Hepatic Stellate Cells as a Target for the Treatment of Liver Fibrosis

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ABSTRACT
Liver fibrosis as the final common end stage of most chronic liver diseases is triggered by chronic liver injury caused by various etiologies including viral infection, cholestasis, metabolic diseases and alcohol abuse. It is reversible wound-healing response characterized by the accumulation of extracellular matrix proteins (ECM) including collagen. Hepatic stellate cells (HSCs) are the major source of extracellular matrix components and are the key mediators of fibrogenesis. Hepatic stellate cells are definitely involved in the pathogenesis of various liver pathologies, besides the well know key role in fibrosis and extracellular matrix remodelling. Potent drugs may not be effective enough in the treatment of liver fibrosis. The inflammatory cytokines produced by kupffer cells, the liver resident macrophage, aggravate liver fibrosis. Transforming growth factor β1 (TGF- β1) is also activator of HSCs. Liver fibrosis is a serious healthcare problem with high morbidity and motility. It is the result of wound healing responses to repeated liver injury irrespective of etiology. With the development of the diseases, excessive extracellular matrix (ECM) components are deposited in the liver, leading to portal hypertension, cirrhosis or hepatocellular carcinoma .Normally HSCs are quiescent in nature but when liver have any injury they get activated and releases extracellular matrix proteins. The review describes the use of HSCs, transforming growth factor β1 for therapeutic purposes in liver fibrosis.

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INTRODUCTION

Liver fibrosis is defined as the accumulation of excessive amounts of extracellular matrix (ECM), also known as scar tissue, in the liver parenchyma [1, 2]. It is the common end stage of most chronic liver diseases and also triggered by various liver injury [3, 2]. Liver damage causes liver stellate cells to be over active and triggers the extra cellular matrix (ECM) synthesis to increase [4]. More than normal amounts of collagen fiber deposits in the extracellular spaces of the liver cells and causes the liver cells to lose blood infusion and to be hardened. Chronic viral hepatitis B and C are the most common causes of liver fibrosis.

During the chronic hepatitis course, fibrosis is a part of the inflammation activities. In the fibrosis stage, there is no lobular regeneration and this distinguishes it from cirrhosis. When fibrosis advances to cause fibrotic separations (or bridging) between the portal areas, the center vein, and the formation of pseudo-lobule, fibrosis enters the final stage, which is cirrhosis. In the fibrotic liver the liver becomes enlarged due to the development of the fibrosis and there is the imbalance between the synthesis and degradation of the ECM molecule. When the liver get injured the quiescent hepatic stellate cells get activated and become proliferate, contractile and fibrogent cells acquiring a myofibroblasts like phenotype [5].

Fibrosis is the first stage of liver scarring, and at the end stage it will lead to cirrhosis. The activation of hepatic stellate cells (HSCs) is important component during the initiation and development of liver fibrosis. The transforming growth factor- β1 (TGF- β1) is also a activator of HSCs. it acts in an

autocrine or pancrine manner and is produced by kupffer cells, sinusoidal endothelilial cells and hepatocytes [6, 7].

Transforming growth factor-β (TGF-β), a prototype of multifunctional cytokine, is a key regulator of extracellular matrix (ECM) assembly and remodeling [8]. TGF-beta is found in all tissues. It helps regulate bone growth. The TGFβ-1 protein is found throughout the body and plays a role in development before birth, the formation of blood vessels, the regulation of muscle tissue and body fat development, wound healing, and immune system function. In the liver, TGF-beta is a very potent profibrogenic mediator of cellular responses leading to tissue repair, ECM production, growth regulation, and apoptosis [9]. During fibrogenesis, tissue and blood levels of active TGF-beta are elevated and over expression of TGF-beta 1.

Transforming growth factor-β1 (TGF-β1) is a pleiotropic cytokine involved in tissue growth regulation, differentiation, ECM production, and the immune response. Three isoforms of this cytokine (β1, β2, andβ3) have been identified, but onlyβ1 has been linked to liver fibrogenesis. TGF-β1 is commonly known as a central component of fibrogenic response in wounds and an over-regulator of different diseases. The correlation between TGF-β1 levels and fibrosis progression is widely accepted [10, 11]. The only effective treatment for end stage liver fibrosis is liver transplantation [11]. Therefore, it is highly desirable to develop novel strategies that prevent the progression of liver fibrosis.

