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PERIBILIARY MYOFIBROBLASTS IN BILIARY TYPE LIVER FIBROSIS

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1. ABSTRACT

Biliary type liver fibrosis develops as part of the wound healing response to bile duct injury in chronic cholestatic liver diseases. The origin of myofibroblasts accumulating together with extracellular matrix around proliferating bile duct structures (referred to as ductular reaction) in the setting of cholestatic injury, has been investigated mostly in the rat bile duct ligation model. Evidence indicates that hepatic stellate cells undergo a myofibroblastic transition following bile duct ligation and that myofibroblastic hepatic stellate cells disclose chemoattraction towards bile duct structures in cholestatic liver. On the basis of morphological studies, nevertheless, the origin of peribiliary myofibroblasts has also been attributed to the activation and proliferation of portal fibroblasts. Bile duct epithelial cells of the ductular reaction actively contribute to the promotion and regulation of biliary type liver fibrogenesis. They synthesize and release a number of paracrine mediators such as transforming growth factor-β, connective tissue growth factor, platelet-derived growth factor–BB, and endothelin-1 that target different liver cell types, including hepatic stellate cells and portal fibroblasts. Through these interactions, bile duct epithelial cells and peribiliary myofibroblasts cause periportal fibrosis in cholestatic and also probably other types of liver diseases.

2. INTRODUCTION

Liver injury, regardless of etiology, is characterized by enhanced deposition of abnormal extracellular matrix and scarring. The production of scarring matrix components in the fibrotic liver has been conclusively ascribed to myofibroblast-like cells, the origin of which remains a subject of debate. Considerable attention has been dedicated to hepatic stellate cells (HSC) since their ability to transdifferentiate into myofibroblasts has been unambiguously demonstrated in culture models (1-4). However, recent reports indicate that myofibroblasts may derive from other cell types in the liver (5, 6), while it was previously suggested that portal fibroblasts can undergo myofibroblastic differentiation (7-9).

Biliary type liver fibrosis arises in the setting of chronic cholestatic liver diseases. Bile duct epithelial cells are major targets in cholestatic injury and their virtually constant response to injury is an intense proliferation referred to as ductular reaction (10). Biliary type fibrosis is initially closely associated with the ductular reaction in portal tracts. In the experimental model of cholestatic liver injury induced by bile duct ligation in rats (11), newly formed bile ducts, derived from proliferating bile duct epithelial cells, are surrounded by fibrosis and myofibroblasts (7-9). Based on morphological investigations and on *in vitro* studies in this model, the accumulation of peribiliary myofibroblasts has been attributed both to the activation and migration of HSC and to the proliferation and phenotypic modulation of portal fibroblasts (7, 8, 12). Increasing attention is drawn to the fact that bile duct epithelial cells are active players in liver fibrogenesis (13). It has been demonstrated that bile duct epithelial cells, in the setting of liver injury, synthesize and secrete a number of cytokines (14-25), which likely enable them to promote activation of both HSC and portal fibroblasts.

