

Conference Paper

MINI REVIEW: LIVER FIBROSIS MECHANISM

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Abstract

Liver fibrosis is the process of wound healing in the liver that caused by a various causes. The longer time of wound healing process, the more severe disorder will be. This chronic condition is characterized by excessive deposition of extracellular matrix then ended with fibrosis. This mini review describes about the phase of liver fibrogenesis. Even though there are many variations of fibrogenesis in animals and human, in general there are three fibrogenic cells that involved in fibrogenesis. These three cells are fibroblast or endogenous fibroblast like-cells, epithelial to mesenchymal transition (EMT), and fibrocytes that derived from bone-marrow.

Keywords: liver, fibrosis, fibroblast, EMT, fibrocytes, collagen.

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Received: 03 October 2017
Accepted: 10 October 2017
Published: 29 November 2017

Publishing services provided
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Selection and Peer-review under the responsibility of the VMIC Conference Committee.

1. Introduction

Wound healing, remodeling, and tissue repair are protective mechanisms that activated in response to stress and/or injury to maintain the integrity of functional organ and body systems. Deregulation of normal healing and exposure to chronic injuries caused fibrosis in tissues and failure of organs function [1].

Liver fibrosis is the process of wound healing due to chronic injuries which is marked by the accumulation of extracellular matrix of proteins especially collagen type I and III, proteoglycan, fibronectin, and also laminin in response to injuries on the liver.

The series of mechanisms that can cause liver fibrosis are the damage of epithelium cells, the release of transforming growth factor- β_1 (TGF- β_1), recruitment of inflammatory cells, induction of reactive oxygen species (ROS) formation, the activation of collagen producing cells, and induction of myofibroblast activation [1].

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2. Epithelial or Endothelial Damages

Vascular damage has an important role on a fibrogenesis process, because it being a trigger for the onset of systemic sclerosis. The systemic sclerosis itself contribute the process of thickening blood vessels, the production of pro-inflammatory cytokines and TGF- β 1, tissue hypoxia, platelets aggregation, decrease the Nitric Oxide (NO) production and caused fibrosis [3, 4]. These vascular damages include the process of vasculogenesis as well as the evidence of apoptotic epithelial cells.

2.1. Vascular Damage and Vasculogenesis

The vascular damage is a critical phase in the fibrosis process. Endothelial cells will secreting many factors that would induce the platelets aggregation, platelets degranulation, blood clot formation, and also accumulation of extracellular matrix. Platelets degranulation will be followed by the release of cytokines that causes dilation so that it will lead to increased permeability of blood vessels and function loss of the barrier. Fibrosis due to vascular damage was caused by damage of blood vessels in the basal membrane and rapid recruitment of inflammatory cells in injury area [5-7].

The recruited macrophages not only phagocytes the products of degradation and cellular debris, but also generate fibrogenic signal and secreted chemotactic factors for endothelial cells. Endothelial cells will subsequently conducted the proliferation and migrated through the basal membrane into the injury center and helped the healing process [4, 8, 9].

In chronic injury, endothelial cells are involved in the process of new formation of blood vessels in condition of tissue damage [8]. Some mediators of angiogenesis are include vascular endothelial growth factor (VEGF), transforming growth factor (TGF- β 1), platelet-derived growth factor (PDGF), tumor necrosis factor- α (TNF- α), interleukin, and fibroblast growth factor (FGF) [10, 11]. Vascular endothelial growth factor as an angiogenesis trigger was in charged to control the ability of endothelial cells to survive and conduct proliferation [4, 12].

2.2. Apoptotic Epithelial Cells

Epithelial and endothelial cells lies to each other, so the injury that occurred in the basal membrane often affected the epithelial cells. The damage on the epithelial and

endothelial cells are also affected on the process of inflammation and fibrogenesis [13, 14].

Epithelial cells that undergone apoptosis secreted the cytokines, which served to recruit and activate macrophages and resulting the transdifferentiation into fibroblast [13]. The activated macrophages are released TGF- β 1 and extracellular ROS, especially H₂O₂, which turned to epithelial cells apoptosis. Hepatocytes released the ROS and fibrogenic factors, such as the chemokine and macrophage protein-inhibitor-2 (MIP-2), which activated the inflammatory cells and collagen producing cells [4].