Cell origin, plasticity and activation

The embryological origin of HSC is still debated. Recent experiments support their origin from either endoderm or septum trasversum. In support of an endodermic origin is the finding of a transient expression of the same cytokeratins of hepatoblasts [12]. In support of a mesodermal origin, common to smooth muscle cells (SMC), is the fact that undifferentiated fetal HSC express α-smooth muscle actin (SMA), an early marker of SMC differentiation but do not store yet vitamin A [13].

Stellate cells can be found within the progenitor cell niche in normal and regenerating liver, near the intrahepatic bile ductules. Moreover, the demonstrated expression of the stem cell marker CD133 by a subset of HSC led to propose these cells as progenitors not only of liver myofibroblasts but also of hepatocytes and bile duct epithelial cells [14].

Analysis of cytoskeletal and cell surface markers has demonstrated a certain degree of heterogeneity and plasticity of HSC in adult liver, depending on actual location in the hepatic lobule, animal species, type of tissue considered, either normal or injured. For instance, desmin, an intermediate filament typical of contractile cells is present in rodent, but not in human HSC [15]. Further, a significant fraction of resting stellate cells is detectable which lacks vitamin A droplets.

Activation of quiescent HSC and subsequent differentiation into myofibroblasts like cells is very reliably indicated by the expression of-smooth muscle actin, an actin iso form which is absent in the other resident liver cells in either normal or injured liver, with the exception of smooth muscle cells surrounding large vessels [16]. Differentiated HSC also express several other marker genes that are in common with smooth muscle cells, like smooth muscle myosin heavy chain, calponin and myocardin [17]. But, activated HSC still differ from myofibroblasts and smooth muscle cells, both in vitro and in vivo , for their vitamin content, contractile activity, and relative responsiveness to cytokines, particularly to transforming growth factor- 1(TGF-1) [18]. Further, the gene expression pattern of HCS keeps evolving, during the cell life, with eventual acquisition of a more inflammatory but less fibrogenic phenotype [19].

Pathophysiology of liver fibrosis

Activation of the hepatic perivascular stellate cells (Ito cells, which store fat) initiates fibrosis. These and adjacent cells proliferate, becoming contractile cells termed myofibroblasts. These cells produce excessive amounts of abnormal matrix (consisting of collagen, other glycoproteins, and glycans) and matricellular proteins. Kupffer cells (resident macrophages), injured hepatocytes, platelets, and leukocytes aggregate. As a result, reactive O_2 species and inflammatory mediators (e.g., platelet-derived growth factor, transforming

growth factors, and connective tissue growth factor) are released. Thus, stellate cell activation results in abnormal extracellular matrix, both in quantity and composition. Myofibroblasts, stimulated by endothelin-1, contribute to increased portal vein resistance and increase the density of the abnormal matrix. Fibrous tracts join branches of afferent portal veins and efferent hepatic veins, bypassing the hepatocytes and limiting their blood supply.

