

Targeting cell-intrinsic metabolism for antifibrotic therapy

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Summary

In recent years, there have been major advances in our understanding of the mechanisms underlying fibrosis progression and regression, and how coordinated interactions between parenchymal and non-parenchymal cells impact on the fibrogenic process. Recent studies have highlighted that metabolic reprogramming of parenchymal cells, immune cells (immunometabolism) and hepatic stellate cells is required to support the energetic and anabolic demands of phenotypic changes and effector functions. In this review, we summarise how targeting cell-intrinsic metabolic modifications of the main fibrogenic cell actors may impact on fibrosis progression and we discuss the antifibrogenic potential of metabolically targeted interventions.

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Introduction

Liver fibrosis and its end-stage, cirrhosis, remain a growing health burden owing to the rising incidence of non-alcoholic fatty liver (NAFLD) worldwide. Liver fibrosis is the common wound healing response associated with chronic liver injury and the consequence of prolonged parenchymal cell injury and/or inflammation.^{1–3} The fibrogenic process is characterised by progressive accumulation of extracellular matrix components following activation of a heterogeneous population of fibrogenic cells mainly derived from hepatic stellate cells (HSCs) and to a lesser extent from portal fibroblasts.^{4,5} Parenchymal cell death, endothelial cell dysfunction and inflammatory signals originating from innate, adaptive and innate-like immune cells, are driving forces of fibrosis progression.^{1,2,6,7} Major advances have been made in the understanding of the mechanisms governing the coordinated dialogue between parenchymal and non-parenchymal cells during chronic liver cell injury, and its impact on fibrosis progression and regression.^{1–3,6} Although these findings have led to the identification of several therapeutic targets, no antifibrotic treatment has been approved. In this context, intrinsic metabolic reprogramming of HSCs, immune cells (immunometabolism) and hepatocytes has emerged as a crucial determinant of the cell phenotype switch that accompanies chronic liver injury and is now considered a potential therapeutic target. Studies on cell-intrinsic metabolism focus on how intracellular metabolic changes will modify effector functions in a given cell. A better understanding of how reprogramming metabolism in each liver cell type may impact on liver fibrosis is essential to predict the antifibrotic efficacy of targeting candidate pathways. In this review, we summarise our current understanding of the role of the intracellular

metabolic machinery on parenchymal and non-parenchymal cells during fibrosis progression and regression, as well as discussing potential therapeutic options.

Reprogramming metabolism of HSCs drives their fibrogenic/immunoregulator functions

In the normal liver, quiescent HSCs display adipocyte-like features characterised by the expression of lipogenic genes and transcription factors, and the presence of abundant cytoplasmic lipid droplets. Following liver injury, HSCs lose their lipid droplets and undergo a phenotypic switch to a myofibroblastic phenotype in which they display fibrogenic, contractile and angiogenic functions. Myofibroblastic HSCs also display features of non-professional immune cells and contribute to the amplification of the inflammatory response by producing reactive oxygen species, pro-inflammatory cytokines and chemokines. They also respond to proinflammatory signals by expressing chemokine receptors and pattern recognition receptors. In addition, they behave as antigen-presenting cells and phagocyte apoptotic bodies, which contributes to activation of their fibrogenic properties.⁴ The acquisition of myofibroblastic features is energetically expensive and relies on intense metabolic reprogramming through activation of glycolysis, glutaminolysis and lipogenesis (Fig. 1 and 2).

Glycolysis drives HSC activation

Glycolysis involves the intracellular processing of glucose to pyruvate, through several steps (Fig. 1). Pyruvate is further converted to lactate (anaerobic glycolysis) or to acetyl CoA that will fuel the tricarboxylic acid (TCA) cycle (aerobic glycolysis).

Keywords:

immunometabolism; lipid metabolism; glucose metabolism; autophagy; nuclear receptors; fibrosis; hepatic stellate cells; hepatocytes; macrophages; innate-like lymphoid cells.

Received 4 November 2020; received in revised form 2 February 2021; accepted 4 February 2021; available online xxx

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<https://doi.org/10.1016/j.jhep.2021.02.012>

