PDGF signaling pathway in hepatic fibrosis pathogenesis and therapeutics (Review)

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Abstract. The platelet-derived growth factor (PDFG) signaling pathway exerts persistent activation in response to a variety of stimuli and facilitates the progression of hepatic fibrosis. Since this pathway modulates a broad spectrum of cellular processes, including cell growth, differentiation, inflammation and carcinogenesis, it has emerged as a therapeutic target for hepatic fibrosis and liver-associated disorders. The present review exhibits the current knowledge of the role of the PDGF signaling pathway and its pathological profiles in hepatic fibrosis, and assesses the potential of inhibitors which have been investigated in the experimental hepatic fibrosis model, in addition to the clinical challenges associated with these inhibitors.

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1. Introduction

Hepatic fibrosis is a common chronic disease caused by long-term stimulation of one or more physical, chemical or microbial factors in the liver. Hepatic fibrosis is characterized by an aberrant accumulation of fibroblasts and excessive extracellular matrix (ECM) deposition, with evident inflammatory lesions and structural alterations (1). Despite the unclear pathogenesis of hepatic fibrosis, early diagnosis and treatment may reduce the mortality rate of patients (2). Therefore, attenuating or reversing hepatic fibrosis has become an important factor to be considered in the prevention and treatment of chronic hepatic injury and cirrhosis.

Hepatic stellate cells (HSCs) are the cells which primarily contribute to fibrogenesis during liver injury. These oval-shaped cells are located in the hepatic sinusoidal space and the space of Disse (3). Numerous retinoid lipid droplets may be observed in the cytoplasm of HSCs, indicating that the primary functions of HSCs are to store and metabolize vitamin A, secrete ECM and produce collagenase. Therefore, these cells are primarily involved in collagen synthesis in the liver. The activation and proliferation of HSCs are important events in hepatic fibrosis (4). A previous study suggested that the activation of HSCs may include three important stages: The initial stage, permanent stage and inflammation resolution stage (5). In the initial stage, ECM and other products, including peroxides, are released from the damaged hepatocytes. In the permanent stage, cellular behaviors are categorized into at least six types: Proliferation, chemotaxis, fibrogenesis, contraction, ECM degradation and vitamin A depletion. In the inflammation resolution stage, HSC apoptosis is promoted, or HSCs may be transformed into a quiescent state. Hepatocyte apoptosis and necrosis caused by various harmful factors, including the inflammatory response in liver tissues, activate Kupffer cells to secrete pro-inflammatory cytokines. These cytokines, in addition to various chemical transmitters, activate and transform HSCs into myofibroblasts, resulting in an alteration to the functional phenotype of HSCs (6). In addition, activated HSCs may promote the proliferation of myofibroblasts through paracrine or autocrine mechanisms, thereby synthesizing large amounts of collagen fibers and other ECM components. During this process, regulators, including tumor

necrosis factor-α (TNF-α) (7,8), transforming growth factor β1 (TGF-β1) (9) and platelet-derived growth factor (PDGF) (10), and the ECM, interact with each other (11,12) to form a sophisticated network and serve roles in hepatic fibrogenesis.

Among these cytokines, PDGF is the most potent factor involved in stimulating HSC proliferation, differentiation, and migration (10). PDGF additionally promotes collagen production and deposition, and transforms HSCs into myofibroblasts. Blocking PDGF signaling inhibits HSC proliferation and ameliorates liver fibrogenesis (12). Clinical studies have additionally confirmed that excessive activation of PDGF and its downstream molecules appears to be associated with the extent of necroinflammation and fibrosis in patients with hepatic damage (13-15). Therefore, the PDGF signaling pathway serves important roles in the development and prognosis of hepatic fibrosis.

2. PDGF and the PDGF receptor (PDGFR)

PDGF family. PDGF is a polypeptide growth factor with a relative molecular weight of 28-35 kDa; PDGF effectively promotes cell division and proliferation. PDGF is primarily produced by platelets, vascular endothelial cells, pericytes and Kupffer cells. PDGF, as the product of oncogenesis, was first identified in platelet α-granules at 1,000 PDGF molecules/platelet. Under physiological conditions, PDGF is primarily secreted by Kupffer cells. Once a tissue has been damaged, various diploid cells may synthesize and secrete PDGF, which is associated with the proliferation of connective tissues, including fibroblasts, and vascular endothelial cells, via autocrine and paracrine mechanisms (16).

A total of four PDGF subunits, termed PDGF-A, -B, -C and -D, have been identified. These subunits contain highly conservative homologous structural domains of PDGF/vascular endothelial growth factor (VEGF), and they produce five homologous or heterogeneous biopolymers, termed PDGF-AA, -BB, -AB, -CC, and -DD, via a disulfide-bond linkage (17,18). Among the dipolymers, PDGF-A, which exhibits a molecular weight of 16 kDa, is composed of 211 amino acids (aa), and located at the chromosomal site 7p22. PDGF-A is highly-expressed in the muscle, aorta and heart (19). PDGF-B, with a molecular weight of 14 kDa, is located at the chromosomal site 22q13 and is highly-expressed in the placenta and heart. These two dipolymers are able to form three homologous or heterologous dipolymers, termed PDGF-AA, -BB and -AB. PDGF-AA primarily binds with PDGFR-αα to control the proliferation and chemotaxis of cells, while PDGF-AB binds with PDGFR-αα and PDGFR-αβ, and PDGF-BB binds with all subunits (PDGFR-αα, -αβ and -ββ); PDGFR-AB and -BB are able to promote collagen synthesis and cellular adhesion (17).

