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MOLLECULAR HEPATIC STELLATE CELLS TARGETS IN ANTIFIBROTIC THERAPY

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ABSTRACT. Hepatic stellate cells (HSC) activation as a result of liver injury, followed by their proliferation and the increase of extracellular matrix (ECM) synthesis, is the main phenomenon in the development of liver fibrosis. In case of chronic liver injury, the negative consequences generated by liver fibrosis on hepatic function and morphology may lead to hepatic insufficiency and portal hypertension. Liver cirrhosis is considered a precancerous stage prior to the development of hepatocellular carcinoma (HCC). Initiation of HSCs activation consists in rapid changes in protein synthesis, followed by alterations in cellular phenotype, amplifying HSCs response to inflammatory cytokines and local stimuli. By blocking activated HSCs response to inflammatory cytokines and growth factors stimulation, antifibrotic therapies aim to inhibit their proliferation, trigger their apoptosis, reduce the collagen synthesis and increase ECM degradation. Future therapeutic strategies target the development of oral drugs with high antifibrotic potential, as well as a series of therapeutic agents with parenteral administration and reduced side effects. Also, a special attention should be given to gene therapy that alters HSCs phenotype and inhibits exacerbated collagen synthesis. In the same time, prophylactic and neoadjuvant therapy for HCC aims to block activated HSCs expression of growth and nuclear factors involved in carcinogenesis.

Keywords: hepatic stellate cells, liver fibrosis, antifibrotic therapy, hepatocellular carcinoma, carcinogenesisnot known.

INTRODUCTION

Liver fibrosis and cirrhosis develop after chronic liver injuries of different ethiologies viral, autoimmune, drugs, cholestasis and metabolic. Excessive accumulation of ECM is present in most chronic liver diseases. In injured hepatic tissue, activated HSCs are the main collagen producing cells. Following liver injury, quiescent HSCs activates and suffer a phenotypic transdifferentiation in proliferative, fibrogenic and contractile myofibroblasts-like cells.

Beside hepatoprotection and treatment of the primary disease, most antifibrotic therapies target the inhibition of HSCs activation by reducing inflammation or host's immune inflammatory response, as well as neutralising HSCs proliferative, profibrogenic, contractile and/or proinflammatory response. A different therapy takes into account triggering HSCs apoptosis.

Other antifibrotic therapies consider direct administration of collagenases like matrix-metalloproteases (MMPs), or stimulation of MMPs producing cells, as well as blocking tisular inhibitors of metalloproteases (TIMPs) which exert an inhibitory effect on MMPs, leading to an increased ECM degradation.

Initiating fibrogenesis

Hepatic injury initiates fibrogenesis through signalling molecules derived from inflammatory cells, hepatocytes and other non-parenchymal cells, especially sinusoidal endothelial cells and Kupffer cells.

Oxidative stress plays an important role in hepatic injury and in initiating liver fibrogenesis through production of reactive oxygen species (ROS). Hepatocytes' necrosis and apoptosis appear following lipids, proteins and DNA oxidation, followed by amplifying inflammatory response and initiating fibrogenesis. ROS stimulates both Kupffer and inflammatory cells in releasing profibrogenic mediators, that in turn stimulates HSCs proliferation which amplifies the production of ECM (Galli et al., 2005).

Hypoxia leads to fibrosis by stimulating the release of Hypoxia-inducible factor 1 (HIF)-1α by HSCs. In turn, (HIF)-1α stimulates the expression of vascular endothelial cell growth factor (VEGF), which leads to an increase of type I collagen synthesis by HSCs (Corpechot et al., 2002). Hypoxia intervenes in autocrine and paracrine control of angiogenesis and fibrosis by increasing the stimulation and synthesis of transforming growth factor (TGF)-β1 (Jeong et al., 2004). Following chronic liver damage, a paracrine loop between fibrosis and hypoxia is created, as they reciprocally stimulate each other and therefore disrupt normal tissue repair.

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Inflammation and immune response play an important role in the initiation and progression of liver fibrosis (Mehal et al., 2007). Chronic liver inflammation leads to the activation of Kupffer cells which locally release proinflammatory cytokines like tumoral necrosis factor (TNF)-α, interleukin-1β (IL-1β) and interleukin-6 (IL-6) (Capuron et al., 2011; Laye et al., 2000).

Electron microscopic examination revealed that the vacuolar juice with anthocyans undergoes a fine granulation of their vacuolar content; witch can be a result of samples preparation with osmic acid. It is not clear if leucoantocians accumulated in vacuolele cells of root tissue undergo this fine granulation, or only those with red anthocyanins. Future researches are needed to elucidate this phenomenon.

Kupffer cells activation emphasizes the activity of nuclear factor kappa-B (NF-kB) with a subsequent release of proinflammatory cytokines, including TNF-α and the monocyte chemoattractant protein (MCP)-1, which initiates HSCs activation (Liu et al., 2010).

In turn, HSCs respond to this stimulation by releasing macrophage colony-stimulating factor (M-CSF), MCP-1 (Czaja et al., 1994) and IL-6, (Tiggelman et al., 1995) which amplifies acute phase response and leads to a supplementary increase of activated macrophages. TNF-α also induces neutrophil infiltration locally in hepatic injury and increases mitochondrial oxidation in hepatocytes, initiating their apoptosis (Pinzani et al., 1992). Hepatocytes apoptosis intervenes in proinflammatory stimulation and in fibrogenesis through apoptotic bodies which are phagocited by Kupffer cells that subsequently synthesizes TNF-α (Canbay et al., 2003). Similarly, HSCs induces profibrogenic response after assimilating apoptotic bodies, through producing oxidative radicals, up-regulating TGF-β1 synthesis and type I collagen expression (Zhan et al., 2006).

Hepatic steatosis indirectly generates liver fibrosis, (Radu et al., 2011), by increasing hepatocyte susceptibility to oxidative stress and viral infection aggression, both leading to HSCs activation followed by an increased accumulation of ECM. A series of different pathways may contribute to steatosis-related fibrogenesis, as increasing oxidative stress response, increasing hepatocytes susceptibility to apoptosis, alteration in cellular response to injury, signalling and activation of peroxisome proliferator-activated receptor (PPAR), as well as dysregulation of leptin expression and signalling (Friedman, 2003).

HSCs activation and myofibroblast transformation

A major breakthrough in understanding the mechanism of liver fibrosis was the identification of HSCs role. HSCs are the main cells involved in producing and deposition of ECM in normal and fibrotic liver.

Chronic hepatic injury generated by hepatitic viruses, toxins, autoimmune diseases stimulates the activation of HSCs through cytokines, which leads to their transformation into myofibroblast-like cells (Bataller et al., 2005). HSCs activation comprises two major phases: initiation (preimflammatory stage) and perpetuation, followed by resolution, if case hepatic injury ends (Friedman, 2004).

Initiation represents the early genetic and structural alteration of HSCs, followed by emphasizing their response to cytokine stimulation, shortly after hepatic injury occurs. Initial paracrine stimulation which includes signals from injured hepatocytes, Kupffer and endothelial cells, leads to early HSCs activation, as well as alteration in surrounding ECM structure. Once the activation process is initiated, perpetuation follows after continuous stimulation which in turn maintains HSCs in active state, involving seven alterations in cellular behaviour: proliferation, chemotaxis, fibrogenesis, contractility, ECM degradation, retinoid loss and leucocite chemoattraction / cytokine release. During perpetuation, a paracrine and autocrine release of proinflammatory, profibrogenic and promitogenic stimuli occurs.

Initiation

Initial alterations in HSCs structure appear following paracrine stimulation generated by Kupffer cells, lymphocytes, hepatocytes, leucocytes and sinusoidal endothelium. A major role in HSCs activation is played by Kupffer cells infiltration and activation (Gressner et al., 1993), which in turn stimulates cellular proliferation, ECM synthesis and retinoid loss, via TGF- (Matsuoka et al., 1990), TNF- and matrix metallo-proteases (MMP)-9 (Winwood et al., 1995). Endothelial cells participate in HSCs activation by converting TGF- from passive to profibrogenic active state, and also by increasing synthesis of cellular fibronectine.

Kupffer cells generate locally, in the injured hepatic tissue, on the one hand ROS which stimulated HSCs activation and collagen I synthesis, and on the other hand they produce nitric oxide (NO) which exerts an antagonist effect of ROS, diminishing HSCs proliferation and contractility. A study conducted on a co-culture of CYP2E1 cells virally infected with HepG2 and HSCs, has shown that hepatocytes-generated ROS can increase the production of collagen by activated HSCs via P4502E1 cytochrome (Nieto et al., 2002). A series of clinical studies have reported a diminishing in activated HSCs number, following a treatment with antioxidant inhibitors of CYP2E1 and hydrogen peroxidase. NADPH oxidase represents another ROS source generated by injured hepatocytes which in turn stimulate fagocites to produce superoxide anions. Hepatocyte apoptosis also stimulates HSCs activation, followed by liver fibrosis development (Canbay et al., 2003).

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An important source of ROS is represented by neutrophiles stimulating collagen I synthesis by activated HSCs via superoxidation. Neutrophiles produce in the same time NO, which reduces superoxide effect, without cancelling it (Casini et al., 1997).

T-hepler (Th) CD4 lymphocytes intervene in HSCs activation by differentiating in subpopulations Th1 and Th2 and also by cytokine synthesis. Th1 cells release - interferon (INF), TNF and IL-2, and also produce cytokines with roles in cellular-mediated immunity. In turn, Th2 cells synthesize IL-4, IL-5, IL-6 and IL-13, promoting humoral-mediated immunity which increases liver fibrosis. During viral aggression, leucocytes presents in the liver join Kupffer cells and synthesize cytokines which modulate HSCs phenotype.

