Chapter

Therapy that Targets Growth Factor Receptors: Novel Approach for Liver Cirrhosis Treatment

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Abstract

The background of liver fibrous degeneration is excessive cell proliferation including hepatic stellate cells, inflammatory cells, fibroblasts and myofibroblasts. Often it is the consequence of increased growth factors and/or their receptors expression. Key contributors to the liver cell proliferation are EGFR, FGFR, PDGFR, VEGFR, TGFβR, the increased expression of which is indicated on *in vitro* and *in vivo* models of liver fibrosis and in patients who experienced fibrosis-accompanied liver diseases. Elimination of growth factors/suppression of their receptors is associated with the weakening/elimination of certain processes responsible for fibrogenesis. This chapter represents the evidences of the efficacy of growth factor receptors signaling downregulation for the suppression of liver fibrosis and cirrhosis and their individual manifestations. The data on established and experimental therapeutics – specific and multikinase growth factor receptor inhibitors which demonstrated antifibrotic and anticirrhotic activity under *in vitro* and *in vivo* models, are also presented.

Keywords: EGFR, VEGFR, PDGFR, FGFR, TGFβR, tyrosine kinase inhibitors

1. Introduction

If organs with high regenerative capacity undergo chronic injury and inflammation, their healing often occurs abnormally - due to replacement of the damaged elements with connective tissue. The most striking example of such distorted regeneration is the development of liver fibrosis and cirrhosis on the background of its chronic damage. Fibrosis is an "exceeding" healing accompanied with the formation of an excessive amount of connective tissue incorporated into liver parenchyma due to extracellular matrix (ECM) overproduction and/or its incomplete degradation.

The main etiological factors of liver fibrosis and cirrhosis are alcohol, storage diseases, hepatitis viruses, hepatotoxic drugs, cholestasis, and autoimmune reactions. The trigger of fibrogenesis is chronic injury accompanied by an inflammatory component, which causes the activation and expansion of mesenchymal cells (including fibroblasts, myofibroblasts, smooth muscle cells) and increased synthesis of ECM molecules, predominantly collagen. Cells involved into the inflammation actively produce soluble factors like pro-inflammatory cytokines, endothelins, growth factors, reactive oxygen and nitrogen species, which also promote fibrogenesis [1, 2]. The final stage of organ's fibrosis is cirrhosis - the

irreversible replacement of a significant part of that by connective tissue, which leads to the organ's failure. The main cells which "trigger" liver fibrosis are hepatic stellate cells (HSC). Under liver injury and if being stimulated with cytokines produced by inflammatory cells, Kupffer cells and hepatocytes, HSCs are activated and transformed into myofibroblasts. The latters are able to migrate to the damaged area and produce a reduced number of matrix metalloproteinases (MMPs) and an increased number of their tissue inhibitors (TIMPs) and ECM proteins, causing the growth of connective tissue in liver and accumulation of fibrillar matrix into Disse spaces. Thick bundles of newly synthesized collagen fibers in the Disse spaces between hepatocytes are surrounded by fibroblasts, macrophages, HSCs, lymphocytes, polymorphonuclear leukocytes, eosinophils and plasmatic cells. These cells produce ROS, inflammatory mediators and growth factors, thus maintaining liver inflammation and promoting substantial disorders followed by cirrhosis development [3].

Cirrhosis is the endpoint of many liver diseases and causes the development of serious complications with possible fatal outcome. Those include: liver failure, gastrointestinal bleeding, portal hypertension, i.e. increased pressure in the portal vein, and hepatic coma. Thus, mortality from liver cirrhosis within 1 year after diagnosis varies from 1 to 57%, depending on the stage [4] and reaches more than 1.2 million deaths annually [5].

2. The role of growth factors and their receptors in fibrogenesis

Growth factor receptors are tightly involved in the pathogenesis of chronic inflammation due to their signaling close relationship with the major proinflammatory pathways. Those include, in particular, nuclear factor kappa B (NFκB), p38 mitogen-activated protein kinase (MAPK), phosphatidylinositol 3-kinase/Protein kinase B (PI3K/Akt), Janus kinase/signal transducer and activator of transcription (Jak/STAT) signaling pathways, which are activated not only by proinflammatory cytokines, but also by individual growth factors, such as transforming growth factor beta (TGFβ), TGFα, hepatocytes growth factor (HGF), epidermal growth factor (EGF), insulin-like growth factor (IGF) [6–9], associated with the "start" of regenerative processes.

