSC). Additionally, the increased ECM impairs the normal exchange of soluble proteins and fluids between sinusoidal blood and adjacent liver cells.

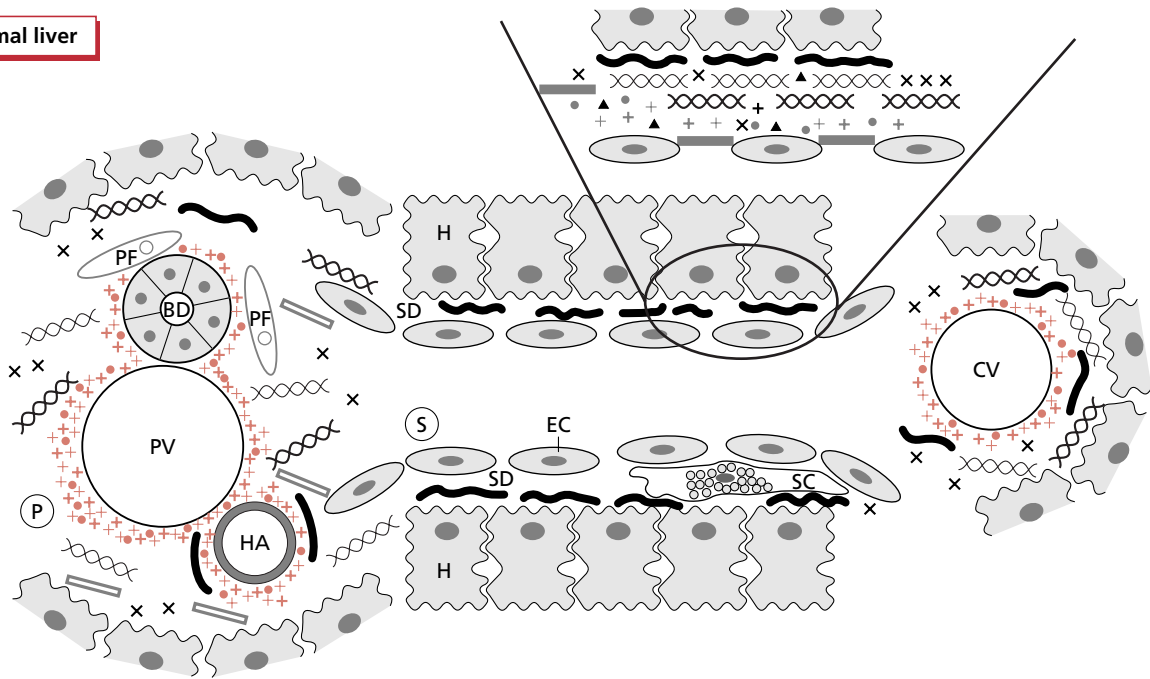
When fibrosis advances to cirrhosis, the normal architecture of the liver is lost with the formation of increasingly dense fibrous septae of fibronectin and collagens I, III, V and VI. These bands of matrix may become progressively stabilized (for example through collagen cross-linking) and protease resistant, impeding remodelling and the resolution of cirrhosis.

