

# We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

**5,300**

Open access books available

**130,000**

International authors and editors

**155M**

Downloads

Our authors are among the

**154**

Countries delivered to

**TOP 1%**

most cited scientists

**12.2%**

Contributors from top 500 universities



**WEB OF SCIENCE™**

Selection of our books indexed in the Book Citation Index  
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?  
Contact [book.department@intechopen.com](mailto:book.department@intechopen.com)

Numbers displayed above are based on latest data collected.

For more information visit [www.intechopen.com](http://www.intechopen.com)



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

*Halyna Kuznietsova and Olexandr Ogloblya*

## 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, TGF $\beta$ R, 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, TGF $\beta$ R, 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 latter 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].

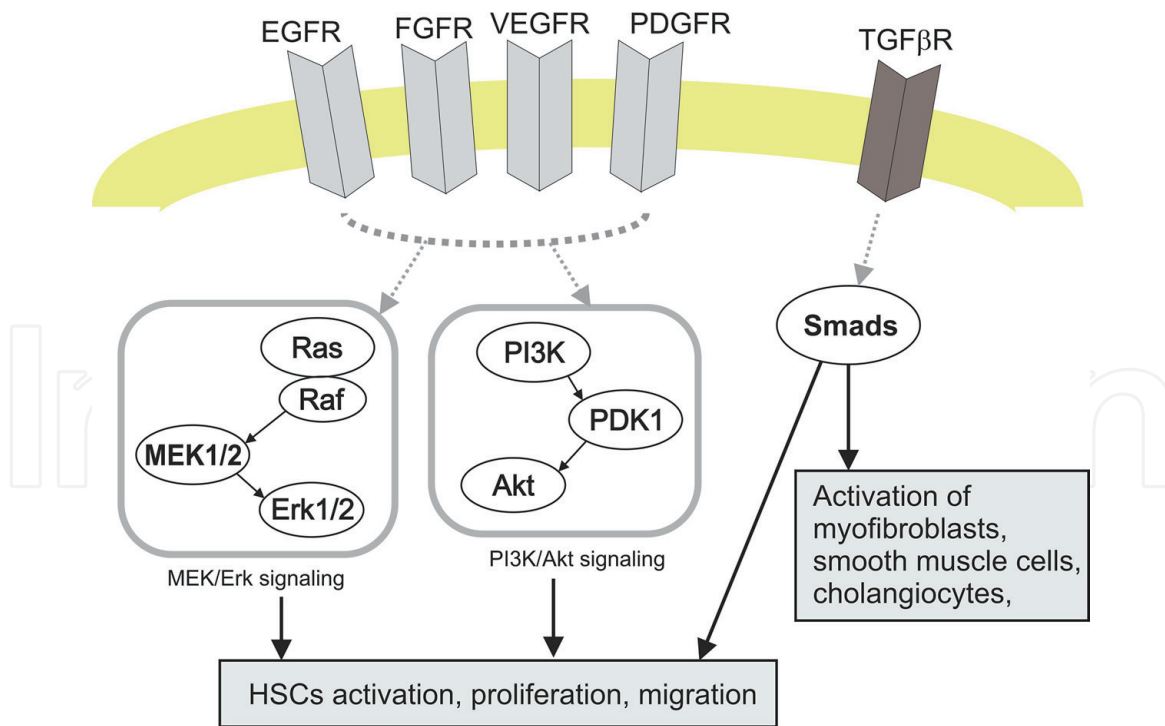
Cirrhosis is the endpoint of many liver diseases and causes the development of serious complications with possible fatal outcome. Those include: liver failure, gastrointestinal bleeding, portal hypertension, i.e. increased pressure in the portal vein, and hepatic coma. Thus, mortality from liver cirrhosis 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 (NF $\kappa$ B), 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 (TGF $\beta$ ), TGF $\alpha$ , 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- $\kappa$ B 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- $\beta$ 1 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- $\beta$ 1 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 sites of injury. With the chronicity of the inflammatory process, these cells are activated and secrete profibrogenic cytokines and growth factors such as TGF- $\beta$ 1, 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- $\beta$  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, TGF $\beta$ R, 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<sub>4</sub>-induced mice liver fibrosis, as evidenced by decrease of fibrous tissue accumulation and hepatic sinusoidal capillarization, and downregulation of  $\alpha$ -smooth muscle actin ( $\alpha$ -SMA), collagen I and TGF- $\beta$ 1 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<sub>4</sub>-, diethylnitrosamine (DEN)- and BDL-induced) rat models [30]. EGFR inhibition also significantly reduced viability and ECM production in activated HSCs, inhibited their proliferation and  $\alpha$ -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 (**Table 1**) [33, 34].