The main proinflammatory pathways are also profibrogenic ones. Thus, NF-κB signaling provides not only survival and inflammatory reaction of Kupffer cells, but also survival, inflammatory response and activation of HSCs. Constitutive activity of this pathway in HSCs and/or hepatic myofibroblasts stimulates fibrous degeneration of the liver due to direct profibrogenic and antiapoptotic effects and by stimulating the secretion of cytokines - macrophage attractants [10]. Another proinflammatory pathway, STAT3, is involved in the control of MMPs and TIMPs transcription, TGF-β1 and ECM molecules synthesis and secretion, myofibroblasts proliferation and resistance to apoptosis, thus enhancing tissue regeneration. Activation of this pathway is observed in many tissues due to their fibrosis [11]. The PI3K/Akt pathway, in addition to its significant role in apoptosis inhibition and cell proliferation and survival, may promote epithelial-mesenchymal transition, thus contributing to fibrogenesis [12] (**Figure 1**). Furthermore, this pathway could be activated by EGF receptor (EGFR), the ligands of which are ones of the main profibrogenic growth factors [13]. P38 MAPK pathway is the one, the effects of the main profibrogenic cytokine TGF-β1 are realized through [14].

Macrophages and neutrophils, the first responders on damage and inducers of acute inflammation, also produce cytokines and chemokines, which serve as mitogens and chemoattractants for endothelial, epithelial and mesenchymal cells

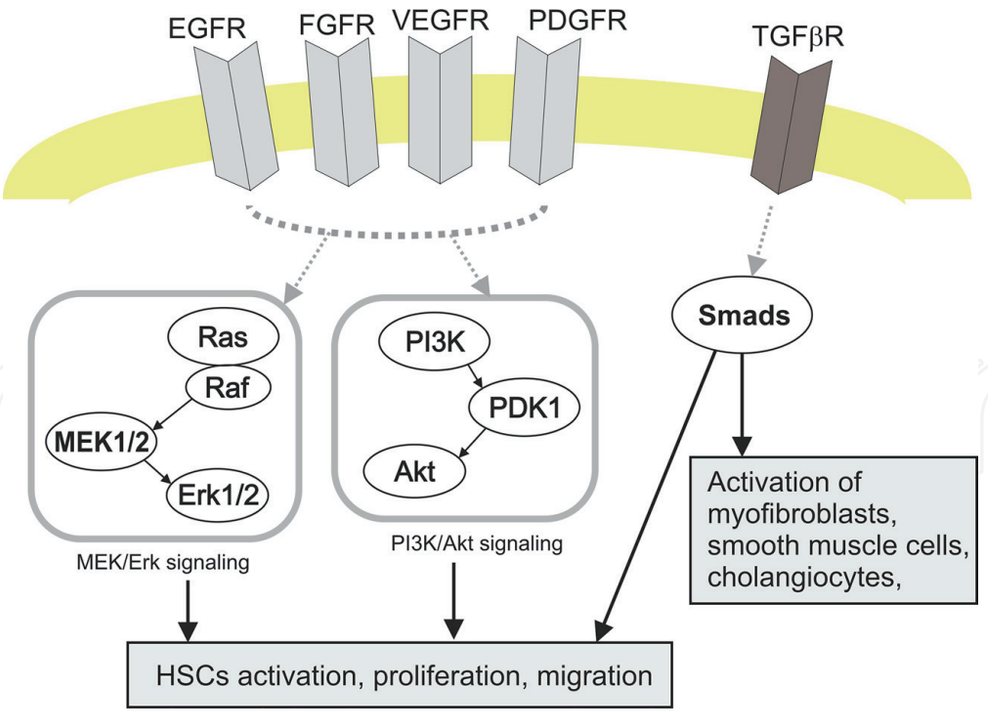

Figure 1.
The role of growth factor receptors in liver fibrogenesis.

(myofibroblasts, HSCs) migrating to the cites of injury. With the chronicity of the inflammatory process, these cells are activated and secrete profibrogenic cytokines and growth factors such as TGF-β1, interleukin 13 (IL-13) and platelet-derived growth factor (PDGF), which further activate macrophages and fibroblasts and promote proliferation of those in addition to epithelial cells. Wound/injury healing also includes ECM synthesis and remodeling. Under chronic inflammation, this process is violated: the synthesis of ECM molecules prevails on their cleavage, leading to accumulation of those, which called fibrosis [15].

