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New Concepts on Pathogenesis and Diagnosis of Liver Fibrosis; A Review Article

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ABSTRACT

Liver fibrosis is a potentially reversible response to hepatic insults, triggered by different chronic diseases most importantly viral hepatitis, alcoholic, and non-alcoholic fatty liver disease. In the course of the chronic liver disease, hepatic fibrogenesis may develop, which is attributed to various types of cells, molecules, and pathways. Activated hepatic stellate cell (HSC), the primary source of extracellular matrix (ECM), is fundamental in pathophysiology of fibrogenesis, and thus is the most attractable target for reversing liver fibrosis. Although, liver biopsy has long been considered as the gold standard for diagnosis and staging of hepatic fibrosis, assessing progression and regression by biopsy is hampered by its limitations. We provide recent views on noninvasive approaches including serum biomarkers and radiologic techniques.

KEYWORDS
Liver Cirrhosis; Fibrosis; Pathogenesis; Diagnosis; Therapeutics; Genetic Therapy

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INTRODUCTION

Liver fibrosis is in fact a healing response to liver injury and is characterized by excessive deposition of extracellular matrix (ECM) proteins as a result of different chronic liver diseases including viral hepatitis, alcoholic or non-alcoholic steatohepatitis.[1-3] Liver fibrosis is beneficial at first because it can encapsulate the injury, and is considered a reversible process at this stage [4-8], but ultimately progresses to advanced fibrosis or cirrhosis, that might be irreversible and causes impaired liver function and subsequent morbidity and mortality.[9]

Cirrhosis may cause serious complications such as hepatocellular carcinoma or bleeding from esophageal varices and invariably leads to death.[10] In the United States alone, cirrhosis is one of the leading causes of death and results in a significant burden as high as $2 billion.[11,12] According to global burden of disease (GBD) study in 2013, cirrhosis is among the 10 most common causes of death in different world areas and the 6th cause of death in developed countries.[13] The burden is expected to rise in the forthcoming years due to increasing prevalence of cirrhotic cases related to non-alcoholic steatohepatitis (NASH) and hepatitis C virus (HCV) infection.[14,15] Thus, cirrhosis and liver transplantation is expected to be among important challenges in the 21st century.[10]

Major advances have occurred in the field of liver fibrosis in recent years. Development of non-invasive strategies to detect liver fibrosis has

enabled clinicians to diagnose at-risk patients rapidly and readily.[16]

The aim of this paper is to review recent achievements in pathogenesis and diagnostic evaluation of liver fibrosis.

Pathogenesis

Liver fibrogenesis is a dynamic interaction between cellular and molecular processes. Although different diseases might result in liver fibrosis, there are common features.[17] Defining mechanisms that contribute to liver fibrosis in each will direct future research toward discovery of therapeutic targets.[18]

Hepatic fibrosis can be regarded as the result of an imbalance between ECM synthesis and degeneration. The balance between matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinase (TIMPs) is crucial for ECM homeostasis.

Cell types

Different populations of cells play roles in fibrogenesis, but activation of hepatic stellate cells (HSCs) is an essential factor in fibrogenesis.[19,20]

Liver myofibroblasts (MF) include a heterogeneous population of highly proliferative cells that accumulate at injury sites and promote ECM accumulation.[21] Myofibroblast pool originates mainly from liver mesenchymal cells, namely HSCs.[22] Although HSCs are the primary source of MFs in liver fibrosis, extrahepatic precursors such as bone marrow derived mesenchymal cells and portal fibroblasts contribute in ECM synthesis.[23-26] Other minor sources of MFs are cells that undergo epithelial-mesenchymal transition (EMT).[27] However, some recent findings raise doubt about potential epithelial origin of ECM-producing cells.[28-30]

Hepatic stellate cells (HSC)

Hepatic stellate cells (HSCs) are one of the non-parenchymal cells of the liver, residing adjacent to sinusoids, in the space of Disse. In liver tissues, HSCs store retinoids such as vitamin A and produce glial fibrillary acidic protein (GFAP), reasoning their former names fat-storing cells or vitamin A-rich cells.[31-35] Since trans-differentiations are seen among HSCs in some pathologic conditions, it has been speculated that HSCs may originate from mesoderm-derived multipotent mesenchymal progenitor cells (MMPCs). These progenitor cells generate several neural cell lineages as well as other mesenchymal cells.[36-38]

