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


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Running title: Dynamics of the extracellular matrix in liver fibrosis
Key Points Box

1. The ECM is key in controlling cell fate and function.
2. The ECM composition and turnover in diseased tissues is different from that of healthy tissue.
3. The ECM proteins may be modified enzymatically to gain signalling functions not present in the intact native proteins, profoundly affecting cell fate and function.
4. There are highly specific interactions between cells and the ECM through e.g. integrins that result in specific ECM-cell signalling, controlling cell fate and functions.
Emerging evidence suggests that altered components and post-translational 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 monogenic disorders leading to severe pathologies; 2) discuss selected pathological post-translational modifications of ECM proteins resulting in altered functional (signalling) 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 harbour signalling functions that can modulate fibrotic liver disease.

The ECM entails functions in addition to anchoring cells and modulating their migratory behaviour. Key ECM components and their post-translational modifications often harbour multiple domains with different signalling potential, in particular when modified during inflammation or wound healing. This signalling by the ECM should be considered as 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 anti-fibrotic treatment strategies for liver fibrosis as well as other fibrotic diseases.
INTRODUCTION

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 due to 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, and 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 biotech and big pharma to the field.

The common denominator of fibroproliferative diseases is a dysregulated tissue remodelling leading to the excessive and abnormal accumulation of extracellular matrix (ECM) components, thereby generating an ECM with different structural and signalling 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). Fig.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, since fibrosis is neither static nor irreversible, but the result of a continuous remodelling 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 have been demonstrated upon treatment of the major underlying cause. Examples are effective anti-viral therapy for chronic hepatitis B (22, 208) or the eradication of chronic hepatitis C with interferon alpha 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 anti-fibrotic 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 signalling. However, the ECM fulfils 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 signalling molecules that affect cell behaviour 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, while their parent molecules are inactive. The ECM thus can control cell phenotype by functioning as a precursor bank of potent signalling 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, 277).

The aims of this review are to 1) explore key structural and functional components of the ECM, in part exemplified by monogenetic disorders leading to severe pathologies; 2) describe select post-translational modifications (PTMs) of ECM proteins that result in altered functional (signalling) properties of the original ECM component, 3) discuss the novel concept that an increasing number of components of the ECM harbour cryptic sigaling functions that may be viewed as endocrine functions, and 4) highlight how this knowledge can be exploited to modulate fibrotic disease.

METHODS

Pubmed was searched using the following keywords: fibrosis, collagen, cytokine, growth factor, laminin, liver, matrix metalloproteinase (MMP), proteoglycan, post translational modification, ECM, neo-epitope. 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 remodelling 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 due to organ failure. Fig. 2 highlights the main cellular and structural components of and differences between healthy and a 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 anti-fibrotic therapies. 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. Early diagnosis for high risk populations combined with targeted, individualised and more rational interventions, including specific anti-fibrotic 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 if 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 hypertention and complications like ascites, bleeding varices or hepatocellular carcinoma (HCC). Further, the heterogeneity within different subtypes of liver diseases is a separate challenge. Less than 20% of chronic alcohol abusers develop fibrosis and of these only few will progress to cirrhosis. Similarly, approximately 50% of patients with hepatitis C virus infection will never develop significant fibrosis, and only 10-20% of patients with non-alcoholic fatty liver disease (NAFLD) will develop non-alcoholic steatohepatitis (NASH) and of these 10-20% will progress towards cirrhosis within 5-15 years. While 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 non-invasive
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 anti-virals the global epidemic of chronic viral hepatitis will likely be harnessed in the near future causally (180, 233). But even in chronic viral hepatitis, anti-fibrotics 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 anti-fibrotic agents, there is a lack of agents that specifically derive from the ECM (287), the fibrotic structure itself. The dynamics of the ECM likely harbour important diagnostic information, but more importantly also clues and pathway for a targeted intervention. Thus, biomarkers of ECM remodelling are the most likely candidates to predict fibrosis progression or regression, or to permit non-invasive monitoring of anti-fibrotic interventions (162). During tissue remodelling, proteases release small protein fragments into the circulation (52, 162, 259, 288) that may serve as biomarkers of the fibrotic process. Further, the dynamic nature of ECM measures versus 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 behaviour and phenotype of cells housed in the ECM, any ECM remodelling will in turn influence adjacent cells and modify their behaviour/phenotype. Consequently, abnormal ECM remodelling may be a prerequisite for fibrogenesis and/or fibrolysis.