HSCs trigger innate immune response through inflammatory signalling of Toll-like receptors (TLR4) which are part of pattern recognition receptors family. TLR4 receptors have been identified on HSCs and Kupffer cells surfaces. An animal model study has shown a reduction in local macrophage infiltration and an improvement in liver fibrosis, which had been experimentally induced by genetic deletion of TLR4 receptor. As a result of TLR4 receptor blockage by liposaccharide ligands (Paik et al., 2003), an intracellular signalling pathway is activated, which includes NF-kB activation (Szabo et al., 2006). Early alterations in ECM structure are consequences of transcriptional events.

GC, GT sequences and CACCC boxes are present in regulatory regions of profibrogenic genes expressed in activated HSCs, where are located genes receptors for 1 (I) collagen, TGF-1 and TGF- type I and type II.

These sequences encode receptors for transcription factor Krupple-like (KLF), which contains terminal DNA C2H2C. This transcription factors family modulates specific tissular genes transcription. In activated HSCs, there have been identified at least three members of this transcriptional type factors Sp1, basic transcription element binding protein (BTEB)-1 and KLF6 which can modulate collagen 1 (I) gene transcription. In vivo and in vitro studies have reported an early presence of KLF-6 gene subsequent to HSCs activation (Bieker, 2001). In activated HSCs, SP1 plays the role of a transcriptional modulator (Rippe et al., 1995) associated to the two regions GC (FP1 and FP2) belonging to collagen 1 (I) promoter gene (Rippe et al., 1999).

Perpetuation

Paracrine stimulation is present during HSCs perpetuation, process which involves simultaneous autocrine cytokines which triggers HSCs activation, like TGF-b1, platelet derived growth factor (PDGF), fibroblastic growth factor (FGF) and endothelin-1 (ET-1), as well as inhibitory cytokines of HSCs activation like hepatocyte growth factor (HGF).

HSCs amplify the inflammation of hepatic lesion by releasing chemoattractants (Marra et al., 1993) for neutrophiles cytokine-induced neutrophil chemoattractant / IL-8 (Maher et al., 1998) and for monocytes MCP-1 (Marra et al., 1998). HSCs synthesize multiple anti-inflammatory cytokines, especially IL-10.

During perpetuation, ECM remodelling continues, low density subendothelial matrix being progressively replaced by an ECM rich in fibril-producing collagen, which leads to an altered behaviour of hepatocytes, sinusoidal endothelium cells and HSCs. In advanced fibrosis stages, in hepatic tissue there has been identified an ECM concentration 6 times greater than normal, including collagen I, III and IV, undulin, laminin, proteoglycans, fibronectinã, elastin and hyaluronan. Fibril-producing ECM accelerates HSCs activation through integrins and by binding receptors located in the discoidin domain receptor (DDR2). DDR2 is a tyrosine kinase receptor, ligand for type I collagen. After binding, a sequence of events is triggered, including src kinase attraction, followed by signal amplification and transcriptional induction of MMP-2 (Ikeda et al., 2002).

HSCs perpetuation

The perpetuation of HSCs activation involved a series of alterations in cellular behaviour, like proliferation, contractility, fibrogenesis, ECM degradation, chemotaxis and retinoid loss.

Proliferation

Local activated HSCs proliferation follows growth factor stimulation. PDGF exerts the most potent mitogen effect towards HSCs. In early stages of HSCs activation, PDGF receptors are expressed on cell surface, amplifying the responsiveness to its stimulation (Wong et al., 1994). Its experimental inhibition has been followed by a reduction in the degree of liver fibrosis. In hepatic injured tissue, HSCs proliferate after PDGF stimulation, accomplished through mitogen activated protein kinase (MAPK) signalling, especially JNK, extracellular signal-regulated kinase (ERK) and p38. JNK and ERK activation induces HSCs proliferation, while p38 activation inhibits proliferative response (Schnabl et al., 2001).

PDGF stimulates the activation of AKt kinase and p70 in activated HSCs, by means of phosphatidylinositol 3 kinase (PI3-K), which leads to an exacerbation of HSCs proliferation and chemotaxis (Reif et al., 2003). ET-1, thrombin, FGF, insulin-like growth factor (IGF) also exerts a mitogen effect on HSCs (Pinzani et al., 2001).

Contractility

The increase in portal resistance in injured liver is generated by activated HSCs contractility. ET-1 and other contraction-stimulating cytokines favour HSCs contraction. An increased production of ET-1 is accompanied by increased levels of the conversion enzyme of ET-1 which activates inactive ET-1. Endothelin stimulates HSCs through two G protein coupled receptors of endothelin type A and B (ETA and ETB) present on quiescent and active HSCs surface (Rockey, 1997). In intralobullar spaces, ET-1 binding points are abundant especially in the juxtaportal region.

An ultrastructural analysis has shown that aproximatively 35% of ET-1 binding points are located on the HSCs surface, and only a part of the other binding points are located on endothelial sinusoidal cells (ESC) and Kupffer cells (Gondo et al., 1993).

The heterogeneity of the ET-1 binding places in hepatic lobules follows blood vessels tracts in hepatic sinusoids. All cell types present in hepatic sinusoids Kupffer cells, ESCs, HSCs and hepatocytes express ETB receptors, but only HSCs and hepatocytes express ETA receptors (Mallat et al., 1995). ET-1 induces HSCs contraction via ETA receptors (Shi-Wen et al., 2004). NO is an ET-1 antagonist, stimulating HSCs relaxation.

Fibrogenesis

Stimulation of collagen synthesis is mediated by transcriptional and posttranscriptional mechanisms, and it represents one of the major molecular responses of activated HSCs following hepatic injury. Collagen I transcriptional activation is mediated by fibrogenic growth factors (Gressner, 1995), adipokines and vasoactive substances, needed in fibrosis development. Following TGF-1 stimulation, activated HSCs increase ECM synthesis (Olaso et al., 1998). HSCs represent a major source of TGF-1, which is also synthesized by Kupffer cells and platelets.

Increased levels of TGF-1 as a result of liver fibrosis stimulates HSCs proliferation through Smad and MAPK signalling pathways (Cao et al., 2002), as well as collagen 1 (I) synthesis by activated HSCs (Dooley et al., 2001).

In activated HSCs, TGF- increases 1 (I) collagen mRNA half-time mediated by increasing stability of 1 (I) collagen mRNA through p38 MAPK pathway (Tsukada et al., 2005). The stability of 1 (I) collagen is increased due to the interaction between a stem loop structure located at the 5' end of 1 (I) collagen with an untranslated 3' region (Lindquist et al., 2004).

Experimentally, TGF- involvement has been reported mainly in perpetuation and less in initiating HSCs activation. Animal model studies have shown that blocking TGF- synthesis or its signalling pathways prevents fibrosis development (Shek et al., 2004).

Leptine is an adipokine mainly synthesised by adipocytes. Its role as a profibrinogenic hormone was emphasized based on an increased synthesis of 1 (I) collagen by activated HSCs, mediated through PI3K/AKt pathway stimulated by activated JAK1 kinases (Niu et al., 2007).

ECM degradation

Fibrosis resolution is characterized by increased collagenolitic activity, through degradation of fibrillar collagen I and III by interstitial MMPs. HSCs contain all components needed for ECM degradation, being a MMP-2 source as well as MMP-3 / stromelysin. During liver fibrosis, due to a reduced level of TIMP-1, the activity of MMPs increases. By TIMP-1 and TIMP-2 stimulation, HSCs inhibits the activity of interstitial collagenases, leading to scar tissue accumulation (Arthur, 1995).

During fibrogenesis, MMP-2, type I membranar MMP, TIMP-1 and TIMP-2 concentrations are increased (Arthur, 2000). On activated HSCs and myofibroblasts there is an increased concentration of TIMPs. Liver macrophages are a source of MMP-13 (Uchinami et al., 2006) and proteases (Hironaka et al., 2000). Clinical studies have emphasized that increased levels of MMPs reduce liver fibrosis, but not biliary fibrosis (Garcia-Banuelos et al., 2002), thus demonstrating MMPs different role depending on type and duration of injury.

Activating protein (AP)-1 modulated genetic remodelling of TIMP and ECM, presenting an increased and persistent activity in activated HSCs). AP-1 contains at least one Jun type protein which may form homodimers or heterodimers with other Jun proteins or with a Fos type protein. JunD protein is an important protein of AP-1, with a major role in genetic stimulation of TIMP-1 and IL-6 synthesis (Smart et al., 2001) in activated HSCs, TIMP-1 transcription being regulated by JunD homodimers operating in parallel with a nuclear RUNX protein (Trim et al., 2000).

As a response to cellular microenvironment signalling, alteration in concentration and activity of different types of AP-1 and in the genes they transcript (MMPs and TIMPs), stimulates liver ECM remodelling by activated HSCs.

HSCs chemotaxis

Following activated HSCs migration, their number increases in the areas of injury. Many chemoattractants are involved in HSCs migration, like PDGF, IGF-1, MCP-1 and ET-1. MCP-1. MCP-1 is a chemokine synthesised only by activated HSCs, and it signals through PI3-K mediated pathways with Ca2+ influx the accumulation of lymphocytes and monocytes in the area of injury.

Retinoid loss

An important feature of HSCs activation is represented by the loss of its intracellular retinoid. Although one cannot firmly state that this loss is necessary or not for HSCs activation, Milliano et al. have reported that HSCs exposed to extracellular retinoid stimulation present a decrease of activity markers, without stopping their activation (Milliano et al., 2005).

Multiple nuclear receptors for retinoids were identified on the surface of activated HSCs (Fiorucci et al., 2005).