3. Release of TGF- β 1 as a Main Fibrogenic Cytokines

Hepatocytes that suffered from injury along with Kupffer cells are the cells that produced TGF- β 1. Transforming growth factor- β 1 was needed for the activation of collagen producing cells as hepatic stellate cells (HSCs) type I on the liver fibrosis. Once it activated, HSCs along with sinusoidal endothelial cells produced TGF- β 1 [4].

Transforming growth factor- β 1 was synthesized in an inactive form and crashed intracellularly by furin endopeptidase. The crashed form still remains in an inactive form because still bounded by two proteins, namely latency-associated peptide (LAP) and latent TGF- β 1 binding protein (LTBP). That complex bounded incurred towards the extracellular matrix by transglutaminase tissue and saved as reservoir without any affects to the tissue. To become active, the TGF- β 1 has to be free from that two bounded proteins [15, 16].

Transforming growth factor- β 1 can be activated by some of protease, for example: plasma TGF- β 1 receptor type I (T β R1) or matrix metalloproteinases (MMPs), which are MMP-2 and MMP-9 that directly induced complex degradation TGF- β 1-LAP/LTBP. Although the activation process of latent TGF- β 1 that become operated biologically and occurred through different ways, but the role of TGF- β 1 in fibrogenesis process has been set up (Figure 1) [17].

The active form of TGF- β 1 will provide a biological effects after their specific receptors bonded. There are three receptors of TGF- β 1 i.e. TGF- β receptor type I (T β RI), TGF- β receptor type II (T β RII) dan TGF- β receptor type III (T β RIII) [18]. TGF- β 1 ligand bonded with T β RII will lead to complex formation of the receptor type I and II, where T β RII will undergone the phosphorylation and activated the T β RI with activity of T β RI kinase [19]. Transforming growth factor beta receptor type III has accessory protein, betaglycan and endoglin, that facilitated the attachment of ligands by T β RII receptor ([18, 20, 21].

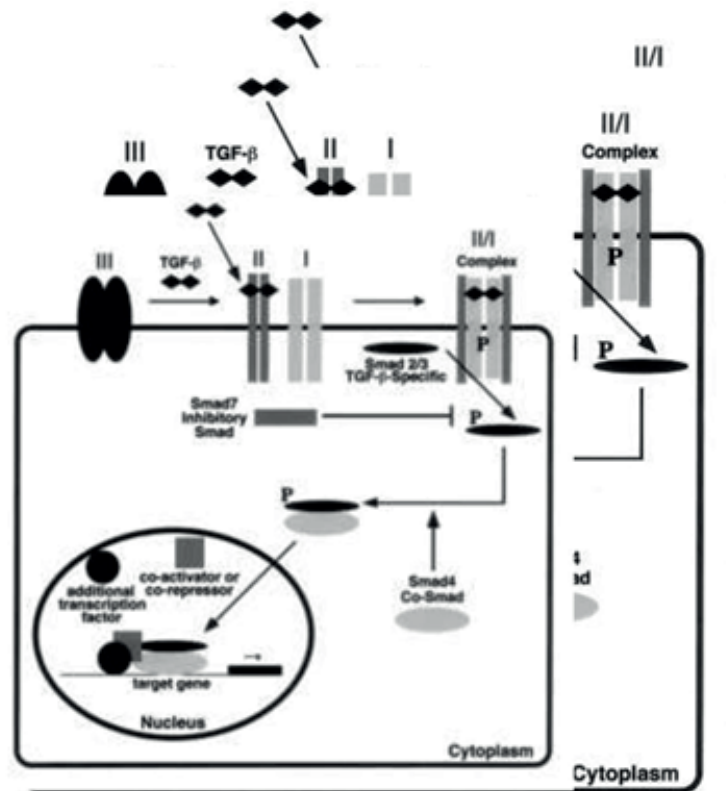


Figure 1: Pathway of Signal Transduction of TGF- β [20]. Note: II = Type II receptor; II/I; A complex of type I and II receptor; P = type II kinase phosphorylates; I = type I receptor; Smad₄ = Co Smad..

The signal of TGF- β ₁ was mediated by Smad and were divided into three groups, which are receptor-regulated Smad (R-smad) consisted of Smad 1, Smad 2, Smad 3, Smad 5 dan Smad 8; common-mediator (co-Smad) which is Smad 4; and antagonistic or inhibitory Smad consisted of Smad 6 dan Smad 7. Smad 3 was proved to have an important role in the fibrogenesis process in the lungs, kidney and liver. The roles of Smad 2 in the fibrosis process did not as much as Smad 3 because of the lethal deletion and Smad 2 regulated different target genes compared with Smad 3 [21, 22].