Common to all fibrotic disorders is the characteristic alterations in ECM remodelling 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 activated stellate cells and (portal) fibroblasts with a phenotype of wounds 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 remodelling 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 maintains 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 signalling (394). The collagen formation observed during fibrosis is also accompanied by an increase in type IV collagen degradation and unfavourable remodelling of the basement membranes, resulting from an increased expression of proteases in the affected tissue (129, 194, 351). This favours myofibroblast activation and a net increase in the deposition of e.g. fibrillar collagens by myofibroblasts (379). This is in part accompanied by a deregulated MMP activity towards 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 e.g. MMP-1, -8, -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. While 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. While 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 which are highly dependant on the ECM and cellular environment and soluble mediators in the host tissue can also phagocytose cellular debris which removes potential pro-inflammatory signals and which can cause increased MMP expression and enhanced matrix degradation (260). In a simplified view M1 macrophages are rather pro-inflammatory and anti-fibrotic, 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 behaviour 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 three-dimensional (3-D) scaffold that supports or encapsulates sessile or migrating cells and defines their microenvironment (11). It consists of a meshwork of proteins and non-protein components (glycosaminoglycans, GAGs) to which soluble factors, such as growth factors and cytokines, can bind, and 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 mono-layers on top of either a plastic substrate or a glass cover slip, 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 signalling 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 as compared to 3-D, which critically regulate cell and tissue behaviour (238, 251, 252). Taken together, in vitro models need to replicate the naturally occurring 3D environment, encompassing a sufficient physiological and pathophysiological variety of ECM components. Thus the effect of 2D vs 3D 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 2D 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 fulfil key functions only in development but may be dispensable later in life, these disease phenotypes provide pivotal information on ECM molecules important for tissue function, and thus give insight into their function and dysfunction in the pathology of non-genomic disorders.

TABLE 1
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), tumour necrosis factor (TNF)-α, Wnt-1 induced secreted protein 1 (WISP1) and bone morphogenic proteins (BMPs) (211), but they possess other signalling properties, both as whole proteins and as protein fragments. Specifically, decorin and biglycan are anti-fibrotic molecules, which by binding active TGF-β1 can interfere with its signalling 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 anti-apoptotic 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 (BDL) models of liver fibrosis (106). The soluble factors are usually bound to specific ECM components via low affinity non-covalent interactions which create stable concentration gradients around the ECM-embedded cells that produce these cytokines and growth factors, guiding e.g. inflammatory cells but also (myo-)fibroblasts towards 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 remodelling. Many of the heparan sulphate (proteoglycan) binding growth factors promote angiogenesis (e.g. fibroblast growth factor (FGF)-1, vascular endothelial growth factor (VEGF)) or epithelial cell proliferation and survival (e.g. epidermal growth factor (EGF), hepatocyte growth factor (HGF), keratinocyte growth factor (KGF)), others that bind to the
abundant collagens regulate fibroblast or immune cell activation (e.g. platelet-derived growth factor (PDGF)-B, oncostatin-M, interleukin (IL)-2, 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 signalling molecules. Other important examples of molecules sequestered in the ECM are 1) the pro-inflammatory 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 (Fn14), known to be overexpressed in chronic liver diseases (148, 243, 333). Lastly, the hedgehog and Wnt/β-catenin pathways together with Notch signalling 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 signalling (307).

TISSUE TURNOVER GENERATES POST-TRANSLATIONAL MODIFICATIONS WITH SIGNALLING FUNCTION AFFECTING CELL PHENOTYPE

To maintain healthy tissue, the ECM must regenerate itself by normal remodelling, 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 signalling potential (216, 230, 268, 305, 358, 388), which will be discussed indept 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 is altered. This transformation from a healthy tissue to a pathological one may cause the matrix to become stiff, which has been shown to enhance tumour 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. Fig.4 illustrates the steps of abnormal ECM remodelling in fibrosis. Healthy ECM consists of a network of fibers organized in a highly ordered fashion, with binding of key growth factors and signalling 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 remodelling 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 isomerisation of aspartate (seen in tissue aging), citrullination of arginine or nitrolysation of cysteine (occurring during inflammation), protease degradation at cleavage hotspots (observed in fibrosis and inflammation), glycosylation (in glycaemia, type II diabetes), and the fibrosis specific modifications made by polysialic acid that modulate the profibrotic ductular reaction in liver injury (62, 64)(339). Each of these modifications may change the function and signalling 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 to 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, isomerisation, 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
non-exhaustive list of ECM proteins (as this list continues to grow) and their exerted effects are shown in Table 3. These examples highlight those ECM proteins that serve as paracrine signalling 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.

POST-TRANSLATIONALLY 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 C-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 lysyl oxidase (LOX) enzyme family that cross-links collagen providing resistance to proteolysis. Similar findings were observed in the lung bleomycin model where E4 inhibited e.g. 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 inhibitory effects on endothelial cell migration, but not on their proliferation (268), while tumour suppression by type XV collagen is independent of the restin domain (225). Vastatin, the noncollagenous C-terminal fragment of type VIII collagen inhibits
endothelial cell proliferation and induces apoptosis in a bovine aortic endothelial (BAE) 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 signalling 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 pre-existing 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. Notable 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 tumours 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, -3 chain, respectively. These peptides are inhibitors of angiogenesis, tumour 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, due to 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 signalling roles in cancer proliferation and dedifferentiation, the increase in cross-links contributes to the stability of collagen accumulation in fibrosis (34, 226, 362). While there is a high constitutive expression of LOX in most tissues, LOXL2 is specifically upregulated in fibrosis and cancer and is tightly linked to a worsening of tumour grade and fibrosis stage (21). The validity of targeting this enzyme and its cross-linking activity to inhibit fibrosis and cancer has been tested using a therapeutic monoclonal antibody (21), and a clinical study in patients with fibrotic NASH and primary sclerosing cholangitis is ongoing.

