HEPATIC STELLATE CELLS IN THE CONTEXT OF LIVER FIBROSIS

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ABSTRACT

Liver fibrosis is a wound healing response caused by either acute or chronic cellular damage. If left untreated, can lead to cirrhosis that progress to liver failure and can causes death. During hepatic fibrosis, the primary cell-type is the hepatic stellate cell (HSC), previously known as Ito cell, responsible for the progressive collagen synthesis in liver. After liver damage, the HSC changes from a quiescent, vitamin A-storing cell to that of an activated myofibroblast-like cell through intricate transformation or an activation process. After HSC activation, a series of intricate cellular cascades are triggered by the stimulation of signaling events. Various signaling pathways include TGF-beta signaling, signaling that favor proliferation, NF-kB signaling, and MAPK signaling that play role in Hepatic stellate cell activation and proliferation. In the present review, an attempt has been madeto add a drop of knowledge in the sea of already available literature on liver fibrosis in order to elaborate the role of hepatic stellate cellsliver fibrosis, its activation, some of the signaling pathways implicated in its activation. And how to control the activation and proliferation of HSCs for prevention of liver fibrosis, has been briefly inked.

KEY WORDS: Hepatic Stellate Cell, Tumor Growth Factor-Beta, Mitogen Activated Protein Kinases, Extracellular Matrix, Liver Fibrosis.

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INTRODUCTION

Liver fibrosis is a reversible wound-healing process that is triggered either by acute or chronic cellular damage which represent a balance between liver regeneration and scare formation. Acute injury is characterized by transient and reversible changes in liver architecture. On the other hand, during chronic injury, scar tissue continuously replaces liver parenchyma. In spite of progressive injury, there is dramatic regenerative potential of liver due to which patients often gradually lead to cirrhosis which takes decades.[1] The advanced stage of liver fibrosis is cirrhosis that is characterized by hepatic vascular distortion.[2] Complications such as ascites, esophageal arices or jaundice once triggered, than there is unpreventable and continued deterioration of liver. The main cause of liver fibrosis is hepatitis viral infections that include hepatitis B and hepatitis C worldwide but the leading cause within the United States is chronic ethanol consumption. Autoimmune disorders, drug-induced, helminthic infection, iron or copper overload and biliary obstruction represent other causes for hepatic fibrosis[3] and nonalcoholic steatohepatitis (NASH) is a major stimulus of fibrosis in emerging research studies.[4]

Fibrosis can be characterized as a wound-healing process in response to various chronic stimuli which can be classified as continues accumulation of extracellular matix (ECM) proteins that comprise of three huge families of proteins-glycoproteins, collagens, and proteoglycans.[5] The role of hepatic stellate cells (HSC) during fibrogenesis as the primary cell responsible for the excessive collagen production have been demonstrated in various experimental animal models of liver disease.[6,7] Although, traditionally it has been considered that during liver fibrosis, HSC as the primary hepatic cell type for the production of progressive accumulation of ECM but increasing evidence demonstrates the contribution of other cells that include fibrogenic cells of mesenchymal origin, myofibroblasts, interstitial fibroblasts, and bile duct epithelial cells.[8]

Hepatic Stellate Cells Activation

Five percent to 10% of the normal liver cells comprise of hepatic stellate cells that are situated between hepatocytes and sinusoidal endothelial cells in the subendothelial space.[9] During hepatic fibrosis, the primary cell-type is the hepatic stellate cell (HSC), previously known as Ito cell, is responsible for the progressive collagen synthesis in liver.[10] After liver damage, the HSC changes from a quiescent, vitamin A-storing cell to that of an activated myofibroblast-like cell through intricate transformation or a activation process.[11] Furthermore, the central role played by HSC is the secretion of cytokines and chemokines that activate an immune response and interact with immune cells. Moreover, HSCs also

play its part in regulation of oxidant stress and angiogenesis process.[12]

HSC are activated into myofibrolast-like phenotype in response to various type of chronic injuries to the liver that include viral hepatitis, toxins, (non-) alcoholic steatohepatitis and autoimmune disorders.[13]

The activation of hepatic stellate cells occurs in a reproducible sequence through a highly pleiotropic but strictly regulated response. The cellular events of hepatic stellate cell activation can be grouped into a defined biologic background by classifying HSC activation into transitory sequential steps. In this context, the cellular events that occur earlier are called initiation (termed proinflammatory stage as well). The Initiation step include all that quick switching in gene expression as well as changes in the phenotype due to which the cells become responsive to cytokines and other local stimuli. Furthermore, transcriptional events and the stimulation of early genes that are in need are linked with initiation. These effects are because of paracrine signaling in response to quick, untoward effects of liver damage on the homeostasis of surrounding cells and from early alterations in the composition of extracellular matrix. Perpetuation include those cellular events that result in the sustained activation of already activated phenotype by sustained cytokine expression and responsiveness; autocrine, paracrine signaling and enhanced ECM remodeling cause the induction of the component.[6]

