Stellate Cells Activation and Extra-Cellular Matrix: New Targets for Therapeutic Intervention in Fibrosis

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Abstract

Hepatic stellate cells are perisinusoidal cells characterized by vitamin A containing lipid droplets and 10 nm desmin filament in their cytoplasm. In an injured liver, stellate cells were activated through two major phase i.e, initiation and perpetuation which response to cytokines. It was postulated that hepatic stellate cell is a central component in the synthesis of large amounts of extra cellular matrix (ECM) that resulting in deposition of scar or fibrous tissue. In repeated injury, extra-cellular matrix altered composition accumulates, which is termed fibrosis. The ECM acts via specific domains that interact with cell receptors for cytokines such as Endothelin-1 and TGFβ that are responsible for stimulating stellate cell activation and ECM production. The regulatory role of stellate cell activation and formation of excess ECM in liver injury offers new targets for therapeutic intervention in fibrosis.

Key word: Stellate cell, ECM, cytokines

Introduction

Hepatic stellate cells known as Ito cells, lipocytes, fat-storing or perisinusoidal cells have emerged as a well-characterized cell type important to response in the process of liver injury. Since the stellate cells are not visible in standard tissue section of normal liver stained with hematoxylin and eosin, then immunocytochemical markers using desmin antibody was used to identify the stellate cells. Their relative abundance has been estimated at one third of the non-parenchymal population or about 15% of the total number of resident cells in normal liver. Major feature of stellate cells activation in liver injury of any type, include: increased proliferation, chemotaxis, enhanced production of interstitial matrix, increased contractility, secretion of leukocyte chemoattractans and cytokines, enhanced matrix degradation and loss of intracellular retinoid. It was reported that granuloma were formed in the pericentral area of Balb/c mice after a single injection of carbon tetrachloride. Direct contact between macrophages and stellate cells were frequently seen within the granulomas stimulated the differentiation of stellate cells to myofibroblast-like cells caharacterized with small lipid droplet, well-developed granular endoplasic reticulum (rER), and thick bundles of intermediate filament in the cytoplasm. Collagen fibril were closely

applied to the stellate cells and connective tissue septa extended between neighboring granulomas after several injection of CCl_4. It was suggested that stellate cells has a role in fibrogenic process by the production of excess extra cellular matrix (ECM). [1]

ECM refers to complexes of collagen and large collagen-associated glycoprotein. It has been reported that hepatic stellate cell is the major cellular source of extra cellular matrix either in normal or injured liver. The excess production of ECM is the most direct way that stellate cell activation generates hepatic fibrosis. The important role of stellate cells in ECM production has been established by combined evidence from in situ evaluation and from culture studies. The phenotype of stellate cells changes, both qualitatively and quantitatively during liver injury. Overall there is a marked increase in cellular matrix production, especially the collagens types I, III that are synthesized and secreted by activated stellate. It has been a major focus of interest because of its importan-ce in the fibrotic scar.

A number of specific antifibrotic therapies have been tried, but the result was poor or mediocre success. However, elucidation of the mechanisms responsible for fibrogenesis, with particular emphasis on stellate cell biology, has highlighted many putative novel therapies. Stellate cell activation and regulatory role of ECM in liver injury offers new targets for therapeutic intervention in fibrosis. This article emphasizes mechanisms underlying fibrogenesis, and reviews current antifibrotic therapies as well as potential future approaches.

