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Review Article

Mouse Models for the Study of Liver Fibrosis Regression *In Vivo* and *Ex Vivo*

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Abstract

This review discussed experimental mouse models used in the pre-clinical study of liver fibrosis regression, a pivotal process in preventing the progression of metabolic dysfunction-associated steatohepatitis to irreversible liver cirrhosis. These models provide a valuable resource for understanding the cellular and molecular processes underlying fibrosis regression in different contexts. The primary focus of this review is on the most commonly used models with diet- or hepatotoxin-induced fibrosis, but it also touches upon genetic models and mouse models with biliary atresia or parasite-induced fibrosis. In addition to emphasizing *in vivo* models, we briefly summarized current *in vitro* approaches designed for studying fibrosis regression and provided an outlook on evolving methodologies that aim to refine and reduce the number of experimental animals needed for these studies. Together, these models contribute significantly to unraveling the underlying mechanisms of liver fibrosis regression and offer insights into potential therapeutic interventions. By presenting a comprehensive overview of these models and highlighting their respective advantages and limitations, this review serves as a roadmap for future research.

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Introduction

Metabolic dysfunction-associated steatotic liver disease (MASLD), previously known as non-alcoholic fatty liver disease (NAFLD), is estimated to affect 25–30% of the world's population and is strongly associated with other metabolic diseases, such as obesity, type 2 diabetes mellitus, and atherosclerotic cardiovascular disease, which are continuously on the rise.[1] Over time, simple hepatic steatosis, characterized by excess lipid deposition in the liver, induces liver inflammation, marking the onset of metabolic dysfunction-associated steatohepatitis (MASH), which affects 25% of individuals with MASLD. Driven by steatohepatitis, scarring and thickening of the liver tissue, known as fibrosis, occurs. This process is reversible early on but may ultimately progress to the irreversible replacement of too much functioning tissue and lead to cirrhosis, a significant impairment of liver function. In addition to MASH, multiple other chronic conditions can cause liver fibrosis, including alcohol abuse, viral or parasitic hepatitis, biliary obstruction, and hemochromatosis.[2,3] End-stage liver disease is currently only treatable with liver transplantation.[4] Coincidentally, liver failure due to end-stage MASH has become the most frequent reason for liver transplantation, while the availability of donor livers has at the same time decreased due to the high rate of organ donors with liver steatosis, highlighting an imminent need for improvement of treatments.[5] Accordingly, this review will primarily discuss fibrosis and fibrosis regression in the context of MASH, although many disease development and regression processes are shared with other chronic liver diseases.

The development of pharmacological treatments for MASH is making great strides, with the first compound, the liver-directed thyroid hormone receptor agonist resmetirom,[6] recently receiving conditional approval from the U.S. Food and Drug Administration for the treatment of adults with noncirrhotic MASH with moderate to advanced fibrosis, to be used in conjunction with diet and exercise.[7] Approval is also on the horizon for other candidates, such as FGF21,[8] combined glucose-dependent insulinotropic polypeptide receptor (GIPR)/glucagon-like peptide-1 receptor (GLP1R) agonists such as tirzepatide,[9] and dual glucagon receptor/GLP1R agonists such as survodutide.[10]

The prevention and reversal of liver fibrosis is an important clinical benchmark, and suitable pre-clinical research models are essential for the development of effective treatments for liver fibrosis. For this purpose, *in vitro* organoids that include fibrosis-mediating hepatic stellate cells (HSCs) are continuously being improved.[11] However, to study the interaction with other metabolic tissues in the body and inflammatory processes, animal studies remain paramount for now. Interestingly, resmetirom, tirzepatide, and survodutide have all been shown to lower the fibrosis score in human MASH, seemingly without directly affecting HSCs.[9,10,12] This suggests that the reduction of intrahepatic lipids and inflammation may be sufficient for the regression of liver fibrosis, underscoring the value of *in vivo* models where these processes can be studied in a whole-body context.

Keywords: Liver fibrosis; Liver steatosis; Metabolic dysfunction-associated steatotic liver disease; Metabolic dysfunction-associated steatohepatitis; Fibrosis regression; Mouse models of liver fibrosis.