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 <sub>4</sub> -induced mice	Fuchs et al. 2014 [36]
Vatalanib	VEGFR	Inhibition of $\alpha$ -SMA, collagen I and TGF- $\beta$ 1 expression	CCl <sub>4</sub> -induced mice	Kong et al. 2017 [26]
Imatinib	PDGFR	Induce of HSC apoptosis, decrease HSC migration	CCl <sub>4</sub> -, TAA-induced mice	Kim et al. 2012 [37]
Sunitinib	VEGFR, PDGFR, c-Kit	Decrease of vascular density, inflammatory infiltrate, $\alpha$ -SMA and collagen expression	CCl <sub>4</sub> -induced rats	Tugues et al. 2007 [25]
Sorafenib	Raf, VEGFR2/3, PDGFR- $\beta$	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- $\beta$ , FGFR	Induce of HSCs apoptosis, inhibition of HSCs activation, $\alpha$ -SMA, MMP-2, TIMP-1 expression	CCl <sub>4</sub> -induced mice	Elshal et al. 2015 [39]
Nilotinib	BCR-ABL, PDGFR, TGF $\beta$ RII	Depression of HSCs activation, proliferation, migration, $\alpha$ -SMA formation, induce of HSCs apoptosis, reduce collagen deposition in activated HSCs and in liver tissues	CCl <sub>4</sub> - 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 <sub>4</sub> -induced mice	Acora et al. 2017 [41]
Regorafenib	VEGFR1-3, PDGFR- $\beta$ and FGFR, TIE2	Reduce portal hypertension, NO effects on HSCs activation and fibrosis progression or regression	BDL-, CCl <sub>4</sub> -induced mice	Uschner et al. 2018 [42]
Brivanib	VEGFR, FGFR	Decrease of HSCs proliferation	BDL-, CCl <sub>4</sub> -, 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  $\alpha 1(I)$ , 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  $\alpha$ -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<sub>4</sub>-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 ( $\alpha$  and  $\beta$ ), 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- $\alpha$  only, and activated ones – predominantly PDGFR- $\beta$ .

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 C $\gamma$  (PLC $\gamma$ ) 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- $\beta$  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- $\beta$  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- $\beta$  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 TGF $\beta$ R

TGF- $\beta$  is a cytokine which plays a prominent role in transformation of HSCs to myofibroblasts. Indeed, many of TGF- $\beta$  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- $\beta$  is EMT in hepatocytes accompanied with loss of cell-cell contacts and polarity [50]. Actually, TGF- $\beta$  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- $\beta$  receptors (TGF $\beta$ RI and TGF $\beta$ RII) are Ser/Thr protein kinases expressed on the membranes of various cells including all above mentioned ones. TGF- $\beta$  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- $\beta$  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- $\beta$ , which is responsible finally for the activation of HSCs, and the higher the level of TGF- $\beta$  the more expressed fibrotic changes in the tissue.

The main mediators of the TGF- $\beta$ -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- $\beta$ 1 also mediates the role of FGF1 and FGF2 in the deposition of ECM, or FGF1 and FGF2 mediate the TGF- $\beta$  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 TGF $\beta$ RII, which contributes to depression of HSCs activation, proliferation, migration, and  $\alpha$ -SMA formation, induction of their apoptosis, reduce collagen deposition in activated HSCs and in liver tissues of CCl<sub>4</sub>- 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- $\beta$ , TGF $\beta$ RII and VEGFR) (**Table 1**).

Treatment of CCl<sub>4</sub>-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- $\beta$  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,  $\alpha$ -SMA and hydroxyproline deposition), however, even its acute administration improved cirrhotic portal hypertension (BDL and CCl<sub>4</sub> 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 PDGFR $\beta$ , which was confirmed *in vitro* and on BDL-, CCl<sub>4</sub>- and TAA-induced mice models and supported by histopathological evidences of liver fibrosis alleviation (**Table 1**) [17].