Participation of Stellate cells in hepatic fibrosis

In normal liver, stellate cells are the primary site for storage of vitamin A, primarily as retinyl esters. Under fluorescence microscope, a strong vitamin A-fluorescence scattered within the hepatic lobules. In CCl_4-induced liver fibrosis, vitamin A auto fluorescence was emitted from degenerating area surrounding central vein and extended septal area (Fig 1). [2]

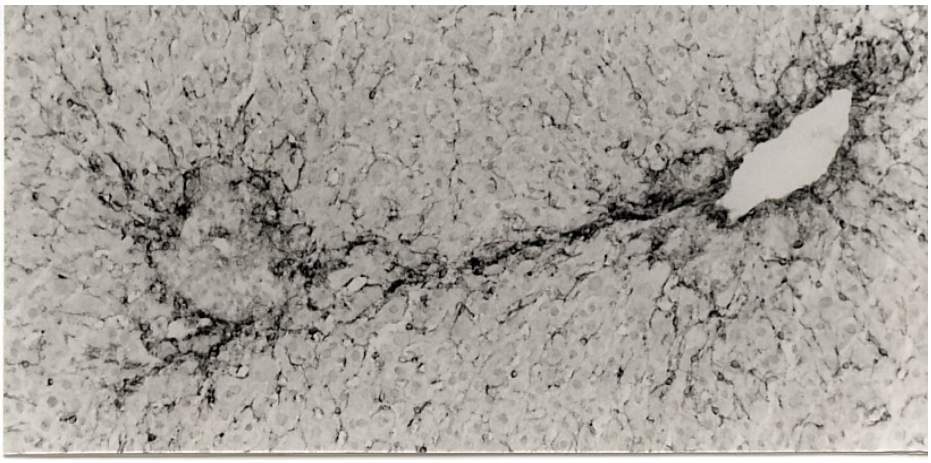

Fig. 1. Vitamin A autofluorescence was emited from stelatte cells surrounding central vein and extended septal area.

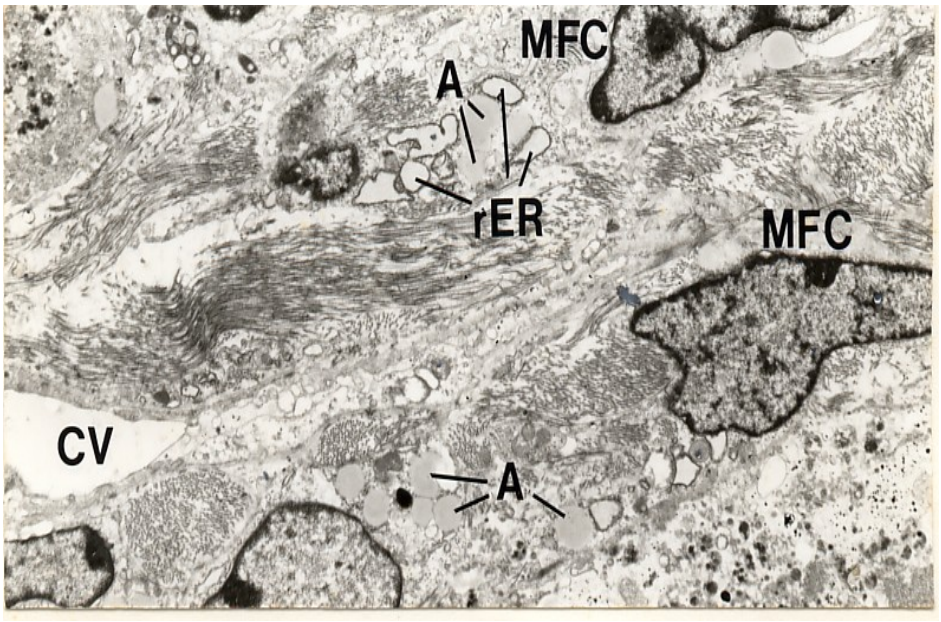

Fig. 2. Myofibroblast-like cells (MFC) with small lipid droplet (A), dilated cysterna of rough endoplasic reticulum (rER) and abundant collagen fibril resided surrounding central vein (CV).

