Is the total amount as important as localization and type of collagen in liver fibrosis due to steatohepatitis?

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Is liver fibrosis just liver fibrosis? Or do the subtype of collagen, its spatial localization in the liver, its cell of origin, and the time point at which it is synthesized also matter? It is important, since the various collagen subtypes hold different informative values regarding reparative processes in the liver, and as collagens have also emerged as important signaling molecules (1). Novel data have challenged our perception of liver fibrosis and collagens, which may have important implications regarding the development of new biomarkers and anti-fibrotic interventions. The traditional histological analysis of liver biopsies using histochemical collagen stains, such as the Masson’s Trichrome stain or the Sirius Red stain, group all triple helical collagen structures into one gross bucket. Importantly, these stains ignore many other quantitatively minor but nonetheless functionally and structurally relevant collagen and non-collagen extracellular matrix (ECM) components.

To increase our understanding of liver fibrosis, we need to examine the specific localization of the individual ECM components related to the time course of fibrosing liver diseases. To fully appreciate the complexity, one should place emphasis upon the biological function of the ECM and the primary cell types responsible for synthesis of each particular collagen component. The most common method of evaluating liver fibrosis in clinical studies is by microscopic analysis of biopsies stained with Masson’s Trichrome or Sirius Red - an approach that may be too simplistic and may have resulted in a range of misunderstandings and simplifications. One major limitation of this approach is that these collagen stains are not able to specifically differentiate between the many different collagen molecules that are characterized by a unique, tightly packed and stable triple helical superstructure (2). This is particularly important because collagens are not just inert structural rods. In fact, most collagens contain only a minor proportion of rigid collagen domains, but are made of rather interrupted triple helices and larger non-collagenous domains which render them flexible and endow them with multifunctional properties, e.g. in cellular interactions (1–4). At present, 28 different collagens have been identified in the collagen superfamily, with 46 genetically distinct collagen chains. Each of these collagen molecules is unique with respect to its structural and signaling properties, its interaction with other ECM components and regarding its localization in the diseased organs (1,3) (Figure 1).

In general, there are two main types of ECM, the basement membrane and the interstitial matrix, which are produced by different cells types and have varied and distinct functions, as depicted in figure 1. The basement membrane is the primary ECM of epithelial and endothelial cells, of which the main constituents are variants of type IV collagen and laminin (Figure 1) (5). Both protein types share the collagen triple helical domains. Typically, type IV collagen is assembled as a loose permeable network,
representing a network forming collagen, allowing for the bidirectional flow of nutrients and metabolites from the blood to the hepatocytes (6). Sinusoidal endothelial cells and biliary epithelial cells have been identified as sources of type IV collagen in the liver, while portal fibroblasts and hepatic stellate cells (HSCs) also contribute to its production (7–11). The interstitial matrix is a supportive scaffold, produced mainly by different types of fibroblasts, and involves the fibrillar collagens such as type I, II, III, V, and XI (1), as seen in figure 1. It contains also other important collagens, such as the micro-filamentous type VI collagen that, when disturbed, may signal danger (12,13) as well as multiple FACIT collagens binding to these dense fibrillar collagens. The fibrillar collagens are very different from the networking collagens, forming a dense diffusion barrier meshwork and are central to tissue structure and integrity (1).

Importantly, the ECM proteins, and prominently the collagens, confer signals of correct position, migration, activation, and metabolic competence to the cells attached to them. Interference with these signals that are transmitted via specific ECM receptors, such as the integrins, can grossly affect cell behavior, as well as fibrosis progression and regression (4,14). Some of these signals are resulting from processing of the networking collagens type IV and VIII. The non-collagen (NC) domains from the 6 different alpha chains of type IV collagen are arresten (α1), canstatin (α2), tumstatin (α3), tetrastatin (α4), penstatin (α5) and hexastatin (α6). The signaling NC domain of type VIII collagen is known as vastatin, have highly anti-migratory effects on endothelial and other cells, and trigger other direct cellular effects that are mediated by certain cellular receptors including integrins (15,16). Another well described collagen fragment with signaling potential is endostatin, a fragment of type XVIII collagen, located in the (sinusoidal) basement membrane, and shown to be anti-fibrotic (17). In addition, the emerging hormone endotrophin, originating from the α3 chain of type VI collagen, is associated with cancer progression, fibrosis and the metabolic syndrome (13,18). Consequently, the type and location, and thereby turnover of the collagens is highly important in the pathophysiology of liver fibrosis. A full list of the collagen signals associated with each collagen molecule is found in figure 1.