Our team developed the set of multikinase inhibitors, and one of them (1-(4-Cl-benzyl)-3-chloro-4-(CF<sub>3</sub>-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<sub>4</sub>- 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 TGF $\beta$ R 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.


IntechOpen

## Author details

Halyna Kuznietsova\* and Olexandr Ogloblya  
Taras Shevchenko National University of Kyiv, Kyiv, Ukraine

\*Address all correspondence to: [biophyz@gmail.com](mailto:biophyz@gmail.com)

## IntechOpen

© 2021 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/3.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. 

## References

- [1] Qiao J. Occurrence, diagnosis and management of hepatic fibrosis and cirrhosis: An updated literature review. *Arch Hepat Res.* 2019;5(1):022-026. DOI: 10.17352/ahr.000023.
- [2] Rieder F, Fiocchi C, Rogler G. Mechanisms, Management, and Treatment of Fibrosis in Patients With Inflammatory Bowel Diseases. *Gastroenterology.* 2017;152(2):340-350. e6. doi:10.1053/j.gastro.2016.09.047
- [3] Zhang CY, Yuan WG, He P, Lei JH, Wang CX. Liver fibrosis and hepatic stellate cells: Etiology, pathological hallmarks and therapeutic targets. *World J. Gastroenterol.* 2016;22:1051210522. doi: 10.3748/wjg.v22.i48.10512
- [4] Li J., Zhao YR, Tian Z. Roles of hepatic stellate cells in acute liver failure: From the perspective of inflammation and fibrosis. *World J Hepatol.* 2019;11(5):412-420. doi: 10.4254/wjh. v11.i5.412
- [5] Mattiuzzi C, Lippi G. Current Cancer Epidemiology. *J Epidemiol Glob Health.* 2019;9(4):217-222. doi: 10.2991/jegh.k.191008.001
- [6] Kostenko S, Dumitriu G, Læg Reid KJ, Moens U. Physiological roles of mitogen-activated-proteinkinase-activated p38-regulated/ activated protein kinase. *World J Biol Chem.* 2011;2(5):73-89. doi: 10.4331/wjbc.v2.i5.73
- [7] Hers I, Vincent EE, Tavaré JM. Akt signalling in health and disease. *Cell Signal.* 2011;23(10):1515-1527. doi: 10.1016/j.cellsig.2011.05.004.
- [8] Neurath MF, Finotto S. IL-6 signaling in autoimmunity, chronic inflammation and inflammation-associated cancer. *Cytokine Growth Factor Rev.* 2011;22(2):83-89. doi: 10.1016/j.cytogfr.2011.02.003
- [9] Hayden MS, Ghosh S. Shared principles in NF-kappaB signaling. *Cell.* 2008;132(3):344-362. doi: 10.1016/j.cell.2008.01.020
- [10] Luedde T, Schwabe RF. NF-κB in the liver--linking injury, fibrosis and hepatocellular carcinoma. *Nat Rev Gastroenterol Hepatol.* 2011;8(2):10818. doi: 10.1038/nrgastro.2010.213
- [11] Kasembeli MM, Bharadwaj U, Robinson P, Tweardy DJ. Contribution of STAT3 to Inflammatory and Fibrotic Diseases and Prospects for its Targeting for Treatment. *Int J Mol Sci.* 2018;19(8):2299. doi: 10.3390/ijms19082299
- [12] Zhang XL, Xing RG, Chen L, Liu CR, Miao ZG. PI3K/Akt signaling is involved in the pathogenesis of bleomycin-induced pulmonary fibrosis via regulation of epithelialmesenchymal transition. *Mol Med Rep.* 2016;14(6):5699-5706. doi: 10.3892/mmr.2016.5960
- [13] Le Cras TD, Korfhagen TR, Davidson C, Schmidt S, Fenchel M, Ikegami M, Whitsett JA, Hardie WD. Inhibition of PI3K by PX-866 prevents transforming growth factor- $\alpha$  induced pulmonary fibrosis. *Am J Pathol.* 2010;176(2):679-686. doi: 10.2353/ajpath.2010.090123
- [14] Choi ME, Ding Y, Kim SI. TGF- $\beta$  signaling via TAK1 pathway: role in kidney fibrosis. *Semin Nephrol.* 2012;32(3):244-252. doi:10.1016/j.semnephrol.2012.04.003
- [15] Wynn TA. Cellular and molecular mechanisms of fibrosis. *J Pathol.* 2008;214(2):199-210. doi:10.1002/path.2277
- [16] Friedman SL. Mechanisms of hepatic fibrogenesis. *Gastroenterology.* 2008;134(6):1655-1669. doi:10.1053/j.gastro.2008.03.003