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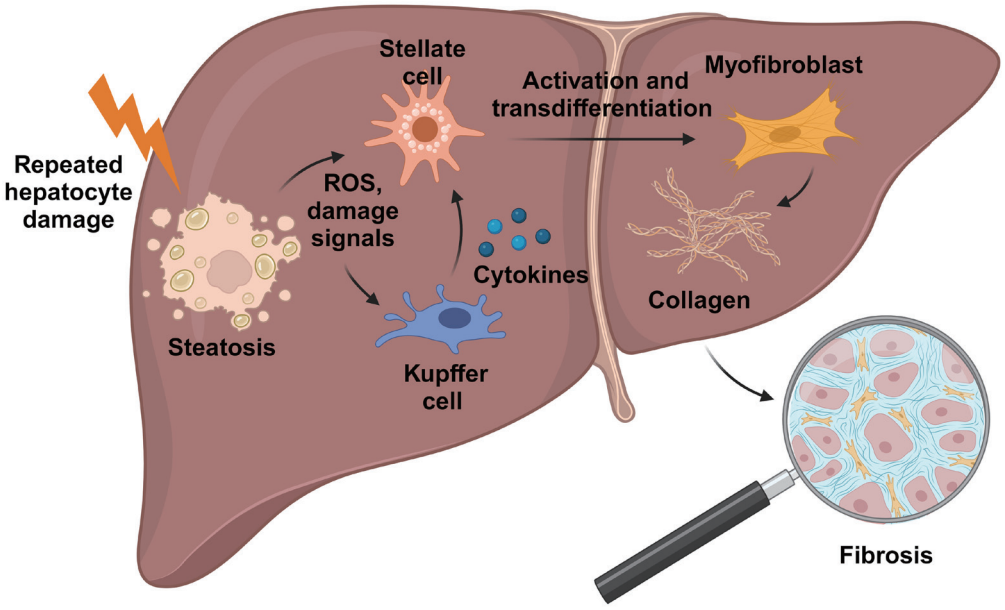

Fig. 1. Schematic overview of the development of liver fibrosis in MASLD. MASLD, Metabolic dysfunction-associated steatotic liver disease; ROS, reactive oxygen species.

A large number of experimental mouse models for MASLD, ranging from diet-induced obesity models to genetic models, are available, though not all models develop all hallmarks of human fibrotic steatohepatitis. This review focuses on mouse models that develop hepatic fibrosis, particularly those in which fibrosis can be reversed, allowing for the study of fibrosis regression.

Liver fibrosis

Fibrogenesis occurs in response to repeated or continuous liver damage from processes such as lipid overload, toxin exposure (e.g., alcohol), infections, bile salt accumulation, or metal poisoning, and it results from insufficient degradation of the fibrillar extracellular matrix produced during the damage repair process. The molecular regulation of fibrogenesis is complex, but in MASH appears to be primarily driven by reactive oxygen species that form, for example, during lipid overload in cells. Reactive oxygen species activate fibroblasts and HSCs, which can transdifferentiate into profibrotic myofibroblasts.[13] This transdifferentiation is also mediated by activated hepatic macrophages through the secretion of pro-inflammatory cytokines such as TGF-β, and clinical trials with compounds that inhibit monocyte migration to the liver have reported anti-fibrotic effects.[14,15] A schematic overview of the development of liver fibrosis in MASLD is shown in Figure 1. Once the inflammation subsides, hepatic macrophages switch to a more fibrolytic phenotype and express matrix metalloproteases that degrade the extracellular matrix, leading to the regression of fibrosis, making this a dynamic process. This indicates that the best models for fibrotic MASH mimic not only the primary cellular contributors but also the inflammatory microenvironment and the metabolic (dys)regulation in the human liver that causes the onset of inflammation in the first place.

The severity of hepatic fibrosis is commonly scored histologically in four stages, which are assessed in addition to the MASLD activity score. This score evaluates the severity of steatosis (graded 0–3), liver cell injury such as ballooning (graded 0–2), and lobular inflammation (graded 0–3).[16] The stages of fibrosis are typically defined as: 0 = no fibrosis, 1 = mild perisinusoidal/pericellular fibrosis (described as resembling chicken wire around the central vein of a liver lobule), 2 = perisinusoidal/pericellular and portal/periportal fibrosis (with dense collagen deposits around the portal vein), 3 = bridging fibrosis (where mature fibrous tissue forms a band connecting the portal area to the central vein), and 4 = cirrhosis (characterized by nodules of cells surrounded by thick, connecting fibrotic septa).[17] In some scoring systems, such as the NASH Clinical Research Network score, stage 1 is further subdivided into 1a–c to distinguish the severity of perisinusoidal fibrosis. The MASLD activity scoring and fibrosis staging systems have been found to be applicable to rodent models and are widely used in mouse studies.[18] During fibrosis regression, fibrous septa thin out and become fragmented, with hepatocytes pushing between the scar tissue. This is followed by vascular remodeling and the reestablishment of the normal architecture of the hepatic trabeculae.[19]