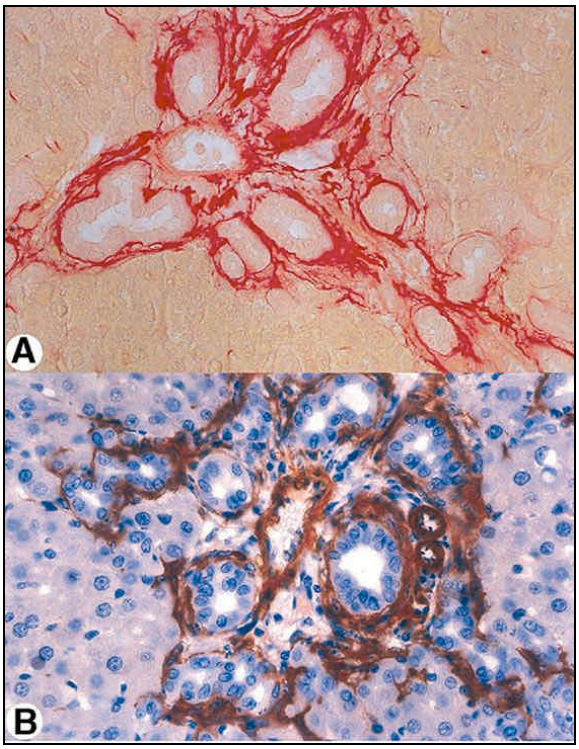

Figure 1. Colocalization of fibrosis and smooth muscle α-actin (α-SMA) immunoreactive myofibroblasts in ductular reaction. Liver tissue from seven day bile duct ligated rats were investigated for fibrosis revealed by sirius red staining (A), and for α-SMA expression by immunoperoxidase method (B). In normal rat liver, α-SMA immunoreactivity is confined to portal vessel walls (not showed). After bile-duct ligation α-SMA-positive myofibroblasts accumulate together with fibrosis around newly formed bile ducts. Original magnification X400.

In this review, the discussion will be focused on the origin of the myofibroblasts accumulating around proliferating bile ducts in the rat model of biliary type fibrosis secondary to bile duct obstruction and the possible role of bile duct epithelial cells in directly promoting the activation and recruitment of fibrogenic cells. This bile duct ligation model has been widely used to investigate the progression of chronic cholestatic liver diseases into the stage of biliary type fibrosis and eventually biliary type cirrhosis.

3. ANIMAL MODEL OF BILIARY TYPE LIVER FIBROSIS

The model of bile duct ligation is characterized by proliferating bile duct structures accompanied by the development of peribiliary fibrosis and the accumulation of peribiliary myofibroblasts (Figure 1). Although neutrophilic infiltration occurs to a certain degree (26), inflammation is not a prominent feature in this model. One can argue that most cholestatic diseases in human are associated with a significant amount of inflammatory infiltrates in the portal tract. Yet there are examples of biliary type fibrosis in human diseases in which inflammation is not a prominent feature. One example is cystic fibrosis-associated liver disease, in which the primary lesion is located in the biliary epithelium (27). Typical findings in cystic fibrosis-associated liver disease include accumulation of myofibroblasts colocalized with fibrosis around bile ducts, without significant inflammatory infiltrates (27-29). These features infer that bile duct epithelial cells can directly promote fibrogenesis through interactions with fibrogenic cells. Bile duct ligation in rat can thus be regarded as a relatively pure model of biliary type fibrosis, in which the absence of major inflammation allows to explore bile duct interactions with fibrogenic cells, and to test pure antifibrotic activities.

4. BILE DUCT EPITHELIAL CELLS – ACTIVE PLAYERS IN BILIARY TYPE FIBROGENESIS

Bile duct epithelial cells may promote fibrogenesis by a number of mechanisms, including not only the synthesis of matrix constituents (30, 31) and the regulation of matrix degradation (32), but also through the release of mediators such as monocyte chemotactic protein-1 (14, 15), interleukin-6 (16, 17), tumor necrosis factor-α (16), platelet-derived growth factor (PDGF)-BB (12, 18), transforming growth factor-β (TGF-β) (19-21), connective tissue growth factor (CTGF) (22), and endothelin-1 (23-25). These, and possibly other mediators released during acute or chronic cholestatic liver injury, likely enable bile duct epithelial cells to communicate with and promote activation of other liver cells, such as inflammatory cells, HSC and portal fibroblasts. Through these mechanisms injured bile duct epithelial cells may directly activate and recruit cells with fibrogenic potential.

TGF-β, which triggers the fibrogenic response in the injured liver, is produced by bile duct epithelial cells (19-21). The highly similar TGF-β isoforms, TGF-β1, TGF-β2, and TGF-β3, act as regulators of cell growth and differentiation (33-35). They are potent inducers of extracellular matrix synthesis in fibroblasts, myofibroblasts, and pericytes and also modulate immune responses (33-36). Several lines of evidence point to TGF-β as a key profibrogenic cytokine in the development of liver fibrosis. *In vitro*, TGF-β1 upregulates HSC activation and stimulates the synthesis of extracellular matrix constituents in HSC (37-39). Increased expression of TGF-β is associated with both experimental fibrosis and hepatic fibrosis in human liver disease (19, 20, 40-46). Direct evidence of a causative role of TGF-β in liver fibrogenesis has been provided by investigations of liver fibrogenesis in transgenic mice overexpressing TGF-β1 (47, 48) and in TGF-β1 knock-out mice (49). Moreover, it has been shown that anti-TGF-β intervention inhibits experimental fibrosis in rat (50-52). Yet, the direct role of TGF-β in the development of biliary type liver fibrosis remains to be established, since in one report, peribiliary fibrosis was unaffected by the administration of soluble TGF-β type II receptor (50).

