UNRAVELLING THE PATHOGENESIS OF LIVER FIBROSIS INSIGHTS, MECHANISMS, AND THERAPEUTIC STRATEGIES

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ABSTARCT

Liver fibrosis represents a complex and dynamic process characterized by the excessive accumulation of extracellular matrix proteins, leading to liver dysfunction and eventual cirrhosis. This review aims to elucidate the intricate pathogenesis of liver fibrosis, offering insights into the underlying mechanisms and discussing emerging therapeutic strategies. The development of liver fibrosis involves a cascade of events triggered by chronic liver injury, inflammation, and the activation of hepatic stellate cells (HSCs). Various cellular and molecular pathways, including transforming growth factor-beta (TGF-β) signalling, oxidative stress, and inflammatory cytokines, contribute to the initiation and progression of fibrosis. Moreover, recent studies have highlighted the role of non-coding RNAs, epigenetic modifications, and the gut-liver axis in modulating fibrogenesis. Understanding the dynamic interplay between different cell types within the liver microenvironment is crucial for developing targeted therapies. Promising therapeutic approaches include antifibrotic agents targeting HSC activation and collagen synthesis, immunomodulatory strategies to attenuate inflammation, and interventions aimed at restoring metabolic homeostasis. Furthermore, advancements in liver imaging techniques and non-invasive biomarkers have facilitated early detection and monitoring of fibrosis progression, enabling timely intervention and personalized treatment strategies. However, challenges remain in translating preclinical findings into effective clinical therapies, emphasizing the need for multidisciplinary collaborations and innovative drug development

platforms. In conclusion, this review provides a comprehensive overview of the pathogenesis of liver fibrosis, highlighting key molecular mechanisms and discussing current and emerging therapeutic avenues. By elucidating the underlying processes driving fibrogenesis, we aim to pave the way for the development of more efficacious and targeted interventions, ultimately improving patient outcomes in liver fibrosis management.

KEYWORD: Liver fibrosis, Pathogenesis, Mechanisms, Hepatic stellate cells, Extracellular matrix, Inflammation, Transforming growth factor-beta, Oxidative stress, non-coding RNAs, Epigenetics, Gut-liver axis, Therapeutic strategies, Antifibrotic agents, Immunomodulation, Biomarkers, Personalized medicine.

INTRODUCTION

Chronic liver disorders pose a formidable challenge to global health, contributing to over 2 million deaths annually worldwide. These conditions stem from various underlying causes, including alcoholic steatohepatitis (ASH), viral chronic liver diseases such as hepatitis B (HBV) and hepatitis C (HCV), immunological and genetic disorders, and non-alcoholic steatohepatitis (NASH). Characterized by progressive organ fibrosis, chronic inflammatory disorders of the liver represent a significant portion, accounting for 45% of global all-cause mortality. The relentless advancement of fibrosis underscores the critical need for effective strategies in diagnosis, treatment, and prevention to mitigate the burden of chronic liver diseases and improve public health outcomes globally. The prognosis and quality of life for individuals with liver-related issues are significantly impacted by the progression of fibrosis in the liver. The degree of fibrosis plays a crucial role as a key risk factor for the development of hepatocellular carcinoma (HCC) and is closely associated with overall liver function. Moreover, chronic portal hypertension, primarily induced by liver fibrosis, gives rise to various clinical complications such as haemoptysis, haemostatic decompensation, and hepatic encephalopathy. Consequently, liver cirrhosis stands as the fourth leading cause of mortality among adults in central Europe and ranks as the 11th most common cause of death globally. The prevalence of hypertension resulting from liver fibrosis underscores the critical importance of addressing and managing fibrotic progression to mitigate severe complications and improve patient outcomes. Efforts aimed at understanding and intervening in the fibrotic process hold significant promise in addressing the broader impact of liver-related disorders on public health. Liver fibrosis, characterized by progressive extracellular matrix (ECM) buildup, poses a significant challenge to the liver's physiological architecture. Various