Mouse models for the study of liver fibrosis

Firstly, due to the highly comparable clinical profiles of NAFLD and MASLD,[20] we regard mouse models previously described for the study of NAFLD as still applicable under the new nomenclature. The most commonly used mouse models in preclinical studies of MASLD are wild-type (C57BL/6) mice on a high-fat diet (HFD; often supplemented with lard, cholesterol, sucrose, or fructose to induce more liver damage[21–23]) and various genetically modified mice on similar diets. All these models have been reviewed extensively before, highlighting their advantages and disadvantages for studying certain disease hallmarks.[24–27] Particularly when focusing on the development of fibrotic MASH, differences between the models become evident.[28,29]

For instance, C57BL/6 mice on HFD develop only minor hepatic fibrosis after 50 weeks of feeding, but more so after 80 weeks.[30–32] At the other end of the spectrum, a methionine- and choline-deficient HFD, which lowers the antioxidant

defense system of the liver and strongly accelerates hepatic lipid retention, induces severe steatosis and fibrosis in just six weeks.[33,34] However, since animals lose weight on the methionine- and choline-deficient diet, the metabolic phenotype is not comparable to that of humans with MASLD. Overall, HFD with a high fructose content and the Gubra Amylin diet (an HFD with high fructose, sucrose, and cholesterol content) were found to most closely resemble human MASLD, with liver fibrosis detectable after 28 weeks of feeding.[35–38]

To study fibrotic MASH in the context of co-morbidities and whole-body metabolic dysfunction associated with the disease in humans, genetic mouse models can be useful. LDL receptor knockout mice and ApoE knockout mice, which are both commonly used to study hypercholesterolemia and atherosclerotic cardiovascular disease, develop periportal fibrosis after 6 to 12 weeks of high-fat, high-cholesterol feeding.[39,40] Similarly, hyperlipidemic APOE*3-Leiden.CETP mice, which have a more humanized lipoprotein metabolism, on a C57BL/6J background, develop stage 3 fibrosis after 16 weeks of high-fat, high-cholesterol feeding, along with obesity and insulin resistance.[41] Leptin-deficient hyperphagic *ob/ob* mice, commonly used in the study of obesity and hyperglycemia, develop fibrotic MASH when fed an HFD rich in cholesterol and trans-fatty acids for 20 weeks.[42,43] Major urinary protein-urokinase-type plasminogen activator mice, a model of chronic ER stress in hepatocytes, develop MASH that closely resembles human pathology, with pericellular and bridging fibrosis and spontaneous progression to hepatocellular carcinoma over 24 weeks of HFD feeding.[44,45] When studying the molecular processes of fibrosis development independently of metabolic dysfunction, organic hepatotoxins (such as ethanol, nitrosamines, tetrachloromethane (CCl_4), or thioacetamide), surgical bile duct ligation to induce cholestasis, and infections (e.g., with the parasitic flatworm *Schistosoma* or hepatitis viruses) are also commonly used to generate animal models of liver fibrosis.[46]

Mouse models of reversible liver fibrosis

As murine livers typically require multiple insults to develop fibrotic MASH, removing these stimuli or restoring homeostasis through genetic tools can lead to fibrosis regression. This section focuses on mouse models in which liver fibrosis has been shown to be reversible. Table 1 lists models with reversible hepatic fibrosis induced by diet, hepatotoxins, genetic predisposition, bile duct atresia, and parasites.[36,47–74]

