Novel insights into the function and dynamics of extracellular matrix in liver fibrosis

Morten A. Karsdal,1,2 Tina Manon-Jensen,1 Federica Genovese,1 © Jacob H. Kristensen,1 Mette J. Nielsen,1 Jannie Marie B. Sand,1 Niels-Ulrik B. Hansen,1 Anne-Christine Bay-Jensen,1 Cecilie L. Bager,1 Aleksander Krag,3 Andy Blanchard,4 Henrik Krarup,5 Diana J. Leeming,1 and Detlef Schuppan6,7

1Nordic Bioscience A/S, Herlev Hovedgade, Herlev, Denmark; 2University of Southern Denmark, SDU, Odense, Denmark; 3Department of Gastroenterology and Hepatology, Odense University Hospital, University of Southern Denmark, Odense, Denmark; 4GlaxoSmithKline, Medicines Research Centre, Gunnels Wood Road, Stevenage, Herfordshire, United Kingdom; 5Section of Molecular Biology, Clinical Biochemistry, Aalborg University Hospital, Aalborg, Denmark; 6Institute of Translational Immunology and Research Center for Immunotherapy, University of Mainz Medical Center, Mainz, Germany; 7Division of Gastroenterology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, Massachusetts

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Karsdal MA, Manon-Jensen T, Genovese F, Kristensen JH, Nielsen MJ, Sand JMB, Hansen N-UB, Bay-Jensen A-C, Bager CL, Krag A, Blanchard A, Krarup H, Leeming DJ, Schuppan D. Novel insights into the function and dynamics of extracellular matrix in liver fibrosis. Am J Physiol Gastrointest Liver Physiol 308: G807–G830, 2015. First published March 12, 2015; doi:10.1152/ajpgi.00447.2014.—Emerging evidence suggests that altered components and posttranslational modifications of proteins in the extracellular matrix (ECM) may both initiate and drive disease progression. The ECM is a complex grid consisting of multiple proteins, most of which play a vital role in containing the essential information needed for maintenance of a sophisticated structure anchoring the cells and sustaining normal function of tissues. Therefore, the matrix itself may be considered as a paracrine/endocrine entity, with more complex functions than previously appreciated. The aims of this review are to 1) explore key structural and functional components of the ECM as exemplified by monogenetic disorders leading to severe pathologies, 2) discuss selected pathological posttranslational modifications of ECM proteins resulting in altered functional (signaling) properties from the original structural proteins, and 3) discuss how these findings support the novel concept that an increasing number of components of the ECM harbor signaling functions that can modulate fibrotic liver disease. The ECM entails functions in addition to anchoring cells and modulating their migratory behavior. Key ECM components and their posttranslational modifications often harbor multiple domains with different signaling potential, in particular when modified during inflammation or wound healing. This signaling by the ECM should be considered a paracrine/endocrine function, as it affects cell phenotype, function, fate, and finally tissue homeostasis. These properties should be exploited to establish novel biochemical markers and antifibrotic treatment strategies for liver fibrosis as well as other fibrotic diseases.

collagen; cytokine; extracellular fibrogenesis; integrin; laminin; matrix metalloproteinase; posttranslational modification; proteoglycan; endocrine

45% OF ALL DEATHS IN THE DEVELOPED WORLD are associated with chronic fibroproliferative diseases (256, 378). Thus there is an increasing need to address fibroproliferative diseases because of their strong impact on the quality of life and health costs consequent to pain and organ failure, with an increased need for organ transplants despite dwindling availability, often followed by death. Moreover, their severity and perceived irreversibility in view of a current paucity of treatment options, coupled with a high prevalence in most and an orphan status in some fibrotic diseases, have just begun to attract biotechnology and big pharmaceutical companies to the field.

The common denominator of fibroproliferative diseases is a dysregulated tissue remodeling leading to the excessive and abnormal accumulation of extracellular matrix (ECM) components, thereby generating an ECM with different structural and signaling properties in the affected tissues (285, 287, 289, 378–380). Fibrosis can affect almost any organ or tissue and is therefore associated with a wide variety of diseases and injuries (287). Figure 1 illustrates the major fibroproliferative diseases with a significant impact on human health (20, 287, 323, 378, 379).
Fibrotic tissue was for a long period of time considered an inactive scaffold, precluding regenerative potential for the affected organ. However, this perception cannot be upheld because fibrosis is neither static nor irreversible but the result of a continuous remodeling process and thereby susceptible to intervention (176, 337, 378). Presently, there are no approved treatments that specifically target the mechanism underlying fibrosis, but, especially in the liver, reversibility of even advanced fibrosis has been demonstrated upon treatment of the major underlying cause. Examples are effective antiviral therapy for chronic hepatitis B (22, 208) or the eradication of chronic hepatitis C with interferon-α-based and interferon-free regimens (94, 263, 264). The major future challenge in hepatology will be to halt fibrogenesis and reverse advanced fibrosis without tissue homeostasis or interfering with normal wound healing. Consequently, our increased understanding of the ECM, its dynamics, and the potential of fibrotic microenvironments to reverse holds promise for the development of highly specific and side effect-free antifibrotic therapies.

Traditionally, growth factors, cytokines, hormones, and certain other small molecules have only been considered as relevant mediators of inter-, para-, and intracellular communication and signaling. However, the ECM fulfills direct and indirect paracrine or even endocrine roles. In addition to maintaining the structure of tissues, the ECM has properties that directly signal to cells. Even conceptual exclusively structural proteins such as fibrillar collagens or proteoglycans are emerging as specific signaling molecules that affect cell behavior and phenotype via cellular ECM receptors. In addition, the ECM can bind multiple otherwise soluble proteins, growth factors, cytokines, chemokines, or enzymes, restricting or regulating their access to cells, apart from specifically attracting and modulating the cells that produce these factors. Moreover, specific proteolysis can generate biologically active fragments from the ECM, whereas their parent molecules are inactive. The ECM thus can control cell phenotype by functioning as a precursor bank of potent signaling fragments in addition to the direct effect on cell phenotype mediated by receptors such as integrins and/or certain proteoglycans (137, 138, 276).

The aims of this review are to 1) explore key structural and functional components of the ECM, in part exemplified by relevant mediators of inter-, para-, and intracellular communication and signaling. However, the ECM fulfills direct and indirect paracrine or even endocrine roles. In addition to maintaining the structure of tissues, the ECM has properties that directly signal to cells. Even conceptual exclusively structural proteins such as fibrillar collagens or proteoglycans are emerging as specific signaling molecules that affect cell behavior and phenotype via cellular ECM receptors. In addition, the ECM can bind multiple otherwise soluble proteins, growth factors, cytokines, chemokines, or enzymes, restricting or regulating their access to cells, apart from specifically attracting and modulating the cells that produce these factors. Moreover, specific proteolysis can generate biologically active fragments from the ECM, whereas their parent molecules are inactive. The ECM thus can control cell phenotype by functioning as a precursor bank of potent signaling fragments in addition to the direct effect on cell phenotype through ECM-cell interactions mediated by receptors such as integrins and/or certain proteoglycans (137, 138, 276).

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Fig. 1. Examples of fibroproliferative diseases in different organs. NASH, nonalcoholic steatohepatitis; HCV, hepatitis C virus; HBV, hepatitis B virus; AMD, age-related macular degeneration; IPF, idiopathic pulmonary fibrosis; COPD, chronic obstructive pulmonary disease; ARDS, acute respiratory distress syndrome; FSGS, focal segmental glomerulosclerosis. [From Karsdal et al. (162).]
monogenetic disorders leading to severe pathologies; 2) describe select posttranslational modifications (PTMs) of ECM proteins that result in altered functional (signaling) properties of the original ECM component; 3) discuss the novel concept that an increasing number of components of the ECM harbor cryptic signaling functions that may be viewed as endocrine functions; and 4) highlight how this knowledge can be exploited to modulate fibrotic disease.

Methods

The PubMed database was searched using the following keywords: fibrosis, collagen, cytokine, growth factor, laminin, liver, matrix metalloproteinase (MMP), proteoglycan, posttranslational modification, ECM, and neoepitope. Each author further selected key publications according to his/her specific expertise.