PPAR receptors , especially PPAR, have been identified on HSCs membrane (Marra et al., 2000), their number increasing proportionally with cellular activity (Hellemans et al., 2003). Marra et al. have shown that ligands blocking the PPAR nuclear receptor reduce HSCs activation, while ligands of PPAR receptor stimulate HSCs proliferation (Marra et al., 2000).

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Resolution

During tisular healing of acute hepatic injury, it has been observed a reduced number of activated HSCs, as a result of reversion into quiescent state, or apoptosis initiation. Due to this observation, antifibrotic therapies of chronic hepatic injuries regard stimulating the pathways which lead to HSCs reversion in quiescent state or inducing their apoptosis.

Reversion

PPARγ is a member of nuclear receptors of steroid or thyroid hormones superfamily which plays a role in genetic transcription control and cellular differentiation. In vivo and in vitro studies have reported a reduction of PPARγ synthesis in activated HSCs (Miyahara et al., 2000). Hazra et al. have created cultured activated HSCs and they have demonstrated their reversion throughout an increased synthesis of PPARγ. PPARγ inhibits the activation of a1 (I) procollagen promoter and it reduces the collagen synthesis, thus determining the reversion of biochemical characteristics of activated HSCs (Hazra et al., 2004).

Apoptosis

In vitro studies have reported the induction of activated HSCs apoptosis following membranar receptors stimulation by apoptosis-inducing membranar proteins TRAIL and CD95-L (Radaeva et al., 2006). Nervous growth factor also stimulates HSCs apoptosis, its effect being antagonised by serotonin (Ruddell et al., 2006).

Natural killer (NK) cells intervene in fibrosis resolution and HSCs apoptosis through the release of TRAIL proteins. NK cells' antifibrotic role was confirmed by clinical trials which demonstrated an intensification of liver fibrosis after the interruption of immunosuppressive therapies (Hudnall, 1991). During liver fibrosis regression, senescent HSCs are more susceptible to NK cells induced apoptosis (Krizhanovsky et al., 2008).

During the healing phase of experimentally induced liver injury, the association between HSCs apoptosis and reduced levels of TIMP-1 synthesis has been demonstrated (Iredale et al., 1998). TIMP-1 generates an anti-apoptotic effect, thus protecting activated HSCs. Blocking TIMP-1 with specific monoclonal antibodies is followed by the reversion of liver fibrosis (Parsons et al., 2004). Subsequently, fibrillar collagen partial degradation is initiated. Therefore, ECM production by activated HSCs is altered, which leads to their apoptosis (Elsharkawy et al., 2005).

In vivo and in vitro studies have demonstrated NF-kB role in blocking activated HSCs apoptosis, through a mechanism involving JNK cascade inhibition and AP-1 pathway (Czaja, 2003)responsible for modulating cellular apoptosis (Oakley et al., 2005). Antagonists of NF-kB activity may represent a therapeutic target in reducing liver fibrosis, by stimulating HSCs apoptosis.

HSCs involvement in hepatocellular carcinoma development

HSCs activation plays an important role in the progression of liver fibrosis, being the main risk factor in HCC development (Elsharkawy et al., 2007).

HSCs transdifferentiation in myofibroblasts-like cells is responsible for an excessive ECM production and for replacing normal liver tissue. An increased number of activates HSCs has been observed around tumor sinusoids, fibrous septa (Schmitt-Griff et al., 1991), as well as in the tumor capsule (Enzan et al., 1994).

HSCs activation plays a major role in hepatocarcinogenesis (Zhao et al., 2011) by inducing autocrine signalling of TGF- and by stimulating -catenin nuclear accumulation in neoplastic hepatocytes (Mikula et al., 2006). TGF- releasing by the activated HSCs stimulates tumor progression in neoplastic hepatocytes. TGF- induces epithelial cells transdifferentiation in mesenchymal cells and it amplifies PDGF signalling in oncogenic Ras transformed hepatocytes. One hypothesis regarding HCC tumorogenesis implies the combined effect of growth factor FGF-1 and -2, PDGF and IGF released by activated HSCs (Bataller et al., 2005).

In HCC, activated HSCs considerably increase NF-kB and ERK activity. NF-kB and MAP kinase / ERK pathways are involved in HCC progression by stimulating tumor cells proliferation and by inhibiting apoptosis (Amann et al., 2009). HSCs intervene in tumoral metastasis via HGF which amplifies tumoral cells invasiveness. A study which used antagonists of tumoral cells HGF receptors, reported that tumoral cells development and migration were impaired (Barnaeva et al., 2007).

Activated HSCs also intervene in tumoral progression, by alterations in ECM remodelling (Théret et al., 2001) and by releasing angiopoietin which induces angiogenesis (Wirz et al., 2008). VEGF released by tumoral cells and activated HSCs is involved in angiogenesis associated with HCC progression (Torimura et al., 1998).

These evidences promote the pathways through which HSCs take part in HCC development and progression as new therapeutic strategies in HCC treatment.

Curing the primary disease

Healing the primary disease represents the ideal therapeutic measure in preventing liver fibrosis development. Antiviral treatment associated with chronic viral hepatitis may lead to viral clearance or suppression, being followed by a reduction in fibrosis and portal pressure (Roberts et al., 2007).

Another therapeutic measure regards stopping alcohol abuse in patients with alcoholic liver disease, or removing iron or copper excess in patients with precirrhotic genetic disease, hemochromatosis and Wilson's disease. A study conducted on a group of patients with nonalcoholic steatohepatitis (NASH) emphasized a correlation between weight loss and a reduced fibrogenesis (Dixon et al., 2004).

Inhibiting HSCs activation

A therapeutic target in fibrotic reaction associated with chronic hepatic disease is represented by inhibiting quiescent HSCs transdifferentiation in activated myofibroblasts. In this direction, a reduction of oxidative stress may represent a possible therapeutic alternative. Thus, a series of antioxidants like vitamin E (Brown et al., 1997), silimarinum (Pietrangelo et al., 1995), phosphatidylcholin and S-adenosyl-L-methionine inhibit HSCs activation, thus playing an antiapoptotic role for hepatocytes and therefore reducing liver fibrosis progression. (Kawada et al., 1998).

An experimental study conducted on an animal model with liver cirrhosis induced by common bile duct ligation, reported an improvement of liver fibrosis after IGF-1 administration an important modulator of intermediary metabolism with role in oxidative stress reduction (Canturk et al., 2003).

Another method of inhibiting HSCs activation implies the administration of interferon-, whose benefic effects in ameliorating liver fibrosis were observed in patients with chronic hepatic viral disease (Rockey et al., 1994).

Therapeutic administration of HGF (Kim et al., 2005) and IGF (Sanz et al., 2005) has determined an inhibition in myofibroblasts' activation, which led to their apoptosis HGF generates an antifibrotic effect via transcriptional Ets-1 factor, which reduces HSCs TGF- β synthesis and stimulates MMP-1 synthesis (Ozaki et al., 2002).

A recent study has identified a new mechanism through which HGF reduces type I collagen synthesis in activated HSCs, by blocking TGF-βmediated activation pathways. HGF inhibits the transduction of TGF-β profibrogenic signal, by increasing the interactions between Gal-7 and phosphorylated Smad3. This leads to p-Smad3 blockage in cellular cytoplasm, followed by type I collagen synthesis inhibition (Inagaki et al., 2008).

Thiazolidinediones are synthetic ligands of PPAR- (Galli et al., 2002) nuclear receptors identified on HSCs membrane (Miyahara et al., 2000). They form a class of antidiabetic drugs with benefic effects in liver disease by reducing HSCs activation (Marra et al., 2000).

Leptin and adiponectin induce insulin resistance, which intervenes in non-alcoholic liver diseases and chronic viral hepatitis pathogenesis and progression.

Leptin is an adipocytokine with profibrogenic properties by stimulating α2 (I) collagen synthesis (Bertolaniet al., 2008), whose excessive accumulation represents the main feature of liver fibrosis (Saxena et al.,2003; Ikejima et al., 2002) On the other hand, leptin inhibits MMP-1 synthesis and activity and it stimulates TIMP-1 expression (Lin et al., 2006).

Leptin maintain HSCs in active state by stimulating proliferation and inhibiting apoptosis via extracellular pathways involving ERK and protein kinase B phosphorylation. Several studies on animal models have reported a decrease in liver fibrosis in animals with leptin deficiency (Saxena et al., 2004).

Adiponectin is a proteic hormone expressed by adipocytes whose seric levels are reduced in obese patients, leading to insulin resistance development. Adiponectin may counteract insulin resistance by TNF-α antagonism and the reduction of serum glucose and triglycerides levels (Balmer et al., 2010). Adiponectin activation of AMPK disrupts leptin-mediated hepatic fibrosis via suppressors of cytokine signalling SOCS-3 (Handy et al., 2010). Chen et al. have demonstrated on animal models that adiponectin administration leads to an improvement of alcoholic-induced hepatic injuries, by reducing lipid droplets present in hepatocytes after alcohol abuse (Chen et al., 2007). Antifibrotic therapy associated to liver disease may target these pathogenic pathways.

The association between pegylated interferon and ribavirin has led to a reduction of liver fibrosis progression (Poynard et al., 2002) in patients with chronic viral hepatitis, by blocking viral replication and stopping hepatic injury (Shiratori et al., 2000). Experimental studies have demonstrated that treatment with -interferon diminishes liver fibrosis in patients with biliary fibrosis, thus demonstrating the existence of an antifibrotic effect associated to the antiviral one. Non the less, the antifibrotic effect of interferon is present only in patients with viral eradication (Shiratori et al., 2000). In vitro and in vivo experiments with corticosteroids have demonstrated an inhibitory effect on HSCs activating signalling molecules. In the past decades, corticosteroid therapy was successfully used to diminish fibrosis associated with autoimmune hepatitis (Czaja et al., 2004).