- [17] Nakamura I, Zakharia K, Banini BA, Mikhail DS, Kim TH, Yang JD, Moser CD, Shaleh HM, Thornburgh SR, Walters I, Roberts LR. Brivanib attenuates hepatic fibrosis in vivo and stellate cell activation in vitro by inhibition of FGF, VEGF and PDGF signaling. *PLoS One*. 2014;9(4):e92273. doi: 10.1371/journal.pone.0092273
- [18] Karlsen TH, Folseraas T, Thorburn D, Vesterhus M. Primary sclerosing cholangitis - a comprehensive review. *J Hepatol*. 2017;67(6):1298-1323. doi: 10.1016/j.jhep.2017.07.022
- [19] Hall C, Sato K, Wu N, Zhou T, Kyritsi K, Meng F, Glaser S, Alpini G. Regulators of Cholangiocyte Proliferation. *Gene Expr*. 2017;17(2):155-171. doi: 10.3727/105221616X692568
- [20] Koyama Y, Xu J, Liu X, Brenner DA. New Developments on the Treatment of Liver Fibrosis. *Dig Dis*. 2016;34(5):58996. doi: 10.1159/000445269
- [21] Qu K, Huang Z, Lin T, Liu S, Chang H, Yan Z, Zhang H, Liu C. New Insight into the Anti-liver Fibrosis Effect of Multitargeted Tyrosine Kinase Inhibitors: From Molecular Target to Clinical Trials. *Front Pharmacol*. 2016;6:300. doi: 10.3389/fphar.2015.00300.
- [22] Glaser SS, Gaudio E, Miller T, Alvaro D, Alpini G. Cholangiocyte proliferation and liver fibrosis. *Expert Rev Mol Med*. 2009;11:e7. doi: 10.1017/S1462399409000994
- [23] Fabris L, Cadamuro M, Fiorotto R, Roskams T, Spirlì C, Melero S, Sonzogni A, Joplin RE, Okolicsanyi L, Strazzabosco M. Effects of angiogenic factor overexpression by human and rodent cholangiocytes in polycystic liver diseases. *Hepatology*. 2006 May;43(5):1001-1012. doi: 10.1002/hep.21143
- [24] Yoshiji H, Kuriyama S, Yoshii J, Ikenaka Y, Noguchi R, Hicklin DJ, Wu Y, Yanase K, Namisaki T, Yamazaki M, Tsujinoue H, Imazu H, Masaki T, Fukui H. Vascular endothelial growth factor and receptor interaction is a prerequisite for murine hepatic fibrogenesis. *Gut*. 2003;52(9):1347-1354. doi: 10.1136/gut.52.9.1347
- [25] Tugues S, Fernandez-Varo G, Muñoz-Luque J, Ros J, Arroyo V, Rodés J, Friedman SL, Carmeliet P, Jiménez W, Morales-Ruiz M. Antiangiogenic treatment with sunitinib ameliorates inflammatory infiltrate, fibrosis, and portal pressure in cirrhotic rats. *Hepatology*. 2007;46(6):1919-1926. doi: 10.1002/hep.21921
- [26] Kong LJ, Li H, Du YJ, Pei FH, Hu Y, Zhao LL, Chen J. Vatalanib, a tyrosine kinase inhibitor, decreases hepatic fibrosis and sinusoidal capillarization in CCl<sub>4</sub>-induced fibrotic mice. *Mol Med Rep*. 2017;15(5):2604-2610. doi: 10.3892/mmr.2017.6325
- [27] Ornitz DM, Itoh N. The Fibroblast Growth Factor signaling pathway. *Wiley Interdiscip Rev Dev Biol*. 2015;4(3):21566. doi: 10.1002/wdev.176.
- [28] Schumacher JD, Guo GL. Regulation of Hepatic Stellate Cells and Fibrogenesis by Fibroblast Growth Factors. *Biomed Res Int*. 2016;2016:8323747. doi: 10.1155/2016/8323747
- [29] Liu Y, Lui EL, Friedman SL, Li L, Ye T, Chen Y, Poon RT, Wo J, Kok TW, Fan ST. PTK787/ZK22258 attenuates stellate cell activation and hepatic fibrosis in vivo by inhibiting VEGF signaling. *Lab Invest*. 2009;89(2):20921. doi: 10.1038/labinvest.2008.127
- [30] Yu C, Wang F, Jin C, Huang X, Miller DL, Basilico C, McKeegan WL. Role of fibroblast growth factor type 1 and 2 in carbon tetrachloride-induced hepatic injury and fibrogenesis. *Am J Pathol*. 2003 Oct;163(4):1653-1662. doi: 10.1016/S0002-9440(10)63522-5