Impaired activity of protein kinases, in particular growth factor receptors such as EGFR, vaso-endothelial growth factor receptor (VEGFR), PDGF receptor (PDGFR), fibroblast growth factor receptor (FGFR), play a significant role in development of numerous non-malignant liver diseases, including diseases associated with its fibrous degeneration [16]. Thus, PDGF is the most important cytokine responsible for the proliferation of HSCs; PDGF, VEGF and FGF2 induce their migration, TGF-β causes HSCs transformation to myofibroblasts, stimulates synthesis of ECM by those and inhibits its degradation. Inhibition of these growth factors receptors downregulates mentioned processes [17]. Furthermore, an excessive proliferation of cholangiocytes which express numerous cytokines, chemokines and growth factors is one of the main mechanisms of fibrogenesis. The proliferating cholangiocytes also involve myofibroblasts, fibroblasts and immune cells in this process [18, 19]. Therefore, activation of biliary proliferation (called ductular reaction) contributes a lot in the initiation and progression of liver fibrosis.

3. Growth factor receptors as the targets of antifibrotic therapy

There is no specific remedy for the liver fibrosis to date. Some compounds having therapeutic activity against liver fibrosis are undergoing preclinical and I-II phases of clinical trials. They include: (1) the monoclonal antibodies and low molecule inhibitors of key signaling pathways involved in the regulation of inflammation, HSCs life cycle and collagen metabolism [20]; (2) the broad-spectrum agents

exhibiting antioxidant, anti-inflammatory, hepatoprotective, antilipotoxic activities such as ursolic, ursodeoxycholic and 24-norursodeoxycholic acids, resveratrol, silymarin [3]. However, the last agents are rather supplements, the positive effect of which is observed only in combination with other therapeutics.

Cytostatics like methotrexate and azathioprine are actively used for the treatment of diseases accompanied by fibrosis. However, due to the nonspecificity of action, they cause the development of numerous side effects. Therefore, the idea of using selective inhibitors of excessive cell proliferation can be fruitful. Impaired activity of tyrosine kinases, in particular growth factor receptors EGFR, VEGFR, PDGFR, TGFβR, and FGFR, contributes significantly to liver diseases associated with its fibrous degeneration [16]. Therefore, these receptors may be potential targets for antifibrotic therapy [21]. Among approved and experimental therapeutics tyrosine kinase inhibitors (TKIs) possess the leading position.

3.1 VEGFR

VEGF is a key regulator of liver cells proliferation. An increased expression of this growth factor and its receptors by the biliary cells was noted under liver biliary pathologies, in particular polycystic liver disease and primary biliary cirrhosis (PBC) [22]. PBC patients also demonstrated over-expression of the angiogenic factors Ang-1, Ang-2 and tyrosine kinase with immunoglobulin-like and EGF-like domains 2 (TIE2) their effects are realized by, in the epitheliocytes and periportal hepatocytes [23], suggesting, therefore, their contribution in fibrosis development. VEGF has been shown to stimulate also proliferation of sinusoidal endothelial cells and activated HSCs *in vitro*, indicating that VEGF-VEGFR interaction in HSCs plays an important role in liver fibrogenesis [24]. VEGFR inhibitor sunitinib significantly reduced the inflammatory infiltrate and collagen expression under liver cirrhosis [25]. Another small molecule tyrosine kinase inhibitor vatalanib, which is effective against all VEGF receptors, inhibited CCl_4-induced mice liver fibrosis, as evidenced by decrease of fibrous tissue accumulation and hepatic sinusoidal capillarization, and downregulation of α-smooth muscle actin (α-SMA), collagen I and TGF-β1 expression as well [26] (**Table 1**). Similar results were demonstrated for pan-VEGFR tyrosine kinase inhibitor PTK787/ZK222584 [27].

3.2 EGFR

The EGFR signaling plays an important role in proliferation of liver progenitor cells and their differentiation into hepatocytes or cholangiocytes during the hepatic regeneration. In liver samples of primary sclerosing cholangitic (PSC) patients, the upregulation of EGFR compared to that of healthy individuals was revealed. EGFR is also required for the induction of active pro-inflammatory response by the cholangiocytes [28]. Indeed, the panitumumab, anti-EGFR antibody, inhibited an excessive proliferation of the bile duct mucosa and accumulation of collagen fibers in chronic proliferative cholangitis [29]. In addition, anti-EGFR antibodies applied at bile duct ligation (BDL) model inhibited biliary epithelium hyperplasia and fibrosis. EGFR inhibitor erlotinib inhibited proliferation of the cholangiocytes and hepatocytes, and prevented activation of HSCs, which was demonstrated on different (CCl_4-, diethylnitrosamine (DEN)- and BDL-induced) rat models [30]. EGFR inhibition also significantly reduced viability and ECM production in activated HSCs, inhibited their proliferation and α-SMA production, but did not affect parenchymal cells [31, 32]. Moreover, inhibition of EGFR signaling by erlotinib and other specific inhibitors effectively prevented the progression of cirrhosis and regressed fibrosis in some animals [33, 34] (**Table 1**).