The key role of lobular zonation in relation to the multitude of metabolic functions of the liver is well established (19). Within the various zones there is a differential expression of functional enzymes, such as cytochrome P450s, according to the metabolic function of the respective zone. For example, zone 3 plays an important role in glycolysis and lipogenesis, and the enzymes vital to these particular functions are therefore highly expressed. It is not surprising that liver fibrosis forms distinct histological patterns dependent on the etiology of the initial tissue damage. The fibrosis pattern of early-stage chronic viral hepatitis is that of a periportal fibrosis (20). Fibrosis due to alcoholic or non-alcoholic steatohepatitis

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(NASH), on the other hand, typically begins around the central veins and usually forms a pericellular and perisinusoidal, “chicken-wire” pattern during its early stage (21). These early stages of mild fibrosis observed in metabolic liver diseases like NASH are characterized by the pericellular accumulation of basement membrane ECM proteins in metabolically stressed areas (zone 3) (Figure 2b) (22). In addition, small amounts of type III and other fibrillar collagens are produced by activated hepatic stellate cells (HSCs) (23). However, the exact location of each type of collagen in fibrotic liver diseases is partly unknown. Remodeling of basement membrane components, such as type IV collagen, has been reported in both early and late liver fibrosis and is dependent on different cell types (24). Such remodeling appears to be due to collagen production by HSC and sinusoidal endothelia that are triggered by products of diseased hepatocytes and by inflammatory cells, to generate a “fibrotic scaffold” rich in basement membrane proteins around which regeneration or further repair with scarring can occur (22–25,28). Notably, hepatocytes, which otherwise do not produce collagens, are the primary source of the minor but functionally important basement membrane collagen type XVIII in normal and fibrotic liver, and activated cholangiocytes also contribute to its production in fibrosing liver disease (10,24). The function of type XVIII is little explored other than it is the parent molecule for the anti-angiogenic polypeptide endostatin (26,27). In addition activated cholangiocytes (cytokeratin 7 or 19 positive ductular cells with properties of epithelial progenitor cells) produce type IV collagen and other basement membrane components (28). Consequently, increased type IV collagen turnover is a first sign of early and possibly reversible fibrosis in the liver tissue in NASH (22). Thus early fibrotic changes are associated with the turnover of the normal fine sinusoidal reticular (Basement membrane collagen) fibers and the initiation of endothelial capillarization (29,30). Capillarization is an important pathological event and occurs in all chronic liver diseases of any etiology. Apart from the formation of a microscopically visible basement membrane, capillarization also includes the loss of endothelial fenestrae and transformation of the sinusoidal to a vascular-type endothelium. The increased deposition of type I, III, IV, V, VI and XVIII collagens widens the Space of Disse, resulting in a perturbed hepatic microcirculation, a compromised exchange of metabolites between blood and hepatocytes, ultimately leading to hepatic dysfunction and usually coupled with portal hypertension (7). Elevated serum levels of type IV collagen or laminin fragments in rodents or patients with liver fibrosis could be correlated with histological capillarization and the resulting increase of type IV collagen turnover products (31,32). At an early stage of fibrosis, this type of basement membrane remodeling may be essential for the reparative capability of the hepatocytes and the regeneration of healthy matrix, whereas in advanced disease it appears to reflect severe derangement of the liver architecture (31,33,34).
In advanced-stage biliary fibrosis and advanced stages of parenchymal liver diseases, also periportal fibroblasts, in addition to HSCs, become activated and begin to produce interstitial matrix, primarily consisting of fibrillar collagens (type I, III, V, XIX collagen) (Figure 2c) (3). Furthermore, elastin is increasingly deposited around the venules of the liver (35). The accumulation of interstitial matrix becomes more prominent and progresses to bridging fibrosis, which is associated with a worse prognosis (Figure 2c) (36). The bridging pattern of fibrosis ultimately leads to encapsulation of liver lobules (regenerative nodules), that are further damaged due to vascular undersupply (37). Hence, the reparative potential and activities of the tissue are fundamentally altered. When regenerative nodules are present, the histological criteria for liver cirrhosis are fulfilled, corresponding to fibrosis stage F4.