Clinical Significance of the ECM in Liver Fibrosis

The common denominator of most chronic liver diseases is altered remodeling of the ECM initiated by inflammation, with both quantitative and qualitative alterations of its composition, thereby gradually disrupting normal function and finally leading to increased morbidity and mortality attributable to organ failure. Figure 2 highlights the main cellular and structural components of and differences between healthy and fibrotic tissue. The ECM is now considered a biologically active system that tends to perpetuate inflammation and fibrosis, rather than a passive consequence of chronic liver injury. This, together with accumulating evidence that fibrosis and even cirrhosis are reversible conditions, has changed the field and paved the path for novel optimism and interest in antifibrotic therapies (287). The novel insights into the pathophysiology and function of the ECM in chronic fibroproliferative diseases in general, and in liver fibrosis in particular, are already beginning to be translated into the clinic (26, 101, 287, 334). Early diagnosis for high-risk populations combined with targeted, individualized, and more rational interventions, including specific antifibrotic drugs, to prevent progressive disease or induce its regression and thus the morbidity, mortality, and excessive cost associated with symptomatic treatment of advanced stage fibrosis is urgently needed. Thus a central question to be addressed in this review is whether and how far our fairly advanced knowledge on the pathophysiology and molecular biology of the ECM can be translated into clinical practice.

Fibrotic liver diseases are usually silent with limited clinical symptoms until development of cirrhosis with portal hypertension and complications like ascites, bleeding varices, or hepatocellular carcinoma (HCC). Furthermore, the heterogeneity within different subtypes of liver diseases is a separate challenge. Fewer than 20% of chronic alcohol abusers develop fibrosis, and of these only few will progress to cirrhosis (293, 319). Similarly, ~50% of patients with hepatitis C virus infection will never develop significant fibrosis (169), and only 10–20% of patients with nonalcoholic fatty liver disease will develop nonalcoholic steatohepatitis (NASH); of these, 10–20% will progress toward cirrhosis within 5–15 yr (290). Although the individual predictors for fast fibrosis progression are diverse, including genetic predisposition, increased age at onset of disease, and especially (hepatic) comorbidities (second hits), there is still a lack of sensitive noninvasive biomarkers to predict the individual risk of fibrosis progression (288).
However, the population at risk for chronic liver diseases is enormous and poses a global health challenge. Thus the prevalence of obesity alone is 10–30% in most Western and developing countries, as is the consumption of harmful quantities of alcohol (1, 61, 232, 393). On the other hand, with the advent of potent antivirals, the global epidemic of chronic viral hepatitis will likely be harnessed in the near future causally (180, 233). However, even in chronic viral hepatitis, antifibrotics will be useful in those patients with advanced disease in whom fast regression is desirable to further decrease the risk of hepatic decompensation and HCC.

Despite the emergence of more effective antifibrotic agents, there is a lack of agents that specifically derive from the ECM (287), the fibrotic structure itself. The dynamics of the ECM likely harbor important diagnostic information but, more importantly, also clues and pathway for a targeted intervention. Thus biomarkers of ECM remodeling are the most likely candidates to predict fibrosis progression or regression or to permit noninvasive monitoring of antifibrotic interventions (162). During tissue remodeling, proteases release small protein fragments into the circulation (52, 162, 259, 288) (Fig. 2) that may serve as biomarkers of the fibrotic process. Furthermore, the dynamic nature of ECM measures vs. current static measures obtained by imaging or histology should synergistically improve diagnostic and prognostic information on fibrotic liver diseases (52, 162, 259, 288).

Fibrosis and the ECM

The matrix composition itself is particularly important for the regulation of fibrosis. Because the ECM composition regulates the behavior and phenotype of cells housed in the ECM, any ECM remodeling will in turn influence adjacent cells and modify their behavior/phenotype. Consequently, abnormal ECM remodeling may be a prerequisite for fibrogenesis and/or fibrolysis.

Common to all fibrotic disorders is the characteristic alterations in ECM remodeling, which results in the excessive accumulation of ECM components in a given organ that can ultimately lead to organ malfunction (117, 151, 352). Fibrosis is also a dynamic condition with accelerated ECM turnover in which both tissue formation and tissue degradation are highly upregulated (15, 16, 297, 350), resulting in pathological collagen deposition and altered MMP expression profiles (353). Moreover, modifications to amino acids or proteolytic cleavage at specific locations by specific PTMs result in both immunologically and functionally different proteins (161).

Fibrogenesis and fibrolysis are driven by many cell types and molecular events. Fibrogenesis is intimately linked to wound healing, serving to prevent tissues from disassembly during inflammation, apoptosis, necrosis, and release of lytic enzymes. In the liver, the major downstream effectors of fibrosis are activatedstellate cells and (portal) fibroblasts with a phenotype of wound myofibroblasts (175), which produce excessive amounts of ECM molecules, such as the prominent collagens, mainly the abundant fibrillar type I and III collagens (131), which are a hallmark of all fibrotic diseases increasing up to tenfold in advanced fibrosis of organs, such as lungs and liver (205, 370). Type IV collagen is also subject to extensive remodeling during fibrosis, and its quantity has been found to increase more than 10-fold during liver fibrogenesis (116, 289). Variants of type IV collagen together with isoforms of laminin, nidogen, and perlecan are the main components of all basement membranes and form a sheet-like scaffold at the basal site of epithelia and endothelia and around interstitial cells (135, 289) that maintain viability and differentiation of these cells (157, 231). Other functions of this network are the provision of interaction sites for other ECM components, inflammatory cells, chemokines, cytokines, and important functions in cellular signaling (394). The collagen formation observed during fibrosis is also accompanied by an increase in type IV collagen degradation and unfavorable remodeling of the basement membranes, resulting from an increased expression of proteases in the affected tissue (129, 194, 351). This favors myofibroblast activation and a net increase in the deposition of, for example, fibrillar collagens by myofibroblasts (379). This is in part accompanied by a deregulated MMP activity toward basement membranes and increased expression of tissue inhibitors of matrix metalloproteinases (TIMPs) by several cell types in the fibrotic liver, especially TIMP-1, which blocks local interstitial collagen degradation by, for example, MMP-1, -8, and -13 and further promotes myofibroblast activation directly (31, 129, 309). Importantly, collagen properties and quality are altered in fibrosis by protein modifications, including increased cross-linking, that result in enhanced tissue stiffness, which contributes to fibroblast activation and compromised hepatocyte function. Although there presently is no consensus on the most important proteins of the ECM that should be specifically addressed in fibrosis, type I and III collagens are the most abundant ones, followed by type IV, V, and VI collagens, nidogen, laminin, fibronectin, biglycan, mimican, versican, decorin, lumican, and elastin, to name a few (117–119, 195, 287, 289), all clearly being altered in quantity and quality in fibrosis. Although myofibroblasts may be the central ECM-producing cells, macrophages are emerging as important upstream regulators of hepatic fibrogenesis and direct effectors of fibrosis resolution, controlled by specific subtypes, such as M1 and variant M2 macrophage subsets (20, 37, 84, 91, 223, 265, 266, 284, 332, 349, 379–381). Specific functional macrophage subtypes that are highly dependent on the ECM and cellular environment and soluble mediators in the host tissue can also phagocytose cellular debris, which removes potential proinflammatory signals and can cause increased MMP expression and enhanced matrix degradation (260). In a simplified view, M1 macrophages are rather proinflammatory and antifibrotic, in contrast to (subtypes of) M2 macrophages, which can assume profibrotic properties.