Drug	Target(s)	Cellular effects	Model/Patients	References
Panitumumab	EGFR	Inhibition of bile duct mucosa excessive proliferation and accumulation of collagen fibers	chronic proliferative cholangitis	Liu et al. 2019 [35]
Erlotinib	EGFR	Reduce the number of activated HSCs	DEN-, BDL-induced rats, CCl_4-induced mice	Fuchs et al. 2014 [36]
Vatalanib	VEGFR	Inhibition of α-SMA, collagen I and TGF-β1 expression	CCl_4-induced mice	Kong et al. 2017 [26]
Imatinib	PDGFR	Induce of HSC apoptosis, decrease HSC migration	CCl_4-, TAA-induced mice	Kim et al. 2012 [37]
Sunitinib	VEGFR, PDGFR, c-Kit	Decrease of vascular density, inflammatory infiltrate, α-SMA and collagen expression	CCl_4-induced rats	Tugues et al. 2007 [25]
Sorafenib	Raf, VEGFR2/3, PDGFR-β	Stimulation of HSCs autophagy and apoptosis, inhibition of HSCs proliferation and collagen deposition	High fat diet-, BDL-, DEN-induced mice	Wang et al. 2010 [38]
Pazopanib	VEGFR1, PDGFR-β, FGFR	Induce of HSCs apoptosis, inhibition of HSCs activation, α-SMA, MMP-2, TIMP-1 expression	CCl_4-induced mice	Elshal et al. 2015 [39]
Nilotinib	BCR-ABL, PDGFR, TGFβRII	Depression of HSCs activation, proliferation, migration, α-SMA formation, induce of HSCs apoptosis, reduce collagen deposition in activated HSCs and in liver tissues	CCl_4- and BDL-induced rats	Liu et al. 2011 [40]
Nintedanib	PDGFR, VEGFR, FGFR	Depression of HSCs activation, contractility, migration, collagen deposition, inhibition of macrophage migration	CCl_4-induced mice	Acora et al. 2017 [41]
Regorafenib	VEGFR1–3, PDGFR-β and FGFR, TIE2	Reduce portal hypertension, NO effects on HSCs activation and fibrosis progression or regression	BDL-, CCl_4-induced mice	Uschner et al. 2018 [42]
Brivanib	VEGFR, FGFR	Decrease of HSCs proliferation	BDL-, CCl_4-, TAA-induced mice	Nakamura et al. 2014 [17]

Table 1.
TKIs which demonstrated antifibrotic effects, their molecular targets and cellular effects.

3.3 FGFR

FGF family includes 7 subfamilies of growth factors (1, 4, 8, 9, 10, 11, 19) and four isoforms of their receptors (FGFR1, FGFR2, FGFR3, FGFR4), and all of them are involved in liver injury and regeneration. There is coordinated regulation of

FGFR activation and FGFs secretion during liver injury and subsequent healing: hepatocyte-derived FGFs activate FGFRs on HSCs, and FGFs produced by HSCs activate FGFRs on hepatocytes [38]. FGF signaling during liver damage enhances liver regeneration, however, its chronic production can also lead to the abnormal regeneration with subsequent fibrosis development.

FGF2, a main FGFR1 binding partner, is a mitogen for HSCs. FGFR1 overexpression has been reported in human liver myofibroblasts and activated HSCs compared to the non-activated ones [37]. Then, FGF2 also induces chemotaxis and chemoinvasion by HSCs and may participate in the recruitment and activation of HSCs in acute liver injury. Thus, Yu et al. demonstrated, that chronic hepatic fibrosis is markedly reduced in FGF1/FGF2-deficient mice. However, the absence of FGF1 and FGF2 did not impair the total number of HSCs and their migration into the areas of injury, but overproduction of matrix components, especially collagen α1(I), by those, and therefore excessive fibrous tissue accumulation. The probable explanation is that FGF1 and FGF2 are not essential activating ligands for proliferation and migration of activated HSCs *in vivo*, but the important ones for fibrosis progression [43].