Autocrine and paracrine secretion of fibrogenic cytokines as a result of pathologic insults to liver cells, promote HSCs to trans-differentiate, changing from a quiescent phenotypes to an activated myofibroblastic state. These fibrogenic cytokines include tumor necrosis factor α (TNF-α), transforming growth factor β (TGF-β), interleukin 1 (IL-1), and platelet-derived growth factor (PDGF).[39-43] Activated HSCs express fibrogenic proteins and α-smooth muscle actin (α-SMA) and lose their lipid and retinoid storages, transitioning from an adipogenic state to a fibrogenic, chemotactic, and mitogenic one.[44,45]

Hepatocytes

Hepatocytes, the parenchymal component of liver, are the main target of hepatitis viruses, alcohol toxicity, steatosis and other hepatotoxic agents. Chronic liver injury promotes hepatocyte apoptosis through TNF-α related apoptosis inducing ligand (TRAIL) and Fas.[46,47] Some reports have shown that hepatocyte-derived apoptotic bodies stimulate secretion of fibrogenic cytokines from macrophages and also promote HSCs activation via interaction of toll-like receptor 9 (TLR9) with DNA, which is released from apoptotic hepatocytes.[48-51] On the other hand, Jiang JX et al. showed that activated HSCs also can act as phagocytes and phagocytize hepatocyte apoptotic bodies which promotes myofibroblasts survival and fibrogenesis.[52] Hypoxic hepatocytes in cirrhotic stages are primary sources for secretion of TGF-β, which may augment liver fibrosis.[53]

Hepatocyte telomerase shortening independent of age, can promote scarring and progression of fibrosis.[54] Studies on telomerase-deficient mice revealed that shortened telomeres due to chronic liver injury are associated with impaired liver regeneration and accelerated fibrosis development.[55]

Liver sinusoidal endothelial cells (LSECs)

The prominent characteristic of liver sinusoidal endothelial cells (LSECs), also known as endothelium, in the normal liver is their fenestrae which control exchange of fluids and particles between hepatocytes and sinusoidal blood.[56] The fenestrated endothelial cells also suppress activation of HSCs through vascular endothelial growth factor (VEGF) stimulated nitric oxide (NO) production.[57]

Upon liver injuries, defenestration and capillarization of LSECs result in disturbances in substrate exchange, the main cause of liver dysfunction in fibrosis.[58] Moreover, capillarized LSECs leads to HSCs activation.[59,60]

Kupffer cells

Kupffer cells (KCs), also called stellate macrophages, are usually activated by multiple injuries such as viral hepatitis and alcohol consumption.[61] Activation of KCs is a key step in initiation and preservation of fibrosis. Activated forms of these cells express chemokine receptors, secret inflammatory cytokines and act as antigen presenting cells in viral hepatitis, which lead to progression of liver fibrosis.[62-64] KCs activate HSCs, and are observed to be engulfing apoptotic particles, which result in fibrogenesis.[65]

Role of cytokines

Liver fibrosis is a consequence of interaction of a complex network of cytokines, which modify activities of circulating immune cells, HSCs, KCs, LSECs, and hepatocytes. In Table 1, we have listed the groups of cytokines which enhance or alleviate liver fibrogenesis. However, the effect of cytokines may differ in pathogenesis of different liver diseases.

