Liver Fibrosis and Hepatic Stellate Cells

Masataka OKUNO¹, Seiji ADACHI¹, Kuniharu AKITA¹, Hisataka MORIWAKI¹,
Soichi KOJIMA² and Scott L. FRIEDMAN³

¹First Department of Internal Medicine, Gifu University School of Medicine
²Tsukuba Research Institute, RIKEN
³Division of Liver Diseases, Mount Sinai Medical Center

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Abstract: Chronic hepatic inflammation leads to fibrosis. Hepatic stellate cells are activated in liver injury, playing a central role in the fibrogenesis under the influence of key cytokines. Transforming growth factor-β and platelet derived growth factor enhance matrix production and proliferation of stellate cells, respectively, and thereby accelerating fibrogenesis. Tumor necrosis factor-α, a major inflammatory cytokine, suppresses matrix production. Endothelin-1 exerts a strong contractile stimulus to stellate cells and may regulate sinusoidal blood flow. Retinoids are released during activation of stellate cells, yet they exert varying effects depending upon their molecular structure. Recent advances in understanding cellular and molecular mechanisms of liver fibrosis will lead to the development of new therapy of the fibrosis.

Key words: liver fibrosis, hepatic stellate cells, transforming growth factor-β, platelet derived growth factor, retinoids

INTRODUCTION

Hepatic fibrosis is a characteristic consequence of chronic liver damage that occurs in response to a long-term inflammation due to vival hepatitis (hepatitis type B and C), alcohol, drug intoxication, autoimmune diseases, parasites and others. Fibrosis can be recognized as one of the processes of wound healing. However, when excess deposition of extracellular matrix occurs, it leads to liver dysfunction and contributes to sustained necrosis of the hepatic parenchymal cells by disturbing blood flow and interfering with exchanges of oxygen and nutrients.

Fibrosis can not be defined merely as a deposition of excess matrices in tissues, because it is also accompanied by a change in the type of matrix molecules (including collagens, glycoproteins and proteoglycans) and histological redistributions of the matrices¹. In the normal liver, 5 types of collagen molecules (type I, III, IV, V and VI) are identified so far. Type I and III collagens account for one third of total collagens in the liver, respectively. When hepatic fibrosis develops, all types of collagens increase their amount, among which type I collagen shows a predominant increase, comprizing a half of all hepatic collagens. Thus, the proportion of matrix molecules alters in the fibrotic liver. Abnormal topographic redistribution of matrices also takes place in the fibrotic liver. Perisinusoidal and pericellular fibrosis is the major cause of hepatocyte dysfunction and altered portal blood flow. It not only blocks portal inflow but interferes with sinusoidal microcirculation and creates diffusion barriers between sinusoidal blood and hepatocytes. This may lead to perpetuating hepatocyte dysfunction and necrosis in the cirrhotic liver.

Activation of Hepatic Stellate Cells

The liver is composed of 5 types of cells; hepatic parenchymal cells, sinusoidal endothelial cells, Kupffer cells, hepatic stellate cells and Pit cells. Among these cells, hepatic stellate cells are the major cellular source of connective tissues in the damaged liver¹⁻⁶. Hepatic stellate cells (also termed as fat-storing cells, lipocytes, vitamin A-storing cells or Ito cells) are located in the perisinusoidal spaces (space of Disse) adjacent to endothelial cells. The cells have hepatocyte-contacting processes that attach to parenchymal cells lining along the Disse space. In normal conditions, hepatic stellate cells contain a large amount of vitamin A (mostly retinyl esters) and function as its major storage site in the body, and have low mitotic and fibrogenic activities. Such phenotypes of hepatic stellate cells are characteristic in the 'quiescent stage'.

In case of liver damage, hepatic stellate cells acquire mitotic, fibrogenic and contractile phenotypes and transform into myofibroblast like cells⁶,⁷. This process is called 'activation' that consists of two stages; initiation and perpetuation (Fig. 1)⁴. Friedman defines initiation as 'rapid changes in gene expression and phenotype that
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render the cells responsive to cytokines and other local stimuli. Disruption of the homeostasis of neighboring cells and alteration of matrices by liver injury trigger this initiation step. Reactive oxygen intermediates products by parenchymal cells and Kupffer cells are some of the factors that trigger initiation step. Perpetuation is defined as 'the stage in which the cells amplify the activated phenotype through enhanced cytokine expression and responsiveness'. In this stage, the cells are stimulated in autocrine and paracrine manners, as well as by continued accumulation of pathologic matrix. Overexpression of cell surface receptors to cytokines seen in activated stellate cells enhances cytokine responsiveness. Intracellular signalling systems downstream of receptor tyrosine kinases and serine/threonine kinase are up-regulated in the activated hepatic stellate cells. Among the cytokines that affect the activation of hepatic stellate cells, transforming growth factor-β (TGF-β) and platelet derived growth factor (PDGF) play major roles in their transformation and mitogenesity.