Signaling pathways in Hepatic stellate cells

After HSC activation, a series of intricate cellular cascades are triggered by the stimulation of signaling events. Regulation of gene expression as well as fibrogenic response of the HSC during liver fibrosis is mainly controlled by these signaling cascades. Several Signaling cascades that have been studied during the cellular activation of HSC are briefly described below.[14]

TGF-b signaling and hepatic stellate cells

Transforming growth factor (TGF)-b is one of the profibrogenic mediators that is more powerful and widely distributed in the body. It modulates both pathological fibrosis and the accumulation of extracellular matrix (ECM) as function of the normal response to tissue injury. The important thing in the fibrotic diseases of multiple tissue is changes in the homeostasis of TGF-b. Furthermore, TGF-b has wide range of possible effects keeping in view the cellular and environmental context that include control of growth and differentiation and modulation of the immune response.[15] The main source of TGF-b is HSCs but this cytokine can be secreted by Kupffer cells, hepatocytes, and platelets also.[16] The most potent fibrogenic cytokine in the liver is TGF-b1 that is triggered by both paracrine and autocrine sources.[17,18] TGFb1 is stored as an inactivated protein bound to a latency-cytokine in the liver. After the activation, TGFb1 leads to the stimulation of collagen production by activating the Smad proteins once it is associated with its respective receptors.[17] Myofibroblasts which secret extracellular matrix, are derived by quiescent HSCs after transdifferentiation by the induction of TGFb1. While HSC fibrogenesis are induced by the apoptosis of hepatocyte and necrosis, during regeneration, TGFb1 probably maintain hepatocyte mass and modulate growth.[18] So therapeutic inhibition of TGFb1 will be counterproductive because some of its actions are important to maintain normal homeostasis of liver such as its anti-inflammatory and growth regulatory roles.[19]

Proliferation of the Hepatic Stellate Cells

Fibrogenesis are further amplified by the increased production of activated HSCs in addition to the cellular events that result in the progressive formation and remodeling of the extracellular martrix.[20,21] Local increase in HSCs contributes in the proliferation of HSCs in damage liver that is stimulated by polypeptide growth factors which mainly signal by tyrosine kinase receptors. Well studied, highly proliferative and most potent is PDGF in liver fibrosis among these factors. Liver injury not only results in the sustained autocrine PDGF but also causes up-regulation of PDGF receptor.[22]

In addition to this, expression of PDGF and PDGF-Rs are modulated by paracrine signaling molecules that are secreted by other liver cells such as endothelial cells, Kupffer cells, and hepatocytes.[23] PDGF is made of two polypeptide chains A and B that combination in different combination (PDGF-AA, -BB, or –AB), that characterizes it as heterodimeric protein. PDGF-AA recognizes PDGF-Rα its only cognate receptor whereas PDGF-Rα and -β, receptors are recognized by PDGF-B that binds with these receptors and dimerizes.[23]

ERK/MAPK pathway is triggered by the signaling molecule Ras that intern activated by the PDGF receptor after binding with its congate ligands. Moreover, high intracellular PH and continues intake of extracellular (Ca2+) is also necessary for proliferative response to PDGF.[22]

In pre-clinical disease models, the inhibition of the PDGFR-b chain has presented a promising anti-fibrotics.[24,25] An inhibitor of multiple receptor tyrosine kinase "Sorafenib" that target the PDGF receptor and the Raf/ERK signaling pathway is not only effective against advanced HCC patients but also shown aniti-fibrotic activity in animal models of fibrosis.[25]

Signal Transduction through MAPK in Hepatic Stellate Cells

Another intracellular pathway that is triggered in progressive HSCs is mitogen-activated protein kinase (MAPK) signaling cascade. Extracellular regulated kinase (ERK), c-jun N-terminal kinase/stress-activated protein kinase-1 (JNK/SAPK-1) are the members of MAPK family that is stimulated by many growth factors and stress which than pass signal to the nucleus.[26]

