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Review Article

A Review on Stellate Cell Responses in Liver Injury and Repair

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ABSTRACT

The hepatic stellate cell has surprised and engaged physiologists, pathologists, and hepatologists for over 130 years yet clear evidence of its role in hepatic injury and fibrosis only emerged following the refinement of methods for its isolation and characterization. The paradigm in liver injury of activation of quiescent vitamin A-rich stellate cells into proliferative, contractile, and fibrogenic myofibroblasts has launched an era of astonishing progress in understanding the mechanistic basis of hepatic fibrosis progression and regression. But this simple paradigm has now yielded to a remarkably broad appreciation of the cell's functions not only in liver injury, but also in hepatic development, regeneration, xenobiotic responses, intermediary metabolism, and immunoregulation. Among the most exciting prospects is that stellate cells are essential for hepatic progenitor cell amplification and differentiation.

INTRODUCTION

Chronic liver injury produces liver fibrosis, and its end stage, cirrhosis, is a major public health problem worldwide owing to life-threatening complications of portalhy pertension and liver failure and to the risk of incident hepato cellular carcinoma. A variety of adverse stimuli may trigger fibrogenesis, including viruses, toxins such as alcohol, autoimmune diseases, chronic biliary stasis, metabolic disorders, genetic defects, or hypoxia. In western countries, the prevailing causes of cirrhosis include chronic alcohol consumption, hepatitis C virus, and non-alcoholic steatohepatitis. Current treatment of hepatic fibrosis is limited to withdrawal of the noxious agent, which not only prevents fibrosis progression but may also induce its regression, as discussed below. Major advances have been made in this respect during the past decade, with the advent of efficient antiviral treatments for hepatitis B and C. Nevertheless, suppression of the cause of hepatic injury is not always feasible, and, therefore, numerous efforts are directed at the development of liver-specific antifibrotic therapies.

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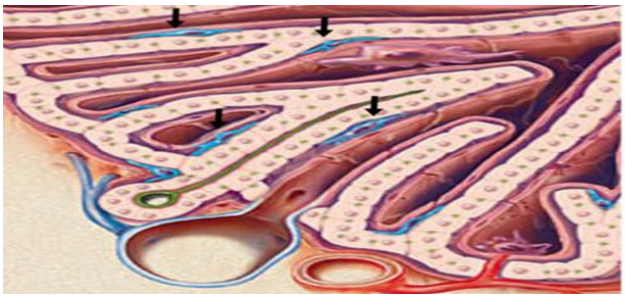
Although effective antifibrotic treatments are not available as yet, several ongoing clinical trials are evaluating molecules identified from the joint efforts of many researchers.

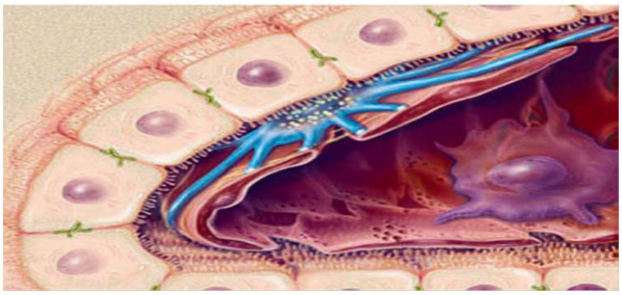
In addition, recent advances in the physiopathology of liver fibrosis are paving the way for the design of new molecules interfering with regulatory pathways in fibrogenic cells. This review highlights recent advances in the molecular mechanisms of liver fibrosis and discusses mechanistically based strategies that have emerged recently (Fig.1).

The hepatic stellate cell has surprised and engaged physiologists, pathologists, and hepatologists for over 130 years yet clear evidence of its role in hepatic injury and fibrosis only emerged following the refinement of methods for its isolation and characterization. The paradigm in liver injury of activation of quiescent vitamin A-rich stellate cells into proliferative, contractile, and fibrogenic myofibroblasts has launched an era of astonishing progress in understanding the mechanistic basis of hepatic fibrosis progression and regression. But this simple paradigm has now yielded to a remarkably broad appreciation of the cell's functions not only in liver injury, but also in hepatic development, regeneration,

xenobiotic responses, intermediary metabolism, and immunoregulation. Among the most exciting prospects is that stellate cells are essential for hepatic progenitor cell amplification and differentiation. Equally intriguing is the remarkable plasticity of stellate cells, not only in their variable intermediate filament phenotype, but also in their functions. Stellate cells can be viewed as the nexus in a complex sinusoidal milieu that requires tightly regulated autocrine and paracrine cross-talk, rapid responses to evolving extracellular matrix content, and exquisite responsiveness to the metabolic needs imposed by liver growth and repair. Moreover, roles vital to systemic homeostasis include their storage and mobilization of retinoids, their emerging capacity for antigen presentation and induction of tolerance, as well as their emerging relationship to bone marrow-derived cells. As interest in this cell type intensifies, more surprises and mysteries are sure to unfold that will ultimately benefit our understanding of liver physiology and the diagnosis and treatment of liver disease.