In liver of Balb/C mice, granulomas were formed on day 5 after a single injection of CCl_4. The granulomas contained several kind of inflammatory cells i.e. monocytes, macrophages, and lymphocytes. On day 7 after injection, within the granulomas, stellate cells and macrophages were increased in number. Bundles of collagen fibers were scattered close to stellate cells. Ultrastructurally examination with Transmission Electron Microscope demonstrated the myofibro-blast-like cells (differentiated from stellate cels) characterized with less lipid droplet, dilated cysterna of rough endo-plasmic reticulum (rER) and abundant collagen fibril resided to the cells (fig 2).[1]

Desmin immunoreactivity in normal liver tissue of ICR mice was identified in smooth muscle in the walls of blood vessel and in perisinusoidal stellate cells surrounding sinusoids. Strong desmin immunoreaction was observed in the hepatic pericentral area and septal area after several injection of CCl_4. (Fig. 3).[2]

The fact that various diseases result in cirrhosis suggests a common pathogenesis. A growing body of literature indicates that the hepatic stellate cell is a central component in the fibrogenic process. Stellate cells undergo a transformation during injury that has been termed activation. Activation is complex and multifaceted, but one of its

most prominent features is the synthesis of large amounts of extracellular matrix, resulting in deposition of scar or fibrous tissue.

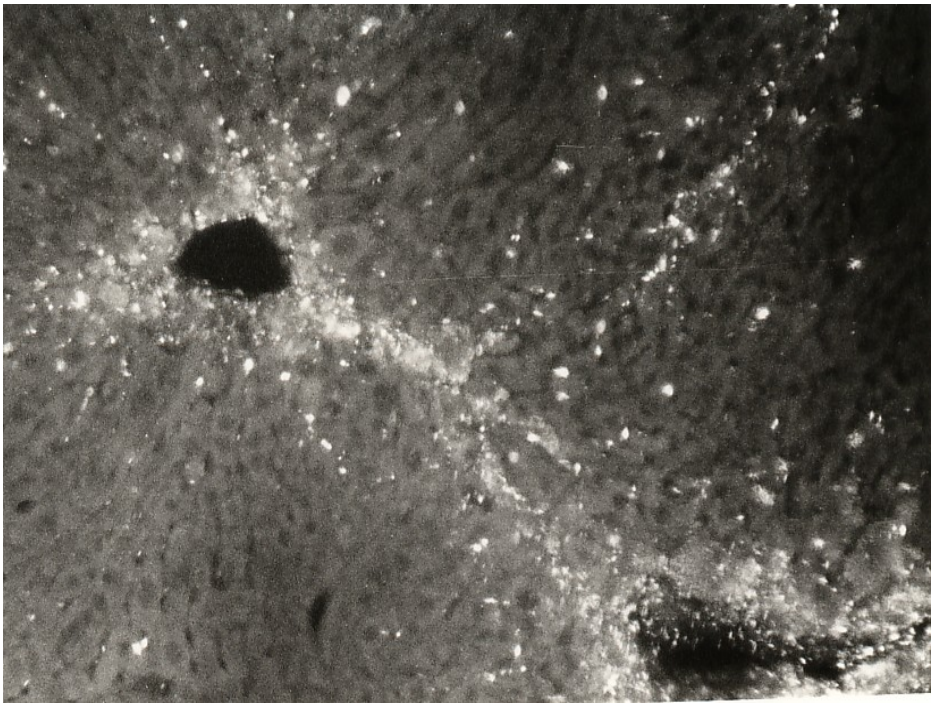

Fig. 3. Desmin-positive stellate cells with long cytoplasmic processus in the pericentral and septal area

A central feature of the fibrogenic response is the transformation of stellate cells from quiescent to an activated state. Although simple in concept, the activation process is remarkably complex and consists of many important cellular changes. Characteristic features of this transition include loss of vitamin A, acquisition of stress bundles, and development of prominent rough endoplasmic reticulum. [3]

Stellate Cell Activation

In liver injury, stellate cells undergo a process known as activation. Stellate cells activation represent a cascade of cellular events common to all form of liver injury , including viral hepatitis, alcohol, toxic injury and genetic liver disease. Activation consists of two major phases, initiation and perpetuation. Initiation refers to early changes in gene expression and phenotype which response to cytokines. Perpetuation is maintaining the activated phenotype and generating fibrosis. Initiation is largely due to paracrine stimulation, where as perpetuation

involves autocrine as well as paracrine loops.