Complex activation of Smad 2/3 formed a hetero-oligomers with another Smad type, which is Smad 4. Furthermore, Smad 2/3 experienced some translocation into the nucleus and initiated the transcription of the target gene TGF- β ₁.

Smad 7 is an inhibitor of TGF- β ₁ that became a signal of some responses to TGF- β ₁ expression and bonded with T β R₁, thus disrupting the activities of TGF- β ₁ [18, 23].

4. Inflammatory Cells Recruitment

Recruitment of inflammatory cells to the injury areas are the part of wound healing process. In this phase, TGF- β ₁ was a potential chemoattractant to macrophages

and monocytes. Adjacent to TGF- β 1, macrophage inhibitory protein-1 and macrophage inhibitory protein-2 (MIP-1 dan MIP-2) together with monocyte chemotactic protein 1 (MCP-1) also plays a role in that process [24, 25].

Early inflammatory response was occurred through endocytosis or phagocytosis mediated by cytokines. Neutrophils are utilized to clear debris cells and phagocytes the apoptotic bodies. The activated neutrophils are gone through degranulation and released fibrogenic and pro-inflammatory cytokines, submitted to apoptosis. Subsequently, the macrophages infiltrated the tissue's injury and performed phagocytosis and secreted fibrogenic cytokines. T and B lymphocytes are also attracted towards injury areas and has role in facilitating the secretion of fibrogenic cytokines [25, 26].

5. Formation of the Induction of Reactive Oxygen Species (ROS)

ROS in the liver was produced by hepatocytes that undergone injury, Kupffer cells, HSCs and activated neutrophils. Oxidative stress due to increased ROS, such as superoxide, hydrogen peroxide and hydroxyl radical has an important role in fibrosis process. End product of lipid peroxidase, 4-hydroxy-2,3-nonenal (HNE) and 4-hydroxy-2,3-alkenals (HAKs) was able to acted as a potential mediators that affected signal transduction, cell proliferation in liver function [27, 28]. In the ROS presence, the endogenous antioxidant levels will declined and the HAKs has contribution in the fibrosis process [25, 29].

6. Activation of Collagen Producing Cells

Collagen was produced through the activation of various cells such as miofibroblast resident, epithelial to mesenchymal transition and bone marrow derived fibroblast (Figure 2).

6.1. Cell Miofibroblast Resident

Miofibroblast resident derived from specific proliferating tissues and activated in response to injury. These cells are believed to be main source of collagen-producing cells in the lungs, skin, kidneys and liver [30].

In normal physiological circumstances, HSCs was on a quiescent situation and has role to regulate the vitamin A homeostasis [31]. As a response to injury, this formation will transformed both its morphology and function. These transformation include the