Hence, fibrosis contributes both to hepatocyte ischemia (causing hepatocellular dysfunction) and portal hypertension. The extent of the ischemia and portal hypertension determines how the liver is affected. For example, congenital hepatic fibrosis affects portal vein branches, largely sparing the parenchyma. The result is portal hypertension with sparing of hepatocellular function.
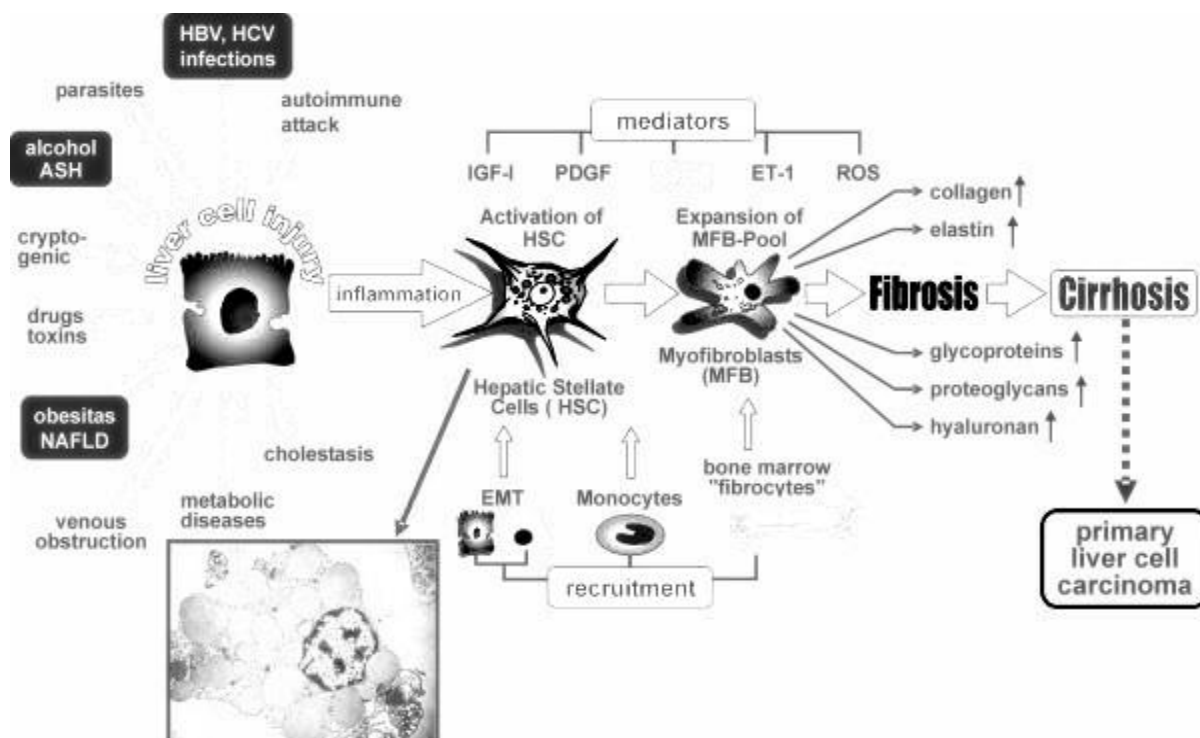

Figure 1: Pathways of HCS activation

Symptoms and Signs
Hepatic fibrosis itself does not cause symptoms. Symptoms may result from the disorder causing fibrosis or, once fibrosis progresses to cirrhosis, from complications of portal hypertension. These symptoms include variceal bleeding, ascites, and port systemic encephalopathy. Cirrhosis can result in hepatic insufficiency and potentially fatal liver failure.
Hepatic stellate cells.
Function
✓The most characteristic feature of resting hepatic stellate cells is vitamin A storage.
✓Following activation by various stimuli, hepatic stellate cells acquire a myofibroblasts-like phenotype.

✓Hepatic stellate cells are key-players in the pathogenesis of liver fibrosis, inducing collagen deposition and abnormal extracellular matrix remodeling.
HSC and its role in the liver
In the normal liver, HSCs comprise approximately 1.4% of total liver volume and are present at a ratio of about 3.6 to 6 cells per 100 hepatocytes (or 1:20). Hepatic stellate cells are typically located in the perisinusoidal space of Disse, a recess between endothelial cells of sinusoids and hepatocytes. Stellate cells (from the Latin stella, which means star) typically have a starlike configuration due to their denditric cytoplasmic processes that partially embrace adjacent endothelial cells 3 in a manner somewhat analogous to astrocytes around

