Targeting the Wnt Signaling Pathway in Liver Fibrosis for Drug Options: An Update

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Abstract

Liver fibrosis is a life-threatening disease, with challenging morbidity and mortality for healthcare systems worldwide. It imparts an enormous economic burden to societies, making continuous research and informational updates about its pathogenesis and treatment crucial. This review’s focus is on the current knowledge about the Wnt signaling pathway, serving as an important pathway in liver fibrosis development and activation of hepatic stellate cells (HSCs).

Keywords: Liver fibrosis; Wnt signaling pathway; Hepatic stellate cell, HSC; Therapeutic solutions.

Abbreviations: α-SM, alpha-smooth muscle actin; ADMA, asymmetric dimethylarginine; ALF, acute liver failure; ALV, alainne amino transferase; APC, adenomatous polyposis coli; AST, aspartate aminotransferase; ß-TrCP, beta-transducin repeat containing protein; CK1α, casein kinase II; CK III, cyclic-AMP response element-binding protein; CD11c, carbon tetrachloride; CK10 cassein kinase 1 alpha; COX-2, cyclo-oxygenase-2; CREB, cyclic-AMP response element-binding protein; CRISPR-Cas9, clustered regularly interspaced short palindromic repeats-associated protein 9; CYP, cytochrome P450; DKK1, dickkopf-related protein 1; Dvl, Disheveled; ECM, extracellular matrix; EMT, epithelial-to-mesenchymal transition; Fz, Frizzled transmembrane receptor; GFRα, glial fibrillary acidic protein; GSK-3β, glycogen synthase kinase-3 beta; HBV, hepatitis B virus; HCV, hepatitis C virus; Hh, hedgehog; HSC, hepatic stellate cell; JNK, Jun N-terminal kinase; LEF, lymphoid enhancer binding factor; LPS, lipopolysaccharide; LRP 5/6, low-density lipoprotein receptor-related protein 5/6; MCP-1, monocyte chemotactic protein-1; MMRPs, matrix-degrading metalloproteinases; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; NDRG1, oxidized-nitro domain-containing protein 1; PCP, planar cell polarity; PDGF, platelet-derived growth factor; PKC, protein kinase C; PLC, phospholipase C; PPAR, peroxisome proliferator-activated receptor; PSH, pinositol bilurate hydrate; sFRP, secreted frizzled-related protein family; TCF, T cell-specific transcription factor; TCF-8, transforming growth factor-beta; TIMP-1, metalloproteinase inhibitor 1; WIF1, Wnt inhibitory factor 1.

Two types of Wnt pathways are distinguished, namely the β-catenin-dependent canonical and non-canonical Ca2+ or planar cell polarity (PCP)-dependent pathway. The dynamic balance of physiologically healthy liver and hepatocytes is disturbed by repeated liver injuries. Activation of the β-catenin Wnt pathway prevents the regeneration of hepatocytes by the replacement of extracellular matrix (ECM), leading to the appearance of scar tissue and the formation of regenerated nodular hepatocytes, lacking the original function of healthy hepatocytes. Therefore, liver function is reduced due to the severely advanced disease. Selective inhibition of β-catenin inhibits inflammatory processes (since chemokines and pro-inflammatory cytokines are produced during Wnt activation), reduces growth of activated HSCs and reduces collagen synthesis and angiogenesis, thereby reducing the progression of liver fibrosis in vivo. While the canonical Wnt pathway is usually inactive in a physiologically healthy liver, it shows activity during cell regeneration or renewal and in certain pathophysiological conditions, such as liver diseases and cancer. Targeted blocking of some of the basic components of the Wnt pathway is a therapeutic approach. These include the frizzled transmembrane receptor (Fz) receptors using the secreted frizzled-related protein family (sFRP), Fz-coreceptors low-density LRP 5/6 through dickkopf-related protein 1 (DKK1) or nicosamide, glycogen kinase-3 beta (GSK-3β) using SB-216763, cyclic-AMP response element-binding protein (CBP) using PRI-724 and ICG-001, the lymphoid enhancer binding factor (LEF)/T cell-specific transcription factor (TCF) system as well as Wnt inhibitory factor 1 (WIF1) and miR-17-5p using pinositol bilurate hydrate (PSH). Significant progress has been made in inhibiting Wnt and thus stopping the progression of liver fibrosis by diminishing key components for its action. Comprehending the role of the Wnt signaling pathway in liver fibrosis may lead to discovery of novel targets in liver fibrosis therapeutic strategies’ development.