**Transglutaminase-mediated cross-linking of the ECM**

In addition to LOX, there is a well established role for transglutaminases in the biochemical modification of ECM proteins (156). Transglutaminases constitute a family of at least 8 related proteins with well characterised transamidation activities forming largely irreversible Nε-(γ-glutamyl) lysine isopeptide bonds between a glutamine residue on one and a lysine (histidine) residue on another protein (88, 204). Transglutaminase 2 (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 tumour 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 the N-terminal portion of 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, since 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 anti-fibrotic effects are likely attributable to inhibition of TG2 cross-linking activity. However, the extracellular functions of TG2 are more complex, including activation of TGF-β1 from its inactive precursor (134), and non-enzymatic 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 pro-proliferative 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 remodelling, which is regularly coupled to deregulated ECM proteolysis, as illustrated in **Figure 5**.

**Perlecan, endorepellin**

The proteoglycan perlecan, a basement membrane heparan sulphate (HS) proteoglycan, has been implicated in fibrosis (86) and has shown to have opposing terminal angiogenic activities. The N-terminus carrying three HS-chains and the C-terminus have pro-
angiogenic and anti-angiogenic properties, respectively (30). The C-terminus is proteolytically processed by bone morphogenetic protein 1 (BMP-1) (110) yielding endorepellin, an 85kDa perlecan domain V fragment (216). The C-terminus V domain of perlecan is similar to another HS proteoglycan, agrin which is the major proteoglycan of the glomerular basement membrane. The C-terminal agrin fragment (CAF) is a known marker of neuronal muscular remodelling, but it has recently been shown to be a potential biomarker for renal function in renal transplant recipients (322). Like other mentioned angiogenesis inhibitors such as arresten, canstatin, endostatin and tumstatin, endorepellin mediates its anti-angiogenesis functions through binding to $\alpha_2\beta_1$ integrin, a key angiogenesis receptor (376). This interaction disintegrates the 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 $\alpha_2\beta_1$ integrin and Src family kinase dependent anti-apoptotic pathways in fibroblasts (188). Thus, the LG3 domain may not only affect angiogenesis, but also collagen deposition 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 SIGNALLING

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 signalling 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 e.g. 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 $\alpha_1\beta_1$ (and to a minor degree through $\alpha_2\beta_1$) while collagen type III engages mainly DDR1 (Table 4). The DDRs are receptor tyrosine kinases (RTKs) that specifically recognize collagens as their ligands (277). Similar to collagen-binding $\beta 1$ 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 2 in particular has demonstrated important functions in tissue homeostasis and cancers (243, 385), in which lack or inhibition of DDR1 attenuated fibrogenesis (35, 97, 120, 272, 276), while 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 due 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 aetiology and consequently 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 non-collagenous 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, e.g., 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 aetiology 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 is highest (329), and 4) smooth muscle cells localized in the larger vessel walls (23, 99, 115, 198). Consequently, the activity of these different cell types result 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 $\alpha$-smooth muscle actin (SMA) and deposit a (peribiliary) ECM (179, 285, 287, 288). The co-proliferation of reactive bile ducts and periductular myofibroblasts results initially in periportal fibrosis, and is followed by portal-portal septa formation surrounding the liver nodules, while 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 due to portal-central bridging necrosis, suggesting that myofibroblasts as well as HSCs migrate from the portal tract and neighbouring 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 due to distal sinusoidal dilation, centrilobular fibrosis and necrosis, endothelialitis and prominent and 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 neighbouring 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 due to different cell phenotypes or specific mechanisms has to our knowledge not been elucidated. However, based on the above mentioned models, one can assume 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 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, since e.g. 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 prior to development of varices and haemorrhages associated with cirrhosis (335).
ECM AND HEPATOCELLULAR CARCINOMA

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 due to the rising prevalence of end stage hepatitis C and NASH (160, 236). HCC is strongly correlated to fibrosis severity (203), with 90% of HCC cases arising in cirrhotic livers (299). Thus, more than 80% of hepatitis B and C patients that have HCC are cirrhotic (140). Similarly, HCC prevalence and progression is related to cirrhosis (93) in alcoholic and NASH (8), 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, while 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 protease activities directed at the ECM or cell associated structures, facilitating ECM remodelling, cancer cell proliferation and migration. As example is MMP-2 which is able to degrade numerous components of the ECM, that are closely correlated with tumour invasion and metastasis. Together with hypoxia-inducible factor 1α (HIF-1α), which regulates proteolytic and angiogenic activities, MMP-2 is overexpressed and its activity enhanced in HCC compared to healthy tissue (359). Additionally, MMP-7 and MMP-26 levels are significantly higher in HCC tissue compared to adjacent healthy hepatic cells from HCC patients (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 are 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, 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. Consequent 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, to date only in preclinical studies. Direct targeting of the ECM and e.g. 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, since the ECM is continuously remodelled, 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. While 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. Several evidences 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 remodelling are emerging (189, 358, 388) -. These examples begin to suggest that matrix molecules themselves may be anti-fibrotic 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 signalling potential in order to grasp the full potential of the ECM and its importance in pathogenesis.

