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JAK-STAT signaling in hepatic fibrosis

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TABLE OF CONTENTS

1. ABSTRACT

Chronic liver injury, liver fibrosis and formation of hepatocellular carcinoma are intimately linked and represent a major medical challenge since treatment options are limited. Therefore, it is important to identify cellular and molecular pathways that promote liver damage or provide hepatoprotection for development of therapeutic approaches. Recently, the transcription factors STAT3 and STAT5 have been implicated in liver fibrosis induced by cholestatic liver damage. In this review, we summarize our current knowledge about STAT proteins in liver fibrosis and focus on common activities that underlie the hepatoprotective mechanisms regulated by IL-6/gp130/STAT3 and GH/STAT5/IGF-1 signaling pathways.

2. INTRODUCTION

2.1. Liver fibrosis and cirrhosis in humans

Chronic liver diseases and its complications constitute one of the major causes of mortality worldwide and are the 12th leading cause of death in the United States (1). Depending on the severity of liver damage, liver architecture can be severely affected resulting in cirrhosis which is characterized by the presence of nodular regeneration and tissue scarring. The underlying etiology of cirrhosis includes chronic liver damage induced by hepatitis B and C virus infections, cholestasis, alcohol abuse, drugs, metabolic, autoimmune and congenital diseases (2). The increasing prevalence of cirrhosis caused by chronic hepatitis C virus and fatty liver disease renders it one of the major medical challenges in the 21st century

(3-7). To date, the only successful therapeutic approach to fibrosis consists in treatment of the underlying conditions. When the underlying disease cannot be treated, therapeutic options are limited to the symptomatic treatment of portal hypertension and liver transplantation for patients with decompensated liver cirrhosis.

2.2. Chronic injury – fibrosis – cirrhosis - HCC axis

Necrosis and inflammation of the liver due to chronic liver damage are important driving forces in the multistep process of hepatocarcinogenesis. Epidemiological studies have shown that ~5% of patients with compensated liver cirrhosis develop liver cancer each year. Moreover, liver cancer accounts for a 50-70% mortality rate among patients with compensated cirrhosis who died due to a liver related cause of death (5, 8-10). Liver cancer summarizes diverse, histologically distinct primary hepatic neoplasms including hepatocellular carcinoma (HCC), intrahepatic bile duct carcinoma (cholangiocarcinoma), hepatoblastoma, bile duct cystadenocarcinoma, haemangiosarcoma and epitheliod haemangioendothelioma. HCC accounts for 85-90% of primary liver cancers in developing countries and is the fifth most common cancer worldwide (11). The 5-year survival rate of patients with HCC is less than 10% despite aggressive conventional therapy, making this malignancy to the second most lethal cancer after pancreatic ductal adenocarcinoma. Surgical resection and liver transplantation represent the only potentially curative treatment approach for liver cancer at present but they can only be offered to a small subgroup of patients with early stages of disease. Moreover, recurrence of HCC following surgery is common and the 5-year survival rate is only 30-40% (12, 13).

2.3. Cellular basis and molecular pathways in liver fibrosis

The liver is a complex organ consisting of parenchymal cells (hepatocytes) and non-parenchymal cells including bile duct epithelial cells (BEC), hepatic stellate cells (HSC), liver sinusoidal endothelial cells, resident macrophages (Kupffer cells) and other immune cells (B-, T- and natural killer (NK) cells). Remarkably, most of these cells can directly or indirectly contribute to liver fibrosis (2, 14). However, activation of HSC represents the "canonical principle" of liver fibrosis since this cell type is the major producer of extracellular matrix (15). Moreover, individual cell types can also exert protective or fibrosis-promoting activities depending on the stage and severity of hepatic fibrosis which has been demonstrated for CD11b-positive cells (16).

There has been substantial progress delineating the molecular mechanisms underlying liver fibrosis in the last decade which have been described in excellent reviews (14, 17, 18). Recently, the renin-angiotensin system has emerged as a key system in tissue remodeling of the liver and represents a promising target for therapeutic intervention (19). Inflammation and activation of the immune system are also critical steps in liver fibrosis. In this respect, it has been shown that gut-derived lipopolysaccharide (LPS) is crucial for the production of interleukin 6 (IL-6) by Kupffer cells thereby linking liver regeneration, fibrosis and cancer development (20, 21). Moreover, recruitment of inflammatory cells from the bone marrow to the injured liver that is mediated by chemokines has been demonstrated to be another critical step for fibrosis progression (22). Additional factors that have emerged as important players in the pathogenesis of liver fibrosis are represented by adipokines (23), in particular leptin that signals through the gp130 co-receptor and activates the JAK-STAT pathway (24).

2.4. Genetic and chemical animal models for liver fibrosis

Several animal models have been employed to identify molecular mechanisms underlying liver fibrosis (25). Commonly used models reflect toxic liver injury and are based on repeated application of hepatotoxic substances such as thioacetamide (TAA), carbon tetrachloride (CCl_4) or dimethylnitrosamine (DMN). Alternatively, surgical ligation of the common bile duct (BDL) or cholic acid (CA) feeding resulting in bile acid overload is used to mimic cholestatic liver injury. In recent years, genetic models for liver fibrosis have been established to identify important effector pathways in hepatic fibrosis such as transforming growth factor beta (TGF-beta) (26-29), platelet-derived growth factor B (PDGF-B). (30), PDGF-C (31) and tissue inhibitor of metalloproteinase 1 (TIMP-1) (32). However, most of these mouse models show only mild fibrosis or lack liver injury and inflammation suggesting that activation of the fibrotic effector pathways is not sufficient to mimic severe hepatic fibrosis observed in human chronic liver diseases. Mice with targeted deletion of the multidrug resistance 2 (Mdr2) gene represent a genetic model for sclerosing cholangitis and cholestasis-induced hepatic fibrosis. The Mdr2 gene encodes a P-type membrane bound glycoprotein that transports phospholipids into the bile. Phospholipids reduce the toxicity of free bile acids because they form mixed micelles with them. Accordingly, genetic disruption of Mdr2 in mice causes accumulation of toxic bile salts in the intrahepatic biliary system associated with inflammatory cholangitis. This is followed by periductal fibrogenesis with obliteration of bile ducts. Although chronic liver injury is rather mild in Mdr2-deficient mice, they develop HCC and represent a genetic model for inflammatory liver cancer (33-35). Recently, Mdr2-deficient mice have been used to identify liver tumor-promoting functions of nuclear factor kappa B (NF-kappaB) (36).