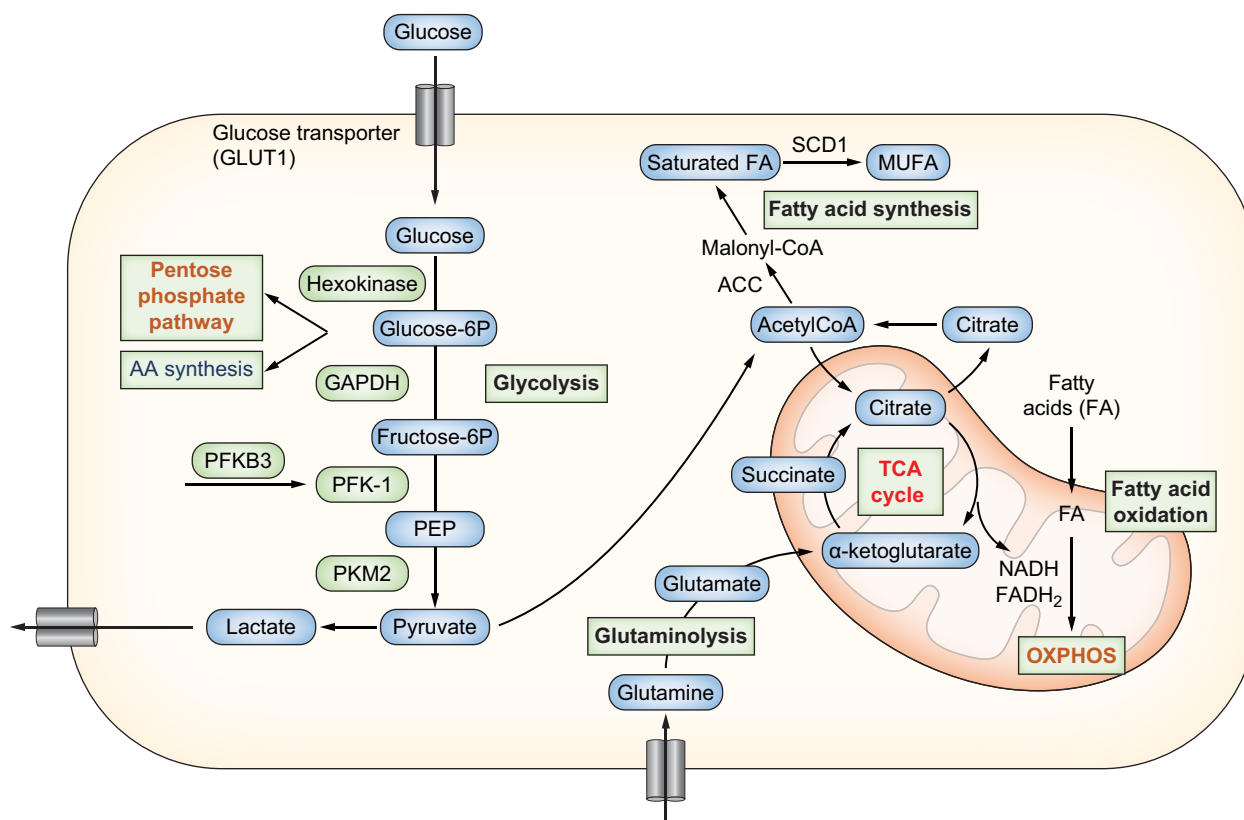


Fig. 1. Summary of the main intrinsic metabolic pathways. Pyruvate generated from glucose through glycolysis is either converted into lactate or is oxidised to acetyl-CoA to fuel the TCA cycle. Two major products of the TCA cycle are NADH and FADH₂ that transfer electrons to the electron transport chain to support OXPHOS. Fatty acid oxidation also produces NADH and FADH₂ which can be used to generate ATP. Fatty acids are synthesised from acetyl-CoA, which is derived from glycolysis or from TCA cycle-derived citrate. The pentose phosphate pathway runs in parallel to glycolysis, generating ribose-5-phosphate required for the synthesis of nucleotides and amino acids. Finally, the TCA cycle can also be fed by amino acids such as glutamine via glutaminolysis for ATP or citrate production. OXPHOS, oxidative phosphorylation; TCA, tricarboxylic acid.

Key point

Cell intrinsic metabolic reprogramming of parenchymal and non-parenchymal cells has recently emerged as a potential therapeutic approach for liver fibrosis.

The glycolytic pathway provides intermediates that are required for the synthesis of nucleotides, fatty acids and amino acids. In addition to its role in proliferation, glycolysis also supports production of extracellular matrix, as shown in the skin or lung.^{8–10} In cultured HSCs, transition to a myofibroblast phenotype is associated with a rise in glucose transporters, particularly GLUT1, and in glycolytic enzymes such as hexokinase 2, pyruvate kinase isoform M2 (PKM2) or 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase-3 (PFKFB3), with concomitant lactate accumulation and downregulation of gluconeogenic genes.¹¹ The resulting increase in glycolysis provides ATP, required for the activation process (Fig. 2). In keeping with *in vitro* studies, similar increases in glycolytic genes have been observed in experimental models of fibrosis and in the livers of patients with chronic liver injury.^{11,12} Interestingly, inhibition of hexokinase 2 (with the inhibitor 2-deoxyglucose), or knockdown/inhibition of PKM2 or PFKFB3, decreases glycolysis and reduces fibrosis following a decrease in the myofibroblastic features of HSCs.^{12,13}

Glutaminolysis supports HSC activation

Besides glycolysis, glutamine metabolism via glutaminolysis has been identified as an additional source of ATP in HSCs that drives their trans-differentiation into myofibroblasts.¹⁴ Glutaminolysis comprises a 2-step reaction, initiated by the conversion of glutamine into glutamate by glutaminase. Glutamate is further metabolised into α-ketoglutarate that fuels the TCA cycle and provides the ATP required for cell anabolism (Fig. 1). Myofibroblastic HSCs show an increase in expression of glutaminase in cell culture,¹⁵ in experimental models of chronic liver injury,^{14,16} and in liver samples of patients with non-alcoholic steatohepatitis (NASH) and advanced fibrosis.¹⁷ Interestingly, pharmacological inhibition of glutaminolysis via a glutaminase inhibitor (BTPES), or glutamine deprivation, is more efficient than glucose deprivation at blocking HSC proliferation and the acquisition of myofibroblastic features, while pharmacological blockade of glutaminase reduces fibrosis progression^{14,16} (Fig. 2). These data suggest that the combined