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Introduction

Liver fibrosis represents a significant global health problem. Due to the fact that fibrosis progression leads to the development of cirrhosis and liver cancer, worldwide mortality related to this condition is 1.5 million deaths per year.1 Numerous epidemiological studies have shown important clinical implications of fibrosis staging.2 Various clinical tools have also been developed in order to better distinguish stages of liver disease and predict mortality with cirrhosis, such as the Child-Pugh score and model for end-stage liver disease score. Etiology of liver fibrosis can be divided into the following two major types of injuries: cholestatic (reduction or obstruction of the gall flow in the liver) and hepatitis (HBV and hepatitis C virus (HCV) infections, metabolic- and alcohol-associated steatohepatitis).3 Common pathophysiological mechanisms involved in fibrosis development, regardless of the etiology, are cytokine release and chronic inflammation, hepatocyte death, HSC activation, and disruption of the endothelial or epithelial barrier.4 Endothelial cells, HSCs and Kupffer cells have various functions, with a particularly important role in fibrosis development. Kupffer cells are liable for the production of different proinflammatory cytokines, and also act as liver macrophages. As the headspring of transforming growth factor-beta (TGF-β) is proven to play a key role in fibrogenesis, Kupffer cells are also an important factor in the development of fibrosis.2,5 Accordingly, liver fibrogenesis represents a complex process which requires extracellular and cellular signaling.2

Recently, reactivation of several signaling pathways, such as Wnt, notch and hedgehog (Hh), has been related with liver injury and regeneration.6 The Wnt signaling pathway is a conserved signal transduction pathway included in the regulation of numerous cellular functions and controlling important aspects of development.7–10 Activation of this signaling pathway is associated to activation of HSCs and fibrogenesis, along with enhanced synthesis of ECM, transformation of epithelial cells or epithelial-to-mesenchymal transition (EMT) or interaction with other profibrogenic mediators.11–13 Through this critical review, we aimed to recapitulate current lore about the Wnt signaling pathway as a part of the underlying pathophysiological mechanism in liver fibrosis development. Also, emerging data about potential molecular treatment options targeting the Wnt signaling pathway are systematically presented.

Wnt signaling pathway

Among several intracellular signaling pathways included in the pathophysiology of liver fibrosis, the Wnt signaling pathway claims a growing share.14 During its activation, it shows fibrotic effects, while poor formation of structure as well as low processes of wound healing occurs during its inactivation.11 As stated above, based on the involvement of β-catenin, the Wnt signaling pathway principally encompasses two classes: canonical and non-canonical. The correlation of these two pathways can also be expressed through the possibility that non-canonical Wnt ligands may have a negative effect on the canonical pathway.11

Canonical pathway

The key component of the canonical Wnt signaling pathway is its downstream actions15 through β-catenin, acting as a protein with dual function (as a transcription factor and an adhesion molecule).16 It is important to emphasize that in healthy liver this protein is localized in the membrane of hepatocyte,17 but in injured liver this location changes to the cytoplasm. In the adhesion cell-cell processes, binding of adhesive and transmembrane component (such as E-cadherin to actin; basically adhesive component-protein)18 within the cytoskeleton is enabled when β-catenin is bound to the plasma membrane; the catenin acts as a bridge between cadherin and actin, as shown in Figure 1. Meanwhile, catenin is the Wnt pathway’s major transducer when localized in the cytoplasm.19 Due to the fact that β-catenin cannot bind directly to DNA in order to make contact with target genes, it relies on coactivators and various transcription factors.19 Its own transcription factor function is mostly regulated by canonical Wnt proteins (e.g., Wnt1, Wnt4, Wnt8b), which are mainly located in the extracellular space. As extracellular signaling molecules, they bind and initiate signaling processes, leading to β-catenin’s cascade reaction.20

When Wnt signaling is off, β-catenin is located in the cytoplasm in the low regime, whereas in the high regime β-catenin is comorbid with either one of the two transcriptional coactivators: cAMP-response element-binding protein (CREB) or p300.28 Decreased degradation of β-catenin results from stabilization caused by the destruction complex disassociation, with absence of β-catenin phosphorylation.25,26 Subsequently, the proportion of unphosphorylated β-catenin increases, followed by its translocation to the nucleus. Although the mechanism of β-catenin’s translocation to nucleus is yet unknown, the main action is to become bound to lymphoid enhancer binding factor (LEF)/T cell-specific transcription factor (TCF) in order to initiate targeted genes’ transcription.27 On the molecular level, it requires engagement with either one of the two transcriptional coactivators: cAMP-response element-binding protein (CREB) or p300.28 Recently, Yu et al.,29 demonstrated a suppressive effect on Wnt by acting through the clustered regularly interspaced short palindromic repeats-associated protein 9 (CRISPR-Cas9) system as an editing system to reduce the effect of LRP 6 gene in mice with alcohol-induced liver injury.