**LIST OF ABBREVIATIONS**

ECM: extracellular matrix  
PTM: post-translational modification  
MMP: matrix metalloproteinase  
NAFLD: non-alcoholic fatty liver disease  
NASH: non-alcoholic steatohepatitis  
HCC: hepatocellular carcinoma  
TIMP: tissue inhibitors of matrix metalloproteinase  
GAG: glycosaminoglycan  
3-D: three-dimensional  
TGF: transforming growth factor  
SLRP: small leucine-rich proteoglycan
TNF: tumour necrosis factor
WISP1: Wnt-1 induced secreted protein 1
BMP: bone morphogenetic protein
IGF: insulin-like growth factor
CCl₄: carbon tetrachloride
BDL: bile duct ligation
FGF: fibroblast growth factor
VEGF: vascular endothelial growth factor
EGF: epidermal growth factor
HGF: hepatocyte growth factor
KGF: keratinocyte growth factor
PDGF: platelet-derived growth factor
IL: interleukin
TWEAK: TNF-like weak inducer of apoptosis
Fn14: fibroblast growth factor-inducible 14
HPCs: hepatic progenitor cells
LOXL2: lysyl oxidase-like 2
LOX: lysyl oxidase
TG2: transglutaminase 2
HS: heparan sulphate
BMP-1: bone morphogenetic protein 1
CAF: C-terminal agrin fragment

LG3: laminin-like globular domain

DDRs: discoidin domain receptors

HSC: hepatic stellate cell

RTK: receptor tyrosine kinase

SMA: smooth muscle actin

HIF-1α: hypoxia-inducible factor 1α

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COMPETING INTERESTS

All authors except HK, AJF, AB and DS are full time employees of Nordic Bioscience. The authors declare that they have no other conflicts of interest. HK, AJF, AB and DS have no conflicts of interest.

AUTHORS’ CONTRIBUTION

MAK made the outline of the manuscript and first draft. DS, TMJ, FG, JHK, MJN, JMLS, NUBH made the final draft of the manuscript, and collected all relevant references for review. HK, DS and DF contributed in all parts of structuring, figure and table design and editing of the manuscript for content and clarity. DJL, DS, AJF, AB, HK and ACBJ made...
contributions to each section of the manuscript in the final draft. DS, MAK, ACBJ and FG
edited the final version. All authors read and approved the final version of the manuscript.
FIGURE LEGENDS

Figure 1. Examples of fibroproliferative diseases in different organs. NASH: non-alcoholic steatohepatitis; AMD: age-related macular degeneration; IPF: idiopathic pulmonary fibrosis; COPD: chronic obstructive pulmonary disease; ARDS: acute respiratory distress syndrome; FSGS: focal segmental glomerulosclerosis. Reproduced with permission from 162.

Figure 2. Illustration depicting the changes that occurs in the extracellular matrix remodelling during the development of fibrosis. The extracellular matrix (ECM) may be divided into the loose basement membrane and the compact interstitial matrix. The basement membrane consists mainly of type IV collagen, laminins, entactin and proteoglycans and functions to anchor cells and connect to the interstitial ECM. The interstitial matrix has a different composition and consists mainly of fibrillar collagens, numerous noncollagenous glycoproteins, proteoglycans and elastin. In healthy tissue, ECM remodelling is tightly controlled to ensure homeostasis; the synthesis of ECM proteins by fibroblasts has a relatively slow metabolic turnover and the proteolytic activity is limited. During fibrogenesis, however, this process is disturbed. As a result of chronic wound healing, immune cells infiltrate the interstitial matrix which helps drive the profibrotic response. Fibroblasts and fibroblast precursors like hepatic stellate cells differentiate into myofibroblasts which deposit excessive levels of interstitial collagens. Initially they may release enhanced levels of ECM degrading matrix metalloproteinases (MMPs), while at later stages most MMPs are down-regulated. This in turn results in a change in the composition of the ECM where high levels of ECM degradation products and increased collagen cross-linking can be observed.

Figure 3: Binding of certain growth factors and cytokines to the extracellular matrix. The figure highlights prominent interactions. Shown is a selection of relevant extracellular matrix (ECM) binding factors (also discussed in the text) and their association with either heparan sulphate or collagen, and their target cells. The liberation of ECM-stored biologically
active growth factors and cytokines 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 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; PDFG, platelet derived growth factor; TGF-β1, transforming growth factor β1; VEGF, vascular endothelial growth factor.

Figure 4: Schematic representation of the high rate of extracellular matrix remodelling in fibrosis. Healthy extracellular matrix (ECM) consists of a network of fibers organized in a highly ordered fashion. During high matrix turnover the ECM is degraded leaving fragments of the ECM in the matrix and releasing other fragments into the circulation, while an accumulation of both new and already existing proteins occur, macroscopically described as fibrosis. ECM remodelling is a delicate equilibrium and a prerequisite for maintenance of a healthy tissue, in which senescent proteins are continuously degraded and replaced by new ones. This delicate balance is disturbed in fibrotic diseases, resulting in an increased ECM turnover (both formation and degradation). Thus, a subset of pathological proteases is overexpressed in the affected tissue, resulting in the release of protease specific fragments of signature proteins of the fibrotic ECM, referred to as protein fingerprints or neoepitopes. These fragments may be used as early diagnostic or prognostic serological markers of tissue degradation and in part formation. PTMs, post-translational modifications. Reproduced with permission from 164.
Figure 5: Type VI collagen as auto- and paracrine activator of fibrogenesis.