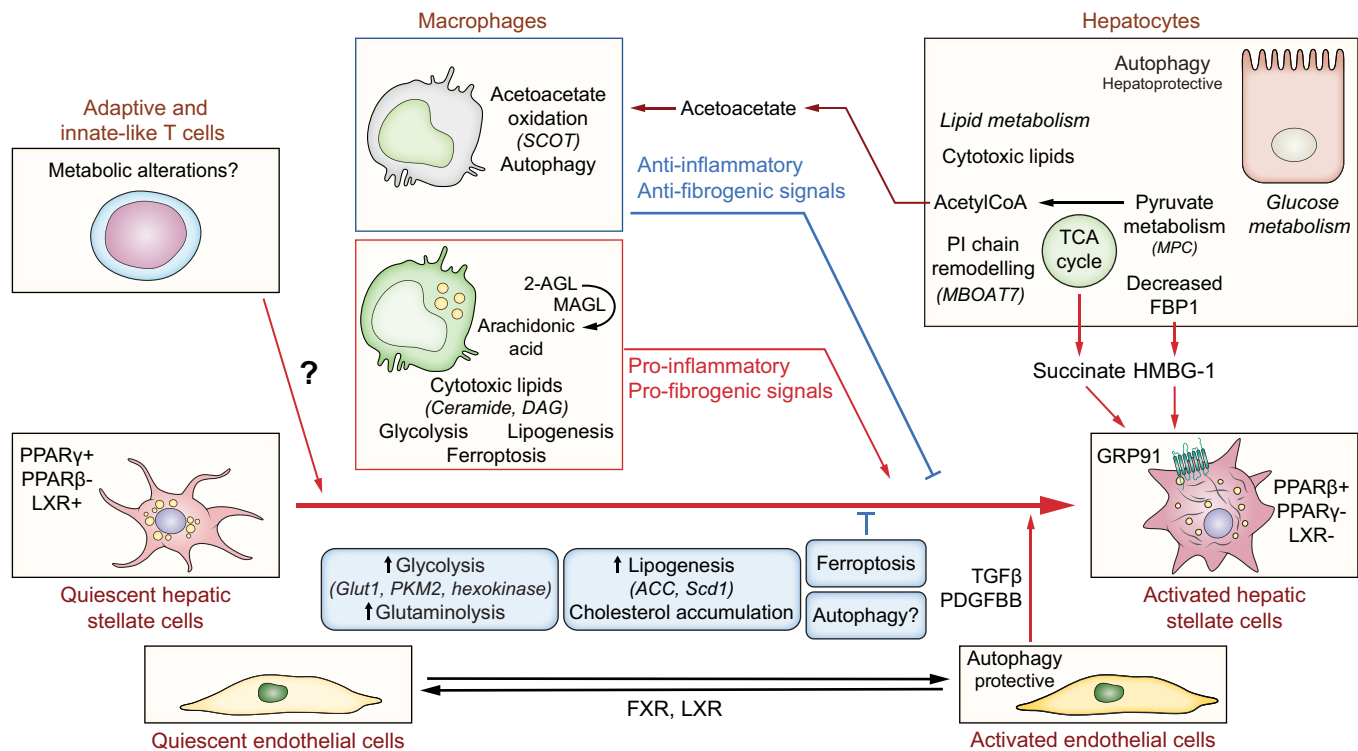


Fig. 2. Targeting intracellular metabolic pathways in hepatocytes, macrophages and hepatic stellate cells: impact on liver fibrosis. Hepatocyte injury and inflammatory signals originating from innate, adaptive and innate-like immune cells contribute to the phenotypic switch from quiescent to activated HSC. Among immune cells, macrophages with proinflammatory and inflammatory properties are major regulators of liver fibrogenesis. In HSC, glycolysis, glutaminolysis, *de novo* lipogenesis, cholesterol accumulation, modulation of nuclear receptor expression support acquisition of myofibroblastic features. Regarding autophagy conflicting data have been reported (see text). In macrophages, cytotoxic lipid accumulation drives polarization toward a pro-inflammatory phenotype as does MAGL. Autophagy and acetoacetate released by hepatocytes is oxidised by macrophages, leading to the release of antifibrogenic signals. The pro- and anti-inflammatory potential of nuclear receptors, glycolysis lipogenesis and ferroptosis are suggested by *in vitro* studies but have not been studied in the context of fibrosis. Hepatocyte injury, as induced by cytotoxic lipid accumulation, activates HSC. Hepatocyte-specific loss of autophagy, MBOAT7 or FBP1 or inhibition of MPC will inhibit HSC activation. FBP1, fructose 1,6-bisphosphatase 1; HSC, hepatic stellate cell; MAGL, monoacylglycerol lipase; MBOAT7, membrane-bound O-acyl-transferase domain containing 7; MPC, mitochondrial pyruvate carrier.

inhibition of glycolysis and glutaminolysis may have synergic antifibrogenic effects. Of note, myofibroblastic HSCs also express the metabotropic glutamate receptor 5 and a recent study has shown that following alcohol exposure, hepatocytes release glutamate with paracrine effects on neighbouring HSCs, leading to the release of steatogenic/fibrogenic mediators.^{18,19}

Lipid metabolism directs activation of HSCs

Though well characterised in hepatocytes, the impact of interfering with lipid metabolism in HSCs has been less well studied. However, recent studies have highlighted the fact that reducing lipid accumulation in HSCs can restrain their activation, directly modulating fibrogenesis.

Targeting lipogenesis

Two recent studies have shown that inhibiting *de novo* lipogenesis inhibits HSC activation and limits fibrosis.^{20,21} Lipogenesis is initiated by the conversion of acetyl-CoA into malonyl-CoA, catalysed by the rate limiting enzyme acetyl-CoA carboxylase (ACC) (Fig. 1). Pharmacological inhibition of ACC by

firsocostat directly suppresses the fibrogenic properties of cultured activated HSCs, and is associated with the blockade of glycolysis and oxidative phosphorylation²⁰ (Fig. 2). Moreover, in 2 mouse models of liver fibrosis (diet-induced NAFLD and diethylnitrosamine), administration of firsocostat is associated with a decrease in liver fibrosis.²⁰ In addition, inhibition of ACC has also been identified as an interesting therapeutic target in NAFLD; indeed, genetic or pharmacological blockade of ACC reduces steatosis and hepatocyte injury in experimental models. The beneficial impact of this approach is also supported by an exploratory study with the ACC inhibitor MK-4074²¹ and a phase II randomised placebo-controlled trial of firsocostat in patients with NASH.²² Both compounds were reported to decrease steatosis, while firsocostat also reduced select markers of fibrosis (Table 1).

Another key lipogenic enzyme is stearoyl-CoA desaturase 1 (SCD1), which converts lipotoxic saturated fatty acids into mono-unsaturated fatty acids (Fig. 1). Inhibitors of SCD1 have been shown to reduce steatogenesis in experimental models. In addition, as described for ACC inhibitors, global or

Table 1. Selected drugs targeting cell metabolism currently evaluated in NASH clinical trials with fibrosis as an endpoint.

Target	Drug	Phase	Results [Ref.]
Glucose metabolism			
SLGT1/2 inhibitor	Empagliflozin	Phase II/III	Decrease hepatic fat.
	Licogliflozin	Phase II	Significant improvement of liver stiffness ¹⁰³
	Dapagliflozin	Phase III	[No significant decrease in fibrosis (Harrison S AASLD: Boston, MA, USA, 2019)]
Hexokinase inhibitor	PF-06835919	Phase IIa	Expected
Lipid metabolism			
ACC1&2 inhibitor	Firsocostat	Phase II	Recruiting
SCD1 inhibitor	Aramchol	Phase IIb	30% reduction of liver fat. Decrease in the fibrosis marker TIMP-1. No decrease in ELF test and magnetic resonance elastography ²²
	Armor	Phase III/IV	Reduction in fibrosis at 600 mg without worsening of NASH [Ratzliff V, AASLD, San Francisco, USA 2018]
	Lanifibranor ($\alpha/\beta/\gamma$)	Phase II	Expected
PPAR	Saroglitazar ($\alpha&\gamma$)	Phase II	Resolution of NASH with no worsening of fibrosis ¹⁰²
	Elafibranor ($\alpha&\delta$)	Phase II	Improvement of liver biochemistry and steatosis. Decrease in APRI and ELF. No clear decrease in liver stiffness [Gawrieh S AASLD: Boston MA, USA, 2019]
	Seladelpar (δ)	Phase III	Not superior to placebo for fibrosis ¹⁰⁷
FXR agonist	Tropifexor	Phase II	Suspended for unexpected effects
	Obeticholic Acid	Phase III	Decrease in steatosis and in ALT. Results on liver biopsies expected
Mitochondrial pyruvate carrier			
MPC inhibitor	MSDC-0602K	Phase IIb Phase III	Improve fibrosis w/o NASH worsening ¹⁰⁵
Autophagy			
ASK1 inhibitor	Selonsertib	Phase III	Reduction in glucose liver enzymes. No improvement of fibrosis ⁶²
AMPK & PG1Ca	Resveratrol	Phase II/III	Not superior to placebo ¹¹⁷
			Prevents liver damage ¹⁰⁶

ALT, alanine aminotransferase; APRI, aspartate aminotransferase-to-platelet ratio index; ELF, enhanced liver fibrosis; NASH, non-alcoholic steatohepatitis.

selective inhibition of SCD-1 in HSCs reduces activation of cultured HSCs and inhibits fibrosis in experimental models²³ (Fig. 2). Similar findings have been obtained with pharmacological SCD1 inhibitors, i.e. A939572 or aramchol in a phase IIb clinical trial (Table 1).^{23,24}

Key point

Glycolysis, glutaminolysis and lipid metabolism control hepatic stellate cell phenotype.