It has also recently been shown that proliferating bile duct epithelial cells are a major source of CTGF in rat

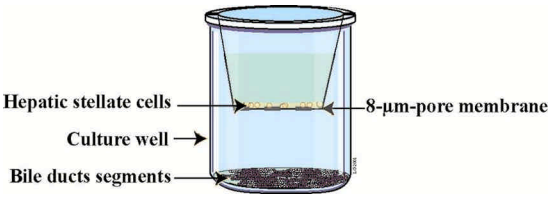

Figure 2. Cell migration assay. A cell culture insert equipped with an 8-micrometer-pore membrane forms the upper compartment, while a culture well forms the lower compartment of the Transwell chemotaxis filter assay. The upper inserts are seeded with hepatic stellate cells (HSC), while the lower wells contain chemotactic factors or cell preparations. After a six hour incubation, HSC remaining on the upper surface of the filters are removed, and HSC that have migrated through the pores to the inferior surface are stained and counted.

biliary type fibrosis (22). CTGF is a cystein-rich peptide originally identified as a growth factor secreted by vascular endothelial cells in culture (53). The physiological function of CTGF has not yet been elucidated, but *in vitro* experiments have shown that CTGF stimulates fibroblast proliferation and migration, and induces overproduction of extracellular matrix constituents (53, 54). It has been proposed that CTGF acts as a downstream effector of TGF-β during liver fibrogenesis (55). Therefore it may be postulated that CTGF released by bile duct epithelial cells acts in conjunction with TGF-β and triggers the peribiliary fibrogenic response.

Bile duct epithelial cells also have the capacity to produce PDGF-BB (12, 18). PDGF-BB has been identified as the most potent mitogen of cultured HSC (56) and is overexpressed during active hepatic fibrogenesis (18, 42, 57, 58), including biliary type fibrogenesis as discussed below.

5. HEPATIC STELLATE CELLS IN BILIARY TYPE FIBROGENESIS

5.1. Hepatic stellate cell activation

Hepaic stellate cells are a population of resident nonparenchymal cells located in the perisinusoidal space as quiescent vitamin A–storing cells secreting low amounts of extracellular matrix. Following liver injury, HSC undergo a pleiotropic response termed “activation” (59). The entire process ultimately leads to the conversion of a quiescent vitamin A-storing cell into a fibrogenic myofibroblastic cell type. Expression of smooth muscle α-actin (α-SMA) is the hallmark of HSC activation both in culture and in the intact liver (60-62). Upon activation, HSC also move from a nonproliferative to a highly proliferative state (56, 61-63). PDGF-BB has been identified as the most potent polypeptide growth factor able to stimulate the proliferation of culture-activated HSC (56).

PDGF isoforms are disulphide-bonded dimers of either AA, AB or BB polypeptide chains. Their effects on target cells are mediated by dimeric transmembrane receptors composed of α and/or β subunits with intrinsic protein-tyrosine kinase activity (64). The α subunit binds both the A and the B chain of PDGF with high affinity, whereas the β subunit binds the B chain only (65, 66). Quiescent HSC exhibit constitutive expression of the α subunit but no detectable transcript for the β subunit (63). Platelet-derived growth factor receptor-β (PDGFR-β) expression is induced in primary cultures of rat HSC within three days after plating, coincident with the onset of cellular proliferation (63). PDGF exerts no mitogenic effect on HSC in very early culture (62), suggesting that *de novo* expression of PDGFR-β is a prerequisite to the growth stimulatory action of PDGF.

It has been demonstrated by the analysis of HSC isolated at different time points after bile duct ligation that cholestatic liver injury induces a number of phenotypic changes characteristic of HSC activation, including increased proliferation, increased expression of extracellular matrix constituents, expression of α-SMA and of PDGFR-β subunit (60, 63, 67, 68). The proliferative response of HSC is induced as early as within 24-48 hours after biliary obstruction, and precedes HSC phenotypic conversion into α-SMA expressing myofibroblasts (68). Furthermore, this early proliferative response to cholestatic injury has been shown to be mediated mainly by PDGF (68).