- [31] Lin N, Chen S, Pan W, Xu L, Hu K, Xu R. NP603, a novel and potent inhibitor of FGFR1 tyrosine kinase, inhibits hepatic stellate cell proliferation and ameliorates hepatic fibrosis in rats. *Am J Physiol Cell Physiol*. 2011;301(2):C469-C477. doi: 10.1152/ajpcell.00452.2010;
- [32] Lou D, Han J, Zhou L, Ma H, Xv J, Shou J, Xu Z, Jiang L, Qian Y. Fibroblast growth factor receptor 1 antagonism attenuates lipopolysaccharide-induced activation of hepatic stellate cells via suppressing inflammation. *Exp Ther Med*. 2018 Oct;16(4):2909-2916. doi: 10.3892/etm.2018.6586
- [33] Trussoni CE, Tabibian JH, Splinter PL, O'Hara SP. Lipopolysaccharide (LPS)-induced biliary epithelial cell NRas activation requires epidermal growth factor receptor (EGFR). *PLoS One*. 2015;10(4):e0125793. doi: 10.1371/journal.pone.0125793
- [34] Breitkopf K, Roeyen Cv, Sawitza I, Wickert L, Floege J, Gressner AM. Expression patterns of PDGF-A, -B, -C and -D and the PDGFR receptors alpha and beta in activated rat hepatic stellate cells (HSC) Cytokine. 2005;31:349-357. doi: 10.1016/j.cyto.2005.06.005
- [35] Liu SH, Chen XF, Xie ZB, Zhou J. EGFR monoclonal antibody panitumumab inhibits chronic proliferative cholangitis by downregulating EGFR. *Int J Mol Med*. 2019;44(1):79-88. Doi: 10.3892/ijmm.2019.4190
- [36] Fuchs BC, Hoshida Y, Fujii T, Wei L, Yamada S, Lauwers GY, McGinn CM, DePeralta DK, Chen X, Kuroda T, Lanuti M, Schmitt AD, Gupta S, Crenshaw A, Onofrio R, Taylor B, Winckler W, Bardeesy N, Caravan P, Golub TR, Tanabe KK. Epidermal growth factor receptor inhibition attenuates liver fibrosis and development of hepatocellular carcinoma. *Hepatology*. 2014;59(4):1577-1590. doi: 10.1002/hep.26898.
- [37] Kim Y, Fiel MI, Albanis E, Chou HI, Zhang W, Khitrov G, Friedman SL. Anti-fibrotic activity and enhanced interleukin-6 production by hepatic stellate cells in response to imatinib mesylate. *Liver Int*. 2012;32:1008-1017. doi: 10.1111/j.1478-3231.2012.02806.x
- [38] Wang Y, Gao J, Zhang D, Zhang J, Ma J, Jiang H. New insights into the antifibrotic effects of sorafenib on hepatic stellate cells and liver fibrosis. *J Hepatol*. 2010;53:132-144. doi: 10.1016/j.jhep.2010.02.027
- [39] Elshal M, Abu-Elsaad N, El-Karef A, Ibrahim TM. The multi-kinase inhibitor pazopanib targets hepatic stellate cell activation and apoptosis alleviating progression of liver fibrosis. *Naunyn Schmiedebergs Arch Pharmacol*. 2015;388(12):1293-1304. doi: 10.1007/s00210-015-1157-7
- [40] Liu Y, Wang Z, Kwong SQ, Lui ELH, Friedman SL, Li FR, Lam RWC, Zhang GC, Zhang H, Ye T. Inhibition of PDGF, TGF- $\beta$ , and Abl signaling and reduction of liver fibrosis by the small molecule Bcr-Abl tyrosine kinase antagonist Nilotinib. *J Hepatol*. 2011;55(3):612-625. doi: 10.1016/j.jhep.2010.11.035
- [41] Akcora BO, Storm G, Prakash J, Bansal R. Tyrosine kinase inhibitor BIBF1120 ameliorates inflammation, angiogenesis and fibrosis in CCl<sub>4</sub>-induced liver fibrogenesis mouse model. *Sci Rep*. 2017;7:44545.
- [42] Uschner FE, Schueller F, Nikolova I, Klein S, Schierwagen R, Magdaleno F, Gröschl S, Loosen S, Ritz T, Roderburg C, Vucur M, Kristiansen G, Lammers T, Luedde T, Trebicka J. The multikinase inhibitor regorafenib decreases angiogenesis and improves portal hypertension. *Oncotarget*. 2018;9(90):36220-36237. doi: 10.18632/oncotarget.26333