The remaining PDGF subunits, PDGF-C and PDGF-D, were discovered by comparing sequence data in the Expressed Sequence Tags database (20,21). The structures and mechanisms of these subunits remain to be elucidated. PDGF-C is composed of 345 aa, exhibits a molecular weight of 36.7 kDa, and consists of three N-linked glycosylation sites at the 22nd, 55th and 254th aa residues. PDGF-D consists of 370 aa, exhibits a molecular weight of 40.2 kDa, and consists of an N-linked glycosylation site at its 276th aa residue.

Unlike the first two isoforms, PDGF-C and PDGF-D only produce homologous biopolymers, termed PDGF-CC and PDGF-DD, the activity of which may be promoted via specific protease cleavage of their CUB domains. PDGF-C and -D are highly-expressed in the kidneys and heart.

PDGFR. PDGFR, which belongs to the receptor tyrosine kinase (RTK) family and serves the function of a protein tyrosine kinase, is primarily located in vascular endothelial cells, fibroblasts and Kupffer cells (22). PDGFs have two types of receptor, termed PDGFR-α and PDGFR-β, thereby forming three subtypes of dipolymers, termed PDGFR-αα, -αβ, and -ββ. PDGFR-α and PDGFR-β each contain five structural domains: Immunoglobulin-like domain, transmembrane domain, ATP binding site, intracellular hydrophilic kinase insert domain, and cytoplasmic tail; these receptors are respectively located at the chromosomal sites 4q12 and 5q33. PDGFR-α and PDGFR-β display different expression patterns and physiological functions. Additionally, the PDGFR-α signal and the PDGF-A, PDGF-B and PDGF-C chains, which exhibit close binding affinities, are associated with the early hematopoietic system and vascularization (23); however, PDGF-B and PDGF-D exhibit high binding tendencies with PDGFR-β (24). Therefore, PDGF-B is more sensitive to PDGFR-α and PDGFR-β compared with other subunits.

3. Biological functions of PDGF and PDGFR

In physiological conditions, PDGF is primarily expressed in the α-granules of platelets. However, when liver damage occurs, PDGF may be highly-expressed in macrophages, injured endothelial cells and activated HSCs. During the early stages of various chronic liver diseases, increased PDGFR expression on the membranes of activated HSCs, in addition to activation of HSCs by the synthesized PDGF via the autocrine mechanism, enhances cellular chemotaxis and decreases the amount of intracellular vitamin A, demonstrating that PDGF is involved in ECM production (22,23). Further studies have also demonstrated that the four PDGF subunits serve different roles in the pathogenesis of hepatic fibrosis. In particular, PDGF-B has been demonstrated to be the most potent factor associated with HSC activation (23,25). PDGF-A mRNA expression exhibits a minor fluctuation during the process of activated HSC transdifferentiation to myofibroblast-like cells; PDGF-B is elevated during the early stage, although it is markedly reduced from day 3 (17). By contrast, PDGF-C and -D mRNA levels continuously rise during the whole process of transdifferentiation (17). In addition, quiescent HSCs only express PDGFR-α, whereas activated HSCs display a marked upregulation of the expression of PDGFR. The expression profile of PDGFR-β mRNA is consistent with that of PDGF-C and -D during transdifferentiation (17). This finding indicated that signal transduction is primarily mediated by PDGF-B at the early stage, and by PDGF-C and PDGF-D at the late stage of hepatic fibrogenesis (17).

A previous study demonstrated that the PDGFR-β-mediated PDGF-B and PDGF-D signaling pathways are the most important proliferation signaling pathways in the development of hepatic fibrosis (26). In a rat model of fibrotic liver disease induced by bile duct ligation (BDL), the expression of

