REVIEW ARTICLE

# Liver fibrosis pathologies and potentials of RNA based therapeutics modalities

Rimpy Diwan[1,2] · Samantha Lynn Gaytan[1,3] · Himanshu Narendrakumar Bhatt[1,2] · Jacqueline Pena-Zacarias[1,4] · Md Nurunnabi[1,2,3,5]

## Abstract

Liver fibrosis (LF) occurs when the liver tissue responds to injury or inflammation by producing excessive amounts of scar tissue, known as the extracellular matrix. This buildup stiffens the liver tissue, hinders blood flow, and ultimately impairs liver function. Various factors can trigger this process, including bloodborne pathogens, genetic predisposition, alcohol abuse, non-steroidal anti-inflammatory drugs, non-alcoholic steatohepatitis, and non-alcoholic fatty liver disease. While some existing small-molecule therapies offer limited benefits, there is a pressing need for more effective treatments that can truly cure LF. RNA therapeutics have emerged as a promising approach, as they can potentially downregulate cytokine levels in cells responsible for liver fibrosis. Researchers are actively exploring various RNA-based therapeutics, such as mRNA, siRNA, miRNA, lncRNA, and oligonucleotides, to assess their efficacy in animal models. Furthermore, targeted drug delivery systems hold immense potential in this field. By utilizing lipid nanoparticles, exosomes, nanocomplexes, micelles, and polymeric nanoparticles, researchers aim to deliver therapeutic agents directly to specific biomarkers or cytokines within the fibrotic liver, increasing their effectiveness and reducing side effects. In conclusion, this review highlights the complex nature of liver fibrosis, its underlying causes, and the promising potential of RNA-based therapeutics and targeted delivery systems. Continued research in these areas could lead to the development of more effective and personalized treatment options for LF patients.

Keywords Liver fibrosis · RNA therapeutics · mRNA · siRNA · Lipid nanoparticle

## Introduction

Liver fibrosis (LF) is characterized as an immune-mediated response leading to the excessive accumulation of extracellular matrix proteins in various chronic liver diseases [1]. As LF progresses, it often leads to liver failure, cirrhosis, and portal hypertension, necessitating liver transplantation in advanced stages [2]. LF, as the 12th leading cause of death in the United States according to 2020 data, underscored its growing prevalence and the urgent need for a deeper understanding of its mechanisms [3]. Given the increasing prevalence of LF, this area has gained widespread attention and has culminated in the discovery of cellular and molecular mechanisms directly involved in LF [4]. Recent studies have highlighted the critical role of bone marrow in LF pathogenesis. Specifically, bone marrow- derived hepatic stellate cells, portal fibroblasts, and myofibroblasts have been identified as key effectors in excessive collagen production characteristic of fibrotic livers [5]. These cells,

Rimpy Diwan and Samantha Lynn Gaytan contributed equally.

✉ Md Nurunnabi
mnurunnabi@utep.edu

1 Department of Pharmaceutical Sciences, School of Pharmacy, The University of Texas El Paso, El Paso, TX 79902, USA

2 Department of Biomedical Engineering, College of Engineering, The University of Texas El Paso, El Paso, TX 79968, USA

3 Department of Interdisciplinary Health Sciences, College of Health Sciences, The University of Texas El Paso, El Paso, Texas 79968, USA

4 Department of Biological Sciences, College of Science, The University of Texas El Paso, El Paso, Texas 79968, USA

5 Border Biomedical Research Center, The University of Texas El Paso, El Paso, TX 79968, USA

upon activation by fibrogenic cytokines such as adipokines, TGF-β1, and angiotensin II, drive the collagen production process [6–8]. However, in chronic liver damage scenarios, the persistent replacement of liver cells with extracellular matrix and collagen compounds the problem, impairing liver function [9]. The type and location of liver injury dictate the deposition pattern of these extracellular components. Efforts to develop drugs targeting these cytokines are underway, with the goal of promoting liver cell regeneration, and reducing scarring in LF patients. In a healthy liver, hepatic stellate cells, star-shaped and containing vitamin A lipid droplets, are in quiescent state [10]. Chronic liver injury, however, disrupts this dormant state, primarily through the action of transforming growth factor (TGF-β1), which is directly activated by extracellular matrix deposits and further expressed by various cells including liver cells, macrophages, and liver sinusoidal endothelial cells. TGF-β1 targets hepatic stellate cells, inducing their transformation into collagen producing myofibroblasts [11]. This excess in extracellular matrix proteins not only alters the livers physical structure, forming distinctive scar tissue, but also impedes blood flow, further damaging healthy liver cells [12, 13].

Current research underscores the TGF-β1 signaling pathway's central role in activating hepatic stellate cells and spurring excessive extracellular matrix production. Portal fibroblasts, integral to the structural integrity of the liver around the portal vein, also contribute to LF in response to chronic liver injury [14]. As LF advances, it leads to the formation of septa - bands of scar tissue – further exacerbating the condition [15, 16].

Chronic liver damage has various origins, including bloodborne pathogens, genetic predispositions, obesity, alcohol abuse, and the use of non-steroidal anti-inflammatory drugs (NSAIDs) [17]. Genese like ABCB4, ALDOB, GBE1, FAH, ASL, SLC25A13, and SERPINA1, when mutated, significantly affect an individual's susceptibility to liver damage [18]. According to the American Liver Foundation, approximately 25% of adults in the United States are diagnosed with non-alcoholic fatty liver disease (NAFLD), and of these, approximately 20% develop non-alcoholic steatohepatitis NASH, conditions often asymptomatic and challenging to diagnose [19, 20]. Individuals at risk include those who are considered obese and those who have been diagnosed with metabolic syndrome and/or type 2 diabetes [21]. There is clear evidence that the consumption of foods containing high fat levels, and a lack of exercise are leading contributors to chronic liver disease and LF [22]. Additionally, excessive alcohol consumption, a primary risk factor, triggers a compensatory response in the liver that accelerates LF and can lead to cirrhosis or liver cancer [23]. The initial pathological indication of alcoholic liver disease, hepatic steatosis, involves the accumulation of fat droplets under liver cells, ultimately resulting in hepatitis [24].

Alcohol, the most commonly abused substance worldwide, is a leading cause of liver disease in the United States [23, 25]. Intriguingly, a study exploring the impact of magnetic field on alcoholic liver disease, found that a 0.1–0.2 T magnetic field directed downward could mitigate the disease by reducing reactive oxygen species, oxidative stress and inflammation [26].

Given the various effectors associated with LF, its rapid progression is a growing concern, highlighting the need for developing effective therapeutics. The review delves into the pathogenesis of LF, the barriers to its treatment, and the potential of RNA-based drug delivery systems and therapeutics in clinical investigations for the treatment of LF.

## Pathogenesis of LF

There are various factors, such as alcohol, severe viral infection, metabolic disorders, high-fat diet, toxins, steatosis, and cholestasis, which are associated with LF [27]. Alcohol metabolism in the body generates acetaldehyde and reactive oxygen species (ROS), which leads to the activation and excessive production of transforming growth factor-β (TGF-β) and increases the expression of type I collagen in hepatic stellate cells [28, 29]. TGF-β1 is a leading factor in alcoholic liver disease, and the liver is damaged by the production of ROS by inducing the necrosis or apoptosis of hepatocytes, which cause LF [30, 31]. Collagen deposition and accumulation of excessive ECM proteins are involved in the pathogenesis of LF [32]. The persistent activation and proliferation of fibroblasts to myofibroblasts is the primary factor for generating fibrous collagen and ECM protein accumulation in damaged liver. LF can be resolved before it turns to cirrhosis and hepatocellular carcinoma [33]. However, there are no standardized treatments available to treat LF to date. Therefore, liver transplantation is the only option once the liver progresses to cirrhosis.

The primary cause of toxins induced LF has been chronic viral infections of the hepatitis B virus (HBV) and hepatitis C virus (HCV) for two decades [34]. Development of novel antiviral molecules has declined HBV and HCV-related liver cirrhosis in the USA [35], while there is an increase in the cases of non-alcoholic steatohepatitis (NASH) and alcohol-associated [36, 36, 37]steatohepatitis and hepatocellular carcinoma [38, 39]. There are several factors that cause NASH, such as obesity, excessive accumulation of triglycerides, fatty acids, or cholesterol in hepatocytes, which further leads to the activation of several transcription factors, such as PPARγ, SERBP1, SERBP2, and expression of caspase 2, which results in inflammation [40]. In addition, there are other important factors associated with NASH, such as the generation of ROS, gut-derived lipopolysaccharide, and release of cytokines such as TGFβ, TNF, IL-6, IL-1β,

IL-17, HNF1B, and leptin, which further lead the activation of HSCs [41–44]. Moreover, the food supplements (e.g., fructose) increase the permeability of the intestine, generate bacterial products (e.g., lipopolysaccharide), and they release these contents in the portal circulation, which further leads to the activation of Toll-like receptor (TLR), inflammation, and LF [36, 37]. Gut microbiota also plays an important role in the development of NASH. For instance, gut microbiota increases the production of IL-17 in NASH patients, demonstrating that there is a connection between NASH, gut microbiota, immune response, and LF [45].

Another major cause of LF is alcoholic liver disease, which eventually leads to steatohepatitis, cirrhosis, and hepatocellular carcinoma [46]. Alcohol over-consumption produces a toxic metabolite (e.g., acetaldehyde) from hepatocytes, and it activates the cytochrome P4502E1 pathway indirectly to induce liver injury. In addition, the formation of a toxic alcoholic metabolite can increase the production of collagen type I by activated HSCs [47]. Alcohol triggers fatty acid and cholesterol synthesis by SREBP1 or SREBP2-dependent signaling, which leads to the accumulation of hepatic fat droplets and the formation of Mallory-Denk bodies in injured hepatocytes [48]. Liver injury by alcohol leads to the upregulation of cytokines and chemokines such as IL-8, IL-17, and CXCL1, and the recruitment of various cells such as neutrophils, bone marrow-derived macrophages which further leads to the activation of myofibroblasts in development of LF [13, 49, 50].

Another important factor that may play a role in LF is impairment in bile flow (e.g., cholestatic LF). The decrease in bile flow is caused by genetic defects (e.g., Alagille syndrome), severe mechanical injury of bile ducts, and impaired immune response during biliary cholangitis. These effects obstruct the bile secretion, lead to liver tissue damage and the bile is refluxed into circulation, which causes inflammation and eventually biliary fibrosis [51]. There are transcription factors, such as BSEP and CYP7A1, which are involved in the pathogenesis and processes in cholestatic LF, such as bile acid synthesis, detoxification, and fibrogenesis [52, 53]. There is another event or disease associated with LF is primary sclerosing cholangitis, which involves the inflammation of the biliary epithelium, which affects the entire biliary system and liver parenchyma, further leading to the development of LF [54].

There are various types of cells, such as myofibroblasts, hepatic stellate cells, inflammatory cells, portal fibroblasts, hepatocytes, and fibrocytes, which are responsible for the fibrogenic transformation of the injured liver and also cultured in 2D or 3D models as spheroids, and organoids which are discussed below in detail [55].

## Role of myofibroblasts in LF development

In response to liver injury, the myofibroblasts, the major source of ECM in LF, are activated. They are characterized by spindle or stellate shape, and they express various intracellular proteins such as vimentin, αSMA, and non-muscle myosin. Myofibroblasts have rough endoplasmic reticulum and Golgi apparatus, which are responsible for collagen [56]. The hepatic myofibroblasts have different origins, such as liver-resident cells, HSCs, portal fibroblasts, and bone marrow-derived cells [1, 57]. However, it is difficult to identify the origin of myofibroblasts in clinical settings. Data obtained from electron microscopy, cell fate mapping, and immunohistochemistry revealed that portal fibroblasts and HSCs are transformed into myofibroblasts, responsible for the accumulation of collagen (> 90%) [58–61]. HSCs are activated in case of toxic liver injury, while portal fibroblasts are activated in periportal liver injury (e.g., cholestatic LF). Thus, the composition of myofibroblasts varies according to the etiology of LF [62]. In addition, it has been revealed that processes such as epithelial-to-mesenchymal transition (EMT) facilitate the differentiation of mature epithelial cells into fully activated mesenchymal cells responsible for the LF [63]. Moreover, several tissue-specific cells, such as bone-marrow-derived progenitor cells, are capable of differentiating into lineage-specific cells, and they were reported to increase the population of tissue myofibroblasts in the injured liver [56, 64]. Some of the overly expressed markers of myofibroblasts in LF are mentioned in Table 1.

**Table 1** Myofibroblasts markers overexpressed in the LF

| Types | Markers | References |
|---|---|---|
| EMT associated | Albumin/CK19, FSP1, COL1A1, αSMA | [63, 65–68] |
| Liver resident or activated HSCs | Desmin, CD146, CD105, GFAP, p75 (NGFR), PDGFRβ1, PPARγ, CD36, LOX, LOXL2, IL-17RA, COL1A1, αSMA | [69, 70] |
| Activated portal fibroblasts | THY1, Elastin, CD105, Cofilin, Mesothelin, IL-18R1, COL1A1, COL15A1, αSMA | [71–75] |
| Bone marrow-derived fibrocytes | COL1A1, CD45, CD34, CD11b, CD80, CD86, CCR2, CCR7, CXCR4, ICAMI, αSMA | [76–79] |

## Role of hepatic stellate cells (HSCs) in LF development

Quiescent HSCs are located in the perisinusoidal space in the liver, and they are known as the pericytes as well as the major storage site of vitamin A [80, 81]. Quiescent HSCs are activated during liver injury, and they start downregulating the expression of vitamin A, Glial fibrillary acidic protein (GFAP), and Peroxisome proliferator-activated receptor gamma (PPARγ). These activated HSCs migrate to the site of injury from the space of Disse and start secreting ECM proteins such as collagen type I and other fibrogenic mediators such as α-SMA and other intracellular microfilaments [57, 58]. Several profibrogenic cytokines, such as TGF-β, activate transcription of collagen type I and activate HSCs in an SMAD2 or SMAD3-dependent manner [82]. It has been reported that the activation of TGF-β, leptin, IL-6, and IL-17 leads to an increase in the expression of several genes such as COL1A1, COL1A2, Activin, Pai1, and JAK-STAT3 [83, 84]. Moreover, the activation of connective tissue growth factor (CTGF) and IL-13 increases the COL1A1 expression in activated HSCs [85]. Some of the molecular and cellular mechanisms related to quiescent HSCs, activated HSCs, inactivated HSCs and HSCs apoptosis are shown in Fig. 1.

## Role of inflammatory cells and cytokines in LF development

Macrophages have a central role in the pathogenesis of LF. For instance, bone marrow-derived macrophages and Kupffer cells are the major sources of TGF-β [86]. However, it has been reported that Kupffer cells have phagocytic and anti-inflammatory properties [69, 87]. It is reported that deletion or blocking of genes such as

IL-6, TNF, and IL-1β reduced LF because these genes can be synergistic with TGF-β, indicating that TGF-β is the key player in LF [88–91]. In addition, TGF-β and IL-6 regulate the differentiation of naive T cells towards T helper 17 (TH17), and it is reported that TH17 cells produce IL-17A and IL-22, which have pro-fibrogenic and anti-inflammatory properties, respectively [83, 88, 92]. The data suggested that the deletion of the IL-17 gene strongly reduced LF, in which the release of IL-22 acts as a survival factor for hepatocytes [83, 93]. These data demonstrated that T cell activation has the potential to reduce LF by releasing the anti-fibrotic cytokine IL-22 [94].

The data revealed that macrophages have a double role in LF e.g., progression and resolution of LF. For instance, the macrophages are recruited in the liver in response to liver injury, and they start producing cytokines and chemokines to trigger the activation of HSCs [80]. During this phenomenon, Kupffer cells and HSCs secrete CCL2, which recruits the specialized macrophages, e.g., monocyte derived LY6C$^{hi}$ macrophages in the liver [95]. The data revealed that deletion of such macrophages in CD11b-DTR transgenic mice ameliorated CCl$_4$-induced LF [96]. In contrast, another type of macrophage (e.g., LY6C$^{low}$) reduces the production of pro-inflammatory cytokines and fibrogenic factors during the resolution of LF [97]. In addition, these macrophages release ECM-degrading enzymes such as matrix metalloproteinases (MMPs) 9 and MMP12 and induce collagenolytic activity [96, 97]. In addition, tissue inhibitor of metalloproteinases (TIMP) levels is elevated in myofibroblasts during fibrosis. The reduction in the myofibroblasts population facilitates the reduction in TIMP levels and increases the MMP activities thereby ECM degradation [98].
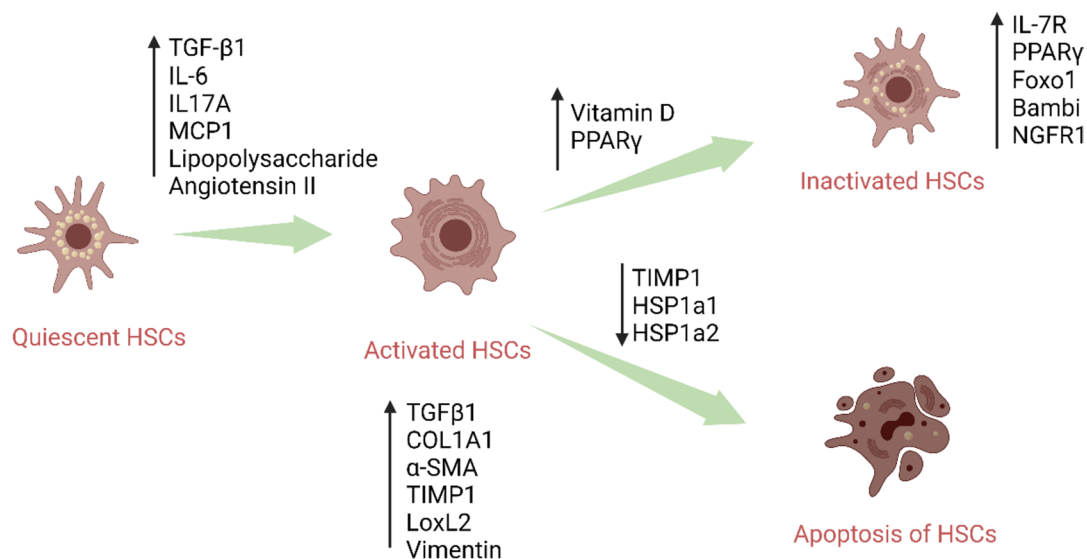


**Fig. 1** Molecular and cellular mechanisms of LF and its regression. Created in BioRender.com

Inflammation is an absolute requirement for the development of fibrosis. Amongst various inflammatory cells, neutrophils are the first responder to liver injury and facilitate the clearance of apoptotic hepatocytes [74, 99]. Neutrophils attract pro-inflammatory cytokines such as IL-8, IL-18 and IL-17, CCL3, CCL4, and CXCL2 and release cell-free DNA [46, 100]. Therefore, the removal of neutrophils or reduction of the above cytokines reduces the severity of LF in mice [101, 102].