Type VI collagen microfibrils serve as sensor of early tissue injury and extracellular matrix (ECM) destruction, usually initiated by release of ECM degrading proteases like the matrix metalloproteinases (MMPs) by inflammatory cells. The thus generated proteolytic fragments activate type VI collagen receptors on (myo-) fibroblasts that promote their fibrogenic activation, in part via integrins, focal adhesion kinase and mitogen activated kinase (Erk1 and Erk2) activation. There is also coactivation of the mitogenic platelet derived growth factor α receptor. The activated myofibroblasts then enhance their deposition of ECM components, including intact type VI collagen whose ongoing degradation maintains a fibrogenic wound healing response.

### TABLES

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

<table>
<thead>
<tr>
<th>Aetiology</th>
<th>Biliary fibrosis</th>
<th>Viral hepatitis</th>
<th>Metabolic/alcoholic liver disease</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Initial histological changes</strong></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><strong>Main fibrogenic cell type</strong></td>
<td>Portal myofibroblasts</td>
<td>Portal myofibroblasts and HSCs</td>
<td>HSCs and later portal myofibroblasts</td>
</tr>
<tr>
<td><strong>Major ECM proteins</strong></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><strong>Fibrosis pattern</strong></td>
<td>Portal-portal septum</td>
<td>Portal-central septum</td>
<td>Chicken wire, later stages central-portal septum</td>
</tr>
</tbody>
</table>
ECM, extracellular matrix; HSC, hepatic stellate cell
Table 1. The relation of matrix components to connective tissue diseases, modified and extended from (164).

<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>(3, 4, 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 mice model (128)</td>
<td>(74)</td>
</tr>
<tr>
<td>Type IX collagen</td>
<td>MED</td>
<td>KO mice model (5, 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 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/Decorin</td>
<td>Osteopenia, skin fragility</td>
<td>Double KO mice (392)</td>
<td>(392)</td>
</tr>
<tr>
<td>Biglycan/Fibromodulin</td>
<td>Osteoarthritis</td>
<td>KO mice (357)</td>
<td>(6)</td>
</tr>
<tr>
<td>Perlecan</td>
<td>Multiple developmental defects, myotonia, Schwartz-Jampel</td>
<td>Transgenic and point mutation mice (324, 348)</td>
<td></td>
</tr>
<tr>
<td>Syndrome</td>
<td>Description</td>
<td>Model/Participants</td>
<td></td>
</tr>
<tr>
<td>--------------------------</td>
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<td>-------------------------------------------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>Nidogen 1 and 2</td>
<td>Lung and kidney development</td>
<td>Single KO mice (224, 296)</td>
<td></td>
</tr>
<tr>
<td>Fibromodulin</td>
<td>Osteoarthritis</td>
<td>KO mice (326)</td>
<td></td>
</tr>
<tr>
<td>Lumican/Fibromodulin</td>
<td>Joint laxity, impaired tendon integrity</td>
<td>Double KO mice (89)</td>
<td></td>
</tr>
<tr>
<td>Lumican</td>
<td>Reduced corneal transparency, skin fragility</td>
<td>KO mice, knockdown zebrafish (89, 390)</td>
<td></td>
</tr>
<tr>
<td>Decorin</td>
<td>Intestinal tumour, Skin fragility, Ehlers-Danlos syndrome-like.</td>
<td>KO mice (75)</td>
<td></td>
</tr>
<tr>
<td>Mimecan</td>
<td>Colorectal cancer early formation</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Fibrillin</td>
<td>Marfan syndrome</td>
<td>Murine and bovine model (27, 199)</td>
<td></td>
</tr>
<tr>
<td>COMP</td>
<td>PSACH, MED</td>
<td>Transgenic mice (172)</td>
<td></td>
</tr>
<tr>
<td>Matrillin-3</td>
<td>MED</td>
<td>KO mice (346)</td>
<td></td>
</tr>
<tr>
<td>Fibronectin</td>
<td>Glomerulopathy (proteinuria, microscopic hematuria, hypertension, renal failure)</td>
<td>Knockdown mice (182)</td>
<td></td>
</tr>
<tr>
<td>Tenascin C</td>
<td>Cardiovascular diseases, liver fibrosis</td>
<td>KO mice (186)</td>
<td></td>
</tr>
</tbody>
</table>

OI, Osteogenesis imperfecta; CIA, collagen-induced arthritis; KO, knock-out; DEB, Dystrophic epidermolysis bullosa; MED, Multiple epiphyseal dysplasia; SMCD, Schmid-type metaphyseal chondrodysplasia; SMD, Spondometaphyseal dysplasia; SVAS, Supravascular aortic stenosis; WBS, William-Beuren syndrome; CL, Cutis laxa; COMP, Cartilage oligomeric matrix protein; PSACH, Pseudoachondroplasia.
### Table 2: Key factors and their downstream effect on signalling molecules and cells.