Pathogenesis of fibrosis in specific liver diseases

Viral hepatitis

Cell death markers such as cytokeratin 18 and alanine aminotransferase (ALT) are found to correlate with fibrosis stage, so hepatocyte death triggered by hepatotropic viruses is considered an essential step in progression of fibrosis in viral hepatitis.[107,108]

Hepatitis B virus (HBV) viral protein, HBx, induces paracrine activation and proliferation of HSCs and enhances the expression of TGF-β, α-SMA and collagen type I .[109]

Nonstructural hepatitis C virus (HCV) proteins, such as NS3 and NS5 may directly activate intracellular pathways within HSCs and promote their activation. HCV infection also causes mitochondrial dysfunction and oxidative stress in hepatocytes.[110] Chronic infection with this virus leads to cell cycle arrest in hepatocyte in G1 stage and inhibits hepatocytes regeneration.[1,111]

-Alcoholic liver disease (ALD)

Liver fibrosis in chronic exposure to ethanol is caused by generation of acetaldehyde. Acetaldehyde stimulates production of TGF-β, TNF-α, IL-1, reactive oxygen species (ROS), and collagen type I .[112] Cytochrome P450 (in particular CYP2E1) has an essential role in production of ROS.[113]

Excessive ethanol consumption leads to reduction of glutathione mediated protection of hepatocytes against ROS and induces hepatocyte apoptosis through downregulation of Bcl-2 signaling pathway.[114,115] Ethanol induces fibrogenic activity of HSCs by inhibition of autophagy, which results in endoplasmic reticulum stresses and inhibits natural killer (NK) cells' anti-fibrogenic functions by enhancing production of TGF-β by HSCs.[116]

Several studies have demonstrated that level of circulating lipopolysaccharide (LPS) is significantly higher in alcohol consumers. LPS promotes activation of TLR4 signaling pathway in HSCs and LSECs leading to liver fibrosis.[117,118]

Non-alcoholic fatty liver disease (NAFLD)

Fibrosis progression in non-alcoholic steatohepatitis (NASH) can be caused by several triggers, some to mention are: 1: oxidative stresses by increased ROS production due to excess accumulation of lipids in the hepatocyte [119]; 2: induction of inflammatory states through release of proinflammatory cytokines from adipose tissue[120]; and 3: activation of TLRs signaling pathway, including TLR2, TLR4, TLR5, and

Table 1: **Role of cytokines involved in regulation of liver fibrosis**

Mediators	Mechanism of action	Reference(s)
Growth Factors		
Platelet-derived growth factor (PDGF)	Stimulates activation and proliferation of HSCs Upregulates expression of TIMP-1 Inhibits collagenase activity	39,66-69
Transforming growth factor-β (TGF-β)	Stimulates activation of HSCs Upregulates type I collagen and α-SMA synthesis Promotes MFs survival through activation of FAK Inhibits DNA synthesis and induces hepatocytes apoptosis Upregulates expression of TIMPs Increases LOXs production	43,70-75
Interleukins		
Interleukin-1 (IL-1)	Activates HSCs and promotes HSCs survival through NF-κB Promotes lipid accumulation in NAFLD Promotes type I collagen synthesis Upregulates TIMP-1	41,42,76-78
Tumor necrosis factor-α (TNF-α)	Promotes activation of HSCs and reduces apoptosis of activated HSCs Upregulates TIMP-1 Stimulates hepatocyte apoptosis	77, 79-83
Interleukin-17 (IL-17)	Upregulates type I collagen, TGF-β, and TNF-α through STAT3 pathway Promotes activation of HSCs	84-86
Interleukin-10 (IL-10)	Inhibits HSCs activity Inhibits expression of TIMP-1 and TGF-β	87-89
Interleukin-22 (IL-22)	Promotes HSCs senescence	90, 91
Interleukin-6 (IL-6)	Attenuates hepatocytes apoptosis and induce regeneration of hepatocytes through NF-κB Induces insulin resistance	92, 93
Interferon		
Interferon-α (IFN-α)	Has an anti-apoptotic effect on activated HSCs	94
Interferon-β (IFN-β)	Inactivates HSCs and decrease production of type I collagen and α-SMA through inhibition of PDGF and TGF-β	94-97
Interferon-γ (IFN-γ)	Inhibits activation of HSCs and reduce type I collagen deposition Induces hepatic HSCs apoptosis and cell cycle arrest	95-97
Chemokine		
CCL2	Promotes migration of bone marrow-derived monocyte to liver Activates HSCs Induces insulin resistance in NAFLD	98-103
CXCL10	Promotes hepatocytes apoptosis Inhibits NK cells-mediated HSCs inactivation Stimulates T-cell chemotaxis to the liver	104, 105
CXCL16	Promotes intrahepatic accumulation of NKT cells	106