Furthermore, blockade of FGFR1 by small molecule inhibitors prevents HSCs activation (as evidenced by diminishing of α-SMA expression by those), inhibits their proliferation and release of the inflammatory cytokines by those both *in vitro* and *in vivo*. *In vivo* experiments also demonstrated that such inhibition significantly ameliorates CCl_4-induced hepatic fibrosis in a rat model [44, 45].

The ability of FGFs to regulate HSCs proliferation, migration, and transdifferentiation makes FGFR signaling an attractive target for the treatment of hepatic fibrosis. Therapeutic agents which are developing now aim to inhibit FGFRs, to modulate FGF expression, are recombinant FGF proteins, therefore achieving to inhibit EGFR signaling in all levels [37].

3.4 PDGFR

PDGF is the most prominent cytokine that regulates HSCs activation, proliferation and migration. Primary producers of PDGF are platelets, vascular endothelial cells, pericytes and Kupffer cells. PDGFR, tyrosine kinase receptor, is primarily located in vascular endothelial cells, fibroblasts and Kupffer cells. Under the liver injury macrophages, injured endothelial cells and activated HSCs synthesize and secrete PDGF which stimulates proliferation of fibroblasts and vascular endothelial cells via autocrine and paracrine mechanisms. Additionally, PDGF promotes HSCs transformation into myofibroblasts and collagen production by those. Marked upregulation of PDGFR expression on the membranes of activated HSCs have been shown under various chronic liver diseases associated with its fibrosis. Hence, PDGFR overexpression contributes to HSCs activation by synthesized PDGF via the autocrine mechanism and enhances cellular chemotaxis [46]. Additionally, clinical studies demonstrated an excessive activation of PDGF and its downstream molecules, and association of those with the extent of fibrosis in patients with hepatic damage.

There are four PDGF subunits (A, B, C and D) and 2 types of PDGFRs (α and β), and all of them are involved in different stages of hepatic fibrogenesis. Thus, PDGF-B is elevated during the early stage of the disease and is the most potent factor associated with HSCs activation, whereas PDGF-C and -D levels continuously rise during the whole process of HSCs transformation into myofibroblasts and demonstrate relatively high level at the late stage of hepatic fibrosis. Then, quiescent HSCs express PDGFR-α only, and activated ones – predominantly PDGFR-β.

The latter is substantially upregulated, and together with PDGF-B and -D serves important role in hepatic fibrosis [46].

Activated PDGFR induces many signaling pathways, which regulate cell proliferation, migration and survival. In particular, activated Ras system through MAPK signaling cascade regulates the expression of collagen type I, MMPs, TIMPs genes responsible for ECM synthesis and degradation; phospholipase Cγ (PLCγ) signaling contributes to HSCs mitosis; PDGFR-activated PI3K/Akt and JAK/STAT pathways promote cell migration, mediate metabolic regulation, stimulate cell growth and inhibit cellular apoptosis.

Blocking of PDGF signaling has been suggested to inhibit HSCs proliferation and to ameliorate liver fibrogenesis, so the strategies aimed to regulate that have been explored in preclinical and clinical investigations. Application of PDGF isoform antagonists, blocking of PDGFR activation and its downstream pathway regulation are considered as those ones. Thus, sorafenib (a first-line oral chemotherapy drug towards advanced hepatocellular carcinoma (HCC)) is a multikinase inhibitor that targets Raf, VEGFR2/3, and PDGFR-β and has been demonstrated to be a potent antifibrotic agent. The mechanisms of its antifibrotic action were revealed on mice models (high fat diet-, BDL- and DEN- induced ones) and include HSCs autophagy and apoptosis induction (through activation of Akt/mTOR and MAPK signaling pathways), suppression of neovascularization and oxidative stress (through PDGF, STAT3 and mitochondrial respiration downregulation), and inhibition of collagen deposition [47]. Imatinib, another selective TKI, which specifically targets PDGFR, attenuates liver fibrosis and additionally inhibits PDGFR-β expression and decreases the levels of proinflammatory cytokines. The ability of imatinib to induce HSCs apoptosis and substantially decrease their migration could contribute a lot to antifibrotic activity of that and was proven *in vitro* and on CCl_4- and thioacetamide (TAA)-induced mice models [35] (**Table 1**). Strong antifibrotic activity under cholestatic liver diseases has been demonstrated for small molecule roseotoxin B, and investigation of its possible mechanisms revealed its ability to block the PDGF-B/PDGFR-β pathway in HSCs directly [48].