Targeting cholesterol metabolism

Free cholesterol activates HSCs, while it has been shown that feeding mice a high-fat high-cholesterol diet leads to accumulation of free cholesterol in HSCs and exacerbates liver fibrosis. Indeed, cholesterol homeostasis is dysregulated in liver diseases, particular during NAFLD (for a review see 25), leading to diet-induced accumulation of free cholesterol in HSCs, which increases Toll-like receptor 4 signalling and results in the activation of the transforming growth factor- β (TGF- β) pathway.^{26,27} In addition, free cholesterol is converted to cholesterol esters by acetyl-CoA acetyltransferase 1 (ACAT1) and accordingly, genetic inactivation of ACAT1 results in increased concentrations of free cholesterol in HSCs and worse fibrosis.^{26,27} A recent study also suggests that HSCs bearing the I148M PNPLA3 variant predisposing to enhanced fibrogenesis show increased levels of free cholesterol following downregulation of ACAT1, and display enhanced fibrogenic properties.²⁸ The beneficial impact of therapeutic strategies aimed at decreasing free cholesterol accumulation in HSCs has also been demonstrated.

Indeed, specific inhibition of lipoprotein lipase in HSCs, or the use of SREBP2 siRNA- or anti-miR-33a-bearing vitamin A-coupled liposomes reduces free cholesterol accumulation in activated HSCs and decreases liver fibrosis in mouse models.²⁹

Targeting succinate

Succinate is an intermediate metabolite produced during the TCA cycle that serves as a final common catabolic pathway of carbohydrates, amino-acids and fatty acids. Succinate is formed from succinyl-CoA by succinyl-CoA synthetase; it is subsequently oxidised to fumarate by succinate dehydrogenase (SDH), which is part of the respiratory electron transport chain (Fig. 1). Normally present in mitochondria, succinate is secreted into the extracellular space and binds to the G-protein coupled receptor, GPR91 (also known as succinate receptor 1). Binding of succinate to GPR91 expressed in HSCs enhances their pro-fibrogenic properties (Fig. 2). Moreover, paracrine succinate shuttle between hepatocytes and HSCs has also been described, involving enhanced succinate release by hepatocytes in stress conditions. Interestingly, inhibition of the succinate/GPR91 pathway has also been shown to have beneficial antifibrogenic effects in experimental models of NASH.^{30,31} However, further studies should evaluate its impact on inflammation, since the succinate/GRP91 pathway displays anti-inflammatory effects on macrophages.³²

Immunometabolic regulation of liver fibrosis

Several studies have emphasised the crucial role of various innate, adaptive or innate-like immune cell subsets in the control of hepatic inflammation, fibrosis progression and regression.^{1,2,6,7} Recent studies also demonstrate the potential beneficial impact of therapeutic strategies targeting their intrinsic metabolism.

Macrophage cell metabolism and fibrogenesis

Among immune cells, a heterogeneous population of macrophages is a major contributor to liver fibrogenesis (Fig. 2).^{7,33} Resident activated Kupffer cells initiate and promote several key steps in the inflammatory response to liver injury. They recruit Ly6Chi macrophages with pro-inflammatory and pro-fibrogenic properties, as well as neutrophils and IL-17-positive cells, which ultimately promote hepatocyte death and induce accumulation and survival of activated HSCs.^{1,2,6,7} Meanwhile, following cessation of liver injury, the recovery phase is characterised by the presence of restorative macrophages with a distinct anti-inflammatory, fibrolytic Ly6C^{lo} phenotype that drive fibrosis resolution (Fig. 2).³⁴ Other innate immune cells also control fibrogenesis, including dendritic and natural killer (NK) cells.^{2,7} However, data regarding the impact of reprogramming the intrinsic metabolism of innate immune cells in liver fibrosis are mainly restricted to macrophages.

It is well established that the macrophage switch to an inflammatory phenotype is tightly controlled by macrophage modifications in glucose and lipid metabolism.^{35,36} In particular, characteristic features of inflammatory macrophages include enhanced glycolysis, activation of the TCA cycle (used to produce citrate for fatty acid synthesis), the pentose phosphate pathway and nitric oxide production. In contrast, anti-inflammatory macrophages use fatty acid oxidation, the TCA cycle (used for oxidative phosphorylation) and the arginase pathway^{35,36} (Fig. 1). These data suggest that manipulation of metabolism in monocytes/macrophages may provide a novel anti-inflammatory and antifibrogenic approach in the liver, and mirrors recent findings in atherosclerosis and diabetes.^{37,38}

Accumulation of lipids in Kupffer cells is proinflammatory

Alterations in lipid metabolism drive the polarisation of Kupffer cells into a pro-inflammatory phenotype. Thus, binding of saturated fatty acid or trapping of oxidised lipoproteins by scavenger receptors such as MSR1 (macrophage scavenger receptor 1), leads to the formation of foamy Kupffer cells with a pro-inflammatory phenotype.^{39,40} In line with these data, mice lacking Msr1 show less hepatic inflammation and greater resistance to

fibrosis in response to a high-fat diet.⁴⁰ Finally, exposure of Kupffer cells to fatty acids leads to accumulation of the cytotoxic lipids diacylglycerol and ceramide, and shifts Kupffer cells into a pro-inflammatory state⁴¹ (Fig. 2).

Targeting lipid metabolism in macrophages: A role for monoacylglycerols

Hydrolysis of monoacylglycerols by monoacylglycerol lipase (MAGL) is the final step in cellular triglyceride breakdown. MAGL hydrolyses 2-arachidonoyl-glycerol to glycerol and fatty acids, which constitutes the major source of arachidonic acid and pro-inflammatory prostaglandins in the liver. We have demonstrated that specific inhibition of MAGL in myeloid cells results in anti-inflammatory and antifibrogenic effects in the liver, via a shift of lipid metabolism in macrophages from an arachidonic acid/prostaglandin proinflammatory phenotype towards an anti-inflammatory 2-arachidonoyl-glycerol-producing phenotype (Fig. 2).⁴² MAGL inhibition also protects mice against cholestatic injury, inflammation and biliary fibrosis.^{43,44} Finally, pharmacological inhibition of MAGL with MJN110 promotes liver fibrosis regression by decreasing the frequency of Ly6C^{hi} and increasing the frequency of Ly6C^{lo} macrophages. Because inhibiting MAGL also decreases hepatocyte injury,⁴² these data suggest that reprogramming lipid metabolism using MAGL inhibitors holds promise as an antifibrogenic approach through its dual effects on macrophages and hepatocytes.