TGF-β

TGF-β is a very potent fibrogenic cytokine, TGF-β is a member of the 'TGF-β superfamily' that includes bone morphogenic proteins (BMPs) and activins. All these growth factors have dimeric polypeptide chains bound by intramolecular disulfide bond. TGF-β regulates the cellular proliferation and differentiation, immune suppression, embryonic development, wound healing and angiogenesis. In liver damage, TGF-β suppresses the parenchymal regeneration and promotes fibrogenesis. Thus, TGF-β plays a central role in the pathogenesis of liver cirrhosis.

Three isoforms are identified for TGF-β (TGF-β1, -β2 and -β3), which are expressed in a tissue-specific manner. In the liver, TGF-β1 and -β2 are constitutively produced, and their expression is up-regulated in the damaged liver. There is only faint expression of TGF-β3 in the liver. TGF-β2 mRNA expression is mainly localized in hepatic stellate cells, sinusoidal endothelial cells and Kupffer cells, but is not in hepatic parenchymal cells. However, since TGF-β protein can be detected in the cytoplasm of parenchymal cells, TGF-β may be taken up by the parenchymal cells.

TGF-β is synthesized as a large latent precursor that contains active TGF-β molecule and latency-associated peptide (LAP). When this latent precursor molecule is secreted from the cells, the molecule binds to another protein called latent TGF-β-binding protein (LTBP) and is stored in the extracellular matrix space. LTBP prevents the binding of latent TGF-β molecule to the TGF-β cell surface receptors. Active TGF-β molecule is cleaved from the precursor complex before it can bind to its cognate receptor and exerts biological functions. Because TGF-β receptors are present in most types of cells, this latent TGF-β activation is key a regulatory step of TGF-β activity. Activation releases the homodimeric active TGF-β molecule from the large

Fig. 1. Activation of hepatic stellate cells

In the quiescent stage, stellate cells contain large amounts of cytoplasmic lipid droplets, possess hepatocyte-contacting processes, and show low mitotic and fibrogenic activities. Upon inflammatory stimulation, the cells start to express cytokine receptors on the cell surface and become responsive to cytokine stimuli, among which TGF-β and PDGF play important roles. Such conditions are termed initiation step. Stellate cells lose lipid droplets from the cytoplasm and begin to express cytoskeletal proteins like smooth muscle actin and desmin. In the perpetuation step, the cells synthesize cytokines and autostimulate themselves to transform into myofibroblast-like cells. Activated stellate cells acquire mitotic and fibrogenic activities and play central roles in hepatic fibrosis.
Fig. 2. Signalling of TGF-β.

TGF-β is synthesized by hepatic stellate cells, Kupffer cells, sinusoidal endothelial cells and platelets in the damaged liver. The cytokine is secreted in a latent form and pooled in the extracellular matrix spaces. Activation of latent TGF-β releases 25 kDa homodimeric peptide from large latent complex. Although physiological mechanisms of latent matrix metalloproteinases (MMPs) and plasmin are suggested as major activators in the body. Thrombospondin-1 and αvβ6 integrin play significant roles in the development of pulmonary and pancreatic fibrosis. We have established a potential role of urokinase type plasminogen activator (uPA)/plasmin system in hepatic fibrosis. Plasmogen activator (uPA) and plasminogen into plasmin. Plasmin activates latent TGF-β in a proteolytic manner. Therefore, plasmin-mediated TGF-β activation occurs in the early stage of hepatic fibrosis. On the other hand, uPA also activates proHGF and plasminogen into plasmin, providing negative feedback systems.

Fig. 3. uPA/plasmin-mediated activation of latent TGF-β and proHGF.

uPA activates proHGF to HGF and plasmin. Plasmin activates latent TGF-β in a proteolytic manner. Active TGF-β suppresses hepatic parenchymal proliferation and stimulates fibrogenesis, whereas HGF inhibits hepatic fibrosis via interfering with the binding of TGF-β to its native receptor. However, those gene therapies still have some difficulties in reaching the clinical setting because of potential toxicity and unwanted side effects.
adverse effects. We are therefore developing alternative approaches using protease inhibitors that block proteolytic activation of latent TGF-β.\(^\text{22}\)

**PDGF**

PDGF is the most potent mitogen for hepatic stellate cells in response to chronic inflammation in the liver.\(^\text{21}\) PDGF is a dimeric polypeptide growth factor, consisting of A- and B-chain. Among the possible dimeric forms of PDGF (-AA, -AB and -BB), PDGF-BB has the strongest mitogenic activity. PDGF-BB binds to PDGF-receptor β that is more abundantly expressed in activated stellate cells than PDGF-receptor α that binds to PDGF-AA.