It is reported that HSC proliferation is also caused by Mitogen-activated protein kinases (MAPK). The stimulation of HSC, both in vitro and in vivo, by PDGF triggers a signaling cascade that includes Ras activation which results in the subsequent activation of Raf, mitogen induced extracellular kinase (MEK) and extracellular signal regulated kinase (ERK).[22,27,28] Inhibiting ERK pharmacologically greatly lowered cell proliferation representing the role of this signaling cascade in the proliferation of HSCs.[29] c-Jun nuclear kinase (JNK) has also been reported to regulate the proliferation of cell positively in different type of cells including HSC.[30,31] Pharmacological inhibition of JNK activity by using negative form of JNK (Ad-TAK) inhibited the proliferation of HSCs in quiescent HSC or in culture-activated HSC.[32] On the other hand, inhibiting another MAPK member, P38, by using the inhibitor SB203580 in both quiescent and activated HSC resulted in the increase of activated HSC cell proliferation demonstrating the inhibitory role of P38 activation on the proliferation of HSC.[32] Inhibitory effect of p38 against cell proliferation may be due to inhibiting the expression of cyclin D1 which has been demonstrated in other cell types.[33] Sustained expression of cyclins D1, D2, and E, are necessary for progression of cell cycle during the proliferative phase, the activation of HSC is associated with these cyclins.[34]

Role of Nuclear Factor-kappaB Activation in Hepatic Stellate Cells

NF-κB is a family of transcription factors that comprise of homo- or hetero-dimeric subunits of the Rel family which include p65 (or RelA), p50, p52, c-Rel, and Rel-B.[35] Various cytokines and mitogens trigger the activation of NF-κB that regulate the expression of different genes involved in immune and inflammatory responses.[35,36] The genes which many regulated by NF-κB include cytokines such as interleukin (IL)-2 and IL-8 and adhesion molecules involve in inflammation such as Eselectin, intercellular adhesion molecule-1 (ICAM-1), Vascular cell adhesion molecule-1 (VCAM-1).[35,36] In the absence of a stimulant, NF-κB is mainly restricted to the cytoplasm in association with an inhibitory protein I-κB. When I-κB stimulated by agents such as tumar necrosis factor-α (TNF-α) or IL-1b, it is phosphorylated, ubiquitinated which then degraded by proteosome machinery.[36] After dissociation from the I-κB, the NF-κB subsequently translocated to the nucleus where it binds with DNA which result in the activation of transcription of specific genes.[37]

NF-κB is activated for a short period in most of the cells whereas in case of HSC activation, there is sustained activation of NF-κB with decrease in the expression of I-κBα.[38] Tumor necrosis factor-α activate NF-κB as its major downstream effector and the up-regulation of this partly associated with hepatic oxidative stress cascade. [39] TNF-α mainly up regulate NF-κB that result in the activation of many target genes in other cell types that include cytokines, chemokines and structural proteins. In HSCs, TNF-α activate transcription factor AP-1, and c-jun kinase which result in the sustained expression of matrix metalloproteinase gene[40] but lowers the expression of collagen genes.[41]

Pro-inflammatory response

In chronic fibrogenesis, prolong inflammatory response seems to be one of the important driving forces. This continued inflammation is due to the release of various pro-inflammatory molecules such as TLR ligands, MCP-1 and several other chemoattractants and chemokines realed by HSC/Myfibroblast (MFs).[13,42] In addition to this, HSC/MF acts as target site for other pro-inflammatory and inflammatory cytokines.

Modulation of Hepatic Stellate Cells Activation

Over the past 2 decade, huge research has been carried out in liver fibrosis that highly helped in our understanding of its mechanisms at molecular level, major chunk of which closely related to hepatic stellate cells. As a consequence, various main events in the stellate cell activation process and fibrogenesis have been characterized that can be target therapeutically which may prove useful clinically in the prevention or treatment of liver fibrosis.[43]

It has been reported recently that increased proliferation of HSCs greatly affect the degree of fibrogenesis in liver diseases.[44,45] Moreover, activated HSCs through its cross-talk in the cancer microenvironment enhance progression of HCC.[46,47]

Therapeutic suppression of the activation and proliferation of HSC has been proposed for hepatic fibrosis treatment and its prevention that result to the irreversible liver cirrhosis and HCC.[48]

It has been increasingly recognized that HSCs are one of the key mediators in the progression of hepatic fibrosis. In vitro and in vivo studies in the past has proposed PDGF as the most potent mitogen of HSCs which implicate as one of the main mediator of sustained prolifera-

tion of HSC during hepatic fibrogenesis in chronic diseases of liver.[42,44,49] The most potent among the three PDGF isoforms is the PDGF-BB[50] and the proliferation of quiescent HSC result in combination with the enhanced expression of PDGF-Rβ having no change in PDGF-Rα.[51] It has been shown that tricin block cell cycle progression by inhibiting PDGF-BB induced cell proliferation either in LI90 cell lines or in activated HSCs through the inhibition of the phosphorylation of PDGF-Rβ.[48] PDGF receptors on the cell surface when bind with its congnate ligand PDGF, this result in the dimerization of the receptor molecules and autphosphorylation at tyrosine residues which stimulate the PDGF effector downstream signaling that include Ras, Raf-1, MEK and ERK. PI3 are recruited by PDGF-R that intern activate Akt which is a requisite for the mitogenesis and chemotaxis triggered by PDGF-BB in HSCs.[42,44,49] Seki et al showed that tricin block the phosphorylation of these downstream signaling molecules that probably due to the inhibitory effects on PDGF-BB in HSCs.[48]