Raetsch et al. have reported a reduction of liver fibrosis by antagonising with pentoxiphylline the effect generated by TNF- (Raetsch et al., 2002). Pentoxiphylline is a metilxantine derivate which reduces activated HSCs synthesis of type α I collagen, via inhibiting I kappa b α degrading, which in turn blocks NF-kB activation (Hernández et al., 2007).

Renin-angiotensin system amplifies inflammation by generating oxidative stress. The therapies with inhibitors of the renin-angiotensin system and with antagonists of type I receptor of type II angiotensinogen were successfully used in diminishing inflammation, which led to an improvement of liver fibrosis (Kurikawa et al., 2003).

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Inducing activated HSCs apoptosis

Activated HSCs apoptosis is the main mechanism in reducing their number during the cure of liver injury (Iredale et al., 1998). Multiple apoptosis mediators, especially Fas/LasL, TNF and Bcl/Bax receptors, were identified on activated HSCs membrane, therefore a new antifibrogenic target may be represented by the stimulation of these receptors which induce HSCs apoptosis (Fallowfield et al., 2004).

Both experimental and clinic evidence have shown the potential reversibility of liver fibrosis and cirrhosis by initiating activated HSCs apoptosis. The effects are thus targeted to the main cell involved in ECM production and in MMPs inhibition by TIMPs expression.

The progressive accumulation of ECM together with an impairment in the mechanism regulating its normal degradation, are responsible for liver fibrosis progression. MMP-1 is the main protease degrading type I collagen, the main collagen form present in fibrotic liver (Milani et al., 1994). MMPs inactivation is accomplished by interaction with TIMPs molecules (Iredale, 2001). Activated HSCs excessively synthesize TIMP-1 and TIMP-250 which inhibit interstitial collagenases, leading to a reduction in ECM degradation and consequently its accumulation. TIMP-1 has an important role in activated HSCs survival by directly inhibiting their apoptosis. Its effect is dependent on MMPs inhibition (Murphy et al., 2002).

An important therapeutic antifibrotic method may be represented by using TIMP antagonists to inhibit type I collagen synthesis and to induce activated HSCs apoptosis (Parsons et al., 2004).

Hepatocytes' apoptosis exerts a profibrogenic inflammatory signalling (Canbay et al., 2005). Caspases are a cistein protease family with multiple major roles in cellular apoptosis and necrosis. Canbay et al. have experimentally emphasized on animal models that caspase inhibitor IDN-6556 attenuates hepatic injury and implicitly reducing fibrosis (Canbay et al., 2004). A major issue in using this therapy is represented by the inhibitory effects on the apoptosis of cells which may have suffered DNA mutations, potentially leading to an increased risk of HCC development.

Farnesoid X receptor (FXR), a member of nuclear transcription factor receptors family, is activated after binding with biliary acids, especially chenodeoxicholic acid (CDCA). FXR controls genes which modulate bile flux and secretion (Rader et al., 2007). After identifying FXR on activated HSCs membrane, its involvement in cellular activity inhibition was also determined. A significant antifibrotic effect was generated experimentally in animal models, by using CDCA - the endogen ligand of FXR.

NR0B2, a small heterodimers partner (SHP), is a member of intracellular nuclear transcription factor receptor family. In vivo and in vitro studies have reported a reduction in type I collagen synthesis by activated HSCs, after FXR ligands stimulation of SHP identified on activated HSCs membrane (Fiorucci et al., 2004). Serial binding of FXR and SHP receptors inhibits TIMP-1 expression in activated HSCs, after SHP's interaction with profibrogenic transcription factor JunD, resulting in preventing JunD's binding with TIMP-1 promoter. At the same time, MMP-2 activity is doubled (Fiorucci et al., 2005). TIMP-1 inhibition is essential: first, metalloproteases activity is no longer blocked, and second, HSCs no longer receive TIMP-1's antiapoptotic signals. Animal model studies have reported an increase synthesis of PPARγ in activated HSCs, after the stimulation of FXR ligands. PPARγ agonists and FXR ligands exert a synergic effect in reducing type I collagen (Fiorucci et al., 2005). The combined effect of FXR ligands and PPARγ agonists therapy diminishes side effects generated by the single use of PPARγ agonists.

Natural killer cells are a major component of innate immunity. They play a role in reducing liver fibrosis mainly by neutralising activated HSCs (Radaeva et al., 2006; Melhem et al., 2006), and also by releasing two proinflammatory cytokines INF and INF (Rockey et al., 1994; Inagaki et al., 2003). NK cells are less efficient in alcohol abusing patients, hence the progression of liver fibrosis is accelerated in their case (Jeong et al., 2008).

Experimental studies conducted on rats have reported that gliotoxin, a fungic metabolite, induces HSCs apoptosis in the absence of oxidative stress, by releasing mitochondrial C cytochrome and by caspase-3 activation and ATP depletion (Anselmi et al., 2007).

Bortesomib, a protease inhibitor, induces HSCs apoptosis by blocking NF-kB activity, and by increasing the half-time of its inhibitors (Anan et al., 2006).

Cytokine therapy may be another therapeutic target in preventing liver fibrosis. Gene therapies with growth factors led to a reduction on liver fibrosis. An animal model study has observed a reduced proliferation and apoptosis initiation in -SMA positive hepatic cells, following HGF administration (Kim et al., 2005).

Stimulation and increasing ECM degradation

Normally, quiescent HSCs produce type III IV collagen, and small quantities of type I collagen (Milani et al., 1989). During liver fibrogenesis, activated HSCs become the main cells which increase the accumulation of ECM, by an increased production of type I collagen (Milani et al., 1989; Maher et al., 1990).

A major role in antifibrotic therapies is represented by existing ECM resorption. TGF- antagonists primary reduce ECM synthesis after the inhibition of activated HSCs, and secondly promotes ECM degradation via modulating TIMP's expression and by increasing interstitial collagenases activity. An in vivo study which used gene therapy with urokinase-type plasminogen activator reported a reduction in collagen synthesis by modulating MMP, HGF and VEGF mediated signalling pathways (Bueno et al., 2006).

In primary biliary cirrhosis, administrating ursodeoxycholic acid generated an anti-inflammatory effect, followed by a reduction of liver fibrosis (Degott et al., 1999). A similarly result was experimentally demonstrated on an animal model, using a nitric oxide-releasing derivative of ursodeoxycholic acid that selectively releases nitric oxide in the liver, which decreased portal pressure (Fiorucci et al., 2003). Another study on mice which used FXR receptors ligands has reported a increased choleresis and an antifibrotic effect (Fiorucci et al., 2004).

A series of studies have demonstrated the importance of TGF- and PDGF in mediating signalling pathways specific for initiating liver fibrosis and tumorogenesis (Liu et al., 2006). These studies have emphasized that genetic alterations in TGF- mediated signalling pathways through Smad7 antagonist lead to a reduction of liver fibrosis and tumoral progression, after interacting with HSCs receptors (Mikula et al., 2006).

Another method to inhibit liver fibrogenesis is based on administrating a powerful tyrosine kinase inhibitor PTK/ZK, which blocks PDGF and TGF- mediated signalling pathways in activated HSCs (Liu et al., 2009).

The wnt pathway, another signalling pathway which mediates renal and pulmonary fibrosis, may also play a role in initiating liver fibrosis. Some authors have reported a reduction in liver fibrosis by blocking this signalling pathway with Dickkopf-1 (DKK-1) an antagonist of Wnt coreceptor (Cheng et al., 2007).

Several studies concentrated on understanding transcriptional regulation have emphasized the possibility of inhibiting activated HSCs by blocking the activity of histone deacetylase (HDACs) an enzyme which plays a major role in chromatin alteration during genetic transcription. Inhibitors with high HDACs specificity may offer a selective method of blocking HSCs activation (Niki et al., 1999).

Neutralizing HSCs proliferative, profibrogenic, contractile and proinflammatory response

Another target for antifibrotic therapies is blocking proliferative, profibrogenic phases and contractile response of activated HSCs, using antagonists of cytokine receptors. Thus, there have been identified inhibitors of -linoleic acid, lipooxigenase (Beno et al., 1995) and for PPAR , PDGF and FGF receptors (Galli et al., 2000).

Several researchers have reported a reduction of activated TGF-b which leads to a diminishing in liver fibrosis progression in rats, after administrating camostat mesilate (Okuno et al., 2001). Yoshiji et al. have demonstrated, in a study conducted on rats, that imatinib mesylate an inhibitor of tyrosine kinase receptor determines a diminution of liver fibrosis due to a significant reduction of activated HSCs proliferation and migration, induced by PDGF-BB, as well as a diminishing in -SMA and mRNA 2 (I) procollagen expression in activated HSCs (Yoshiji et al., 2005).

TGF-antagonists, like monoclonal antibodies and protease inhibitors, determine both the inhibition of ECM production and also the acceleration of its degradation. A soluble recombined protease inhibitor mannose 6-phosphate (M-6-P) is in direct competition with TGF in binging on M-6-P receptor located on HSCs membrane, stopping their activation (de Bleser et al., 1995). Long term administration of TGF- antagonists in humans may lead to HCC development, by altering inflammatory and immune response, with a loss in growth inhibition mediated by TGF- (Matsuzaki et al., 2000).

Renin-angiotensin system inhibitors are commonly used as antifibrotic agents in chronic kidney and heart disease, especially by having reduced adverse effects in long term administration. A study conducted on two patient groups with chronic viral hepatitis C and non-alcoholic steatohepatitis have emphasized the benefic effects generated by renin-angiotensin inhibitors in preventing liver fibrosis development (Yokohama et al., 2004).

Son et al. have reported a diminution in activated HSCs proliferation, migration, collagen synthesis, as well as a reduced activity of additional profibrogenic constituent genes, after stimulation with an adenovirus which encodes a negative form dominated by PI3K, controlled by an -SMA promoter. This adenovirus also induces cellular apoptosis (Son et al., 2009).