Initiation phase
The intimate association between stellate cells and neighboring sinusoidal cells, including sinusoidal endothelium, Kupffer cells, hepatocytes, and platelets may facilitate paracrine stimulation by soluble mediators. The paracrine and/ or autocrine secretion of various cytokines modulate the inflammatory response in liver injury. Recent studies suggest that activations of stellate cells is provoked by generation of free radicals in culture and is blocked by antioxidants. These activations may involve the transcription factor NF kβ.[4]

Perpetuation phase
Perpetuation of stellate cell activation involves at least seven distinct activationdependent functional changes i.e: proliferation, migration into regions of injury, contractility, fibrogenesis, cytokine release, matrix protease release/activity, and retinoid loss. Either directly or indirectly, the net effect of these changes is to increase accumulation of extra cellular matrix. [4]

Regulatory factors of
Stellate cell fibrogenesis

Multiple factors play a key pathogenic role in stellate cell fibrogenesis. Prominent among these factors are cytokines, small peptides, and the extracellular matrix itself. Transforming growth factor-B-1 (TGF-B1) appears to be the most profibrogenic cytokine present in the liver. TGF-B1 is produced in a paracrine manner by Kupffer cells, sinusoidal endothelial cells, bile duct epithelial cells, hepatocytes, or by stellate cells themselves. TGF-B1 production by stellate cells is important, and thus points to this cytokine as a classic autocrine factor. When over expressed in the liver, it leads to fibrosis, and when inhibited during experimental liver injury, fibrosis is decreased.

Cytokines and growth factors that drive stellate cell included platelet-derived growth factor (PDGF), monocyte chemotactic factor, insulin-like growth factors-1 and -2, interleukin-6, hepatocyte growth factor, and vasoactive peptides : endothelin-1 and angiotensin II.[3] Increased activity of cytokines may be critical for both autocrine and paracrine perpetuation of stellate cell activation.

Stellate Cell in Extracellular
Matrix production

ECM refers collectively to complexes of collagen and large collagen-associated glycoprotein[5]. ECM complexes are quiet diverse, the ECM around the biliary epithelium is different from that associated with hepatocytes and within the the space of Disse associated with stellate cells and sinusoidal endothelial cells. The ECM is highly dynamic and capable of undergoing rapid change in response to injury. The hepatic stellate cell is the major cellular source of extra-cellular matrix (ECM) in normal and injured liver. The important role of stellate cells in ECM production has been established by combined evidence from in situ evaluation by immunohisto chemistry and nuclei acid hybridization, from culture studies, and from analysis of freshly isolated stellate cells from normal or injured liver.[6,7] The matrix phenotype of stellate cells changes, both qualitatively and quantitatively during liver injury. Overall there is a marked increase in cellular matrix production, and a shift from basement membrane-like matrix containing non-fibril forming collagens e.g, type IV, VI

to so-called interstitial matrix rich in fibril-forming collagens types I, III. Synthesis and secretion of collagen type I by activated stellate has been a major focus of interest because of its importance in the fibrotic scar, and because regulatory mechanisms for collagen I gene expression have been well characterized in other systems.[7] Direct effects on stellate cell matrix production and contractility have been attributed to autocrine TGFβ and endothelin-1, respectively.[8,9] The inflammatory component may be amplified by increased MCP-1 production by activated stellate cells.[10]

The ECM and Wound repair

The response to injury is one of wound healing and, subsequently, fibrosis. This response is generalized, occurring in diverse organ systems. Injury and wounding in the liver ultimately lead to cirrhosis in many patients. The change in ECM that follow injury to the liver, as well as other epithelia, funda-mentally represent wound repair. Their role as ECM producers is to bind the wound and possibly to restrict spread of the injury. Pathological fibrosis, or "scar", represents on-going or multiple cycles of repair and contraction in response to chronic or repetitive injury.

