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Review

Origins and functions of liver myofibroblasts ☆

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ABSTRACT

Myofibroblasts combine the matrix-producing functions of fibroblasts and the contractile properties of smooth muscle cells. They are the main effectors of fibrosis in all tissues and make a major contribution to other aspects of the wound healing response, including regeneration and angiogenesis. They display the *de novo* expression of α-smooth muscle actin. Myofibroblasts, which are absent from the normal liver, are derived from two major sources: hepatic stellate cells (HSCs) and portal mesenchymal cells in the injured liver. Reliable markers for distinguishing between the two subpopulations at the myofibroblast stage are currently lacking, but there is evidence to suggest that both myofibroblast cell types, each exposed to a particular microenvironment (*e.g.* hypoxia for HSC-MFs, ductular reaction for portal mesenchymal cell-derived myofibroblasts (PMFs)), expand and exert specialist functions, in scarring and inflammation for PMFs, and in vasoregulation and hepatocellular healing for HSC-MFs. Angiogenesis is a major mechanism by which myofibroblasts contribute to the progression of fibrosis in liver disease. It has been clearly demonstrated that liver fibrosis can regress, and this process involves a deactivation of myofibroblasts, although probably not to a fully quiescent phenotype. This article is part of a Special Issue entitled: Fibrosis: Translation of basic research to human disease.

1. Introduction

Myofibroblasts are the main effectors of fibrosis in all tissues. They also make a major contribution to other aspects of the wound healing response, including regeneration, inflammation, angiogenesis, normal tissue repair after acute injury and to the stromal reaction in tumors. They combine phenotypic features of fibroblasts, such as the production of extracellular matrix, with the contractile functions of the smooth muscle cells involved in tissue architecture distortion. Myofibroblasts may be defined as cells that develop contractile force and stress fibers, *de novo*, and *in vivo* [1]. The most widely used and accessible marker of these cells is the *de novo* expression of α-smooth muscle actin (α-SMA), although this is not an absolute requirement for the identification of a cell as a myofibroblast. Other markers of myofibroblasts (endosialin, P311, integrin α11β1, osteopontin, periostin) have been proposed, but all were identified in specific conditions, and it remains unclear whether they could serve as general markers [1]. The precursors of myofibroblasts have also yet to be identified. In most tissues, myofibroblasts are thought to originate from resident fibroblasts, but they may also be derived from other cell types, mostly of mesenchymal origin, such as vascular smooth muscle cells, pericytes and adipocytes. There is also evidence to suggest that myofibroblasts may be derived from circulating fibrocytes or resident epithelial cells, through epithelial-to-mesenchymal transition, although recent studies argue against a significant contribution of these mechanisms to fibrosis. In addition, resident fibroblasts are themselves heterogeneous and may even include antifibrotic subpopulations, such as lung Thy-1-expressing fibroblasts [2].

2. Origins of myofibroblasts in liver fibrosis

Myofibroblasts are absent from the normal liver, but they accumulate at sites of injury, in patients with chronic liver diseases. They are the major source of extracellular matrix constituents in the injured liver, as clearly demonstrated by clinical and experimental studies. However, although myofibroblasts appear to be necessary for the development of fibrosis, they may not be sufficient. For example, in scleroderma [1] and focal nodular hyperplasia (unpublished personal observation), the liver may contain large numbers of myofibroblastic cells without significant fibrosis. In the liver, as in

Abbreviations: α-SMA, α-smooth muscle actin; HSC, hepatic stellate cell; PMF, portal myofibroblast

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other tissues, the origin of myofibroblasts is a matter of debate. During liver development, the septum transversum-derived mesothelium, which signals to induce hepatogenesis from the foregut endoderm, gives rise to sinusoidal pericytes, called hepatic stellate cells (HSCs), and perivascular mesenchymal cells, including portal fibroblasts, smooth muscle cells and fibroblasts around the central veins [3]. All these cells therefore have a common mesodermal origin, different from that of sinusoidal endothelial cells, Kupffer cells and hepatoblasts. It is now more than 35 years since the initial demonstration by Hans Popper and coworkers that transitional cells with the morphologic characteristics of vitamin A-containing cells (*i.e.* HSCs) and fibroblasts, overproduce fibrillar collagen in rats with carbon tetrachloride-induced liver injury [4]. The paradigm of hepatic stellate cell (HSC) activation giving rise to myofibroblasts has since dominated the focus of research on liver fibrosis [5]. HSC was the first major cell type in the liver to be identified as a prominent source of collagen production in the injured liver [6], and to be shown to acquire a myofibroblastic phenotype in culture, with the ability to overproduce extracellular matrix, to contract and to undergo chemoattraction [7–10]. The factors and mechanisms triggering the myofibroblastic differentiation of hepatic stellate cells have been reviewed extensively elsewhere [5]. They include biological (*e.g.* lipopolysaccharide) [11], physicochemical (*e.g.* hypoxia) [12] and mechanical (*e.g.* substrate stiffness) [13] stimuli. There is now both *in vitro* and *in vivo* evidence for the existence of more than one origin of liver myofibroblasts. In preparations of cells isolated from the liver, HSCs are recognized by the fluorescence of their retinoid droplets under UV excitation at a wavelength of 328 nm. Culture studies have clearly demonstrated that other liver cell types, without fluorescent retinoid droplets, can give rise to myofibroblasts [14,15]. *In situ* ultrastructural studies have shown that fibroblasts reside in the portal mesenchyme and accumulate, with fibrosis, around bile ducts in bile duct-ligated rats [16], precisely in the zones in which α-SMA can be detected [15]. Moreover, immunohistochemical studies have shown that, in fibrotic human or rat liver, portal and septal myofibroblasts have expression profiles different from those of interface myofibroblasts or sinusoidally located HSCs, suggesting that at least two subpopulations of myofibroblasts — HSC-derived myofibroblasts (HSC-MFs) and portal mesenchymal cell-derived myofibroblasts (PMFs) — populate the injured liver [17]. The possible contribution of epithelial-to-mesenchymal transition to renal fibrosis *in vivo* has been challenged by studies based on genetic cell lineage tracing in mice [18]. Likewise, studies based on genetic cell fate tracking have strongly challenged the concept that either hepatocytes or cholangiocytes acquire a mesenchymal phenotype *in vivo* through epithelial-to-mesenchymal transition to produce the extracellular matrix in liver fibrosis [19–21]. A number of studies have also suggested that circulating cells from the bone marrow can function as stem cells, contributing to the liver myofibroblast population [22]. However, a recent study based on a system for the exclusive detection of bone marrow-derived collagen-producing cells, showed that bone marrow-derived cells played a limited role in collagen production during liver fibrosis [23].