Structure and key features of specific ECM proteins

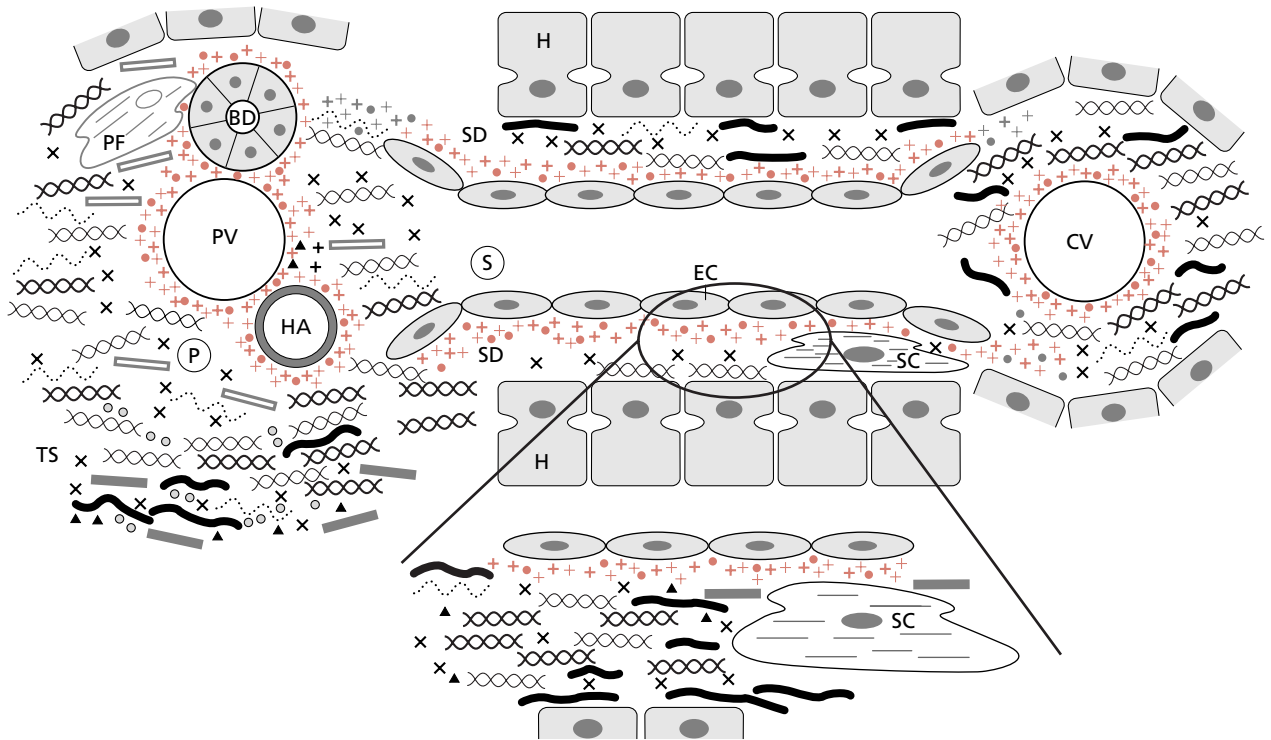
The ECM and its individual components are multifunctional. They have architectural and barrier functions, regulate growth factors and are themselves signalling molecules (Table 1). There are multiple complex interactions between different matrix components, making it difficult to understand the function of individual proteins. A variety of related mesenchymal cells in the liver synthesize normal and pathological matrix, suggesting that fibrosis is best understood as a generalized process rather than one limited to specific ECM molecules or specific fibrogenic cells such as HSC (see also Chapters 6.1 and 6.2).

Fig. 1 (*opposite*) Distribution of major matrix components in normal and fibrotic liver. A liver acinus is shown graphically to demonstrate changes in extracellular matrix (ECM) distribution in normal and diseased liver. Note that continuity between portal, parenchymal and central structures is not shown due to controversy regarding their relative anatomy. The size of the space of Disse compared with the sinusoidal space is greatly exaggerated, particularly in the normal liver. The interrelationship between different ECM components is not well understood and is shown only schematically. The organized basement membrane shown contains collagens VIII, XIX, XV, XIV and XVIII in addition to collagen IV, laminin, perlecan, and entactin. Proteoglycans other than perlecan are not shown. PV, portal vein; CV, central vein; HA, hepatic artery; SC, hepatic stellate cell; EC, sinusoidal endothelial cell; PF, portal fibroblast; SD, space of Disse; BD, bile duct; H, hepatocyte; TS, thick, organized septa; S, sinusoid; P, portal tract.

Normal liver



Fibrotic liver



xxxxxx	Collagen I	} often + Laminin + Collagen IV • Perlecan	x	Collagen VI	~	Fibronectin	—	Fibrillin
xxxxxx	Collagen III		+	Organized basement membrane	oo	Collagen XIV	—	Fibrillin/elastin fibre, associated with collagen VIII
.....	Collagen V		+		▲	Tenascin-C		

Table 1 Functions of ECM proteins in the liver.

