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Extracellular matrix degradation in liver fibrosis: Biochemistry and regulation ☆,☆☆

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ABSTRACT

Fibrosis is a highly conserved wound healing response and represents the final common pathway of virtually all chronic inflammatory injuries. Over the past 3 decades detailed analysis of hepatic extracellular matrix synthesis and degradation using approaches incorporating human disease, experimental animal models and cell culture have highlighted the extraordinarily dynamic nature of tissue repair and remodelling in this solid organ. Furthermore emerging studies of fibrosis in other organs demonstrate that basic common mechanisms exist, suggesting that bidirectionality of the fibrotic process may not solely be the preserve of the liver. In this review we will examine the cellular and molecular mechanisms that govern extracellular matrix degradation and fibrosis resolution, and highlight how manipulation of these processes may result in the development of effective anti-fibrotic therapies. This article is part of a Special Issue entitled: Fibrosis: Translation of basic research to human disease.

1. Background

Chronic inflammation invariably gives rise to tissue fibrosis, which can be considered a dysregulated fibroproliferative response ultimately impacting on tissue architecture and function. Fibrosis results from the interplay of a number of different cell types including macrophages, myofibroblasts and epithelial cells and results in the accumulation of fibrillar collagens (predominantly collagens I and III) as a result of both changes in matrix synthesis and in the pattern of extracellular matrix (ECM) degradation. It has been estimated that inflammation and fibrosis contribute to 45% of deaths in the western hemisphere [1].

Hepatic fibrosis, the common final pathway of virtually every chronic inflammatory liver injury, represents a particularly well investigated model of the generic inflammation–fibrosis–progression/resolution pathological continuum [2]. Research interest in liver fibrosis continues to grow particularly because the burden of chronic liver disease is increasing; cirrhosis, the end-stage of fibrotic liver disease, is currently the fifth commonest cause of mortality in the UK [3]. Liver fibrosis can be considered a paradigm for the generic aspects of this pathological process and as such it is at the vanguard of studies of ECM and ECM turnover in experimental pathology [2]. Indeed, hepatic fibrosis in both progression and resolution has arguably been studied in greater detail than any other organ model system. This is in part because of the tractability of animal models of liver fibrosis where induction of sterile inflammatory injury is relatively straightforward, consistent and can be studied in a predictable and practical timeframe. But there has also been an increase in our detailed knowledge of both the natural history of human liver disease and the response of the fibrotic human liver to well characterised therapeutic interventions (particularly interferon in the treatment of chronic hepatitis B and C) which has established a powerful model illuminating critical aspects of matrix synthesis and degradation across relevant species [2,4–9]. For these reasons this review will focus primarily on the pathogenesis of liver fibrosis, but with reference to other organ systems where appropriate.

2. Introduction of key concepts

At a cellular level the perisinusoidal hepatic stellate cell (HSC) has been extensively studied as a key effector of fibrogenesis [10–12]. In acute and chronic injury this vitamin A storing cell sheds its retinoid and lipid droplets and transforms to an "activated" myofibroblast-like phenotype. Activation of this cell to an extracellular matrix-secreting myofibroblast phenotype is associated with fibrillar collagen production and fibrotic matrix deposition in vivo. Activated HSCs also express tissue inhibitors of metalloproteinases (TIMPs) [13–15] as well as chemotactic and vasoactive factors. The major secreted TIMP, TIMP-1 inhibits the endogenous matrix degrading activities of a wide range of matrix metalloproteinases (MMPs) favouring scar deposition. Furthermore, using a host of different model systems has allowed us to gain a deeper understanding of the complexity of liver inflammation and repair. In particular the dynamic interplay between the epithelial, inflammatory, myofibroblast and extracellular matrix components of tissue repair are becoming increasingly well defined [16]. Other key emerging

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concepts include the idea that inflammatory cell phenotypes demonstrate plasticity and that specific inflammatory cell types may contribute to matrix degradation not only in the development but also in the spontaneous resolution of liver fibrosis [17], and the observation that liver myofibroblasts may arise from a number of different cell lineages [2]. Perhaps most promising and of direct relevance to this review is the growing body of in vivo and in vitro evidence that suggests that liver fibrosis is a bidirectional process [2]. These data challenge the traditional dogma that fibrosis is at best irreversible and importantly are supported by robust clinical observations in humans. Understanding fibrosis resolution has the potential to highlight the essential attributes of an antifibrotic, or more accurately a pro-resolution therapy applicable to the liver and potentially other organs.

3. Matrix components of the liver in health and disease

The extracellular matrix within the normal liver is composed of a series of classes of macromolecules which include collagens (types I, III, IV, V and VI), the non- collagenous glycoproteins which encompass laminin and fibronectin amongst others and proteoglycans [18,19]. In the normal liver sinusoid, there is a non-electron dense basement membrane matrix which comprises laminin and type IV collagen. During the development of fibrosis this matrix becomes progressively replaced by one rich in interstitial collagens particularly collagens I and III [19–21]. Initially, and by virtue of changes in cell-matrix interactions, this accumulation of fibrillar ECM is associated with capillarization of the sinusoids; a loss of the sinusoidal endothelial fenestrae and physical change in the hepatocytes which lose their microvilli [19–21]. Ultimately the accumulation of collagens increases until vascular structures are linked and the architecture of the liver is disrupted significantly. Furthermore, areas of hepatocyte regeneration form spheres which further distort the structure and angiogenesis may occur in dense areas of scarring [19,22].