3. STATS IN LIVER REGENERATION, CANCER AND PHYSIOLOGY

Liver cells persistently encounter extracellular signals that subsequently lead to the activation of various signal transduction pathways. The JAK-STAT pathway is activated in response to cytokines, growth factors and hormones, mediating a plethora of cellular functions including defense against pathogens, differentiation, proliferation, apoptosis, metabolism and cellular transformation (37, 38). Several components of the JAK-STAT pathway and its negative regulators have been under intense investigation over the last decades (39). Upon ligand binding to cognate receptors, the JAK-STAT

pathway is activated by phosphorylation of the Janus protein tyrosine kinases (JAK1, 2, 3, TYK2), followed by phosphorylation and activation of STAT1, 2, 3, 4, 5a, 5b and 6. Subsequently, signal transducers and activators of transcription (STATs) form dimers and translocate to the nucleus where they modulate expression of target genes (38). Animal models have tremendously contributed to our understanding of JAK-STAT components that regulate liver regeneration and physiologic functions. In addition, JAK-STAT signaling is activated in a variety of human tumors including HCC (40-42).

3.1. STAT1

STAT1 signaling is mainly activated by Interferon-alpha/beta (IFN-alpha/beta) and Interferon-gamma (IFN-gamma) and has crucial functions in immune responses (43, 44). It is well established that STAT1-deficient mice display increased sensitivity towards viral and microbial pathogens due to their inability to respond to Interferons. Transgenic mice overexpressing IFN-gamma developed chronic hepatitis (45), whereas disruption of IFN-gamma, the IFN-gamma receptor or STAT1 genes abolished liver damage and necrosis in Concanavalin A– (Con A) and LPS/Galactosamine-induced liver injury. Consistently, Hep3B cells overexpressing STAT1 were more susceptible to IFN-gamma-induced cell death. However, CCl_4 treatment did not cause significant STAT1 activation in the liver (46-48).

Several experiments have been performed to study STAT1 functions in liver regeneration. It was shown that the known suppressive effect of poly I:C treatment (which mimics viral infections) on liver regeneration after partial hepatectomy (PHx) was associated with STAT1 activation and increased expression of interferon regulatory factor 1 (IRF-1) and p21 (49). In a follow up study, these data were evaluated in $STAT1^{-/-}$, $IRF\text{-}1^{-/-}$ and $p21^{-/-}$ mice using combined PHx plus poly I:C treatment (50). Importantly, disruption of STAT1 abolished the negative effect of poly I:C treatment suggesting that STAT1 is a negative regulator of liver regeneration (50).

STAT1 was also suggested to integrate major antitumor activities through Interferon signaling (51). Recently, polymorphisms in the STAT1 gene were associated with increased HCC risk (52).

3.2. STAT2

STAT2 is an essential transcription factor in immune responses through which mainly type I Interferons signal (53) and is activated in primary hepatocytes in response to IFN-alpha treatment (54). Recently, IL-27 was shown to induce STAT1 and STAT2 in an IFN-gamma-like response leading to the induction of Interferon-regulated proteins such as IRF-1, IRF-9, myxovirus resistance A and guanylate binding protein 2 thereby contributing to the antiviral response in hepatocytes and hepatoma cells (55). Studies on STAT2 functions in liver metabolism and regeneration have not yet been reported. Similarly, STAT2 was not yet reported to have a role in liver cancer development but tumor promoting activities have been demonstrated in murine colorectal– and skin carcinogenesis models (56).

3.3. STAT3

The cytokine IL-6 activates STAT3 and the mitogen-activated protein kinase (MAPK) signaling pathway through the gp130–IL-6R complex (57). IL-6/gp130/STAT3 functions have been extensively analyzed in the liver since it was observed that IL-6 stimulates hepatocytes to produce a variety of acute-phase proteins (58) in response to liver injury. It is currently believed that IL-6 is mainly released from Kupffer cells (macrophages of the liver) upon liver damage but hepatocytes have not been excluded as origin since hepatoma cells have been shown to produce this cytokine (59). IL-6-deficient mice are more susceptible to liver injury induced by ethanol (60), Con A (46, 61) acetaminophen (62, 63) and bile acids (64), suggesting that IL-6 has protective functions (65, 66). Indeed, most of these protective functions have been linked to the downstream signaling molecule STAT3 that protects from CCl4 (67), Fas (68) bile acid (64), and ischemic reperfusion–induced liver injury (69). Important target genes that mediate the hepatoprotective activity of STAT3 have been identified during Fas–induced injury. These include Bcl-2, Bcl-xL, Mcl-1, FLIP, Ref-1, cyclin D1 and c-myc (68).

Targeted disruption of IL-6 in mice also resulted in a delay of hepatocyte proliferation after PHx suggesting that IL-6 signaling is important for liver regeneration (70) although controversial data have also been published (71). Additional evidence from liver-specific STAT3 knock-out and gp130 knock-in mice that harbor mutant alleles for selective activation of STAT3 or MAPK strengthened the importance for IL-6/gp130/STAT3 signaling in hepatocyte proliferation after PHx (72, 73).

Recently, it was shown that STAT3 suppresses the expression of genes that control gluconeogenesis by a mechanism involving the fasting–activated longevity protein SIRTUIN 1 (74). Hepatic STAT3 ablation causes insulin resistance associated with increased expression of gluconeogenic genes, whereas overexpression of constitutively activated STAT3 reduces blood glucose, plasma insulin concentrations and hepatic gluconeogenic gene expression in diabetic mice (75).

STAT3 is involved in cancer development of various tissues and organs. It was demonstrated that STAT3 is activated in a wide range of human tumors including HCC. Besides well established noxae that may be causative for HCC development (76), inflammatory conditions have been shown to promote hepatocyte cycling and carcinogenesis, in part through paracrine IL-6 release by macrophages. Accordingly, IL-6-deficiency inhibited DEN-initiated proliferation and HCC development, which was associated with diminished activation of STAT3 in hepatocytes (21). In addition, it was demonstrated that IL-6 and tumor necrosis factor alpha (TNF-alpha) are key factors in liver tumorigenesis in genetically and dietary obese mice (77). Recently, gain of function mutations in gp130 co-receptors that persistently activate STAT3 in HCC have been reported (78), supporting the oncogenic role of STAT3 in liver cancer.