Mechanical and architectural
Tensile strength
Resilience
Scaffolds for supramolecular assemblies
Anchors and connectors for blood vessels, nerves, other cells and other matrix components
Filtration barrier
Migration substrates and barriers
Signalling
Ligands for integrins and other signalling receptors
Signalling receptors and coreceptors
Sources of biologically active fragments (regulation of growth and apoptosis)
Sequestration and targeting of growth factors
Damage sensors

Collagens

Collagen structure and synthesis

The major structural proteins in both normal and fibrotic liver are collagens, ECM proteins with sizeable domains of Gly-Xaa-Yaa repeats, where X and Y are often proline and hydroxyproline respectively. This basic structure, with a central glycine and a rotation-limiting proline, allows the formation of a rigid triple helix (Fig. 2). Variations in chain composition among collagen family members are important for function, allowing differences in structural characteristics and network formation as well as different interactions with proteoglycans, receptors, growth factors and other ECM proteins in supramolecular assemblies (Table 2).

The fibrillar collagens (see below) are the most abundant collagens in the liver, and their synthesis is potentially regulated at multiple steps. Although the topic is beyond the scope of this

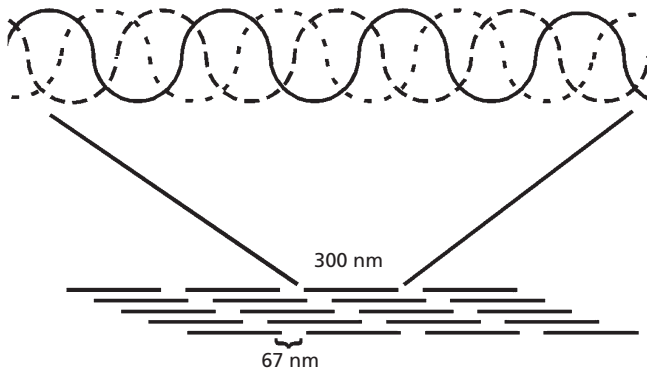


Fig. 2 Fibril arrangement of the fibrillar collagens. Collagen chains made up of repeating Gly-Xaa-Yaa motifs form right-handed triple helices 300 nm long (top). The hydrogen atom side-chains of the glycine residues pack into the centre of the triple helix; the rigid helical geometry is maintained by the proline and hydroxyproline residues, which usually occupy the second and third positions of the repeat. Fibrils of collagens I, III and V are formed by the staggered, parallel packing of many triple helices with a periodicity of 67 nm (bottom).

chapter, the fibrillar collagens are subject to significant transcriptional and posttranscriptional regulation; transcription of the collagen $\alpha 1(I)$ chain in particular is regulated by complex interactions involving both 5' stem-loop and 3' untranslated region (UTR) binding proteins [7]. The fibrillar collagens are synthesized with N- and C-terminal propeptide sequences, the latter playing an important role in protein folding. Nascent chains undergo several kinds of posttranslational modification: vitamin C-dependent proline hydroxylation by prolyl-4-hydroxylase on the polypeptides, which is essential for the stability of the triple helix, hydroxylation on lysine residues by lysyl hydroxylase, which is important for fibril packing, and N- and O-linked glycosylation. After secretion into the extracellular space, collagen chains are cleaved by N- and C-propeptidases and form fibrils. Fibril formation is also modulated at multiple levels including retention of the N-terminal propeptides of collagens III and V, which can regulate lateral fibril growth, and association with the FACIT collagens or decorin (see below), which can regulate fibril thickness [4].

Collagen cross-linking enzymes

Mature collagen fibrils undergo interchain cross-linking, which is essential for mechanical stability. There are three major families of enzymes important in collagen cross-linking; these enzymes also participate in cross-linking of other matrix molecules including elastin and fibronectin. Tissue transglutaminase is a multifunctional protein which mediates the formation of ϵ -(γ -glutamyl lysine) cross-links in collagens and other ECM molecules, rendering them resistant to proteolysis [8]. It is increased in transdifferentiating HSC in culture and is found in myofibroblasts in the fibrotic liver [9–11]. Cross-links typical of tissue transglutaminase action have been demonstrated in the residual fibrous septae of rats with partially resolved fibrosis, suggesting that cross-linking modulates matrix degradation and fibrosis resolution [12] (see also Chapter 6.1).

The lysyl oxidases (LOX) catalyse the oxidative deamination of lysine and hydroxylysine in collagen and of lysine in elastin, leading to the formation of aldehydes, which then condense with neighbouring groups to form covalent cross-links. Although present in the normal liver, LOX family members increase dramatically in fibrosis, including very early after injury and before fibrogenic myofibroblasts appear [13,14]. LOX-mediated cross-links render collagens resistant to degradation and may also play a causal role in the initiation of fibrosis [13,15].