Consequently, not only the amount and pattern of fibrosis, but also the localization and type of collagens change during progression of NASH from stage F1 to F4. The initial phase and stages encompass active generation of basement membrane components with a loose network of type IV collagen and a still delicate reticular meshwork of mainly type III collagen, with a high potential of reversibility (fibrolysis) depending on, e.g. lifestyle changes or pharmacological therapy (38). In later stages with bridging fibrosis, the dense fibrillar collagens type I and III are mainly produced by portal fibroblasts and to some extent by activated HSCs. The understanding of the individual expression of different types of collagens and their localization and regulatory role is of great importance for the identification of potentially reversible or progressive phenotypes of fibrosis, even when cirrhosis is already present. We hypothesize that the zonation of the liver parenchyma with its spatially different localization of fibrogenic cells, most importantly HSCs and portal fibroblasts, is related to the synthesis of different ECM proteins that play variant functional roles and differentially affect reversibility of fibrosis.

In conclusion, further studies are urgently needed to evaluate the expression of specific ECM components and specific collagen types in liver fibrosis, with special emphasis on their time-dependent and spatial expression patterns, their cell(s) of origin, their different function, and their prognostic roles in fibrotic liver diseases. As discussed, different normally cryptic signaling fragments are emerging from proteolytic degradation of collagens (1,3), that have potent anti-angiogenic, cell migration promoting, or metabolic effects, and some of which have even anti-fibrotic properties (1,39). The fibroblast-derived type VI collagen fragment endotrophin, on the other hand, promotes fibrosis (13). Thus, the collagens are more than passive rods or bricks of the ECM but key elements in the maintenance of understanding of the complexity of fibrotic liver diseases. Further understanding of the differential expression of collagen types and their functional aspects may in turn pave the way for the development of tailored and personalized anti-fibrotic therapies in the future (14).
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Figure 1

Structure, organization and cellular association of the 28 known collagens, originating from 46 different unique chains. To date, 13 of these collagens have been found in normal and/or fibrotic livers: type I, III, IV, V, VI, VIII, X, XII, XIV, XV, XVI, and XVIII. The scheme illustrates the different molecular design and supramolecular organization of these collagens, resulting in numerous functions far beyond a mere structural role in the maintenance of tissue integrity. In addition, a range of these collagens are subject to proteolytic processing that results in cryptic collagen signals, some of which hold anti-angiogenic and pro-fibrotic signaling potential, listed below. Importantly, the basement membrane and the interstitial matrix both are composed of a range of unique collagens with specific function at their location. While the basement membrane collagens are structured rather loosely and thereby allow for diffusion, the structurally more rigid fibrillar collagens provide the backbone of the tissue. Collagens type IV, VIII and X are network forming collagens. Collagens type I, II, III, V, XI, XXIV, XXVI and XXVII are fibrillar collagens, with the latter two being short fibrils. Type XIII, XVII, XXIII and XXV are membrane bound collagens. Collagen type IX, XII, XIV, XVI, XIX, XX, XXI and XXII are fibrillar associated collagens with an interrupted triple helix (FACIT).
Figure 2

Schematic illustration of the liver architecture in healthy individuals, in the early regenerative phase and in late stage fibrotic NASH. Early-stage fibrosis is prominently located pericellularly around hepatocytes and underneath (sinusoidal) endothelial cells. It consists mainly of a loose network of basement membrane components, and a delicate meshwork of type networking collagens dominated by type IV collagen. The portal-to-central vein bridging fibrosis is produced by activated HSCs and different types of (myo-) fibroblasts and consists mainly of fibrillar collagens type I, III, and, although to a lesser extent, the filamentous microfibrillar type VI collagen that form a dense ECM. Moreover, a dense meshwork of type IV collagen and other basement membrane components like laminins now promote and sustain sinusoidal capillarization that compromises hepatocyte function. Inspired by (40).
Healthy Liver

Early Regenerative Phase

Late stage liver fibrosis