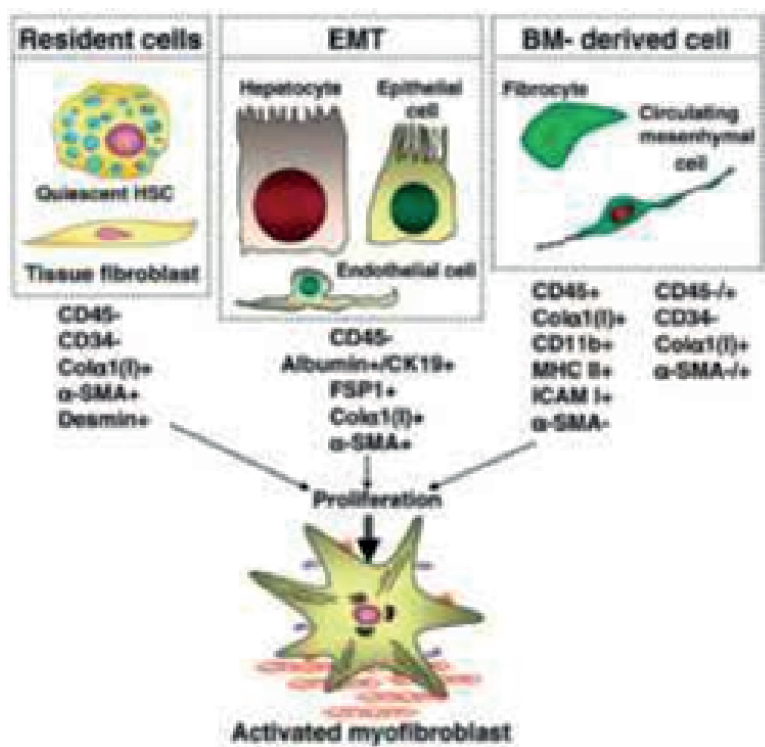


Figure 2: The Origin of Collagen Producing Cells [1] Note: There are three sources of collagen-producing cells, i.e. the endogenous fibroblast or fibroblast-like cells, epithelial to mesenchymal transition and fibrocyte derived from bone marrow. Hepatic stellate cells (HSCs); α -smooth muscle actin (α -SMA); collagen I alpha 1 (Col I α 1); endothelial to mesenchymal transition (EMT); fibroblast-specific protein 1 (FSP-1).

loss of vitamin A, activated miofibroblast, the expression of fibroblast markers (collagen tipe I, α -SMA, desmin and vimentin) dan has ability to phagocyte and turned as antigen presenting cells (APC) [32–34]. Fibroblast portal as a part of the endogenous liver cells population also has an implication on liver fibrogenesis [25, 35].

6.2. Epithelial-to-mesenchymal Transition (EMT)

In response to injury, the epithelium has a role in the fibrosis process of epithelial-to-mesenchymal transition (EMT). Epithelial-to-mesenchymal transition is a process when the epithelial cells differentiated completely in phenotypes become mesenchyme cell [36]. Some of the important process in EMT are basal membranes disruption, loss of epithelial cells adhesion, synthesis of α -SMA, the formation of cytoskeletal protein and transmigration of epithelial cells through interstitial spaces [37]. The basal membrane damages due to activity loss of MMPs and released growth factors that accelerated the EMT [38, 39].

The epithelium cells normally located along the basal membrane and bound to each other through intercellular adhesion molecule named E-cadherin. In response to injury,

epithelial cells that gone through the activation and separated the basal membrane. After that, the cells will start to secrete cytokines then stimulating the process of EMT [38]. Epithelial cells will elude an expression of epithelial markers, E-cadherin and zonula occludens-1 (ZO-1) and changed to polygonal form and apical-basal polarity become elongated. The epithelial cells altered to resemble fibroblasts and will start expressing fibroblast-specific protein (FSP-1), extracellular matrix proteins (fibronectin, collagen type I and III) and α -SMA. The cells have the ability on high contractility and will migrated through the basement membrane to the interstitial tissue, then they will power a proliferation. This process causes an increase in the number of miofibroblas that will secrete collagen [37, 40].

6.3. Fibrocyte And Bone Marrow Derived Fibroblast

Fibrocyte released cytokines and growth factors, such as TGF- β 1 and monocyte chemotactic protein-1 (MCP-1), which plays a role in the process of deposition of extracellular matrix in the area under the injury [41]. Fibrocyte also expressed a number of chemokine receptors, ie. cys-cys receptor 2, 3, 5, 7 (CCR2, CCR3, CCR5, CCR7) and cys-X-cys receptor 4 (CXCR4). Migration of fibrocyte on the organ that resulted to injury requiring interaction between chemokine and its receptor [42, 43].

Bone marrow-derived cell (BM-derived cell) have been shown to have any contribution in the process of fibrosis [8]. On the liver injury which is caused by intoxication CCl₄, BM-derived collagen, expressing the myofibroblast α -smooth muscle actine (α -SMA), desmin and vimentin as a sign of the onset of fibrosis [44, 45].

7. Induced Activation of Miofibroblast by Matrix

Depending on the composition of the extracellular matrix, fibroblasts can be in a secured position as fibroblasts or activated turned into miofibroblast. With the presence of laminin, which is an important component of the basement membrane, it will be in a state quiescence fibroblasts. Instead of stress, fibroblasts caused of abnormal matrix extracellular, it will proliferated and changed the phenotype into miofibroblast. Increased of abnormal extracellular matrix deposition will accelerated the activation of collagen-producing cells [46, 47].

In response to presence of injury, there will be an alteration in the extracellular matrix composition that have numerous fibrillar collagen, promotes acceleration of the activation process of HSCs [48].

8. Conclusion Remark

The liver fibrosis is a very complex processes. The knowledge of the liver fibrosis can increase the awareness of fibrosis that occurred in the liver that can also developed in other organs. Therefore, it is required to collaborate the several science disciplines from traditional to modern methods to understand the process and how to overcome this problem. Several science disciplines might include biology, immunology, pathology, biochemistry, pharmacology, toxicology, transgenic technology, and others.

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