Lysyl hydroxylases catalyse the conversion of lysine into hydroxylysine and thereby determine the route of collagen cross-linking by LOX. The hydroxylysine route appears to be particularly important in diminishing the susceptibility of collagen to proteolysis and is the major pathway for cross-link formation in human cirrhosis [7,16,17].

Collagens in the liver

Ten collagens have been identified in the adult liver. These are divided into two groups based on structure, the fibrillar and the

Table 2 Collagens of the liver.

Type	Composition	Structure
Fibrillar		
I	$[\alpha 1(I)]_2[\alpha 2(I)]$	300-nm rigid fibrils, 67-nm periodicity Uninterrupted triple helices Forms composite structures with collagens III and V Non-collagenous N- and C-terminal propeptides cleaved before fibril assembly
III	$[\alpha 1(III)]_3$	Same as for collagen I
V	$[\alpha 1(V)]_2[\alpha 2(V)]$	Same as for collagen I
Non-fibrillar		
Network forming		
IV	$[\alpha 1(IV)]_2[\alpha 2(IV)]$	400-nm-long chains Triple helix with multiple interruptions Forms filamentous network via N- and C-terminal and lateral associations
VI	$[\alpha 1(VI)][\alpha 2(VI)][\alpha 3(VI)]$	105-nm triple helix Large N- and C-terminal globular domains with von Willebrand factor (VWF) A and fibronectin (Fn) type 3 repeats Forms antiparallel dimers, then tetramers and networks
VIII	$[\alpha 1(VIII)]_2[\alpha 2(VIII)]$	130-nm-long chains Interrupted triple helices Large C-terminal and small N-terminal globular domains Forms hexagonal lattices in some tissues
FACITS (fibril associated with interrupted triple helices)		
XIV	$[\alpha 1(XIV)]_3$	Two short collagenous domains with three non-collagenous domains Glycosaminoglycan (GAG) modified N-terminal domain with Fn type 3 repeats and VWF A domains Associates with surface of collagen fibrils
XIX	$[\alpha 1(XIX)]_3$	Five short collagenous and six non-collagenous domains Associates with surface of collagen fibrils
Multiplexins (multiple triple-helical domains and interruptions)		
XV	$[\alpha 1(XV)]_3$	Interrupted triple helix Large N- and C-terminal globular domains Chondroitin sulphate GAGs Highly homologous to collagen XVIII
XVIII	$[\alpha 1(XVIII)]_3$	Interrupted triple helix Large N- and C-terminal globular domains Heparan sulphate GAGs Cleavage of C-terminal domain NC 1 generates endostatin

non-fibrillar collagens (see Table 1). Although the fibrillar collagens, particularly collagens I and III, are generally regarded as the most important in fibrosis on the basis of their quantity as well as mechanical characteristics, it is now understood that other collagens also contribute to fibrosis in important ways.

Fibrillar collagens (I, III and V)

The fibrillar collagens I, III and V all increase significantly in fibrosis; together with collagen IV, they are the most abundant ECM proteins in the liver. They are mechanically important, contributing tensile strength. The fibrillar collagens do not form separate structures but, rather, may be incorporated into

composite fibrils, with the relative contribution of each collagen determining the diameter and mechanical properties of the fibril as well as the sensitivity of the fibril to different proteases [4,18,19].

Collagen I has few interruptions in the Gly-Xaa-Yaa structure, enabling it to form rigid triple-helical fibrils, with three amino acids per turn. Many other matrix molecules (including collagens III, V, VI, fibronectin and proteoglycans) are found on the surface of collagen I. Collagen III is structurally similar. It is the first collagen to increase in chronic liver disease; although later replaced in part by collagen I, it remains highly expressed [20]. Collagen V is an abundant collagen that acts as a connector to

other collagen types and may be important in initiating the growth of other collagen fibrils [21,22].

Non-fibrillar collagens

The collagens in this group do not form fibrils but, instead, contain combinations of collagenous and non-collagenous domains that enable them to form a variety of different three-dimensional structures. The non-fibrillar collagens in the liver can be divided into three groups based on structure: the network-forming collagens, the FACITS (fibril-associated with interrupted triple helices) and the multiplexins (multiple triple-helical domains and interruptions).