## Portal fibroblasts, hepatocytes, and fibrocytes

Portal fibroblasts are located surround the portal vein and they provide the support to maintain the integrity of portal circulation. They have a role in the pathogenesis of cholestatic liver injury [73]. TGF-β is a key player in the activation of portal fibroblasts, and they have few defining fibrotic markers such as COL1A1, αSMA, TIMP1, LoxL2, TGFβR1, PDGFRb, Vimentin, Spp1 [103]. For instance, the interaction between mesothelin (MSLN), mucin 16 (MUC16), and THY1 surface receptors facilitates the regulation of TGFB1–TGFBRI–SMAD2/3-induced fibrogenic pathway via MSLN-AKT-FGFR1-dependent signaling [74]. Therefore, MSLN and MUC16 play a role in the activation of portal fibroblasts. In addition, IL-25-triggered activated portal fibroblasts secrete IL-13, which induces the release of CTGF and HSCs activation [58, 85]. These results demonstrated that targeting activated portal fibroblasts has the potential to treat LF.

Hepatocytes start changing the expression of genes such as Notch, osteopontin, TGF-β, NADPH oxidase 4, transcriptional coactivator with PDZ-binding motif (TAZ), and Hedgehog, in response to liver injury [104–106]. Interestingly, it is reported that injured hepatocytes activate HSCs by secreting exosomes that contain microRNA [107]. Nevertheless, studies suggested that injured hepatocytes are not sufficient to activate HSCs, indicating that inflammation is still a necessary requirement for the development of fibrosis.

Fibrocytes are secreted from hematopoietic stem cells, and they express CD45 and collagen type I and regulate tissue repair [79]. Fibrocytes are differentiated into myofibroblasts, and they play a role in the pathogenesis of LF [108]. They express various pro-fibrogenic factors in ECM, such as TGF-β, monocyte chemoattractant protein-1 (MCP1), fibronectin, and vimentin [109]. They have the ability to proliferate and migrate to injured sites and start recruiting themselves, which contributes to 3–5% of LF [78, 110].

## Barriers to LF treatment

Though there is a tremendous improvement in understanding LF, there is no standardized treatment available to treat LF. The liver perfusion is hampered due to the excessive accumulation of ECM proteins, which decrease the internalization of anti-fibrotic drugs or macromolecules, thereby reducing the efficacy of treatment. Liver sinusoidal capillary has discontinuous fenestration or endothelium, where the mixing of blood carrying oxygen from the hepatic artery happens with nutrient-rich blood from the portal vein. In adults, liver sinusoidal endothelial cells express several markers, such as embryonic and endothelial cell markers. In a healthy liver, these cells maintain their quiescent state and regulate blood flow and pressure in the sinusoidal region and the portal region, respectively. In addition, these cells allow several molecules (e.g., metabolites, plasma proteins, drug molecules, chylomicrons, viruses, and exosomes) to pass into the perisinusoidal space. In case of liver injury, these cells are de-differentiated, resulting in the activation of HSCs and macrophages [111]. After de-differentiation and excessive ECM deposition, the fenestration disappears, and the space of Disse starts decreasing the liver uptake of anti-fibrotic molecules [112]. Therefore, the specific delivery of anti-fibrotic drugs into cells such as activated HSCs or Kupffer cells in the liver becomes challenging. For example, targeting HSC cells is difficult because HSCs constitute a small portion (10–15%) of liver tissue, and the majority of delivery carriers are taken up by activated Kupffer cells that abide with HSCs, resulting in decreased therapeutic efficacy [113].

## Liver fibrosis animal models

Animal models play an important role in studying the pathogenesis of liver fibrosis and in evaluating the therapeutic effect of anti-fibrotic drugs. These models are widely used in basic research, drug discovery, and regenerative medicine in liver fibrosis research. We discussed a few important current rodent models of liver injuries in this section.

### Toxin-induced liver fibrosis models

The most used animal model of liver fibrosis is the carbon tetrachloride ($CCl_4$) induced animal model. This toxin is administered at a dose of 0.5 to 2 mL/kg of body weight in mice via intraperitoneal or oral route twice or thrice a week [114]. In this model, HSCs are activated following ECM deposition after 4–6 weeks from the first injection of $CCl_4$. This treatment increases the level of serum enzymes such as aspartate aminotransferase and alanine aminotransferase and initiates liver oxidative stress [115]. Administration of $CCl_4$ generates toxic trichloromethyl ($CCl_3$) radicals by metabolizing the $CCl_4$ via the cytochrome P450 2E1 pathway, which promotes liver fibrosis [116]. Data showed that inflammation induced by $CCl_4$ in the liver recruited several cells, such as CD4^{+} T, CD8^{+} T, and B cells, which broke down the liver tolerance and triggered the autoimmune

response [117]. This model has numerous advantages, such as relatively cheaper, simple, fast induction of disease, and huge pathological changes in the liver tissue [118]. In addition, it was also observed that stopping the administration of CCl$_4$ leads to the regression of liver fibrosis [119, 120].

Another classic model is the thioacetamide (TAA)-induced model of liver fibrosis, which induces severe oxidative stress and inflammation, resulting in acute or chronic liver injury [121]. TAA is administered via the intraperitoneal route at a dose of 150–200 mg/kg body weight three times per week or by oral route at a dose of 200 mg/L [122]. It has been observed that this toxin causes fibrosis and cirrhosis in 12–16 weeks in rats and 16–24 weeks in mice [123, 124]. TAA is metabolized by cytochrome P450 2E1 and generates toxic metabolites such as S, S-dioxide, which bind to the lipid and proteins, affects hepatocytes, produces fibrinogen and growth factors, and cause hepatotoxicity [125, 126]. This model mimics the human liver fibrosis environment in several aspects, such as hemodynamics, morphology, and biochemical metabolism [127]. Additionally, TAA interrupts DNA, RNA, and protein synthesizing enzymes in hepatocytes and creates a disturbance in metabolism, due to which the fibrosis lasts for more than two months after TAA withdrawal [123].

## Models of biliary fibrosis

Cholestatic liver diseases such as primary biliary cholangitis (PBC) and primary sclerosing cholangitis (PSC) involve injury in the biliary epithelium and bile duct, which results in liver fibrosis and, eventually, cirrhosis. The most common experimental rodent model of cholestatic liver injury is bile duct ligation, which involves the ligation of the common extrahepatic bile duct, resulting in biliary fibrosis after 21–28 days [128]. This method needs abdominal laparotomy in the middle portion of the liver, isolation of the bile duct above the duodenum, and ligation and dissection of the bile duct [129]. This surgical procedure starts the proliferation of biliary epithelial cells and differentiation of portal fibroblasts, which induce high expression of ECM proteins [130, 131]. This model is used to understand the pathogenesis of biliary fibrosis and inflammation. The drawback of this model is high mortality due to bile leakage and rupturing of the gall bladder during the ligation procedure, resulting in severe pain [132, 133].

## Models of non-alcoholic steatohepatitis (NASH)-induced liver fibrosis

During the development of NASH, the liver becomes fatty, and hepatocytes become bigger in size, which causes inflammation and fibrosis [134]. There is no animal model that can fully mimic the histology and pathogenesis of human NASH to date. The commonly used model in NASH is the diet-based model, known as the methionine- and choline-deficient diet (MCDD) model [135]. After feeding the MCDD diet in animals, the secretion of very low-density protein is altered, and they develop steatohepatitis and fibrosis in the space of Disse by 7–10 weeks [136]. There are factors that need to be considered for this model, such as weight loss and insulin hypersensitivity, which are not observed in human NASH [137]. Another model is the ob/ob (ob = obese) mouse model of NASH [138]. These genetically modified mice are leptin-deficient and become hyperphagic, obese, hyperglycemic, and hepatic steatosis. Several other genetic models of NASH have been explored by Larter et al. in detail [139].

## Models of alcohol-induced fibrosis

Several animal models for alcoholic liver disease have been explored. The Lieber-De Carli model was developed by Iseri and co-workers which involved animal feeding with an alcohol-containing liquid diet [140]. The animal developed mild liver steatosis and inflammation after 4–12 weeks which mimic the chronic drinking pattern in humans. However, it was concluded that there was no fibrosis observed in this model [141]. In addition, the mice naturally died due to alcohol consumption. Later on, another model was developed by Tsukamoto et al. to overcome the above issue in which the high blood alcohol level was obtained [142, 143]. Although currently there are no animal models able to mimic all features of alcoholic liver disease (ALD), several animal models for ALD have been generated. During the study, the animals were administered alcohol using an intragastric cannula, and fibrosis was observed in 6–8 weeks [144]. The drawback is it requires surgical procedures and intensive medical care, which poses a limit to the use of this model. Chiang et al. and Jeong et al. developed a model in which the alcohol-based liquid diet was combined with intraperitoneal injections of CCl$_4$ [145, 146]. The HSCs activation and fibrosis were observed after 5–8 weeks of feeding. However, there is lack of animal models that can fully mimic human alcohol-induced fibrosis to date.

## Fumarylacetoacetate hydrolase-knock out (Fah-KO) mice model

Patients with inborn tyrosinemia are prone to fulminant liver failure, resulting in death or an increased risk of hepatocellular carcinoma [147, 148]. This error is caused by a defect in the enzyme fumarylacetoacetate hydrolase (Fah), accumulating toxic metabolites such as fumarylacetoacetate and maleylacetoacetate [147]. This enzyme is mainly present in the liver and kidney, and it is required to hydrolyze fumarylacetate into fumarate and acetoacetate [148, 149].

In addition, it has been reported that Fah-/- mice have died within 12 h of birth due to hypoglycemia and liver dysfunction in response to the development of inflammation and fibrosis generated owing to the toxic metabolite fumarylacetoacetate [150, 151]. Hence, the FAH-KO (Fah-/-) mouse model is important for inducible liver injury and repopulation [152]. 2-(2-Nitro-4-trifluoromethylbenzoyl)-1,3-cyclohexanedione (NTBC) is one of the inhibitors that block the accumulation of toxic metabolites such as fumarylacetoacetate and maleylacetoacetate that can control this hepatic inflammation and fibrosis [153]. FAH-KO (Fah-/-) mice and pigs are maintained using NTBC, and both models are able to induce progressive liver failure and renal dysfunction when NTBC treatment is withdrawn [154]. Interestingly, Fah-/- mice can survive by wild-type (WT) hepatocyte transplantation, and hence, this model is important to evaluate the in vivo functions of lately identified liver progenitor cells and hepatocyte-like cells [155–157]. In addition, the Fah-/- mice model can also be utilized to evaluate the therapeutic efficacy of transplanted cells from hepatic sources (e.g., hepatocytes) and non-hepatic sources (e.g., bone marrow and pancreas) [158, 159]. Moreover, it has been shown using this model that the immune system is not required for liver regeneration, and CD8$^+$ T cells are responsible for liver carcinogenesis in chronic liver injury [151, 160].

## Delivery strategies for RNA based therapeutics

The conventional strategy of anti-fibrotic therapy is not useful at the clinical level because of the non-specific distribution of drugs in the body. To solve this problem, nano-medicine-based strategies are promising to load the active molecules. The nanocarriers can accumulate in the liver with or without active targeting in the fibrotic area. For instance, nanocarriers are internalized by hepatocytes with the help of different transporters present at the sinusoidal site, and for this, active targeting is not mandatory. In LF, the approaches of targeting depend on the type of cells in the liver because each type of cell in the liver has specific receptors. There are four types of cells that coordinate the function of the liver: Kupffer cells, hepatocytes, liver sinusoidal endothelial cells (LSECs), and hepatic stellate cells (HSCs) [161]. The nanoparticles with lipophilic surfaces are cleared rapidly by Kupffer cells. Furthermore, the Kupffer cells and liver sinusoidal endothelial cells recognize the nanoparticles according to ligands present over the surface (e.g., LDL, human serum albumin). For example, negatively charged particles are taken up by scavenger receptors, while positively charged particles are taken up by hepatocytes [113].

Collagen deposition in the perisinusoidal space impedes the delivery of nanocarriers to treat LF. LF is diagnosed at a later stage, and therefore, ECM protein deposition is prominent in liver tissue, which reduces the nanoparticle entry and diffusion inside the condensed ECM network. Moreover, the blood vessels and lymphatic vessels are compressed due to a dense ECM network, which leads to high interstitial fluid pressure and prevents nanoparticle convection and transport inside tissue [162]. Hence, targeting ECM facilitates the penetration of nanoparticles in poorly perfused fibrotic liver. For example, the nanocarriers carrying ECM degrading agents such as collagenase, hyaluronidase, and MMPs could enhance the nanoparticle transport.

Hepatocyte targeting is difficult because these cells do not express specific receptors for targeting. These cells express extracellular glycoprotein receptors (ASGPR), and expression of these receptors in LF is stage dependent. Galactose has been recognized as a specific ligand of ASGPR. Hence, galactose modification over the nanoparticle surface can be a useful strategy to target hepatocytes in LF [163]. LSECs are another target for the immunomodulatory effect and their role in antigen presentation [164]. The mannose receptors are highly expressed in LSECs which can bind with endogenous glycoproteins and pathogens. Hence, mannose functionalization over nanoparticle surface enhances the targeting of LSECs in LF. In response to liver injury, HSCs are differentiated into myofibroblasts like cells and start secreting collagen and MMPs to create scar liver. HSCs are reservoirs of vitamin A in the body [165]. Hence, vitamin A could be the potential hallmark of LF by incorporating vitamin A into nanoparticles, which can target HSCs.

Amongst various types of nanoparticles, presently, LNPs are the most advanced delivery system for the successful delivery of nucleic acid-based therapeutics (e.g., mRNA, siRNA, miRNA, oligonucleotides, lnc) to LF. One of the primary challenges in the development of RNA-based treatments is the effective delivery of these molecules to target cells in the liver. Recent advancements in lipid nanoparticle (LNP) technology have emerged as a pivotal solution to this challenge by significantly improving the delivery and efficacy of RNA therapeutics. Comprising a combination of ionizable lipid, cholesterol, phospholipid, and polyethylene glycol (PEG), LNPs have proven to be effective in stabilizing RNA molecules, facilitating their entry into cells, and ensuring their release into the cytoplasm [166]. The ionizable lipid component is particularly crucial, as it not only protects the RNA but also aids in the escape from endosomes, ensuring the RNA reaches its target within the cell. The cholesterol and phospholipid components contribute to the stability of LNPs and facilitate membrane fusion, while the PEG-lipid stabilizes LNPs during formulation and promotes cellular uptake upon intravenous injection. For instance, lipid nanoparticles have been utilized to enhance the delivery of RNA therapeutics to liver cells, including hepatocytes and hepatic stellate cells (HSCs), the primary effector cells in liver fibrogenesis [167].

We have summarized some LNP-based strategies to reduce LF in Table 2.

There are other types of nanocarriers (30–150 nm in diameter), known as cell-derived vesicles or exosomes, consisting of lipid bilayers secreted by cells [168]. Various types of nucleic acids, such as DNA, mRNA, miRNA, and other non-coding RNAs, are present in exosomes [169]. The exosomes are secreted from the plasma membrane, which contains proteins and other molecules that possess the transcription behavior of cells of origin [170]. After release into the intercellular space, exosomes are fused to recipient cells and deliver the informative cargoes. After that, those recipient cells undergo epigenetic reprogramming and phenotypic alternations depending on the information received [171]. We have summarized some exosome-based strategies to reduce LF in Table 2.

There are other types of nanocarriers that can be used for targeting LF, such as polymeric nanoparticles. Polymers are large molecules having multiple repeating units called monomers. Polymers are chemically flexible delivery platforms because tuning their size, structure, and functionality can alter their physicochemical properties. The optimum selection of polymers, as well as supplemental excipients, can influence nanoparticle potency, stability, and targeting abilities [172]. Polymeric nanoparticles can be assembled into several structures, such as solid matrix systems, micelles, and polyplex nanoparticles [173]. Solid matrix systems are formed by hydrophobic interactions between individual polymer molecules. Micelles are amphiphilic system which is formed by a hydrophobic core with a hydrophobic shell to reduce unwanted interactions with an aqueous environment [174]. Polyplex nanoparticles are formed by electrostatic interactions between the polymeric material and oppositely charged macromolecules (e.g., nucleic acids), which can be further stabilized through the incorporation of other molecular components [175]. Though there are advanced discoveries of various polymers and co-polymers, there are very few polymer-based delivery of nucleic acids in the clinic compared to LNPs. This allows further research in developing materials with new properties, such as cell and tissue tropism and endosomal escape, for the tissue-specific delivery strategies to deliver nucleic acid-based therapeutics [176, 177]. We have summarized some polymeric nanoparticle-based strategies to reduce LF in Table 2. Some of the delivery systems to deliver RNA therapeutics are shown in Fig. 2.