pathogenic factors such as toxic, metabolic, or viral insults lead to hepatocyte destruction and immune cell infiltration, triggering the differentiation of hepatic stellate cells (HSCs) into myofibroblasts, which in turn produce collagen. Under normal circumstances, after initial tissue damage, there is a balanced process of tissue regeneration and inhibition of anti-fibrotic pathways, leading to the clearance of myofibroblasts and resolution of scarring. However, in chronic liver diseases, an imbalance between pro- and anti-fibrogenic pathways perpetuates the activation of myofibroblasts. These myofibroblasts become persistent, exhibiting migratory, contractile, and proliferative characteristics, ultimately leading to the excessive deposition of ECM and the progression of fibrosis. This chronic activation of myofibroblasts underscores the importance of understanding and targeting the intricate molecular mechanisms involved in liver fibrogenesis to develop effective therapeutic interventions **(Roehlen** *et al.,* **2020).**

Non-parenchymal cells (NPCs), such as Kupffer cells and various immune cells, play a pivotal role in steering the liver toward either an unrestrained fibrosis-promoting state or an anti-fibrotic scar-dissolving stage. This fate is influenced not only by their direct activation of Hepatic Stellate Cells (HSCs) but also by the process of hepatocyte apoptosis and the release of damage-associated patterns (DAMPs), which attract and activate macrophages and lymphocytes. These immune cells, in turn, foster HSC trans-differentiation and myofibroblast activation by generating pro-inflammatory and pro-fibrogenic cytokines. Certain macrophage subpopulations express matrix-metalloproteinases (MMPs), which contribute to the resolution of fibrosis. The regulation of pro-fibrogenic cell interactions involves a complex network of signalling pathways triggered by cytokines at the molecular level. Notably, the caspase1-NLRP3-inflammasome pathway, platelet-derived growth factor (PDGF), and transforming growth factor-beta (TGF-β) are key players in this intricate process. Additionally, the WNT/β-catenin signalling pathway and the inflammasome (NLRP3)-Caspase1 pathway have been identified as significant signalling cascades linked to HSC activation and the progression of fibrosis. **(Elpek** *et al.,* **2014).**
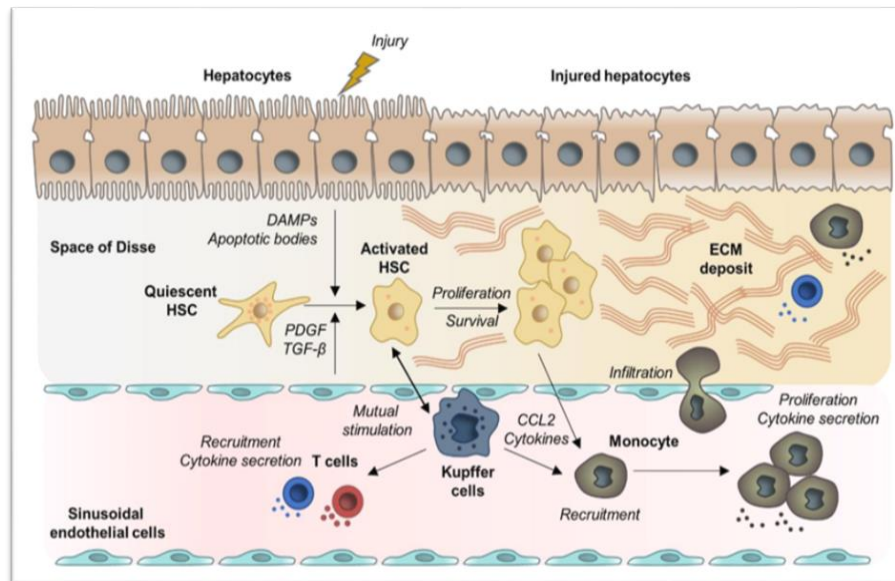
Figure 1: depicts the broad spectrum of etiology-independent cell interactions contributing to the development of fibrosis.