Network-forming collagens (IV, VI, and VIII) The network-forming collagens have many interruptions in their triple-helical chains, which give them flexibility. This flexibility enables them to make linear, axial and lateral associations and so form networks, not fibres [23]; it also allows them to interact with multiple other proteins and macromolecules.

Collagen IV, the main component of most basement membranes, has many variants, although in liver it occurs almost exclusively in the form $[\alpha 1(IV)]_2[\alpha 2(IV)]$. The ends of this collagen cross-link to enable the formation of a three-dimensional network, which anchors and stabilizes other basement membrane components, including perlecan and laminin. Collagen IV is synthesized primarily by endothelial cells, suggesting an active role for these cells in the capillarization of the sinusoids [24].

Collagen VI is a heterotrimeric collagen that forms branching filamentous networks and binds to many additional matrix proteins [4,23,25]. It surrounds the fibres of loosely packed collagens I and III and may anchor structures such as nerves, blood vessels and other cells into place, in part by interconnections with collagen IV in endothelial cell basement membranes [25,26]. Collagen VI is increased up to 10-fold in liver fibrosis. Soluble collagen VI is increased in the circulation of patients with chronic liver disease, which can promote mesenchymal cell (and potentially HSC) proliferation, and is also a potent inhibitor of apoptosis. For these reasons, collagen VI has been proposed as a sensor for tissue damage, with the release of the soluble form stimulating proliferation of surrounding cells and wound healing [4,27,28].

Collagen VIII forms tetrahedral assemblies and, in some cases, hexagonal lattices [23]. It appears to be associated with the basement membrane as well as the elastic fibres of the portal triad, and may serve as a bridge between other matrix molecules. It is especially important in the vasculature, playing a role in angiogenesis [29–31]. Although collagen VIII has been minimally studied in the liver, in other injury models it stimulates smooth muscle cell migration and matrix metalloproteinase (MMP) synthesis and thus may be involved in differentiation and remodelling in wound healing [29,32].

FACIT collagens (XIV, XIX) The FACITs consist of alternating short triple-helical domains and non-triple-helical domains

and, as a result, are highly flexible. Rather than forming fibrils themselves, they associate with pre-existing fibrils of other collagens.

Collagen XIV (also known as undulin) is widely expressed in liver, particularly on the surface of mature, dense fibrils of collagens I and III; it is not seen in the disorganized tissue of actively fibrogenic regions [33,34]. Interestingly, the procollagen I N-proteinase (which cleaves the N-propeptide of collagen I) is bound to collagen XIV, raising the possibility that collagen XIV provides spatial control in regulating the growth of the fibrillar collagens [35]. In the liver, collagen XIV is absent from the sinusoidal space, but is abundant in the portal tract. It increases in fibrosis and can suppress proliferation of HSC and fibroblasts; these effects, as well as its association with dense collagen fibres, suggest a role in established rather than ongoing fibrosis [4]. The other FACIT collagen in the liver, collagen XIX, is primarily found in basement membranes and, to a small extent, in the sinusoids [36]. It has a significant vascular association and may be involved in angiogenesis.

Multiplexin collagens (XV, XVIII) The multiplexin collagens XVIII and XV are structurally homologous proteoglycans with central triple-helical domains and multiple non-collagenous regions which give them flexibility. Collagen XVIII derived from tissues is heparan sulphate linked, whereas collagen XV has chondroitin sulphate modifications [37–39]. Both are found in basement membranes, where they may organize the basement membrane and link it to the underlying matrix.

Collagen XVIII is unusual in that it is increased only twofold in diseased liver compared with normal liver. It is synthesized primarily by hepatocytes and biliary epithelial cells in both states, and is localized in a continuous distribution in both basement membranes and the perisinusoidal space [36,40,41]. Most of the increase in collagen XVIII in disease is along sinusoids that have undergone capillarization, suggesting that it may contribute to this process. Collagen XVIII is also found in the diseased liver in the basement membrane surrounding ductular hepatocytes, proliferating bile ductules and cirrhotic nodules; this distribution implies a potential role in ECM breakdown and remodelling, as well as the ductular reaction and angiogenesis [40,41]. Intact collagen XVIII supports endothelial cell survival, migration and proliferation, and also has the potential to sequester heparan sulphate and heparan sulphate-binding growth factors [31]. The C-terminal globular domain of collagen XVIII can be cleaved by a variety of proteases including MMPs and elastases to generate endostatin, a heparin-binding protein that inhibits angiogenesis and mediates apoptosis [39].