- [43] Wang X, Gao Y, Li Y, Huang Y, Zhu Y, Lv W, Wang R, Gou L, Cheng C, Feng Z, Xie J, Tian J, Yao R. Roseotoxin B alleviates cholestatic liver fibrosis through inhibiting PDGF-B/PDGFR- $\beta$  pathway in hepatic stellate cells. *Cell Death Dis.* 2020;11(6):458. doi: 10.1038/s41419-020-2575-0
- [44] Ying HZ, Chen Q, Zhang WY, Zhang HH, Ma Y, Zhang SZ, Fang J, Yu CH. PDGF signaling pathway in hepatic fibrosis pathogenesis and therapeutics (Review). *Mol Med Rep.* 2017;16(6):7879-7889. doi: 10.3892/mmr.2017.7641
- [45] Wells RG. Cellular sources of extracellular matrix in hepatic fibrosis. *Clin Liver Dis.* 2008;12:759-768. Doi: 10.1016/j.cld.2008.07.008
- [46] Lazaridis KN, Strazzabosco M, Larusso NF. The cholangiopathies: disorders of biliary epithelia. *Gastroenterology.* 2004;127:1565-1577. Doi: 10.1053/j.gastro.2004.08.006
- [47] Pellat A, Vaquero J, Fouassier L. Role of ErbB/HER family of receptor tyrosine kinases in cholangiocyte biology. *Hepatology.* 2018;67(2):762773. doi: 10.1002/hep.29350
- [48] Fabregat I, Caballero-Díaz D. Transforming Growth Factor- $\beta$ Induced Cell Plasticity in Liver Fibrosis and Hepatocarcinogenesis. *Front Oncol.* 2018;8:357. Doi: 10.3389/fonc.2018.00357
- [49] Wynn TA, Barron L. Macrophages: master regulators of inflammation and fibrosis. *Semin Liver Dis.* 2010;30:24557. Doi: 10.1055/s-0030-1255354
- [50] Hassan S, Zil-E-Rubab, Shah H, Shawana S. Dysregulated epidermal growth factor and tumor growth factorbeta receptor signaling through GFAPACTA2 protein interaction in liver fibrosis. *Pak J Med Sci.* 2020;36(4):782787. doi: 10.12669/pjms.36.4.1845
- [51] Wilhelm SM, Dumas J, Adnane L, Lynch M, Carter CA, Schütz G, Thierauch KH, Zopf D. Regorafenib (BAY 73-4506): a new oral multikinase inhibitor of angiogenic, stromal and oncogenic receptor tyrosine kinases with potent preclinical antitumor activity. *Int J Cancer.* 2011;129(1):245-255. doi: 10.1002/ijc.25864
- [52] Xu H, Liu L, Cong M, Liu T, Sun S, Ma H, You H, Jia J, Wang P. EGF neutralization antibodies attenuate liver fibrosis by inhibiting myofibroblast proliferation in bile duct ligation mice. *Histochem Cell Biol.* 2020;154(1):107116. doi: 10.1007/s00418-020-01867-9.
- [53] Arabpour M., Poelstra K., Helfrich W., Bremer E., Haisma H.J. Targeted elimination of activated hepatic stellate cells by an antiepidermal growth factor-receptor single chain fragment variable antibody-tumor necrosis factor-related apoptosisinducing ligand (scFv425-sTRAIL) *J Gene Med.* 2014;16:281-290. doi: 10.1002/jgm.2776
- [54] Liang D, Chen H, Zhao L, Zhang W, Hu J, Liu Z, Zhong P, Wang W, Wang J, Liang G. Inhibition of EGFR attenuates fibrosis and stellate cell activation in diet-induced model of nonalcoholic fatty liver disease. *Biochim Biophys Acta Mol Basis Dis.* 2018;1864(1):133-142. doi: 10.1016/j.bbadis.2017.10.016
- [55] Kuznietsova H, Dziubenko N, Byelinska I, Hurmach V, Bychko A, Lynchak O, Milokhov D, Khilya O, Rybalchenko V. Pyrrole derivatives as potential anti-cancer therapeutics: synthesis, mechanisms of action, safety. *J Drug Target.* 2020;28(5):547-563. doi: 10.1080/1061186X.2019.1703189
- [56] Kuznietsova HM, Yena MS, Kotlyar IP, Ogloblya OV, Rybalchenko VK. Anti-Inflammatory Effects of Protein Kinase Inhibitor Pyrrol Derivate. *ScientificWorldJournal.*