The Importance of the ECM for Tissue Function: The ECM Is Controlling Cell Phenotype

The first evidence of a central functional role of the ECM in controlling cellular behavior was obtained when a malignant cellular genotype and phenotype could be repressed by a normal mouse embryonic ECM (81, 214). Generally the ECM is a 3-D scaffold that supports or encapsulates sessile or migrating cells and defines their microenvironment (11). It consists of a meshwork of proteins and nonprotein components (glycosaminoglycans) to which soluble factors, such as growth
factors and cytokines, can bind, which regulates accessibility of common nutrients. The importance and the role of ECM in cell phenotype, tissue-specific differentiation is exemplified by the fact that cells grown as 2-D monolayers on top of either a plastic substrate or a glass coverslip, with or without ECM ligand, fail to assemble into the same tissue-like structures as those growing in or on top of the normal 3-D ECM. Cells grown on plastic or glass are also unable to express differentiated proteins upon stimulation (247) or to respond to growth factors or protease inhibitors in the same way as cells growing in a 3-D setting (163). These phenotypic disparities can be explained, at least in part, by ECM-derived signals that living tissues in 3-D emit and that are transmitted into the cell via ECM receptors such as integrins and via receptors for ECM-bound proteins such as growth factors. This temporospatial 3-D signaling is absent or altered in 2-D substrata. This has been convincingly shown for both epithelial and interstitial cells (122). The architecture of the interstitial matrix in vivo also differs substantially from that offered to or found typically in cells cultured on plastic (163). As an example, osteoblasts grown on plastic in 2-D do not rely on MMPs for survival, whereas osteoblasts embedded in an interstitial 3-D matrix containing type I collagen are critically dependent on MMP activation of latent transforming growth factor (TGF)-β for their survival (163). Moreover, the orientation and function of cells and collagen fibers are lost when cells are grown in 2-D compared with 3-D, which critically regulate cell and tissue behavior (238, 251, 252). Taken together, in vitro models need to replicate the naturally occurring 3-D environment, encompassing a sufficient physiological and pathophysiological variety of ECM components. Thus the effect of 2-D vs. 3-D ECM environments on central biological features of fibroblasts and myofibroblasts, e.g., contraction, migration, proliferation, ECM synthesis, and degradation, is remarkable, usually with a much higher fibrogenic activation under 2-D conditions (44, 71, 76, 149).

The Relation of Structural Proteins to Pathologies

Important information on the functional role of structural components of the ECM has been obtained from mutations in ECM genes that lead to pathologies. Table 1 contains a summary of key structural proteins and their known mutations leading to matrix and tissue failure. With the caveat that some of these components fulfill key functions only in development but may be dispensable later in life, these disease phenotypes provide pivotal information on ECM molecules important for

Table 1. The relation of matrix components to connective tissue diseases

<table>
<thead>
<tr>
<th>Protein</th>
<th>Disease</th>
<th>Animal Models</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type I collagen</td>
<td>OI, Ehlers-Danlos syndrome type VII</td>
<td>OI model (303)</td>
<td>(328, 368)</td>
</tr>
<tr>
<td>Type II collagen</td>
<td>Several chondrodysplasias, osteoarthritis</td>
<td>CIA model (39, 336)</td>
<td>(4, 5, 95, 246)</td>
</tr>
<tr>
<td>Type III collagen</td>
<td>Ehlers-Danlos syndrome type IV, aortic aneurysms</td>
<td>KO/haploinsufficiency mice model (201, 312)</td>
<td>(183, 338)</td>
</tr>
<tr>
<td>Type IV collagen</td>
<td>Kidney fibrosis, Alport syndrome</td>
<td>Canine and murine models (166)</td>
<td>(18, 331, 347, 348)</td>
</tr>
<tr>
<td>Type V collagen</td>
<td>Ehlers-Danlos syndrome type I and II</td>
<td>—</td>
<td>(269, 369)</td>
</tr>
<tr>
<td>Type VI collagen</td>
<td>Bethlem myopathy, Ullrich congenital muscular dystrophy</td>
<td>KO murine models (32)</td>
<td>(187)</td>
</tr>
<tr>
<td>Type VII collagen</td>
<td>Epidermolysis bullosa dystrophica</td>
<td>DEB mouse model (128)</td>
<td>(74)</td>
</tr>
<tr>
<td>Type IX collagen</td>
<td>MED</td>
<td>KO mouse model (6, 132)</td>
<td>(70)</td>
</tr>
<tr>
<td>Type X collagen</td>
<td>SMCD, Japanese-type SMD</td>
<td>KO and transgenic mice models (145)</td>
<td>(206, 373)</td>
</tr>
<tr>
<td>Type XV collagen</td>
<td>Cardiac and muscle phenotypes</td>
<td>KO mice (87)</td>
<td>(348)</td>
</tr>
<tr>
<td>Type XVII collagen</td>
<td>Growth retardation</td>
<td>KO and nude mice (113, 235)</td>
<td>(348)</td>
</tr>
<tr>
<td>Type XVIII collagen</td>
<td>Renal filtration defects, Knobloch syndrome</td>
<td>KO and loss-of function mice (103, 344)</td>
<td>(302, 348)</td>
</tr>
<tr>
<td>Elastin</td>
<td>Lung, skin, and arterial defects, SVAS, WBS, CL</td>
<td>Various mice models (245)</td>
<td>(147, 170, 213)</td>
</tr>
<tr>
<td>Laminin</td>
<td>Alport syndrome</td>
<td>Murine and canine models (167)</td>
<td>(18)</td>
</tr>
<tr>
<td>Biglycan</td>
<td>Cardiovascular disease, osteoporosis</td>
<td>KO mice (392)</td>
<td>(56, 127, 387)</td>
</tr>
<tr>
<td>Biglycan/Fibromodulin</td>
<td>Osteopenia, skin fragility</td>
<td>Double KO mice (392)</td>
<td>(392)</td>
</tr>
<tr>
<td>Perlecan</td>
<td>Osteoarthritis</td>
<td>KO mice (357)</td>
<td>(6a)</td>
</tr>
<tr>
<td>Nidogen 1 and 2</td>
<td>Lung and kidney development</td>
<td>Single KO mice (224, 296)</td>
<td>(347)</td>
</tr>
<tr>
<td>Fibromodulin</td>
<td>Osteoarthritis</td>
<td>KO mice (326)</td>
<td>(108)</td>
</tr>
<tr>
<td>Lumican/Fibromodulin</td>
<td>Joint laxity, impaired tendon integrity</td>
<td>Double KO mice (89)</td>
<td>(150, 326)</td>
</tr>
<tr>
<td>Lumican</td>
<td>Reduced corneal transparency, skin fragility</td>
<td>KO mice, knockdown zebrafish (89, 390)</td>
<td>(54)</td>
</tr>
<tr>
<td>Decorin</td>
<td>Intestinal tumor, skin fragility, Ehlers-Danlos syndrome-like</td>
<td>KO mice (75)</td>
<td>(28, 68, 75)</td>
</tr>
<tr>
<td>Mimecan</td>
<td>Colorectal cancer early formation</td>
<td>—</td>
<td>(364)</td>
</tr>
<tr>
<td>Fibrillin</td>
<td>Marfan syndrome</td>
<td>Murine and bovine model (27, 199)</td>
<td>(126)</td>
</tr>
<tr>
<td>COMP</td>
<td>Transgenic mice (172)</td>
<td>KO mice (346)</td>
<td>(42)</td>
</tr>
<tr>
<td>Matrilin-3</td>
<td>MED</td>
<td>Knockdown mice (182)</td>
<td>(295, 310)</td>
</tr>
<tr>
<td>Fibronecrtin</td>
<td>Glomerulopathy (proteinuria, microscopic hematuria, hypertension, renal failure)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Tenasin C</td>
<td>Cardiovascular diseases, liver fibrosis</td>
<td>KO mice (186)</td>
<td>(59, 60)</td>
</tr>
</tbody>
</table>

[Modified and extended from Karsdal et al. (164).] OI, osteogenesis imperfecta; CIA, collagen-induced arthritis; KO, knockout; DEB, dystrophic epidermolysis bullosa; MED, multiple epiphyseal dysplasia; SMCD, Schmid-type metaphyseal chondrodysplasia; SMD, spondometaphyseal dysplasia; SVAS, supravalvar aortic stenosis; WBS, William-Beuren syndrome; CL, cutis laxa; COMP, cartilage oligomeric matrix protein; PSACH, pseudoachondroplasia.