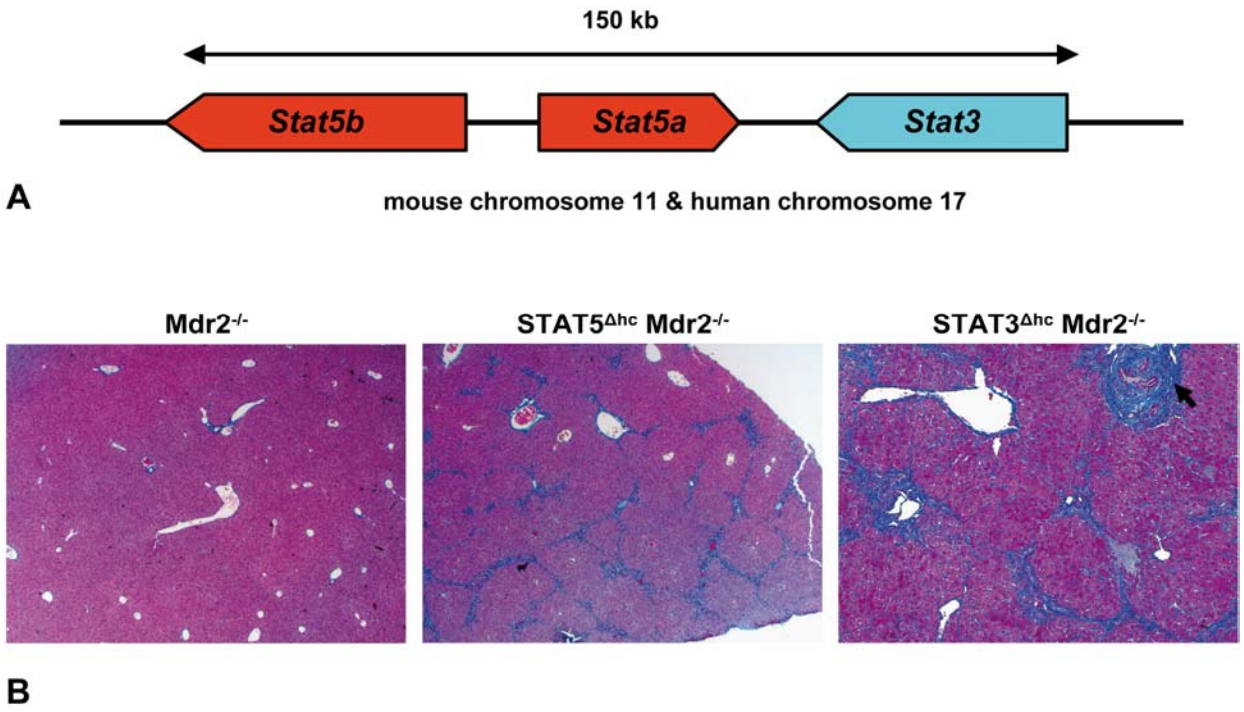

Figure 1. (A) The scheme shows the genomic organization of STAT5a, STAT5b and STAT3 genes. They are located at a common locus on chromosome 11 of mice and chromosome 17 of humans and have the same organization. Therefore, simultaneous conditional deletion of STAT5 and STAT5 would require introduction of loxP sites that are 150kb apart. (B) Bridging fibrosis in $Mdr2^{-/-}$ mice upon loss of STAT5 or STAT3. Conditional inactivation of STAT5 or STAT3 in hepatocytes and cholangiocytes using the AlfpCre transgene strongly aggravates fibrosis in $Mdr2^{-/-}$ mice (a model for sclerosing cholangitis) as demonstrated by chromanilinblue-staining (collagen in blue). Fibrosis starts in the periportal area and progresses to bridging fibrosis thereby insulating liver lobes. The right image represents $STAT3^{\Delta hc}$ $Mdr2^{-/-}$ mice at higher magnification showing a bile duct with onion skin type fibrosis (arrow) which is typical for sclerosing cholangitis (adapted from references (64, 137)).

3.4. STAT4

STAT4 is mainly activated by IL-12 (79, 80) and is expressed exclusively in myeloid cells, thymus, and testes. STAT4 is responsible for the differentiation of T_h0 to T_h1 cells (81). In the liver, STAT4 has been shown to be activated during Con A- and ischemia/reperfusion-induced liver injury (46, 82). Data on STAT4 in liver cancer are currently not available.

3.5. STAT5

The STAT5 proteins STAT5a and STAT5b are encoded by two different genes that are located in close vicinity on the same chromosome together with STAT3 (Figure 1A). STAT5 proteins are activated by cytokines, growth hormones and interleukins such as IL-2, IL-3, IL-5, erythropoietin (EPO), thrombopoietin (TPO) and granulocyte-macrophage colony-stimulating factor (GM-CSF). Growth hormone (GH) and STAT5b display pivotal functions in liver physiology and regeneration. Generation of mice with conditional deletion of STAT5a and STAT5b (83) offered the possibility to study the functions of these proteins in detail in the liver and other organs (84, 85). Using these mice, it was demonstrated that liver regeneration after PHx is impaired in the absence of GH or STAT5 (84). Moreover, loss of STAT5 caused hepatosteatosis, glucose intolerance and insulin resistance which was mechanistically linked to defective GH/STAT5/IGF-1 signaling and increased STAT1 activity (84, 86, 87). STAT5 deficiency had also an impact on the expression of acute phase proteins such as haptoglobulin, hemopexin a-1, antitrypsin, a1-antichymotrypsin and fibrinogen, which was most likely due to compensatory STAT3 activation observed in STAT5-deficient livers (84). It is well established that GH mediates postnatal body growth via STAT5b activation that controls insulin-like growth factor 1 (IGF-1) expression in hepatocytes and muscle cells (88, 89). IGF-1 expression was reduced in STAT5-deficient animals, leading to reduced circulating IGF-1 levels, reduced body growth and a compensatory increase of GH levels (88). The regulatory function of body growth depends on STAT5b but not STAT5a (89, 90). Interestingly, male but not female STAT5b-null mice displayed reduced body growth indicating a sexual dimorphism (89).

STAT5 has been described as key factor in hematopoietic malignancies but was also linked to development of solid tumors. In HCC development, STAT5b activation correlated with more aggressive stages due to the induction of epithelial-mesenchymal transition (EMT) of liver tumor cells (91). Interestingly, liver-specific conditional deletion of STAT5 led to enhanced tumor