Figure 1. Examples for mechanistic concepts for liver fibrosis. Chronic hepatocyte injury causes release of damage-associated patterns (DAMPs) and apoptotic bodies that activate Hepatic stellate cells (HSCs) and recruit immune cells. Complex multidirectional interactions between activated HSCs and Kuper Figure 1. Examples for mechanistic concepts for liver fibrosis. Chronic hepatocyte injury causes release of damage-associated patterns (DAMPs) and apoptotic bodies that activate Hepatic stellate cells (HSCs) and recruit immune cells. Complex multidirectional interactions between activated HSCs and Kupffer cells, as well as innate immune cells promote trans-differentiation into proliferative and extracellular matrix (ECM) producing myofibroblasts. Abbreviations: PDGF: Platelet Derived Growth Factor; TGF-β: Transforming Growth Factor Beta; CCL2: chemokine (C-C motif) ligand 2. cells, as well as innate immune cells promote trans-di erentiation into proliferative and extracellular matrix (ECM)producing myofibroblasts. Abbreviations: PDGF: Platelet Derived Growth Factor; TGF-: Transforming Growth Factor Beta; CCL2: chemokine (C-C motif) ligand 2 **(Nishikawa *et al.*, 2018).**

PATHOGENESIS OF LIVER FIBROSIS

When extracellular matrix (ECM) accumulates within the Disse space, the endothelial lining's characteristic fenestrations diminish. This alteration hampers the typical bidirectional metabolic exchange between hepatocytes and portal venous flow, a phenomenon termed as capillarization of the sinusoids. According to the Hernandez-Gea etiology, the site of injury

(e.g., portal or central), the origin of fibrogenic cells implicated, and the principal fibro genic mechanisms, diverse patterns of fibrosis progression have been delineated (refer to Figure 2).
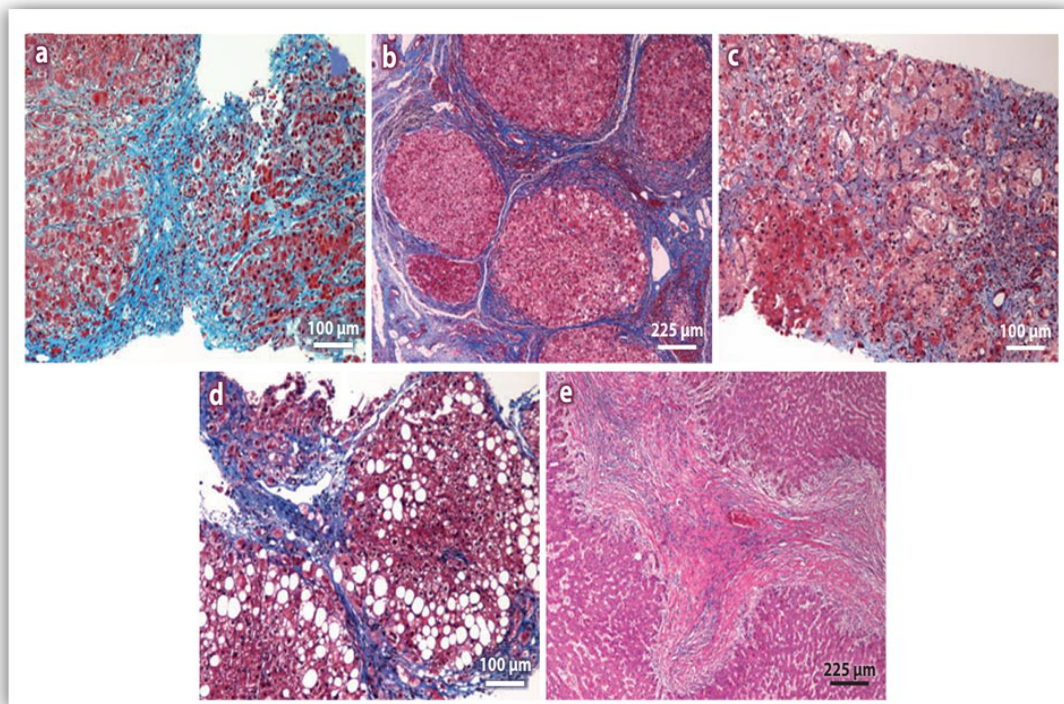
Figure-2 Photomicrographs illustrating various fibrosis patterns across different etiologies of liver disease are as follows: (a) Autoimmune hepatitis: Portal-central vein bridging necrosis. (b) Chronic viral hepatitis C: Trichrome staining reveals portal-central fibrotic septa and nodule formation. (c) Acute alcoholic hepatitis: Deposition of extracellular matrix around hepatocytes (referred to as the "chicken-wire pattern") and ballooning degeneration of hepatocytes. (d) Non-alcoholic steatohepatitis: Trichrome staining highlights macrovesicular steatosis and pericellular fibrosis. (e) Biliary cirrhosis: Portal-portal fibrotic septa and proliferation of bile ductulus. These images are reproduced with courtesy from Dr. M. Isabel Fiel, Mount Sinai School of Medicine (Annu *et al.,* 2011).