Cho et al. have emphasized the benefic effect of vasodilatatory substances like prostaglandine E2 and NO in antifibrotic therapies. These act by blocking endotheline A receptors, thus stopping collagen synthesis and accumulation which leads to a diminishing in liver fibrosis (Cho et al., 2000).

Liver fibrogenesis is inhibited by small molecular weight molecules which block intracellular signalling pathways. These molecules represent a selective inhibitor of Rho mediated focal adhesion (Tada et al., 2001) and also an antisense for PDGF B-chain, with a benefic effect in preventing experimental liver fibrosis (Borkham-Kamphorst et al., 2004).

HSP 47 is a collagen chaperone which is found in activated HSCs, whose concentration in endoplasmic reticulum (Nagata, 1998) is correlated with an increased production of 1 (I) and 1 (III) collagen (Masuda et al., 1994). Collagen synthesis in activated HSCs may be blocked by administration of liposomes containing vitamin A and siRNA which exerts inhibitory effects on HSP 47 (Sato et al., 2008).

Rapamycin an immunosupressor administrated after liver transplant may represent a new therapeutic agent which inhibits HSCs proliferation (Zhu et al., 1999). Its long term administration increases the risk to develop hepatic artery thrombosis (Trotter, 2003).

Bennett et al. have observed in vivo and in vitro a reduction in collagen synthesis by activated HSCs, associated with an increase in ECM degradation, after administrating relaxin a natural peptide hormone, whose receptors are present on activated HSCs membrane (Bennett et al., 2005).

Gene therapies targeting activated HSCs

In the following period, specific gene therapies acting directly on activated HSCs may be developed along existing pharmacologic ones, targeting deficit correction or stimulating transcription of cellular essential proteins. Adenoviral or lipidic nonviral vectors may be the carries for these genes. Up till now, few studies regarding gene therapies have been carried out.

Studies conducted in vitro have evaluated several methods of HSCs transfection. Notable results have been obtained after using an adenoviral vector (Ad5), as it allowed a 100% transfection via coxackie-adenovirus (CAR) receptor present on myofibroblasts and activated HSCs membrane, thus demonstrating the possibility to induce genes in cells. When developing a therapy, one must take into consideration the fact that, in the absence of specificity, genetic material carried by the adenovirus may also enter in hepatocytes, through CAR receptors found on their membrane (Weiskirchen et al., 2000).

Further studies have demonstrated that adenoviral particle internalisation is also stimulated by integrins present on HSCs, endothelial cells and myofibroblasts membrane. Weiskirchen et al. have improved adenoviral transduction specificity by adding a promoter recognized only by receptors located on activated HSCs membrane, like CRP-2 (Weiskirchen et al., 2001).

Another therapeutic method may concern the use of telomerase. Experimental studies on animal model have reported a reduced degree of liver fibrosis after telomerase gene delivery. One of the main factors responsible in triggering organ insufficiency in chronic diseases like liver cirrhosis is a reduced cellular level of telomerase (Rudolph et al., 2000). A study conducted on lab animals with induced telomerase deficiency has reported a diminishing in liver fibrosis after administrating telomerase RNA using adenoviruses, due to a recovery in telomerase's activity and function.

Future therapeutic strategies

Antifibrotic therapies cannot be developed without knowing the major phenomena which modulate physiopathological mechanisms involved in early stages of hepatic injury, implicitly contributing to HSCs activation.

An ideal therapy should correspond to the fundamental therapeutic principles, consisting in oral drugs, well tolerated in long term administration, which next to preventing fibrosis, also stimulates ECM degradation, thus stabilising and improving liver function.

Besides oral therapy, we could also consider parenteral administration of drugs, with weekly or monthly administration. The most promising agents from this category may be the monoclonal antibodies which can exert benefic and certain effects.

REFERENCES

Amann T, Bataille F, Spruss T, Mühlbauer M, Gäbele E, Schölmerich J, Kiefer P, Bosserhoff AK, Hellerbrand C, Activated hepatic stellate cells promote tumorigenicity of hepatocellular carcinoma, Cancer Sci, 100(4), 646-653, 2009.

Anan A, Baskin-Bey ES, Bronk SF, Werneburg NW, Shah VH, Gores GJ, Proteasome inhibition induces hepatic stellate cell apoptosis, Hepatology, 43, 335-344, 2006.

Anselmi K, Stolz DB, Nalesnik M, Watkins SC, Kamath R, Gandhi CR, Gliotoxin causes apoptosis and necrosis of rat Kupffer cells in vitro and in vivo in the absence of oxidative stress: exacerbation by caspase and serine protease inhibition, J Hepatol, 47, 103-113, 2007.

Arthur MJ, Collagenases and liver fibrosis, J Hepatol, 22;Suppl 2, 43-48, 1995.

Arthur MJ, Fibrogenesis II. Metalloproteinases and their inhibitors in liver fibrosis, Am J Physiol, 279, G245-249, 2000.

Balmer ML, Joneli J, Schoepfer A, Stickel F, Thormann W, Dufour JF, Significance of serum adiponectin levels in patients with chronic liver disease, Clin. Sci, 119, 431-436, 2010.

Barnaeva E, Nadezhda A, Hannappel E, Sjogren MH, Rojkind M, Thymosin beta4 upregulates the expression of hepatocyte growth factor and downregulates the expression of PDGF-beta receptor in human hepatic stellate cells, Ann N Y Acad Sci, 1112, 154-160, Sep. 2007.

Bataller R, Brenner DA, Liver fibrosis, J Clin Invest, 115, 209-218, 2005.

Bennett RG, Mahan KJ, Gentry-Nielsen MJ, Tuma DJ, Relaxin receptor expression in hepatic stellate cells and in cirrhotic rat liver tissue, Ann N Y Acad Sci, 1041, 185-189, 2005.

Beno DW, Mullen J, Davis BH, Lipoxygenase inhibitors block PDGF-induced mitogenesis: a MAPK-independent mechanism that blocks fos and egr, Am J Physiol, 268, C604-610, 1995.

Bertolani C, Marra F, The role of adipokines in liver fibrosis, Pathophysiology, **15**, 91-101, 2008.

Bieker JJ, Kruppel-like factors: three fingers in many pies, J Biol Chem, 276, 34355-34358, 2001.

Borkham-Kamphorst E, Stoll D, Gressner AM, Weiskirchen R, Antisense strategy against PDGF B-chain proves effective in preventing experimental liver fibrogenesis, Biochem Biophys Res Commun, 321(2), 413-423, 2004.

Brown KE, Poulos JE, Li L, et al, Effect of vitamin E supplementation on hepatic fibrogenesis in chronic dietary iron overload, Am J Physiol, 272, G116-G123, 1997.

Bueno M, Salgado S, Beas-Zárate C, Armendariz-Borunda J, Urokinase-type plasminogen activator gene therapy in liver cirrhosis is mediated by collagens gene expression down-regulation and up-regulation of MMPs, HGF and VEGF, J Gene Med, 8, 1291-1299, 2006.

Canbay A, Feldstein A, Baskin-Bey E, Bronk SF, Gores GJ, The caspase inhibitor IDN-6556 attenuates hepatic injury and fibrosis in the bile duct ligated mouse, J Pharmacol Exp Ther, 308 (3), 1191-1196, 2004.

Canbay A, Kip SN, Kahraman A, Gieseler RK, Nayci A, Gerken G, Apoptosis and fibrosis in nonalcoholic fatty liver disease, Turk J Gastroenterol, 16(1), 1-6, 2005.

Canbay A, Taimr P, Torok N, Higuchi H, Friedman S, Gores GJ, Apoptotic body engulfment by a human stellate cell line is profibrogenic, Lab Invest, 83(5), 655-663, 2003.

Canturk NZ, Canturk Z, Ozden M, Dalcik H, Yardimoglu M, Tulubas F, Protective effect of IGF-1 on experimental liver cirrhosis - induced common bile duct ligation, Hepatogastroenterology, 50, 2061-2066, 2003.

Cao Q, Mak KM, Lieber CS, DLPC decreases TGF-beta1-induced collagen mRNA by inhibiting p38 MAPK in hepatic stellate cells, Am J Physiol Gastrointest Liver Physiol, 283, G1051-G1061, 2002.

Capuron L, Miller AH, Immune system to brain signaling: Neuropsychopharmacological implications, *Pharmacology and Therapeutics,* 130, 226-238, 2011.

Casini A, Ceni E, Salzano R, Biondi P, Parola M, Galli A, et al., Neutrophil-derived superoxide anion induces lipid peroxidation and stimulates collagen synthesis in human hepatic stellate cells: role of nitric oxide, Hepatology, 25, 361-367, 1997.

Cheng JH, She H, Han YP, Wang J, Xiong S, Asahina K, et al., Wnt antagonism inhibits hepatic stellate cell activation and liver fibrosis, Am J Physiol Gastrointest Liver Physiol, 15, 2007.

Cho JJ, Hocher B, Herbst H, Jia JD, Ruehl M, Hahn EG, et al., An oral endothelin-A receptor antagonist blocks collagen synthesis and deposition in advanced rat liver fibrosis, Gastroenterology, 118, 1169-1178, 2000.

Corpechot C, Barbu V, Wendum D, et al., Hypoxia-induced VEGF and collagen I expressions are associated with angiogenesis and fibrogenesis in experimental cirrhosis, Hepatology, 35(5), 1010-1021, 2002.

Czaja AJ, Carpenter HA, Progressive fibrosis during corticosteroid therapy of autoimmune hepatitis, Hepatology, 39(6), 1631-1638, 2004.