Targeting acetoacetate metabolism: a two-tale hepatocyte-macrophage story

Mitochondrial metabolism of acetyl-CoA by HMG-CoA synthase in hepatocytes produces ketone bodies in 2 forms, acetoacetate and β -hydroxybutyrate. However, hepatocytes are unable to metabolize ketones due to the lack of expression of succinyl-CoA-oxoacid transferase (SCOT), an enzyme that prepares acetoacetate for terminal oxidation in the TCA cycle. In contrast, SCOT is highly expressed in macrophages. A recent study unveiled a protective metabolic response of macrophages to liver fibrogenesis, via a hepatocyte-macrophage acetoacetate shuttle. In brief, acetoacetate released by hepatocytes is further oxidised by mitochondrial SCOT in alternatively polarised macrophages, thereby reducing hepatic fibrogenesis (Fig. 2).⁴⁵ Indeed, locally produced or systemically administered acetoacetate attenuates the hepatic fibrogenic response induced by a high-fat diet. In contrast, SCOT-KO macrophages exhibit perturbations of glycosaminoglycan metabolism, known to be associated with expansion of extracellular matrix. Accordingly, mice lacking SCOT in macrophages are predisposed to accelerated fibrogenesis.⁴⁵

Key point

In macrophages, targeting lipid or acetoacetate metabolism by inhibiting monoacylglycerol lipase has antifibrogenic consequences.

Metabolism of adaptive immune cells and innate/innate-like lymphoid cells and liver fibrosis

Adaptive (CD4⁺, T helper [Th]1, 2 and 17) and innate/innate-like (ILC2, mucosal-associated invariant T [MAIT] and $\gamma\delta$ T) lymphoid cells are major regulators of the hepatic immune response and consequently the fibrogenic process, with positive or negative outcomes depending on their phenotype (Fig. 2).^{2,7} In particular, IL-17 produced by Th17 and MAIT cells directly activates the fibrogenic functions of HSCs, while the anti-fibrogenic properties of $\gamma\delta$ T cells and Th1 lymphocytes are related to their apoptotic effects on HSCs and IFN γ production, respectively (for extensive reviews see^{2,7,46}). It is well established that metabolic changes in adaptive immune cells and innate-like T cells support their functions and drive their differentiation. In particular, shifting to aerobic glycolysis, lipogenesis and arginine and glutamine metabolism is fundamental for differentiation of naïve CD4⁺ T cells into Th1 and Th17 cells, of $\gamma\delta$ T into $\gamma\delta$ T1 and $\gamma\delta$ T17 cells, and to drive proliferation and cytokine production in ILCs.⁴⁷ Gut dysbiosis is also a characteristic feature of chronic liver injury and is a major driver of liver fibrosis. Interestingly, accumulating data demonstrate that gut-derived bacterial metabolites modify immune cell metabolism⁴⁸ and support their activation. This could be a factor in the activation of MAIT cells, as these innate-like lymphoid cells are activated by bacterial ligands and display fibrogenic properties.^{49,50} Data are still lacking, but whether intrinsic metabolic changes in adaptive and innate immune cells govern their differentiation toward a pro-inflammatory and pro-fibrogenic phenotype, and the resulting impact on fibrosis progression or regression, merits further investigation.

Hepatocyte metabolism and liver fibrosis

Omics approaches related to changes in hepatocyte metabolism have been the focus of many studies in the context of NASH, but the link between the accumulation of cytotoxic lipids in hepatocytes and the development of liver fibrosis remains elusive. Hepatocyte injury is a major driver of fibrosis, via direct effects on HSC functions and indirect effects on immune cell activation.^{2,3,6} Changes in intrinsic hepatocyte metabolism may lead to inhibition of fibrogenesis, by reducing steatosis and liver injury or through indirect modifications of the macrophage-HSC dialogue, as described above for the acetoacetate shuttle. However, in the next paragraph we will focus on preclinical studies demonstrating that metabolic reprogramming of hepatocytes can directly impact on the profibrogenic functions of HSCs. Among metabolic pathways preserving hepatocyte integrity, key proteins involved in glucose or lipid metabolism are potential antifibrogenic candidates (Fig. 2).

Changes in glucose metabolism through hepatocyte-specific loss of the gluconeogenic enzyme fructose 1,6-bisphosphatase 1 (FBP1) result in hepatocyte secretion of the non-histone nuclear protein high-mobility group protein B1 (HMGB1) which causes HSC activation⁵¹ (Fig. 2). Future studies should address whether crosstalk between hepatocyte glucose metabolism and HSC activation could contribute to liver fibrosis in chronic liver diseases and become a target for novel therapeutic approaches.

The accumulation of cytotoxic lipids (*i.e.* free fatty acids, ceramides, diacylglycerol, cholesterol and phospholipids) in hepatocytes is a hallmark of NASH;^{52–55} thus, it has been suggested that therapeutic interventions, either pharmacological or dietary (*e.g.* caloric restriction), may result in a reduction of liver fibrosis following a decrease in hepatocyte lipid accumulation or prevention of lipid-induced apoptosis. Another link between alterations in hepatocyte lipid metabolism and liver fibrosis was recently provided by a study showing that disturbance of phosphatidylinositol (PI) chain remodelling, following inhibition of membrane-bound O-acyltransferase domain containing 7 (MBOAT7) in hepatocytes, increases fibrosis in response to a NASH-inducing diet in mice⁵⁶ (Fig. 2). Interestingly, the rs641738C>T MBOAT7 variant which is associated with reduced hepatic levels of MBOAT7, has been identified as a risk locus for alcohol-related cirrhosis,⁵⁷ NAFLD and viral hepatitis-related fibrosis.^{58–60}

Finally, transport of pyruvate into the mitochondrial matrix is critical for lipid and glucose metabolism. Modulating pyruvate metabolism in hepatocytes by inhibiting mitochondrial pyruvate carrier (MPC) prevents fibrosis, not only because of its anti-steatogenic properties but also by inhibiting HSC activation (Fig. 2),⁶¹ although no improvement in fibrosis was observed in a clinical trial.⁶²

Endothelial cells

Loss of liver sinusoidal endothelial cell (LSEC) fenestration (*i.e.* capillarisation of sinusoids) is an early characteristic feature of the fibrogenic process. Activated endothelial cells contribute to HSC activation, by producing TGF- β and platelet-derived growth factor (PDGF)-BB, and secreting components of the extracellular matrix, including collagen 1. Restoration of LSEC differentiation also accounts for fibrosis regression by promoting HSC quiescence.¹¹ However, data regarding the impact of intrinsic metabolic reprogramming of LSECs during liver fibrosis are scarce and limited to the description of the beneficial role of autophagy on fibrosis progression, of liver X receptors (LXRs) on the restoration of endothelial cell capillarisation and of farnesoid X receptor (FXR) on endothelial dysfunction (see below, Fig. 2 and Fig. 3).