## RNA based therapeutics delivery for treatment of LF

LF, a critical stage in chronic liver disease, represents a major healthcare challenge worldwide due to the lack of effective antifibrotic therapies. However, RNA-based therapeutics have emerged as promising strategies for the treatment of LF [178]. The basis of RNA-based therapies marks a significant leap forward from traditional treatment modalities. One of the most remarkable advantages of RNA therapeutics is their targeting abilities. Unlike broad-spectrum drugs that can affect multiple pathways and systems, RNA therapies can precisely target and modulate specific genes involved in LF, leading to more effective and efficient treatment outcomes. These therapies aim to silence specific genes involved in the fibrotic process, potentially reversing or halting the progression of the disease [179]. Some of the RNA therapeutics explored for their delivery in treatment of LF are shown in Fig. 2. RNA-based therapeutics, particularly small interfering RNA (siRNA) and microRNA (miRNA), operate through a mechanism of gene silencing. siRNA functions by binding to a specific mRNA sequence in the cell, leading to the degradation of this mRNA and thus preventing the translation of the target protein of interest. This process, known as RNA interference (RNAi), allows for the selective silencing of genes implicated in disease processes [180]. Similarly, miRNA regulates gene expression post-transcriptionally. It typically binds to the 3' untranslated region of target mRNA, blocking its translation or initiating its degradation [181]. This ability to target specific mRNA sequences is key in addressing the pathogenesis of diseases like LF, where overexpression or abnormal activity of certain genes drives disease progression.

The specificity of these RNA molecules in targeting only the desired genes minimizes off-target effects, as the therapy does not interfere with unrelated biological pathways making RNA therapeutics a highly precise approach in disease management. Additionally, RNA therapeutics offer the potential for reversal of fibrosis, a challenge that conventional treatments often fail to address. This is particularly promising in the context of liver diseases, where the ability to reverse fibrosis can significantly improve patient outcomes and quality of life. Moreover, RNA therapies are adaptable and can be quickly designed to target newly discovered genes involved in disease processes, offering a flexible approach to emerging health challenges.

## Advantages of RNA based therapeutics for treatment of LF

In addressing the comparative effectiveness of RNA therapeutics in the treatment of liver fibrosis, it is important to contrast it with other prevalent treatment modalities. Liver fibrosis, a consequence of chronic liver injury, has been traditionally managed through methods such as antifibrotic drugs, lifestyle modifications, and, in severe cases, liver transplantation. However, these approaches often address the symptoms or consequences of liver fibrosis rather than

Table 2 Formulation approaches for RNA therapeutics for the treatment of LF

| Delivery System | RNA therapeutic | Route of administration | Inference | Reference |
|---|---|---|---|---|
| Lipid nanoparticle | mRNA; HNF4A | Intravenous administration; 2 mg/kg | HNF4A mRNA loaded hepatocyte-targeted biodegradable lipid nanoparticles were developed to evaluate the restoration of the metabolic activity of fibrotic murine and human hepatocytes in vitro. Results form in vitro and in vivo studies revealed the inhibition of fibrogenesis. They have also discovered the direct target of HNF4A which is paraoxonase 1 which helps in attenuation of LF by modulating liver macrophages and hepatic stellate cells. | [194] |
| Lipid nanoparticle | mRNA; erythropoietin | Intravenous administration; 2 mg/kg | Erythropoietin based mRNA was encapsulated in lipid nanoparticles consisting of ionizable lipid iBL0713 and evaluated for its therapeutic efficacy both in vitro and in vivo. In vitro studies showed that the developed mRNA-based formulations were expressed in human hepatocellular carcinoma cells and hepatocytes. In vivo studies revealed the maximum protein expression at 6 h post administration and the developed formulations were found to be safe as signs of toxicity and immunogenicity were not observed in vivo. | [215] |
| Exosomes | miRNA; miRNA-148a | Intravenous administration; 150 μg of Exosomes diluted in 150μL PBS | MiRNA-148a loaded mesenchymal stem cells derived exosomes were administered intravenously in carbon tetrachloride induced LF animal model to assess the therapeutic efficacy of developed exosomes. Exosomes were found to target kruppel like factor-6 thereby suppressed pro-inflammatory macrophages and promoted anti-inflammatory macrophages & resulted in amelioration of LF. | [216] |

**Table 2** (continued)

| Delivery System | RNA therapeutic | Route of administration | Inference | Reference |
|---|---|---|---|---|
| Micelles | Modified miRNA-29b1 | Intravenous administration; 2 mg/kg | Modification of miRNA-29b1 was done by modifying its antisense strands with phosphorothioate (PS-miR-29b1), 2'-O-methyl-phosphorothioate (OMe-miR-29b1), locked nucleic acid (LNA-miR-29b1), and N,N'-diethyl-4-(4-nitronaphthalen-1-ylazo)-phenylamine (ZEN-miR-29b1). OMe-miR-29b1 was found to be highly stable in 50% FBS as compared to other modified miRNAs. The injury markers were reduced, and liver functions were improved in fibrotic mice after treatment with OMe-miR-29b1. The protein levels of collagen, α-SMA, and TIMP-1 were significantly decreased when treated with OMe-PS-miR-29b1-loaded micelles compared to miR-29b1-loaded micelles thereby could provide a treatment for LF. | [217] |
| Lipid nanoparticles | siRNA; High mobility group box-1 (HMGB1) protein | Intravenous administration; 0.1 and 0.75 mg/kg | HMGB1 siRNA loaded, and peptide modified lipid nanoparticles showed active targeting towards HSCs, inhibiting the activation and proliferation of HSCs. The targeted LNP was found to be highly therapeutic active as it effectively silenced the HMGB1 gene, reduced the release of HMGB1 protein, inhibited collagen deposition and fibrosis formation in the liver, and significantly prolonged the survival time of cirrhotic mice models. | [199] |
| Polypeptide modified lipid nanoparticles | siRNA | Intravenous administration; 0.023 mg/kg of siRNA | Modified nanoparticles showed increased cellular uptake by LX-2 cells and HSCs and biodistribution study revealed the higher uptake by liver in as compared to unmodified nanoparticles. | [218] |

**Table 2** (continued)

| Delivery System | RNA therapeutic | Route of administration | Inference | Reference |
|---|---|---|---|---|
| Lipid nanoparticles | siRNA - siColl$\alpha$1, siTIMP | Intravenous administration; 0.3 mg/kg | HSC-targeting lipid nanoparticles were developed using amphiphilic cationic hyperbranched lipoids (C15-PA) and helper lipoids (cholesterol-polyethylene glycol-vitamin A, Chol-PEG-VA) for dual siRNA delivery. Fibrotic mice treated with dual siRNA loaded VLNPs showed a great reduction in the collagen accumulation in liver. No signs of toxicity or inflammation were observed after repeated doses of nanoparticles indicated the biocompatibility of developed nanoparticles | [219] |
| Lipid nanoparticles | siRNA | Intravenous administration; 0.25 mg/kg | siRNA loaded lipid nanoparticles were prepared using two ionizable lipids-CL15A6 and CL15H6 and evaluated for their antifibrotic activity in mice. Results showed that the developed LNP were able to reverse the LF and normalize the liver function by knocking down the knockdown of Hedgehog (Hh) and TGF$\beta$1 signaling pathways simultaneously. | [220] |
| Carbon nitride based nanosheet | siRNA - HIF-1$\alpha$ | Intravenous administration | HIF-1$\alpha$ small interfering RNA encapsulated VA-PEG-modified CNs-based nanosheets were developed for targeted delivery of nanosheets to the HSCs. In vivo studies revealed the improvement in hypoxic environment at the LF site and downregulation of HIF-1$\alpha$ expression was observed in fibrotic mice. | [221] |
| Nanopolyplex | siRNA- siPDGFR-$\beta$ | Intravenous administration | Dual sensitive nanoplexes were prepared by complexing siPDGFR-$\beta$ with vitamin A-modified crosslinking nanopolyplex. siPDGFR-$\beta$-vitamin A complex reduced the activation of HSCs followed by the production of pro-fibrogenic proteins. | [222] |
| Exosomes | siRNA- Osteopontin | Intravenous administration 5 $\mu$g siRNAof $1 \times 10^9$ engineered exosome | siRNA- Osteopontin was loading in exosomes prepared by electroporation technique and were evaluated in vitro and in vivo. Exosomes suppressed activation of HSCs and ECM deposition, inhibited TGF-$\beta$ signaling and improved liver function as compared to naked siRNA-Osteopontin. | [223] |

**Table 2** (continued)

| Delivery System | RNA therapeutic | Route of administration | Inference | Reference |
|---|---|---|---|---|
| Nanocomplex | siRNA of plasminogen activator inhibitor 1 | Intravenous administration 4 mg/kg for nanocomplex, 1 mg/kg siRNA | CXCR4-inhibiting nanocomplex composed of polymeric CXCR4 antagonism, clodronate and siPAI-1 was developed to improve the targeting and penetration ability of nanocomplex. Nanocomplex showed antifibrotic activity by synergistic effects of Kupffer cells apoptosis, ECM degradation and HSC inactivation. | [224] |
| Nanocomplex | siRNA-poly (r C)-binding protein 2 | Intravenous administration; 1 mg/kg | A nanocomplex of siRNA/peptide nucleic acid was prepared and evaluated for its therapeutic efficacy in vitro and in vivo. The siRNA nanocomplex showed high serum stability, and high cellular uptake in activated HSCs. Treatment of fibrotic rats with nanocomplex inhibited the mRNA expressions of PCBP2 and type I collagen in fibrotic liver. | [225] |
| Polyplex | siRNA- TGFβ | Intravenous administration; siTGFβ 0.5 mg/kg | A polyplex based delivery system was developed using Cyclam-modified PEI and siRNA. In vivo studies revealed the reduction in inflammation, collagen deposition and cells proliferation in fibrotic liver. | [226] |
| Exosomes | siRNA-STAT3 or antisense oligonucleotide targeting STAT3 | Intraperitoneal administration;5 µg siRNA-STAT3 in 100 µL of PBS | Exosomes derived from fibroblast-like mesenchymal stem cells were prepared for carrying siRNA and oligonucleotides. In vivo study revealed the suppression in STAT3 levels and ECM deposition in LF. | [227] |
| Exosomes | Oligonucleotide-RBP-J | Intraperitoneal administration;200 µg | HEK293T- derived exosomes were developed using Hairpin-type decoy oligodeoxynucleotide-RBP-J and evaluated for the therapeutic efficacy in mitigating LF in mice. Data revealed the exosomes were taken up by hepatic macrophages and thereby inhibited Notch signaling in macrophages and ameliorated LF. | [228] |
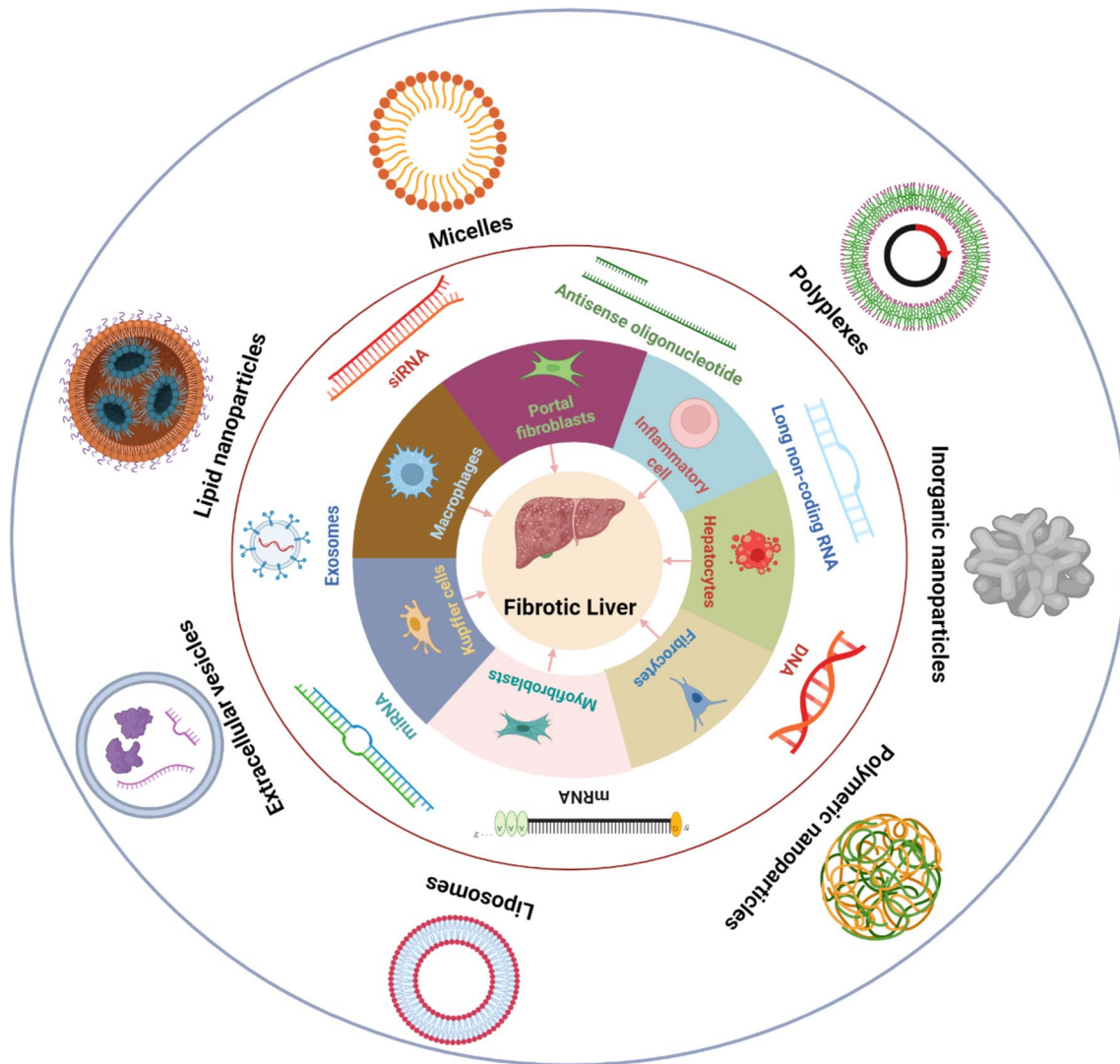
**Fig. 2** RNA-based therapeutics and drug delivery systems for the treatment of LF. Created in BioRender.com

targeting the underlying molecular pathways directly involved in the progression of the disease. RNA therapeutics present a novel approach by specifically targeting the genetic basis of liver fibrosis. This method contrasts with conventional pharmacological treatments that typically act on proteins or enzymes involved in fibrosis. The specificity of RNA therapeutics allows for a more targeted approach, potentially reducing side effects associated with the use of broader systemic drugs. The specificity of RNA-based treatments in targeting pathogenic genes can minimize interactions with other cellular pathways, thereby reducing unintended side effects [182]. For example, RNA therapeutics, such as siRNA and miRNA, specifically target genes implicated in the fibrogenic process, offering a level of precision that conventional pharmacotherapies cannot achieve. This gene-targeting capability of RNA therapeutics is particularly relevant in the complex pathophysiology of liver fibrosis,

where multiple pathways might be involved [167]. The ability to design RNA therapeutics for virtually any gene makes them highly versatile. This versatility is crucial in liver fibrosis, a condition driven by various molecular pathways. Additionally, emerging research suggests that RNA therapeutics might not only halt the progression of liver fibrosis but could also potentially reverse fibrotic changes [183]. This is a significant advantage over most current treatments, which primarily focus on slowing disease progression or managing symptoms. RNA therapeutics, particularly siRNA and miRNA, offer a novel and promising approach in the treatment of liver fibrosis by specifically targeting the genetic drivers of the disease. This contrasts with traditional treatments that focus more on symptom management and disease progression. RNA therapeutics' ability to potentially reverse fibrotic changes, combined with their high specificity and versatility, positions them as

a significant advancement over existing conventional liver fibrosis treatments. In the context of RNA therapeutics for fibrosis treatment, it is crucial to establish an understanding of RNA molecules, as they play a pivotal role in the development and application of such therapies. RNA, or Ribonucleic Acid, is a versatile biomolecule that serves as an intermediary between the genetic information stored in DNA and the production of functional proteins [184]. By understanding the structural and compositional characteristics of these RNA types, we can appreciate their roles in gene regulation and their potential in RNA therapeutics for fibrosis treatment. In the following sections, we will explore how these RNA molecules are harnessed to address the pathogenesis of fibrosis and their therapeutic implications.

## Structure and composition of RNA

At its core, RNA comprises nucleotide units, serving as the fundamental building blocks of the molecule. The sugar component in RNA is ribose, characterized by the presence of a hydroxyl group (-OH) at the 2' carbon position. This distinguishes ribose from the deoxyribose sugar found in DNA, where this oxygen atom is absent. Ribose's presence is fundamental to RNA's versatility in various cellular functions [185]. Additionally, a phosphate group is attached to the 5' carbon of the ribose sugar. These phosphate groups link adjacent nucleotides in the RNA strand through phosphodiester bonds, forming the structural backbone of the RNA molecule, which provides stability and integrity. RNA's uniqueness is further accentuated by its nitrogenous bases, including adenine (A), cytosine (C), guanine (G), and uracil (U). The sequence of these bases along the RNA strand carries the genetic code and determines the functional properties of RNA molecules, facilitating a wide array of biological processes [186]. Unlike the double-stranded helical structure of DNA, RNA typically exists as a single-stranded molecule. This single-stranded configuration provides RNA with remarkable flexibility, allowing it to adopt diverse secondary and tertiary structures that are vital for various functions. These structural configurations play integral roles in processes such as translation, splicing, and RNA folding [187]. Understanding the composition and structural nuances of RNA establishes the groundwork for comprehending its diverse functions, including its pivotal role in fibrosis treatment through RNA therapeutics.

## mRNA based therapeutics for treatment of LF

Messenger RNA (mRNA) assumes a central role in the intricate process of translating genetic information into functional proteins. Its structural composition plays a pivotal role in this fundamental biological function. Firstly, the 5' untranslated region (UTR) of mRNA serves as an essential regulatory region that influences translation efficiency and initiation. It contains sequences and structural elements that guide the ribosome to the proper location on the mRNA strand for protein synthesis [188]. Secondly, the coding sequence of mRNA carries the genetic code, providing instructions for the precise sequence of amino acids that constitute the protein to be synthesized. The coding sequence is central to the entire translation process, as it directs the ribosome in assembling the protein accurately. Thirdly, mRNA possesses a 3' UTR at its other end which is significant for post-transcriptional regulation, mRNA stability, and localization [189]. It contains elements that can interact with regulatory molecules and proteins, influencing the fate and function of the mRNA. In addition to these critical structural elements, mRNA features distinct end modifications. At the 5' end, mRNA exhibits a cap structure that plays an indispensable role in mRNA stability and translation initiation [190]. This cap structure also facilitates the recognition of the mRNA by the ribosome. At the 3' end, mRNA is distinguished by a poly-A tail, which contributes to mRNA stability, preventing rapid degradation. The combination of these structural features equips mRNA with the necessary attributes for efficient translation, stability, and regulation [191]. Understanding the architecture of mRNA is fundamental to comprehending its role as an intermediary between the genetic code stored in DNA and the synthesis of functional proteins. A few examples of mRNA-based treatment have been discussed in the following section and some of the other treatments have been mentioned in Table 2.