Bridging fibrosis primarily occurs due to chronic viral hepatitis B and C. Fibrosis, characterized by the formation of fibrotic septa between the portal and central areas, results from interface hepatitis and necrosis bridging between portal and central veins. Perisinusoidal or pericellular fibrosis is commonly seen in conditions related to alcohol use, such as non-alcoholic fatty liver disease. Alcohol-related fibrosis is distinguished by the deposition of extracellular matrix (ECM) in the space around sinusoids or hepatocytes, forming a "chicken-wire pattern." In biliary fibrosis, myofibroblasts (MF) around bile ductules proliferate,

leading to the formation of fibrotic septa around liver nodules. Centrilobular fibrosis, characterized by fibrotic septa in the central area, is primarily caused by conditions affecting venous outflow. Cirrhosis, a condition marked by persistent fibrogenesis, is not merely the final stage of scar buildup but a complex progression of liver disease. This development involves significant vascular architecture alterations and parenchymal deformations. The primary pathological hallmark of cirrhosis is the formation of regenerating parenchymal nodules encircled by fibrotic septae. In cases where these nodules are particularly large, they may encompass both portal tracts and terminal hepatic venules, resulting in macronodular cirrhosis. Portal hypertension emerges due to frequent venous blockages and the presence of porto-systemic shunts, compromising liver function. Angiogenesis, the formation of new blood vessels, plays a crucial role in the development of vascularized fibrous septa. These septa connect portal tracts and central veins, facilitating porto-systemic shunting. This process diverts blood flow away from the liver parenchyma, contributing to the progression of cirrhosis. (Figure-3).
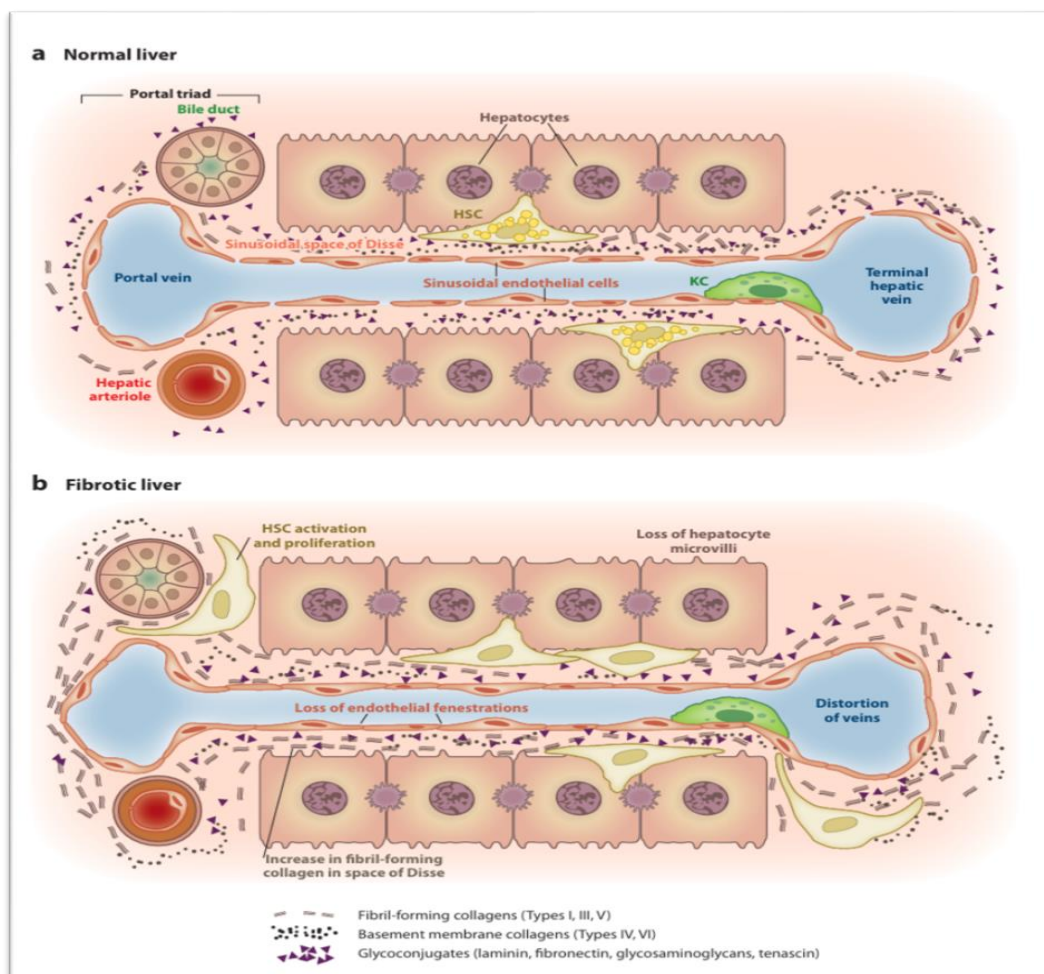
Figure 3: Represent the Normal Liver and Fibrotic Liver.