The finding that liver "myofibroblasts" represent for the most part activated stellate cells, focusing attention on the regulation of activation. It is clear, moreover, that this represents a common response to injury of diverse etiology. Chronic biliary obstruction and alcoholic hepatitis cause pathology initially in different areas of the lobule, but in both cases the activation of stellate cells is prominent. The ECM has a central role in contraction, providing the fiber net to which stellate cells attach and through which their contractile force is transmitted. Endothelin 1 induces contraction, proliferation, and collagen synthesis of hepatic stellate cells in vitro, which may be mediated via the endothelin A receptor. It is speculated if specific blockade of the endothelin A receptor inhibits hepatic fibrosis in vivo. [11]

New targets for therapeutic intervention in fibrosis

The current understanding of stellate cell-ECM interaction in liver injury, while far from complete, has yielded several new targets for therapeutic manipulation of fibrogenesis. Although stellate cell activation occurs after both acute and chronic liver injury, only chronic and not acute injury result in progressive fibrosis. Stellate cell activation is a key therapeutic target in treating hepatic fibrosis and associated portal hypertention. Antifibrotic and hemodynamic effects of the early and chronic administration of octreotide in two models of liver fibrosis in rats were reported. The early and chronic administration of octreotide prevents the development of portocollateral blood flow without reducing portal pressure in the CCl_4 model liver fibrosis.[12] A new category of antifibrotic agents are those that act directly on stellate cells to modulate their activation. Given their global effects on the activation response, they are assumed to act at the level of the nucleus, therefore interferon is one such compound[13]. In conjuction with these studies, the drug was noted to elicit a greater response in female animals than in males. Moreover, this difference was demonstrable with stellate cells isolated from females and males and placed in

primary culture. The suggestion of a sexual dimorphism of wound repair may be relevant to the fact that in chronic liver injury, the progression of fibrosis to cirrhosis and its complications such as hepatocellular carcinoma is more rapid in men than in women. The data suggest further that it may be necessary to devise different anti-fibrotic regimens for men and women, and different intensity of the treatment.

Finally, contractile antagonists are attractive therapeutically because of the dynamic nature of contraction. In theory they are capable of reversing an injury-related increase in intrahepatic pressure and thereby reducing the risk of complication such as variceal bleeding. Selective endothelin-A receptor blockade can dramatically reduce collagen accumulation in rat secondary biliary fibrosis, a model refractory to most potential antifibrotic agents. Endothelin-A receptor antagonists are promising antifibrotic agents in chronic liver disease.[14] Antagonists of mediators that regulate both activating and contractile stimuli are already undergoing testing in experimental models of liver injury, and will likely emerge in clinical trials in the coming decade. Study of anti fi-brotic effects in vitro and in vivo models of liver fibrosis in rats demonstrated that inhibition of PDGF and TGF-beta1 actions on hepatic stellate cell provide a possible mechanism of its antifibrotic activities. [15]

Conclusion

Progress continues on defining of two major phases of stellate cells activation i.e., initiation and perpetuation. The injury response of the liver is increasingly well understood with regard to the role of individual cell types such as sinusoidal endothelial cells and stellate cells. Furthermore cytokines such as TGF-B1 and PDGF play a key pathogenic role in stellate cell fibrogenesis. Transforming growth factor-B-1 (TGF-B1) appears to be the most profibrogenic cytokine present in the liver. The targets for antifibrotic therapy are receptors for those cytokines such as TGFβ and endothelin 1 that are responsible for stimulating stellate cell activation and ECM production. Thereby Endothelin-A receptor antagonists are promising antifibrotic agents in chronic liver disease. Their regulation at a molecular level is a topic of current investigation.

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