In a recent study, a nanoplatform has been developed for the delivery of mRNA to the activated hepatic stellate cells (aHSCs) and evaluated in vitro and in vivo. The lipid CL15A6, which possesses affinity towards aHSCs was selected based on pKa, selectivity and biosafety for the preparation of mRNA loaded LNP. The cellular uptake study revealed the pKa dependent uptake of LNP in aHSCs by Clathrin-mediated endocytosis through the Platelet-derived growth factor receptor beta. Biodistribution of developed FLuc mRNA loaded LNPs revealed the superior luciferase activity in fibrotic liver tissues. The enhanced green fluorescence protein-mRNA loaded LNP were injected intravenously in wild type mice at a dose of 2 mg/kg and formulations were found to be safe even in chronic administration mode. The levels of IL-6 and TNF-α following acute or chronic administration of LNPs confirmed the absence of systemic inflammation. Therefore, developed nanoplatform holds a significant potential in developing LNPs for clinical applications [192]. Similarly, promising data from a research study for the biodistribution of mRNA-based lipid nanoparticles and immunohistochemistry of fibrotic markers in fibrotic liver tissue is shown in Fig. 3.

Extracellular vesicles have also been explored for the delivery of mRNA. Left-right determination factor 1 (lefty

1) loaded hepatic stellate cells derived extracellular small vesicles have been developed to inhibit fibrosis by blocking TGF-β1 signaling pathway. The developed extracellular vesicles were evaluated in vitro and in vivo to assess the therapeutic efficacy. In vitro study revealed their effective uptake by cells and reduced the activation of hepatic stellate cells. Data from in vivo studies in $CCl_4$ induced LF suggested that treatment with developed formulations reduced the ECM production and promoted ECM degradation significantly by downregulating α-SMA, collagen I, TIMP-1, and MMP-1. Therefore, the developed extracellular vesicles could be developed to deliver mRNA for the treatment of LF [193].

## siRNA based therapeutics for treatment of LF

Small interfering RNA (siRNA) represents a crucial subset of small RNA molecules actively engaged in RNA interference (RNAi) pathways. These molecules are typically double stranded, comprising approximately 21–25 nucleotides in length. One distinctive feature of siRNA is its dual-stranded structure, consisting of two complementary strands. This duplex arrangement is vital for its function in RNA interference [195, 196]. Among these two strands, one is known as the guide strand, while the other is termed the passenger strand. The guide strand holds the key to siRNA's remarkable specificity in target recognition. It serves as the "guide" determining the precise mRNA sequence that siRNA will target for degradation [197]. This strand's complementary binding to the target mRNA sequence is a hallmark of RNA interference. In contrast, the passenger strand, though essential for the formation of the siRNA duplex, does not actively participate in target recognition. Instead, it often becomes displaced during the assembly of the RNA-induced silencing complex (RISC), which is responsible for carrying out the RNA interference process [197]. Once the RISC is
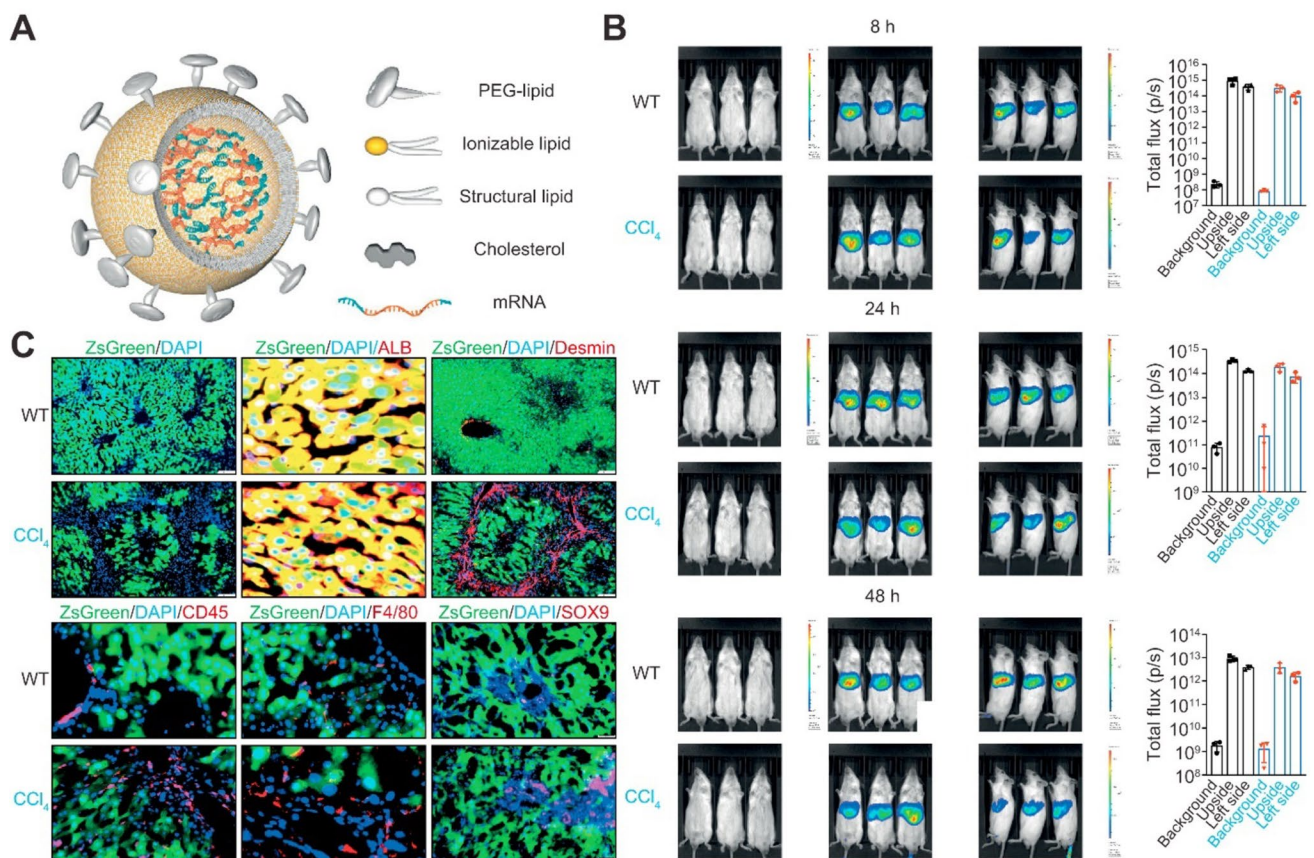


**Fig. 3** Successful mRNA delivery into hepatocytes of fibrotic BALB/c mice by LNP. **A** Illustration of LNPs designed to deliver mRNA into hepatocytes. **B** Bioluminescence analyses at 8 h, 24 h, and 48 h in WT control and CCl4-induced fibrotic mice (n = 3 mice per group) injected i.v. with 40 µg luciferase mRNA-encapsulated LNP (Luc/LNP). **C** Immunofluorescence images of ZsGreen expression and co-staining with hepatocyte marker (ALB), HSC marker (desmin), leukocyte marker (CD45), KC marker (F4/80) and cholangiocyte marker (SOX9) in WT control and CCl4-induced fibrosis in mice, injected i.v. with 40 µg ZsGreen/LNP. Scale bars: 100 µm for DAPI and Desmin, 50 µm for ALB, CD45, F4/80 and SOX9 images. CCl4, carbon tetrachloride; HSC, hepatic stellate cell; KC, Kupffer cell; LNP, lipid nanoparticle; WT, wild type. Reproduced with permission [194]
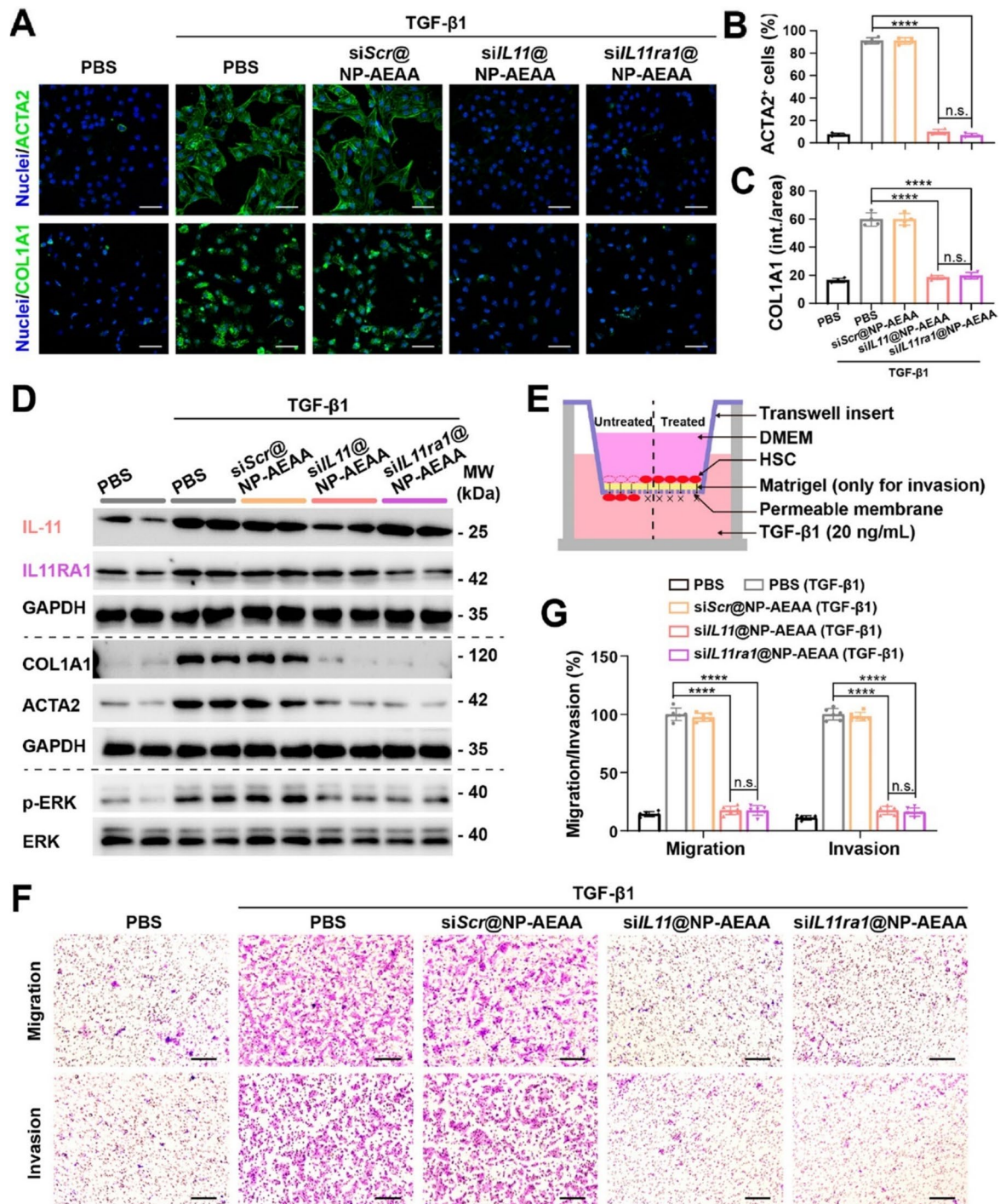
loaded with the guide strand, it becomes the effector complex, guiding the precise degradation of the target mRNA or inhibiting its translation. The distinct characteristics of siRNA, including its double-stranded nature and the functional role of its guide strand, make it a powerful tool in post-transcriptional gene silencing [198]. Researchers harness these attributes to target specific mRNA sequences, making siRNA an indispensable component of RNA therapeutics and gene regulation studies.

Among the various LNP formulations, anisamide ligand-tethered lipid nanoparticles (AA-LNPs) have shown considerable promise for targeted RNA delivery. One notable example is the development of AA-LNPs in targeting activated HSCs, the central players in LF. This approach involves the incorporation of anisamide, a ligand with high affinity for activated HSCs, into the lipid nanoparticle structure. The development of the AA-T3A-C12 lipidoid, a component of these LNPs, represents a significant advancement

◄**Fig. 4** Suppression of IL-11 or IL11RA1 using NP-AEAA siRNA therapeutics inhibits the activation, migration, and invasion of HSCs in vitro. **A** Representative images of ACTA2 and COL1A1 immunostaining in HSCs treated with TGF-β1 (20 ng/mL) for 48 h in the presence of PBS, siScr@NP-AEAA, siIL11@NP-AEAA, or siIL11ra1@NP-AEAA. HSCs treated with PBS and without any stimulation served as the "basal" state. Scale bars represent 50 μm. **B**, **C** Quantification analysis of ACTA2+cells (**B**) and COL1A1 immunofluorescence intensity (**C**) in HSCs as shown in **A**. n=4. **D** Western blots of IL-11, IL11RA1, COL1A1, ACTA2, phosphorylation, and total expression of ERK in HSCs treated with PBS, siScr@NP-AEAA, siIL11@NP-AEAA, or siIL11ra1@NP-AEAA followed by TGF-β1 stimulation (20 ng/mL) for 48 h. n=2 samples per group. GAPDH was used as a loading control to normalize protein levels, with HSCs treated with PBS alone serving as a negative control. MW, molecular weight. **E** Schematic illustration of the HSC migration and invasion assay using a two-compartment Boyden chamber system. Cells were incubated with PBS, siScr@NP-AEAA, siIL11@NP-AEAA, or siIL11ra1@NP-AEAA in the top chamber, and the medium in the bottom chamber was supplemented with chemotactic stimuli. For the invasion assay, the upper side of the polycarbonate membrane in the upper chamber was coated with a Matrigel matrix enriched with type IV collagen. HSCs migrate and invade through the membrane into the bottom chamber. **F**, **G** Microscopy images (**F**) and quantitative analysis (**G**) of stained HSCs that invaded through the Matrigel matrix and migrated from the top chamber to the bottom chamber in response to TGF-β1 stimulation (20 ng/mL) following the indicated treatments (n=3). Images from two randomly selected fields per well were acquired, and three independent wells were measured, resulting in a total of six measurements. Migration (%) or invasion (%) was expressed as a percentage of NP-treated cells passing through the membrane relative to the number of PBS-treated cells that traversed the membrane following stimulation with TGF-β1. Scale bars represent 100 μm. Statistical significance was determined via a one-way ANOVA with Tukey test (**B**, **C**, and **G**). Results are presented as means±SD. ****P<0.0001, n.s., not significant, P>0.05. Reproduced with permission [202]

in the field [167]. This lipidoid has been meticulously designed to improve targeting and transfection efficiency of the therapeutic RNA, especially targeting heat shock protein 47 (HSP47), a key molecule in the pathogenesis of LF [199]. AA-LNPs have shown promising results in preclinical models, achieving significant gene silencing in activated fibroblasts, and reducing collagen deposition, which is a hallmark of LF.

Systematic in vitro and in vivo studies confirmed that AA-T3A-C12 LNP mediated greater RNA delivery and gene knockdown than non-targeted LNPs [167]. Specifically, in a mouse model of CCl4-induced LF, AA-T3A-C12 LNP loaded with siHSP47 remarkably outperformed the benchmark MC3 LNP in silencing HSP47, reducing collagen deposition, and alleviating LF without further exacerbating liver damage. This demonstrates the potential of this targeted LNP platform for anti-fibrotic therapy and opens avenues for developing new ligand-tethered lipidoids for cell and tissue types that are challenging to target using traditional LNP technologies. The progress in clinical trials of siRNA-based therapies further highlights the potential of RNA-based treatments for LF. Several siRNA therapeutics

have reached clinical trials, and some, such as those targeting HSP47, have shown promising results in reducing fibrosis in preclinical models, demonstrating the potential of this therapeutic approach [200]. Additionally, the recent FDA approvals of siRNA-based drugs for other liver diseases, such as patisiran and givosiran, reflect the growing confidence in RNA-based therapeutics and their potential applicability to LF [201].

siRNA loaded PEGylated polymeric nanoparticles have been prepared to target overexpressed IL-11 on activated HSCs (siIL11@NP-AEAA) and its cognate receptor IL11ra1 (siIL11ra1@NP-AEAA). The developed PLGA-PEG based nanoparticles have been evaluated for their physicochemical characterization to confirm the particle characteristics, in vitro cell based studies to evaluate the inhibition of HSC activation and in vivo studies to demonstrate the resolution of fibrosis in mice. Results from this research study revealed that both the nanoparticles were able to inhibit HSCs activation and resolve fibrosis effectively but siIL11ra1@NP-AEAA showed superior therapeutic effects in reducing LF and steatosis and also improving liver function. The data generated from immunostaining, migration assay and western blot analysis are shown in Fig. 4 [202].

## miRNA based therapeutics for treatment of LF

MicroRNA (miRNA) constitutes another significant class of small RNA molecules within the realm of RNA-based regulation. These molecules are characterized by their single-stranded structure [203]. miRNAs typically consist of approximately 22 nucleotides. The fundamental role of miRNAs lies in post-transcriptional gene regulation, where they exert fine-tuned control over gene expression. This regulation is primarily achieved through the recognition of specific target mRNA sequences. miRNAs form base-pairing interactions with these target mRNAs, facilitating their modulation. The target recognition process is highly precise and occurs primarily in the 3' UTR of the target mRNA. miRNAs, through complementary base pairing, bind to sequences within the 3' UTR of their target mRNAs. This binding can lead to two main outcomes: translation inhibition or mRNA degradation. The degree of complementarity between the miRNA and its target mRNA determines the specific regulatory mechanism employed. Due to their pivotal roles in regulating gene expression, miRNAs have emerged as key players in various biological processes, ranging from development and homeostasis to disease pathogenesis [204]. The ability to fine-tune gene expression post-transcriptionally underscores the significance of miRNAs in the intricate web of cellular regulation and their potential in RNA therapeutics and disease management.