(A)



(B)

Figure1: Morphology of hepatic stellate cells in normal liver. **(A)** Diagram of the hepatic sinusoid demonstrating the relative orientation of stellate cells (in blue, indicated with arrows) within the sinusoidal architecture. **(B)** Higher resolution drawing of stellate cells situated within the subendothelial space.

A. Stellate Cell Activation: Features, Regulation, and Reversibility

The clarification of stellate cell responses in hepatic injury and repair has been a significant turning point in understanding the basis of hepatic fibrosis. In particular, the identification of stellate cell activation as a key event in fibrogenesis has provided an important framework for conceptualizing the liver's response to injury [1-6].

Stellate cell "activation" refers to the conversion of a resting vitamin A-rich cell to one that is proliferating, fibrogenic, and contractile. While it is increasingly clear that other mesenchymal cell populations also contribute to extracellular matrix accumulation, stellate cell activation remains the most dominant pathway leading to hepatic fibrosis (see Fig. 1). Moreover, stellate cell activation represents a continuum, such that early changes in cellular phenotype may be distinct from those occurring with progressive injury and activation in terms of growth characteristics, response to soluble mediators, inflammatory signalling, and apoptotic potential [7- 12].

Activation consists of two major phases: initiation and perpetuation, followed by resolution of fibrosis if injury subsides (Fig. 2).

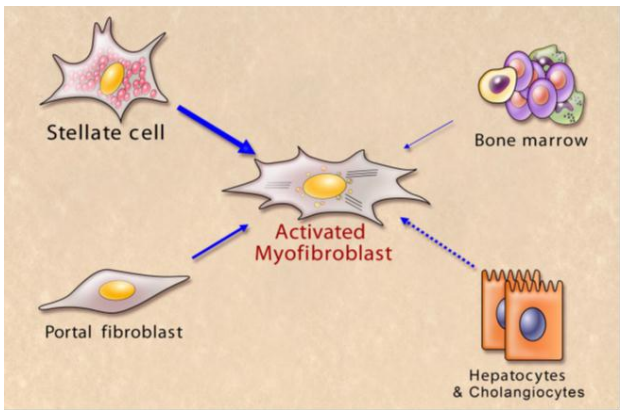


Figure 2: Sources of myofibroblasts in liver injury. Multiple sources of fibrogenic myofibroblasts are likely in liver injury depending on the site and nature of the injury. While resident stellate cells appear to be the most likely source, periportal fibroblasts may be especially prominent in biliary injury, whereas bone marrow and possible epithelial-mesenchymal transition may contribute as well.

1. Initiation (also called a "pre inflammatory stage") refers to early changes in gene expression and phenotype that render the cells responsive to other cytokines and

stimuli. Initiation results mostly from paracrine stimulation, primarily due to changes in surrounding extracellular matrix, as well as exposure to lipid peroxides and products of damaged hepatocytes

2. Perpetuation results from the effects of these stimuli on maintaining the activated phenotype and generating fibrosis. Perpetuation involves autocrine as well as paracrine loops. It is comprised of several discrete responses including proliferation, contractility, fibrogenesis, matrix degradation, retinoid loss, and inflammatory cell infiltration.
3. Resolution of fibrosis refers to pathways that either drive the stellate cell to apoptosis, or contribute to their reversion to a more quiescent phenotype (Fig.2).