<table>
<thead>
<tr>
<th>Growth Factor Signalling</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, anti-fibrotic.</td>
<td>(105, 174, 382)</td>
</tr>
<tr>
<td>IGFs</td>
<td>MAPK, PI3K, ERK</td>
<td>Anti-fibrotic, HSC proliferation.</td>
<td>(49, 313, 325)</td>
</tr>
<tr>
<td>TNF-α</td>
<td>NF-κB, JNK, ERK</td>
<td>Hepatocyte proliferation, inflammation, fibrogenic.</td>
<td>(262, 330)</td>
</tr>
<tr>
<td>Chemokines</td>
<td>CXCL1, CXCL9, CXCL10</td>
<td>Inflammation, anti-fibrotic, chemotactant.</td>
<td>(130, 191, 366, 395)</td>
</tr>
<tr>
<td></td>
<td>CXCR2, CXCR3, TGF-β</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>MCP1-3</td>
<td>HSC activation, NK cell activation, inflammation, chemotactant.</td>
<td>(202) (301)</td>
</tr>
<tr>
<td></td>
<td>CCR5, Pi3k/Akt</td>
<td>NK cell activation, HSC migration and proliferation, inflammation, chemotactant.</td>
<td>(173, 191, 202, 300)</td>
</tr>
<tr>
<td>Innate immune interactions</td>
<td>TLR2, TLR4</td>
<td>TNF-α, NFκB, JNK, Bambi</td>
<td>Chemotactic/inflammatory.</td>
</tr>
<tr>
<td></td>
<td>CD40</td>
<td>JNK, NF-κB</td>
<td>Secretion of MCP-1 and IL-8, inflammation, chemotactant.</td>
</tr>
<tr>
<td></td>
<td>IL-6</td>
<td>JAK/STAT, ERK1/2, Akt, NF-κB, MAPK</td>
<td>Regulation of inflammatory response, anti-fibrotic.</td>
</tr>
<tr>
<td></td>
<td>IL-8 (CXCL8)</td>
<td>CXCR1, CXCR2</td>
<td>Chemo attractant, inflammation.</td>
</tr>
<tr>
<td></td>
<td>IL-10</td>
<td>JAK/STAT</td>
<td>Anti-fibrotic.</td>
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<tr>
<td>Adipokine pathways</td>
<td>Leptin</td>
<td>OB-R, JAK/STAT, TGF-β</td>
<td>Fibrogenic, HSC activation.</td>
</tr>
<tr>
<td></td>
<td>Adiponectin</td>
<td>AMPK</td>
<td>Anti-fibrotic.</td>
</tr>
<tr>
<td>Developmental</td>
<td>Hedgehog</td>
<td>Ptc, Smo, Gli family</td>
<td>Fibrogenic, HPC differentiation, epithelial-mesenchymal transition.</td>
</tr>
<tr>
<td></td>
<td>Notch</td>
<td>NICTD, RBP-Jk</td>
<td>Fibrogenic, HSC activation and proliferation.</td>
</tr>
<tr>
<td></td>
<td>Wnt</td>
<td>Wnt/β-catenin, Wnt/β-catenin/Ca²⁺</td>
<td>Fibrogenic, HSC activation.</td>
</tr>
</tbody>
</table>
Adenosine monophosphate-activated protein kinase; Ptc, Patched; Smo, Smoothened; NICD, Notch intracellular domain; RBP, Recombinant signal binding protein.
Table 3. The matri-cellular effects of extracellular matrix components, extended and modified from (164).