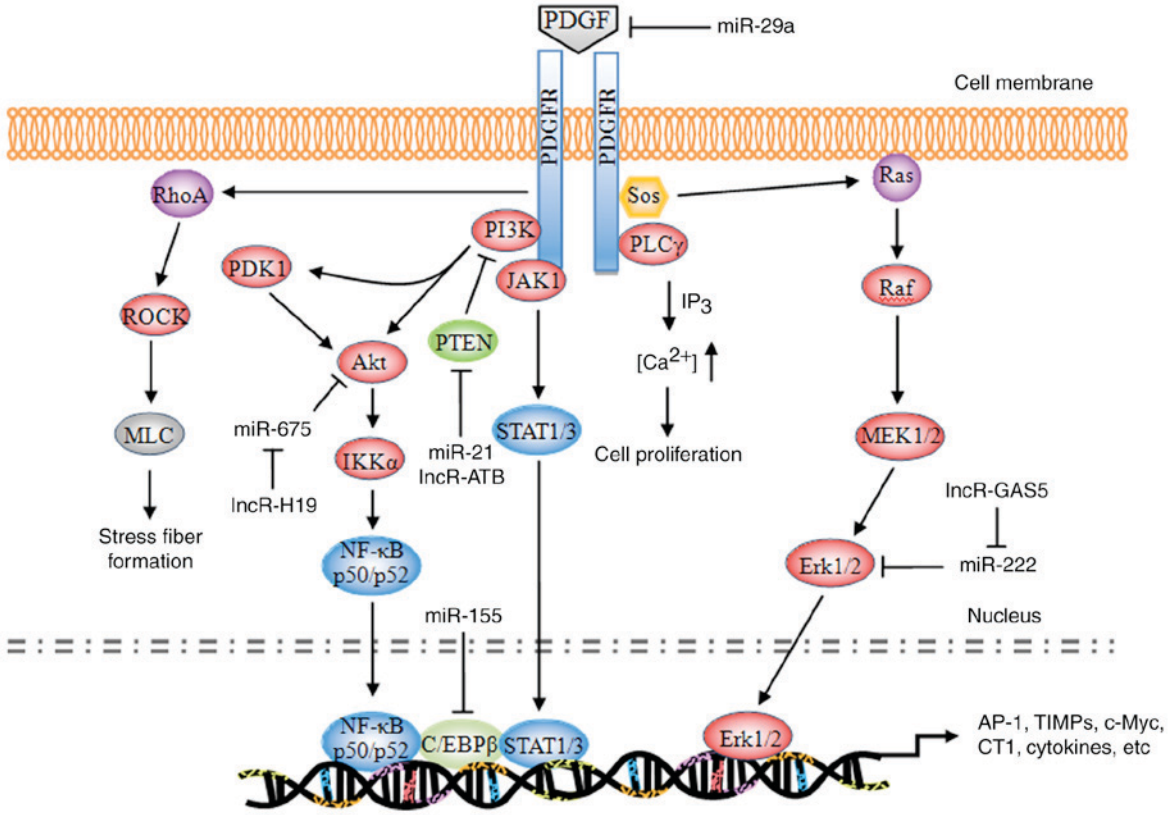

Figure 1. Schematic representation of the PDGF signaling pathway. PDGF, platelet-derived growth factor; PDGFR, PDGF receptor; miR, microRNA; PI3K, phosphatidylinositol 3-kinase; PLCγ, phospholipase Cγ; Sos, son of sevenless homolog; JAK1, tyrosine-protein kinase JAK1; PDK1, 3-phosphoinositide-dependent protein kinase 1; Akt, RAC-α serine/threonine protein kinase; PTEN, phosphatidylinositol 3,4,5-trisphosphate 3-phosphatase and dual-specificity protein phosphatase PTEN; ROCK, Rho-associated protein kinase 1; MLC, membrane protein MLC; NF-κB, nuclear factor-κB; IKKα, inhibitor of NF-κB kinase subunit α; C/EBPβ, CCAAT/enhancer-binding protein β; STAT, signal transducer and activator of transcription; MEK, dual specificity mitogen-activated protein kinase kinase; Erk, extracellular signal-regulated kinase; AP-1, transcription factor AP-1; TIMP, metalloproteinase inhibitor; c-Myc, myc proto-oncogene protein; CT1, cardiotrophin 1.

PDGF-B, PDGF-D, and PDGFR-β mRNA were respectively increased compared with the other isotypes; PDGF-B- and PDGF-D-stimulated HSC activation and proliferation markedly altered, exhibiting apparent activation morphologically. By contrast, the proliferation of cells treated with PDGF-A or PDGF-C did not alter (27). This previous result indicated that PDGF-B and -D may induce more apparent hepatic fibrosis compared with PDGF-A and -C. In addition, hepatic fibrosis and HSC activation have been demonstrated to be markedly increased in transgenic mice overexpressing PDGF-BB compared with normal controls; the expression levels of α-smooth muscle actin (α-SMA) and PDGFR-β in the liver were also elevated, demonstrating that PDGF-B and PDGFR-β may promote fibrosis in an animal model and at the cellular level (28). Therefore, PDGFR-β expression may be most apparent in the cytomembranes of HSCs. PDGFR-β may additionally exhibit relatively strong affinities with PDGF-B and -D, and these three factors serve important roles in hepatic fibrosis.

4. PDGF signaling pathway

The majority of the substrates of PDGF exhibit similar structures to the Src homology 2 (SH2) domain. These substrates bind to a corresponding phosphorylation site of the activated PDGFR, enabling receptor dimerization and autophosphorylation, and leading to phosphorylation of tyrosine residues at specific intracellular sites (29). The currently identified substrates primarily include phospholipase Cγ (PLCγ), Ras, phosphatidylinositol 3-kinase (PI3K), and the signal transducer and activator of transcription (STAT) pathway (Fig. 1).