Non-canonical pathway

Non-canonical signaling pathways are β-catenin-independent.20 They encompass non-the canonical Wnt/Ca2+ pathway and PCP pathway1 as shown on Figure 2. In the Wnt/Ca2+ signaling pathway, binding of a non-canonical Wnt protein (e.g., Wnt5a) results in activation of the cytoplasmic protein Dvl, which increases the concentration of cytoplasmatic Ca2+ and subsequently activates the protein kinase C (PKC) and calcium sensitive enzymes calmodulin kinase II (CamKII).20 It is also important to emphasize its role in increasing the activity of phospholipase C (PLC) and the nuclear factor related to T cells’ activation in transcrip-
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Fig. 1. Activated and inactivated canonical Wnt signaling. In an inactivated state, β-catenin in the hepatocyte membrane forms a bridge between actin and E-cadherin. When Wnt signaling is off, β-catenin (in a multiprotein complex with GSK-3β, axin, CK1α, β-TrCP and APC) is phosphorylated by GSK-3β and CK1α and ubiquitinated by β-TrCP. In the end, β-catenin is degraded by the proteasome. When Wnt signaling is on, Wnt-Fz and LRP coordinate the activation of Dvl, leading to dissociation of the multiprotein complex and resulting in the inactivation of GSK-3β (no phosphorylation anymore). Excessive free β-catenin translocates to the nucleus and binds to TCF/LEF transcription factors, resulting in a transcriptional activation of Wnt target genes. GSK-3β, glycogen synthase kinase-3 beta; CK1α, casein kinase 1 alpha; β-TrCP, beta-transducin repeat containing protein; Wnt-Fz, Wnt-Frizzled transmembrane receptor; LRP, low-density lipoprotein receptor-related protein; Dvl, Disheveled gene; TCF/LEF, T cell-specific transcription factor/lymphoid enhancer binding factor.
Liver fibrosis

HSCs

HSCs belong to the group of specialized liver pericytes located in the space of Disse, between endothelial sinusoidal cells and hepatocytes. Their major role relates to molecular mechanisms of transdifferentiation, itself representing a key role in liver fibrosis. Numerous investigations have attempted to discover the key role of the Wnt signaling pathway in HSCs and liver fibrosis. Nevertheless, its role in HSC biology still remains to be fully elucidated.

In the physiological condition, HSCs are in a quiescent state and store retinoids. Also, in the inactive stage, they...
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synthesize glialfibrillaryacidic protein (GFAP), a component of the ECM.

The matrix itself may be degraded by numerous different enzymes, but the matrix-degrading metalloproteinases (MMPs) have a key role in the degradation process. Due to that, ECM does not accumulate to cause fibrosis. In healthy liver, HSC’s major function is to maintain extracellular matrix integrity and accumulation of retinyl esters forms of vitamin A in cytoplasmic lipid droplets. In conditions of liver damage, their transformation into myofibroblasts occurs as a part of wound healing response. If the injury is prolonged, activated HSCs stimulate the production of ECM components, while reducing their degrada-
tion and therefore becoming the major fibrogenic type of liver cell.41 Mechanical or inflammatory processes may cause liver damage and subsequent HSCs’ activation, including Wnt signaling pathway on a molecular level. As a matter of fact, activated HSCs result in increased α-smooth muscle actin (α-SMA) and collagens type I and II (i.e. the ECM components). Moreover, growth regulation by platelet-dele-
grown factor (PDGF) and TGF-β1 are also increased.42 On the other hand, activation of HSCs decreases retinoids and GFAP. The profibrogenic cytokine TGF-β1 affects MMP pathways, particularly down-regulating interstitial collagenase expression, simultaneously up-regulating expression of metallopeptidase inhibitor 1 (TIMP-1), collagen I and gelatinase A.43 Hence, the binding of TIMPs to activated MMPs causes irreversible deposition of ECM, which leads to liver fibrosis.44

For these mechanisms, it has been hypothesized that HSC activation and proliferation can be hampered, or the rate of apoptosis enhanced, the progression of liver fibrosis may be inhibited as well.45

Even though HSC cells cultures have shown their potential in observation of fibrogenesis and in the estimation of complex toxicity responses, the deficiency of cultures and reliable sources of HSCs restrict their utilization.46,47 A few published findings show correlation among liver fibrosis and activation of the Wnt signaling pathway in HSCs. Antagonism of the Wnt signaling pathway with an inhibitor of interactions among β-catenin and CREB-binding protein suppresses and reverses HSC activation, resulting in attenuation of liver fibrogenesis.48,49 In 2008, Kordes et al.50 demonstrated that the Wnt/β-catenin pathway negatively regulates HSC activation. On the other hand, a larger number of studies have provided evidence to support a positive correlation between activation of the canonical Wnt signaling pathway, fibrosis and the process of HSC activation.49,51,52 However, simultaneously, for non-canonical Wnt signaling, β-catenin (independent) and its components have been observed as contributors of HSC activation and as compounds of fibrotic livers.53,54