2016;2016:2145753. doi:  
10.1155/2016/2145753

[57] Kuznietsova HM, Lynchak OV, Danylov MO, Kotliar IP, Rybal'chenko VK. [Effect of dihydropyrrrol and maleimide derivatives on the state of the liver and colon in normal rats and those with colorectal carcinogenesis induced by dimethylhydrazine]. *Ukr Biokhim Zh* (1999). 2013;85(3):74-84. Ukrainian. PMID: 23937051.

[58] Chávez E, Reyes-Gordillo K, Segovia J, Shibayama M, Tsutsumi V, Vergara P, Moreno MG, Muriel P. Resveratrol prevents fibrosis, NF-kappaB activation and TGF-beta increases induced by chronic CCl4 treatment in rats. *J Appl Toxicol*. 2008;28(1):35-43. doi: 10.1002/jat.1249

[59] Li W, Ma X, Li N, Liu H, Dong Q, Zhang J, Yang C, Liu Y, Liang Q, Zhang S, Xu C, Song W, Tan S, Rong P, Wang W. Resveratrol inhibits Hexokinases II mediated glycolysis in non-small cell lung cancer via targeting Akt signaling pathway. *Exp Cell Res*. 2016;349(2):320-327. doi: 10.1016/j.yexcr.2016.11.002

[60] Hu WH, Duan R, Xia YT, Xiong QP, Wang HY, Chan GK, Liu SY, Dong TT, Qin QW, Tsim KW. Binding of Resveratrol to Vascular Endothelial Growth Factor Suppresses Angiogenesis by Inhibiting the Receptor Signaling. *J Agric Food Chem*. 2019;67(4):1127-1137. doi: 10.1021/acs.jafc.8b05977

[61] Vera-Ramirez L, Pérez-Lopez P, Varela-Lopez A, Ramirez-Tortosa M, Battino M, Quiles JL. Curcumin and liver disease. *Biofactors*. 2013;39(1):88100. doi: 10.1002/biof.1057

[62] Yao QY, Xu BL, Wang JY, Liu HC, Zhang SC, Tu CT. Inhibition by curcumin of multiple sites of the transforming growth factor-beta1 signalling pathway ameliorates the progression of liver fibrosis induced

by carbon tetrachloride in rats. *BMC Complement Altern Med*. 2012;12:156. doi: 10.1186/1472-6882-12-156

[63] Starok M, Preira P, Vayssade M, Haupt K, Salomé L, Rossi C. EGFR Inhibition by Curcumin in Cancer Cells: A Dual Mode of Action. *Biomacromolecules*. 2015;16(5):163442. doi: 10.1021/acs.biomac.5b00229

[64] Tang Y. Curcumin targets multiple pathways to halt hepatic stellate cell activation: updated mechanisms in vitro and in vivo. *Dig Dis Sci*. 2015;60(6):1554-1564. doi: 10.1007/s10620-014-3487-6