The most commonly used protocol to induce severe reversible hepatic fibrosis in mice involves the repeated intraperitoneal injection of CCl_4, allowing for the study of fibrosis regression after the withdrawal of the hepatotoxin. Severe fibrosis can be achieved in a short timeframe (four to eight weeks) with this approach, and depending on the mouse strain, nearly complete fibrosis regression can be observed in a similar timeframe following CCl_4 withdrawal. Fibrosis induced through thioacetamide treatment, on the other hand, appears less reversible. Lee *et al*. previously concluded that this model is less suitable for studying fibrosis regression.[75] The general drawback of chemically induced liver fibrosis is that the metabolic phenotype, as well as the structure of the rapidly accumulating collagen deposits, may differ from human MASLD. Consequently, CCl_4 exposure and withdrawal is a useful method to study the effects of gene therapy [47] or pharmacological treatments [48] targeting fibrogenic and fibrinolytic processes outside the context of the hepatic metabolic impairments seen in human MASLD. The choice of the genetic background of the mice hereby depends on prior data on the treatment in question and personal preference, as near-complete fibrosis regression can be achieved in both C57BL/6J and BALB/c mice with CCl_4 withdrawal (see Table 1).

To mimic the slow development of fibrosis through hepatic metabolic dysfunction-induced inflammation seen in humans, modified HFDs are suitable in mice. However, the reversal of fibrosis through a switch from HFD to a regular chow diet is often incomplete and varies between individual animals, even after several months. Nevertheless, since incomplete and highly variable fibrosis regression is also observed clinically, this may not be a disadvantage of these mouse models and may instead mimic the clinical treatment response in addition to disease development.[76,77] Accordingly, diets rich in fat, sucrose, fructose, and cholesterol, such as the Gubra Amylin diet—most commonly fed to mice on a C57BL/6J genetic background, are particularly useful for studying the effects of metabolic or inflammatory modulators on fibrosis regression. This may also include treatments that do not act directly on the liver but, for example, influence food intake.[78] Unless particularly relevant to the research question, methionine-choline-deficient diets are less recommended for studying treatments in a human-like metabolic context of fibrotic MASLD, as they induce substantial weight loss due to adverse reactions.[49] The applicability of genetic models, as well as biliary atresia and parasite-induced fibrosis models, highly depends on the research question, with the downside that advanced molecular gene-editing tools or surgical techniques are often required to induce and reverse fibrosis development. Among genetic models, major urinary protein-urokinase-type plasminogen activator mice are particularly versatile, as they allow for the study of liver fibrosis regression in the context of advanced liver disease and hepatocellular carcinoma, which is an important clinical outcome.[26] The high mortality associated with parasitic infections makes these mouse models most useful for studying specific antiparasitic treatments, where hepatic fibrosis regression may be a desirable side effect. Similarly, the effects of antiviral treatments on fibrosis may be best tested in models where hepatic fibrosis was induced by viral infections. In summary, the choice of the most appropriate mouse model for studying fibrosis regression depends heavily on the research question and whether the molecular processes of fibrosis regression are being studied in the context of inter-organ crosstalk, metabolic dysfunction, or other comorbidities.

Ex vivo and *in vitro* models to study fibrosis regression

To test anti-fibrotic compounds, the *ex vivo* treatment of human or murine precision-cut liver slices (PCLS) offers a well-controlled experimental setup.[79,80] It was recently demonstrated that 250 µm thick PCLS from various diet-induced and hepatotoxin-induced liver fibrosis models remain stable in culture for up to 72 h, and gene expression responses to anti-fibrotic drugs matched those observed in liver tissue *in vivo*.[81] Ongoing work focuses on extending the viability of these slices *in vitro*, as well as optimizing culturing conditions to induce and study the regression of fibrosis in PCLS.[82] While this technique does not account for drug-induced changes in interorgan crosstalk or the liver's physiological complexity—such as the influx of inflammatory cells or the modulation of the gut-liver axis through bacterial drug metabolism—it surpasses cell cultures of individual cell types, such as stellate cells, in terms of mechanistic insights. Similarly, organoids containing multiple or all cell types found in the liver are continuously being improved to accurately recapitulate human (patho)physiology on a miniature scale. Since these