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tissue function and thus give insight into their function and dysfunction in the pathology of nongenomic disorders.

Mutations within these structural proteins clearly suggest that these proteins have capacities that are important for the maintenance of a healthy ECM phenotype.

The ECM as Regulator of Cytokine and Growth Factor Activities

In addition to the direct effect of ECM structural molecules on cell phenotype and tissue function, the ECM also serves as a storage site for multiple otherwise soluble cytokines and growth factors (306). In particular, the small leucine-rich proteoglycans (SLRPs), which act as matricellular proteins, are active components of the ECM with a specific role in direct or indirect modulation of the cell-matrix crosstalk. These molecules are modulators of growth factor and cytokine functions, such as TGF-β1 (considered the most potent profibrotic cytokine), tumor necrosis factor (TNF)-α, Wnt-1 induced secreted protein 1, and bone morphogenic proteins (BMPs) (211), but they possess other signaling properties, both as whole proteins and as protein fragments. Specifically, decorin and biglycan are antifibrotic molecules, which by binding active TGF-β1 can interfere with its signaling and neutralize its activity (106). The biological activity of TGF-β1 is attenuated by binding to the core proteins of the SLRPs decorin and biglycan (280–282). The SLRPs are also potent antiproliferative molecules of tubular epithelial and endothelial cells, acting through binding to the insulin-like growth factor (IGF) type I receptor (211, 280). Notably, biglycan degradation was recently shown to be highly correlated to fibrosis in carbon tetrachloride (CCL4) and bile duct ligation models of liver fibrosis (106). The soluble factors are usually bound to specific ECM components via low-affinity noncovalent interactions, which create stable concentration gradients around the ECM-embedded cells that produce these cytokines and growth factors, guiding, for example, inflammatory cells but also (myo)fibroblasts toward the target site of inflammation and often excess ECM production. Moreover, ECM binding serves as acellular storage sites, from which these factors will be released during tissue injury, when inflammatory cells degrade the ECM, promoting tissue regeneration and remodeling. Many of the heparan sulfate (HS) (proteoglycan) binding growth factors promote angiogenesis [e.g., fibroblast growth factor (FGF)-1 and vascular endothelial growth factor (VEGF)] or epithelial cell proliferation and survival [e.g., epidermal growth factor (EGF), hepatocyte growth factor (HGF), and keratinocyte growth factor]; others that bind to the abundant collagens regulate fibroblast or immune cell activation [e.g., platelet-derived growth factor (PDGF)-B, oncostatin-M, interleukin-2, and HGF]. Most of these interactions have been studied in detail (289, 291, 315–317), which is schematically drawn in Fig. 3 and listed in Table 2 and highlights that the ECM is a key specific storage facility for potent signaling molecules. Other important examples of molecules sequestered in the ECM are 1) the proinflammatory cytokine TNF-like weak inducer of apoptosis (TWEAK) that induces proliferation of hepatic progenitor cells (HPCs) directly through its receptor fibroblast growth factor-inducible 14, known to be overexpressed in chronic liver diseases (148, 243, 333). Lastly, the hedgehog and Wnt/β-catenin pathways together with Notch signaling have been documented to be important in HPC activation and differentiation (37, 154, 307, 320), as HPCs maintain viability via autocrine and/or paracrine Hedgehog signaling (307).

Tissue Turnover Generates Posttranslational Modifications with Signaling Function Affecting Cell Phenotype

To maintain healthy tissue, the ECM must regenerate itself by normal remodeling, in which aged or damaged proteins are broken down in a specific sequence of proteolytic events and replaced by new proteins. However, during pathological conditions, such as fibrosis and inflammation, the delicate repair response balance is disturbed (142, 289). The constituents of the “aged” ECM are degraded, which in these disease states results in an array of protein fragments and altered proteins, some of which have documented signaling potential (216, 230, 268, 305, 358, 388), which will be discussed in depth on an individual protein level in the following sections. The original proteins of the ECM are replaced by different constituents, and, consequently, the composition and quality of the matrix are altered. This transformation from a healthy tissue to a pathological one may cause the matrix to become stiff, which has been shown to enhance tumor cell migration, myofibroblast activation, and collagen deposition (19, 21, 29, 66, 143, 171, 219, 253, 271), thereby linking the actual matrix quality to disease progression and changing cell phenotypes. Figure 4 illustrates the steps of abnormal ECM remodeling in fibrosis. Healthy ECM consists of a network of fibers organized in a highly ordered fashion, with binding of key growth factors and signaling molecules at specific interaction sites in the network. During high matrix turnover, the ECM is degraded, leaving fragments of the ECM in the matrix and releasing other fragments into the circulation. Multiple enzymes are released into the matrix by both resident and invading cells, modulating the ECM and generating an altered microenvironment. Consequently, the altered cell-ECM interactions result in altered cellular phenotypes. Different steps during ECM remodeling and fibrogenesis are likely characterized by unique patterns of protein formation, deposition, and degradation, which generates unique protein fingerprints, part of which should be released into the circulation to be exploited as stage-specific serum markers of liver fibrogenesis and/or fibrolysis (259, 288).

One group of modifications affecting protein quality is PTMs. PTMs are non-DNA-encoded modifications and are a consequence of tissue physiology and pathophysiology (63, 64). Examples of physiological PTMs are isomerization of aspartate (seen in tissue aging), citrullination of arginine, nitrolysisation of cysteine (occurring during inflammation), protease degradation at cleavage hotspots (observed in fibrosis and inflammation), glycosylation (in glycemia, type II diabetes), and the fibrosis-specific modifications made by polylysic acid that modulate the profibrotic ductular reaction in liver injury (62, 64, 339). Each of these modifications may change the function and signaling of the modified protein. Several lines of independent evidence suggest that PTMs to specific proteins contribute to abnormal cellular proliferation, adhesion, and morphology (185) and may cause many of the differences in fibrotic compared with normal tissue (36, 125, 185, 209, 279, 321). These specific PTMs made to specific proteins, in particular to structural proteins including collagens, have been
shown to be an integrated part of disease progression, exemplified by citrullination of type II collagen in rheumatoid arthritis, cross-linking of collagens in fibrosis and acetylation, citrullination, isomerization, and phosphorylation of myelin basic protein in systemic lupus or multiple sclerosis (63, 164). Similar PTMs made to other collagens or key structural proteins could be important determinants of fibrosis progression, and evidence has recently been obtained for this role, as exemplified by modifications to type XVIII collagen, as discussed later.

There is a growing list of ECM molecules with documented effect on tissue function. This effect may be referred to as a newly discovered paracrine function of ECM proteins. A nonexhaustive list of ECM proteins (as this list continues to grow) and their exerted effects is shown in Table 3. These examples highlight those ECM proteins that serve as paracrine signaling molecules, often revealed during pathological processes that, in addition to cytokines, growth factors, and hormones, become essential players in tissue homeostasis, apart from their roles to anchor cells and transmit positional information and differentiation signals. Notably, some proteins do not change the cellular phenotypes in their native conformation, whereas, subsequent to a specific PTM, a highly potent and novel function of the same protein is revealed.