[65] Cerezo AB, Hornedo-Ortega R, Álvarez-Fernández MA, Troncoso AM, García-Parrilla MC. Inhibition of VEGF-Induced VEGFR-2 Activation and HUVEC Migration by Melatonin and Other Bioactive Indolic Compounds. *Nutrients*. 2017;9(3):249. doi: 10.3390/nu9030249

[66] Hu C, Zhao L, Tao J, Li L. Protective role of melatonin in early-stage and end-stage liver cirrhosis. *J Cell Mol Med*. 2019;23(11):7151-7162. doi: 10.1111/jcmm.14634

[67] Wang X, Wu X, Zhang A, Wang S, Hu C, Chen W, Shen Y, Tan R, Sun Y, Xu Q. Targeting the PDGF-B/PDGFR- $\beta$  Interface with Destruxin A5 to Selectively Block PDGF-BB/PDGFR- $\beta$  Signaling and Attenuate Liver Fibrosis. *EBioMedicine*. 2016;7:146-156. doi: 10.1016/j.ebiom.2016.03.042

[68] Seki N, Toh U, Kawaguchi K, Ninomiya M, Koketsu M, Watanabe K, Aoki M, Fujii T, Nakamura A, Akagi Y, Kusakawa J, Kage M, Shirouzu K, Yamana H. Tricin inhibits proliferation of human hepatic stellate cells in vitro by blocking tyrosine phosphorylation of PDGF receptor and its signaling pathways. *J Cell Biochem*. 2012;113(7):2346-2355. doi: 10.1002/jcb.24107

- [69] Wang R, Zhang H, Wang Y, Song F, Yuan Y. Inhibitory effects of quercetin on the progression of liver fibrosis through the regulation of NF- $\kappa$ B/I $\kappa$ B $\alpha$ , p38 MAPK, and Bcl-2/Bax signaling. *Int Immunopharmacol.* 2017;47:126-133. doi: 10.1016/j.intimp.2017.03.029
- [70] Fridrich D, Teller N, Esselen M, Pahlke G, Marko D. Comparison of delphinidin, quercetin and (-)-epigallocatechin-3-gallate as inhibitors of the EGFR and the ErbB2 receptor phosphorylation. *Mol Nutr Food Res.* 2008;52(7):815-822. doi: 10.1002/mnfr.200800026
- [71] Kuznietsova HM, Lynchak OV, Dziubenko NV, Osetskyi VL, Ogloblya OV, Prylutskyi YuI, Rybalchenko VK, Ritter U, Scharff P. Water-soluble C60 fullerenes reduce manifestations of acute cholangitis in rats. *Appl Nanosci.* 2019;9:601-608. Doi: 10.1007/s13204-018-0700-5
- [72] Kuznietsova H, Lynchak O, Dziubenko N, Herheliuk T, Prylutskyi Y, Rybalchenko V, Ritter U. Water-soluble pristine C60 fullerene attenuates acetaminophen-induced liver injury. *Bioimpacts.* 2019;9(4):227-237. doi: 10.15171/bi.2019.28
- [73] Kuznietsova HM, Dziubenko NV, Lynchak OV, Herheliuk TS, Zavalny DK, Remeniak OV, Prylutskyi YI, Ritter U. Effects of Pristine C60 Fullerenes on Liver and Pancreas in  $\alpha$ -Naphthylisothiocyanate Induced Cholangitis. *Dig Dis Sci.* 2020;65(1):215-224. doi: 10.1007/s10620-019-05730-3
- [74] Kuznietsova H, Dziubenko N, Herheliuk T, Prylutskyi Y, Tauscher E, Ritter U, Scharff P. WaterSoluble Pristine C60 Fullerene Inhibits Liver Alterations Associated with Hepatocellular Carcinoma in Rat. *Pharmaceutics.* 2020;12(9):794. doi: 10.3390/pharmaceutics12090794
- [75] [Kuznietsova H, Dziubenko N, Hurmach V, Chereschuk I, Motuziuk O,
- Ogloblya O, Prylutskyi Y. Water-Soluble Pristine C60 Fullerenes Inhibit Liver Fibrotic Alteration and Prevent Liver Cirrhosis in Rats. *Oxid Med Cell Longev.* 2020;2020:8061246. doi: 10.1155/2020/8061246