MECHANISM OF LIVER FIBROSIS

Activation of the hepatic stellate cell

In hepatic fibrosis, the primary cellular entity responsible for the excessive synthesis of collagen in the liver is the hepatic stellate cell (HSC), also referred to as the Ito cell. The HSC undergoes a complex process of activation following liver injury, transitioning from a quiescent state characterized by vitamin A storage to an activated state resembling myofibroblasts. This metamorphosis involves intricate molecular and cellular changes that drive the HSC towards a profibrogenic phenotype, contributing significantly to the progression of hepatic fibrosis. During the activation of hepatic stellate cells (HSCs), several morphological changes occur, signalling their transition to a more active state. One significant transformation is the emergence of the cytoskeletal protein smooth muscle alpha-actin (α-SMA), which reflects the increased contractility and fibrogenic potential of the activated HSCs. Concurrently, there is a decrease in the amount of stored vitamin A within the cells, a hallmark characteristic of quiescent HSCs. Additionally, activated HSCs exhibit an increase in the rough endoplasmic reticulum, indicating heightened protein synthesis and secretory activity. Following HSC activation, there is a notable metabolic surge characterized by enhanced DNA synthesis and cellular proliferation. This increased cellular activity contributes to the progression of hepatic fibrosis, a key feature of chronic liver diseases. Moreover, the pattern of gene expression undergoes significant changes post-activation, with a pronounced upregulation of types I and III collagens. This elevation in collagen expression underscores the pivotal role of HSCs in the deposition of extracellular matrix proteins during liver fibrosis. Notably, the heightened collagen synthesis is facilitated by the upregulation of heat shock protein 47 (HSP47), a collagen-binding stress protein that serves as a molecular chaperone during collagen formation. The increased expression of HSP47 in activated HSCs further underscores their pivotal role in driving the fibrotic process within the liver parenchyma. Collectively, these morphological and functional alterations highlight the pivotal role of activated HSCs in hepatic fibrosis progression and emphasize potential therapeutic targets for mitigating fibrotic liver diseases. Furthermore, the production of all three isoforms of the potent fibrogenic cytokine, transforming growth factor-beta (TGF-β), is explained in the context of Hepatic Stellate Cells (HSCs). Following HSC activation, there is an upregulation of its receptors. Additionally, post-activation, there is an escalation in the synthesis of PDGF-BB, which acts as the most potent mitogen for HSCs, along with an increase in PDGF receptors. HSC activation is associated with various cellular alterations, yet two fundamental processes significantly enhance their proliferative capacity. Firstly, HSCs