PDGF receptors have tyrosine kinase activities in the intracellular domain. Upon binding to PDGF, the receptors dimerize and are autophosphorylated at tyrosine residues. Phosphorylated receptors acquire high affinity to several downstream molecules and activate Ras, which subsequently activate ERK (extracellular-signal regulated kinase)/MAPK (mitogen activated protein kinase) pathway. In addition, phosphoinositol 3-kinase (PI 3-K) pathway is also necessary for mitogenic activity of PDGF, which is independent of ERK activation. Moreover, PDGF-stimulation increases both intracellular Ca\(^{2+}\) concentrations and pH, which are required for proliferation of stellate cells. Activated hepatic stellate cells increase both PDGF-regulated Na\(^+\)/H\(^+\) and Na\(^+\)/Ca\(^{2+}\) exchangers.

**TNF-α**

Tumor necrosis factor-α (TNF-α) is a multifunctional cytokine that induces apoptosis while triggers proliferation of hepatic parenchymal cells.\(^\text{22}\) TNF-α as well as interleukin-6 (IL-6) are the major cytokines that promote acute phase response in inflammation. Both induce the production of acute phase proteins by parenchymal cells. In fibrogenesis, IL-6 up-regulates type I procollagen expression, whereas TNF-α down-regulates the expression in hepatic stellate cells. Recently, TNF-α-responsive element (TaRE) of the type I procollagen gene has been colocalized with TGF-β-responsive element (ThRE), which might be an important mechanism of antagonism between TNF-α and TGF-β.\(^\text{26}\)

**Endothelin-1**

One of the characteristic features of activated hepatic stellate cells is the expression of α-smooth muscle actin. This cytoskeletal protein is directly involved in the contractility of stellate cells. Contraction of the cells in vivo may regulate sinusoidal blood flow and might contribute to portal hypertension.\(^\text{23}\)

Endothelin-1 (ET-1) is a key contractile stimulus to stellate cells, and is antagonized by arginine vasopressin, adrenomedullin and eicosanoids. ET-1 also enhances α-smooth muscle actin expression in stellate cells, which provides a positive feedback mechanism.

**Retinoids**

In the quiescent stage, hepatic stellate cells contain a large amount of retinoids (mostly retinyl esters) in the cytoplasmic lipid droplets. Loss of retinoids is a typical feature of stellate cell activation. However, it still remains unresolved whether retinoids prevent or accelerate stellate cell activation.\(^\text{24}\) There are several molecular species of retinoids; retinyl esters are a storage form, retinol is a delivery form that is transported through blood stream, and retinoic acid is an active form that binds to nuclear retinoid receptors and modulates gene transcription. Hepatic stellate cells take up retinyl esters when added in culture, and the cytoplasm is occupied by lipid droplets, showing a quiescent phenotype.\(^\text{25,26}\) On the contrary, when retinoic acid added, it may activate latent TGF-β via inducing urokinase type plasminogen activator (uPA)/plasmin system, and thereby accelerate stellate cell activation.\(^\text{15,16}\) Recently, a novel isomer of retinoic acid, 9, 13-di-cis-retinoic acid, has been identified in the fibrotic liver, and may be synthesized in activated stellate cells.\(^\text{27}\) This isomer of retinoic acid also exerts a fibrogenic effect.

**CONCLUSIONS**

A large body of information is accumulating regarding cellular and molecular mechanisms of liver fibrosis. However, one of the critical issues that remain to be settled is the prevention and therapy of hepatic fibrosis. The only way to treat the fibrosis thus far is using anti-inflammatory or anti-viral drugs such as interferon α and β (IFN α and β).\(^\text{21}\) These agents may indirectly improve fibrosis in some patients. Because there are no direct antifibrotic drugs, many attempts are now under-way to develop effective therapies for hepatic fibrosis, some of which seem promising. These trials include conventional therapies using TJ-9,\(^\text{22}\) antiooxidating agents,\(^\text{24}\) xanthin analogs,\(^\text{36}\) a propryl hydroxylase inhibitor,\(^\text{36}\) and protease inhibitors.\(^\text{21}\) In addition, gene therapies are also possible, employing truncated TGF-β receptor,\(^\text{31}\) soluble TGF-β receptor\(^\text{22}\) and HGF.\(^\text{36}\) The establishment of a new therapy is a goal of the worldwide research on hepatic fibrosis and will hopefully be achieved in the near future.

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