The great potency of PDGFR inhibitors was demonstrated on numerous animal and *in vitro* models. However, it is difficult and often impossible to distinguish the antifibrotic activity from anticancer one due to analysis of clinical trials outcomes. The first reason is that these agents are tested as anti-HCC therapeutics, and outcomes important for anticancer assessment only (like overall survival, disease-free survival etc.) are considered. The second possible reason is strong stratification of HCC patients involved in clinical trial according to their cirrhotic stage, and, despite "anticancer-important" outcomes are monitored thoroughly, the level of cirrhosis is not reassessed. So anticancer activity of the chemicals might be accompanied with antifibrotic one, however, it should be checked additionally. Furthermore, due to high similarity of the homologous domains of PDGFR and VEGFR, applied TKIs like sorafenib, sunitinib and pazopanib could not only inhibit PDGFR activation but also downregulate VEGFR (**Table 1**). It could indicate the complex and therefore more powerful action of these drugs on liver fibrogenesis, but, on the other hand, could also lead to non-target cells impairment and additional toxicity [49].

3.5 TGFβR

TGF-β is a cytokine which plays a prominent role in transformation of HSCs to myofibroblasts. Indeed, many of TGF-β pathological effects could be related with its ability to regulate cell plasticity – change of cell phenotype and function due to genetic and epigenetic changes and cytoskeleton remodeling. One of the

most striking events of cell plasticity is epithelial-mesenchymal transition (EMT). Activation of HSCs and their transformation to myofibroblasts is an example of that one. Moreover, another example of cell transformation caused by TGF-β is EMT in hepatocytes accompanied with loss of cell–cell contacts and polarity [50]. Actually, TGF-β stimulates almost of all liver cell populations (portal and resident fibroblasts, bone marrow-derived fibrocytes, endothelial cells, vascular smooth muscle cells, pericytes and cholangiocytes additionally to hepatocytes and HSCs) to change into a more fibroblastic phenotype [40] and to release profibrogenic transcriptional program manifested by upregulation of collagen expression [41] and disturbances in ECM turnover through imbalance between MMPs and TIMPs. TGF-β receptors (TGFβRI and TGFβRII) are Ser/Tre protein kinases expressed on the membranes of various cells including all above mentioned ones. TGF-β is secreted by these cells and regulates their activity by autocrine and paracrine mechanisms. Moreover, both monocyte-derived macrophages and Kupffer cells (liver resident macrophages) produce this cytokine and some other profibrogenic factors like PDGF and connective tissue growth factor (CTGF), contributing, therefore, to HSCs activation and transdifferentiation, and promoting fibrosis [39]. Thus, TGF-β plays a master role in the activation of HSCs to myofibroblasts. In fact, some of the previous factors stimulate the expression, production and activation of TGF-β, which is responsible finally for the activation of HSCs, and the higher the level of TGF-β the more expressed fibrotic changes in the tissue.

The main mediators of the TGF-β-induced fibrogenic transcriptional program are SMADs (*Caenorhabditis elegans* Sma genes and the *Drosophila* Mad, Mothers against decapentaplegic) [41] (**Figure 1**). Moreover, proteins enriched in TGFR signaling involve Src, cAMP response element-binding protein (CREBP) and others, and some of them belong to EGFR signaling, indicating the crosstalk between these pathways [51]. Additionally, TGF-β1 also mediates the role of FGF1 and FGF2 in the deposition of ECM, or FGF1 and FGF2 mediate the TGF-β activity, or both factors play independent roles through convergent signaling pathways *in vivo* [43].

4. Multikinase inhibitors

Some TKIs have been shown to release antifibrotic activity do not demonstrate exact specificity against their targets and could inhibit more than one receptor. So, it is difficult to explain the mechanism of their action precisely. Nevertheless, these agents attract the attention and reveal the antifibrotic potency even more than specific inhibitors because of multiplicity of mechanisms and downregulated signaling pathways, and therefore, ability to avoid drug resistance through the compensatory mechanisms and signaling crosstalk.