Pathophysiological mechanism of fibrosis

Pathophysiology of liver fibrosis involves a complex interplay of many mechanisms, including the intracellular signal-
ing pathways of Wnt/β-catenin, GAS6/Axl and TGF-β/Smad,55 and other preserved morphogenetic developmental signaling pathways, such as of notch and Hh.56 Repeated liver injuries, massive accumulation of ECM leading to cell stiffening, and the appearance of scar tissue disrupts liver homeostasis.57 In the absence of hepatocyte regeneration, the cells are replaced by ECM and the formation of regenerating nodular hepatocytes.56 The Wnt pathway has been shown to be activated in the process of liver fibrosis involving β-catenin, Fz receptors and LRP coreceptor upregulation.57 Downregulation or selective inhibition of the Wnt/β-catenin pathway significantly inhibits activation, proliferation and migration of HSCs in vitro, also processes of fibrosis, inflammation and angiogenesis are attenuated in vivo.58 Selective inhibition of the Wnt/β-catenin pathway using ICG-001 inhibits HSCs activation, differentiation, contractility and migration in vitro, as well as collagen deposition and processes of fibrosis, inflammation and angiogenesis in vivo.59 It is known that activation of HSCs also results in the secretion of CXCL12, which stimulates macrophage infiltration of the liver and HSCs’ activation, thus promoting fibrosis, inflammation and angiogenesis.60 When inhibiting the Wnt/β-catenin pathway, production of CXCL12 and the processes of fibrosis, inflammation and angiogenesis are attenuated in the liver.61

Studies have shown the connection between Wnt5a and TGF.62 When Wnt5a expression was studied in fibrotic livers of mice and human, upregulation of Wnt5a gene and protein was found in comparison to that in healthy livers, and the level of Wnt5a was also found to have decreased after therapeutic intervention in mice.63 In vitro studies have shown that expression of Wnt5a and Wnt receptors Fz2 and Fz8 were significantly enhanced by TGF.64 Levels of collagen type I and fibronectin in TGF-stimulated myofibroblasts are increased, along with Wnt, and decreased when Wnt5a is suppressed by antifibrotic cytokine in vitro and in vivo.65 In addition, suppression of Wnt5a in activated LX2 cells decreases production of both collagen type I and TGF-β.66 A recent study showed that establishment of fibrosis is affected by the induction of EndMT,67 which is crucial for the production of myofibroblasts in fibrous organs and tissues.60,61 Increased TGF-β expression increases asymmetric dimethylarginine (ADMA) and factors of inflammation, while a decrease is expected in nitric oxide secretion and nitric oxide synthase activity, as well as in dimethyl arginine dimethylaminohydrido-
lase-1 (DDAH1), Nrf2 and VE-cadherin; all, together are defined as factors for fibrosis improvement through EndMT.68 Levels of Wnt5a in serum follow hepatosteatosis, nonalco-
holic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH).62 It is supposed that suppression of Wnt5a, as it has proinflammatory effects, can reduce NASH; based on that hypothesis, studies using celecoxib, an inhibitor of cyclooxygenase-2 (COX-2), were performed.69 In a rat model of NASH, the expression of Wnt5a, COX-2, JNK1 and NF-κB p65 were higher, as observed by levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT), and histologically; however, the administration of celecoxib suppressed their expression and inflammation in liver.65 It was concluded by the authors that it is possible to amelio-
rate NASH by suppressing the Wnt5a/JNK1 pathway.63 In another study, pharmacologically-increased Wnt3a and β-
catenin, along with suppressed Wnt5a, produced an anti-
flammatory effect in hepatosteatosis, NAFLD, and NASH.20

Hepatic fibrosis used to be considered an irreversible pro-
cess, taking into account that hepatic parenchyma is de-
structed and replaced with fibrotic tissue; however, laborato-
ry and clinical studies have revealed possible reversibility of progressive Wnt signaling pathway-related liver fibrosis.64 Studies performed in vitro and in vivo have also lent support to the hypothesis that β-catenin protects hepatocytes through the inhibition of apoptosis associated with FoxO3; importantly, such findings can be relevant for future therapeu-
tic interventions in the field of liver injury protection, repair, and regeneration.65 A recent study confirmed that increased EMT due to Dact2 (an antagonist of β-catenin) deficiency helps to promote healing processes in the liver.66 Dact2 inhibits binding of Lef1 to β-catenin and promotes degradation of Dvl; by establishing re-expression of Dact2, T-cell factor 4 (its transcriptional activity) and downstream signaling of Wnt are stimulated, which is known to play a role in gene suppression.66