Key point

In hepatocytes, accumulation of cytotoxic lipids or alterations of lipid or glucose metabolism result in hepatic stellate cell activation and the development of fibrosis.

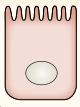


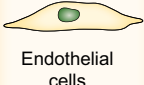
	Glycolysis	Glutaminolysis	Lipogenesis	PPAR	FXR	LXR	Ferroptosis	Autophagy
 Hepatocytes	Controls of ATP homeostasis	ND	Accumulation of cytotoxic lipids; pro-apoptotic and pro-fibrogenic	α : Increases fatty acid oxidation β/δ : Increases lipogenesis; hepatoprotective	Represses TG synthesis Increases cholesterol	Steatogenic LXR(a) bile acid report	Promotes hepatocyte necrosis	Protective against steatosis and hepatocyte injury
 Macrophages	Potential source of pro-inflammatory mediators	ND	Pro-inflammatory	α , β/δ , γ : Anti-inflammatory	Anti-inflammatory	Anti-inflammatory	Pro-inflammatory	Anti-inflammatory
 Hepatic stellate cells	Enhances fibrogenic functions	Enhances fibrogenic functions	Enhances fibrogenic functions	β/δ : Promotes HSC activation γ : Inhibits HSC activation	Indirect inhibition of fibrogenic functions	Inhibits fibrogenic functions	Inhibits fibrogenic functions	Promotes HSC activation but inhibits the release of profibrogenic extracellular vesicles Pro or anti-fibrogenic?
 Endothelial cells	ND	ND	ND	α , γ : Improve endothelial dysfunction	Improves endothelial dysfunction	Suppresses endothelial capillarization	ND	Anti-fibrogenic
Resulting effect of drugs in animal models	Inhibitors reduce fibrosis	Inhibitors reduce fibrosis	Inhibitors reduce fibrosis	Agonists reduce fibrosis	Agonists reduce fibrosis	Increased fibrosis in LXRα and β KO models	?	Activators reduce fibrosis
Targets-drugs	Hexokinase2 inhibitor PKM2 inhibitor PFKFB3 inhibitor	Gis1 inhibitor BPTES	ACC inhibitor: MK-4074/firsocostat SCD1 inhibitor: aramchol/A939572	α : Wy-14,643 β/δ : GW501516 γ : Pioglitazone, rosiglitazone	Obeticholic acid WAY362450 PX20696			(Rapamycin-carbamazepin)

Fig. 3. Targeting metabolic pathways in different liver cell types: expected outcomes. The different outcomes resulting from the activation of cell intrinsic metabolic pathways on the cellular actors of liver fibrogenesis are depicted. ND, not determined

Targeting common metabolic pathways in different liver cell types: Benefits and pitfalls

As depicted in the first part of the review, metabolic reprogramming is emerging as a potential therapeutic in the field of chronic liver diseases. However, given the pleiotropic role of these metabolic targets in various liver cells, the following sections focus on some of the pathways for which studies in different liver cell types are available.

Autophagy

One of the main functions of autophagy is to regulate cellular metabolism and energy, through lipophagy, mitophagy, amino acid pool refuelling, or degradation of proteins involved in glucose metabolism.^{63,64} The role of autophagy in liver fibrosis has been delineated using mice with cell-specific deletions in autophagic genes.⁶⁵ Mice with *Atg5* or *Atg7* deletions in hepatocytes or endothelial cells show increased fibrosis either spontaneously (hepatocytes,⁶⁶) or in response to a fibrogenic insult (endothelial cells⁶⁷) (Fig. 3). In monocytes/macrophages, a non-canonical form of autophagy (i.e. LC3-associated phagocytosis) protects against inflammation-driven liver fibrosis.^{68,69} Regarding HSCs, conflicting results have been reported. Pharmacological inhibition of

autophagy with 3-methyladenine or chloroquine or *in vivo* deletion of ATG7 in HSCs results in inhibition of their fibrogenic properties.^{70,71} However, this view has recently been challenged, as autophagy in HSCs was shown to have an anti-fibrogenic effect related to its capacity to inhibit the release of fibrogenic extracellular vesicles.⁷² Although further studies are required, it is anticipated that the resulting global impact of autophagy activators will result in a reduction in fibrosis. Along these lines, preclinical studies have demonstrated the antifibrogenic effects of autophagy inducers such as rapamycin or carbamazepine (Fig. 3).^{73,74}

Ferroptosis

Ferroptosis is a form of iron-dependent cell death. The execution of ferroptosis relies on modulation of intracellular metabolism, particularly lipid peroxidation, amino acid metabolism (glutamate and glutamine) and autophagy. Antifibrogenic properties of ferroptosis inhibitors have been demonstrated in the heart and lung,^{75,76} but may depend on the targeted cell type in the context of liver fibrosis. Thus, in HSCs, ferroptosis is induced following inhibition of the cysteine/glutamate antiporter xCT, leading to a reduction in fibrosis.⁷⁷ Accordingly, induction of HSC ferroptosis by the anti-malaria drug artemether attenuates hepatic

injury and liver fibrosis.⁷⁸ In contrast, ferroptosis reprograms macrophages to a pro-inflammatory and profibrogenic phenotype⁷⁹ (Fig. 3). Likewise, ferroptosis triggers inflammation in NASH, while iron chelators protect parenchymal cells from necrotic death and suppress the infiltration of inflammatory cells.⁸⁰ From a therapeutic perspective, the cell-specific effects of inhibiting ferroptosis must be considered.

Nuclear receptors

LXR

LXRs are central in lipogenesis and cholesterol metabolism.⁸¹ LXR α is highly expressed in hepatocytes, Kupffer cells⁸² and endothelial cells.⁸³ While LXR β is predominant in HSCs, its expression decreases upon activation.⁸⁴ LXR displays anti-inflammatory properties in Kupffer cells, anti-fibrogenic effects in HSCs, and suppresses endothelial cell capillarisation, as shown in different cell culture and mouse models of chronic liver diseases^{83–85} (Fig. 3). Interestingly, disrupting LXR α phosphorylation at the inhibitory Ser196 site reduces hepatic inflammation and fibrosis despite enhanced steatosis in mice fed a high-fat high-cholesterol diet.⁸⁶ Moreover, the overexpression of LXR β has recently been shown to suppress fibrosis and HSC activation, without inducing steatosis, in a mouse model of carbon tetrachloride-induced fibrosis.⁸⁷ However, to further consider the potential therapeutic value of LXR agonists, the specific contribution of LXR α vs. LXR β needs to be addressed, considering the lipogenic properties of LXR α and the potential steatogenic side effects of non-selective LXR $\alpha\beta$ -targeting molecules.