For example, multikinase TKI nilotinib, which is a breakpoint cluster region protein (Bcr)-tyrosine-protein kinase ABL (Abl) inhibitor, also significantly inhibited PDGFR and TGFβRII, which contributes to depression of HSCs activation, proliferation, migration, and α-SMA formation, induction of their apoptosis, reduce collagen deposition in activated HSCs and in liver tissues of CCl_4- and BDL-induced rats experienced liver fibrosis [52]. Moreover, the effects of nilotinib also include diminished expression of VEGF and VEGFR, which, however, is expected due to high similarity of PDGFR and VEGFR kinase domains. These results indicated that nilotinib may represent a putative antifibrotic treatment due to its combined inhibition of non-receptor tyrosine kinases (nonRTK) (Abl) and RTK (PDGFR-β, TGFβRII and VEGFR) (**Table 1**).

Treatment of CCl_4-induced fibrotic mice with nintedanib that blocks PDGFR, VEGFR and FGFR, in addition to depression of HSCs activation, contractility,

migration, and collagen deposition, inhibited macrophage migration, intrahepatic inflammation and angiogenesis as well [36]. Another oral multitargeted TKI pazopanib (approved for renal cell sarcoma treatment) directly inhibits PDGFRs, FGFRs, mast/stem cell growth factor receptor (KIT) and selectively suppresses VEGFR-mediated angiogenesis. The drug can halt liver fibrosis progression through modulating inflammatory cytokines, suppressing HSCs activation, inducing their apoptosis, and regulating angiogenesis [53]. Regorafenib could affect similar targets (VEGFR1–3, PDGFR-β and FGFR) and also potently inhibits another angiogenic RTK TIE2. This drug has recently been approved as a second-line therapy for HCC and demonstrated depression of cirrhotic-associated systemic changes and portal hypertension in HCC patients. Moreover, regorafenib might also be beneficial towards fibrosis and portal hypertension even in absence of HCC [42]. Despite regorafenib treatment had no direct observable effect on HSCs activation and fibrosis progression or regression (as evidenced by liver histopathology, α-SMA and hydroxyproline deposition), however, even its acute administration improved cirrhotic portal hypertension (BDL and CCl_4 models of liver fibrosis) and also hemodynamic circulation in an animal model mimicking portal vein thrombosis [54] (**Table 1**). These findings might explain the anticirrhotic effects of the drug in HCC patients by normalization of liver blood circulation in fibrotic liver and therefore exhausting the inflammatory microenvironment which leads to fibrosis progression.

Brivanib is a selective inhibitor of VEGFR and FGFR and also affects liver fibrosis through multiple signaling pathways. Nakamura et al. demonstrated that brivanib decreased HSCs proliferation induced by PDGF, VEGF and FGF treatment, and also abrogated the phosphorylation of PDGFRβ, which was confirmed *in vitro* and on BDL-, CCl_4- and TAA-induced mice models and supported by histopathological evidences of liver fibrosis alleviation [17] (**Table 1**).

Our team developed the set of multikinase inhibitors, and one of them (1-(4-Cl-benzyl)-3-chloro-4-(CF3-phenylamino)-1H-pyrrole-2,5-dione, called MI1) demonstrated high inhibitory activity against EGFR, VEGFR1,2,3 (the most prominent results), FGF-R1, IGF1-R, spleen associated tyrosine kinase (Syk), 3-phosphoinositide-dependent protein kinase-1 (PDK1), and Src [55]. Besides anticancer and anti-inflammatory activity having been revealed in our previous investigations [56, 57], we showed that MI1 could inhibit liver fibrosis development on rat acute (3 days) and chronic (28 days) cholangitis models, as evidenced by substantially depleted connective tissue deposits in liver and improved liver general state (according to plasma biochemical tests). Moreover, antifibrotic effects of MI1 preserved through at least 28 days since the interventions were terminated (unpublished data, under consideration).

Thus, multikinase inhibitors might be more potent antifibrotic treatments through their impact on several signaling pathways. However, this task should be explored in more detail because of high probability of adverse effects due to multiplicity of these drugs' targets.

5. Small molecule inhibitors of RTK signaling – "noncanonical" approach

Inhibitors of RTK signaling include not only molecules designed to block ATP-binding sites of the kinase, but also small therapeutic molecules with different activities, which, however, could additionally inhibit RTK. For example, natural antioxidant of polyphenol origin resveratrol despite of different therapeutic activities (anti-inflammatory, antitumor, antiaging, protective etc.) demonstrated also

strong antifibrotic effect against liver cirrhosis (CCl_4- model) [58]. The mechanisms of its action are different and include predominantly antioxidant capability, but also impact on gene expression and ability to modulate different signaling pathways through interaction with their key molecules. Among others, resveratrol could downregulate EGFR/Akt/ERK1/2 signaling pathway particularly by decrease of EGFR activation [59]. Furthermore, this polyphenol could scavenge VEGF, altering, therefore, its binding with VEGFR and activation of the latter [60]. Of course, this action could not be interpreted as direct impact on VEGFR. However, it deserves to be considered as an approach for modulation of this signaling activity on its initial stages.