In a recent study, miR-27b-3p loaded exosomes derived from mesenchymal stem cells have been prepared and

evaluated for their antifibrotic activity in CCl$_4$ induced LF in rats and the data related to qRT-PCR analysis, western blot analysis and immunofluorescence staining are shown in Fig. 5. Systemic administration of developed exosomes reduced the expression of Lysyl oxidase-like 2 (LOXL 2) and collagen crosslinking. Results from in vitro and in vivo studies also revealed the downregulation of Yes-associated protein (YAP)/LOXL2 and inhibition of aHSC after treatment with miR-27b-3p loaded exosomes. The developed liposomes also showed the prominent inhibitory effect on YAP/LOXL2 expression than blank exosomes. The inference from this research work confirms the role of miR-27b-3p in YAP/LOXL2 expression of HSC [205].

## Long non-coding RNA (lncRNA)based therapeutics for treatment of LF

Long non-coding RNA (lncRNA) represents a versatile and diverse category of RNA molecules that significantly deviates from the well-defined structure of messenger RNA (mRNA). One defining characteristic of lncRNAs is their extensive length, often spanning thousands of nucleotides [206]. One notable feature of lncRNAs is their structural diversity. Unlike the relatively uniform structure of mRNA, lncRNAs exhibit a wide range of structural variations. This structural variability enables lncRNAs to engage in an array of interactions and functions within the cell [207]. Moreover, lncRNAs partake in various cellular processes, such as cell differentiation, development, and response to environmental cues. They can act as scaffolds for protein complexes, participate in RNA splicing, and serve as molecular guides for directing cellular machinery to specific targets. The intricate roles of lncRNAs in both normal cellular processes and disease pathogenesis, with the potential to uncover new avenues for therapeutic interventions.

There is no reported research work available for the delivery of lncRNA in the form of lipid nanoparticles, liposomes, or exosomes though a lot of markers have been identified which can be target using lncRNA to treat LF. lncRNAs act as suppressors and can be used to downregulate the LF by inhibiting the activation of migration of HSC. Maternally expressed gene 3, Growth arrested specific transcript 5, Gm5091, and, Antisense noncoding RNA in the INK4 Locus are some the lncRNA discovered for the treatment of LF [208–211].

## Oligonucleotides based therapeutics for treatment of LF

Oligonucleotides are short, single-stranded sequences of nucleotides that hold immense promise in the field of RNA therapeutics. These molecules, typically composed of 20–25 nucleotides, have garnered significant attention for their role

in gene regulation and RNA-based therapies [212]. Oligonucleotides can be designed to specifically target and modulate gene expression at the transcriptional or post-transcriptional level. They offer versatility to researchers and clinicians to intervene in disease processes by silencing or modifying the expression of target genes. Notable classes of oligonucleotides include antisense oligonucleotides (ASOs), small interfering RNAs (siRNAs), and aptamers [213]. The development and application of oligonucleotides represent a rapidly growing area of research, with the potential to revolutionize the treatment of genetic disorders, cancer, and other diseases.

In a recent study, a targeted delivery system for oligonucleotide therapeutics has been developed by cholesterol-mediated seeding of protein corona on DNA nanostructures. DNA tetrahedron with trivalent cholesterol conjugation has been done to induce interactions with lipoproteins in serum, which generates the lipoprotein-associated protein corona on a DNA nanostructure in situ. The developed delivery system was developed to target TGF-β1 mRNA to ameliorate LF. In vivo study revealed the significant distribution of developed delivery system in liver compared to kidney and was found to be taken up by the hepatocytes compared to nonconjugated DNA tetrahedrons. The developed hepatocyte-preferred delivery system based on interaction with lipoproteins using the concept of protein adsorption-derived targeting could be effective in treatment of LF [214].

## Efficiency and efficacy of RNA therapeutics delivery in LF

The application of RNA therapeutics for liver fibrosis hinges critically on the efficient and effective delivery of RNA molecules to the liver. The efficacy of RNA targeted delivery is influenced by several factors, including the choice of delivery material, the specific type of RNA therapeutic (siRNA, miRNA), and the pathological state of the liver. In the context of RNA delivery for liver fibrosis therapeutics, the use of lipid nanoparticles (LNPs) has shown significant promise. Studies have demonstrated the effectiveness of LNPs in targeting liver cells, particularly in the delivery of siRNA therapeutics. Currently, LNPs are among the most advanced non-viral delivery systems for RNA therapeutics, particularly in liver-targeted applications. The liver's natural propensity to uptake lipids makes LNPs an ideal vehicle for delivering RNA molecules to hepatic cells [229]. LNPs are typically composed of an ionizable lipid, which helps encapsulate the RNA and facilitates endosomal escape, along with helper lipids, cholesterol, and polyethylene glycol (PEG)-lipids, which collectively enhance stability and distribution [230]. The efficiency of RNA delivery using LNPs has been demonstrated in several studies. For instance, in preclinical models of liver fibrosis, LNPs encapsulating siRNA
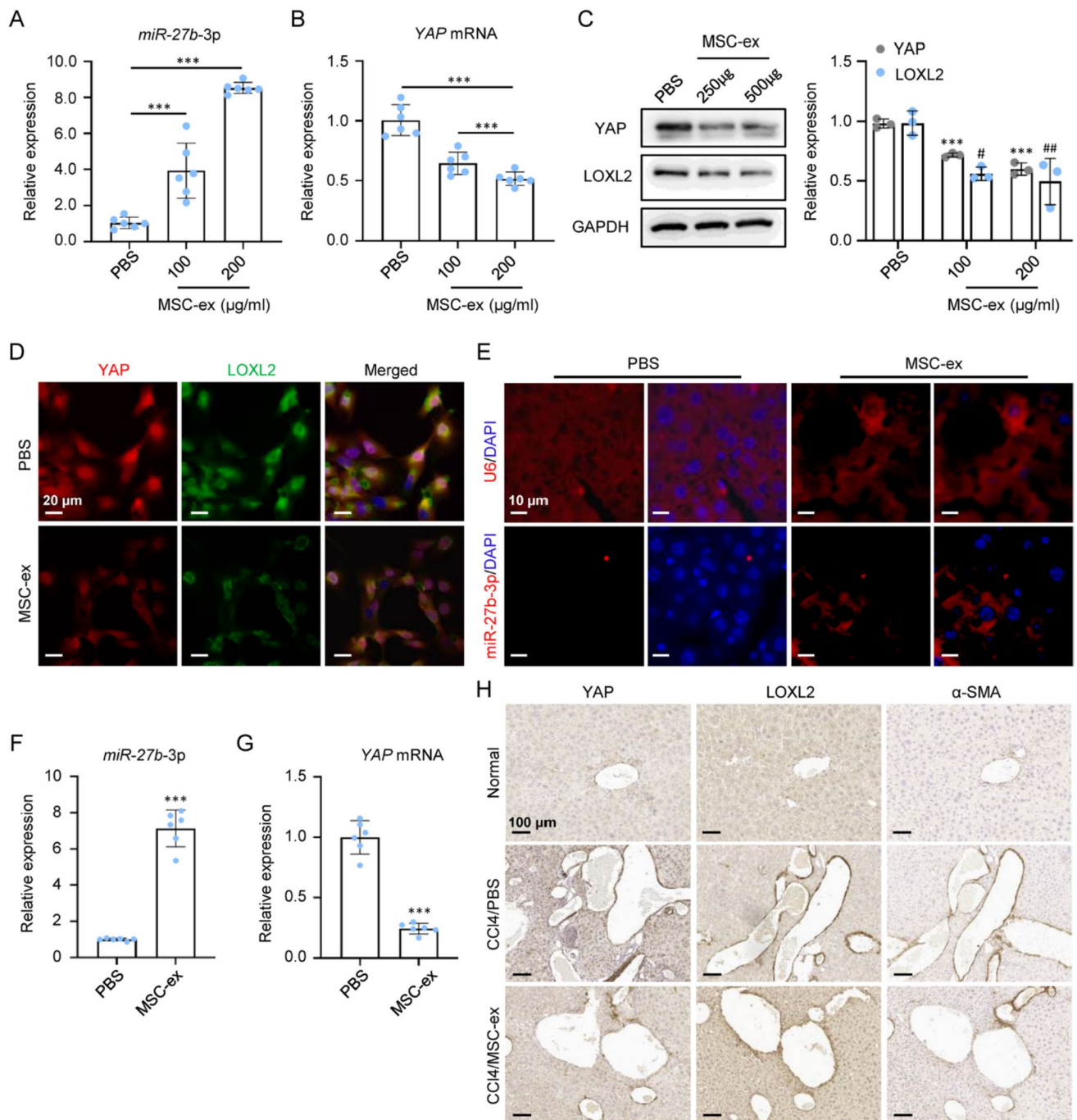
**Fig. 5** MSC-ex increased miR-27b-3p expression and downregulated YAP/LOXL2 expression. **A** qRT-PCR analysis of miR-27b-3p in PBS or MSC-ex (100 or 200 μg/mL) treated LX-2 cells (n=6; ***p < .001). **B** qRT-PCR analysis of YAP mRNA in PBS or MSC-ex (100 or 200 μg/mL) treated LX-2 cells (n=6; ***p < .001). **C** Western blot analysis and quantification of YAP protein in PBS or MSC-ex (100 or 200 μg/mL) treated LX-2 cells (n=3; ***p < .001, #p < .05, and ##p < .01). **D** Immunofluorescence staining images of YAP (Red) and LOXL2 (Green) protein in PBS or MSC-ex treated LX-2 cells. Scale bars, 20 μm. **E** FISH analysis of U6 and miR-27b-3p in PBS or MSC-ex treated fibrotic livers. Scale bars, 10 μm. **F** qRT-PCR analysis of miR-27b-3p in PBS or MSC-ex treated fibrotic livers (n=6; **p < .01). **G** qRT-PCR analysis of YAP mRNA in PBS or MSC-ex treated fibrotic livers (n=6; ***p < .001). **H** Immunohistochemistry staining for YAP, LOXL2, and α-SMA in PBS or MSC-ex treated fibrotic livers. Scale bars, 50 μm. Reproduced with permission [205]

targeting key fibrogenic genes have shown significant reductions in fibrosis markers and collagen deposition. This is indicative of effective delivery and gene silencing within hepatic cells [167]. Furthermore, the recent clinical success of mRNA-based vaccines, which utilize LNP technology, underscores the high potential of this delivery system for liver applications [231]. In summary, LNPs currently demonstrate the best efficacy in RNA delivery to the liver due to their biocompatibility, ease of uptake by the liver, and ability to encapsulate various types of RNA. Future research and ongoing clinical trials will likely continue to refine these delivery methods, improving the efficiency and efficacy of RNA therapeutics for liver fibrosis.

## RNA based therapeutics for LF in clinical investigation

Liver fibrosis is associated with other liver diseases such as non-alcoholic steatohepatitis (NASH), elevated hepatic fat content, non-alcoholic fatty liver disease (NAFLD), and liver cancer. The therapeutics treatments available in the clinic are based on small molecule drugs and large molecule drugs. Due to unavailability of a proper treatment to treat or reverse the disease, many drug delivery systems based on both small molecules and large molecules are under preclinical and clinical investigation. The clinical investigation of developed and preclinical phase passed formulations is currently in progress to bring a promising therapeutic to the clinic to treat LF. The biomarkers involved in LF have been identified and therapeutics are in development stage to target the identified biomarkers. On the other hand, research is still in progress to identify the potent biomarkers involved in fibrogenesis. Scientific community has already found some key mediators and cellular pathways which are responsible in the generation of LF. RNA based approaches are also under clinical investigation which will potentiate the degradation of fibrosis related extracellular matrix components and helpful in improving the liver functions in humans. Most of the clinical trials which are based on RNA therapeutics are in the early stages so it will be too early to judge the potential outcomes of later phases. Nevertheless, it is remarkable progress in the treatment of LF that RNA based therapeutics reach the phase of clinical investigations. Lipid nanoparticles or exosomes-based on siRNA and other RNAi therapeutics have shown their therapeutic efficacy in preclinical investigations and some of them have also been proven to be therapeutic efficacious in clinical investigations.

A RNAi therapeutic named ALN-HSD is under phase 2 of clinical investigations for its efficacy and safety evaluation in adult participants with non-alcoholic steatohepatitis with fibrosis. The incidence, progression, and changes in qFibrosis score are measured on biopsied liver by two photon excitation microscopy technique. The changes in the level of serum alanine aminotransferase, serum aspartate aminotransferase, enhanced liver fibrosis, N-terminal type III collagen propeptide, non-invasive fibrosis biomarkers, hepatic hydroxysteroid 17β dehydrogenase 13, and liver quantitative ballooning etc. from baseline to week 52 will be evaluated to examine the efficacy and safety of developed RNAi therapeutic [232].

A Vitamin A-coupled lipid nanoparticle containing siRNA against HSP47 is in phase 1b/2 of clinical trials wherein the injection formulation (ND-L02-s0201) of siRNA is being evaluated for its safety, tolerability, biological activity and pharmacokinetics in subjects with moderate to extensive hepatic fibrosis [233]. Another formulation based on siRNA targeting PNPLA3 is also under phase 1 for the evaluation of its safety and tolerability. The pharmacokinetics, potential major metabolites, immunogenicity, and effect of formulation on blood lipid profile will be assessed after single administration via subcutaneous route. The change in levels of low-density lipoprotein, high-density lipoprotein, triglyceride, and apolipoprotein B will be examined and compared with the baseline levels [234]. Two RNAi therapeutics; VIR 2218 and VIR 3434 are under phase 1 in which these therapeutics will be assessed for their pharmacokinetics, safety, and tolerability as a monotherapy or in combination in subjects with cirrhosis and Hepatic Impairment. The pharmacokinetic parameters will be estimated along with documenting the treatment-emergent adverse events and serious adverse events observed post treatment [235].

Some of the RNA therapeutics-based treatments in the clinical investigation are listed in Table 3.

## Patents for RNA based therapies to treat liver fibrosis

There are very few patents available on application of RNAi therapeutics in treatment of liver fibrosis. In a patent, US 2023/0190955 A1, a MiniVector has been synthesized which can be encapsulated in nanoparticle delivery systems and administered via intravenous route to upregulate p53 or relaxin and downregulate FOXM1, CAD11, MDM2, MDM4 and STATS for the treatment of liver fibrosis [236]. In another patent, US 2017/0101442 A1, a polypeptide ligand has been configured to bind to insulin-like growth factor 2 receptor. A nanocomplex has been developed using siRNA, cholesterol, vitamin A and IGF2R-specific peptide 431 and administered by tail vein at a siRNA dose of 0.065 mg/kg in rats with liver fibrosis. The developed peptide targeted nano delivery system to selectively target hepatic stellate cells showed higher uptake in HSCs and found to be localized in fibrotic liver in biodistribution study [237].

**Table 3** RNA based therapeutics in clinical investigation for the treatment of LF

| RNA type | Phase | Sponsor, NCT ID | Delivery system/ Route of administration | Study | Reference |
|---|---|---|---|---|---|
| siRNA (ND-L02-s0201) | 1b | Bristol-Myers Squibb, NCT02227459 | Vitamin A-coupled lipid nanoparticle containing siRNA against HSP47 / Route of administration is not available | An open label, repeat dose, dose escalation study of injection in subjects with moderate to extensive fibrosis for the evaluation of safety, tolerability, biological activity, and pharmacokinetics was performed. | [233] |
| RNAi therapeutic targeting HSD17B13 (ALN-HSD) | 2 | Regeneron Pharmaceuticals, NCT05519475 | Data related to formulation is not available/ Subcutaneous | A randomized, double-Blind, placebo-controlled, phase 2 studies of siRNA gene silencing for the treatment of non-alcoholic steatohepatitis (NASH) in participants with genetic risk factors was evaluated in patients. | [232] |
| siRNA Targeting PNPLA3 (ALN-PNP) | 1 | Regeneron Pharmaceuticals, NCT05648214 | Data related to formulation is not available/ Subcutaneous | A phase 1, randomized, double-blind, placebo-controlled, single ascending dose study was performed for the safety, tolerability, and pharmacokinetics of ALN-PNP, an siRNA targeting PNPLA3, in healthy adult participants. | [234] |
| RNAi therapeutics VIR 2218, VIR 3434 | 1 | Vir Biotechnology, Inc., NCT05484206 | Data related to formulation is not available/ Subcutaneous; VIR-2218 up to 200 mg or VIR-3434 at 300 mg or in a combination of both as monotherapy | A phase 1 open-label, single-dose, parallel-group study of the pharmacokinetics and safety of VIR-2218 and VIR-3434 monotherapy and combination therapy in adult participants with hepatic impairment. | [235] |

## Conclusions and future perspectives

This review provides a comprehensive overview of liver Fibrosis (LF), including its current status, the underlying mechanisms of its development (pathogenesis), the challenges in treating it, and promising therapeutic strategies. Specifically, it focuses on RNA-based therapeutics and the different delivery systems being developed to deliver them to their target site in the liver. The discussion on pathogenesis delves into the key players involved in LF, such as myofibroblasts, stellate cells, and inflammatory cells. This deep dive helps us understand the complex biological processes driving the disease. Furthermore, the review goes beyond the basics of RNA therapeutics and showcases relevant case studies on delivery systems like lipid nanoparticles, exosomes, and micelles. These case studies illuminate how these systems can effectively deliver RNA-based therapies to the affected areas. Animal models used to evaluate the therapeutic efficacy of these RNA-based treatments are explored in detail, alongside data from ongoing clinical trials. This highlights the promising potential of this approach. It is clear that, current treatments for LF are limited, often focusing on managing the root cause rather than directly addressing the fibrotic process. This review emphasizes the urgent need for innovative solutions that target the underlying mechanisms and potentially even reverse the damage, offering new hope for LF management. RNA-based therapeutics, particularly those utilizing advanced delivery systems, represent a groundbreaking avenue for LF treatment. These therapies hold tremendous promise based on their ability to target key pathways and genes involved in the disease progression, as demonstrated in preclinical models and early clinical trials.