Table 1. Mouse models demonstrating STAT functions in liver fibrosis

Strain	Description	Protocol	Phenotype	Ref.
IL-6/gp130/STAT3				
IL-6$^{-/-}$	IL-6 ko	BDL [1]	Liver injury and biliary fibrosis	102
IL-6$^{-/-}$	IL-6 ko	CCl_4 [2] CCl_4/Pb [3]	Liver injury and pericentral fibrosis	174 (174)
IL-6$^{-/-}$ gp130$^{\Delta hepa}$ gp130$^{\Delta hepaRas}$ gp130$^{\Delta hepaSTAT}$	IL-6 ko; conditional gp130 ko in the liver (AlfpCre); knock-in for selective activation of MAPK or STAT3	CDE [4]	Steatosis and beginning signs of liver fibrosis that depend on STAT3	175 (175)
gp130$^{\Delta hepa}$ gp130$^{\Delta hepaRas}$ gp130$^{\Delta hepaSTAT}$	Conditional gp130 ko in the liver (AlfpCre); knock-in for selective activation of MAPK or STAT3	DDC [5]	Cholestatic liver injury and periportal fibrosis that depend on STAT3	104
STAT3$^{\Delta hc}$	Conditional STAT3 ko in the liver (AlfpCre)	Mdr2$^{-/-}$ [6] CA [7]	Liver injury and periportal fibrosis	64
GH/STAT5/IGF-1				
STAT5-LKO	Conditional STAT5 ko in the liver (AlbCre)	CCl_4	Periportal fibrosis and liver tumors	92
STAT5$^{\Delta hep}$	Conditional STAT5 ko in the liver (AlfpCre)	Mdr2$^{-/-}$	Liver injury and periportal fibrosis	137
Others				
STAT1$^{-/-}$	STAT1 ko	CCl_4	Pericentral fibrosis and HSC proliferation	155
IFN-gamma$^{-/-}$	Interferon-gamma ko	DDC	Periportal fibrosis and HSC activation	159
IFN-gamma$^{-/-}$	Interferon-gamma ko	CCl_4	Liver fibrosis	160
SOCS1$^{+/-}$	Heterozygous for SOCS1	DMN [8] CDE	Periportal and pericentral fibrosis	167
SOCS3$^{+/-}$ AdCre-SOCS3$^{fl/fl}$	Heterozygous for SOCS3; Conditional SOCS3 ko in the liver (AdenoCre)	ConA [9] DMN	Liver fibrosis with STAT3 activation in hepatocytes and non-parenchymal cells	168

formation upon CCl_4 treatment suggesting an anti-oncogenic function of STAT5 in liver cancer. It is likely that the observed compensatory upregulation of STAT3 in the liver-specific STAT5-deficient mice promotes hepatocarcinogenesis (92).

3.6. STAT6

STAT6 is mainly activated by IL-4, IL-12 and IL-13 and has important roles in T_h2 lymphocyte differentiation (93, 94). In the liver, STAT6 functions have been studied in various injury models including ischemia/reperfusion-, Con A- and LPS- treatment (95-98). It turned out that IL-4/STAT6 signaling has important functions in Con A–induced T-cell mediated hepatitis. Consistently, IL-4$^{-/-}$ and STAT6$^{-/-}$ mice were resistant to Con A-induced hepatitis (99). Data on STAT6 functions in liver cancer are currently not available.

4. STATS IN HEPATIC FIBROSIS: FOCUS ON IL-6/gp130/STAT3

Several liver functions such as liver regeneration, expression of acute phase response genes, hepatoprotective effects and metabolic activities that could influence the severity of hepatic fibrosis during chronic liver injury are regulated by the STAT3 signaling pathway and its negative regulator suppressor of cytokine signaling 3 (SOCS3). These regulatory functions were predominantly investigated in hepatocytes but there is evidence that STAT3 signaling regulates protective mechanisms in BEC and profibrogenic processes in non-parenchymal cells as well. STAT3 is activated by hepatic inflammation and various other insults (100). Important activators are growth factors such as epidermal growth factor (EGF) and the inflammatory cytokine IL-6. This multifunctional cytokine binds to membrane-anchored IL-6R/gp130 heterodimers, which activates the JAK-STAT, Ras and PI3K pathways. IL-6 stimulation leads to phosphorylation of STAT3 at Tyr-705 (pY-STAT3) and subsequent formation of STAT3 homodimers or STAT1/STAT3 heterodimers that enter the nucleus and activate gene transcription (101).

The IL-6/gp130/STAT3 pathway protects from cholestasis-induced hepatic damage as demonstrated by BDL studies in IL-6-deficient (IL-6$^{-/-}$) mice. BDL resulted in elevated bilirubin levels, reduced compensatory hepatocyte proliferation and a more advanced stage of biliary fibrosis with pronounced collagen deposition in IL-6$^{-/-}$ mice when compared to IL-6$^{+/+}$ controls (Table 1). These pathogenic changes could be partly reversed by recombinant IL-6 treatment (102). The hepatoprotective activity of IL-6 in cholestasis induced by BDL is most likely mediated through activation of STAT3, although, this has not been proven directly in the original study (102). However, we have performed BDL experiments in STAT3$^{\Delta hc}$ mice (mice with conditional inactivation of

STAT3 in hepatocytes and cholangiocytes using the alpha-fetoprotein (Alfp) Cre transgene) and observed a substantial increase in liver fibrosis and collagen deposition when compared to $STAT3^{+/+}$ mice (M. Mair, unpublished) indicating that STAT3 mediates the hepatoprotective effect of IL-6 after BDL. Additional model systems and experimental approaches have been employed to prove the hepatoprotective activity of IL-6/gp130/STAT3 signaling in cholestasis-induced liver injury. These include feeding with CA or 3,5-diethoxycarbony-1,4-dihydrocollidine (DDC) and loss of the Mdr2 gene as a genetic model for sclerosing cholangitis (64, 103). As mentioned above, $Mdr2^{-/-}$ mice suffer from cholestasis-induced liver injury (33, 35) that does, however, not affect their lifespan, is macroscopically inconspicuous and leads only to mild liver fibrosis. In contrast, liver fibrosis is strongly aggravated in $IL\text{-}6^{-/-}$ $Mdr2^{-/-}$ and $STAT3^{\Delta hc}$ $Mdr2^{-/-}$ mice (Figure 1B) indicating that IL-6/gp130/STAT3 signaling protects from cholestatic liver injury in sclerosing cholangitis (64). Moreover, mice lacking gp130 in hepatocytes and cholangiocytes ($gp130^{hepa}$) were more sensitive to DDC feeding (a mouse model of hepatic liver injury resembling human sclerosing cholangitis) resulting in severe liver fibrosis and collagen deposition (104). The hepatoprotective activity of gp130 signaling could be attributed to gp130-mediated STAT activation using specific gp130 knock-in mutant alleles that lack either the region for STAT1/3 activation ($gp130^{\Delta hepaSTAT}$) or carry a Y757F mutation that impedes activation of the Ras/MAPK pathway ($gp130^{\Delta hepaRas}$) (104).