undergo direct fibrogenesis by altering their gene expression pattern, leading to an abrupt increase in the synthesis and deposition of extracellular matrix proteins. Secondly, post-activation, the proliferation rate of HSCs escalates, effectively augmenting the population of fibrogenic cells within the liver. Thus, it is believed that effective therapy aimed at reducing or inhibiting the fibrogenic or proliferative responses of hepatic stellate cells (HSCs) will mitigate the adverse effects of their activation in the initiation and progression of liver fibrosis. Apart from the alterations directly contributing to the fibrogenic nature of HSCs, several additional changes following their activation also contribute to their role in liver disease development. HSC activation is associated with an increase in cell contractility, leading to elevated portal pressure by contracting both the cirrhotic liver overall and specific sinusoids. The primary stimulant for HSC contractility is endothelin-1 (ET-1). Moreover, activated HSCs produce nitric oxide (NO), which acts as a physiological antagonist of ET-1. **(Tsukada *et al.*, 2005).**

STIMULI OF LIVER FIBROSIS

As previously mentioned, hepatic fibrosis can be influenced by a variety of stimuli that operate through distinct pathways. Among these, non-alcoholic steatohepatitis (NASH) and alcohol consumption have garnered significant attention.

Alcohol: In the United States, excessive ethanol intake stands as the primary cause of liver fibrosis, a condition characterized by the excessive accumulation of scar tissue in the liver. This phenomenon is intricately linked to the emergence of oxidative stress within the liver. Reactive oxygen intermediates (ROIs), generated notably by cytochrome P450 2E1 (CYP2E1), play a significant role in the fibrogenic effects of ethanol consumption. These ROIs exert a direct impact on hepatic stellate cells (HSCs), prompting an increase in the production of extracellular matrix (ECM), a key component of scar tissue formation. Studies conducted in mice have shown that inhibiting CYP2E1 activity in ethanol-fed subjects effectively reduces ROI formation and the production of lipid peroxidation products, indicating a direct correlation between CYP2E1 activity, ROIs, and liver fibrosis. Additionally, ROIs resulting from ethanol metabolism can also be generated by the NADPH oxidase of Kupffer cells, specialized macrophages within the liver. Activation of Kupffer cells by ROIs further enhances their secretion of profibrogenic and proinflammatory cytokines, exacerbating the fibrogenic process and contributing to the progression of liver fibrosis associated with ethanol consumption. Acetaldehyde, the principal metabolite of

ethanol, plays a significant role in encouraging fibrogenesis, although the exact mechanism by which it enhances collagen transcription remains unclear. Nevertheless, extensive research is currently underway to elucidate this intricate process. Acetaldehyde is known to form covalent bonds with proteins via Schiff bases, particularly with the alpha-amino group of lysine, resulting in the formation of adducts referred to as acetaldehyde-protein adducts. These adducts have been identified in the livers of rats subjected to ethanol consumption, and a noteworthy observation is that most individuals with alcoholism exhibit antibodies to these adducts in their serum. This suggests a potential link between acetaldehyde-protein adducts and the pathogenesis of liver fibrosis in alcohol-related liver disease. Further investigations are warranted to comprehensively understand the underlying mechanisms and implications of acetaldehyde-induced fibrogenesis in liver pathology. **(Tsukada *et al.,* 2005).**