Non-collagenous proteins of the ECM

Fibronectin

Fibronectin is an abundant and widely distributed ECM glycoprotein with multiple domains, including a heparin-binding N-terminus, a collagen-binding domain and an Arg-Gly-Asp

(RGD) motif-containing cell-binding domain with multiple repeated sequences termed type 3 repeats. Unlike many other matrix components, assembly of fibronectin is driven by interaction with its integrins (via the cell-binding domain), suggesting that it plays a particularly important role in the interaction between cells and the surrounding ECM. In the normal liver, fibronectin is found in the portal region around basement membranes and also in a perisinusoidal, speckled pattern; it is the most abundant ECM protein in the normal space of Disse, coating hepatocytes as well as normal collagen fibrils [2]. With its multiple domains, fibronectin probably functions to connect the surfaces of both endothelial cells and hepatocytes to collagen bundles. Fibronectin is one of the first matrix proteins to increase in fibrosis, with significantly increased deposition in the space of Disse, the portal region and fibrotic bands; as a result, it has been called a fibrotic ‘pacemaker’ [2,42,43].

There are multiple splice variants of fibronectin. Variant EIIIA is expressed primarily during repair of liver injury. It stimulates the activation of fibroblasts and HSC to myofibroblasts in culture [44]. Transforming growth factor (TGF)- β induces production of EIIIA by endothelial cells, suggesting one mechanism among many whereby TGF- β induces fibrosis [45].

Tenascin-C

The tenascins are oligomeric glycoproteins with complex, multidomain structures including fibronectin type 3 repeats. Although the tenascins are highly conserved across vertebrate species and are tightly regulated, their functions are not well understood. Tenascin-C is the best characterized member of the family and the only one known to be expressed in the liver. It antagonizes cell attachment to fibronectin and appears to block fibronectin-mediated signalling at the level of focal adhesion kinase and Rho-mediated pathways; it stimulates growth pathways, including Wnt pathways [46–48]. Tenascin-C also binds multiple additional proteins including integrins, a variety of proteoglycans, adhesion molecules and collagens [49].

Tenascin-C is upregulated in liver fibrosis. In the normal liver, it is found as discontinuous deposits in the sinusoids, but is absent from portal tracts [50–52]. In rat models of fibrosis, tenascin-C is expressed transiently and is found in early septae (at the interface between the parenchyma and the scar) but not in organized areas of fibrosis, suggesting that it has a role in early deposition of ECM. Tenascin-C in the fibrotic liver is synthesized and secreted by HSC [13,50–53].

Laminins

The laminins are a family of large heterotrimeric (α , β , γ chains) glycoproteins typically found in basement membranes associated with collagen IV, perlecan and a variety of other molecules including the glycoprotein nidogen (entactin). They self-assemble into a mesh-like structure and play an important role in the architecture of the basement membrane. Laminins interact with cell surface receptors to regulate additional functions including development and differentiation [54].

Several laminin chains are found in the liver, although their distributions vary [55,56]. In the normal liver, laminins are found in the basement membrane structures of the portal region, with small, focal deposits in the sinusoidal space [1,2]. In the cirrhotic liver, there is a marked increase in laminin deposition, which is found surrounding single or small groups of hepatocytes. Laminin is heavily deposited in the space of Disse in the fibrotic liver along with collagen IV and perlecan, eventually forming a continuous basement membrane [2]. HSC are important laminin-producing cells [56].

Elastin and fibrillin

Elastin and the fibrillins are the major components of elastic fibres in the body and are responsible for many of the mechanical properties of tissues, in particular resilience. Elastic fibres are formed from fibrillin microfibrils with or without a core of elastin. Assembly begins with fibrillin arrays forming transglutaminase cross-linked beaded microfibrils. Elastin binds to these microfibrils, undergoes ordered self-assembly and is then stabilized by LOX-mediated cross-links, which render it highly protease resistant [57]. The function of the different elastic fibres in the liver is not well understood. Both fibrillin and elastin bind to multiple other ECM components including collagens and proteoglycans. Fibrillin can mediate signalling by binding to integrin $\alpha_v\beta_3$; it also binds to TGF- β and may regulate TGF- β targeting [57]. Fibrillin may be cleaved into antiadhesive fragments by proteases, potentially modulating cell/matrix interactions and enhancing cell migration [58].