Several cellular and molecular mechanisms might contribute to the hepatoprotective activity of the IL-6/gp130/STAT3 pathway during cholestatic liver injury and fibrosis. In hepatocytes, activation of STAT3 by IL-6 is considered to be an essential event for hepatoprotection (105). We have demonstrated that STAT3 regulates the expression of epidermal growth factor receptor (EGFR) and IGF-1 in hepatocytes (64). These two signaling molecules are downregulated in the liver of $STAT3^{\Delta hc}$ mice at the RNA and protein levels when compared to $STAT3^{+/+}$ mice (64). Both molecules are components of signaling pathways that prevent from bile-acid-induced hepatocyte death *in vitro* (106-109). Therefore, regulation of EGFR and IGF-1 by STAT3 might be an important hepatoprotective mechanism in cholestasis-induced hepatic injury (Figure 2) and fibrosis which was confirmed in $STAT3^{\Delta hc}$ mice that are hypersensitive to CA feeding (64). As mentioned above, liver fibrosis is strongly aggravated in the $Mdr2^{-/-}$ mouse model of sclerosing cholangitis when IL-6 or STAT3 are absent. However, reconstitution of IGF-1 signaling by daily injection of recombinant IGF-1 had only a mild effect on the severity of fibrosis in $STAT3^{\Delta hc}$ $Mdr2^{-/-}$ mice indicating that signaling via the EGFR is required as well (64). The qualitative importance of both signaling pathways in protection from bile acid induced liver injury has to be evaluated in $Mdr2^{-/-}$ mice with conditional deletion of EGFR or IGF-1 in hepatocytes.

Besides regulation of hepatoprotective pathways, STAT3 can influence the severity of cholestasis-induced fibrosis via regulation of bile acid biosynthesis genes such as Cytochrome P450 family 7 member a1 (Cyp7a1) and Cyp27a1 in hepatocytes (Figure 2). These genes encode key enzymes for classical and alternative bile acid biosynthesis pathways (110). Increased bile acid biosynthesis gene expression was observed in $STAT3^{\Delta hc}$ mice resulted in hypercholanemia and adaptive expression of bile acid transporters (64). This deregulation of bile acid biosynthesis might sensitize $STAT3^{\Delta hc}$ mice to cholestasis, thereby aggravating cholestatic liver damage and fibrosis under challenging conditions.

In addition to hepatocytes, the IL-6/gp130/STAT3 signaling pathway displays a protective function in cholangiocytes and maintains the integrity of the biliary tree via regulation of cholangiocyte-protective small proline-rich proteins (SPRRs) and trefoil factors (TFF) (111). The SPRR proteins display protein-protein crosslinking functions and influence the barrier function of epithelia. TFF proteins have been implicated in the restitution phase of wound healing and protection of the intestinal mucosa from injury through their ability to increase mucous viscosity. Among the trefoil proteins, TFF3 is prominently expressed in mucin secreting BEC (111). In particular, microarray analysis of cultured BEC lacking the IL-6 gene and real-time PCR analysis of wild-type BEC stimulated with recombinant IL-6 have demonstrated that expression of SPRR2A, SPRR2B, SPRRE and SPRRI as well as TFF3 are regulated by the IL-6/gp130/STAT3 pathway (112, 113). Adenoviral gene transfer of a dominant negative STAT3 allele into BEC demonstrated that this regulatory effect is mediated via STAT3. Consistent with these *in vitro* data, *in vivo* analysis demonstrated that the observed upregulation of SPRR2 family members after BDL was blunted in $IL\text{-}6^{-/-}$ mice which were xassociated with impaired barrier function (113). Expression of TFF3 is also induced by BDL in an IL-6-dependent manner as demonstrated in $IL\text{-}6^{-/-}$ mice (112). Interestingly, expression of TFF3 in cultured BEC is reciprocally regulated by STAT3 and MAPK downstream effectors of IL-6/gp130 signaling. Whereas STAT3 is a positive regulator, activation of MAPK signaling has a suppressive effect on TFF3 expression (112). Consistently, $gp130^{\Delta hepaSTAT}$ knock-in mice are sensitive to dextran-sulfate-induced colitis because of decreased TFF3 expression (114). Taken together, these data suggest that activation of the IL-6/gp130/STAT3 pathway protects cholangiocytes from bile-acid-induced injury and triggers proper bile duct wound healing responses through SPRR2 and TFF3 proteins (Figure 2).

The discovery that leptin, a 16kd adipokine that regulates many metabolic functions and is predominantly expressed in adipose tissue, is a profibrogenic factor in the liver has drawn the attention to STAT3 functions in stellate cells. *In vivo* analysis in homozygous ob/ob mice which lack leptin expression demonstrated that they are more resistant to development of CCl_4-induced hepatic fibrosis and harbor less alpha-smooth muscle actin (alpha-SMA) - positive collagen producing cells. Importantly, decreased CCl_4-induced fibrogenesis is reverted by administration of exogenous leptin (115). Leptin signals via six isoforms of leptin receptors (ObRa through ObRf) that are expressed in