NASH: Non-alcoholic steatohepatitis (NASH) is a hepatic disease that is increasingly prevalent, particularly in the United States. It represents a distinct entity within a spectrum of interconnected liver conditions termed non-alcoholic fatty liver disease (NAFLD), characterized by non-specific inflammation and hepatic steatosis. NAFLD affects 5–10% of the general population, making it the most prevalent liver disease in the United States. Its prevalence is markedly higher—up to 70%—among individuals who are obese and/or afflicted with type II diabetes. Furthermore, NASH appears to disproportionately affect middle-aged women, although there is an emerging trend of NASH cases in younger individuals. The escalating prevalence of unhealthy dietary patterns and insufficient physical activity among children today has sparked concerns regarding various health issues, including Non-Alcoholic Fatty Liver Disease (NAFLD). This condition has been associated with a range of documented patient histories, such as hyperglycaemia, hypertension, polycystic ovarian syndrome, and hyperuricemia. Of particular concern is Non-Alcoholic Steatohepatitis (NASH), which has recently garnered significant attention due to its potential complications. Studies indicate that 20–40% of individuals diagnosed with NASH progress to liver fibrosis and/or cirrhosis, whereas those with steatosis alone or steatosis with non-specific inflammation do not exhibit such severe outcomes. In experimental rat models comparing NASH to steatosis alone, distinct differences have emerged. NASH animals demonstrated heightened lipid peroxidation, increased expression of KLF6 and TGF-h1 mRNA, activation of hepatic stellate cells (HSC), and greater collagen deposition compared to their steatosis counterparts. These findings underscore the need for heightened awareness and proactive

measures to address the rising incidence of NAFLD and its potentially severe consequences, especially among vulnerable populations like children **(Tsukada _et al._, 2005).**

ANTI-FIBROTIC THERAPEUTIC STRATEGIES

Liver fibrosis, which serves as the final common pathway for various chronic liver diseases, commonly stems from factors such as alcohol consumption and viral infections like hepatitis B and C. Removing the underlying causes is often deemed the most effective approach to treating the illness. Treatment strategies for liver damage encompass abstaining from alcohol, eliminating excess iron or copper from the blood in individuals with conditions like hemochromatosis or Wilson's disease, and administering antiviral medications such as lamivudine or interferon alpha (IFN-a) combined with ribavirin to combat hepatitis. Historically, liver transplantation has been the only truly successful treatment for advanced cirrhosis and hepatic fibrosis. Despite advancements in transplantation, patients still face potential postoperative complications like pleural effusion, pulmonary edema, pneumonia, and recurrence of diseases such as hepatitis C virus infection. Furthermore, primary biliary cirrhosis can lead to chronic ascites. Mental disorders like anxiety and despair, along with morbidity due to rejection or infection, are also concerns. Hence, research endeavours seek alternative methods to prevent early-stage liver fibrosis (Table-1).

Table 1: Therapeutic strategies for the treatment of liver fibrosis.

TYPE	AGENT
Anti-inflammatories	Prednisone Colchicine + ursodeoxycholic acid + methotrexate Malotilate Octreotide IL-1 receptor antagonists
Antioxidants	Vitamin E/C Silymarin Dilineolylphosphatidylcholine N-acetylcysteine S-adenosyl-L-methionine Polyenylphosphatidylcholine
Cytokine/signal transduction molecules	TGF-β receptor competitors Halofuginone Hepatocyte growth factor Interferon-α Interferon-γ AT receptor inhibitors (losartan, olmesartan) ACE inhibitors (peridinopril, captopril) TNP-470 Carbenoxolone Tyrosine kinase inhibitors (genistein, imatinib mesylate)

	Soluble PDGF receptor Farnesyl-/geranylgeranyltransferase inhibitors S-farnesylthiosalicyclic acid PD 98059 (ERK inhibitor) Y-27632 (ROCK inhibitor)
ECM-Targeted	Antisense TIMP-1 TIMP-1 antibody MMP gene therapy Prolyl-4-hydroxylase inhibitors (HOE077, S4682)
Promoters of HSC Apoptosis	Sulfasalazine Gliotoxin Anandamide
Herbal medicines	Sho-saiko-to (TJ-9) Inchin-ko-to (TJ-135) Glycyrrhizin Han-dan-gan-le