Posttranslationally Modified Proteins Affect Cell Phenotype: Examples of a Paracrine Function of the ECM in Relation to Fibrosis

Fragments of type XVIII collagen: endostatin. In line with the PTMs of ECM molecules being able to control cell phenotype (388), a peptide derived from endostatin by MMP activity (239) was shown to ameliorate organ fibrosis. Here, peptide E4 (endostatin), derived from the noncollagenous COOH terminus of type XVIII collagen, which is present in the liver sinusoidal and basement membrane (286), prevented TGF-β1-induced dermal fibrosis and bleomycin-induced dermal and pulmonary fibrosis in mouse models and ex vivo in human skin. In addition, E4 significantly reduced existing fibrosis in these preclinical models. E4 amelioration of fibrosis was accompanied by reduced cell apoptosis and lower levels of lysyl oxidase-like 2 (LOXL2), a disease-related member of the LOX enzyme family that cross-links collagen providing resistance to proteolysis. Similar findings were observed in the lung bleomycin model where E4 inhibited the TGF-β and TNF-α pathways (358). Along the same line of thinking is restin, which is a close homolog to E4 derived from type XV collagen, another basement membrane collagen with predominant localization in the portal ECM of the liver (123). Restin has

![Fig. 3. Binding of certain growth factors and cytokines to the extracellular matrix. The figure highlights prominent interactions. Shown is a selection of relevant ECM binding factors (also discussed in the text) and their association with either heparan sulfate or collagen and their target cells. The liberation of ECM-stored biologically active growth factors can either trigger (inflammatory) cells to further degrade ECM or promote excess ECM deposition by (myo)fibroblasts (as is the case with decorin/biglycan-bound transforming growth factor, TGF-β1). EGF, epidermal growth factor; FGF, fibroblast growth factor; HGF, hepatocyte growth factor; IL-2, interleukin-2; KGF, keratinocyte growth factor; OsM, oncostatin-M; PDGF, platelet-derived growth factor; VEGF, vascular endothelial growth factor.](http://ajpgi.physiology.org/)

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Table 2. Key factors and their downstream effect on signaling molecules and cells

<table>
<thead>
<tr>
<th>Growth Factor Signaling</th>
<th>Downstream</th>
<th>Effect</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>PDGFs</td>
<td>Ras-MAPK, PI3K-Akt/ PKB, PKC</td>
<td>HSC activation</td>
<td>(33, 90, 168, 242, 375)</td>
</tr>
<tr>
<td>TGFs</td>
<td>JNK, NF-κB, CTGF, Smad</td>
<td>HSC proliferation, fibrogenic/inhibitory</td>
<td>(53, 191, 200, 287)</td>
</tr>
<tr>
<td>EGFs</td>
<td>STATs, EGFR, ERK1/2</td>
<td>HSC proliferation, polypeptide mitogen</td>
<td>(107, 159, 191, 270)</td>
</tr>
<tr>
<td>VEGFs</td>
<td>MAPK, Akt</td>
<td>HSC activation, hepatic angiogenesis</td>
<td>(67, 191, 397)</td>
</tr>
<tr>
<td>HGFs</td>
<td>JAK/STAT, MAPK, c-Met</td>
<td>Hepatocyte mitogen, antifibrotic</td>
<td>(105, 174, 382)</td>
</tr>
<tr>
<td>IGFs</td>
<td>MAPK, PI3K, ERK</td>
<td>Antifibrotic, HSC proliferation</td>
<td>(49, 313, 325)</td>
</tr>
<tr>
<td>TNF-α</td>
<td>NF-κB, JNK, ERK</td>
<td>HSC proliferation, inflammation, fibrogenic</td>
<td>(262, 330)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Chemokines</th>
<th>Downstream</th>
<th>Effect</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>CXCL1, CXCL9, CXCL10</td>
<td>CXCR2, CXCR3, TGF-β</td>
<td>Inflammation, antifibrotic, chemooactive</td>
<td>(130, 191, 366, 395)</td>
</tr>
<tr>
<td>MCP1-3</td>
<td>CCR2, p47</td>
<td>HSC activation, NK cell activation, inflammation, chemooactive</td>
<td>(202, 301)</td>
</tr>
<tr>
<td>CCL5 (RANTES)</td>
<td>CCR5, PI3K/Akt</td>
<td>NK cell activation, HSC migration and proliferation, inflammation, chemooactive</td>
<td>(173, 191, 202, 300)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Innate immune interactions</th>
<th>Downstream</th>
<th>Effect</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>TLR2, TLR4</td>
<td>TNF-α, NF-κB, JNK, BAMBI</td>
<td>Chemotactics/inflammatory</td>
<td>(101, 191, 287)</td>
</tr>
<tr>
<td>CD40</td>
<td>JNK, NF-κB</td>
<td>Secretion of MCP-1 and IL-8, antifibrotic</td>
<td>(100, 101, 292)</td>
</tr>
<tr>
<td>IL-6</td>
<td>JAK/STAT, ERK1/2, Akt, NF-κB, MAPK</td>
<td>Regulation of inflammatory response, antifibrotic</td>
<td>(229, 270)</td>
</tr>
<tr>
<td>IL-8 (CXCL8)</td>
<td>CXCR1, CXCR2.</td>
<td>Chemotaxant, inflammation</td>
<td>(398)</td>
</tr>
<tr>
<td>IL-10</td>
<td>JAK/STAT</td>
<td>Antifibrotic</td>
<td>(22, 105, 396)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Adipokine pathways</th>
<th>Downstream</th>
<th>Effect</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adiponectin</td>
<td>AMPK</td>
<td>Antifibrotic</td>
<td>(46, 92, 158)</td>
</tr>
<tr>
<td>Hedgehog</td>
<td>Ptc, Smo, Gli family</td>
<td>Fibrogenic HSC activation and proliferation</td>
<td>(255, 287, 389)</td>
</tr>
<tr>
<td>Notch</td>
<td>NICD, RBP-Jα</td>
<td>Fibrogenic, HPC differentiation, epithelial-mesenchymal transition</td>
<td>(57, 217)</td>
</tr>
<tr>
<td>Wnt</td>
<td>Wnt/β-catenin, Wnt/Ca^2+</td>
<td>Fibrogenic, HSC activation</td>
<td>(58, 197)</td>
</tr>
</tbody>
</table>

inhibitory effects on endothelial cell migration but not on their proliferation (268), whereas tumor suppression by type XV collagen is independent of the restin domain (225). Vastatin, the noncollagenous COOH-terminal fragment of type VIII collagen, inhibits endothelial cell proliferation and induces apoptosis in a bovine aortic endothelial cells (386). These protein fragments are all derived from the processing of collagen and are consequently PTMs of collagens involved in organ fibrosis. They add to the evidence that ECM molecules have signaling properties, which can be considered an endocrine function.

**Fragments of type IV collagen, fibronectin, and plasminogen.**

Arresten, canstatin, tumstatin, angostatin, and anastellin. Angiogenesis generally precedes fibrosis, and consequently these early events are of pivotal importance for progression of fibrosis. Several molecules of the ECM modified by proteolysis affect angiogenesis by ECM-cell interactions through the fibrosis structure itself. The sprouting of new vessels from preexisting vasculature may enhance the deregulated and amplified ECM composition, support fibroblast proliferation, and constrain normal tissue repair. Numerous endogenous inhibitors of angiogenesis are derived from proteolysis of the ECM and vascular basement membrane. Notably, angiotatin, a fragment derived from blood coagulation factor plasminogen, is one of the most potent antagonists of angiogenesis and is shown to inhibit liver fibrosis in mice (356). In a similar fashion, anastellin, a peptide derived from the first type III repeat of fibronectin, is an example of a matrix-derived inhibitor of angiogenesis. It prevents angiogenesis and growth of human tumors when injected into mice (391). Anastellin binds to full-length fibronectin, augmenting formation of high polymerized fibronectin multimers termed superfibronectin (220). Additionally, it remodels already assembled fibronectin matrix, affecting apoptosis, cellular differentiation, and cell cycle progression, being thus critical for cell growth and survival. Other matrix-derived fragments are arresten, canstatin, and tumstatin, all derived from MMP cleavage of the NC1 domain of type IV collagen, α-1, -2, and -3 chain, respectively. These peptides are inhibitors of angiogenesis, tumor growth, and endothelial cell proliferation and migration (124, 222). Despite the fact that numerous matrix-derived fragments are angiogenesis inhibitors, they inhibit distinct aspects of angiogenesis. Endostatin and anastellin fragment of type XVIII collagen and fibronectin, respectively, both inhibit endothelial cell migration in response.
to VEGF, but only anastellin completely inhibited dermal endothelial proliferation (230).