Delivery systems play a crucial role in ensuring RNA therapeutics reach their target site effectively. Therefore, the review dives into targeted delivery systems under development, such as those employing ligands to specifically guide the therapeutic molecules. Additionally, it discusses advancements in nanomedicine, suggesting how formulating stimuli-responsive and biocompatible delivery systems can further improve clinical applications. As research in this field continues to progress, RNA-based therapeutics have the potential to become a cornerstone in the treatment of LF and other liver diseases, offering a brighter future for patients.

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## Declarations

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## References

1. Bataller R, Brenner DA. Liver fibrosis. J Clin Invest. 2005;115:209–18.
2. Friedman SL. Liver fibrosis – from bench to bedside. J Hepatol. 2003;38:38–53.
3. How Many People Have Liver Disease? [Internet]. 2022 [cited 2023 Dec 11]. Available from: https://liverfoundation.org/about-your-liver/facts-about-liver-disease/how-many-people-have-liver-disease/.
4. Elpek GÖ. Cellular and molecular mechanisms in the pathogenesis of liver fibrosis: An update. World J Gastroenterol. 2014;20:7260–76.
5. Zhang C-Y, Yuan W-G, He P, Lei J-H, Wang C-X. Liver fibrosis and hepatic stellate cells: Etiology, pathological hallmarks and therapeutic targets. World J Gastroenterol. 2016;22:10512–22.
6. Gressner AM, Weiskirchen R, Breitkopf K, Dooley S. Roles of TGF-beta in hepatic fibrosis. Front Biosci. 2002;7:d793–807.
7. Naito M, Hasegawa G, Ebe Y, Yamamoto T. Differentiation and function of Kupffer cells. Med Electron Microsc. 2004;37:16–28.
8. Kamada Y, Tamura S, Kiso S, Matsumoto H, Saji Y, Yoshida Y, et al. Enhanced carbon tetrachloride-induced liver fibrosis in mice lacking adiponectin. Gastroenterology. 2003;125:1796–807.
9. Arriazu E, Ruiz de Galarreta M, Cubero FJ, Varela-Rey M, Pérez de Obanos MP, Leung TM, et al. Extracellular matrix and liver disease. Antioxid Redox Signal. 2014;21:1078–97.
10. Acharya P, Chouhan K, Weiskirchen S, Weiskirchen R. Cellular Mechanisms of Liver Fibrosis. Front Pharmacol. 2021;12:671640.
11. Dewidar B, Meyer C, Dooley S, Meindl-Beinker AN. TGF-β in Hepatic Stellate Cell Activation and Liver Fibrogenesis-Updated 2019. Cells. 2019;8:1419.
12. McQuitty CE, Williams R, Chokshi S, Urbani L. Immunomodulatory Role of the Extracellular Matrix Within the Liver Disease Microenvironment. Front Immunol. 2020;11:574276.
13. Dominguez M, Miquel R, Colmenero J, Moreno M, García-Pagán J, Bosch J, et al. Hepatic Expression of CXC Chemokines Predicts Portal Hypertension and Survival in Patients With Alcoholic Hepatitis. Gastroenterology. 2009;136:1639–50.

14. Karin D, Koyama Y, Brenner D, Kisseleva T. The characteristics of activated portal fibroblasts/myofibroblasts in liver fibrosis. Differentiation. 2016;92:84–92.

15. Arthur MJP. Fibrogenesis II. Metalloproteinases and their inhibitors in liver fibrosis. Am J Physiol Gastroint Liver Physiol. 2000;279:G245-9.

16. Bataller R, Gäbele E, Schoonhoven R, Morris T, Lehnert M, Yang L, et al. Prolonged infusion of angiotensin II into normal rats induces stellate cell activation and proinflammatory events in liver. Am J Physiol Gastrointest Liver Physiol. 2003;285:642–51.

17. Sharma A, Nagalli S. Chronic Liver Disease. StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023 [cited 2023 Dec 12]. Available from: http://www.ncbi.nlm.nih.gov/books/NBK554597/.

18. Pinto RB, Schneider ACR, da Silveira TR. Cirrhosis in children and adolescents: An overview. World J Hepatol. 2015;7:392–405.

19. Fibrosis: Development [Internet]. American Liver Foundation. 2022 [cited 2023 Dec 12]. Available from: https://liverfoundation.org/about-your-liver/how-liver-diseases-progress/fibrosis-scarring/.

20. Zhu Z, Chen Y, Qin X, Liu S, Wang J, Ren H. Multidimensional landscape of non-alcoholic fatty liver disease-related disease spectrum uncovered by big omics data: Profiling evidence and new perspectives. Smart Medicine. 2023;2:e20220029.

21. Chiang DJ, Pritchard MT, Nagy LE. Obesity, diabetes mellitus, and liver fibrosis. Am J Physiol Gastrointest Liver Physiol. 2011;300:G697-702.

22. van der Windt DJ, Sud V, Zhang H, Tsung A, Huang H. The Effects of Physical Exercise on Fatty Liver Disease. Gene Expr. 2018;18:89–101.

23. Osna NA, Donohue TM, Kharbanda KK. Alcoholic Liver Disease: Pathogenesis and Current Management. Alcohol Res. 2017;38:147–61.

24. Brunt EM. Pathology of fatty liver disease. Mod Pathol. 2007;20:S40–8.

25. Patel R, Mueller M. Alcoholic Liver Disease. StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023 [cited 2023 Dec 12]. Available from: http://www.ncbi.nlm.nih.gov/books/NBK546632/.

26. Song C, Chen H, Yu B, Zhang L, Wang J, Feng C, et al. Magnetic Fields Affect Alcoholic Liver Disease by Liver Cell Oxidative Stress and Proliferation Regulation. Research. 2023;6:0097.

27. Asrani SK, Devarbhavi H, Eaton J, Kamath PS. Burden of liver diseases in the world. J Hepatol. 2019;70:151–71.

28. Ma X. Liver Fibrogenesis in Non-Alcoholic Steatohepatitis. Front Physiol [Internet]. 2012 [cited 2023 Dec 13];3. Available from: https://doi.org/10.3389/fphys.2012.00248.

29. Carpino G, Morini S, Ginanni Corradini S, Franchitto A, Merli M, Siciliano M, et al. Alpha-SMA expression in hepatic stellate cells and quantitative analysis of hepatic fibrosis in cirrhosis and in recurrent chronic hepatitis after liver transplantation. Dig Liver Dis. 2005;37:349–56.

30. Souza-Mello V. Peroxisome proliferator-activated receptors as targets to treat non-alcoholic fatty liver disease. World J Hepatol. 2015;7:1012–9.

31. Giby VG, Ajith TA. Role of adipokines and peroxisome proliferator-activated receptors in nonalcoholic fatty liver disease. World J Hepatol. 2014;6:570–9.

32. Seki E, Brenner DA. Recent advancement of molecular mechanisms of liver fibrosis. J Hepatobiliary Pancreat Sci. 2015;22:512–8.

33. Chen R-J, Wu H-H, Wang Y-J. Strategies to prevent and reverse liver fibrosis in humans and laboratory animals. Arch Toxicol. 2015;89:1727–50.

34. El-Serag HB. Epidemiology of Viral Hepatitis and Hepatocellular Carcinoma. Gastroenterology. 2012;142:1264-1273.e1.

35. Tornesello ML, Buonaguro L, Izzo F, Buonaguro FM. Molecular alterations in hepatocellular carcinoma associated with hepatitis B and hepatitis C infections. Oncotarget. 2016;7:25087–102.

36. Stanhope KL, Schwarz JM, Keim NL, Griffen SC, Bremer AA, Graham JL, et al. Consuming fructose-sweetened, not glucose-sweetened, beverages increases visceral adiposity and lipids and decreases insulin sensitivity in overweight/obese humans. J Clin Invest. 2009;119:1322–34.

37. Jang C, Hui S, Lu W, Cowan AJ, Morscher RJ, Lee G, et al. The Small Intestine Converts Dietary Fructose into Glucose and Organic Acids. Cell Metab. 2018;27:351-361.e3.

38. Mazzanti R, Arena U, Tassi R. Hepatocellular carcinoma: Where are we? World J Exp Med. 2016;6:21–36.

39. Machado MV, Diehl AM. Pathogenesis of Nonalcoholic Steatohepatitis. Gastroenterology. 2016;150:1769–77.

40. Kim JY, Garcia-Carbonell R, Yamachika S, Zhao P, Dhar D, Loomba R, et al. ER Stress Drives Lipogenesis and Steatohepatitis via Caspase-2 Activation of S1P. Cell. 2018;175:133-145.e15.

41. Maricic I, Marrero I, Eguchi A, Nakamura R, Johnson CD, Dasgupta S, et al. Differential Activation of Hepatic Invariant NKT Cell Subsets Plays a Key Role in Progression of Nonalcoholic Steatohepatitis. J Immunol. 2018;201:3017–35.

42. Malaguarnera M, Di Rosa M, Nicoletti F, Malaguarnera L. Molecular mechanisms involved in NAFLD progression. J Mol Med. 2009;87:679–95.

43. Di Rosa M, Malaguarnera L. Genetic variants in candidate genes influencing NAFLD progression. J Mol Med. 2012;90:105–18.

44. Chen J, Zhou Z-C, Yan Y, Wu S-Z, Ma T, Xuan H, et al. Characterization of aggrephagy-related genes to predict the progression of liver fibrosis from multi-omics profiles. Biomedical Technology. 2024;5:46–59.

45. Zhou D, Pan Q, Shen F, Cao H, Ding W, Chen Y, et al. Total fecal microbiota transplantation alleviates high-fat diet-induced steatohepatitis in mice via beneficial regulation of gut microbiota. Sci Rep. 2017;7:1529.

46. Gao B, Bataller R. Alcoholic Liver Disease: Pathogenesis and New Therapeutic Targets. Gastroenterology. 2011;141:1572–85.

47. Ikenaga N, Peng Z-W, Vaid KA, Liu SB, Yoshida S, Sverdlov DY, et al. Selective targeting of lysyl oxidase-like 2 (LOXL2) suppresses hepatic fibrosis progression and accelerates its reversal. Gut. 2017;66:1697–708.

48. Practice Guidelines | AASLD [Internet]. [cited 2023 Dec 13]. Available from: https://www.aasld.org/practice-guidelines.

49. Lemmers A, Moreno C, Gustot T, Maréchal R, Degré D, Demetter P, et al. The interleukin-17 pathway is involved in human alcoholic liver disease. Hepatology. 2009;49:646–57.

50. Maltby J, Wright S, Bird G, Sheron N. Chemokine levels in human liver homogenates: Associations between GRO α and histopathological evidence of alcoholic hepatitis. Hepatology. 1996;24:1156–60.

51. O'Leary JG, Pratt DS. Cholestasis and cholestatic syndromes. Curr Opin Gastroenterol. 2007;23:232.

52. Vavassori P, Mencarelli A, Renga B, Distrutti E, Fiorucci S. The Bile Acid Receptor FXR Is a Modulator of Intestinal Innate Immunity1. J Immunol. 2009;183:6251–61.

53. Wagner M, Zollner G, Trauner M. Nuclear Receptor Regulation of the Adaptive Response of Bile Acid Transporters in Cholestasis. Semin Liver Dis. 2010;30:160–77.

54. Eaton JE, Talwalkar JA, Lazaridis KN, Gores GJ, Lindor KD. Pathogenesis of Primary Sclerosing Cholangitis and Advances in Diagnosis and Management. Gastroenterology. 2013;145:521–36.

55. Huang D, Zhang X, Fu X, Zu Y, Sun W, Zhao Y. Liver spheroids on chips as emerging platforms for drug screening. Engineered Regeneration. 2021;2:246–56.

56. Kisseleva T, Brenner DA. Mechanisms of Fibrogenesis. Exp Biol Med (Maywood). 2008;233:109–22.

57. Fallowfield JA, Mizuno M, Kendall TJ, Constandinou CM, Benyon RC, Duffield JS, et al. Scar-Associated Macrophages Are a Major Source of Hepatic Matrix Metalloproteinase-13 and Facilitate the Resolution of Murine Hepatic Fibrosis1. J Immunol. 2007;178:5288–95.

58. Iwaisako K, Jiang C, Zhang M, Cong M, Moore-Morris TJ, Park TJ, et al. Origin of myofibroblasts in the fibrotic liver in mice. Proc Natl Acad Sci. 2014;111:E3297–305.

59. Schmitt-Gräff A, Krüger S, Bochard F, Gabbiani G, Denk H. Modulation of alpha smooth muscle actin and desmin expression in perisinusoidal cells of normal and diseased human livers. Am J Pathol. 1991;138:1233–42.

60. Ueno T, Sata M, Sakata R, Torimura T, Sakamoto M, Sugawara H, et al. Hepatic stellate cells and intralobular innervation in human liver cirrhosis. Hum Pathol. 1997;28:953–9.

61. Goddard CJR, Smith A, Hoyland JA, Baird P, McMahon RFT, Freemont AJ, et al. Localisation and semiquantitative assessment of hepatic procollagen mRNA in primary biliary cirrhosis. Gut. 1998;43:433–40.

62. Nishio T, Hu R, Koyama Y, Liang S, Rosenthal SB, Yamamoto G, et al. Activated hepatic stellate cells and portal fibroblasts contribute to cholestatic liver fibrosis in MDR2 knockout mice. J Hepatol. 2019;71:573–85.

63. Zavadil J, Bitzer M, Liang D, Yang Y-C, Massimi A, Kneitz S, et al. Genetic programs of epithelial cell plasticity directed by transforming growth factor-β. Proc Natl Acad Sci. 2001;98:6686–91.

64. Russo FP, Alison MR, Bigger BW, Amofah E, Florou A, Amin F, et al. The Bone Marrow Functionally Contributes to Liver Fibrosis. Gastroenterology. 2006;130:1807–21.

65. Kalluri R, Weinberg RA. The basics of epithelial-mesenchymal transition. J Clin Invest. 2009;119:1420–8.

66. Okada H, Danoff TM, Kalluri R, Neilson EG. Early role of Fsp1 in epithelial-mesenchymal transformation. American Journal of Physiology-Renal Physiology. 1997;273:F563–74.

67. Kalluri R. EMT: When epithelial cells decide to become mesenchymal-like cells. J Clin Invest. 2009;119:1417–9.

68. Chu AS, Diaz R, Hui J-J, Yanger K, Zong Y, Alpini G, et al. Lineage tracing demonstrates no evidence of cholangiocyte epithelial-to-mesenchymal transition in murine models of hepatic fibrosis. Hepatology. 2011;53:1685.

69. Seki E, De Minicis S, Österreicher CH, Kluwe J, Osawa Y, Brenner DA, et al. TLR4 enhances TGF-β signaling and hepatic fibrosis. Nat Med. 2007;13:1324–32.

70. Friedman SL. Hepatic Stellate Cells: Protean, Multifunctional, and Enigmatic Cells of the Liver. Physiol Rev. 2008;88:125–72.

71. Bosselut N, Housset C, Marcelo P, Rey C, Burmester T, Vinh J, et al. Distinct proteomic features of two fibrogenic liver cell populations: Hepatic stellate cells and portal myofibroblasts. Proteomics. 2010;10:1017–28.

72. Tuchweber B, Desmoulière A, Bochaton-Piallat ML, Rubbia-Brandt L, Gabbiani G. Proliferation and phenotypic modulation of portal fibroblasts in the early stages of cholestatic fibrosis in the rat. Lab Invest. 1996;74:265–78.

73. Desmoulière A, Darby I, Costa AM, Raccurt M, Tuchweber B, Sommer P, et al. Extracellular matrix deposition, lysyl oxidase expression, and myofibroblastic differentiation during the initial stages of cholestatic fibrosis in the rat. Lab Invest. 1997;76:765–78.

74. Koyama Y, Wang P, Liang S, Iwaisako K, Liu X, Xu J, et al. Mesothelin/mucin 16 signaling in activated portal fibroblasts regulates cholestatic liver fibrosis. J Clin Invest. 2017;127:1254–70.

75. Dranoff JA, Wells RG. Portal fibroblasts: Underappreciated mediators of biliary fibrosis. Hepatology. 2010;51:1438–44.

76. Pilling D, Fan T, Huang D, Kaul B, Gomer RH. Identification of Markers that Distinguish Monocyte-Derived Fibrocytes from Monocytes, Macrophages, and Fibroblasts. PLoS ONE. 2009;4:e7475.

77. Strieter RM, Keeley EC, Burdick MD, Mehrad B. The Role of Circulating Mesenchymal Progenitor Cells, Fibrocytes, in Promoting Pulmonary Fibrosis. Trans Am Clin Climatol Assoc. 2009;120:49–59.

78. Scholten D, Reichart D, Paik YH, Lindert J, Bhattacharya J, Glass CK, et al. Migration of Fibrocytes in Fibrogenic Liver Injury. Am J Pathol. 2011;179:189–98.

79. Bucala R, Spiegel LA, Chesney J, Hogan M, Cerami A. Circulating Fibrocytes Define a New Leukocyte Subpopulation That Mediates Tissue Repair. Mol Med. 1994;1:71–81.

80. Iredale JP. Models of liver fibrosis: exploring the dynamic nature of inflammation and repair in a solid organ. J Clin Invest. 2007;117:539–48.

81. Geerts A. History, Heterogeneity, Developmental Biology, and Functions of Quiescent Hepatic Stellate Cells. Semin Liver Dis. 2001;21:311–36.

82. Xu F, Liu C, Zhou D, Zhang L. TGF-β/SMAD Pathway and Its Regulation in Hepatic Fibrosis. J Histochem Cytochem. 2016;64:157–67.

83. Meng F, Wang K, Aoyama T, Grivennikov SI, Paik Y, Scholten D, et al. Interleukin-17 Signaling in Inflammatory, Kupffer Cells, and Hepatic Stellate Cells Exacerbates Liver Fibrosis in Mice. Gastroenterology. 2012;143:765-776.e3.

84. Dooley S, Delvoux B, Streckert M, Bonzel L, Stopa M, ten Dijke P, et al. Transforming growth factor β signal transduction in hepatic stellate cells via Smad2/3 phosphorylation, a pathway that is abrogated during in vitro progression to myofibroblasts: TGFβ signal transduction during transdifferentiation of hepatic stellate cells. FEBS Lett. 2001;502:4–10.