Table 1. Studies in mouse models of reversible liver fibrosis

Mouse strain	Fibrosis induction	Fibrosis stage	Regression method	Consideration	Ref.
Diet models					
C57BL/6	HFD with 40% fat, 25% fructose, 2% cholesterol for eight months	2	Switch to a chow diet for 16 weeks	Regression of portal but not perisinusoidal fibrosis (assessed via H&E staining) along with reduced steatosis and inflammation	50
C57BL/6J	Gubra Amylin (GAN) diet (46% fat, 22% fructose, 10% sucrose, 2% cholesterol) for nine months	≥1	PPAR agonist lanifibranor (30 mg/kg orally per day) for another 12 weeks while still on the GAN diet	Fibrosis regression in only half of the lanifibranor-treated cohort (assessed via collagen I staining) along with reduced steatosis	36
C57BL/6J	Choline-deficient L-amino-defined (CDAA)-HFD for 8 weeks	1	Switch to a chow diet and a daily injection of MAIT cell inhibitor acetyl-6-formylpterin (Ac-6-FP) for eight days		51
C57BL/6J	HFD with 40% fat, 20% fructose, 10% sucrose, 2% cholesterol for 12 weeks	1–2	Nitro-oleic acid (OA-NO_2) via minipump for another 12 weeks while still on HFD		52
C57BL/6N	Methionine-choline deficient (MCD) diet for seven weeks	1	PPARα agonist Wy-14,643 in the MCD diet for 12 days	MCD diet generally induces weight loss	49
C57BL/6J with 129S1/SvImJ	HFD with 42% kcal from fat, 0.1% cholesterol, and drinking water with 23.1 g/L fructose and 18.9 g/L glucose for 40 to 52 weeks	2–3	Switch to a chow diet and normal drinking water for four weeks	Also known as diet-induced animal model of non-alcoholic fatty liver disease (DIAMOND mouse)	53
Hepatotoxins					
C57BL/6J	single s.c. injection of streptozotocin two days after birth followed by HFD feeding from four weeks of age	1 (at nine weeks of age)	Galectin-3 protein inhibitors (intravenously) for four weeks starting at nine weeks of age while still on HFD	Only modest fibrosis regression (assessed via Sirius Red staining) along with reduced steatosis	54
C57BL/6	CCl_4 (0.5 mL/kg in olive oil, i.p. twice per week for 4 weeks)	3	Removal of CCl_4 for one month		55
C57BL/6J	CCl_4 (0.6 µL/g, 15% with olive oil i.p.) for six weeks	3	Removal of CCl_4 for up to 14 days		56
C57BL/6J	Escalating doses of CCl_4 (up to 1.25 ml/kg in corn oil, orally three times per week for eight weeks)	3	Removal of CCl_4 for four weeks		57
C57BL/6J	CCl_4 (1 mL/kg in olive oil, i.p. twice per week for four weeks)	3	Removal of CCl_4 for six weeks		58
C57BL/6JOlaHsd	CCl_4 (0.5 mg/kg in corn oil, i.p. twice per week for seven weeks)	3	Indoline derivative AN1284 (1 mg/kg/day for three weeks, four weeks after removal of CCl_4)	Reversal of more than 50% of fibrosis (assessed via Sirius Red and collagen IV staining)	48
BALB/c	CCl_4 (diluted 1:8 v/v in corn oil, i.p. twice per week for 10 weeks)	3	Removal of CCl_4 and treatment with macrophage-targeted PPARα agonist GW1929 (i.v. every three days for 10 days)	Reversal of more than 70% of fibrosis (assessed via Sirius Red staining)	59

(continued)