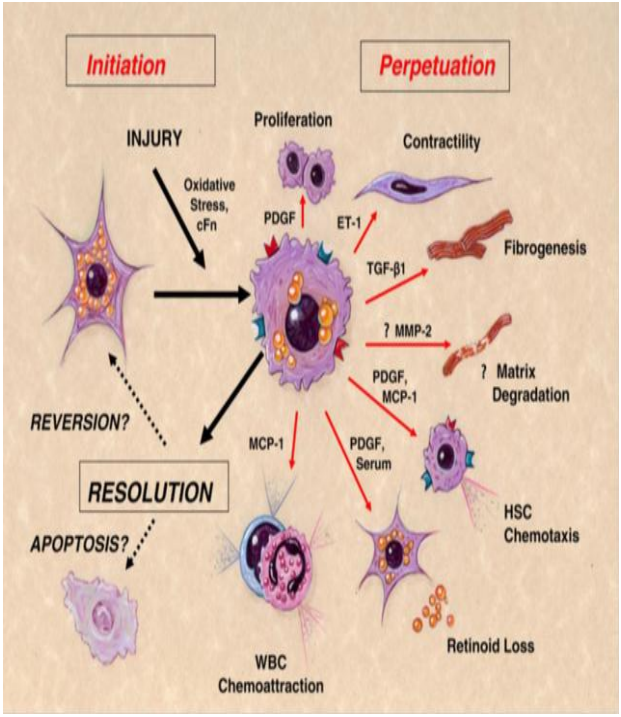


Figure 3: Pathways of stellate cell activation and resolution.

Following liver injury, hepatic stellate cells undergo "activation," which connotes a transition from quiescent vitamin A-rich cells into proliferative, fibrogenic, and contractile myofibroblasts. The major phenotypic changes after activation include proliferation, contractility, fibrogenesis, matrix degradation, chemot axis, retinoid loss, and WBC chemo attraction. Key mediators underlying these effects are shown. The fate of activated stellate cells during resolution of liver injury is uncertain but may include reversion to a quiescent phenotype and/or selective clearance by apoptosis.

1. Initiation

The earliest changes observed during stellate activation result from paracrine stimulation by all neighboring cell types, including sinusoidal endothelium, Kupffer cells, hepatocytes, and platelets. As noted above, early injury to endothelial cells stimulates production of cellular fibronectin, which has an activating effect on stellate cells [13]. Endothelial cells are also likely to participate in conversion of TGF-β from the latent to active, profibrogenic form. Platelets are another important source of paracrine stimuli, including PDGF, TGF-β, and EGF [14].

Kupffer cell infiltration and activation also contribute to stellate cell activation. Kupffer cells stimulate matrix synthesis, cell proliferation, and release of retinoids by stellate cells through the actions of cytokines (especially TGF-β) and reactive oxygen intermediates/lipid peroxides [15].

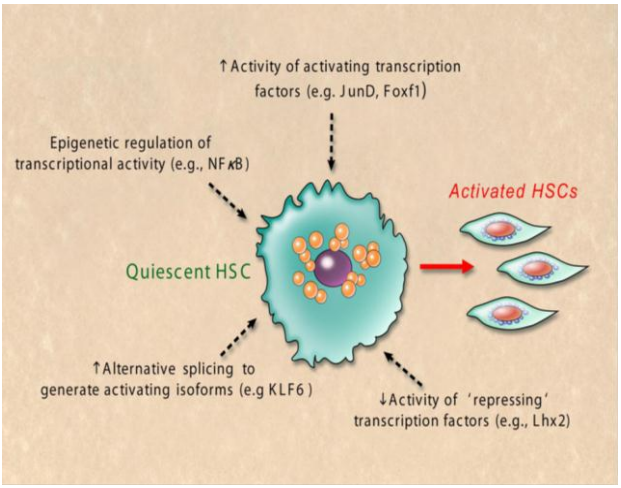


Figure 4: Mechanisms of transcriptional regulation of stellate cell activation

Hepatocytes are a potent source of fibrogenic lipid peroxides, although effects on stellate cell collagen synthesis and proliferation may be dose dependent [16]. Hepatocyte apoptosis following injury also promotes stellate cell initiation through a process mediated by F as [17, 18]. This process may involve the TNF-related apoptosis inducing ligand (TRAIL) [17, 18]. Whereas hepatocyte necrosis associated with lipid peroxidation is considered a classical inflammatory and fibrogenic stimulus, recent findings also implicate apoptosis, or programmed cell death, in the fibrogenic response. Apoptotic fragments released from

hepatocytes are fibrogenic towards cultured stellate cells [19] and activate Kupffer cells [20]. Also, F as-mediated hepatocyte apoptosis is fibrogenic *in vivo* in experimental animals [18].

2. Perpetuation

Perpetuation of stellate cell activation involves at least seven discrete changes in cell behaviour: proliferation, chemot axis, fibrogenesis, contractility, matrix degradation, retinoid loss, and WBC chemo attractant/cytokine release. The net effect of these changes is to increase accumulation of extracellular matrix. As an example, proliferation and chemot axis lead to increased numbers of collagen-producing cells. Cytokine release by stellate cells can amplify the inflammatory and fibrogenic tissue responses, and matrix proteases may hasten the replacement of normal matrix with one typical of the wound "scar."