FXR

FXR is the main regulator of bile acid synthesis expressed in hepatocytes, Kupffer cells, endothelial cells and to a far lesser extent in HSCs.^{88–91} In hepatocytes, FXR plays an important role in lipid metabolism, inducing genes involved in lipoprotein metabolism and repressing hepatic genes involved in the synthesis of triglycerides, but increasing cholesterol.⁸¹ Experimental models have also shown that FXR displays anti-inflammatory and antifibrogenic properties related to its effects on hepatocytes, macrophages and endothelial dysfunction, but most likely unrelated to direct effects on HSCs^{88–93} (Fig. 3). Accordingly, obeticholic acid, an FXR agonist, which has been approved as a second-line treatment for primary biliary cholangitis, has undergone clinical development in patients with NASH (see clinical perspectives).

PPARs

Peroxisome proliferator-activated receptors (PPARs) are a family of nuclear transcription factors (α , β/δ and γ) regulating lipid and glucose metabolism.⁹⁴ The PPAR α isoform is predominantly

found in hepatocytes and to a lesser extent in Kupffer cells and endothelial cells. The PPAR β/δ isoform is expressed in all liver cells, whereas PPAR γ is expressed in macrophages and quiescent HSCs (Fig. 2). Activation of PPAR α results in increased fatty acid oxidation in hepatocytes, shifts the macrophage phenotype toward an anti-inflammatory state and improves endothelial cell function.⁹⁵ In animal models, activation of PPAR α by Wy-14,643 normalises histological changes by preventing intrahepatic lipid accumulation, liver inflammation, and fibrosis⁹⁶ (Fig. 3). The role of PPAR β/δ is more complex, since it increases *de novo* lipogenesis and protects against lipotoxicity, displays anti-inflammatory properties in Kupffer cells but increases activation of HSCs (Fig. 3). Nevertheless, the beneficial hepatoprotective and anti-fibrogenic effect of a PPAR β/δ agonist (GW501516) has not been confirmed in clinical trials with seladelpar (Table 1). The PPAR γ isoform displays anti-inflammatory properties in macrophages by downregulating inflammatory gene expression, and it is essential for maintaining the quiescent phenotype of HSCs⁹⁷ (Fig. 3). Interestingly, in response to PPAR γ overexpression or stimulation with PPAR γ agonists, such as thiazolidinediones, activated HSCs can revert to a quiescent phenotype.⁹⁸ Although encouraging results were obtained in preclinical models with pioglitazone or rosiglitazone,⁹⁹ no amelioration of liver fibrosis has been obtained in humans, following 1- or 2-year treatment with rosiglitazone.^{100,101} Combination strategies using pan-PPAR agonists have been developed, based on encouraging results obtained in animal models. In particular, the pan-PPAR $\alpha/\delta/\gamma$ agonist lanifibranor improves all features of steatohepatitis, *i.e.* steatosis, inflammation and fibrosis in experimental models of NASH (choline-deficient, amino acid-defined high-fat diet) and of chronic toxic injury (chronic carbon tetrachloride administration).^{102,103}

Targeting cell intrinsic metabolism for antifibrotic therapy – clinical perspectives

The antifibrotic effects of strategies targeting lipid or glucose metabolism are increasingly being evaluated in patients with NASH (Table 1).^{104–106} Drugs targeting metabolism may display antifibrogenic properties by reducing hepatocyte injury. In addition, results from the aforementioned preclinical studies suggest that they may also reduce fibrosis via direct effects on HSCs and/or changes in inflammatory cell phenotype (Fig. 4).

Several phase II and III trials with these new classes of drugs are underway and interesting results have been reported for a variety of compounds in the past 2 years. Fibrosis endpoints in phase II trials may rely on non-invasive measures, such as enhanced liver fibrosis test or fibroscan. Phase III trials use the surrogate liver histology endpoints currently required for accelerated

Key point

Targeting common metabolic pathways such as autophagy, ferroptosis or nuclear receptors is an interesting therapeutic approach but may have different impacts on different parenchymal or non-parenchymal liver cell types.

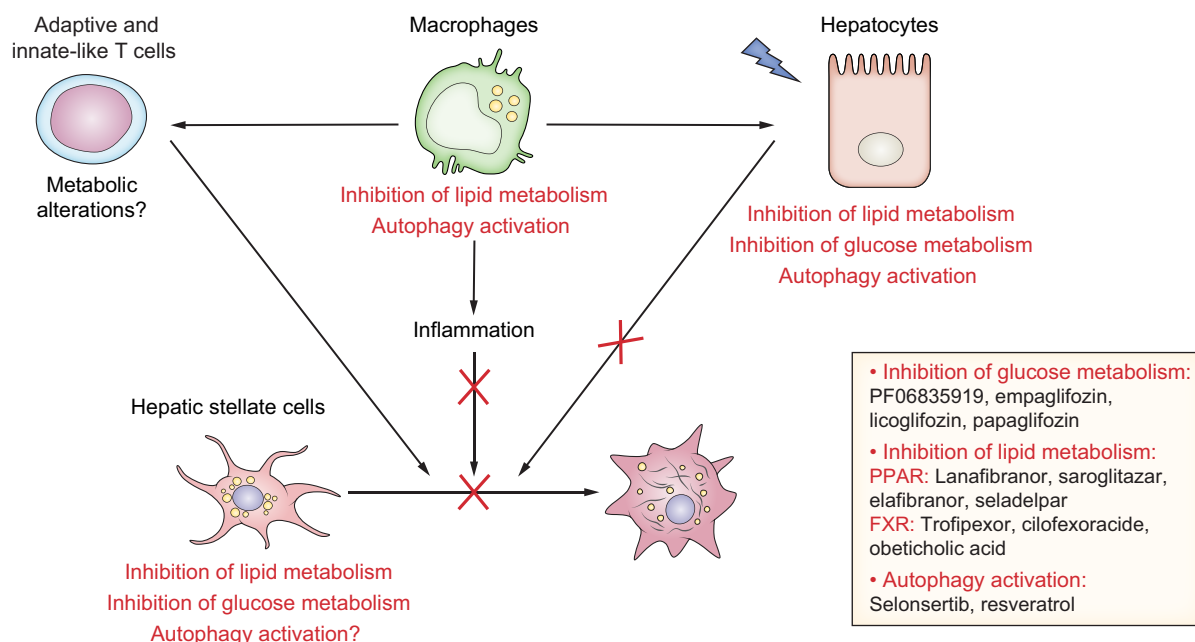


Fig. 4. Cellular targets of molecules reprogramming intrinsic metabolism currently under development for NASH. In addition to directly targeting HSC metabolism, molecules tested in clinical trials as antifibrogenic compounds may also indirectly impact on hepatocyte injury, or on inflammation via changes in macrophage or adaptive and innate-like immune cell phenotype. HSC, hepatic stellate cell; NASH, non-alcoholic steatohepatitis.

approval in NASH: i) a decrease of fibrosis stage of at least 1 point with no worsening of NASH (hepatocyte ballooning and inflammation scores), and ii) NASH resolution (hepatocyte ballooning and inflammation scores 0-1) with no worsening of fibrosis.