terminal cerebral vessels or podocytes around renal capillaries, extending into the spaces in between hepatocytes and reaching the cytoplasmic processes of other stellate cells [20]. In its quiescent state, HSCs show large perinuclear lipid droplets, which serve as the main storage site for vitamin A and are essential in the regulation of retinoic acid homeostasis [21]. Stellate cells also seem to play a key role in the maintenance of steady-state levels of basement membrane–like matrix (mostly types IV and VI collagen) in normal hepatic sinusoids, [22] as well as in regulation of hepatic blood flow and portal venous pressure [20, 22].
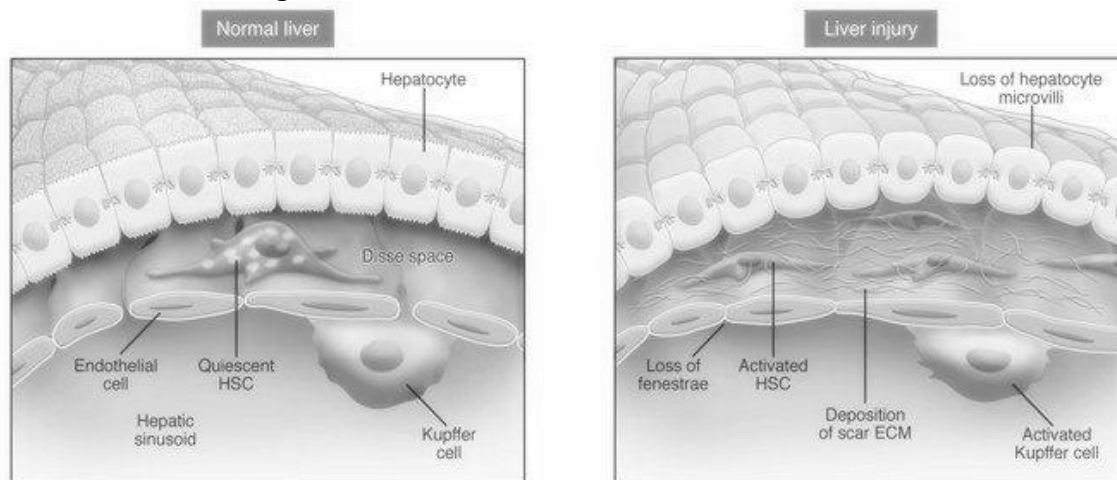

Figure 2: Normal and Injured Liver

Injury to hepatocytes results in the recruitment and stimulation of inflammatory cells, as well as the stimulation of resident inflammatory cells (including Kupffer cells). Factors released by these inflammatory cells lead to transformation of HSCs into a myofibroblasts-like phenotype. HSC activation leads to accumulation of scar (fibrillar) ECM. The presence of a fibrillar ECM in the Disse space has consequences for hepatocyte function, leading to the loss of microvilli and endothelial fenestrae. Therefore, the loss of normal tissue architecture contributes to impairment of organ function.

The cytokines secreted by HSCs that are involved in the liver response to injury and regeneration, the large variety of cytokines secreted by HSC, TGF-1 most likely represents that with the highest impact on collagen over-production and accumulation in liver fibrosis [24].TGF-β1 binds to specific receptors (TGFRI and TGFRII) present on cell surface then signals to the nucleus through several intracellular mediators belonging to the Smad family. Modulation and inhibition of TGF-β1 signalling appears as a promising approach to anti-fibrotic therapy [25].

Drug targeting to hepatic stellate cells
HSCs, which comprise about 5-10% of the total amount of liver cells, play a important role in fibrosis. Fibrosis occurs when the hepatic stellate cells and portal fibroblasts produce excessive amounts of extracellular matrix proteins like collagen types I and III. Eventually this leads to portal hypertension due to the increased intrahepatic resistance [26, 27]. Liver fibrosis has been considered as an irreversible process for a long time, research has shown that the process is reversible in animal models [28]. However, in most cases the only option for therapy is a liver transplantation [29]. Effective antifibrotic drugs are not available. Hepatic stellate cell is the major producer of scar tissue. HSCs cell is the main target cell for antifibrotic therapies. To optimize the therapeutic success of potential antifibrotic drugs, targeting to hepatic stellate cells has been explored [30].

DISCUSSION
Chronic liver disease and fibrosis sent a major global health concern. Hepatic stellate cells represent a highly versatile cytotype that plays a significant role in liver development and differentiation, regeneration, xenobiotic response,

immunoregulation, control of hepatic blood flow and inflammatory reactions. Hepatic stellate cells (HSCs) as the main matrix producing cells in the process of liver fibrosis. Liver injury of any etiology will ultimately lead to activation of HSCs. transforming growth factor beta is thought to be the main mediator of fibrogenesis and platelet-derived growth factor is the major inducer of HSC proliferation. The majority of liver diseases cannot be treated at all by conventional approaches. Sometime drug have beneficial effect on one cell type but adverse effect on other adjacent liver cells. Therefore, targeting to HSCs is a novel strategy that prevents the progression of liver fibrosis.

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