It is therefore evident that, following cholestatic liver injury, HSC move from a quiescent into an activated state. Yet, the contribution of activated HSC to the population of peribiliary myofibroblasts would further require that they migrate from the perisinusoidal space, where they are normally located, to bile duct structures located in the portal tracts. The anatomical connection between the space of Disse and the portal interstitial space documented by electron microscopical studies of human and rat liver (69) is consistent with such a possibility.

5.2. Hepatic stellate cell migration

Chemotactic factors are produced during wound healing, and play an important role in the recruitment of different cell types involved in tissue repair to the sites of injury. In keeping with an important role of HSC in liver wound healing, it has been proposed that following necrotic injury, HSC detach from the sinusoidal wall, where they are normally located, and move into postnecrotic areas (70). This assumption has been reinforced by the demonstration of human and rat HSC migratory capacities *in vitro*, particularly in reponse to PDGF-BB (12, 71-76). Other chemotactic mediators active on HSC include endothelin-1 (12, 77), monocyte chemotactic protein-1 (78), and insulin-like growth factor-1 (79), but the list is likely to grow longer as the response of HSC to other chemotactic mediators is investigated.

Results from dynamic studies using a Transwell chemotaxis filter assay (Figure 2) have shown that HSC may also migrate in cholestatic liver injury and accumulate within the ductular reaction as a result of PDGF-mediated chemoattraction by bile duct structures (12). The major findings supporting this evidence were that bile duct segments isolated from bile duct-ligated rats exhibited high levels of PDGF-B chain mRNA and protein, that they were

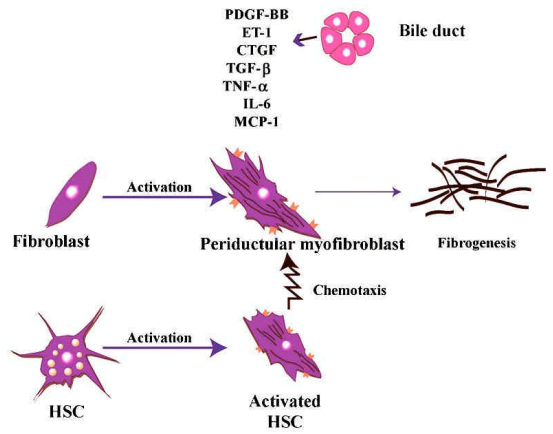

Figure 3. Origin of peribiliary myofibroblasts. Peribiliary myofibroblasts could derive from either the activation and proliferation of portal fibroblasts, or from activated hepatic stellate cells (HSC) that are recruited to the portal tracts by chemotaxis. Bile duct epithelial cells likely play a pivotal role in these processes by secreting cytokines that could directly act on both portal fibroblasts to stimulate their activation and proliferation, or on HSC to stimulate their recruitment by chemotaxis. Cytokines also enable bile duct epithelial cells to communicate with inflammatory cells (not shown), which in turn could regulate and stimulate the fibrogenic response. MCP-1: monocyte chemotactic protein-1; IL-6: interleukin-6; TNF-α: tumor necrosis factor-α; PDGF-BB: platelet-derived growth factor-BB; TGF-β: transforming growth factor-β; CTGF: connective tissue growth factor; ET-1: endothelin-1.

very active in inducing chemotaxis of myofibroblastic HSC, and that this effect was inhibited by blocking PDGF-BB ligand or receptor activity. The possibility that in human chronic liver disease, newly formed bile ducts are, in addition to inflammatory cells and activated HSC, a major source of PDGF, is supported by investigations of human cirrhotic liver tissue (42, 57).

Endothelin-1 is a vasoactive peptide that exerts an array of biological effects on HSC and modulates liver fibrogenesis (25, 67, 80-84). A number of findings point to proliferative bile duct epithelial cells, in addition to endothelial cells and myofibroblastic HSC, as a major source of endothelin-1 within the liver (24, 25). Yet, even though a chemotactic effect of endothelin-1 on HSC has been demonstrated (12, 77), the role of endothelin-1 in mediating bile duct-induced migration of HSC is probably minor as compared to that of PDGF-BB (12).