Studies have demonstrated a 4- to 7-fold increase in the content of collagen and glycosaminoglycans in the cirrhotic liver compared with normal liver. There is a disproportionate increase in the fibrillar collagen, collagen type I, and there are also increases in laminin and proteoglycans [19], with cross-linking of matrix also documented [22]. End-stage cirrhosis is associated with swathes of dense extracellular matrix rich in elastin in addition to the fibrillar collagens described above. Indeed, elastin is used as a pathological benchmark for chronicity of fibrotic change. The accumulation of matrix is associated with an impairment of hepatic function and predisposition to the development of hepatocellular cancer, although the mechanisms governing neoplastic change in the fibrotic liver are still incompletely understood [23]. This profound architectural disruption of liver anatomy results in the well-known complications of liver cirrhosis, particularly the development of portal hypertension, which is a major cause of death in patients with cirrhosis.

This review is necessarily brief, and therefore we will focus on the changes that occur to the cellular and extracellular matrix components in progressive fibrosis, fibrosis resolution and irreversible end-stage cirrhosis, with particular emphasis on collagens I and III and elastin as critical determinants of disease outcome.

4. Sources of extracellular matrix within the liver

With respect to the fibrillar collagens and elastin, the major source of these matrix components is the hepatic myofibroblast. In turn, this cell type has typically been described as representing an activated phenotype of the hepatic stellate cell [24–26]. Hepatic stellate cells are mesenchymal cells which lie in the space of Disse between the specialised hepatic sinusoidal endothelium and the palisades of hepatocytes. Rich in vitamin A in health, which is stored in the form of retinol esters within cytoplasmic droplets, these cells lie in close proximity to the normal basement membrane matrix consisting of type IV collagen, laminin and heparin sulphate proteoglycans. During liver injury stellate cells proliferate, become activated to a myofibroblast-like phenotype expressing alpha smooth muscle actin, and secrete fibrillar collagens, elastin and matrix proteins [24–27]. Recent interest has focused on the specific origins of these cells. Whilst there is evidence that portal myofibroblasts, circulating fibrocytes and mesenchymal stem cells in addition to peritoneal mesoepithelial cells may all give rise to a myofibroblast population [28–33], the relative contribution of each lineage is currently moot. Indeed, the relative contributions of individual cell lineages likely depend on the site and duration of injury and current evidence still supports hepatic stellate cells as being the predominant source of liver myofibroblasts. Of note, recent interest in epithelial–mesenchymal transition (EMT) as a major process deriving myofibroblasts from hepatocytes appears to have been misplaced [34–36]. Certainly, in genetically modified animal models in which robust lineage tracing can be undertaken no clear cut evidence of EMT has been demonstrated in in vivo models of hepatic fibrogenesis, or recently in models of renal fibrosis. We have recently demonstrated in unpublished data that the PDGFRβ gene (platelet derived growth factor receptor beta) can be used to effectively drive both marker proteins and gene deletion strategies identifying a commonality between hepatic stellate cells and pericytes seen elsewhere in the body, including the kidney, lung and heart. The mechanisms underpinning stellate cell activation have been studied in enormous detail using genetically modified mice in addition to tissue culture models and these studies have recently been reviewed extensively by Friedman [37]. A detailed discussion is beyond the scope of this article, but critical activating stimuli include TGFβ1 to promote a fibrogenic collagen-secreting phenotype and PDGF stimulation which promotes a proliferative phenotype [38,39]. Additionally stellate cells appear exquisitely sensitive to the extracellular components that they are in direct contact with, demonstrating profound changes in their behaviour in response to the physical environment provided by the matrix [40].

5. Extracellular matrix degradation during liver fibrosis

Even during progressive liver fibrosis there is evidence of a potential for matrix degradation. Matrix may be degraded by a number of enzymatic families, but foremost are the matrix degrading metalloproteinases (MMPs). These are a family of zinc and calcium dependent endopeptidases which are produced by connective tissue cells and inflammatory cells and have a range of activity against the major constituents of ECM including fibrillar and non-fibrillar collagens and elastin [41]. Individual MMPs are more or less promiscuous with respect to their direct substrate specificity with certain enzymes demonstrating a wide range of substrate specificity. Expression of MMPs has been demonstrated in a spectrum of liver cells which includes hepatocytes, hepatic stellate cells, kupffer cells and neutrophils and recruited hepatic macrophages [41]. Interestingly, for both HSC and macrophages the repertoire of MMPs expressed by the cells appears to alter with specific changes in phenotype that accompany fibrogenesis in vivo.

MMPs can be grouped according to enzymatic substrate; collagenases are central to the process of remodelling fibrotic tissue because they cleave the native helix of fibrillar collagens rendering the product (a gelatin) susceptible to degradation by other MMPs. Neutrophil collagenase, MMP-8, is expressed by both neutrophils and macrophages (both populations are well represented in the inflammatory stages of liver injury) [42]. Interstitial collagenase or MMP-1 has been described, particularly in inflammatory cells in human liver [15], and its counterpart in rodents MMP-13 has been shown to be expressed by stellate cells and macrophages [14,43]. Whereas in stellate cells MMP-13 is a feature of early activation and the fully activated fibrogenic stellate cell phenotype downregulates MMP-13, expression in macrophages appears to be relatively constant regardless of the stage or stimulus for activation in models of liver injury. It is always considered axiomatic that

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