During the development of hepatic fibrosis, autophosphorylated PDGFR primarily activates the Ras system, which in turn activates the RAF proto-oncogene serine/threonine protein kinase (Raf-1), dual-specificity mitogen-activated protein kinase kinaseN (MEK) and extracellular signal-regulated protein kinase (ERK) signaling pathways. The activated signaling pathways enhance the phosphorylation of cytoplasmic target proteins, regulate the activity of various proteases, promote the phosphorylation of a number of transcription factors [including activator protein 1 (AP-1) and nuclear factor-κB (NF-κB)] and enable them to bond with the target gene promoters of the corresponding response elements. Consequently, these downstream elements increase the transcriptional activity, regulate the expression levels of the products of target genes [including type I collagen (CT I), metalloproteinase inhibitors (TIMPs), matrix metalloproteinases (MMPs), apoptosis regulator Bcl-2 (Bcl-2), and E3 ubiquitin-protein ligase XIAP (XIAP)], resulting in cell division, proliferation and apoptosis (30-32).

Ras pathway. Ras is a small GTPase with a relative molecular weight of 21 kDa. Ras has been demonstrated to be a 'crossing

point' or molecular switch for a variety of cell signal transduction processes. The protein possesses endogenous GTP enzyme activity, facilitating extracellular-to-intracellular signaling, and is additionally an upstream protein of the Raf-MEK-ERK pathway (33). Ras, combined with c-Raf-1, facilitates the transport of Raf from the cytoplasm to the cytomembrane; Raf is subsequently activated by the corresponding kinase on the cytomembrane and its C-end catalytic domain bonded with MEK phosphorylates (via MAPK) two Ser residues in the MEK catalytic domain, thereby activating MEK.

PDGF, combined with a corresponding receptor on the cytomembrane, induces the phosphorylation of intracellular tyrosine residues of the receptor; the phosphorylated tyrosine may recruit GF receptors containing SH structural domains from the cytoplasm to bond with the protein 2 (Grb2); by virtue of its SH2 structural domain, Grb2 binds with the guanine nucleotide exchange factor [son of sevenless (SOS)] and forms a Grb2-SOS compound, which dissociates the GDP of Ras to bind with GTP and converts the quiescent Ras-GDP into an active Ras-GTP to activate Ras. The activated Ras in turn activates Raf1, MEK1/2 and ERK1/2 and transfers the corresponding signals into the cell nucleus, thereby promoting the phosphorylation of various transcription factors, increasing transcriptional activity, and triggering cell growth, differentiation, migration, angiogenesis, anti-apoptosis, drug tolerance and other processes (34,35). During the induction of hepatic fibrosis by PDGF, ERK1/2 primarily regulates the mitosis and chemotaxis of HSCs. The ERK1/2 pathway inhibitor is able to completely inhibit mitosis in HSCs and decrease the mitogenesis and chemotaxis of the cells, thus reducing the concentration of inflammatory sites (36-38).

PLCγ pathway. PLCγ is a 145-kDa enzyme consisting of two SH2 domains and one SH3 domain. In hepatic fibrosis, PDGF mediates the cell proliferation via the regulatory role of PLCγ in the mitosis of HSCs (39). The primary mechanism of action is as follows: When PDGF is bonded with PDGFR, PLCγ is activated by a protein tyrosine kinase; the activated PLCγ hydrolyzes phosphatidylinositol diphosphate, producing inositol triphosphate (IP3) and diacylglycerol (DAG). IP3 primarily acts on the Ca^{2+} repository of the endoplasmic reticulum, promoting the release of cytoplasmic Ca^{2+}; the intracellular Ca^{2+} thus increases and induces mitosis in HSCs. DAG, together with Ca^{2+}, acts on the protease protein kinase C (PKC) and induces the phosphorylation of Ser and Thr residues of the ribosome, in addition to the proliferation of cells (40,41). In addition, DAG is able to activate the intracellular Na^+/H^+ proton pump, leading to the frequent intracellular H^+ and extracellular Na^+ interchanges; DAG additionally decreases the quantity of cellular H^+, which is conducive to mitosis (42,43).

PI3K pathway. PI3K is a heterogeneous dipolymer consisting of two subunits, termed p85 and p110. p85 is the subunit which regulates the primary functions of PI3K (44). The RAC-α serine/threonine protein kinase (Akt/PKB) pathway is currently considered to be the primary downstream signaling pathway of PI3K. PKB is a serine/threonine protease and it encompasses three domains: The N-terminal regulatory domain, the catalytic domain, and the C-terminal tail domain. The specific signaling pathway is as follows: The p85 subunit, which is bonded with the phosphorylated PDGFR, phosphorylates the third group of the phosphatidylinositol ring, producing PI (3,4) P2, PI (3-5) P3, and certain secondary messengers for downstream signaling; the latter may bind with the group via the N-terminal regulatory domain of PKB, transporting it to the cytomembrane or partially activating it. Notably, phosphorylated sites respectively activate 3-phosphoinositide-dependent protein kinase 1 (PDK1) and PDK2; subsequently, PDK1 and PDK2, or an unknown Ser473 kinase, phosphorylates the Ser473 and Thr308 ion of the PKB protein, thereby completely activating the group. The PDGFR-activated PI3K/Akt pathway may promote actin recombination, increase cell migration, mediate metabolic regulation, stimulate cell growth and inhibit cellular apoptosis (45). ERK signaling is involved in PI3K/Akt-mediated HSC chemotaxis and proliferation (46).