Czaja MJ, Geerts A, Xu J, et al., Monocyte chemoattractant protein 1 (MCP-1) expression occurs in toxic rat liver injury and human liver disease, J Leukoc Biol, 55, 120-126, 1994.

Czaja MJ, JNK/AP-1 regulation of hepatocyte death, Am J Physiol Gastrointest Liver Physiol, 284, G875-G879, 2003.

de Bleser PJ, Jannes P, van Buul-Offers SC, et al., Insulinlike growth factor-II/mannose 6-phosphate receptor is expressed on CCl4-exposed rat fat-storing cells and facilitates activation of latent transforming growth factor-beta in cocultures with sinusoidal endothelial cells, Hepatology, 21, 1429-1437, 1995.

Degott C, Zafrani ES, Callard P, Balkau B, Poupon RE, Poupon R, Histopathological study of primary biliary cirrhosis and the effect of ursodeoxycholic acid treatment on histology progression, Hepatology, 29(4), 1007-1012, 1999.

Dixon JB, Bhathal PS, Hughes NR, O'Brien PE, Nonalcoholic fatty liver disease: Improvement in liver histological analysis with weight loss, Hepatology, 39(6), 1647-1654, 2004.

Dooley S, Streckert M, Delvoux B, Gressner AM, Expression of Smads during in vitro transdifferentiation of hepatic stellate cells to myofibroblasts, Biochem Biophys Res Commun, 283, 554-562, 2001.

Elsharkawy AM, Mann DA, Nuclear factor-kappaB and the hepatic inflammation-fibrosis-cancer axis, Hepatology, 46, 590-597, 2007.

Elsharkawy AM, Oakley F, Mann DA, The role and regulation of hepatic stellate cell apoptosis in reversal of liver fibrosis, Apoptosis, 10, 927-939, 2005.

Enzan H, Himeno H, Iwamura S, Onishi S, Saibara T, Yamamoto Y, Hara H, c~-smooth muscle actin-positive perisinusoidal stromal cells in human hepatocellular carcinoma, Hepatology, 19, 895-903, 1994.

Fallowfield JA, Iredale JP, Targeted treatments for cirrhosis, Expert Opin Ther Targets, 8(5), 423-435, 2004.

Fiorucci S, Antonelli E, Morelli A, Nitric oxide and portal hypertension: a nitric oxide-releasing derivative of ursodeoxycholic acid that selectively releases nitric oxide in the liver, Dig Liver Dis, 35 Suppl, 61-69, 2003.

Fiorucci S, Antonelli E, Rizzo G, et al., The nuclear receptor SHP mediates inhibition of hepatic stellate cells by FXR and protects against liver fibrosis, Gastroenterology, 127(5), 1497-1512, 2004.

Fiorucci S, Rizzo G, Antonelli E, et al., Crosstalk between farnesoid X-receptor (FXR) and peroxisome proliferator-activated receptor (PPAR){gamma} contributes to the anti-fibrotic activity of FXR ligands in rodent models of liver cirrhosis, J Pharmacol Exp Ther, 315, 58-68, 2005.

Fiorucci S, Rizzo G, Antonelli E, et al., A FXR-SHP regulatory cascade modulates TIMP-1 and MMPs expression in HSCs and promotes resolution of liver fibrosis, J Pharmacol Exp Ther, 2005.

Friedman SL, Liver fibrosis - from bench to bedside, J Hepatol, 38 Suppl 1, S38-S53, 2003.

Friedman SL, Mechanisms of hepatic fibrosis and therapeutic implications, Nat Clin Pract Gastroenterol Hepatol, 1, 98-105, 2004.

Galli A, Crabb D, Price D, et al., Peroxisome proliferator-activated receptor gamma transcriptional regulation is involved in platelet-derived growth factor-induced proliferation of human hepatic stellate cells, Hepatology, 31, 101-108, 2000.

Galli A, Svegliati-Baroni G, Ceni E, et al., Oxidative stress stimulates proliferation and invasiveness of hepatic stellate cells via a MMP2-mediated mechanism, Hepatology, 41(5), 1074-1084, 2005.

Garcia-Banuelos J, Siller-Lopez F, Miranda A, Aquilar LK, Auilar-Cordova E, Armendariz-Borunda J, Cirrhotic rat livers with extensive fibrosis can be safely transduced with clinical-grade adenoviral vectors. Evidence of cirrhosis reversion, Gene Ther, 9, 127-134, 2002.

Gondo K, Ueno T, Sakamoto M, Sakisaka S, Sata M, Tanikawa K, The endothelin-1 binding site in rat liver tissue: light-and electron-microscopic autoradiographic studies, Gastroenterology, 104, 1745-1749, 1993.

Gressner AM, Lotfi S, Gressner G, Haltner E, Kropf J, Synergism between hepatocytes and Kupffer cells in the activation of fat storing cells (perisinusoidal lipocytes), J Hepatol, 19, 117-132, 1993.

Gressner AM, Cytokines and cellular crosstalk involved in the activation of fat-storing cells, J Hepatol, 22, 28-36, 1995.

Handy JA, Saxena NK, Fu P, Lin S, Mells JE, Gupta NA, Anania FA, Adiponectin activation of AMPK disrupts leptin-mediated hepatic fibrosis via suppressors of cytokine signaling (SOCS-3), J. Cell. Biochem, 110, 1195-1207, 2010.

Hazra S, Xiong S, Wang J, et al., Peroxisome proliferatoractivated receptor gamma induces a phenotypic switch from activated to quiescent hepatic stellate cells, J Biol Chem, 279, 11392-11401, 2004.

Hellemans K, Michalik L, Dittie A, et al., Peroxisome proliferator-activated receptor-beta signaling contributes to enhanced proliferation of hepatic stellate cells, Gastroenterology, 124, 184-201, 2003.

Hernández E, Bucio L, Souza V, Escobar MC, Gómez-Quiroz LE, Farfán B, et al., Pentoxifylline downregulates alpha (I) collagen expression by the inhibition of I kappa b alpha degradation in liver stellate cells, Cell Biol Toxicol, 20, 2007.

Hironaka K, Sakaida I, Matsumura Y, Kaino S, Miyamoto K, Okita K, Enhanced interstitial collagenase (matrix metalloproteinase-13) production of Kupffer cell by gadolinium chloride prevents pig serum-induced rat liver fibrosis, Biochem Biophys Res Commun, 267(1), 290-295, 2000.

Hudnall SD, Cyclosporin A renders target cells resistant to immune cytolysis, Eur J Immunol, 21 (1), 221-226, 1991.

Ikeda K, Wang LH, Torres R, Zhao H, Olaso E, Eng FJ, et al., Discoidin domain receptor 2 interacts with Src and Shc following its activation by type I collagen, J Biol Chem, 277, 19206-19212, 2002.

Ikejima K, Takei Y, Honda H, Hirose M, Yoshikawa M, Zhang YJ, Lang T, Fukuda T, Yamashina S, Kitamura T, Sato N, Leptin receptor-mediated signaling regulates hepatic fibrogenesis and remodeling of extracellular matrix in the rat, Gastroenterology, 122, 1399-1410, 2002.

Inagaki Y, Higashi K, Kushida M, et al., Hepatocyte growth factor suppresses profibrogenic signal transduction via nuclear export of Smad3 with galectin-7, Gastroenterology, 134(4), 1180-1190, 2008.

Inagaki Y, Nemoto T, Kushida M, et al., Interferon alfa down-regulates collagen gene transcription and suppresses experimental hepatic fibrosis in mice, Hepatology, 38(4), 890-899, 2003.

Iredale JP, Benyon RC, Pickering J, McCullen M, Northrop M, Pawley S, et al., Mechanisms of spontaneous resolution of rat liver fibrosis. Hepatic stellate cell apoptosis and reduced hepatic expression of metalloproteinase inhibitors, J Clin Invest, 102, 538-549, 1998.

Iredale JP, Hepatic stellate cell behavior during resolution of liver injury, Semin Liver Dis, 21 (3), 427-436, 2001.

Jeong WI, Do SH, Yun HS, et al., Hypoxia potentiates transforming growth factor-beta expression of hepatocyte during the cirrhotic condition in rat liver, Liver Int, 24(6), 658-668, 2004.

Jeong WI, Park O, Gao B, Abrogation of the antifibrotic effects of natural killer cells/interferongamma contributes to alcohol acceleration of liver fibrosis, Gastroenterology, 134(1), 248-258, 2008.

Kawada N, Seki S, Inoue M, Kuroki T, Effect of antioxidants, resveratrol, quercetin, and N-acetylcysteine, on the functions of cultured rat hepatic stellate cells and Kupffer cells, Hepatology, 27, 1265-1274, 1998.

Kim WH, Matsumoto K, Bessho K, Nakamura T, Growth inhibition and apoptosis in liver myofibroblasts promoted by hepatocyte growth factor leads to resolution from liver cirrhosis, Am J Pathol, 166(4), 1017-1028, 2005.

Krizhanovsky V, Yon M, Dickins RA, et al., Senescence of activated stellate cells limits liver fibrosis, Cell, 134(4), 657-667, 2008.

Kurikawa N, Suga M, Kuroda S, Yamada K, Ishikawa H, An angiotensin II type 1 receptor antagonist, olmesartan medoxomil, improves experimental liver fibrosis by suppression of proliferation and collagen synthesis in activated hepatic stellate cells, Br J Pharmacol, 139(6), 1085-1094, 2003.

Laye S, Gheusi G, Cremona S, Combe C, Kelley K, Dantzer R, Parnet P, Endogenous brain IL-1 mediates LPS induced anorexia and hypothalamic cytokine expression, *Am J Physiol Regulatory Integrative Comp Physiol,* 279, R93-R98, 2000.