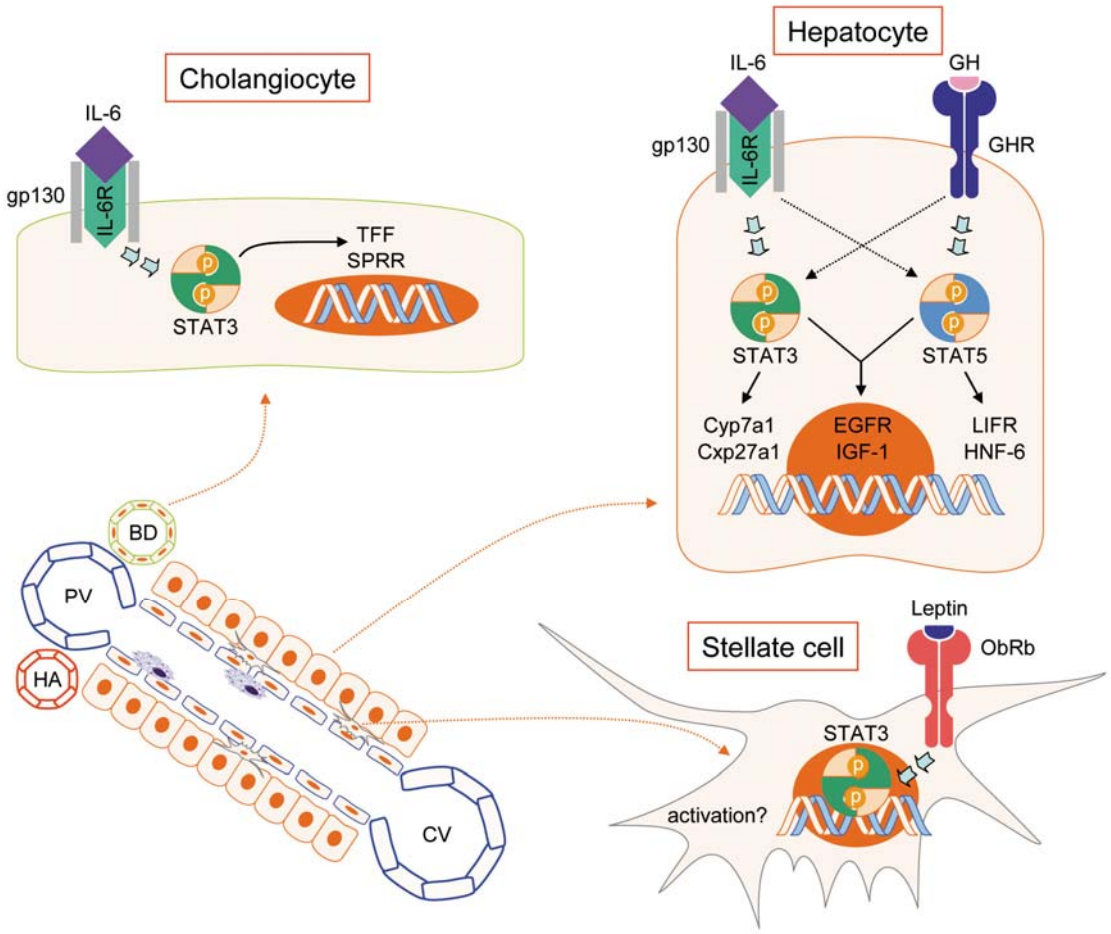

Figure 2. Schematic of STAT3 and STAT5 functions in hepatic cell types that are implicated in cholestasis-induced liver fibrosis. In cholangiocytes, IL-6/gp130/STAT3 signaling regulates the expression of cholangioprotective trefoil factors (TFF) and small proline-rich proteins (SPRR). In hepatocytes, IL-6/gp130/STAT3 and GH/STAT5 signaling pathways regulate expression of EGFR and IGF-1. In addition, STAT3 and STAT5 have individual functions. STAT3 regulates bile acid biosynthesis genes thereby changing the susceptibility to cholestasis. STAT5 regulates additional hepatoprotective factors that might prevent hepatocyte cell death upon cholestatic liver injury. STAT3 is also activated in HSC cells via adipokines such as leptin. The implication of this event in hepatic fibrosis and HSC activity has to be evaluated. BD: bile duct; PV: branch of portal vein; HA: branch of hepatic artery; CV: central vein.

the central nervous system and peripheral tissues (116). HSC express predominantly ObRb which signals through Jak2 and STAT3 (23). Leptin is usually not expressed by quiescent HSC at high levels (as is ObRb) but is induced when they become activated (117). Therefore, leptin might establish an autocrine activation loop in activated HSC that leads to increased STAT3 activity and expression of extracellular matrix components such as alpha2 (I) collagen (118). This has been demonstrated by stimulation of cultured HSC with leptin (118). However, *in vivo* evidence using application of a fibrosis-inducing protocol in a mouse model with conditional deletion of STAT3 in HSC is missing.

5. STATS IN HEPATIC FIBROSIS: FOCUS ON GH/STAT5/IGF-1

The growth hormone receptor (GHR), a class 1 cytokine receptor (119), is a potent activator of STAT5 in the liver. GH is secreted by somatotrophic cells in the anterior pituitary gland and secreted into the blood stream. Hepatocytes have growth hormone receptors and GH-GHR binding results in dimerization of the receptor and recruitment of JAK2 to its cytoplasmic domain. The receptor is phosphorylated and STAT5 is activated (120). GH-GHR binding not only triggers transcriptional activity of STAT5, but also of STAT1 and STAT3 as well as activation of PI3K, extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK) and Src (121). In the liver, STAT5b is the predominantly found STAT5 isoform and it controls modulation of cellular metabolism and hepatocyte physiology (84), production of bioactive IGF-1 (120) and acid labile subunit (ALS) (122). Therefore, the effects of bioactive GH in liver epithelial cells (123) are predominantly conferred by STAT5b.

High serum levels of GH and IGF-1 deficiency are two phenomena that are often associated with human

liver cirrhosis (124, 125). Studies have reported that the degree of IGF-1 deficiency correlates with disease progression. Low levels of IGF-1 have been attributed to decreased liver mass with hepatocellular dysfunction (126) which inhibits IGF-1 synthesis and lack of GHR expression (124, 127). The latter explains why administration of recombinant human GH to cirrhotic patients does not significantly increase IGF-1 serum levels (128). The lack of bioavailable IGF-1 was also partially attributed to the deregulation of IGF-1 binding proteins (IGF1BP) as shown in human patients and *in vivo* animal models of liver fibrosis. IGFBP3 increases the bioavailability of IGF-1 and IGFBP1 counteracts IGF-1 functions through direct binding to IGF-1 (129). Interestingly, a low ratio of IGFBP3/IGF1BP1 serum levels has been correlated with the degree of liver dysfunction (130). Another IGFBP that was found to be deregulated in human liver cirrhosis is IGFBP5. The precise mechanism how IGFBP5 influences liver cirrhosis remains to be determined but might include direct inhibition of IGF-1 effects (131) on HSC or hepatocytes (132).

Several *in vivo* studies have been performed to demonstrate the importance of IGF-1 in liver functions and regeneration. Treatment with recombinant IGF-1 has been shown to improve liver fibrosis in rats undergoing CCl_4 treatment (133). Oxidative liver damage was reduced and mitochondrial function was increased. A similar beneficial effect was achieved by application of a Simian virus 40 (SV40) vector encoding IGF-1 in CCl_4 treated rats which resulted in increased expression of several matrix metalloproteinases (MMP) thereby promoting fibrolysis (134). Moreover, IGF-1 therapy improved liver fibrosis in rats that underwent BDL (135). Interestingly, hepatocytes express only little IGF-1R and were believed to be non-responsive for IGF-1 signals (136). This suggests that the beneficial effects of IGF-1 on liver fibrosis are mediated by a cellular mechanism that is independent of hepatocytes. However, we have recently demonstrated that isolated hepatocytes of mice lacking STAT5 prominently undergo apoptosis in response to bile acids *in vitro* which was ameliorated after administration of recombinant IGF-1 (137). This suggests that hepatocytes can directly respond to IGF-1 at least *in vitro*.