Tyrosine protein kinase JAK (JAK)/STAT pathway. STAT signaling is a process wherein an 84-113-kDa cytoplasmic protein, combined with the regulatory domain DNA of the target gene, regulates transcription. A total of seven STAT family members have been identified, termed STAT1, STAT2, STAT3, STAT4, STAT5a, STAT5b, and STAT6. STAT is an intracellular signal transduction protein, and additionally acts as a transcription factor (47). Following phosphorylation by tyrosine protein kinases of the JAK family, STATs produce homologous or heterogeneous dipolymers; the dipolymers are subsequently translocated, enter the nucleus, and trigger gene transcription following DNA-binding (48). Activated PDGFR primarily triggers the phosphorylation of the C-terminal tyrosine residues (Tyr705) and serine residues (Ser727) of the STAT protein via JAKs, thereby activating STAT5, leading to the production of homologous or heterologous STAT protein dipolymers. The dipolymers are subsequently translocated, enter the nucleus, activate the target genes, and promote cell growth and division (49,50).

Rho pathway. Using two intracellular signaling pathways (PKC and RhoA), PDGF alters the cytoskeleton distribution in HSCs, stimulates the transformation of HSCs into muscle-like fibroblasts, and promotes type I and III collagen formation (51).

Non-coding RNA (ncRNA) regulation. Altered profiles of ncRNAs, including microRNAs (miRNAs/miRs), long non-coding RNAs (lncRNAs) and circular RNAs (circRNAs), have been demonstrated to be associated with hepatic fibrosis. Different alterations of miRNA expression have been observed. Several specific miRNAs (including let-7d, miR-29a, miR-21, miR-155 and miR-214) (52-56) and lncRNAs (lncRNA-ATB, GAS5 and H19) (57-59), have been identified to be diagnostic biomarkers and the therapeutic targets in the progression of hepatic fibrosis. miRNAs induced or repressed by PDGF challenge exhibit a feedback mechanism balancing multiple growth factor receptor signaling in HSC activation (52-56,60,61), while lncRNAs are able to competitively bind to anti-fibrogenic miRNAs leading to increased collagen I production (57-59). Since a single ncRNA may affect the expression of various mRNAs, it may simultaneously modulate various cellular events. At present, the multiple molecular mechanism through which ncRNAs may serve a role in hepatic fibrogenesis have not been completely elucidated.

5. Targeting the PDGF signaling pathway in hepatic fibrosis treatment

With the increased understanding of the biological functions of the PDGF signaling pathway in liver fibrogenesis, antifibrotic drugs have become a focus of research. A number of strategies to regulate the PDGF signaling pathway have been employed in preclinical and clinical settings (Table I). These may be categorized as: i) PDGF isoform antagonists; ii) blocking of PDGFR activation; and iii) downstream regulation of the PDGF pathway.

PDGF-B kinoid vaccines, prepared using PDGF-B-derived polypeptides bonded to carrier protein heterocomplexes, displayed marked antifibrotic effects in CCl_4-induced hepatic fibrosis (62). Notably, the normal function of the hepatocytes was not altered by the vaccine. Although the side effects were not observed in this previous study and in other reports (63-65), the safety of PDGF vaccine requires investigation in a systematic toxicity assay.

Sorafenib is a first-line oral chemotherapy drug for patients with advanced hepatocellular carcinoma. As an RTK inhibitor, sorafenib may target a number of kinases on the cytomembrane, including Raf, VEGFR2/3, and PDGFR-β (66,67). Despite the narrow therapeutic window and adverse effects (68,69), sorafenib has previously been demonstrated to be a potential antifibrotic agent, due to its multi-targeting of the Ras/MEK/ERK pathway (70). Sorafenib may induce HSC autophagy and apoptosis through activation of the Akt/protein kinase mTOR/p70S6K and MAPK signaling pathways (71). It may additionally suppress collagen deposition, neovascularization and oxidative stress through PDGF downregulation, STAT3 inhibition and mitochondrial respiration in fibrosis mouse models consuming a high fat diet, and undergoing BDL and dimethylnitrosamine injection (72,73).

Nilotinib, a selective breakpoint cluster region protein (Bcr)-tyrosine-protein kinase ABL (Abl) non-receptor tyrosine kinase (nRTK) inhibitor, is well-tolerated and has been approved for the treatment of leukemia. Since RTKs may be activated by nRTKs, the interaction between RTKs and nRTKs is involved in the regulation of HSC differentiation and proliferation (74). In addition to its anticancer activity, nilotinib therefore exerts numerous beneficial therapeutic outcomes, including neuroprotective, vasodilatory and antifibrotic effects (75-78). Nilotinib inhibited α-SMA, procollagen-(I), TIMP-1, phosphorylated (p)-ERK and p-Akt expression, and reduced collagen deposition in activated HSCs and in the liver tissues of CCl_4- and BDL-induced fibrosis rats (79-81). Nilotinib inhibited PDGFR activation, in addition to TGFβ receptor type II via Src (81). These previous results indicated that nilotinib may represent a putative antifibrotic treatment due to its combined inhibition of nRTK and RTK.