A) PROLIFERATION. PDGF is the most potent stellate cell mitogen identified [21, 22]. Induction of PDGF receptors early in stellate cell activation increases responsiveness to this potent mitogen [23]. Downstream pathways of PDGF signalling have been carefully characterized in stellate cells and include PI 3-kinase, among others [24, 25]. In addition to proliferation, PDGF stimulates Na+/H+ exchange, providing a potential site for therapeutic intervention by blocking ion transport [26].

B) CHEMOTAXIS. Stellate cells can migrate towards cytokine chemoattractants [27-29]. Explaining in part why stellate cells align within inflammatory septae *in vivo*. A number of chemoattractants have been identified, among which are prominent PDGF [30-31] MCP-1 [32] and CXCR3 [33]. In contrast, adenosine [34] blunts chemot axis and may immobilize cells once they reach the site of injury. The mechanical features of stellate cell chemot axis have recently been explored, revealing that PDGF-stimulated chemot axis is associated with cell spreading at the tip, movement of the cell body towards the stimulant, and retraction of trailing protrusions associated with transient myosin phosphorylation [35].

C) FIBROGENESIS. Stellate cells generate fibrosis not only by increased cell numbers, but also by increasing matrix production per cell. The best-studied component of hepatic scar is collagen type I, the expression of which is regulated both transcriptionally and post transcriptionally in hepatic stellate cells by a growing number of stimuli and pathways [36–45].

The most potent stimulus for production of collagen I and other matrix constituents by stellate cells is TGF-β , which is derived from both paracrine and autocrine sources [46]. Signals downstream of TGF-β include a family of bifunctional molecules known as S mads, upon which many extra cellular and intracellular signals converge to fine-tune and enhance TGF-β's effects during fibrogenesis [47]. TGF-β also stimulates the production of other matrix components including cellular fibronectin and proteoglycans [48]. In addition to a major role for S mad proteins, TGF-β1 stimulates collagen in stellate cells through a hydrogen peroxide- and C/EBPβ-dependent mechanism [49]. The response of S mads in stellate cells differs between acute and chronic injury to further favour matrix production [50- 52].

D) CONTRACTILITY. Contractility of stellate cells may be a major determinant of early and late increases in portal resistance during liver fibrosis. The collagenous bands typical of end-stage cirrhosis contain large numbers of activated stellate cells. These impede portal blood flow by constricting individual sinusoids and by contracting the cirrhotic liver. The acquisition of a contractile phenotype during stellate cell activation has been documented in culture and *in vivo* and is mediated in part by receptors that interact with the extracellular matrix and are driven by calcium signalling. As noted in section v D, endothelin-1 and nitric oxide are major counter regulators controlling stellate cell contractility, in addition to a growing list of additional mediators including angiotensinogen II, eicosanoids, atrial natriuretic peptide, somatostatin, and carbon monoxide, among others.

E) MATRIX DEGRADATION. Fibrosis reflects a balance between matrix production and degradation. The degradation of extracellular matrix is a key event in hepatic fibrosis. Early disruption of the normal hepatic matrix by matrix-degrading proteases hastens its replacement by scar matrix, which has deleterious effects on cell function.

Disruption of the normal liver matrix is also a requirement for tumor invasion and desmoplasia and may be particularly relevant to pancreatic stellate cells. Degradation in these contexts is referred to as being "pathological." On the other hand, resorption of excess matrix in patients with chronic liver disease provides the opportunity to reverse hepatic dysfunction and portal hypertension.

F) RETINOID LOSS: Activation of stellate cells is accompanied by the loss of the characteristic perinuclear retinoid (vitamin A) droplets. In culture, retinoid is stored as retinyl esters, whereas the form of retinoid released outside the cell during activation is retinol, suggesting that there is intracellular hydrolysis of esters prior to export. Whether retinoid loss is required for stellate cells to activate, and which retinoids might accelerate or prevent activation are not clarified.

RESOLUTION

As attention has turned to the treatment of liver fibrosis, the issue of how stellate cell activation resolves has become quite critical. Two potential pathways account for reduction in activated stellate cells, either reversion to a quiescent phenotype or clearance through apoptosis. Although reversion can be accomplished in cultured stellate cells through transfer of activated cells to a basement membrane matrix, this has not been validated *in vivo*. To do so, genetic lineage tracing would be required to confirm that cells once activated have resumed a quiescent phenotype; however, such studies have not yet been reported. In contrast, a large amount of evidence supports the importance of stellate cell apoptosis during regression of liver fibrosis. In culture, stellate cells are sensitive to CD95-L and TRAIL-mediated apoptosis, and NK cells can induce apoptosis of stellate cells by a TRAIL mediated mechanism (see sect. VC). NGF derived from hepatocytes is also apoptotic towards stellate cells and is antagonized by serotonin receptor signalling. Apoptosis requires an intact proteosomal degradation pathway, since its inhibition prevents stellate cell apoptosis. In a recent study, an antifibrotic effect of NK cells was indicated by the presence of increased fibrosis in mice depleted of NK cells by anti-asialo-GM1 antibody and by decreased fibrosis after NK cell activation by a TLR3 ligand poly I: C. The NK cell-induced stellate cell apoptosis was specific for activated stellate cells that expressed the NK cell activating receptor NKG2D.