The exact mechanism how IGF-1 influences liver fibrosis remains unclear. It was suggested that its main anti-fibrotic effects depend on suppression of HSC activity. This was demonstrated in transgenic mice expressing IGF-1 under control of the alpha-smooth muscle actin promoter (138). Fibrotic lesions induced by CCl_4 treatment were strongly reduced in these transgenic mice when compared to control animals. Interestingly, IGF-1 rather increased proliferation and collagen synthesis of HSC *in vitro* (139, 140), whereas HSC undergo apoptosis in response to IGF-1 *in vivo* (138). IGF-1-stimulated production of hepatocyte growth factor (HGF) by HSC seems to mediate the apoptotic response. Consistently, administration of HGF to DMN treated rats promoted apoptosis of HSC (141) thereby establishing an anti-fibrotic signaling loop.

Recently, disruption of the STAT5 locus in the liver of mice using the AlbuminCre transgene has been shown to result in liver fibrosis and HCC formation after CCl_4 administration. A direct interaction between STAT5 and TGF-beta proteins which mediates prolongation of the TGF-beta half-life in hepatocytes was suggested as molecular mechanism for increased fibrosis. Moreover, enhanced induction of STAT3 activity by GH was observed in the absence of STAT5 which could promote HCC formation (92). We have recently demonstrated that IGF-1 is a crucial downstream target of GH/STAT5 signals in cholestasis-induced liver fibrosis (137). In this study, STAT5 was conditionally inactivated in hepatocytes and cholangiocytes of Mdr2-knock-out mice. Loss of STAT5 strongly promoted cholestasis-induced hepatic fibrosis in this model (Figure 1B). However, no major change of TGF-beta protein expression was observed upon hepatic deletion of STAT5 in the Mdr2 knock-out model. Alternatively, several STAT5 target genes have been identified in a microarray screen that might contribute to the hepatoprotective activity of STAT5 in cholestasis-induced liver fibrosis (Figure 2). IGF-1 and IGF-1 signaling components were identified in this screen confirming the importance of the GH/STAT5/IGF-1 axis in hepatoprotection. Moreover, additional changes in gene expression profiles revealed deregulation of the known STAT5 target genes hepatocyte nuclear factor 6 (HNF6) and prolactin receptor (PRLR) as well as additional targets such as leukemia inhibitory factor receptor (LIFR) and EGFR. They have all been implicated in the protection of hepatocytes from experimentally induced liver damage (142-145). Although not proven directly, additional data in the literature support the importance of these STAT5 targets in cholestasis-induced liver fibrosis. The expression of HNF6 is regulated by GH/STAT5 (146) and a recent report has demonstrated that GH-induced HNF6 supports liver regeneration and repair in mice upon bile acid-induced liver injury (144). Infection of mice with a HNF6 expressing adenovirus or GH injection into untreated mice suggested that the Cyp7a1 gene is regulated at the transcriptional level by GH/HNF6. The Cyp7a1 gene encodes one of the major enzymes that control the conversion of cholesterol to bile acids (147) and explains the cholesterol clearance function of HNF6 in response to hepatic injury after BDL. Only little information exists about the role of PRLR in liver fibrosis. When mice were treated with prolactin (PRL) and subjected to PHx, they displayed premature cell cycle entry of hepatocytes leading to increased numbers of proliferating liver cells when compared to untreated mice (143). PRLR mRNA is expressed at higher levels in explanted human fibrotic and cirrhotic liver tissue than in normal liver tissue (148). These data led to the conclusion that PRLR conveys regenerative and protective signals in fibrotic and cirrhotic livers (148).

Interestingly, GH treatment of mice induced significant binding of STAT5 to the LIFR promoter (137). In healthy human livers, LIFR expression is hardly detectable in the portal tracts but is upregulated in cirrhotic livers (149). Interestingly, LIFR expression was also detected in oval cells (145) which partially reconstitute the hepatocyte cell mass that is lost during liver injury. It could be speculated that STAT5-mediated upregulation of LIFR in oval cells might lead to expansion and differentiation of

the liver stem cell compartment to replace damaged hepatocytes. Activation of LIFR by LIF usually facilitates phosphorylation and activation of STAT3 (150). However, LIF can also activate phosphorylation of STAT5b in hepatoma cells *in vitro* (151).

The EGFR was shown to be a crucial player in hepatocyte regeneration in partially hepatectomized mice (142). Deletion of the EGFR gene using the AlfpCre recombinase delayed hepatocyte proliferation and resulted in increased mortality. Additionally, EGFR was shown to activate STAT3 (152) and functional STAT3 is crucial for hepatocyte proliferation in the process of liver regeneration (73). A comparison of global gene expression data from several mouse models deficient in functional GH or IGF-1 signaling unveiled that the EGFR mRNA is commonly downregulated in these mouse strains (153). Accordingly, mutation of several GHR domains resulted in downregulation of EGFR expression (154). These data indicate that EGFR gene expression correlates with functional GH and IGF-1 signaling.

6. OTHER STATS IN LIVER FIBROSIS

Apart from STAT3 and STAT5, STAT1 has been shown to protect from liver fibrosis (Table 1). Mice lacking STAT1 are more prone to development of liver fibrosis upon treatment with CCl_4 (155). The cellular mechanisms behind this phenotype are a pivotal HSC activation upon STAT1 ablation in response to CCl_4 and reduced NK cell-mediated killing of activated HSC. On the other hand, *in vitro* treatment of HSC with IFN-gamma, a major activator of STAT1, inhibits activation and proliferation of wild-type HSC, but not HSC lacking STAT1. These controversial data might be reconciled by the assumption that STAT1 influences the severity of fibrosis by modulating functions in HSC and immune cells. Consistent with the aggravated fibrosis in CCl_4 treated $STAT1^{-/-}$ mice, treatment with IFN-alpha and gamma ameliorates liver fibrosis in mice treated with CCl_4 or DMN (156-158). Similarly, treatment of wild-type mice with poly I:C, which mimics Interferon effects, improves CCl_4 induced liver fibrosis, a beneficial effect that is abrogated in $STAT1^{-/-}$ animals. Moreover, mice lacking the IFN-gamma receptor are more susceptible to develop liver fibrosis in response to DDC or CCl_4 treatment (159, 160). The hepatoprotective activity of Interferon/STAT1 signaling has been conferred to human therapy and clinical data have shown that treatment of cirrhosis patients with IFN-alpha or gamma improves cirrhosis associated parameters (161, 162). Taken together these data suggest that the Interferon/STAT1 signaling pathway plays a critical role in liver cirrhosis by controlling the activation of HSC and NK cells. The use of more sophisticated genetic models, such as conditional STAT1 knock-out mice will allow a further clarification of the role of STAT1 in different cell types regarding liver fibrosis.