DISCUSSION

Liver fibrosis represents a significant challenge in global healthcare, given its association with various liver diseases such as viral hepatitis, non-alcoholic fatty liver disease (NAFLD), alcoholic liver disease (ALD), and autoimmune liver diseases. Understanding the underlying mechanisms driving liver fibrosis is crucial for the development of effective therapeutic strategies and improving patient outcomes. The dysregulated wound healing response to chronic liver injury characterizes liver fibrosis, with hepatic stellate cells (HSCs) playing a central role in fibrogenesis. Activation of HSCs triggers the deposition of extracellular matrix proteins, leading to scar tissue formation and organ dysfunction. Recent advancements in molecular biology and imaging techniques have enhanced our understanding of the complex interplay between immune cells, cytokines, growth factors, and matrix remodelling enzymes in driving fibrogenesis. Moreover, emerging research on the gut-liver axis and the role of gut microbiota-derived metabolites highlights new avenues for therapeutic intervention. Several signalling pathways and cellular processes contribute to liver fibrosis pathogenesis, with the transforming growth factor-beta (TGF-β) signalling pathway playing a central role in promoting HSC activation and collagen synthesis. Other profibrotic mediators such as platelet-derived growth factor (PDGF), connective tissue growth factor (CTGF), and nuclear factor-kappa B (NF-κB) also contribute to fibrogenesis. Hepatocyte apoptosis, dysfunctional liver regeneration, and alterations in extracellular matrix turnover dynamics further exacerbate liver fibrosis progression. The complex crosstalk between parenchymal and non-parenchymal cells within the liver microenvironment underscores the multifactorial nature of fibrotic liver diseases. Current therapeutic approaches target key molecular pathways

implicated in liver fibrosis progression. Antifibrotic agents, including small molecule inhibitors, monoclonal antibodies, and gene therapies, show promise in halting or reversing fibrosis. Lifestyle modifications such as dietary interventions, weight management, and alcohol cessation are integral components of fibrosis management, particularly in metabolic liver diseases like NAFLD and ALD. Liver transplantation remains the ultimate therapeutic option for end-stage liver fibrosis/cirrhosis refractory to medical therapy. However, challenges such as donor shortages and post-transplant complications underscore the need for continued research into alternative therapeutic modalities and preventive strategies for liver fibrosis. In conclusion, a comprehensive understanding of the pathogenesis of liver fibrosis and the development of novel therapeutic interventions are essential for addressing the significant global health burden posed by this condition. Collaborative efforts involving clinicians, researchers, and policymakers are necessary to improve patient outcomes and reduce the morbidity and mortality associated with liver fibrosis.

CONCLUSION

In conclusion, the unravelling of the pathogenesis of liver fibrosis has provided invaluable insights into the intricate mechanisms underlying this complex condition. Through extensive research and clinical studies, several key factors have been identified as contributors to the progression of liver fibrosis, including chronic inflammation, oxidative stress, hepatic stellate cell activation, and dysregulated extracellular matrix deposition. The understanding of these mechanisms has paved the way for the development of innovative therapeutic strategies aimed at halting or reversing the progression of liver fibrosis. Targeted interventions such as antifibrotic agents, immunomodulatory therapies, and lifestyle modifications hold promise in mitigating the detrimental effects of liver fibrosis and improving patient outcomes. Moreover, advancements in diagnostic modalities, including non-invasive imaging techniques and biomarker assays, have facilitated early detection and monitoring of liver fibrosis, enabling timely intervention and personalized treatment approaches. Moving forward, continued interdisciplinary collaboration and translational research efforts will be essential in further elucidating the pathogenesis of liver fibrosis and refining therapeutic strategies. By addressing the multifactorial nature of this disease and harnessing the potential of emerging technologies, we can strive towards more effective management and prevention of liver fibrosis, ultimately improving the quality of life for affected individuals worldwide.

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