HSC: hepatic stellate cell; NF-κB: nuclear factor-κB; NAFLD: non-alcoholic fatty liver disease; TIMP: tissue inhibitors of metalloproteinase; STAT: signal transducer and activator of transcription; SMA: smooth muscle actin; NKT: natural killer T cell; FAK: focal adhesion kinase; LOX: lysyl oxidase; CXCL: chemokine (C-X-C motif) ligand; CCL: chemokine (C-C motif) ligand

TLR9, in KCs and HSCs. As a result of downstream signaling pathways, KCs produce CCL2 that recruits LY6Chi monocytes into the liver. LY6Chi monocytes produce IL-1 and TNF-α and intensify NASH-related

fibrosis .[78, 99,121,122]

Studies in animal models have shown that in non-alcoholic steatohepatitis, hepatic stellate cells are increasingly activated. Moreover, association between decreased insulin sensitivity and advanced liver fibrosis in NAFLD, suggests that insulin resistance besides high fructose and sodium consumption play role in HSCs activation and development of fibrosis.[123-125] Recently, it has been suggested that free cholesterol accumulation in HSCs as a consequence of Acyl-coenzyme A: cholesterol acyltransferase 1 (ACAT1) deficiency enhances TLR4 signaling and liver fibrosis in the mouse model of NASH.[126]

Diagnosis of liver fibrosis

Defining the disease state is essential for deciding on therapeutic choices and predicting prognosis. Although liver biopsy is considered the standard reference for assessing liver fibrosis, it has important limitations including invasiveness[127]; hence, the need is growing for alternative accurate and noninvasive methods for diagnosis and staging of hepatic fibrosis. Over the last few decades, non-invasive approaches to diagnosis of liver fibrosis have been developed, which overcome some limitations of liver biopsy.

Liver biopsy

Apart from being invasive, liver biopsy has other limitations including sampling error as well as intra- and inter-observer variability. Since fibrosis is heterogeneous in distribution and samples taken by biopsy correspond to roughly 1/50,000 of hepatic parenchymal mass, the tissue taken might not represent the major liver pathology.[128]

Based on the underlying fibrogenic factor, liver fibrosis could have diverse patterns, and thus histologic examination is important in distinguishing between different causes of liver fibrosis. Generally, histopathological assessments can show necro-inflammatory activity (grade) and fibrosis stage of chronic hepatitis. There are different scoring systems for staging liver fibrosis such as METAVIR score, the most widely used scoring system for interpretation of fibrosis stage, histology activity index (HAI) proposed by Knodell in 1981, and modified HAI of Ishak et al.[129-131]

Recently pathologists have developed a new approach, named "morphometry", which can be used to quantify fibrosis by measurement of collagen proportional area (CPA).[132,133] Though previous fibrosis scores are semi-quantitative and cannot be treated as linear values with statistical tests, CPA enables linear assessment of the amount of fibrosis.[134]

Immunohistochemical analysis of cellular markers such as cytokeratin 7 as a marker of ductular proliferation, α-SMA as a marker of HSCs activation, and CD34 as a marker of LSEC capillarization provides functional and dynamic information about fibrogenesis.[135,136]

Laboratory tests

In search of noninvasive markers of fibrosis, a constellation of biochemical and hematological serum markers have been suggested as predictors of liver fibrosis. Serum markers of fibrosis are classified as direct and indirect markers. Direct biomarkers measure components of the hepatic ECM as well as the enzymes, which regulate the matrix; they include MMPs, subtypes of collagen, and hyaluronic acid among others.[137,138] Indirect markers refer to parameters such as platelet count which might reflect hypersplenism due to portal hypertension, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) as markers of liver cell inflammation, cytokeratin 18 as an indicator of hepatocyte apoptosis, and international normalized ratio (INR) as an index of hepatocyte malfunction. [107,139] Various combinations of these markers are used in the diagnosis of hepatic fibrosis.[140] Combination of serum markers with clinical findings and defining new measures may improve accuracy of liver fibrosis prediction. Some of these predictors are presented in table 2.