85. Liu Y, Meyer C, Müller A, Herweck F, Li Q, Müllenbach R, et al. IL-13 Induces Connective Tissue Growth Factor in Rat Hepatic Stellate Cells via TGF-β–Independent Smad Signaling. J Immunol. 2011;187:2814–23.

86. Karlmark KR, Weiskirchen R, Zimmermann HW, Gassler N, Ginhoux F, Weber C, et al. Hepatic recruitment of the inflammatory Gr1+ monocyte subset upon liver injury promotes hepatic fibrosis. Hepatology. 2009;50:261–74.

87. Krenkel O, Tacke F. Liver macrophages in tissue homeostasis and disease. Nat Rev Immunol. 2017;17:306–21.

88. Sudo K, Yamada Y, Moriwaki H, Saito K, Seishima M. Lack of tumor necrosis factor receptor type 1 inhibits liver fibrosis induced by carbon tetrachloride in mice. Cytokine. 2005;29:236–44.

89. Miura K, Kodama Y, Inokuchi S, Schnabl B, Aoyama T, Ohnishi H, et al. Toll-Like Receptor 9 Promotes Steatohepatitis by Induction of Interleukin-1β in Mice. Gastroenterology. 2010;139:323-334.e7.

90. de Gouville A-C, Boullay V, Krysa G, Pilot J, Brusq J-M, Loriolle F, et al. Inhibition of TGF-β signaling by an ALK5 inhibitor protects rats from dimethylnitrosamine-induced liver fibrosis. Br J Pharmacol. 2005;145:166–77.

91. Hellerbrand C, Stefanovic B, Giordano F, Burchardt ER, Brenner DA. The role of TGFβ1 in initiating hepatic stellate cell activation in vivo. J Hepatol. 1999;30:77–87.

92. Kolls JK, Lindén A. Interleukin-17 Family Members and Inflammation. Immunity. 2004;21:467–76.

93. Zenewicz LA, Yancopoulos GD, Valenzuela DM, Murphy AJ, Karow M, Flavell RA. Interleukin-22 but Not Interleukin-17

Provides Protection to Hepatocytes during Acute Liver Inflammation. Immunity. 2007;27:647–59.

94. Radaeva S, Sun R, Pan H, Hong F, Gao B. Interleukin 22 (IL-22) plays a protective role in T cell-mediated murine hepatitis: IL-22 is a survival factor for hepatocytes via STAT3 activation. Hepatology. 2004;39:1332.

95. Baeck C, Wehr A, Karlmark KR, Heymann F, Vucur M, Gassler N, et al. Pharmacological inhibition of the chemokine CCL2 (MCP-1) diminishes liver macrophage infiltration and steatohepatitis in chronic hepatic injury. Gut. 2012;61:416–26.

96. Duffield JS, Forbes SJ, Constandinou CM, Clay S, Partolina M, Vuthoori S, et al. Selective depletion of macrophages reveals distinct, opposing roles during liver injury and repair. J Clin Invest. 2005;115:56–65.

97. Ramachandran P, Pellicoro A, Vernon MA, Boulter L, Aucott RL, Ali A, et al. Differential Ly-6C expression identifies the recruited macrophage phenotype, which orchestrates the regression of murine liver fibrosis. Proc Natl Acad Sci. 2012;109:E3186–95.

98. Iredale JP. Hepatic Stellate Cell Behavior during Resolution of Liver Injury. Semin Liver Dis. 2001;21:427–36.

99. Mridha AR, Wree A, Robertson AAB, Yeh MM, Johnson CD, Van Rooyen DM, et al. NLRP3 inflammasome blockade reduces liver inflammation and fibrosis in experimental NASH in mice. J Hepatol. 2017;66:1037–46.

100. Meier A, Chien J, Hobohm L, Patras KA, Nizet V, Corriden R. Inhibition of Human Neutrophil Extracellular Trap (NET) Production by Propofol and Lipid Emulsion. Frontiers in Pharmacology [Internet]. 2019 [cited 2023 Dec 13];10. Available from: https://doi.org/10.3389/fphar.2019.00323.

101. Gehrke N, Nagel M, Straub BK, Wörns MA, Schuchmann M, Galle PR, et al. Loss of cellular FLICE-inhibitory protein promotes acute cholestatic liver injury and inflammation from bile duct ligation. Am J Physiol Gastrointest Liver Physiol. 2018;314:G319-33.

102. Moles A, Murphy L, Wilson CL, Chakraborty JB, Fox C, Park EJ, et al. A TLR2/S100A9/CXCL-2 signaling network is necessary for neutrophil recruitment in acute and chronic liver injury in the mouse. J Hepatol. 2014;60:782–91.

103. Kisseleva T, Brenner D. Molecular and cellular mechanisms of liver fibrosis and its regression. Nat Rev Gastroenterol Hepatol. 2021;18:151–66.

104. Zhu C, Kim K, Wang X, Bartolome A, Salomao M, Dongiovanni P, et al. Hepatocyte Notch activation induces liver fibrosis in nonalcoholic steatohepatitis. Sci Transl Med. 2018;10:0344.

105. Lan T, Kisseleva T, Brenner DA. Deficiency of NOX1 or NOX4 Prevents Liver Inflammation and Fibrosis in Mice through Inhibition of Hepatic Stellate Cell Activation. PLoS ONE. 2015;10:e0129743.

106. Xie G, Karaca G, Swiderska-Syn M, Michelotti GA, Krüger L, Chen Y, et al. Cross-talk between Notch and Hedgehog regulates hepatic stellate cell fate in mice. Hepatology. 2013;58:1801.

107. Lee Y-S, Kim SY, Ko E, Lee J-H, Yi H-S, Yoo YJ, et al. Exosomes derived from palmitic acid-treated hepatocytes induce fibrotic activation of hepatic stellate cells. Sci Rep. 2017;7:3710.

108. Abe R, Donnelly SC, Peng T, Bucala R, Metz CN. Peripheral Blood Fibrocytes: Differentiation Pathway and Migration to Wound Sites1. J Immunol. 2001;166:7556–62.

109. Karhadkar TR, Pilling D, Cox N, Gomer RH. Sialidase inhibitors attenuate pulmonary fibrosis in a mouse model. Sci Rep. 2017;7:15069.

110. Xu J, Cong M, Park TJ, Scholten D, Brenner DA, Kisseleva T. Contribution of bone marrow-derived fibrocytes to liver fibrosis. Hepatobiliary Surg Nutr. 2015;4:34–47.

111. Miyao M, Kotani H, Ishida T, Kawai C, Manabe S, Abiru H, et al. Pivotal role of liver sinusoidal endothelial cells in NAFLD/NASH progression. Lab Invest. 2015;95:1130–44.

112. Varin F, Huet PM. Hepatic microcirculation in the perfused cirrhotic rat liver. J Clin Invest. 1985;76:1904–12.

113. Kumar V, Xin X, Ma J, Tan C, Osna N, Mahato RI. Therapeutic targets, novel drugs, and delivery systems for diabetes associated NAFLD and liver fibrosis. Adv Drug Deliv Rev. 2021;176:113888.

114. Liedtke C, Luedde T, Sauerbruch T, Scholten D, Streetz K, Tacke F, et al. Experimental liver fibrosis research: update on animal models, legal issues and translational aspects. Fibrogenesis Tissue Repair. 2013;6:19.

115. Kurdi A, Hassan K, Venkataraman B, Rajesh M. Nootkatone confers hepatoprotective and anti-fibrotic actions in a murine model of liver fibrosis by suppressing oxidative stress, inflammation, and apoptosis. J Biochem Mol Toxicol. 2018;32:e22017.

116. Slater TF, Cheeseman KH, Ingold KU, Rice-Evans C, Sies H, Norman ROC, et al. Carbon tetrachloride toxicity as a model for studying free-radical mediated liver injury. Philosophical Transactions of the Royal Society of London B, Biological Sciences. 1997;311:633–45.

117. Chi G, Pei J, Ma Q, Ru Y-X, Feng Z. Chemical induced inflammation of the liver breaks tolerance and results in autoimmune hepatitis in Balb/c mice. Immunol Lett. 2020;218:44–50.

118. Scholten D, Trebicka J, Liedtke C, Weiskirchen R. The carbon tetrachloride model in mice. Lab Anim. 2015;49:4–11.

119. Iredale JP, Benyon RC, Pickering J, McCullen M, Northrop M, Pawley S, et al. Mechanisms of spontaneous resolution of rat liver fibrosis. Hepatic stellate cell apoptosis and reduced hepatic expression of metalloproteinase inhibitors. J Clin Invest. 1998;102:538–49.

120. Kisseleva T, Cong M, Paik Y, Scholten D, Jiang C, Benner C, et al. Myofibroblasts revert to an inactive phenotype during regression of liver fibrosis. Proc Natl Acad Sci. 2012;109:9448–53.

121. Eissa LA, Kenawy HI, El-Karef A, Elsherbiny NM, El-Mihi KA. Antioxidant and anti-inflammatory activities of berberine attenuate hepatic fibrosis induced by thioacetamide injection in rats. Chem Biol Interact. 2018;294:91–100.

122. Ding Z, Zhuo L. Attenuation of hepatic fibrosis by an imidazolium salt in thioacetamide-induced mouse model. J Gastroenterol Hepatol. 2013;28:188–201.

123. Reif S, Aeed H, Shilo Y, Reich R, Kloog Y, Kweon YO, et al. Treatment of thioacetamide-induced liver cirrhosis by the Ras antagonist, farnesylthiosalicylic acid. J Hepatol. 2004;41:235–41.

124. Salguero Palacios R, Roderfeld M, Hemmann S, Rath T, Atanasova S, Tschuschner A, et al. Activation of hepatic stellate cells is associated with cytokine expression in thioacetamide-induced hepatic fibrosis in mice. Lab Invest. 2008;88:1192–203.

125. Kang JS, Wanibuchi H, Morimura K, Wongpoomchai R, Chusiri Y, Gonzalez FJ, et al. Role of CYP2E1 in thioacetamide-induced mouse hepatotoxicity. Toxicol Appl Pharmacol. 2008;228:295–300.

126. Hajovsky H, Hu G, Koen Y, Sarma D, Cui W, Moore DS, et al. Metabolism and Toxicity of Thioacetamide and Thioacetamide S-Oxide in Rat Hepatocytes. Chem Res Toxicol. 2012;25:1955–63.

127. Yang M-C, Chang C-P, Lei H-Y. Induction of liver fibrosis in a murine hepatoma model by thioacetamide is associated with enhanced tumor growth and suppressed antitumor immunity. Lab Invest. 2010;90:1782–93.

128. Scholten D, Österreicher CH, Scholten A, Iwaisako K, Gu G, Brenner DA, et al. Genetic Labeling Does Not Detect Epithelial-to-Mesenchymal Transition of Cholangiocytes in Liver Fibrosis in Mice. Gastroenterology. 2010;139:987–98.

129. Tag CG, Sauer-Lehnen S, Weiskirchen S, Borkham-Kamphorst E, Tolba RH, Tacke F, et al. Bile Duct Ligation in Mice: Induction of Inflammatory Liver Injury and Fibrosis by Obstructive Cholestasis. J Vis Exp. 2015;10:e52438.

130. Faccioli LAP, Dias ML, Paranhos BA, dos Santos Goldenberg RC. Liver cirrhosis: An overview of experimental models in rodents. Life Sci. 2022;301:120615.

131. Chilvery S, Bansod S, Saifi MA, Godugu C. Piperlongumine attenuates bile duct ligation-induced liver fibrosis in mice via inhibition of TGF-β1/Smad and EMT pathways. Int Immunopharmacol. 2020;88:106909.

132. Tang G, Seume N, Häger C, Kumstel S, Abshagen K, Bleich A, et al. Comparing distress of mouse models for liver damage. Sci Rep. 2020;10:19814.

133. Panasevich MR, Meers GM, Linden MA, Booth FW, Perfield JW, Fritsche KL, et al. High-fat, high-fructose, high-cholesterol feeding causes severe NASH and cecal microbiota dysbiosis in juvenile Ossabaw swine. American Journal of Physiology-Endocrinology and Metabolism. 2018;314:E78-92.

134. Rinella ME. Nonalcoholic Fatty Liver Disease: A Systematic Review. JAMA. 2015;313:2263–73.

135. Ghoshal AK. New Insight into the Biochemical Pathology of Liver in Choline Deficiency. Crit Rev Biochem Mol Biol. 1995;30:263–73.

136. Ip E, Farrell G, Hall P, Robertson G, Leclercq I. Administration of the potent PPARalpha agonist, Wy-14,643, reverses nutritional fibrosis and steatohepatitis in mice. Hepatology. 2004;39:1286–96.

137. Rinella ME, Green RM. The methionine-choline deficient dietary model of steatohepatitis does not exhibit insulin resistance. J Hepatol. 2004;40:47–51.

138. Lindström P. The Physiology of Obese-Hyperglycemic Mice [*ob/ob* Mice]. Sci World J. 2007;7:666–85.

139. Larter CZ, Yeh MM. Animal models of NASH: Getting both pathology and metabolic context right. J Gastroenterol Hepatol. 2008;23:1635–48.

140. Iseri OA, Lieber CS, Gottlieb LS. The ultrastructure of fatty liver induced by prolonged ethanol ingestion. Am J Pathol. 1966;48:535–55.

141. Bala S, Petrasek J, Mundkur S, Catalano D, Levin I, Ward J, et al. Circulating microRNAs in exosomes indicate hepatocyte injury and inflammation in alcoholic, drug-induced, and inflammatory liver diseases. Hepatology. 2012;56:1946–57.

142. Rouach H, Fataccioli V, Gentil M, French SW, Morimoto M, Nordmann R. Effect of chronic ethanol feeding on lipid peroxidation and protein oxidation in relation to liver pathology. Hepatology. 1997;25:351–5.

143. Tsukamoto H, French SW, Benson N, Delgado G, Rao GA, Larkin EC, et al. Severe and progressive steatosis and focal necrosis in rat liver induced by continuous intragastric infusion of ethanol and low fat diet. Hepatology. 1985;5:224–32.

144. Iimuro Y, Ikejima K, Rose ML, Bradford BU, Thurman RG. Nimodipine, a dihydropyridine-type calcium channel blocker, prevents alcoholic hepatitis caused by chronic intragastric ethanol exposure in the rat. Hepatology. 1996;24:391–7.

145. Chiang DJ, Roychowdhury S, Bush K, McMullen MR, Pisano S, Niese K, et al. Adenosine 2A Receptor Antagonist Prevented and Reversed Liver Fibrosis in a Mouse Model of Ethanol-Exacerbated Liver Fibrosis. PLoS ONE. 2013;8:e69114.

146. Jeong W, Park O, Gao B. Abrogation of the Antifibrotic Effects of Natural Killer Cells/Interferon-γ Contributes to Alcohol Acceleration of Liver Fibrosis. Gastroenterology. 2008;134:248–58.

147. Van Spronsen FJ, Thomasse Y, Smit GPA, Leonard JV, Clayton PT, Fidler V, et al. Hereditary tyrosinemia type I: A new clinical classification with difference in prognosis on dietary treatment. Hepatology. 1994;20:1187–91.

148. Tanguay RM, Valet JP, Lescault A, Duband JL, Laberge C, Lettre F, et al. Different molecular basis for fumarylacetoacetate hydrolase deficiency in the two clinical forms of hereditary tyrosinemia (type I). Am J Hum Genet. 1990;47:308–16.

149. Lindblad B, Lindstedt S, Steen G. On the enzymic defects in hereditary tyrosinemia. Proc Natl Acad Sci. 1977;74:4641–5.

150. Endig J, Buitrago-Molina LE, Marhenke S, Reisinger F, Saborowski A, Schütt J, et al. Dual Role of the Adaptive Immune System in Liver Injury and Hepatocellular Carcinoma Development. Cancer Cell. 2016;30:308–23.

151. Grompe M, al-Dhalimy M, Finegold M, Ou CN, Burlingame T, Kennaway NG, et al. Loss of fumarylacetoacetate hydrolase is responsible for the neonatal hepatic dysfunction phenotype of lethal albino mice. Genes Dev. 1993;7:2298–307.

152. Wangensteen KJ, Wilber A, Keng VW, He Z, Matise I, Wangensteen L, et al. A facile method for somatic, lifelong manipulation of multiple genes in the mouse liver. Hepatology. 2008;47:1714–24.

153. Lindstedt S, Holme E, Lock EA, Hjalmarson O, Strandvik B. Treatment of hereditary tyrosinaemia type I by inhibition of 4-hydroxyphenylpyruvate dioxygenase. Lancet. 1992;340:813–7.

154. Hickey RD, Mao SA, Glorioso J, Lillegard JB, Fisher JE, Amiot B, et al. Fumarylacetoacetate hydrolase deficient pigs are a novel large animal model of metabolic liver disease. Stem Cell Res. 2014;13:144–53.

155. Tarlow BD, Pelz C, Naugler WE, Wakefield L, Wilson EM, Finegold MJ, et al. Bipotential adult liver progenitors are derived from chronically injured mature hepatocytes. Cell Stem Cell. 2014;15:605–18.

156. Huang P, Zhang L, Gao Y, He Z, Yao D, Wu Z, et al. Direct Reprogramming of Human Fibroblasts to Functional and Expandable Hepatocytes. Cell Stem Cell. 2014;14:370–84.

157. Azuma H, Paulk N, Ranade A, Dorrell C, Al-Dhalimy M, Ellis E, et al. Robust expansion of human hepatocytes in Fah−/−/Rag2−/−/Il2rg−/− mice. Nat Biotechnol. 2007;25:903–10.

158. Lagasse E, Connors H, Al-Dhalimy M, Reitsma M, Dohse M, Osborne L, et al. Purified hematopoietic stem cells can differentiate into hepatocytes in vivo. Nat Med. 2000;6:1229–34.

159. Wang X, Al-Dhalimy M, Lagasse E, Finegold M, Grompe M. Liver Repopulation and Correction of Metabolic Liver Disease by Transplanted Adult Mouse Pancreatic Cells. Am J Pathol. 2001;158:571–9.

160. Marhenke S, Lamlé J, Buitrago-Molina LE, Cañón JMF, Geffers R, Finegold M, et al. Activation of nuclear factor E2-related factor 2 in hereditary tyrosinemia type 1 and its role in survival and tumor development. Hepatology. 2008;48:487–96.