Imatinib (also termed Gleevec® and STI-571) is a selective tyrosine kinase inhibitor (TKI), which is able to specifically target PDGFR, Abl, Abelson tyrosine-protein kinase 2, mast/stem cell growth factor receptor, and their oncogenic forms Bcr-Abl (82). At concentrations required for Bcr-Abl inhibition, imatinib is additionally able to attenuate hepatic fibrosis by blocking the expression of PDGFR-β and decreasing the levels of proinflammatory cytokines. Therefore, imatinib has been demonstrated to induce HSC apoptosis *in vitro* and to control liver fibrosis in CCl_4- and thioacetamide (TAA)-treated mice (80,82-84). Compared with a decreased of 60% mediated by anti-PDGF antibodies, imatinib has been demonstrated to exert an 85% decrease in HSC migration triggered by bile duct segments (27). However, unlike nilotinib, animals treated with imatinib (20 mg/kg) exhibited a degree of hepatotoxicity evidenced by increased levels of serum aminotransferases and total bilirubin (79).

Other TKIs, including pazopanib, regorafenib, AG1295 and AG1296, may selectively inhibit the tyrosine phosphorylation of PDGFR-β (85-87) and the PDGF-BB-induced activation of its downstream signaling pathway in HSCs (85). additionally, AG1295 inhibits PDGF-induced thymidine uptake by pulmonary myofibroblasts *in vitro* (88).

Celecoxib, etoricoxib and DFU, selective cyclooxygenase-2 (COX-2) inhibitors (coxibs), are widely-used in the management of osteoarthritis and rheumatoid arthritis, in addition to the treatment of colon cancer, atherosclerosis and Alzheimer's disease, due to their analgesic, anticoagulant and anti-inflammatory effects (89-91). During the development of steatohepatitis and hepatic fibrosis, COX-2 and its products prostaglandin E_2 (PGE_2) and prostacyclin (PGI) may upregulate the expression of VEGF, PDGF and fibroblast growth factor receptor 1, resulting in ERK activation, and COX-2 may be activated by these factors (92). COX-2 inhibitors may alter the metabolism of arachidonic acid and, subsequently, PGE_2 and PGI. Therefore, coxibs may inhibit PDGF-induced HSC proliferation; however, in contrast to NS-398 and DFU, only celecoxib (≥50 mM) is able to induce HSC apoptosis and inhibit Akt activation (93). Oral administration of celecoxib significantly decreased hepatic collagen deposition and α-SMA expression in CCl_4- and TAA-treated rats due to its dual inhibitory effects on intrahepatic fibrosis and angiogenesis (94).

A number of active substances from traditional herbal and ethnobotanical medicine [e.g., silymarin, quercetin, proanthocyanidins, curcumin and salvianolic acid B (Sal B)] have come into use as putative treatments for liver disease. Silymarin, a mixture of flavonolignans obtained from the edible milk thistle plant [*Silybum marinaum* (L) Gaenrt], has been used as a natural medicine for the treatment of liver diseases. The four principal isomers within silymarin are silybin, isosilybin, siliehristin and silydianin (95). Among these isomers, silybin is the most bioactive substance, which is able to directly inhibit the phosphorylation of the Raf/MEK/ERK pathway, decrease the activation of sodium/hydrogen exchanger 1 and inhibitor of NF-κBα phosphorylation, thereby preventing oxidative anomalies and fibrosis (96,97). Treatment with silybin or silybin-vitamin E phospholipid complexes has been demonstrated to ameliorate hepatic fibrosis in patients with chronic hepatitis C, who have been treated previously with pegylated-interferon a and ribavirin (98,99).

Curcumin, the principal component of the spice turmeric and certain herbal medicines (*Zedoariae* rhizome and *Radix Curcumae*), is able to inhibit epithelial-to-mesenchymal transition and affect numerous intracellular targets, involving certain miRNAs, and the estrogen receptor, nuclear factor-like 2, insulin-like growth factor-1 and PI3K/Akt signaling pathways (100-103). Due to its numerous potential effects, including anti-inflammatory, hypolipidemic, hypoglycemic and chemopreventive activity, curcumin may increase antioxidant enzyme activities, activate cytochrome P4502E1 and concomitantly

Table I. PDGF inhibitors and downregulators.