Sp1 is a member of Kruppel-like factors that is greatly linked with GC-rich promoters.[6] Along with its role in the regulation of large number of different housekeeping genes, there is increasing evidence of the regulatory role of Sp1 in other different cellular events such as cell proliferation and fibrosis.[52,53] The expression of many genes are regulated by Sp1 that is associated with fibrosis process that include TGF-β1, VEGF, COLIA2, and the downstream effecter of TGF-β1 including PAI-1 fibronectin and MMPs.[54,55] In addition to this, other group has previously reported that binding activity of SP1 with DNA enhanced in different form of fibrosis including activated HSCs.[56] The striking new findings of the Chen et al work is the optimal transfection of the Sp1 decoy ODN that greatly caused the inhibition of the expression of fibrotic genes and the proliferation of HSC-T6 and probably functions in the sustained activation of HSC-T6.[57] But, Sp1 gene manipulation required special attention as there may chances of serious side effects in development, differentiation and metabolism and most importantly if targeting is not sufficient.[58,59] Development of cell-specific gene modulation for the HSCs will be necessary for the establishment of Sp-1 directed gene therapy for hepatic fibrosis.[57]

Snail1 is a member of gene gamily Snai1(zinc-finger transcription factors) that are necessary for the formation of mesoderm and neural crest, cell fate and survival decisions, identification of left-right.[60] The members of this gene family play role as transcriptional repressors that bind to E-box sequences, which is the consensus sequence of the core binding site of basic helix-loop-helix transcription factors. The best known role of snai1 genes is that, that induce the epithelial-mesenchymal transition (EMT) in the process of embryonic development and tumor progression, while cell adhesion migration can also be regulated by these genes and have survival properties.[61,62] There is evidence that in vertebrates, transcriptional regulation of Snai1is because of the action of various signaling pathways that include ERK2, NF-κB, phosphatidylinositol 3-kinase (PI 3-kinase) (78-80)[63,64] all of which have been implicated in the HSC activation process.[14] Scarpa et al demonstrated HSC is Snail1$^+$, which is one of major sources of myofibroblast in the liver. Both in vitro and in vivo, the expression of this transcription factor greatly up-regulated at the mRNA level in the activated HSC but on the other hand, at the protein level, it is localized in nucleus in activated and trans-differentiating cells.[65] This shows the significance of Snail1 post-translational regulation, that regulate the intracellular location of the transcription factor through phosphorylation of various enzymes such as glycan synthase kinase 3β (GSK-3β),[66] P21-activated kinase-1[67] and LIV-1[68], all of these has not yet been studied in the HSC.[69]

In addition to this, it has been reported that lysyl oxidase-like2 and 3 (LOXL2/3) enzymes, which have been implicated in the biogenesis of connective tissue, stabilize the nuclear localization of Snail1 by modifying at lysines K98 and K137,[69] Surprisingly, in the fibrotic septa of human samples of HCV and HBV-related chronic hepatitis both of these enzymes, LOXLL2 and LOXL3 have been detected[70] indicating their possible function in the regulation of hepatic fibrogensis.[65]

In regression of liver fibrosis, apoptosis of activated HSC is a key factor.[71] Activated version HSC is more venerable to the process of apoptosis that lead to spontaneous cell death or cell death by death receptor meditation due to deprivation of serum or signaling by cytokines. In response to antiviral drug therapy or lowered fibrogenic signals, there is enhanced expression of Fas receptor (Fas) or TNF receptor 1 (TNFR1) and their ligands in the HSC which lead to caspase8/caspase3-dependent apoptosis. On the other hand, pro-apoptotic proteins over expression e.g. P53, Bax and Bcl-2 result in caspase-9-mediated programmed cell death.[72,73] Program cell death is a striking mechanism that can lower proliferation and enhance regeneration of liver parenchymal cells.[74]

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CONFLICT OF INTEREST

Author declares no conflict of interest

GRANT SUPPORT AND FINANCIAL DISCLOSURE

NIL