Another plant-derived polyphenol curcumin among various types of biological activities (anticancer, antiviral, antioxidant, anti-inflammatory ones) had beneficial effects in animal models of liver injury and cirrhosis [61]. While studying the possible mechanisms of its action, substantial reduce of TGFβRII levels and its downstream molecules Smad2/3 phosphorylation in response to added TGF-β was found [62]. Furthermore, curcumin revealed anti-EGFR activity: firstly, it was able to inhibit directly the enzymatic activity of the EGFR intracellular domain, and, secondly, it could influence the cell membrane environment of the receptor [63, 64].

Ability to affect the membrane environment of the receptor and thus alter its binding with ligand and subsequent activation has been shown for biologically active indolic related compounds including melatonin, 3-indoleacetic acid, 5-hydroxytryptophol, and serotonin. These chemicals are proven to significantly inhibit VEGF-induced VEGFR2 activation in human umbilical vein endothelial cells through interacting with the cell surface components in a way that prevents VEGF from activating the receptor [65]. This property could contribute to the hepatoprotective and antifibrotic efficacy of melatonin realizing by inhibition of inflammation, HSCs proliferation and hepatocyte apoptosis [66]. The similar mechanism of RTK inhibition has been considered for natural cyclopeptide destruxin A5, that effectively downregulate PDGF-B-induced PDGFR-β signaling. Destruxin A5 does not bind to the ATP-binding pocket of PDGFR-β, so the inhibitory mechanism of that is distinct from the mechanism of "canonical" TKIs. It looks like this chemical selectively targets PDGF-β/PDGFR-β interaction interface and blocks this signaling [67].

However, some non-specific small molecules are able to inhibit RTK by "classical" mechanism – through binding to receptor and preventing its activation by ligand. A naturally occurring flavone 4′,5,7-trihydroxy-3′,5′-dimethoxyflavone (tricin) is one of them. Tricin affected HSCs *in vitro* exploring its potential as antifibrotic therapeutic, as evidenced by inhibiting of human HSC line LI90 and culture-activated HSCs proliferation and migration by that. This flavone reduced the phosphorylation of PDGFRβ and downstream signaling molecules ERK1/2 and Akt, which might be due to its TKI properties rather than inhibition of the direct binding between PDGF-B and its receptor [68]. Flavonoid quercetin was reported to exhibit a wide range of pharmacological properties, including its ability to attenuate liver fibrosis by multiple mechanisms involving several signaling pathways [69]. In particular, quercetin was found to suppress the phosphorylation of EGFR by direct binding with its ATP-binding site [70]. A powerful free radical scavenger carbon-based nanoparticle C60 fullerene could be considered as another unusual RTK inhibitor. It explores wide range of biological activities including antifibrotic and anticirrhotic ones [71–75] probably realized by its antioxidant capacity. However, we also demonstrated its ability to bind to ATP-binding pockets of EGFR and FGFR and to avoid interaction of those with ATP [75], which could be an alternative mechanism of this nanoparticle's antifibrotic action.

6. Conclusions

Growth factor receptors, in particular EGFR, VEGFR, PDGFR, FGFR, and TGFβR are proven to be key regulators of various liver cell populations behavior under hepatic injury and reparation, and subsequent fibrosis development if "something has been going wrong". Upregulation of related signaling pathways has been shown in numerous *in vitro* and *in vivo* models, and for patients who experienced liver diseases accompanied by its fibrosis as well. Inhibiting of those by specific and non-specific compounds followed by fibrosis depression. Above mentioned suggests the potency of RTK inhibition as an antifibrotic treatment. However, all the clinical evidences dedicated to that are rather "concomitant" to TKIs anticancer activity because of predominant focus of these studies on the therapy of liver malignancies developed on cirrhotic background. However, we should remember that liver fibrosis and subsequent cirrhosis are severe high-morbidity diseases themselves. And our knowledge about mechanisms of liver fibrosis development and essential RTKs involvement in that, as well as our achievements in the field of liver fibrosis therapy by TKIs should not be neglected.

Conflict of interest

The authors declare that they have no conflict of interest.

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