Author, year	Drug	Targets	Action	Applications	Groups	(Refs.)
Gounder *et al*, 2011; Zahavi *et al*, 2016; Lin *et al*, 2016; Hong *et al*, 2013; Hao *et al*, 2016; Liu *et al*, 2015	Sorafenib	Raf kinase, PDGFR-β, VEGFR2/3, c-kit	Receptor tyrosine kinase inhibitor	Advanced renal cell carcinoma and unresectable hepatocellular carcinoma	Approved	(66-69,71,72)
Qu *et al*, 2016; Karuppagounder *et al*, 2014; Elsherbiny *et al*, 2015; Shaker *et al*, 2011; Lemos *et al*, 2015	Imatinib	Bcr-Abl, PDGFR-β, c-kit	Protein-tyrosine kinase inhibitor	Chronic myelogenous leukemia	Approved	(74-78)
Moawad, 2015; Kuo *et al*, 2012; Kim *et al*, 2012	Nilotinib	PDGFR-α/β	Tyrosine kinase inhibitor	Chronic myelogenous leukemia	Approved	(82-84)
Hutson *et al*, 2010	Pazopanib	PDGFR-α/β, VEGFR1/2/3, c-kit	Protein-tyrosine kinase inhibitor	Advanced renal cell cancer and advanced soft tissue sarcoma	Approved	(86)
Eisen *et al*, 2012	Regorafenib	PDGFR-α/β, VEGFR1/2/3, RET, BRAF, FGFR1/2	Multiple membrane-bound and intracellular kinases inhibitor	Metastatic colorectal cancer and advanced gastrointestinal stromal tumors	Approved	(87)
Iwamoto *et al*, 2000; Rice *et al*, 1999	AG1295/1296	PDGFR-β	Tyrosine kinase inhibitor	Unknown	Preclinical	(85,88)
Venè *et al*, 2016; Soininen *et al*, 2007; Raval *et al*, 2010; Gao *et al*, 2013	Celecoxib	COX-2, Akt	COX-2 inhibitor	Rheumatoid arthritis, osteoarthritis, acute pain, colon and familial adenomatous polyposis	Approved	(89-92)
Trappoliere *et al*, 2009; Serviddio *et al*, 2014	Silymarin	Phosphorylation of IkB and Raf/MEK/ERK	Unknown	Liver related diseases including hepatic fibrosis and cirrhosis	Approved	(96,97)
Zhou *et al*, 2015; Lian *et al*, 2015; Taverna *et al*, 2015; El-Bahr, 2013; Zhang *et al*, 2015	Curcumin	PI3K/Akt, ERK	Unknown	Edible pigment; aging, irradiation, hepatic fibrosis and hepatitis	Approved	(100-104)
Wang *et al*, 2012; Lv *et al*, 2010; Gao *et al*, 2012; Xu *et al*, 2012	Salvianolic acid B	JAK2/STAT3, TGF-β1 and RhoA	Unknown	Myocardial infarction, cerebral ischemia, hepatic fibrosis and hepatitis	Approved	(110-113)

PDGF, platelet-derived growth factor; PDGFR, PDGF receptor; VEGFR, vascular endothelial growth factor receptor; c-kit, mast/stem cell growth factor receptor; Bcr, breakpoint cluster region protein; Abl, tyrosine-protein kinase Abl-1; RET, proto-oncogene tyrosine-protein kinase receptor Ret; BRAF, serine/threonine-protein kinase B-raf; FGFR, fibroblast growth factor receptor; COX-2, cyclooxygenase-2; Akt, RAC-α serine/threonine protein kinase; IκB, inhibitor of nuclear factor-κB; MEK, dual specificity mitogen-activated protein kinase kinase; ERK, extracellular signal-regulated kinase; PI3K, phosphatidylinositol 3-kinase; JAK2, tyrosine-protein kinase JAK2; STAT3, signal transducer and activator of transcription 3; TGF-β1, transforming growth factor-β1.

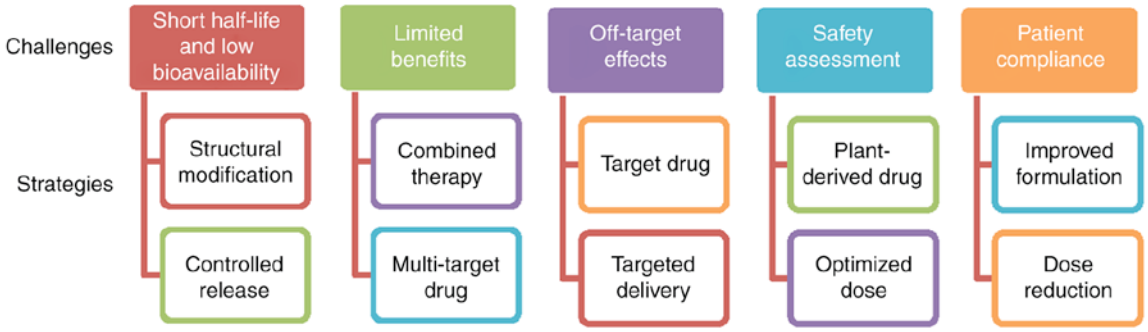

Figure 2. Recent challenges and future strategies for the treatment of hepatic fibrosis.

suppress the activity of fatty acid synthase, β-catenin transactivation and DNA-binding (104,105). In addition, curcumin has been demonstrated to markedly reverse PDGF-BB-induced hepatic myofibroblast cell proliferation and the expression of collagen I and collagen IV mRNA. Curcumin may alleviate hepatic fibrosis by modulating lipid metabolism and inducing HSC apoptosis, in part via the PDGF- and ERK-dependent pathway (101,106).