Following promising phase II data, the FXR agonist obeticholic acid has been evaluated in a pivotal placebo-controlled phase III trial assessing the impact of a 10 or 25 mg dose. An interim analysis in a subgroup of 660 patients reported positive results, with improvement of fibrosis and prevention of fibrosis progression in the 25 mg group (23% vs. 12% on placebo). However, the NASH resolution endpoint was not met (12% vs. 8%).¹⁰⁵ Thus, the drug was not granted accelerated approval by the FDA, pending submission of additional efficacy and safety data. Encouraging results have also been reported for the stearyl CoA desaturase inhibitor aramchol. Preliminary data indicate that 1 year of treatment with a 600 mg dose of aramchol significantly decreases fibrosis stage without worsening NASH and a phase III/IV trial evaluating the safety and efficacy of a 300 mg dose is underway (NCT04104321) (Table 1). In contrast, other potential drugs, such as the PPAR γ agonist elafibranor,¹⁰⁷ have failed to demonstrate clinical efficacy.

Overall, therapeutic strategies based on a single target seem to have limited antifibrogenic efficacy in NASH, hence multiple targeting is emerging as an attractive alternative.¹⁰⁶ Thus, a recent study has shown that activated HSCs display limited

response to obeticholic acid due to progressive FXR sumoylation during the activation process.¹⁰⁸ Interestingly, combined treatment with a sumoylation inhibitor and obeticholic acid downregulated the fibrogenic phenotype in cultured HSCs and reduced experimental liver fibrosis.¹⁰⁸ Whether this combined strategy proves useful in patients with NASH remains to be determined. The validity of multiple targeting has also been shown with the use of a pan-PPAR $\alpha/\delta/\gamma$ agonist, as initially shown in preclinical studies.¹⁰² Interestingly, a recent press release – based on a phase IIb placebo controlled-trial – indicated that a 1,200 mg dose of lanifibranor met its fibrosis endpoints.¹⁰³ Future studies should also evaluate whether combining drugs targeting cell-intrinsic metabolism and compounds targeting other antifibrogenic mechanisms, such as inflammation, lead to better outcomes. Along this line, the TANDEM phase II trial will assess the efficacy of combining the FXR agonist trofipexor with the CCR2/5 antagonist cenicriviroc.^{106,109}

Towards cell-specific metabolic targeting of antifibrotic agents

Drugs targeting metabolism in hepatocytes may display antifibrogenic properties, by reducing liver injury. However, results from aforementioned preclinical studies suggest that they may also have antifibrogenic effects by directly targeting HSCs, or indirectly by changing inflammatory cell phenotypes (Fig. 4). Nevertheless, the efficacy of strategies targeting a metabolic pathway in a given cell

Key point

The antifibrotic effects of strategies targeting lipid or glucose metabolism are increasingly being evaluated in patients with NASH, with fibrosis as a surrogate primary endpoint.

type may be limited by opposite effects in another liver cell type or by safety concerns due to interferences with extrahepatic metabolic pathways. Targeted delivery of antifibrotic agents to a specific liver cell type has thus emerged as an attractive option and surface markers or receptors expressed by specific liver cell types have been targeted using si-RNA, antisense oligonucleotides, non-lipid nanoparticles or vitamin A-conjugated liposomes (reviewed in¹¹⁰). A number of protein markers can be used, such the asialoglycoprotein receptor in hepatocytes^{111,112} or the PDGFR, IGFIIR, or type VI collagen receptor for activated HSCs.¹¹³ Cell-based macrophage therapies are more challenging, due to the heterogeneity of the cell population, but folate or mannose-scavenger receptors have been identified as potential targets. Nanoparticle-based medicine has also been suggested for the specific targeting of macrophages, HSCs, hepatocytes or LSECs.^{114,115} Further studies will need to adapt these cell-based approaches to the fibrotic liver, since hepatic accumulation of extracellular matrix and closure of endothelial fenestrae may profoundly affect targeting efficiency.¹¹⁶

Conclusion

Concordant preclinical studies have undoubtedly demonstrated that targeting cell-intrinsic metabolism has a beneficial effect on liver fibrosis through the combined reprogramming of hepatocyte, HSC and macrophage phenotypes. Clinical translation remains challenging, although promising results have emerged in the context of NASH-related fibrosis. Whether these metabolic approaches can also be extended to fibrosis originating from other aetiologies remains uncertain.

Abbreviations

ACAT1, acetyl-CoA acetyltransferase; ACC, acetyl-CoA carboxylase; BPTES, Bis-2-(5-phenylacetamido-1,2,4-thiadiazol-2-yl)ethyl sulfide; CCR2/5, C-C chemokine

receptor type 2/5; FBP1, fructose 1,6-bisphosphatase 1; FXR, farnesoid X receptor; GPR91, G-protein coupled receptor 91; HMGB1, high-mobility group protein B1; HSCs, hepatic stellate cells; ILCs, innate lymphoid cells; KCs, Kupffer cells; LSECs, liver sinusoidal endothelial cells; LXR, liver X receptors; MAGL, monoacylglycerol lipase; MAIT, mucosal-associated invariant T cells; MBOAT7, membrane-bound O-acyltransferase domain containing 7; MPC, mitochondrial pyruvate carrier; MSR1, macrophage scavenger receptor 1; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; NK, natural killer; PDGF, platelet-derived growth factor; PFKFB3, 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase-3; PKM2, pyruvate kinase isoenzyme M2; PNPLA3, patatin Like phospholipase domain containing 3; PPAR, peroxisome proliferator-activated receptor; SCD1, stearoyl-CoA desaturase 1; SCOT, succinyl-CoA-oxoacid transferase; SDH, succinate dehydrogenase; SREBP, sterol regulatory element-binding protein; TCA, tricarboxylic acid cycle; TGF- β , transforming growth factor- β .

Financial support

This work was supported by grants from INSERM (France), the Université de Paris, Labex Inflammex and National Research Agency to SL (ANR-10-LABX-17, ANR-18-CE14-0006, ANR-19-CE14-0041).

Conflict of interest

The authors declare no competing financial interests.

Please refer to the accompanying ICMJE disclosure forms for further details.

Authors' contributions

HG, AM RM and SL all contributed to writing.

Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jhep.2021.02.012>.

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