Fibrillin-1, the only family member studied in the liver, is found associated with elastin in vessel walls and portal tract connective tissue. Unlike elastin, fibrillin-1 is also found adjacent to the limiting plate and lining the sinusoids, forming a continuous network in the space of Disse, where it may have a mechanical role in sinusoidal wall adaptation to variations in blood flow [59]. Fibrillin-1 expression increases in fibrosis where it is found in areas of newly developing matrix (surrounding myofibroblasts invading the parenchyma), around the ductular basement membrane and in the dense septae of organized matrix [58, 59]. Elastin, unlike fibrillin, is not found around invading myofibroblasts or in the space of Disse [58,60,61]. Fibrillin is thought to be produced by both quiescent and activated HSC and portal fibroblasts; elastin, in contrast, appears to be produced only by portal fibroblasts and myofibroblasts [58,59].

Proteoglycans and hyaluronic acid

Proteoglycans are proteins with glycosaminoglycan (GAG) modifications. They have multiple functions including maintaining the structural integrity of the ECM, forming a highly charged barrier to the passage of other molecules and regulating growth factor signalling by binding to and sequestering many growth factors including fibroblast growth factor (FGF)-2. Some proteoglycans, in particular the syndecans, heparan sulphate proteoglycans that increase dramatically in fibrosis, are now recognized as signalling receptors [62–65]. Additionally,

certain proteases copurify with proteoglycans; this is increased in cirrhosis. There is a fivefold increase in proteoglycan expression in fibrosis. Given the many functions of proteoglycans, they are likely to play a significant role in regulating liver function [66].

Heparan, dermatan and chondroitin sulphate proteoglycans are found in the normal liver, with heparan sulphate proteoglycans comprising approximately 60% of the total [66]. In the cirrhotic liver, chondroitin and dermatan sulphate proteoglycans are disproportionately increased; there are also subtle changes in the side-chain composition of individual proteoglycans, which may affect their function [62,66]. Heparan sulphate proteoglycans are primarily synthesized by hepatocytes in the normal and diseased liver while, in contrast, increases in chondroitin and dermatan sulphate proteoglycans in disease are mediated largely by HSC [66].

The basement membrane proteoglycans include perlecan and collagens XVIII and XV (see above). Perlecan is a large, modular heparan sulphate proteoglycan that interacts with a variety of other proteins including other basement membrane proteins (laminin, collagen IV), heparan sulphate-binding growth factors and fibrillin-1. Perlecan serves as an architectural component of the basement membrane as well as a reservoir for growth factors and a filter for soluble proteins [67]. In the normal liver, perlecan is present in the portal and central venous basement membranes as well as the sinusoids. In fibrosis, it is increased up to eightfold and is modified with the addition of chondroitin sulphate as well as heparan sulphate. Perlecan is increased around reactive bile ductules and markedly increased in sinusoids and the space of Disse, where it is incorporated into the basement membrane, reflecting the capillarization of the sinusoids [62–64,68]. SEC are a major source of perlecan *in vivo* [43].

Decorin, a small chondroitin sulphate proteoglycan, is also increased in cirrhosis. Decorin binds to collagen VI, fibronectin and thrombospondin and appears to be required for the assembly of collagen XIV to the surface of collagen I [4]. Normally located in the space of Disse and portal region, decorin is found prominently in the fibrotic septae of the diseased liver [68,69].

Hyaluronic acid (HA) is a pure carbohydrate polymer composed of repeating disaccharide units present in the extracellular matrix. HA has a high rate of turnover for an ECM molecule, and liver SEC are responsible for a significant amount of its catabolism [70]. It is a minor component of the liver ECM, although significantly increased in fibrotic livers [71,72]; HA and one of its receptors, CD44, increase in parallel in liver fibrosis [73]. An ECM rich in HA is highly hydrated, promotes migration and is specifically important in the migration of HSC in liver disease [74].