Lin S, Saxena NK, Ding X, Stein LL, Anania FA, Leptin increases tissue inhibitor of metalloproteinase I (TIMP-1) gene expression by a specificity protein 1/signal transducer and activator of transcription 3 mechanism, Mol. Endo (Baltimore) Md. **20**, 3376-3388, 2006.

Lindquist JN, Parsons CJ, Stefanovic B, Brenner DA, Regulation of alpha 1(I) collagen messenger RNA decay by interactions with alphaCP at the 3′-untranslated region, J Biol Chem, 279, 23822-23829, 2004.

Liu C, Tao Q, Sun M, et al., Kupffer cells are associated with apoptosis, inflammation and fibrotic effects in hepatic fibrosis in rats, Lab Invest, 90, 1805-1816, 2010.Liu X, Hu H, Yin JQ, Therapeutic strategies against TGFbeta signaling pathway in hepatic fibrosis, Liver Int, 26, 8-22, 2006.

Liu Y, Wen XM, Lui EL, Friedman SL, Cui W, Ho NP, Li L, Ye T, Fan ST, Zhang H, Therapeutic targeting of the PDGF and TGF-beta-signaling pathways in hepatic stellate cells by PTK787/ ZK22258, Lab Invest, 89, 1152-1160, 2009.

Maher JJ, Lozier JS, Scott MK, Rat hepatic stellate cells produce cytokine-induced neutrophil chemoattractant in culture and in vivo, Am J Physiol, 275, G847G853, 1998.

Maher JJ, McGuire RF, Extracellular matrix gene expression increases preferentially in rat lipocytes and sinusoidal endothelial cells during hepatic fibrosis in vivo, J Clin Invest, 86, 1641-1648, 1990.

Mallat A, Fouassier L, Préaux AM, Mavier P, Lotersztajn S, Antiproliferative effects of ET-1 in human liver Ito cells: an ETBand a cyclic AMP-mediated pathway, J Cardiovasc Pharmacol, 26(Suppl 3), S132-S134, 1995.

Marra F, DeFranco R, Grappone C, Milani S, Pastacaldi S, Pinzani M, et al., Increased expression of monocyte chemotactic protein-1 during active hepatic fibrogenesis: correlation with monocyte infiltration, Am J Pathol, 152, 423-430, 1998.

Marra F, Efsen E, Romanelli RG, et al., Ligands of peroxisome proliferator-activated receptor gamma modulate profibrogenic and proinflammatory actions in hepatic stellate cells, Gastroenterology, 119, 466-478, 2000.

Marra F, Valente AJ, Pinzani M, Abboud HE, Cultured human liver fat-storing cells produce monocyte chemotactic protein-1. Regulation by proinflammatory cytokines, J Clin Invest, 92, 1674-1680, 1993.

Masuda H, Fukumoto M, Hirayoshi K, Nagata K, Coexpression of the collagen-binding stress protein HSP47 gene and the alpha 1(I) and alpha 1(III) collagen genes in carbon tetrachloride induced rat liver fibrosis, J Clin Invest, 94(6), 2481-2488, 1994.

Matsuoka M, Tsukamoto H, Stimulation of hepatic lipocyte collagen production by Kupffer cell-derived transforming growth factor beta: implication for a pathogenetic role in alcoholic liver fibrogenesis, Hepatology, 11, 599-605, 1990.

Matsuzaki K, Date M, Furukawa F, Tahashi Y, Matsushita M, Sakitani K, Yamashiki N, Seki T, Saito H, Nishizawa M, Fujisawa J, Inoue K, Autocrine stimulatory mechanism by transforming growth factor beta in human hepatocellular carcinoma, Cancer Res, 60, 1394-1402, 2000.

Mehal WZ, Friedman SL, The role of inflammation and immunity in the pathogenesis of liver fibrosis. In: Gershwin ME, Veirling JM, Manns MP, editors, Liver Immunology, Totowa, New Jersey: Humana Press, 99-109, 2007.

Melhem A, Muhanna N, Bishara A, et al., Anti-fibrotic activity of NK cells in experimental liver injury through killing of activated HSC, J Hepatol, 45(1), 60-71, 2006.

Mikula M, Proell V, Fischer AN, Mikulits W, Activated hepatic stellate cells induce tumor progression of neoplastic hepatocytes in a TGF- dependent fashion, J Cell Physiol, 209, 560-567, 2006.

Milani S, Herbst H, Schuppan D, et al., Cellular localization of laminin gene transcripts in normal and fibrotic human liver, Am J Pathol, 134, 11751182, 1989.

Milani S, Herbst H, Schuppan D, et al., In situ hybridization for procollagen types I, III and IV mRNA in normal and fibrotic rat liver: evidence for predominant expression in nonparenchymal liver cells, Hepatology, 10, 84-92, 1989.

Milani S, Herbst H, Schuppan D, et al., Differential expression of matrix-metalloproteinase-1 and -2 genes in normal and fibrotic human liver, Am J Pathol, 144(3), 528537, 1994.

Milliano MT, Luxon BA, Rat hepatic stellate cells become retinoid unresponsive during activation, Hepatol Res, 33, 225-33, 2005.

Miyahara T, Schrum L, Rippe R, Xiong S, Yee HF Jr, Motomura K, et al., Peroxisome proliferator-activated receptors and hepatic stellate cell activation, J Biol Chem, 275, 35715-35722, 2000.

Murphy FR, Issa R, Zhou X, et al., Inhibition of apoptosis of activated hepatic stellate cells by tissue inhibitor of metalloproteinase-1 is mediated via effects on matrix metalloproteinase inhibition: implications for reversibility of liver fibrosis, J Biol Chem, 277(13), 11069-11076, 2002.

Nagata K, Expression and function of heat shock protein 47: a collagen-specific molecular chaperone in the endoplasmic reticulum, Matrix Biol, 16(7), 379-386, 1998.

Nieto N, Friedman SL, Cederbaum AI, Stimulation and proliferation of primary rat hepatic stellate cells by cytochrome P450 2E1- derived reactive oxygen species, Hepatology, 35, 62-73, 2002.

Niki T, Rombouts K, De Bleser P, et al., A histone deacetylase inhibitor, trichostatin A, suppresses myofibroblastic differentiation of rat hepatic stellate cells in primary culture, Hepatology, 29, 858-867, 1999.

Niu L, Wang X, Li J, Huang Y, Yang Z, Chen F, et al., Leptin stimulates alpha 1(I) collagen expression in human hepatic stellate cells via the phosphatidylinositol 3-kinase/Akt signalling pathway, Liver Int, 27, 1265-1272, 2007.

Oakley F, Meso M, Iredale JP, Green K, Marek CJ, Zhou X, et al., Inhibition of IκB kinases stimulates hepatic stellate cell apoptosis and accelerates recovery from liver fibrosis, Gastroenterology, 128, 108-120, 2005.

Okuno M, Akita K, Moriwaki H, Kawada N, Ikeda K, Kaneda K, et al., Prevention of rat hepatic fibrosis by the protease inhibitor, camostat mesilate, via reduced generation of active TGFbeta, Gastroenterology, 120, 1784-1800, 2001.

Olaso E, Friedman SL, Molecular mechanisms of hepatic fibrogenesis, J Hepatol, 29, 836-847, 1998;

Ozaki I, Zhao G, Mizuta T, et al., Hepatocyte growth factor induces collagenase (matrix metalloproteinase-1) via the transcription factor Ets-1 in human hepatic stellate cell line, J Hepatol, 36(2), 169-178, 2002.

Paik YH, Schwabe RF, Bataller R, Russo MP, Jobin C, Brenner DA, Toll-like receptor 4 mediates inflammatory signaling by bacterial lipopolysaccharide in human hepatic stellate cells, Hepatology, 37, 1043-1055, 2003.

Parsons CJ, Bradford BU, Pan CQ, Cheung E, Schauer M, Knorr A, et al., Antifibrotic effects of a tissue inhibitor of metalloproteinase-1 antibody on established liver fibrosis in rats, Hepatology, 40, 1106-1115, 2004.

Pietrangelo A, Borella F, Casalgrandi G, et al., Antioxidant activity of silybin in vivo during long-term iron overload in rats, Gastroenterology, 109, 1941-1949, 1995.

Pinzani M, Abboud HE, Gesualdo L, et al., Regulation of macrophage colony-stimulating factor in liver fat-storing cells by peptide growth factors, Am J Physiol, 262, C876-C881, 1992.

Pinzani M, Marra F, Cytokine receptors and signaling during stellate cell activation, Semin Liver Dis, 21, 397-416, 2001.

Poynard T, McHutchison J, Manns M, et al., Impact of pegylated interferon alfa-2b and ribavirin on liver fibrosis in patients with chronic hepatitis C, Gastroenterology, 122(5), 1303-1313, 2002.

Weiskirchen R, Abriss B, Arias M, Kneifel J, Van De Leur E, Weiskirchen S, Gressner AM, Experimental approaches to antifibrotic strategies using gene transfer. Falk Symposium - Progress in Gastroenterology and Hepatology - Cytokines in liver injury and repair, 30 sept-1 oct. Hannover, Germany, 60, 2001.

Weiskirchen R, Kneifel J, Weiskirchen S, Van De Leur E, Kunz D, Gressner AM, Comparative evaluation of gene delivery devices in primary cultures of rat hepatic stellate cells and rat myofibroblasts, BMC Cell Biology, 1, 4, 2000.

Weiskirchen R, Moser M, Weiskirchen S, Erdel M, Dahmen S, Buettner R, Gressner AM, LIM-domain protein cysteine- and glycine-rich protein 2 (CRP2) is a novel marker of hepatic stellate cells and binding partner of the protein inhibitor of activated STAT1, Biochem J, 359, 485-496, 2001.