<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. Proliferation of fibroblasts and smooth muscle cells. 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>(112), (7), (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, tumour growth and endothelial cell proliferation and migration. Induction of apoptosis</td>
<td>Various integrins</td>
<td>(222), (124)</td>
</tr>
<tr>
<td>Endostatin – fragment of collagen type XVIII</td>
<td>Inhibition of endothelial proliferation, angiogenesis and tumour growth. Induction of endothelial cell apoptosis</td>
<td>Glypicans, Nucleolin</td>
<td>(239), (78), (165), (305)</td>
</tr>
<tr>
<td>RGD motif – present in collagens, laminin and fibronectin</td>
<td>Cell adhesion, angiogenesis, apoptosis</td>
<td>Various integrins</td>
<td>(278), (45)</td>
</tr>
<tr>
<td>Fibromodulin</td>
<td>Proliferation, migration, and chemotaxis of HSCs</td>
<td>Unknown</td>
<td>(221)</td>
</tr>
<tr>
<td>Laminin-332 – elastase-generated fragment of γ2</td>
<td>Neutrophil chemotaxis</td>
<td>Unknown</td>
<td>(227)</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 chemotaxis</td>
<td>Unknown receptors. SIKVAV interacts with integrins α1, α6, and β1 in salivary gland carcinoma cell line.</td>
<td>(2), (98)</td>
</tr>
<tr>
<td>Laminin</td>
<td>Chemotactic migration of malignant</td>
<td>67LR (LamR)</td>
<td>(318), (79)</td>
</tr>
<tr>
<td></td>
<td>Regulation of inflammation and innate immunity.</td>
<td>CD14, Fas ligand, CXCL1</td>
<td>Fas</td>
</tr>
<tr>
<td>--------</td>
<td>-------------------------------------------------</td>
<td>------------------------</td>
<td>-----</td>
</tr>
<tr>
<td><strong>Lumican</strong></td>
<td>Apoptosis induction</td>
<td>(377), (104), (50)</td>
<td>(355)</td>
</tr>
<tr>
<td><strong>Biglycan</strong></td>
<td>Regulation of inflammation and innate immunity and effect on adhesion and migration</td>
<td>TLR2, TLR4, P2X4/P2X7, selectin L/CD44, C1q</td>
<td>(281), (12), (178), (311)</td>
</tr>
<tr>
<td></td>
<td>Cytokine modulation (PDGF, TGF-β, TNF-α, WISP-1, BMP-4).</td>
<td>RhoA, Rac1</td>
<td>(234), (181), (341), (121), (144), (218), (55)</td>
</tr>
<tr>
<td><strong>Decorin</strong></td>
<td>Signal transduction.</td>
<td>LRP-1, c-MET</td>
<td>(40), (109)</td>
</tr>
<tr>
<td></td>
<td>Cytokine modulation (PDGF, TGF-β, TNF-α, VWF, WISP-1).</td>
<td>TGF-β, C1q</td>
<td>(234), (181), (341), (121)</td>
</tr>
<tr>
<td></td>
<td>Regulation of inflammation and innate immunity.</td>
<td>IGF-IR</td>
<td>(77)</td>
</tr>
<tr>
<td></td>
<td>Anti-apoptotic effects.</td>
<td>EGF-R, VEGF-R2</td>
<td>(65), (311)</td>
</tr>
<tr>
<td></td>
<td>Antioncogenic effects.</td>
<td>IGF-IR, integrin α2β1, RhoA, Rac1</td>
<td>(282)</td>
</tr>
<tr>
<td></td>
<td>Adhesion and migration effects.</td>
<td></td>
<td>(298), (139)</td>
</tr>
<tr>
<td><strong>Fibronectin</strong></td>
<td>A 40 KDa fragment prevents PDL cell spreading, thereby inducing anoikis</td>
<td>Various integrins</td>
<td>(17, 72)</td>
</tr>
<tr>
<td></td>
<td>The 29- and 50-kd amino terminal fragments mediate release of proteoglycan from articular cartilage by RGD-independent mechanisms.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fn fragments can induce fibroblast gene expression of MMPs or can act as proteinases themselves</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Tenascin-C</strong></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>

PTM, Post-translational modification; DDR, Discoidin domain receptor; CXCL/R, CXC chemokines ligand/receptor; HSC, Hepatic stellate cell; PDGF, Platelet-derived growth factor; TGF, Transforming growth factor; TNF, Tumour necrosis factor, WISP, Wnt1-inducible signalling pathway protein; BMP, Bone morphogenic protein; TLR, Toll-like receptor; VWF, Von Willebrand factor; LRP, Lipoprotein
receptor-related protein; IGF-IR, Insulin-like growth factor-I receptor; EGF-R, Epidermal growth factor receptor; VEGF-R; Vascular endothelial growth factor receptor; PDL, Peridontal ligament; MMP, Matrix metalloproteinase.