**LOX**: cross-linking of the ECM. Modifications such as cross-linking of ECM proteins by LOX family members have gained increasing attention in fibrosis because of their role in generating tissue stiffness, which promotes fibrogenesis (21). LOX is highly overexpressed in the local fibrotic microenvironment, in particular by myofibroblasts (374). Focus has been directed especially to LOXL2, which is the most expressed of the nine members of the LOX family (141), predominantly at advanced disease stages (69, 343). LOXL2 activity and expression correlate with the derangement of the ECM microenvironment of tissues that are associated with cancer and fibrosis (43). Apart from direct signaling roles in cancer proliferation and dedifferentiation, the increase in cross-links contributes to the stability of collagen accumulation in fibrosis because of their role in generating tissue stiffness, which promotes fibrogenesis (21).

**Transglutaminase-mediated cross-linking of the ECM.** In addition to LOX, there is a well-established role for transglutaminases (TGs) in the biochemical modification of ECM proteins (156). TGs constitute a family of at least eight related proteins with well-characterized transamidation activities forming largely irreversible $\text{N}^2(\gamma\text{-glutamyl})$ lysine isopeptide bonds between a glutamine residue on one and a lysine (histidine) residue on another protein (88, 204). TG2 is the most ubiquitously expressed enzyme of this family, also in liver, but is largely retained intracellularly in an inactive state. Its secretion is carefully regulated, in particular by myofibroblasts and endothelial cells, increasing dramatically following cellular damage, where it becomes activated by the high extracellular calcium concentrations (193, 308). Extracellular TG2-dependent matrix cross-linking has been closely linked with the pathogenesis of fibrotic disease of kidneys, liver, and lungs (152, 215, 244) and also with tumor progression, cardiovascular diseases, and intestinal inflammation, where it plays a central role in celiac disease (38, 80, 207). TG2 has been shown to cross-link certain ECM proteins, like type III procollagen, fibronectin, and elastin, conferring increased stability, rigidity, and resistance to degradation of the ECM, similar to the effects of LOXL2 (88, 354). However, a recent study comparing liver fibrosis progression and regression with the extent of collagen cross-linking in several mouse models demonstrated that TG2 does not significantly affect fibrosis in mice deleted of TG2 vs. wild-type controls (261). This may be different when TG2 activity is blocked in wild-type mice because the irreversible TG2 inhibitor NTU281 ameliorated the development of fibrosis and kidney failure in a model of diabetic nephropathy (133, 134). Given that this inhibitor acts extracellularly, its antifibrotic effects are likely attributable to inhibition of TG2 cross-linking activity. However, the extracellular functions of TG2...
Table 3. The matricellular effects of ECM components

<table>
<thead>
<tr>
<th>Protein or PTM</th>
<th>Cellular Phenotype</th>
<th>Responsible Receptor</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elastin-derived peptides</td>
<td>Chemotaxis of monocytes, fibroblasts, endothelial cells&lt;br&gt;Proliferation of fibroblasts and smooth muscle cells&lt;br&gt;Protease release from fibroblasts and leukocytes</td>
<td>Elastin-binding protein in complex with protective protein/cathepsin A and neuraminidase-1</td>
<td>(83)</td>
</tr>
<tr>
<td>Thrombospondin</td>
<td>Inhibition of angiogenesis</td>
<td>CD36 and CD47</td>
<td>(7, 112, 363)</td>
</tr>
<tr>
<td>Type I collagen</td>
<td>Fibroblast migration</td>
<td>DDR2</td>
<td>(277)</td>
</tr>
<tr>
<td>Acetylated Pro-Gly-Pro (acPGP), fragment of type I collagen</td>
<td>Neutrophil chemotaxis</td>
<td>CXCR1 and CXCR2</td>
<td>(254, 367)</td>
</tr>
<tr>
<td>Arresten, canstatin and tumstatin, fragments of type IV collagen</td>
<td>Inhibition of angiogenesis, tumor growth, and endothelial cell proliferation and migration</td>
<td>Various integrins</td>
<td>(124, 222)</td>
</tr>
<tr>
<td>Endostatin, fragment of collagen type XVIII</td>
<td>Induction of apoptosis</td>
<td>Glypicans, nucleolin</td>
<td>(239)</td>
</tr>
<tr>
<td>RGD motif, present in collagens, laminin and fibronectin</td>
<td>Inhibition of endothelial proliferation, angiogenesis, and tumor growth&lt;br&gt;Induction of endothelial cell apoptosis</td>
<td>Various integrins</td>
<td>(78, 165, 305)</td>
</tr>
<tr>
<td>Fibromodulin</td>
<td>Cell adhesion, angiogenesis, apoptosis</td>
<td>Various integrins</td>
<td>(45, 278)</td>
</tr>
<tr>
<td>Laminin-332, elastase-generated fragment of γ2</td>
<td>Proliferation, migration, and chemotaxis of HSCs</td>
<td>Neutrophil chemotaxis</td>
<td>Unknown</td>
</tr>
<tr>
<td>SIKVAV and ASKVKV (sequences in linker regions between coiled-coil and globular domains of laminin α1 and α5 chains)</td>
<td>Neutrophil and macrophage chemotaxis</td>
<td>Unknown receptors. SIKVAV interacts with integrins α1, α6, and β1 in salivary gland carcinoma cell line</td>
<td>(3, 98)</td>
</tr>
<tr>
<td>Laminin</td>
<td>Chemotactic migration of malignant cells toward laminin</td>
<td>67LR (LamR)</td>
<td>(79, 318)</td>
</tr>
<tr>
<td>Lumican</td>
<td>Regulation of inflammation and innate immunity&lt;br&gt;Apoptosis induction</td>
<td>CD14, Fas ligand, CXCL1</td>
<td>(50, 104, 355, 377)</td>
</tr>
<tr>
<td>Biglycan</td>
<td>Regulation of inflammation and innate immunity and effect on adhesion and migration&lt;br&gt;Cytokine modulation (PDGF, TGF-β, TNF-α, WISP-1, BMP-4)</td>
<td>Fas&lt;br&gt;TLR2, TLR4, P2X4/P2X7, selectin L/CD44, C1q</td>
<td>(12, 55, 121, 144, 178, 181, 218, 234, 281, 311, 341, 342)</td>
</tr>
<tr>
<td>Decorin</td>
<td>Signal transduction&lt;br&gt;Cytokine modulation (PDGF, TGF-β, TNF-α, VWF, WISP-1)</td>
<td>RhoA, Rac1&lt;br&gt;LRP-1, c-MET&lt;br&gt;TGF-β, C1q</td>
<td>(40, 109)</td>
</tr>
<tr>
<td></td>
<td>Regulation of inflammation and innate immunity&lt;br&gt;Antiproliferative effects&lt;br&gt;Antioncogenic effects</td>
<td>IGF-IR</td>
<td>(65, 311)</td>
</tr>
<tr>
<td></td>
<td>Adhesion and migration effects&lt;br&gt;The 29- and 50-kDa amino terminal fragments mediate release of proteoglycan from articular cartilage by RGD-independent mechanisms&lt;br&gt;Fn fragments can induce fibroblast gene expression of MMPs or can act as proteinases themselves</td>
<td>EGF-R, VEGF-R2&lt;br&gt;IGF-IR, integrin α2β1, RhoA, Rac1</td>
<td>(139, 298)</td>
</tr>
<tr>
<td>Fibronectin</td>
<td>A 40-kDa fragment prevents PDL cell spreading, thereby inducing anoikis&lt;br&gt;The 29- and 50-kDa amino terminal fragments mediate release of proteoglycan from articular cartilage by RGD-independent mechanisms&lt;br&gt;Fn fragments can induce fibroblast gene expression of MMPs or can act as proteinases themselves</td>
<td>Various integrins</td>
<td>(96, 342)</td>
</tr>
<tr>
<td>Tenascin-C</td>
<td>Fragments are highly upregulated in arthritic cartilage, where they mediate cartilage degradation by the induction of aggrecanase activity</td>
<td>αv-integrins and unknown</td>
<td>(314)</td>
</tr>
</tbody>
</table>

[Extended and modified from Karsdal et al. (164).] ECM, extracellular matrix; PTM, posttranslational modification; DDR, discoidin domain receptor; WISP, Wnt1-inducible signaling pathway protein; BMP, bone morphogenic protein; VWF, Von Willebrand factor; LRP, lipoprotein receptor-related protein; PDL, periodontal ligament; MMP, matrix metalloproteinase. RGD, arginine-glycine-aspartic acid; Fn, fibroblast growth factor-inducible.
are more complex, including activation of TGF-β1 from its inactive precursor (134) and nonenzymatic interactions between ECM proteins such as fibronectin and cell surface growth factor receptors and integrins (80).