Sal B, and its metabolite danshensu, the major active substances from *Salvia miltiorrhiza*, are widely-used as commercial anticoagulant drugs in the treatment of myocardial infarction and cerebral ischemia (107-109). The two substances exhibit hepatoprotective effects against CCl_4-induced lipid peroxidation, collagen accumulation and necroinflammation in liver tissues, the probable mechanism underlying which is associated with the regulation of the intrahepatic JAK2/STAT3 and TGF-β1/mothers against decapentaplegic homolog pathways for maintaining collagenic homoeostasis (110-112). *In vitro*, Sal B has been demonstrated to inhibit endothelin-induced HSC activation by regulating RhoA/cardiac myosin light chain 2 signaling activation and the phosphorylation of downstream protein phosphatase 1 regulatory subunit 12A at Thr(696) (113).

6. Future challenges and prospects

PDGF-B-mediated PDGFR-β signaling has been demonstrated to serve an important role in hepatic fibrosis (17,22). Although the numerous approaches mentioned above have been applied to modulate this pathway, no ideal treatment for liver fibrosis has been employed in clinical practice at present (Fig. 2). TKIs, coxibs and natural products exhibit short half-lives and low bioavailabilities, and therefore require long-time repeated administration to achieve therapeutic benefits (114,115). In addition, the majority of TKIs are only approved for cancer therapy, and coxibs for arthritis. Although the effectiveness of TKIs and coxibs has been demonstrated in animal models and cultured HSCs, the outcomes of patients treated in early-phase clinical trials have not been systematically analyzed. Notably, due to the high similarity of the homologous domain, TKIs, including sorafenib, sunitinib and pazopanib, may inhibit PDGFR expression and regulate the expression of VEGF receptors (VEGFR), which are involved in the maintenance of vessel diameters and the integrity of endothelial cells (116); therefore, the inhibition of PDGFR by these approved targeted drugs may impair non-target normal cells or tissues. The primary concerns about the safety of TKIs and coxibs are bleeding and myocardial ischemia. Congestive liver failure, QT prolongation and severe fatigue induced by TKIs have additionally been observed in certain clinical trials (117-119). With the development of molecular biotechnology methods, gene therapy may be used to transport genetic materials to the specific cells for correcting or targeting the genetic defects, thereby achieving the goal of disease treatment. However, there are significant potential safety and target-specific concerns associated with small interfering RNA- and adenovirus-mediated gene therapies involving genetic modification (120).

An additional challenge is that the inhibition of a single profibrotic molecule may exert little or no impact on fibrogenesis and overall recovery. Hepatic fibrogenesis is a complex process involving a variety of pathogenic and host-specific signal transduction processes (4). Certain stimuli, including TGF-β1, TNF-α and hepatitis viruses, serve roles in HSC activation. Different stages of fibrosis development may depend on different growth factors (7-9). Notably, PDGF mediates the actions of these stimuli in liver cells, including the regulation of hepatocyte growth and death, in addition to the differentiation of activated HSCs to myofibroblasts; conversely, TGF-β1, TNF-α and hepatitis-mediated oxidative stress may upregulate PDGF expression (121,122). In particular, hepatitis B virus- or hepatitis C virus-associated fibrosis is considered to be irreversible prior to the control of the ongoing viral replication and inflammation by combined treatment (123). In addition, it remains unknown whether the normal biological functions of the liver may be affected by the inhibition of PDGF signaling. Considering that that liver is a non-immunological organ engaged primarily in metabolism, nutrient storage and detoxification, novel therapies are required to deliver longer lasting benefits directly to the targeted cells. Combination strategies (including multi-agent regimens and improved drug delivery) are the future of antifibrotic therapy, and the primary focus for medicinal chemistry is the reduction of toxicity and the maintenance (or enhancement) of potency. Novel combinations may simultaneously target multiple profibrotic factors to induce HSC apoptosis, improve the microenvironment, prolong drug release and reverse drug resistance. Co-delivery systems modified by nanotechnology may be a promising strategy to maximize the additive or synergistic effects of drugs, since various agents may specifically bind to the target together. These multi-target delivery systems, which may more specifically deliver inhibitors to the target, may be able to increase the antifibrotic action and decrease the exposure of

normal and non-targeted cells or tissues. However, epidemiological evidence for the association between plant remedies (involving the mixed herbal formulations) and the long latency of hepatic fibrosis progression has led to the active investigation of natural chemicals from different naturally-occurring substances in various preclinical and clinical studies. In general, the potential of natural compounds to exhibit low toxicity and low drug-resistance, with pleiotropy and synergistic action, makes them promising candidates for the treatment of hepatic fibrosis. A number of plant-derived active compounds have been approved for the treatment of hepatic fibrosis and may provide valuable resources for the development of novel formulations and treatment modalities in future.

7. Conclusion

In conclusion, excessive PDGF expression is an important factor in HSC activation. Hepatic fibrosis may be effectively reversed by inhibiting PDGF and PDGFR expression, or by inhibiting the phosphorylation of its downstream signaling pathways, although complete elucidation of the mechanisms underlying signaling transduction and crosstalk between the different pathways requires additional research. A number of PDGF antagonists and receptor inhibitors have been previously investigated for their potential to treat hepatic fibrosis, although further clinical trials to examine their safety and effectiveness may be considered.

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