Radaeva S, Sun R, Jaruga B, Nguyen VT, Tian Z, Gao B, Natural killer cells ameliorate liver fibrosis by killing activated stellate cells in NKG2D-dependent and tumor necrosis factor-related apoptosis inducing ligand-dependent manners, Gastroenterology, 130(2), 435-452, 2006.

Radu CI, Crişan DA, Grigorescu M, How can we predict steatosis using biochemical markers (SteatoTest and Fatty Liver Index)? Human & Veterinary Medicine 3(2):89-96, 2011.

Rader DJ, Liver X receptor and farnesoid X receptor as therapeutic targets, Am J Cardiol, 100 (11 A), n15n19, 2007.

Raetsch C, Jia JD, Boigk G, et al., Pentoxifylline downregulates profibrogenic cytokines and procollagen I expression in rat secondary biliary fibrosis, Gut, 50(2), 241-247, 2002.

Reif S, Lang A, Lindquist JN, Yata Y, Gabele E, Scanga A, et al., The role of FAK-PI3-K-Akt signaling in hepatic stellate cell proliferation and type I collagen expression, J Biol Chem, 278, 8083-8090, 2003.

Rippe RA, Almounajed G, Brenner DA, Sp1 binding activity increases in activated Ito cells, Hepatology, 22, 241-251, 1995.

Rippe RA, Schrum LW, Stefanovic B, Solis-Herruzo JA, Brenner DA, NF-κB inhibits expression of the α1(I) collagen gene, DNA Cell Biol, 18, 751-761, 1999.

Roberts S, Gordon A, McLean C, et al., Effect of sustained viral response on hepatic venous pressure gradient in hepatitis C-related cirrhosis, Clin Gastroenterol Hepatol, 5(8), 932-937, 2007.

Rockey D, The cellular pathogenesis of portal hypertension: stellate cell contractility, endothelin, and nitric oxide, Hepatology, 25, 2-5, 1997.

Rockey DC, Chung JJ, Interferon gamma inhibits lipocyte activation and extracellular matrix mRNA expression during experimental liver injury: implications for treatment of hepatic fibrosis, J Investig Med, 42, 660-670, 1994.

Ruddell RG, Oakley F, Hussain Z, et al., A role for serotonin (5-HT) in hepatic stellate cell function and liver fibrosis, Am J Pathol, 169(3), 861-876, 2006.

Rudolph KL, Chang S, Millard M, Schreiber-Agus N, DePinho RA, Inhibition of experimental liver cirrhosis in mice by telomerase gene delivery, Science, 287, 1253-1258, 2000.

Sanz S, Pucilowska JB, Liu S, et al., Expression of insulin-like growth factor I by activated hepatic stellate cells reduces fibrogenesis and enhances regeneration after liver injury, Gut, 54(1), 134-141, 2005.

Sato Y, Murase K, Kato J, et al., Resolution of liver cirrhosis using vitamin A-coupled liposomes to deliver siRNA against a collagen-specific chaperone, Nat Biotechnol, 26(4), 431-442, 2008.

Saxena NK, Saliba G, Floyd JJ, Anania FA, Leptin induces increased alpha2(I) collagen gene expression in cultured rat hepatic stellate cells, J. Cell. Biochem, 89, 311-320, 2003.

Saxena NK, Titus MA, Ding X, Floyd J, Srinivasan S, Sitaraman SV, Anania FA, Leptin as a novel profibrogenic cytokine in hepatic stellate cells: mitogenesis and inhibition of apoptosis mediated by extracellular regulated kinase (Erk) and Akt phosphorylation, Faseb J, 18, 1612-1614, 2004.

Schmitt-Griff A, Krtiger S, Bochard F, Gabbiani G, Denk H, Modulation of alpha smooth muscle actin and desmin expression in perisinusoidal cells of normal and diseased human livers, Am J Pathol, 138, 1233-1242, 1991.

Schnabl B, Bradham CA, Bennett BL, Manning AM, Stefanovic B, Brenner DA, TAK1/JNK and p38 have opposite effects on rat hepatic stellate cells, Hepatology, 34, 953-963, 2001.

Shek FW, Benyon RC, How can transforming growth factor beta be targeted usefully to combat liver fibrosis, Eur J Gastroenterol Hepatol, 16, 123-126, 2004.

Shiratori Y, Imazeki F, Moriyama M, et al., Histologic improvement of fibrosis in patients with hepatitis C who have sustained response to interferon therapy, Ann Intern Med, 132(7), 517-524, 2000.

Shi-Wen X, Chen Y, Denton CP, Eastwood M, Renzoni EA, Bou-Gharios G, et al., Endothelin-1 promotes myofibroblast induction through the ETA receptor via a rac/phosphoinositide 3-kinase/Akt-dependent pathway and is essential for the enhanced contractile phenotype of fibrotic fibroblasts, Mol Biol Cell, 15, 2707-2719, 2004.

Smart DE, Vincent KJ, Arthur MJ, Eickelberg O, Castellazzi M, Mann J, et al., JunD regulates transcription of the tissue inhibitor of metalloproteinases-1 and interleukin-6 genes in activated hepatic stellate cells, J Biol Chem, 276, 24414-24421, 2001.

Son G, Hines IN, Lindquist J, Schrum LW, Rippe RA, Inhibition of phosphatidylinositol 3-kinase signaling in hepatic stellate cells blocks the progression of hepatic fibrosis, Hepatology, 50, 1512-1523, 2009.

Szabo G, Dolganiuc A, Mandrekar P, Pattern recognition receptors: a contemporary view on liver diseases, Hepatology, 44, 287-298, 2006.

Tada S, Iwamoto H, Nakamuta M, et al., A selective ROCK inhibitor, Y27632, prevents dimethylnitrosamine-induced hepatic fibrosis in rats, J Hepatol, 34(4), 529-536, 2001.

Théret N, Musso O, Turlin B, Lotrian D, Bioulac-Sage P, Campion JP, Boudjéma K, Clément B, Increased extracellular matrix remodeling is associated with tumor progression in human hepatocellular carcinomas, Hepatology, 34, 82-88, 2001.

Tiggelman AM, Boers W, Linthorst C, et al., Interleukin-6 production by human liver (myo)fibroblasts in culture. Evidence for a regulatory role of LPS, IL-1 beta and TNF alpha, J Hepatol, 23, 295-306, 1995.

Torimura T, Sata M, Ueno T, Kin M, Tsuji R, Suzaku K, Hashimoto O, Sugawara H, Tanikawa K, Increased expression of vascular endothelial growth factor is associated with tumor progression in hepatocellular carcinoma, Hum Pathol, 29, 986-991, 1998.

Trim JE, Samra SK, Arthur MJ, Wright MC, McAulay M, Beri R, et al., Upstream tissue inhibitor of metalloproteinases-1 (TIMP-1) element-1, a novel and essential regulatory DNA motif in the human TIMP-1 gene promoter, directly interacts with a 30-kDa nuclear protein, J Biol Chem, 275, 6657-6663, 2000.

Trotter JF, Sirolimus in liver transplantation, Transplant Proc, 35(3 Suppl), 193S-200S, 2003.

Tsukada S, Westwick JK, Ikejima K, Sato N, Rippe RA, SMAD and p38 MAPK signaling pathways independently regulate alpha 1(I) collagen gene expression in unstimulated and transforming growth factor-beta-stimulated hepatic stellate cells, J Biol Chem, 280, 10055-10064, 2005.

Uchinami H, Seki E, Brenner DA, D'Armiento J, Loss of MMP 13 attenuates murine hepatic injury and fibrosis during cholestasis, Hepatology, 44, 420-429, 2006.

Winwood PJ, Schuppan D, Iredale JP, Kawser CA, Docherty AJ, Arthur MJ, Kupffer cell-derived 95 kd type IV collagenase/ gelatinase B: characterization and expression in cultured cells, Hepatology, 22, 304-315, 1995.

Wirz W, Antoine M, Tag CG, Gressner AM, Korff T, Hellerbrand C, Kiefer P, Hepatic stellate cells display a functional vascular smooth muscle cell phenotype in a three-dimensional co-culture model with endothelial cells, Differentiation, 76, 784-794, 2008.

Wong L, Yamasaki G, Johnson RJ, Friedman SL, Induction of beta-platelet-derived growth factor receptor in rat hepatic lipocytes during cellular activation in vivo and in culture, J Clin Invest, 94 (4), 1563-1569, 1994.

Chen X, Sebastian B, Nagy L, Chronic ethanol feeding to rats decreases adiponectin secretion by subcutaneous adipocytes, J Physiol Endocrinol Metab, 292, E621-E628, 2007.

Yokohama S, Yoneda M, Haneda M, Okamoto S, Okada M, Aso K, et al., Therapeutic efficacy of an angiotensin II receptor antagonist in patients with nonalcoholic steatohepatitis, Hepatology, 40, 1222-1225, 2004.

Yoshiji H, Noguchi R, Kuriyama S, Ikenaka Y, Yoshii J, Yanase K, et al., Imatinib mesylate (STI-571) attenuates liver fibrosis development in rats, Am J Physiol Gastrointest Liver Physiol, 288, G907-G913, 2005.

Zhan SS, Jiang JX, Wu J, et al., Phagocytosis of apoptotic bodies by hepatic stellate cells induces NADPH oxidase and is associated with liver fibrosis in vivo, Hepatology, 43(3), 435-443, 2006.

Zhao W, Zhang L, Yin Z, Su W, Ren G, Zhou C, You J, Fan J, Wang X, Activated hepatic stellate cells promote hepatocellular carcinoma development in immunocompetent mice, Int J Cancer, 6, 2011.

Zhu J, Wu J, Frizell E, et al., Rapamycin inhibits hepatic stellate cell proliferation in vitro and limits fibrogenesis in an in vivo model of liver fibrosis, Gastroenterology, 117(5), 1198-1204, 1999.