Two other important regulators of the JAK/STAT pathway associated with liver fibrosis are the SOCS1 and SOCS3. They are transcribed in response to several cytokines and they act as negative regulators of JAK/STAT signaling, preferentially STAT1 and STAT3. SOCS1 inhibits JAK signaling by directly binding to the activation loop of the JAK, while SOCS3 blocks JAK by binding with its src-homlogy 2 (SH2) domains to phosphotyrosine motifs of the cytokine receptor (163-165). Homozygous $SOCS1^{-/-}$ animals die at 3 weeks of age from severe hepatitis (166) but mice lacking only one allele of SOCS1 ($SOCS1^{+/-}$) are viable and more prone to development of DMN-induced liver fibrosis when compared to wild-type mice (167). Mechanistically, it has been suggested that haploinsufficiency of SOCS1 results in reduced STAT3 activity, downregulation of the STAT3 target gene Bcl-xL and increased activity of STAT1. Importantly, the methylation pattern in the SOCS1 promoter leading to silencing of SOCS1 expression has been directly correlated with the severity of liver fibrosis in human samples underlining the importance of SOCS1-mediated STAT regulation in this disease (167).

Similar to SOCS1, SOCS3 has also been shown to protect from liver fibrosis (168). $SOCS3^{-/-}$ mice are embryonic lethal (169) but heterozygous $SOCS3^{+/-}$ are viable and show increased liver fibrosis upon challenge with DMN when compared to wild-type mice. Moreover, Ogata and colleagues have generated mice with a conditional allele for SOCS3 ($SOCS3^{fl/fl}$). By intravenous application of adenoviruses expressing Cre recombinase, SOCS3 was deleted in parenchymal and non-parenchymal cells of the liver (168). Resulting AdCre-$SOCS3^{fl/fl}$ mice are more prone to development of liver fibrosis in response to DMN and Con A than AdCre-$SOCS3^{fl/fl}$ mice. Mechanistically, loss of SOCS3 results in upregulation of activated STAT3 and TGF-beta. Interestingly, overexpression of a dominant negative version of STAT3 in the liver of AdCre-$SOCS3^{fl/fl}$ mice by adenoviral gene delivery ameliorated DMN-induced liver fibrosis. This indicates that STAT3 promotes DMN-induced liver fibrosis via regulation of TGF-beta (168). Consistently, IL-6 induced TGF-beta expression at the mRNA level in cultured primary hepatocytes and this effect was blocked using a dominant negative version of STAT3. These data somehow contradict the aforementioned hepatoprotective functions of STAT3 in liver fibrosis.

Finally, recent data obtained from a genome wide association screening have identified single nucleotide polymorphisms (SNPs) that strongly link the JAK/STAT pathway to biliary cirrhosis in humans. SNPs in the IL12a, IL12RB2 and STAT4 genes showed to be significantly associated with primary biliary cirrhosis, thus emphasizing the importance of this pathway in cholestasis-induced liver fibrosis (170).

7. CONCLUSIONS AND PERSPECTIVES

Liver fibrosis is a complex disease that involves multiple cell types. Despite the presence of data and animal models that sometimes seem to give contradictory results, the implication of JAK-STAT signaling in hepatic fibrosis has been proven unequivocally (Table 1). One major problem in the interpretation of data is the known compensatory activation of other STAT members in STAT-specific knock-out and conditional knock-out mice (101).

Fore example, deletion of STAT1 leads to a substantial activation of STAT3 by Interferons that is usually not observed (171, 172). Alternatively, deletion of STAT3 renders STAT1 responsive to IL-6 signals leading to IL-6-induced missexpression of Interferon-response genes (173). Similarly, upregulation of STAT1 and STAT3 activities has been observed in liver-specific STAT5-deficient mice (84). Another difficulty is the dissection of cell-specific functions of STATs in liver fibrosis. For example, STAT3 might have protective functions not only in hepatocytes but also cholangiocytes and it can be hypothesized that it could also modulate activation and matrix production by HSC. Mouse Cre-lines for conditional deletion of STATs in the various hepatic cell lineages might be helpful to address this question. Despite these difficulties, multiple genetic models with disturbed STAT expression or JAK-STAT signaling are prone to develop liver fibrosis (Table 1). In particular, STAT3 and STAT5 genes seem to share common hepatoprotective functions in liver fibrosis thereby integrating the GH and IL-6 signaling pathways (Figure 2). These findings, together with the unbiased identification of SNPs in the JAK-STAT pathway that are associated with primary biliary cirrhosis in humans highlight the tight link between JAK-STAT signaling and liver fibrosis.

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Abbreviations: Alfp: Alpha-fetoprotein, Alpha-SMA: Alpha-smooth-muscle actin, ALS: Acid labile subunit, BDL: Bile duct ligation, BEC: Bile duct epithelial cell, CA: Cholic acid, CCl_4: Carbon tetrachloride, Con A: Concanavalin A, Cyp: Cytochrome P450, CDE: cholin-deficient, ethionine-supplemented diet, DDC: Dihydrochollidine, DEN: Dimethylnitrosamine, DMN: Dimethylnitrosamine, EGF: Epidermal growth factor, EGFR: Epidermal growth factor receptor, EMT: Epithelial-mesenchymal transition, EPO: Erythropoietin, ERK: Extracellular signal-regulated kinase, GH: Growth hormone, GHR: Growth hormone receptor, GM-CSF: Granulocyte-macrophage colony-stimulating factor, HCC: Hepatocellular carcinoma, HGF: Hepatocyte growth factor, HNF6: Hepatocyte nuclear factor 6, HSC: Hepatic stellate cell, IFN: Interferon, IGF-1: Insulin-like growth factor 1, IGF1BP: IGF-1 binding protein, IL: Interleukin, IRF: Interferon regulatory factor, Jak: Janus kinase, JNK: C-Jun N-terminal kinase, LIFR: Leukemia inhibitory factor receptor, LPS: Lipopolysaccharide, MAPK: Mitogen-activated protein kinase, Mdr2: Multidrug resistance 2, MMP: Matrix metalloproteinase, NF-kappaB: Nuclear factor kappa B, NK: Natural killer cell, Pb: Phenobarbital, PDGF: Platelet-derived growth factor, PHx: Partial hepatectomy, PRL: Prolactin, PRLR: Prolactin receptor, SH2: Src-homology 2 , SNP: Single nucleotide polymorphism, SOCS: Suppressor of cytokine signaling, SPRR: Small proline-rich proteins, STAT: Signal transducer and activator of transcription, SV40: Simian virus 40, TAA: Thioacetamide, TFF: Trefoil factor, TGF: Transforming growth factor, TIMP: Tissue inhibitor of metalloproteinase, TNF: Tumor necrosis factor, TPO: Thrombopoietin

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