<table>
<thead>
<tr>
<th>Protein</th>
<th>Receptor</th>
<th>Associated pathways</th>
<th>Effect</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collagen type 1</td>
<td>$\alpha_1\beta_1$, $\alpha_2\beta_1$, DDR1, DDR2</td>
<td>ShcA, Nck2, Shp-2, STAT5, NFkB, p37 MAPK, Src, Erk1/2, AP-1</td>
<td>HSC activation, proliferation and migration.</td>
<td>(73, 153, 275, 384)</td>
</tr>
<tr>
<td>Collagen type 3</td>
<td>DDR1, DDR2</td>
<td>ShcA, Nck2, Shp-2, STAT5, NFkB, p37 MAPK, Src, Erk1/2, AP-1</td>
<td>HSC activation, proliferation and migration.</td>
<td>(73, 275, 384)</td>
</tr>
<tr>
<td>Collagen type 4</td>
<td>$\alpha_1\beta_1$, DDR1</td>
<td>ShcA, Nck2, Shp-2, STAT5, NFkB, p37 MAPK, Src, Erk1/2, AP-1</td>
<td>HSC activation, proliferation and migration.</td>
<td>(73, 275, 384)</td>
</tr>
<tr>
<td>Collagen type 6</td>
<td>NG2</td>
<td>Paxillin, FAK, erk2.</td>
<td>HSC and myofibroblast activation and proliferation.</td>
<td>(289)</td>
</tr>
<tr>
<td>Collagen type 18</td>
<td>$\alpha_1\beta_1$</td>
<td>ILK, Akt.</td>
<td>Hepatocyte survival.</td>
<td>(85)</td>
</tr>
<tr>
<td>Fibronectin</td>
<td>$\alpha_1\beta_1$, $\alpha_5\beta_3$, $\alpha_5\beta_1$</td>
<td>ERK, JNK.</td>
<td>HSC activation</td>
<td>(262)</td>
</tr>
<tr>
<td>Fibronectin</td>
<td>$\alpha_5\beta_6$</td>
<td>ERK, JNK.</td>
<td>Cholangiocyte proliferation, local TGF$\beta_1$-activation</td>
<td>(248, 258, 262)</td>
</tr>
<tr>
<td>Angiotensin II</td>
<td>AT1R, AT2R</td>
<td>JAK2, Rho/Rho</td>
<td>Inflammation, HSC activation.</td>
<td>(22, 114, 177)</td>
</tr>
<tr>
<td>Luminican</td>
<td>TLR4, $\beta_2$ integrins</td>
<td>Under investigation</td>
<td>Collagen fibrillogenesis, requisite for hepatic fibrosis.</td>
<td>(184, 190)</td>
</tr>
<tr>
<td>Laminin</td>
<td>$\alpha_1\beta_1$, $\alpha_6\beta_1$, $\alpha_7\beta_1$</td>
<td>FAK, ILK</td>
<td>Fibrogenic, HSC activation.</td>
<td>(136, 249)</td>
</tr>
<tr>
<td>Fibromodulin</td>
<td>Under investigation</td>
<td>Under investigation</td>
<td>HSC activation, collagen type 1 deposition, anti-fibrotic.</td>
<td>(136, 192, 221)</td>
</tr>
<tr>
<td>Decorin</td>
<td>EGFR</td>
<td>Met</td>
<td>Anti-fibrotic by binding of TGF-β, collagen-assembly.</td>
<td>(13, 14)</td>
</tr>
<tr>
<td>Biglycan</td>
<td>TLR2, TLR4</td>
<td>P38, ERK, NFkB</td>
<td>Collagen assembly, proinflammatory, anti-fibrotic by binding of TGF-β.</td>
<td>(106, 281)</td>
</tr>
<tr>
<td>Syndecans</td>
<td>CD148, $\alpha_5\beta_3$</td>
<td>PI3K, Src, MAPK.</td>
<td>Hepatocyte proliferation, differentiation and adhesion. HCV attachment, angiogenesis.</td>
<td>(10, 24, 25, 212, 371)</td>
</tr>
<tr>
<td>TWEAK</td>
<td>Fn14</td>
<td>TRAF, JNK, NFkB</td>
<td>HSC proliferation, liver progenitor cell, proinflammatory.</td>
<td>(148, 333, 372)</td>
</tr>
</tbody>
</table>

DDR, Discoid domain receptor; STAT, Signal transducer and activator of transcription; NF, Nuclear factor; MAPK, Mitogen-activated protein kinase; ERK, Extracellular signal-regulated kinase; AP, Activator protein; HSC, Hepatic stellate cell; FAK, Focal adhesion kinase; ILK, Integrin-linked kinase; JNK, c-Jun N-terminal kinase; AT1/2R, Angiotensin type 1/2 receptor; JAK, Janus kinase; TLR, Toll-like receptor; EGFR, Epidermal growth factor receptor; TGF, Transforming growth factor; PI3K.
Phosphoinositide 3-kinase; HCV, Hepatitis C virus; TWEAK, TNF-like weak inducer of apoptosis; Fn, Fibroblast growth factor-inducible; TRAF, TNF receptor associated factor.


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**Heart**
1. Congestive heart failure
2. Endomyocardial fibrosis
3. Myocardial infarction

**Eyes**
1. Glaucoma
2. Diabetic retinopathy
3. Diabetic macular edema
4. AMD
5. Dry eye disease

**Liver**
1. NASH
2. Alcoholic liver disease
3. Schistosomiasis
4. Idiopathic portal hypertension
5. Congenital hepatic fibrosis
6. HCV/HBV
7. Autoimmune hepatitis
8. Primary sclerosing cholangitis
9. Primary biliary cirrhosis

**Lung**
1. IPF
2. Asthma
3. COPD
4. ARDS
5. Pulmonary arterial hypertension

**Intestine**
1. Inflammatory bowel diseases

**Kidney**
1. FSGS
2. Diabetic nephropathy
3. IgA nephropathy
4. Lupus nephritis
5. Transplant nephropathy

**Systemic**
1. Systemic sclerosis
2. Atherosclerosis
3. Nephrogenic systemic fibrosis
4. Cystic fibrosis
5. Chronic graft vs. host disease

**Skin**
1. Hypertrophic scars
2. Scleroderma
3. Eosinophilic fasciitis
4. Keloids
5. Dermatomyositis

**Iatrogenic fibrosis**
1. Surgery
2. Radiation therapy
3. Chemotherapy
Connective tissue
Tissue turnover equilibrium

Connective tissue
Increased tissue remodeling

Tissue formation & degradation
Release of protein fingerprints

Normal cells
Myofibroblasts
Proteolytical enzymes
ECM proteins
New ECM proteins
Neoeptopes, PTMs

Fibrosis
ECM deposition