Therapeutic solutions

Scientists have been steadily working for years on elucidat-
ing the underlying molecular mechanisms responsible for liver fibrosis development and to develop adequate therapeu-
tic strategies (which have to be validated in preclinical and clinical trials). 67–72 Alleviation of the Wnt signaling path-
way is possible, due to its members Wnt1, Wnt3a and Wnt10b, as well as Fz2 and S; WIF is influential, in that it joins to ei-
ther its ligand, the sFRP family or antagonist to prevent association between the LRP coreceptor and Fz. Agents that exer-
it inhibitory effects on the Wnt signaling pathway are being considered to have preventive or therapeutic ef-
fects in liver fibrosis; these are DKK1, niclosamide, sFRP5, SB-216763, omoepas, PRI-724 and pinoctilbene hydrate (PSH), as shown in Figure 3.

**DKK1**

DKK1 is among the best-characterized inhibitors of the Wnt/β-catenin signaling pathway. It prevents binding between Wnt and the LRP5/6 component of the receptor complex, resulting in a disruption of Fz-LRP6 dimerization. 74 DKK1 was used to prove the role of the Wnt canonical pathway in ac-

**sFRP5**

sFRP5 is declared as an adipocytokine with anti-inflamma-
tory and anti-fibrotic effects, that plays a regulatory role in metabolic homeostasis. In mouse models, it has been shown that sFRP5 can inhibit effects of Wnt3a/Fz2 on proliferation, migra-
tion and activation of Kupffer cells and inflammatory fac-
tor expression of adipokines in hepatic tissues (also shown in a different NASH model). 87 Intervention with recombinant sFRP5 significantly lowered levels of I16, monocyte chem-

**Niclosamide**

Antifibrotic effect of the Food and Drug Administration-ap-
proved antihelminthic niclosamide was recently confirmed by El Ashmawy et al. 56 in carbon tetrachloride (CCL4)-in-
duced fibrosis in mice. Niclosamide suppressed expression of TGF-β1 and the Dvl2 genes 56,79 and activity of β-
catenin. 56,80 Serum levels of ALT, AST, L-hydroxyproline and L-glutaminase activity and total bilirubin were significantly reduced by niclosamide and CCL4 compared to a mouse group treated with CCL4 only. 56 Significant reduction of α-SMA expression, which confirmed niclosamide’s inhibi-
tory effect of S100A4 81 and was responsible for induction of α-SMA, 82 was also proven. Niclosamide achieved a weak-
ened effect of autophagy-induced apoptosis, 83,84 which can be explained by the fact that apoptosis may be one of the main mechanisms of liver fibrosis resolution, despite its as-

**SB-216763**

SB-216763 (3-(2,4-Dichlorophenyl)-4-(1-methyl-1H-indol-
3-yl)-1H-pyrrole-2,5-dione) is a selectable and potent GSK-3ß inhibitor, capable of exerting an effective role in therapy of many diseases and currently under investigation. GSK-3ß has a known regulatory role in the inflammatory response and cytokine production, as well as in cellular proliferation. Over the years, GSK-3ß has gained importance among researchers, for its role in the Wnt/β-catenin signaling pathway. Studies have shown that inhibition of GSK-3ß

![Diagram of Activated and Inactivated Canonical Wnt Signaling](image_url)