**Type VI collagen as regulator of fibrogenesis.** Type VI collagen forms microfilaments that traverse interstitial connective tissues. These microfibrils are among the first ECM structures to be degraded upon tissue injury, resulting in larger fragments that, via engagement of integrins and perhaps NG2 proteoglycan, prevent apoptosis and induce fibrogenic activation of surrounding (myo)fibroblasts via activation of proproliferative mitogen-activated kinases and PDGF receptor-α (9, 273, 274). This mechanism likely serves to trigger an immediate wound-healing response, with type VI collagen as a sensor for ECM destruction. Modulation of type VI collagen degradation or blocking its receptors may prevent an overshooting wound-healing response, as occurs in fibrotic tissue remodeling, which is regularly coupled to deregulated ECM proteolysis, as illustrated in Fig. 5.

**Perlecan, endorepellin.** The proteoglycan perlecan, a basement membrane HS proteoglycan, has been implicated in fibrosis (86) and has shown to have opposing terminal angiogenic activities. The NH₂ terminus carrying three HS chains and the COOH terminus have proangiogenic and antiangiogenic properties, respectively (30). The COOH terminus is proteolytically processed by BMP-1 (110), yielding endorepellin, an 85-kDa perlecan domain V fragment (216). The COOH terminus V domain of perlecan is similar to another HS proteoglycan, agrin, which is the major proteoglycan of the glomerular basement membrane. The COOH-terminal agrin fragment is a known marker of neuronal muscular remodeling, which is regularly coupled to deregulated ECM proteolysis, as illustrated in Fig. 5.

**Actin, actin cytoskeleton and focal adhesions (237). The laminin-like globular domain (LG3) of endorepellin can be released by further proteolytic processing with BMP-1 (111). The LG3 has been associated with end-stage renal failure (241) and shown to be implicated in renal allograft rejection (240). Caspase-3 activation triggers cathepsin-L proteolytic processing of endorepellin to release the LG3 domain (48). LG3 induces αβ1-integrin and Src family kinase-dependent antiapoptotic pathways in fibroblasts (188). Thus the LG3 domain may affect, not only angiogenesis, but also collagen depositions and overall tissue stiffness, known hallmarks of fibrosis.

**The ECM as Regulator of Cell Function: The ECM Interacts with Cells Through Integrins and DDRs and Induces Specific Signaling**

Integrins and discoidin domain receptors (DDRs) are part of a network that enables the cell to sense and interact with the microenvironment and adapt to changes (102), through specific interactions and signaling, as outlined in Table 4. Integrins are involved in cell-cell interactions and the attachment of cells to the ECM. They are vital in development and tissue homeostasis, where they transmit “outside-in signals” and “inside-out signals,” which are important in the regulation of cellular proliferation, apoptosis, adhesion, migration, and growth (294). Their extracellular domain is responsible for sensing the microenvironment by ECM binding, which also modulates hepatocyte differentiation (283). Several proteins of the ECM, also the abundant collagens, signal through integrins and DDRs. In hepatic stellate cells (HSC), collagen type I signals through integrin-α1β1 (and to a minor degree through α2β1), whereas collagen type III engages mainly DDR1 (Table 4). The DDRs are receptor tyrosine kinases that specifically recognize collagens as their ligands (276). Similar to collagen-binding β1-integrins, the DDRs bind to specific secondary structural motifs within the collagen triple helix (383). DDRs are involved in tissue homeostasis...
and transduce signals regulating cell polarity, tissue morphogenesis, and cell differentiation (345). Here, DDR1 and DDR2, in particular, have demonstrated important functions in tissue homeostasis and cancers (243, 385), in which lack or inhibition of DDR1 attenuated fibrogenesis (35, 97, 120, 272, 276), whereas DDR2 deficiency promoted experimental liver fibrosis (101).

Liver Pathologies Associated with the ECM

Common to all underlying causes of fibrosis is the disruption of the normal ECM pattern attributable to activation of (myo)fibroblasts (activated HSCs) in the portal tracts initiated by tissue injury and inflammation. This enables proliferation and migration of these fibrogenic effector cells into the parenchyma along the sinusoids, which is the hallmark of incipient septum formation (289). The activated HSCs deposit excess ECM, serving to “close the wound” and to provide a scaffold for orderly liver regeneration in case of a short-term insult. However, with ongoing (inflammatory) insults, the excess ECM, which becomes dominated by fibrillar collagen (mainly type I and type III), generates scar tissue that merely maintains organ integrity but has lost the guiding function for orderly tissue regeneration (267). The deposition of ECM is dependent on etiology and consequent on activation of different subtypes of cells, including mainly myofibroblasts of variant activation state, suggesting that “fibrosis is not just fibrosis.” During fibrosis progression, the quantity and quality of the hepatic ECM changes with an up to 10-fold increase in collagenous and noncollagenous components followed by a shift in matrix composition from the low-density basement membrane-like matrix to an interstitial matrix containing mainly fibril-forming collagens (289). A specific characteristic of fibrosis development in the liver is the presence of specific growth points of the scar tissue, i.e., portal zone vs. central zone, and space of Disse, determining the development of portal, central, or pericellular fibrosis, with different clinical consequences as to development of liver failure, i.e., with relative preservation of liver function in portal fibrosis despite massive collagen accumulation (285). Thus the developmental pattern of fibrosis is dependent on the underlying etiology causing the fibrosis (51, 285).

The structural representation of fibrosis depends on the cell types and injury involved. Today, several fibroblast-like cell types have been identified in the liver, all of which also contribute to the development of fibrosis, including 1) septal myofibroblasts present in the inner part of fibrous septa, 2) activated HSCs located in capillarized sinusoids adjacent to expanded portal tracts, 3) interface myofibroblasts located at the edge of fibrous septa derived from activated HSCs (or portal fibroblasts) recruited at the site of injury where the ECM turnover (synthesis and degradation) and accompanying cell damage and inflammatory infiltration are highest (10, 24, 25, 212, 371), and 4) smooth muscle cells localized in the larger vessel walls (2, 99, 115, 198). Consequently, the activity of these different cell types results in distinct fibrosis patterns.
with formation of 1) portal-portal, 2) portal-central, or 3) central-central septa (Table 5).

**Portal-portal septa.** In biliary fibrosis, portal fibroblasts residing next to the bile duct epithelium (or to biliary progenitors) are the major myofibroblast source. After injury, mainly to the bile ducts (but also severe damage to hepatocytes, which generates biliary progenitors), these fibroblasts undergo rapid activation, express prominent α-smooth muscle actin, and deposit a peribiliary ECM (179, 285, 287, 288). The coproliferation of reactive bile ducts and periductular myofibroblasts results initially in periportal fibrosis and is followed by portal-portal septa formation surrounding the liver nodules, whereas the central vein and the sinusoids that connect from it to the portal tracts are anatomically preserved until later stages where HSCs and to a lesser extent fibrocytes contribute to disease progression.

**Portal-central septa.** The initial histological changes in chronic viral hepatitis are characterized by inflammatory cell infiltration and matrix deposition around the portal tracts. The fibrotic pattern develops as portal-central septa because of portal-central bridging necrosis, suggesting that myofibroblasts as well as HSCs migrate from the portal tract and neighboring sinusoids into the developing septa (267, 285, 287, 288). In alcoholic and metabolic liver diseases, the fibrosis is characterized as the “chicken wire” pattern, in which the fibrillar matrix is deposited around groups of hepatocytes and sinusoids (51, 257).

**Central-central septa.** These can be found secondary to venous outflow obstruction, such as in Budd Chiari syndrome. The central-central septa develop because of distal sinusoidal dilation, centrilobular fibrosis and necrosis, endothelialitis, and prominent platelet activation (196, 228).