Thus, it has been conclusively demonstrated that cholestatic liver injury induces several feaures of HSC activation, and that dynamic interactions between bile duct epithelial cells and HSC may occur. This is in accordance with the view that peribiliary myofibroblasts accumulating within collagen deposits in biliary type fibrosis may in part derive from HSC. However, it has also been proposed that portal fibroblasts may play a major role in biliary fibrogenesis, in particular at early stages of cholestatic injury.

6. PORTAL FIBROBLASTS IN BILIARY TYPE FIBROGENESIS

Although myofibroblastic HSC are considered as the main fibrogenic cell type in hepatic fibrosis, it has been proposed that myofibroblasts with fibrogenic potential may derive from other cell types of the fibroblast lineage (5, 6). In particular, based on morphological investigations of the cellular response to cholestatic injury induced by bile duct ligation in rats, the origin of peribiliary myofibroblasts has been attributed to portal fibroblasts (7, 8). These *in situ* studies showed that in the early stages of biliary type fibrosis, only a minority of α-SMA-positive peribiliary myofibroblasts also expressed desmin, a cytoskeletal protein generally expressed by myofibroblastic HSC. Seventy-two hours after bile duct ligation, peribiliary myofibroblasts expressing both α-SMA and desmin appear, and their proportion increases until day seven (7). Thus, it is likely that in the early stages of biliary type fibrosis, portal fibroblasts, or possibly other cell types located in the portal tract, are activated into myofibroblasts and constitute the majority of peribiliary myofibroblasts accumulating around bile ducts. At later stages following cholestatic injury it is likely that HSC contribute to the peribiliary myofibroblast population, since an increased number of α-SMA-positive/desmin-positive cells can be noted in peribiliary areas after prolonged times of bile duct ligation (7, 85, 86).

Little is known about the myofibroblastic conversion of portal fibroblasts, but mechanisms similar to those seen in HSC activation are likely involved. HSC activation is considered to be initiated by paracrine stimuli from neighbouring cells (2). These stimuli include disruption of the normal extracellular membrane pattern and exposure to cytokines. TGF-β, TNF-α and endothelin-1 have all been shown to promote HSC activation into myofibroblasts *in vitro* (37, 67). Interestingly, these mediators may be produced by bile duct epithelial cells in liver injury (16, 19-21, 23, 25). PDGF-BB is another cytokine produced by bile duct epithelial cells (12, 18) that has been shown to induce the transformation of fibrocytes to myofibroblasts (87). We could recently report from *in vitro* and *in vivo* studies that PDGF-BB could play an important role in the conversion of portal fibroblasts into α-SMA-expressing myofibroblasts (88).

7. SUMMARY AND PERSPECTIVE

It is apparent that cholestatic liver injury elicits an early proliferative response in HSC, which may be part of a cascade of reversible events leading to hepatic wound healing. With ongoing or recurrent injury, HSC will eventually complete their transition into fibrogenic myofibroblasts, and move into peribiliary regions in portal areas, in response to chemotactic factors, mainly PDGF, released by bile duct epithelial cells. However, in the early stages of biliary type fibrosis the accumulation of peribiliary myofibroblasts could be mainly due to the activation and proliferation of portal fibroblasts. It is obvious that bile duct epithelial cells must be looked upon as active players in the promotion and modulation of fibrogenesis in the setting of cholestatic injury. The release of a number of mediators, including PDGF and TGF-β, permits the crosstalk of bile duct epithelial cells with other liver cells, including HSC and portal fibroblasts (Figure 3). These mechanisms can explain the

accumulation of myofibroblasts and collagen deposits around bile ducts even in the absence of marked inflammation, such as in cystic fibrosis-associated liver disease (27-29), and could be further amplified by the inflammatory reaction in biliary diseases such as primary biliary cirrhosis and primary sclerosing cholangitis.

As our knowledge of the pathogenesis of biliary type fibrosis is growing, new targets for anti-fibrotic therapy are emerging. Among these, inhibition of mediators of biliary type fibrogenesis, such as PDGF, endothelin, and TGF-β, could constitute new targets for anti-fibrotic intervention. Understanding the mechanisms by which bile duct epithelial cells promote fibrogenesis is a major prerequisite for the development of effective therapies in chronic cholestatic liver diseases.

8. ACKNOWLEDGEMENT

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