Table 1. *(continued)*

Mouse strain	Fibrosis induction	Fibrosis stage	Regression method	Consideration	Ref.
BALB/c	Escalating doses of CCl_4 (up to 1 mL/kg in olive oil, i.p. twice per week for four weeks)	3	Removal of CCl_4 and treatment with mIFNγ for two weeks	Nearly complete regression (assessed via collagen I gene expression and WNT-5A staining)	60
BALB/c	CCl_4 (0.4 mL/kg in olive oil, i.p. twice per week for 6 weeks)	2	Removal of CCl_4 and treatment with luteolin for two weeks	Nearly complete regression (assessed via Mallory trichrome staining)	61
BALB/c	TAA (200 mg/kg, i.p. every three days for four weeks)	4	Deactivating stellate cells using siRNA nanoparticle cocktail targeting Hedgehog and TGFβ1 (i.v. every three days, five times)	Limited spontaneous regression (assessed via Mason's Trichrome staining)	47
MMP9-/- (FVB background)	TAA (0.1 mg/g, i.p. every two days for eight weeks)	n.d.	Withdrawal of TAA for up to nine days	Improved regression after transplantation of wild-type Kupffer cells (assessed via Western blotting for collagen I, III, and IV)	62
129/Sv	α-naphthylisothio-cyanate (ANIT; 75 mg/kg via the diet for 14 days)	2	PPARα activator fenofibrate (25 mg/kg via oral gavage, twice per day for 14 days while still on the ANIT diet)	Cholestatic fibrosis model, stage 3 fibrosis after 28 days of ANIT diet (assessed via Sirius Red staining)	63
Genetic models					
NOD-Inflammation Fibrosis (N-IF, 24αβNOD. Rag2-/-)	Spontaneous development of chronic inflammation and liver fibrosis	3	Anti-inflammatory Paquinimod (25 mg/kg body weight/ day in the drinking water) for 10 weeks	Rag2-/- mice are severely immunodeficient and produce no mature B and T cells	64
Conditional liver-specific expression of TGF-β1 (inhibited by doxycycline)	10 cycles of TGF-β1 induction	1	TGF-β1 inhibition with doxycycline for 21 days	Fluctuating body weight through doxycycline cycles	65
Glycogen storage disease IIIa (Agl-/-)	Progressive liver fibrosis associated with glycogen storage disease	3	AAV expressing the bacterial glycogen debranching enzyme pullulanase, reversal over 10 weeks	Only applicable to glycogen storage disease	66
Peroxidasin knockout (Pxdn-/-)	Choline-deficient L-amino-defined (CDAA)-HFD for 16 weeks	1	Switch to a chow diet for two weeks	Peroxidasin deficiency results in atypical fibrosis formation (assessed via Sirius Red staining, collagen I staining, and electron microscopy)	67
MUP-uPA	HFD for six months	HCC	Lorsatan (30 mg/kg in the drinking water) for another two months while still on HFD	85% of MUP-uPA mice spontaneously progress to HCC when fed HFD	68
Ldlr-/-.Leiden	HFD with 45% fat from lard, 35% carbohydrates, 20% casein for 30 weeks	1	Running wheel access or switch to chow diet or a combination of both for 20 weeks	Ldlr-/- mice are also a model for atherosclerosis development	69
Aryl hydrocarbon receptor knockout (AHR-/-)	Fibrotic phenotype due to increased liver retinoid content	1	Vitamin A (retinol)-deficient diet for up to 18 weeks		70

(continued)

Table 1. *(continued)*

Mouse strain	Fibrosis induction	Fibrosis stage	Regression method	Consideration	Ref.
Mdr2-/- on FVB/NJ background (also called *Abcb4* mice)	Spontaneous sclerosing cholangitis	2 (at 52 weeks of age)	Hedgehog pathway inhibitor GDC-0449 (40 mg/kg, i.p., daily for nine days)	Biliary fibrosis development (assessed via Sirius Red staining) from four weeks of age, spontaneous occurrence from six months on	71
Biliary atresia					
Collagen 1(α)1-GFP	Bile duct ligation for 14 days	2	Surgical gall bladder-jejunum shunt to bypass bile duct ligation	Requires two surgical procedures	72
Parasites					
C57BL/6	*Schistosoma japonicum* infection	Fibro-cellular granulo-mas	150–350 mg/kg praziquantel for five consecutive days seven weeks after the infection	No steatosis development, mortality of 10-20% following infection	73
Swiss albino CD-1	*Schistosoma mansoni* infection	Fibro-cellular granulo-mas	Single intra-hepatic injection of Wharton's jelly-derived mesenchymal stem cells combined with anti-helminth drug praziquantel (PZQ) eight to sixteen weeks after the infection	No steatosis development, mortality of 10-20% following infection	74