**Fig. 3. Activated and inactivated canonical Wnt signaling.** When Wnt signaling is off, β-catenin (in a multiprotein complex with GSK-3ß, axin, CK1α, β-TrCP and APC) is phosphorylated by GSK-3ß and CK1α and ubiquitinated by β-TrCP. In the end, β-catenin is degraded by the proteosome. When Wnt signaling is on, Dvl inhibits β-catenin phosphorylation by inhibiting GSK-3ß activity. The Wnt-Fz-LPR5/6 heterotrimer coordinates Dvl activation, dissociating the multiprotein complex and resulting in the inactivation of GSK-3ß (no phosphorylation anymore). Unphosphorylated β-catenin increases, then migrates to the nucleus and links to TCF/LEF and CBP. The TCF/β-catenin complex links DNA and leads to transcription of Wnt target genes. Antifibrotic compounds that inhibit Wnt signaling and prevent liver fibrosis are DKK1, niclosamide, sFRP5, SB-216763, PRI-724, ICG-001, and PSH. GSK-3ß, glycogen synthase kinase-3 beta; CK1α, casein kinase 1 alpha; β-TrCP, beta-transducin repeat containing protein; APC, adenomatous polyposis coli; β-TrCP, beta-transducin repeat containing protein; APC, adenomatous polyposis coli; β-TrCP; Dvl, Disheveled gene; Wnt-Fz-LPR 5/6, Wnt-Frizzled transmembrane receptor-low-density lipoprotein receptor-related protein 5/6; TCF/LEF, T cell-specific transcription factor/lymphoid enhancer binding factor; CBP, cyclic-AMP response element-binding protein; DKK1, dickkopf-related protein 1.
results in anti-inflammatory cytokine production. Inhibition of GSK-3β has also been studied in pulmonary fibrosis for which administration of SB-216763 has produced beneficial effects on the inflammatory and profibrotic milieu by inhibiting the production of MCP-1 and TNFα in pulmonary macrophages; significant reduction in bleomycin-induced apoptosis of alveolar epithelial cells has also been noted. In vivo administration of SB-216763 to mice has been proven as safe for preventing bleomycin-induced respiratory distress syndrome, as there were no toxic effects on heart, liver or kidney, and in improving survival of treated mice. These results positioned GSK-3β as a potential molecular target in pulmonary fibrosis treatment, and it has been further investigated in diabetes mellitus, bipolar disorder, Huntington’s disease, Alzheimer’s disease, Parkinson’s disease and septic shock.

It is well known that liver dysfunction can occur in cases of sepsis. Studies suggest that activation of GSK-3β is involved in apoptosis and excessive inflammation in acute liver failure (ALF). Zhang et al. investigated the inhibitory effects of GSK-3β on polymicrobial sepsis in a liver injury model (induced by cecal ligation and puncture). Inhibition of GSK-3β in an animal model, by means of SB-216763 administration, resulted in reduced in mortality, amelioration of liver injury and suppression of hepatic apoptosis. On the molecular level, decreases in leukocyte infiltration, expression and release of inflammatory cytokines in the liver were noted. NF-κB transcriptional activity was enhanced. The authors concluded that inhibition of GSK-3β reduces inflammatory cytokine production through modulation of the NF-κB and CREB signaling pathways in macrophages simulated by lipopolysaccharide (LPS). As the studies have shown, GSK-3β was activated during ALF progression, and its inhibition mitigated inflammation of the liver and ameliorated ALF in the mouse model. In the study by Ren et al., the coinjection of D-galactosamine and LPS was used to induce ALF. Inhibition of GSK-3β by administration of SB-216763 resulted in increased autophagy and decreased liver inflammation. GSK-3β inhibition by SB-216763 protected against aldosterone-induced renal and cardiac injuries by activating autophagy. However, for cholestatic liver disease in a mouse model, a study by Zhuang et al. demonstrated that SB-216763 therapy aggravated liver fibrosis.

IGC-001

IGC-001 is characterized as a selective first-generation CBP inhibitor, capable of disrupting β-catenin’s interaction with CREB and thereby inducing the expression of the anti-proliferative and anti-fibrotic effect on survivin significantly; survivin is one of the inhibitors of cyclin D1 and apoptosis. This therapeutic solution was primarily designed for targeting colon carcinoma cells, in order to induce apoptosis; although, in physiologically normal (epithelial) cell lines, the effect was not noticed. There are reports on the beneficial and inhibitory effects of ICG-001 in fibrosis; meanwhile, another report indicated that mRNA levels of collagen I, Wnt3a, a-SMA, LRP6 and Wnt10 stimulated with TGF-β in mouse fibroblasts and human HSCs were inhibited with this CBP inhibitor. Attenuation of lung fibrosis (as induced by bleomycin) in mice was observed with ICG-001 treatment, as was prevention of fibrosis when simultaneously administering bleomycin and ICG-001, which subsequently reversed the pathogenic effect at later stages of the disease and positively impacted survival rate. A similar outcome was achieved with renal fibrosis, which is regulated expression of the corresponding genes in vitro models of TGF-β-induced LX2 cells (HSCs). Furthermore, due to HSC migration during fibrogenesis and differentiation into myofibroblasts (contractile), scientists discovered an inhibitory effect with ICG-001 at 48 h and maximum inhibition in 72 h. The alamarBlue cell viability assay (ThermoFisher, Waltham, MA, USA) was used to confirm the absence of a relationship between migration of cells and proliferation differences. Confirmatory investigations of the findings from the previous study on LX2 cells (all parameters included, except vimentin) resulted in the same outcome on primary human HSCs, and ICG-001 was shown to inhibit expression of peristin. ICG-001-related downregulation of Axin-2 and β-catenin was also noted in a mouse liver injury model induced by CCl₄. The beneficial effects on fibrosis, as measured by ICG-001 were enhanced when it was used as a prototype in the development of PRI-724, a novel promising and effective therapeutic solution for liver fibrosis.