Other matrix proteins

A variety of other matrix proteins are present in the liver and upregulated during fibrogenesis including, although not limited to, entactin, thrombospondin, vitronectin, secreted protein

acidic and rich in cysteine (SPARC; osteonectin), the GPI-linked protein glypican and the proteoglycans aggrecan and betaglycan.

Receptors for ECM proteins

Many of the functions of ECM molecules are mediated by their signalling receptors, heterodimeric (α , β) membrane proteins termed integrins. Matrix-bound integrins initiate cell signalling through a variety of pathways and thereby regulate cell growth, differentiation and migration; in the liver, they are important regulators of fibrosis and tissue remodelling. Integrin expression by the different cells of the liver reflects the specific composition of the underlying matrix, such that hepatocytes and SEC express a unique panel of integrins while biliary epithelial cells express integrins typical of most epithelial cells. In fibrosis, hepatocyte and SEC integrin expression changes to a more epithelial-like pattern and may contribute to the development of fibrosis [75,76]. Integrins may play a role in initiating and maintaining the activation of HSC; they also potentially regulate contractility, proliferation and apoptosis in activated cells [77].

A newly identified class of membrane proteins, the discoidin domain receptors (DDR), are non-integrin tyrosine kinase receptors for fibrillar collagens. In HSC, these receptors may mediate collagen-induced MMP upregulation and proliferation, although their role in liver fibrosis is not known [78].

Matrix homeostasis and degradation in the liver

Matrix deposition in the normal liver is a dynamic process reflecting ongoing synthesis and degradation. Because the collagens and other matrix proteins of the fibrotic liver are highly cross-linked (see above) and relatively resistant to proteolysis, their degradation requires a specialized group of more than 25 proteases, the MMPs. The MMPs are divided into five families, although they show significant functional overlap [79] (see also Chapter 6.1).

Several MMPs deserve note. The interstitial collagenases (MMPs -1, -8, -13 and possibly -2 and -14) are the only MMPs able to cleave native collagens I and III in their triple-helical domains, and they play a key role in fibrosis and its resolution. The gelatinases (MMPs -2 and -9), however, are also important as they degrade collagen IV, elastin, fibronectin and laminin in addition to denatured fibrillar collagens that have been partially unfolded by interstitial collagenases. MMP-2 (gelatinase A) demonstrates increased activity in parallel with the development of fibrosis, particularly at intermediate stages; it degrades the normal perisinusoidal matrix as part of the parenchymal remodelling involved in progression of disease, enabling HSC migration [80–83].

Regulation of matrix degradation is important in both normal and diseased tissue. In the normal liver, avoiding excess degradation is critical for avoiding tissue injury. In fibrosis, there are changes in both deposition and degradation of matrix

components. Resolution of fibrosis requires remodelling of the abnormal matrix; mice expressing a collagenase-resistant collagen I have decreased resolution of fibrosis even after removal of the original profibrogenic insult [84]. The expression and proteolytic activation of the MMPs are subject to complex regulation by growth factors, other proteases including other MMPs, and the matrix itself via integrin-mediated signalling. An important level of regulation is derived from a family of inhibitors, the tissue inhibitors of metalloproteinases (TIMPs), which bind in a non-covalent and reversible manner to the active sites of the MMPs. TIMP-1 and -2 have both been shown to be highly upregulated in fibrosis [81,85,86]. Their importance is illustrated by the demonstration that matrix deposition is enhanced in a CCl₄ model of fibrosis in mice overexpressing TIMP-1 in the liver [87]. HSCs are a key cellular source of the MMPs, their activators and the TIMPs [83].

Conclusion

The ECM is now recognized to be a complex and dynamic rather than a static component of the liver. Although the fibrillar collagens and fibronectin are justifiably the subject of much attention, there is increasing appreciation for the role of other collagens, proteoglycans and additional minor components of the ECM in regulating liver function. Liver cells and the ECM have a bidirectional relationship: almost all liver cells produce some matrix, and most are in turn phenotypically regulated by the ECM. Recent research has focused on the role of fibrogenic cells in addition to HSC and will probably yield important insights in the future. A further area of intense research will be on new functions of the matrix including mechanisms of ECM-mediated regulation of cell phenotype.

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