HFD, high-fat diet; H&E, hematoxylin & eosin; GAN, Gubra Amylin; PPAR, Peroxisome proliferator-activated receptor; CDAA, Choline-deficient L-amino-defined; MAIT, Mucosal-associated invariant T cell; Ac-6-FP, Acetyl-6-formylpterin; OA-NO_2, Nitro-oleic acid; MCD, Methionine-choline deficient; s.c., subcutaneous; CCl_4, Carbon tetrachloride; i.p., intraperitoneal; i.v., intravenous; BALB/c, mIFNγ, mature interferon γ; WNT-5A, Wingless/integrase 1 5A; TAA, Thioacetamide; MMP9, Matrix metalloproteinase-9; FVB, Friend leukemia virus B; ANIT, α-naphthylisothio-cyanate; NOD, Non-obese diabetic; Rag2, Recombination activating gene 2; TGF-β1, Transforming growth factor β1; AAV, Adeno-associated virus; Pxdn, Peroxidasin knockout; MUP-uPA, Major urinary protein-urokinase type plasminogen activator; HCC, Hepatocellular carcinoma; Ldlr, Low density lipoprotein receptor; AHR, Aryl hydrocarbon receptor; Mdr2, Multidrug resistance-2; GFP, Green fluorescent protein; CD-1, Cluster of differentiation 1; PZQ, Praziquantel.

organoids can be generated from patient-derived induced pluripotent stem cells, they bypass the need for animal models and allow the development of personalized therapies. Organoids cultured using extracellular scaffolding matrices and self-assembling spheroids, both incorporating hepatic stellate cells, have demonstrated the development of fibrosis characterized by collagen deposits.[83–85] However, as of now, there has been no documented regression of fibrosis in these models, possibly due to an altered response of stellate cells *in vitro* compared to *in vivo*, the limited representation of the liver's immune environment, and the lack of vascularization, which provides essential oxygen and nutrient gradients in tissues. Lastly, profibrotic markers can be induced in MASH-on-chip models, where hepatocytes, HSCs, Kupffer cells, and endothelial cells are co-cultured under microfluidic dynamics.[86,87] Modulating the stiffness of the hydrogel in which the cells are cultured has been shown to affect the activity of stellate cells, with a reduction in the expression of profibrotic markers such as α-smooth muscle actin as hydrogel stiffness decreases.[88] This suggests that modeling fibrosis regression in organ-on-chip models may be achievable and cost-effective in setups where mechanical features are modifiable.[89] In summary, *ex vivo* PCLS that preserve *in vivo* tissue structure are currently the most suitable models for studying the local response to anti-fibrotic treatments in the liver over short periods. However, the systemic interactions are not fully reflected in these models.

Outlook

Several technical advancements will allow for further refinement of *in vivo* studies on fibrosis regression in mice and potentially reduce the need for animal models through optimized *in vitro* setups. First, since MASLD and fibrosis grading in mice generally take place histologically in whole liver tissues collected postmortem, the non-invasive fibrosis quantification techniques used clinically, along with blood markers of liver fibrosis, need to be further adapted for mice. For this, the combination of magnetic resonance imaging (hereinafter referred to as MRI) and MRI-based elastography has been found to reliably predict hepatic collagen content in mice with CCl_4-induced liver fibrosis, as well as fibrosis regression after CCl_4 withdrawal.[90] Additionally, the assessment of visco-elastic parameters using MRI-based elastography alone in a 5-minute scan in mice has shown good diagnostic performance for detecting substantial fibrosis, though it is less effective for diagnosing MASH.[91,92] A serological miRNA-based scoring algorithm, developed for the clinical diagnosis of significant liver fibrosis in blood, was validated in mice and revealed a pronounced plasma enrichment of the miRNAs 451a, 142-5p, Let-7f-5p, and 378a-3p in mice with CCl_4-induced liver fibrosis compared to mice with healthy livers. This suggests that the quantification of circulating miRNAs may aid in grading developing or regressing fibrosis.[93] Lastly, while more invasive, repeated needle biopsies of the mouse liver for histological grading are possible, though they carry the downside of tissue damage at the biopsy site, which may locally accelerate fibrosis development.[94] Together, these advancements are expected to enable reliable and repeated fibrosis assessments in the same mouse over time, thereby reducing the number of animals needed for these studies. Additionally, further refinement of mouse models is desirable, such as combining genetic and diet models to more

closely mimic human liver fibrosis development and regression, particularly in the context of common comorbidities such as obesity, type 2 diabetes, and cardiovascular diseases in the case of MASH-induced liver fibrosis.

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Conflict of interest

The authors have no conflict of interests related to this publication.

Author contributions

MS wrote the original draft, and MS and PCNR revised and edited the manuscript. All authors have approved the final version and the publication of the manuscript.

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