PRI-724

PRI-724 is selective CBP second-generation inhibitor and influences β-catenin interaction. It acts through HSC inhibition and disruption of the macrophage-inflammation system, exerting antifibrotic effects that have been confirmed in murine models of fibrosis. In a murine model of CCl₄-induced liver fibrosis, administration of PRI-724 showed accelerating effects on the resolution of fibrosis in liver followed by an increasing effect on MMPs in intrahepatic leukocytes. It is significant that this inhibitor reduced CCL₄-induced liver fibrosis in mice without affecting levels of serum ALT and proved incoherence with reparation of hepatic fibrosis. Effects of C-82, an active PRI-724 metabolite, was demonstrated through a mRNA-mediated suppression of α-SMA, TIMP-1 and collagen 1, as well as down-regulation of proteins such as Kι67, α-SMA and cyclin D. Gene expressions in activated HSCs were abrogated by PRI-724 and ECM production of C-82, an active PRI-724 metabolite, was demonstrated, which led to antifibrotic effects. HCV transgenic mice are another model system used to determine effects of PRI-724 on liver fibrosis, collagen production, and levels of α-SMA. A study using this model showed attenuation of the previous increased collagen. Levels of α-SMA, reflecting HSC activation, were reported to be higher in the transgenic mice in comparison with controls and the administration of PRI-724 attenuated this effect. Another study showed decreased levels of TIMP-1 and elevated levels of MMP-8 as another line of evidence supporting PRI-724’s anti-fibrotic effect.

A NASH mouse model was used to investigate the antifibrotic effects of PRI-724; the administration of PRI-724 inhibited hepatocyte apoptosis and hepatic fibrosis. PRI-724 was also used in clinical trials that included advanced stages of myeloid malignancies, pancreatic cancer and solid tumors, and according to the available literature there has been a phase I clinical trial (single-center, open-label) on the tolerance and safety of PRI-724 in HCV cirrhosis patients, in which it showed good tolerability. Reduction of hepatic lobule fibrosis by PRI-724 improved liver histology (in a dose-dependent manner) as well as Child-Pugh scores (in some individuals). In a pre-clinical study of PRI-724 administration to dogs for 28 days at the dosage of 120 mg/
kg/day showed that this therapeutic solution did not yield any adverse effect; in addition, when given to 18 participants with solid tumors (dose range: 40–1,280 mg/m²/day), hyperbilirubinemia (reversed) was recorded as an event not directly correlated. Overall, PRI-724 has been very well tolerated, and no fatalities have been reported. Taking into account a small sample of patients (n=14 in total) in one study, the following side effects were noted: fatigue (n=3) and nausea (n=4), vomiting (n=2), constipation (n=2), and reaction at the injection site (n=4). Other less common side effects included pruritus, rash, headache, fever, vertigo, insomnia, and bleeding; no and biochemical results showed the most common to be elevations in total bilirubin and thrombocytopenia. Two other serious adverse events not directly related to the study were reported, namely bacillemia due to injection site infection and hemorrhage due to liver biopsy. However, a phase Ia/Ib clinical trial (NCT01302405) was discontinued due to low patient response. Currently, PRI-714 (new drug name: OP-724) is in a phase I clinical trial (NCT04688034) for patients with liver cirrhosis caused by human immunodeficiency virus/HCV co-infection with hemophilia.

Of note, the previous clinical studies investigating PRI-724 have included a small number of patients. As such, further and more detailed studies with larger samples are needed to gain a clearer understanding of its safety and efficacy. We hope that one of the aforementioned clinical trials will yield significant results.

**PSH**

PSH, a methylated derivative of resveratrol, has been previously evidenced to have antioxidant, anti-inflammatory and anticarcinogenic effects, and has been observed to act as reducing HSC activity. The inhibitory effect was achieved by suppressing the Wnt/β-catenin pathway via miR-17-5p (acting as an oncogene) and also that it could enhance WIF1 expression. Since activation of HSCs is at the core of liver fibrosis development, it is rational to develop a therapeutic strategy that would block or limit their activation. The inability of β-catenin translocation into the nucleus and reduction of TCF activity is due to inhibition of resveratrol on the Wnt/β-catenin-dependent pathway. MicroRNAs, as a group of non-coding RNA molecules, can significantly affect the course of liver fibrosis development, as they regulate the action of HSCs. A study by Yu et al. showed how resveratrol can reduce the progression of liver fibrosis, while one by Chao et al. demonstrated that the PSH, as a methylated form of resveratrol, is more stable than other forms. As previously mentioned, the imbalance between ECM synthesis and its degradation is at the core of the liver fibrosis process. The presence of myofibroblasts derived from HSCs form the basis for the production of ECM that contributes to the development of liver fibrosis.