161. Trefts E, Gannon M, Wasserman DH. The liver. Curr Biol. 2017;27:R1147–51.

162. McHutchison J, Goodman Z, Patel K, Makhlouf H, Rodriguez-Torres M, Shiffman M, et al. Farglitazar Lacks Antifibrotic Activity in Patients With Chronic Hepatitis C Infection. Gastroenterology. 2010;138:1365-1373.e2.

163. Peng W, Cheng S, Bao Z, Wang Y, Zhou W, Wang J, et al. Advances in the research of nanodrug delivery system for targeted treatment of liver fibrosis. Biomed Pharmacother. 2021;137:111342.

164. Wohlleber D, Knolle PA. The role of liver sinusoidal cells in local hepatic immune surveillance. Clin Transl Immunol. 2016;5:e117.

165. Senoo H, Yoshikawa K, Morii M, Miura M, Imai K, Mezaki Y. Hepatic stellate cell (vitamin A-storing cell) and its relative – past, present and future. Cell Biol Int. 2010;34:1247–72.

166. Kamanzi A, Gu Y, Tahvildari R, Friedenberger Z, Zhu X, Berti R, et al. Simultaneous, Single-Particle Measurements of Size and Loading Give Insights into the Structure of Drug-Delivery Nanoparticles. ACS Nano. 2021;15:19244–55.

167. Han X, Gong N, Xue L, Billingsley MM, El-Mayta R, Shepherd SJ, et al. Ligand-tethered lipid nanoparticles for targeted RNA delivery to treat liver fibrosis. Nat Commun. 2023;14:75.

168. He C, Zheng S, Luo Y, Wang B. Exosome Theranostics: Biology and Translational Medicine. Theranostics. 2018;8:237–55.

169. Zhang J, Li S, Li L, Li M, Guo C, Yao J, et al. Exosome and Exosomal MicroRNA: Trafficking, Sorting, and Function. Genomics Proteomics Bioinformatics. 2015;13:17–24.

170. Tkach M, Théry C. Communication by Extracellular Vesicles: Where We Are and Where We Need to Go. Cell. 2016;164:1226–32.

171. Chen L, Brenner DA, Kisseleva T. Combatting Fibrosis: Exosome-Based Therapies in the Regression of Liver Fibrosis. Hepatol Commun. 2018;3:180–92.

172. Cheng CJ, Tietjen GT, Saucier-Sawyer JK, Saltzman WM. A holistic approach to targeting disease with polymeric nanoparticles. Nat Rev Drug Discov. 2015;14:239–47.

173. Dilliard SA, Siegwart DJ. Passive, active and endogenous organ-targeted lipid and polymer nanoparticles for delivery of genetic drugs. Nat Rev Mater. 2023;8:282–300.

174. Hwang D, Ramsey JD, Kabanov AV. Polymeric micelles for the delivery of poorly soluble drugs: From nanoformulation to clinical approval. Adv Drug Deliv Rev. 2020;156:80–118.

175. Hall A, Lächelt U, Bartek J, Wagner E, Moghimi SM. Polyplex Evolution: Understanding Biology. Optimizing Performance Molecular Therapy. 2017;25:1476–90.

176. Kowalski PS, Capasso Palmiero U, Huang Y, Rudra A, Langer R, Anderson DG. Ionizable Amino-Polyesters Synthesized via Ring Opening Polymerization of Tertiary Amino-Alcohols for Tissue Selective mRNA Delivery. Adv Mater. 2018;30:1801151.

177. Liu S, Wang X, Yu X, Cheng Q, Johnson LT, Chatterjee S, et al. Zwitterionic Phospholipidation of Cationic Polymers Facilitates Systemic mRNA Delivery to Spleen and Lymph Nodes. J Am Chem Soc. 2021;143:21321–30.

178. Holm A, Løvendorf MB, Kauppinen S. Development of siRNA Therapeutics for the Treatment of Liver Diseases. In: Ditzel HJ, Tuttolomondo M, Kauppinen S, editors. Design and Delivery of SiRNA Therapeutics [Internet]. New York, NY: Springer US; 2021 [cited 2023 Dec 13]. p. 57–75. Available from: https://doi.org/10.1007/978-1-0716-1298-9_5.

179. Mukai H, Ogawa K, Kato N, Kawakami S. Recent advances in lipid nanoparticles for delivery of nucleic acid, mRNA, and gene editing-based therapeutics. Drug Metab Pharmacokinet. 2022;44:100450.

180. Alshaer W, Zureigat H, Al Karaki A, Al-Kadash A, Gharaibeh L, Hatmal MM, et al. siRNA: Mechanism of action, challenges, and therapeutic approaches. Eur J Pharmacol. 2021;905:174178.

181. Fabian MR, Sonenberg N. The mechanics of miRNA-mediated gene silencing: a look under the hood of miRISC. Nat Struct Mol Biol. 2012;19:586–93.

182. Woitok MM, Zoubek ME, Doleschel D, Bartneck M, Mohamed MR, Kießling F, et al. Lipid-encapsulated siRNA for hepatocyte-directed treatment of advanced liver disease. Cell Death Dis. 2020;11:1–14.

183. Dammes N, Peer D. Paving the Road for RNA Therapeutics. Trends Pharmacol Sci. 2020;41:755–75.

184. Moore PB, Steitz TA. The Roles of RNA in the Synthesis of Protein. Cold Spring Harb Perspect Biol. 2011;3:a003780.

185. Cech TR, Steitz JA. The noncoding RNA revolution-trashing old rules to forge new ones. Cell. 2014;157:77–94.

186. Zhang J, Fei Y, Sun L, Zhang QC. Advances and opportunities in RNA structure experimental determination and computational modeling. Nat Methods. 2022;19:1193–207.

187. Ganser LR, Kelly ML, Herschlag D, Al-Hashimi HM. The roles of structural dynamics in the cellular functions of RNAs. Nat Rev Mol Cell Biol. 2019;20:474–89.

188. Mauger DM, Cabral BJ, Presnyak V, Su SV, Reid DW, Goodman B, et al. mRNA structure regulates protein expression through changes in functional half-life. Proc Natl Acad Sci. 2019;116:24075–83.

189. Leppek K, Byeon GW, Kladwang W, Wayment-Steele HK, Kerr CH, Xu AF, et al. Combinatorial optimization of mRNA structure, stability, and translation for RNA-based therapeutics. Nat Commun. 2022;13:1536.

190. Leppek K, Das R, Barna M. Functional 5′ UTR mRNA structures in eukaryotic translation regulation and how to find them. Nat Rev Mol Cell Biol. 2018;19:158–74.

191. Jia L, Mao Y, Ji Q, Dersh D, Yewdell JW, Qian S-B. Decoding mRNA translatability and stability from the 5′ UTR. Nat Struct Mol Biol. 2020;27:814–21.

192. Younis MA, Sato Y, Elewa YHA, Kon Y, Harashima H. Self-homing nanocarriers for mRNA delivery to the activated hepatic stellate cells in liver fibrosis. J Control Release. 2023;353:685–98.

193. Zhao Y, Yu Y, Wang S, Li J, Teng L. Small extracellular vesicles encapsulating lefty1 mRNA inhibit hepatic fibrosis. Asian J Pharm Sci. 2022;17:630–40.

194. Yang T, Poenisch M, Khanal R, Hu Q, Dai Z, Li R, et al. Therapeutic HNF4A mRNA attenuates liver fibrosis in a preclinical model. J Hepatol. 2021;75:1420–33.

195. Fire A, Xu S, Montgomery MK, Kostas SA, Driver SE, Mello CC. Potent and specific genetic interference by double-stranded RNA in Caenorhabditis elegans. Nature. 1998;391:806–11.

196. Hannon GJ. RNA interference. Nature. 2002;418:244–51.

197. Reynolds A, Leake D, Boese Q, Scaringe S, Marshall WS, Khvorova A. Rational siRNA design for RNA interference. Nat Biotechnol. 2004;22:326–30.

198. Valdés JJ, Miller AD. New opportunities for designing effective small interfering RNAs. Sci Rep. 2019;9:16146.

199. Zhang J, Shen H, Xu J, Liu L, Tan J, Li M, et al. Liver-Targeted siRNA Lipid Nanoparticles Treat Hepatic Cirrhosis by Dual Antifibrotic and Anti-inflammatory Activities. ACS Nano. 2020;14:6305–22.

200. Zhao Z, Lin C-Y, Cheng K. siRNA- and miRNA-based therapeutics for liver fibrosis. Transl Res. 2019;214:17–29.

201. Kulkarni JA, Cullis PR, van der Meel R. Lipid Nanoparticles Enabling Gene Therapies: From Concepts to Clinical Utility. Nucleic Acid Ther. 2018;28:146–57.

202. Zhang C, Teng Y, Li F, Ho W, Bai X, Xu X, et al. Nanoparticle-Mediated RNA Therapy Attenuates Nonalcoholic Steatohepatitis and Related Fibrosis by Targeting Activated Hepatic Stellate Cells. ACS Nano. 2023;17:14852–70.

203. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell. 2004;116:281–97.

204. Krol J, Loedige I, Filipowicz W. The widespread regulation of microRNA biogenesis, function and decay. Nat Rev Genet. 2010;11:597–610.

205. Cheng F, Yang F, Wang Y, Zhou J, Qian H, Yan Y. Mesenchymal stem cell-derived exosomal miR-27b-3p alleviates liver fibrosis via downregulating YAP/LOXL2 pathway. Journal of Nanobiotechnology. 2023;21:195.

206. Qi P, Du X. The long non-coding RNAs, a new cancer diagnostic and therapeutic gold mine. Mod Pathol. 2013;26:155–65.

207. Mercer TR, Dinger ME, Mattick JS. Long non-coding RNAs: insights into functions. Nat Rev Genet. 2009;10:155–9.

208. Chen M-J, Wang X-G, Sun Z-X, Liu X-C. Diagnostic value of LncRNA-MEG3 as a serum biomarker in patients with hepatitis B complicated with liver fibrosis. Eur Rev Med Pharmacol Sci. 2019;23:4360–7.

209. Yu F, Zheng J, Mao Y, Dong P, Lu Z, Li G, et al. Long Non-coding RNA Growth Arrest-specific Transcript 5 (GAS5) Inhibits Liver Fibrogenesis through a Mechanism of Competing Endogenous RNA *. J Biol Chem. 2015;290:28286–98.

210. Zhou B, Yuan W, Li X. LncRNA Gm5091 alleviates alcoholic hepatic fibrosis by sponging miR-27b/23b/24 in mice. Cell Biol Int. 2018;42:1330–9.

211. Yang J-J, Yang Y, Zhang C, Li J, Yang Y. Epigenetic silencing of LncRNA ANRIL enhances liver fibrosis and HSC activation through activating AMPK pathway. J Cell Mol Med. 2020;24:2677–87.

212. Dias N, Stein CA. Antisense Oligonucleotides: Basic Concepts and Mechanisms. Mol Cancer Ther. 2002;1:347–55.

213. Egli M, Manoharan M. Chemistry, structure and function of approved oligonucleotide therapeutics. Nucleic Acids Res. 2023;51:2529–73.

214. Kim K-R, Kim J, Back JH, Lee JE, Ahn D-R. Cholesterol-Mediated Seeding of Protein Corona on DNA Nanostructures for Targeted Delivery of Oligonucleotide Therapeutics to Treat Liver Fibrosis. ACS Nano. 2022;16:7331–43.

215. Yang T, Li C, Wang X, Zhao D, Zhang M, Cao H, et al. Efficient hepatic delivery and protein expression enabled by optimized mRNA and ionizable lipid nanoparticle. Bioactive Materials. 2020;5:1053–61.

216. Tian S, Zhou X, Zhang M, Cui L, Li B, Liu Y, et al. Mesenchymal stem cell-derived exosomes protect against liver fibrosis via delivering miR-148a to target KLF6/STAT3 pathway in macrophages. Stem Cell Res Ther. 2022;13:330.

217. Kumar V, Kumar V, Luo J, Mahato RI. Therapeutic Potential of OMe-PS-miR-29b1 for Treating Liver Fibrosis. Mol Ther. 2018;26:2798–811.

218. Jia Z, Gong Y, Pi Y, Liu X, Gao L, Kang L, et al. pPB Peptide-Mediated siRNA-Loaded Stable Nucleic Acid Lipid Nanoparticles on Targeting Therapy of Hepatic Fibrosis. Mol Pharmaceutics. 2018;15:53–62.

219. Qiao J-B, Fan Q-Q, Zhang C-L, Lee J, Byun J, Xing L, et al. Hyperbranched lipoid-based lipid nanoparticles for bidirectional regulation of collagen accumulation in liver fibrosis. J Control Release. 2020;321:629–40.

220. Younis MA, Sato Y, Elewa YHA, Harashima H. Reprogramming activated hepatic stellate cells by siRNA-loaded nanocarriers reverses liver fibrosis in mice. J Control Release. 2023;361:592–603.

221. Liu M-X, Xu L, Cai Y-T, Wang R-J, Gu Y-Y, Liu Y-C, et al. Carbon Nitride-Based siRNA Vectors with Self-Produced O2 Effects for Targeting Combination Therapy of Liver Fibrosis via HIF-1α-Mediated TGF-β1/Smad Pathway. Adv Healthcare Mater. 2023;12:2301485.

222. Huang J, Huang H, Wang Y, Xu B, Lin M, Han S, et al. Retinol-binding protein-hijacking nanopolyplex delivering siRNA to cytoplasm of hepatic stellate cell for liver fibrosis alleviation. Biomaterials. 2023;299:122134.

223. Tang M, Guo C, Sun M, Zhou H, Peng X, Dai J, et al. Effective delivery of osteopontin small interference RNA using exosomes suppresses liver fibrosis via TGF-β1 signaling. Front Pharmacol [Internet]. 2022 [cited 2023 Dec 14];13. Available from: https://doi.org/10.3389/fphar.2022.882243.

224. Wu P, Luo X, Sun M, Sun B, Sun M. Synergetic regulation of kupffer cells, extracellular matrix and hepatic stellate cells with versatile CXCR4-inhibiting nanocomplex for magnified therapy in liver fibrosis. Biomaterials. 2022;284:121492.

225. Jain A, Barve A, Zhao Z, Fetse JP, Liu H, Li Y, et al. Targeted Delivery of an siRNA/PNA Hybrid Nanocomplex Reverses Carbon Tetrachloride-Induced Liver Fibrosis. Advanced Therapeutics. 2019;2:1900046.

226. Ullah A, Chen G, Hussain A, Khan H, Abbas A, Zhou Z, et al. Cyclam-Modified Polyethyleneimine for Simultaneous TGFβ siRNA Delivery and CXCR4 Inhibition for the Treatment of CCl4-Induced Liver Fibrosis. Int J Nanomed. 2021;16:4451–70.

227. Tang M, Chen Y, Li B, Sugimoto H, Yang S, Yang C, et al. Therapeutic targeting of STAT3 with small interference RNAs and antisense oligonucleotides embedded exosomes in liver fibrosis. FASEB J. 2021;35:e21557.

228. He F, Li W-N, Li X-X, Yue K-Y, Duan J-L, Ruan B, et al. Exosome-mediated delivery of RBP-J decoy oligodeoxynucleotides ameliorates hepatic fibrosis in mice. Theranostics. 2022;12:1816–28.

229. Hou X, Zaks T, Langer R, Dong Y. Lipid nanoparticles for mRNA delivery. Nat Rev Mater. 2021;6:1078–94.

230. Let's talk about lipid nanoparticles. Nat Rev Mater. 2021;6:99–99.

231. Wang MM, Wappelhorst CN, Jensen EL, Chi Y-CT, Rouse JC, Zou Q. Elucidation of lipid nanoparticle surface structure in mRNA vaccines. Sci Rep. 2023;13:16744.

232. Regeneron Pharmaceuticals. A Master Protocol of Randomized, Double-Blind, Placebo-Controlled, Phase 2 Studies of siRNA Gene Silencing for the Treatment of Non-Alcoholic Steatohepatitis (NASH) in Participants With Genetic Risk Factors [Internet]. clinicaltrials.gov; 2023 Dec. Report No.: NCT05519475. Available from: https://clinicaltrials.gov/study/NCT05519475.

233. Bristol-Myers Squibb. A Phase 1b/2, Open Label, Randomized, Repeat Dose, Dose Escalation Study to Evaluate the Safety, Tolerability, Biological Activity, and Pharmacokinetics of ND-L02-s0201 Injection, A Vitamin A-coupled Lipid Nanoparticle Containing siRNA Against HSP47, in Subjects With Moderate to Extensive Hepatic Fibrosis (METAVIR F3-4) [Internet]. clinicaltrials.gov; 2017 May. Report No.: NCT02227459. Available from: https://clinicaltrials.gov/study/NCT02227459.

234. Regeneron Pharmaceuticals. A Phase 1, Randomized, Double-blind, Placebo-Controlled, Single Ascending Dose Study of the Safety, Tolerability, and Pharmacokinetics of ALN-PNP, an siRNA Targeting PNPLA3, in Healthy Adult Participants [Internet]. clinicaltrials.gov; 2023 Jan. Report No.: NCT05648214. Available from: https://clinicaltrials.gov/study/NCT05648214.

235. Vir Biotechnology, Inc. A Phase 1 Open-Label, Single-Dose, Parallel-Group Study of the Pharmacokinetics and Safety of VIR-2218 and VIR-3434 Monotherapy and Combination Therapy in Adult Participants With Hepatic Impairment [Internet]. clinicaltrials.gov; 2023 Apr. Report No.: NCT05484206. Available from: https://clinicaltrials.gov/study/NCT05484206.

236. Zechiedrich EL, Arevalo-Soliz LM, JR DJC, FOGG JM, Coker CE, Agarwal S. Treatment of liver cancer or liver fibrosis [Internet]. 2023 [cited 2024 Jan 22]. Available from: https://patents.google.com/patent/US20230190955A1/en?oq=US20230190955A1.

237. Cheng K, Chen Z. Peptide Ligands for Hepatic Stellate Cells [Internet]. 2017 [cited 2024 Jan 22]. Available from: https://patents.google.com/patent/US20170101442A1/en?oq=US20170101442A1.