The activated NK cells deliver a lethal blow to stellate cells by inducing apoptosis with TRAIL. In this study, NK cell function was dependent on interferon-$\gamma$and providedan explanation for earlier experiments demonstrating animportant antifibrotic role for interferon-γ. The antifibrotic role of NK cells was further supported by evidence of their direct adhesion to stellate cells in mouse livers, and by the development of greater fibrosis in mice genetically deficient in NK cells. Most recently, these findings have been reinforced by studies in humans with HCV. In addition to NK cells, activated Kupffer cells can also provoke stellate cell apoptosis by a unique caspase 9- and a receptor-interacting protein-dependent mechanism.

The antifibrotic role of NK cells is also consistent with the clinical data of increased liver fibrosis in the setting of therapeutic immune suppression. The effect of single immunosuppressive agents on NK cell function is minimal, but the combination of cyclosporine and cortico steroids results in significant loss of NK cell cytotoxicity. In addition, cyclosporine renders some cells resistant to NK cell-mediated cytotoxicity. The effect of HIV infection on NK cell number and function is more complex. Some NK cell subsets co express CD4 and HIV co receptors and are targets for infection with HIV. NK cells from HIV-infected patients have reduced cytolytic activity and decreased production of cytokines. The hypothesis that NK cells limit liver fibrosis by inducing stellate cell apoptosis predicts that NK cell function will be relatively impaired in individuals with rapid progression of fibrosis compared with those in whom liver fibrosis progresses slowly. It may also explain why fibrosis accelerates with aging, since NK cell function declines with age.

B. Transcriptional Regulation of Stellate Cell Behaviour

Evolving concepts of transcriptional gene regulation have now been applied to stellate cell biology and fibrosis Both genetic and epigenetic regulation are critical to stellate cell responses and are reviewed extensively in several recent articles. In addition, evidence of posttranscriptional control has been described in stellate cells as well. Stellate cell activation may result from either "activating" event, such as induction of transcription factor splice forms, as

well as loss of repressive signalling. These complex cascades illustrate how transcriptional regulation in stellate cells is finely tuned and involves several interdependent layers of transcriptional, translational, posttranslational, and epigenetic control. In addition, the identification of micro RNAs has emerged as a major new pathway of gene regulation in many systems. Including cancer; however, this area has not yet been explored in stellate cells.

A growing number of transcription factors have been identified in stellate cells, yet these only represent a small number of the total number of factors contributing to transcriptional control. Many target genes of these transcription factors in stellate cells have been reported, but those target genes most intensively evaluated have included type I collagen (α1-and α-2-chains), TGFβ 1 and TGF-β receptors, MMP-2, TIMPs 1 and 2, and α-SMA.

The following four examples illustrate how stellate cell activation may be controlled by widely divergent regulatory pathways, including transcription factors that contribute to stellate cell activation directly and whose deletion attenuates fibrosis (e.g., Foxf1 and JunD), alternative splicing of a growth inhibitory transcription factor (e.g., KLF6), epigenetic regulation of a factor regulating stellate cell survival (e.g., NFκB), and regulation of a transcription factor whose expression maintains stellate cell quiescence (e.g., Lhx2).

CONCLUSION

Equally intriguing is the remarkable plasticity of stellate cells, not only in their variable intermediate filament phenotype, but also in their functions. Stellate cells can be viewed as the nexus in a complex sinusoidal milieu that requires tightly regulated autocrine and paracrine cross-talk, rapid responses to evolving extracellular matrix content, and exquisite responsiveness to the metabolic needs imposed by liver growth and repair. Moreover, roles vital to systemic homeostasis include their storage and mobilization of retinoids, their emerging capacity for antigen presentation and induction of tolerance, as well as their emerging relationship to bone marrow-derived cells. As interest in this cell type intensifies, more surprises and mysteries are sure to unfold that will ultimately benefit our understanding of liver physiology and the diagnosis and treatment of liver disease.

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