PSH’s beneficial effects involve reducing cell proliferation, collagen production and α-SMA expression, and partially inhibiting HSC activation. The inability of β-catenin translocation into the nucleus and reduction of TCF activity is due to PSH caused by an increased amount of APC, phosphorylated-β-catenin, GSK-3β and WIF1 (acting as Wnt antagonist) that inhibit the Wnt β-catenin-dependent pathway. This therapeutic solution needs to be further investigated in correlation in situations of liver fibrosis development and treatment; however, the potential adverse effects of resveratrol may limit its usage.

At a dose of 100 µM, resveratrol reduces the regulation of vascular endothelial growth factor and inhibits the formation of major blood vessels in zebrafish; in this model, it also reduces survival and hatching rate of eggs, and causes teratogenic deformities and cardiac edema. Administration of resveratrol to rats at doses of 300/1,000/3,000 mg/kg/day led to hepatic impairment (confirmed by aberrant liver gene expression); at a dose of 1,000 mg/kg/day, it increased bilirubin levels. A dose of 1,000–3,000 mg/kg/day, administered for 4 weeks, induced renal toxicity. Guha et al. showed that resveratrol can delay the healing of gastric ulcers in mice. At very high doses, resveratrol has been shown to cause severe adverse effects. Lethal outcomes have been regarded in rats, due to acute inflammation of the pelvic region, renal tubular dilatation, papillary necrosis, severe nephropathy, and cardiac inflammation. In addition, increased levels of liver enzymes as well as blood urea nitrogen and creatinine can be a significant problem.

It is important to emphasize that resveratrol can act as a substrate for tyrosinase (an enzyme essential for the production of melanin) for the production of toxic o-quinones, which cause cytotoxicity of melanocytes (thiol protein production) and may have an adverse effect on the skin. The adverse effect on the skin can be explained by the fact that resveratrol is very similar to rhododendrol, which is used for whitening and lightening in cosmetics and is basically also a tyrosinase inhibitor that can increase the incidence of leukoderma skin toxicity, so a similar effect of resveratrol can be assumed. Resveratrol is known to exert pleiotropic effects on humans, and so far adverse effects involving nephrotoxicity and gastrointestinal problems have been reported. Although there is little information on human clinical trials (several are awaiting publication), it is known that resveratrol can cause an increase in plasma ALT and decreases in white blood cell count and plasma IL-6 and TNF. Resveratrol at a dose of 1,000 mg/day or higher can cause significant interactions with other drugs because it activates the cytochrome P450 (CYP) isofrom CYP1A2 and inactivates CYP3A4, CYP2C9 and CYP2D6. At the same dose, an increase in markers of cardiovascular disease has been observed. Administration of resveratrol in high doses (2,000–5,000 mg/day) may cause nausea, hypersensitivity, anal pruritus, and episodes of diarrhea (light and mild), which is a more important consideration for sick persons than for healthy ones. The aforementioned has been confirmed by a phase II clinical trial in humans with refractory multiple myeloma, in which 5,000 mg of resveratrol was administered; one patient had a lethal outcome, probably due to the adverse effects (renal toxicity, fatigue, nausea, diarrhea).

The agents discussed above have evidenced potential therapeutic effects in liver fibrosis, acting through numerous molecular mechanisms and signaling cascades, including inflammation and fibrogenesis.

**Conclusions**

Liver fibrosis is a significant global health problem and economic burden, with potential to progress to liver cirrhosis and cancer. Its worldwide mortality is 1.5 million deaths per year. Regardless of etiology, the pathophysiology of fibrosis includes chronic inflammation, hepatocyte death, HSC activation, and endothelial or epithelial barrier disruption, all mediated by various proinflammatory cytokines and other chemokines, and including several signaling pathways, such as those of Wnt, notch and Hh. The Wnt signaling pathway is activated in the process of ongoing liver fibrosis. It acts through canonical and non-canonical pathways. Several agents that have shown inhibitory effects on Wnt signaling are being considered to impart preventive or therapeutic effects on liver fibrosis. These are DKK1, niclosamide, sFRP5, SB-216763, ICG-001, PRI-724 and PSH. Their validation and utility with regards to potential adverse effects has yet to be proven, but the future remains promising. In this review, the current lore about the Wnt signaling pathway,
which notably participates in liver fibrosis development, was gathered and summarized. Considering the critical points of the Wnt signaling pathway in liver fibrosis, the findings on potential therapeutic targets in liver fibrosis and agents that are being considered for preventive or therapeutic effects in liver fibrosis were presented.

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Conflict of interest
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Author contributions
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