3. Portal myofibroblasts

In almost all types of chronic liver disease, including biliary (*i.e.* primary biliary cirrhosis, biliary atresia), viral, alcoholic and non-alcoholic fatty liver diseases, fibrosis develops predominantly in the portal area and appears to progress from this area, even if the primary targets of injury are intralobular hepatocytes [24–28]. This observation suggests that the contribution of PMFs to liver fibrosis may be more important than generally assumed. Furthermore, in chronic liver diseases of various origins, including viral hepatitis and non-alcoholic fatty liver disease, fibrogenesis is associated with the occurrence of a ductular reaction, in which duct-like cells with progenitor features proliferate, expanding outwards from the portal area. The correlation between the extents of the ductular reaction and of replicative senescence in hepatocytes suggests that ductular/progenitor cells may be recruited in situations in which the regeneration of mature hepatocytes is impaired. One possible explanation for this is that ductular/oval cells are less sensitive to TGF-β-induced growth inhibition than hepatocytes [29]. Several potential mechanisms by which cholangiocytes or hepatic progenitor cells may promote fibrogenesis (Fig. 1) have been reviewed elsewhere [30] and new mechanisms have recently been put forward. Ductular/progenitor cells may act on matrix accumulation directly, through the release of tissue inhibitor metalloprotease 1 (TIMP1), for example [31]. They may act on myofibroblasts by releasing promitogenic, profibrogenic, chemotactic or anti-apoptotic factors. Hepatic progenitor cells have been shown to increase hepatic fibrogenesis, in an experimental model in which rat liver fibrosis is induced by chronic treatment with a combination of carbon tetrachloride and acetylaminofluorene, promoting activation of the hepatic progenitor cell compartment [32]. Chronic treatment with carbon tetrachloride alone caused liver fibrosis, which began around the central veins, eventually extending to form incomplete centro-central septa with sparse fibrogenic cells expressing α-smooth muscle actin. In acetylaminofluorene/carbon tetrachloride-treated animals, the fibrogenic response was strongly amplified and an expansion of the subpopulation of hepatic progenitor cells expressing transforming growth factor-β (TGF-β) was observed. In this model, hepatic progenitor cells, through their production of TGF-β, contributed to the accumulation of α-SMA-positive myofibroblasts in the ductular reaction, enhancing fibrosis but also leading to disease progression and a pattern of fibrosis similar to that observed in humans. Furthermore, ductular cells produce much more αvβ6 integrin than normal cholangiocytes. This molecule is closely linked to periductal fibrogenesis, through the activation of TGF-β [33]. Ductular cells secrete growth arrest-specific protein 6 (Gas6), which protects myofibroblasts against apoptosis [34]. A role for the hedgehog pathway in the interaction between ductular cells and portal myofibroblasts was also recently highlighted. Both cell types produce hedgehog ligands, thereby enhancing each other's viability and proliferation, and the activation of this pathway in mice amplifies both the ductular and fibrogenic responses triggered by bile duct ligation [35]. Notch signaling is essential to the development of tubular epithelial cells in the kidney, and activation of this pathway in tubular cells has been implicated in renal fibrosis [36]. Notch signaling is also essential in biliary differentiation and has recently been shown to be activated in rat experimental liver fibrosis. In this context, high levels of Notch3, Jagged1 (a Notch ligand) and Hes1 (a downstream target gene) were observed and the blocking of Notch signaling activation by a γ-secretase inhibitor significantly attenuated portal fibrosis [37]. In Alagille syndrome, a disease caused by genetic defects of Notch signaling and characterized by severe ductopenia, reactive ductular cells and hepatic progenitor cells are very rare and liver fibrosis is much less severe than in biliary atresia, a disease in which an intense ductular reaction is associated with rapid progression to biliary cirrhosis [38]. The importance of the Notch pathway in liver fibrosis has also been demonstrated in double-heterozygous mice haploinsufficient for both Jagged1 and another gene (Lunatic) altering ligand–receptor affinity. In this model, intense ductular proliferation contrasted with low levels of fibrosis [39]. Finally, hepatic progenitor cells have been shown to produce adipokines. In pediatric non-alcoholic fatty liver disease, the degree of fibrosis is related to the production of resistin [28], an adipokine with proinflammatory effects on HSCs [40] and hepatic progenitor cells.

No reliable markers have yet been identified for distinguishing between HSCs and portal mesenchymal cells at the myofibroblast stage. We have established a culture model for PMFs obtained by outgrowth from rat bile duct preparations [15]. These cells have several features in common with rat liver myofibroblasts [14,41]