Imaging modalities

Imaging diagnostic modalities, especially ultrasound (US), have played significant role in chronic

Table 2: **Selected biomarkers for assessment of liver fibrosis**

Biomarkers	Components	Reference(s)
AST to Platelet Ratio Index (APRI)	AST/platelet count	139
BARD score	BMI, AST/ALT ratio, diabetes	141
Enhanced Liver Fibrosis Score (ELF)	Age, hyaluronic acid, TIMP-1 level, N-terminal propeptide of type I collagen	142
FIB-4	AST, ALT, age, platelet count	143
Fibrometer	Platelet count, prothrombin index, AST, α2-macroglobulin, hyaluronate, urea, age	144
Fibrotest	α2-macroglobulin, haptoglobin, apolipoprotein A1, γGT, total bilirubin, age, gender	145
Hepascore	Bilirubin, α2-macroglobulin, hyaluronate, γGT, age, gender	146
NAFLD Fibrosis Score (NFS)	Age, IFG/diabetes, BMI, platelet count, albumin, AST/ALT ratio	147

AST: aspartate aminotransferase; ALT: alanine aminotransferase; BMI: body mass index; γGT: gamma glutamyl transferase; IFG: impaired fasting glucose

liver disease management. In addition to US, magnetic resonance imaging (MRI), computed tomography (CT), and other modalities derived from these basic methods have enabled accurate estimation of liver fibrosis and findings associated with portal hypertension.

Liver stiffness (LS) results from fibrotic tissue deposition and liver inflammation, which associates with staging of liver fibrosis.[148] Liver stiffness can be measured by using principles of elastography, which are based on propagation of mechanical shear wave in tissues. Shear waves propagate more rapidly in stiffer tissue corresponding to advanced fibrosis. Here we review US- and MR-based LS measurement.

US-based LS measurement: transient elastography (TE)

Transient elastography (TE; Fibroscan), the first commercialized elastography technique, was introduced in 2003.[149] Transient elastography calculates LS and displays the value as the median of 10 validated measurements in kilopascals (kPa) and closely correlates with the stage of liver fibrosis. The technique is noninvasive, replicable, fast, and has high inter- and intra-observer repeatability; however it has limitations, including probable sampling errors, since it assesses approximately 1/100 liver parenchyma, confounding effect of a meal on its accuracy, and measurement failure in presence of ascites and severe obesity. Therefore, its accuracy decreases in patients with NAFLD many of whom are obese.[150,151]

MR-based LS measurement: magnetic resonance elastography (MRE)

Magnetic resonance elastography has been widely evaluated and demonstrated as a precise modality with high diagnostic performance for detection of advanced fibrosis.[152] Recently, a meta-analysis by Singh et al, that included 12 retrospective studies, identified the area under receiver-operating curve (AUROC) value of MRE as 0.84 for diagnosis of any fibrosis (stage 1), 0.88 for significant fibrosis (stage 2), 0.93 for advanced fibrosis (stage 3), 0.92 for cirrhosis (stage 4), and the overall failure rate of MRE as 4.3%.153 Moreover, three-dimensional (3D)-MRE, a newly emerging technique, has been introduced with higher diagnostic accuracy under some circumstances than two-dimensional (2D)-MRE.[154]

MRE might be time consuming and expensive, but has the ability to analyze the entire liver tissue and is not affected by severe obesity and presence of ascites.[155]

CONCLUSION

New findings have revealed novel cellular and molecular pathways in the development of liver fibrosis. These achievements are the basic step in determination of biological targets and development of pharma-

cologic agents to stop the process of fibrogenesis and reverse it towards less stages of liver fibrosis. As the concept of liver fibrosis reversibility becomes more at hand, there is a need for better diagnostic tools for evaluating the severity of liver fibrosis in order to evaluate the effectiveness of novel interventions. Advanced imaging techniques are being developed in this regard and there is much hope to see significant steps forward in the area of research on liver fibrosis in coming years.

CONFLICT OF INTEREST

The authors declare no conflict of interest related to this work.

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