How the organization of the structural and functional unit of the hepatic lobule is related to function and disease has been intensively discussed during the past decade. Several models describing the functional unit of the liver have been proposed. One model describes the hepatic acinus with the portal tract as its axis and its peripheral boundary circumscribed by an imaginary line connecting the neighboring terminal hepatic venules. The acinus is divided into three zones, each with different oxygen content and metabolic function (155). Another model describes the classic lobule as subdivided into several primary lobules, the so-called hepatic microvascular subunits. The subunits consist of a group of sinusoids supplied by a single venule and its associated termination of a branch of the hepatic arteriole from the adjacent portal space accompanied by hepatic parenchymal cells and associated cholangioles. Furthermore, a hepatocellular metabolic gradient has also been demonstrated in this proposed functional unit model (210).

Whether the distinct fibrosis patterns develop because of different cell phenotypes or specific mechanisms has to our knowledge not been elucidated. However, on the basis of the above mentioned models, one can assume that a combination of both phenotype and mechanisms, dependent on the local metabolic activity and oxygen content at the specific site of injury, is operative.

**Primary cholangiopathies** are of particular interest. Here changes are confined to the periportal area, with a mixed inflammatory cell infiltrate leading to periportal fibrosis. Interestingly, inflammation and fibrosis are not necessarily closely associated because the risk of biliary dysplasia and malignancy is not correlated with disease duration or severity (47, 327). Additionally, primary sclerosing cholangitis can lead to sinusoidal hypertension before development of varices and hemorrhages associated with cirrhosis (335).

**ECM and HCC**

Worldwide, HCC is the fifth most common cancer and the third most common cause of cancer-related death (304). In the US, the occurrence of HCC is increasing, mainly because of the rising prevalence of end-stage hepatitis C and NASH (160, 236). HCC is strongly correlated to fibrosis severity (203), with a yearly HCC incidence of 1.7% (93) and 2.6%, respectively (8). Mechanisms linking fibrosis and HCC are in need of further exploration, whereas there is increasing evidence that the fibrotic/cirrhotic and associated (immunosuppressive) inflammatory liver microenvironment appears to be a major player in HCC pathology, with several molecular pathways being shared between fibrogenesis and carcinogenesis (340). Thus, apart from direct carcinogens such as hepatitis B virus, microenvironmental stimuli include intrahepatic cell subpopulations, such as progenitor, immune and stellate cells, proliferative or differentiation-modulating cytokines, and growth factors like TGF-β1, EGF, IGF-1, and VEGF and altered ECM molecules and ECM receptors like the integrins. These interact and increase proaese activities directed at the ECM or cell-associated structures, facilitating ECM remodel-

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Table 5. The initial histological changes and developmental patterns of fibrosis in biliary fibrosis, viral hepatitis, and metabolic (nonalcoholic steatohepatitis) and alcoholic liver disease

<table>
<thead>
<tr>
<th>Etiology</th>
<th>Biliary Fibrosis</th>
<th>Viral Hepatitis</th>
<th>Metabolic/Alcoholic Liver Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial histological changes</td>
<td>Bile duct obstruction → fibroblast activation, enlargement of portal tracts with massive collagen deposition and mild inflammation</td>
<td>Portal and parenchymal (hepatocellular) fibrogenesis → ECM deposition around portal vein and surrounding sinusoids, inflammatory cell infiltrate, bridging necrosis</td>
<td>Perivenular and perihepatocellular fibrosis → HSC activation and collagen deposition in the space of Disse; advanced stages with extension and involvement of portal areas</td>
</tr>
<tr>
<td>Main fibrogenic cell type</td>
<td>Portal myofibroblasts</td>
<td>Portal myofibroblasts and HSCs</td>
<td>HSCs and later portal myofibroblasts</td>
</tr>
<tr>
<td>Major ECM proteins</td>
<td>Laminin, fibronectin, tenasin, collagens (type I, III, IV, V, VI, XV, XIX), elastin, fibrillin</td>
<td>Laminin, fibronectin, tenasin, collagens (type I, III, IV V, VI, XV, XIX), elastin, fibrillin</td>
<td>Collagens (type I, III, IV, V, VI, XVIII), fibronectin, tenasin C, fibrillin</td>
</tr>
<tr>
<td>Fibrosis pattern</td>
<td>Portal-portal septum</td>
<td>Portal-central septum</td>
<td>Chicken wire, later stages central-portal septum</td>
</tr>
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ing, cancer cell proliferation, and migration. An example is MMP-2, which is able to degrade numerous components of the ECM that are closely correlated with tumor invasion and metastasis. Together with hypoxia-inducible factor 1α, which regulates proteolytic and angiogenic activities, MMP-2 is over-expressed and its activity enhanced in HCC compared with healthy tissue (359). Additionally, MMP-7 and MMP-26 levels are significantly higher in HCC tissue compared with adjacent healthy hepatic cells from patients with HCC (361) and strongly correlated to phosphorylated FGF receptor 2, supporting a direct link to angiogenesis (361).

Another class of enzymes that is linked to HCC development is LOXL2, which catalyzes collagen cross-linking, enhancing matrix stiffness and resistance to proteolysis, and promotes HCC metastasis (374). Induction of LOXL2 occurs via multiple regulators, including hypoxia, TGF-β1, and microRNAs (374). Matrix stiffness alone appears to determine fibrosis and HCC progression, as expression of procollagen, LOXL2, VEGF and the endothelial marker CD31 is highly correlated (82). Therefore, the ECM remains a yet insufficiently exploited target for HCC therapies.

**Do We Need to Target the ECM?**

We have highlighted the importance of the ECM in cell differentiation and function, fibrosis, and HCC development. The normal and especially fibrotic ECM affects cell phenotype through cell-ECM receptor interactions and liberated and stored growth factors/cytokines/chemokines and vice versa. Moreover, we have highlighted specific PTMs made to the ECM that themselves affect cell phenotype and fate differently than the parent molecules. Consequently to these observations, it should be possible to ameliorate progression of and reverse established fibrosis without crucially disrupting beneficial cell-ECM interactions by differentially targeting pathogenic ECM structures, PTMs, and protein fragments.

Clearly, interfering with the usual suspects TGF-β, PDGF, or VEGF may ameliorate fibrogenesis in certain settings, only in preclinical studies, to date. Direct targeting of the ECM and cells whose fibrogenic activation or fibrolytic activity depends on specific ECM structures that are prevalent in fibrosis would render a fibrosis-specific and potentially fibrolysis-inducing therapy. This appears feasible because the ECM is continuously remodeled, even in cirrhosis (289, 291, 301). Such therapy would ideally be coupled to elimination or suppression of the major cause of fibrosis, such as viral hepatitis B or C. Although this seems a long way to go, the advanced understanding of the pathophysiology of the ECM in fibrosis is already beginning to translate into the clinic, as exemplified in an ongoing clinical study with a LOXL2-blocking antibody.

**Conclusions**

There is a growing body of evidence that modifications made to the structural proteins of the matrix may be both a consequence of the disease as well as drivers of disease progression. Consequently, PTMs within specific ECM proteins (such as degradation products) may be more integrated in pathogenesis than previously thought. Evidence for the use of biomimetic peptides from the ECM, such as types IV and XVIII collagen, as anti-ECM therapeutics, which block fibrogenesis and ECM remodeling, is emerging (189, 358, 388). These examples begin to suggest that matrix molecules themselves may be antibioptic agents in addition to kinase inhibitors and receptor blockers. A further example on how PTMs regulate ECM function is cross-linking of collagens or elastin by LOXL2, which prevents degradation and increases stiffness of the tissue. These combined observations clearly suggest that the ECM is more than just a structural framework for tissues and may perform paracrine functions affecting cell phenotype and fate. It is of key interest to understand the role of each of the major ECM components and their PTMs as well as their signaling potential to grasp the full potential of the ECM and its importance in pathogenesis.

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**DISCLOSURES**

All authors except H. Krarup, A. Blanchard, and D. Schuppan are full-time employees of Nordic Bioscience. The authors declare that they have no other conflicts of interest.

**AUTHOR CONTRIBUTIONS**


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