REVIEW

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STAT3 in hepatocellular carcinoma: new perspectives

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Practice Points

- Hepatocellular carcinomas (HCCs) have a heterogeneous etiology and are promoted by chronic inflammation and liver injury. Treatment of HCCs includes resection of solitary tumors, liver transplantation, radiofrequency ablation, transarterial chemoembolization and application of the tyrosine-kinase inhibitor sorafenib.
- Although modern screening techniques have identified a plethora of epigenetic and genetic changes, development of molecular therapies is difficult because drugs display enhanced hepatocyte toxicity in cirrhotic livers and no obvious oncogene addiction loops have been identified in HCCs.
- Cytokine receptor signaling activates the JAK/STAT pathway, which consists of four Janus kinases (JAK1–3 and TYK2) and seven STAT transcription factors (STAT-1, -2, -3, -4, -5a, -5b and -6), through phosphorylation of STATs. Moreover, unphosphorylated U-STAT proteins can display noncanonical functions in gene regulation, metabolism and oncogenesis.
- In the liver, STAT3 is mainly activated by IL-6 or IL-22 and regulates acute phase response proteins, liver regeneration, hepatoprotection and gluconeogenesis. STAT3 has been considered as a *bona fide* oncogene that counteracts tumor-suppressive STAT1 activities. Experimental HCC studies in mice lacking IL-6, IL-22 or STAT3 in hepatocytes have underlined the oncogenic activity in liver cancer.
- The oncogenic role of STAT3 in tumorigenesis has to be revised since several studies have demonstrated dual functions. STAT3 has hepatoprotective activities that ameliorate chronic liver injury and act tumor suppressive during early HCC formation. Moreover, STAT3 displays tumor-suppressive functions in HCCs that have lost the p19ARF/p14ARF cyclin-dependent kinase inhibitor.
- The application of STAT3 inhibitors requires personalized approaches and characterization of additional genetic changes in HCCs that can modulate oncogenic or tumor-suppressive activities.

SUMMARY Chronic liver damage and inflammation are strong promoters of hepatocellular carcinoma (HCC) formation. HCC cells communicate with inflammatory and stromal cells via cytokine/chemokine signals. These heterotypic interactions inhibit immunologic anticancer activities and promote protumorigenic activities, such as

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angiogenesis or invasiveness. STAT3 mediates several reciprocal interactions between liver cancer cells and stromal cells and modulates preconditions of tumor formation such as chronic inflammation. Therefore, activation of STAT3 is considered as a tumor-promoting event in HCC formation. However, the oncogenic role of STAT3 in cancers has been challenged by several reports that suggest a tumor-suppressive activity. Here we discuss tumor-promoting and tumor-suppressive effects of cytokine-activated STAT3 in HCC.

Etiology of HCC

Hepatocellular carcinoma (HCC) is a malignant disease with heterogeneous etiology. Many insults that lead to chronic liver damage, such as intoxications, viral infections, cholestasis or metabolic diseases, increase the risk for HCC formation [1]. Recently, a contribution of the intestinal microbiota to HCC formation has been described [2]. Chronic liver damage is accompanied by immigration of inflammatory cells that promote hepatocyte proliferation through the production of cytokines. Moreover, inflammation-induced formation of reactive oxygen species increases DNA damage and mutagenesis rates in hepatocytes [3]. Subsequent DNA replication and cell proliferation lead to expansion of hepatocyte populations with fixed genetic alterations. This increases the likelihood for accumulation of additional tumor-promoting mutations and establishes a vicious circle for HCC formation [4].

The first morphologically apparent lesions eventually leading to progressed HCCs are dysplastic foci. They appear either as small cell foci or large cell foci and harbor already pre-cancerous changes, such as high proliferative activity, telomere shortening, p21 checkpoint inactivation or chromosomal instability [5,6]. These prestages may progress directly to HCCs or through the intermediary stage of dysplastic nodules [6]. HCCs can also originate from hepatocellular adenomas (HCAs), a heterogeneous entity of benign liver tumors that are classified as HNF1 α -mutated HCAs, β -catenin-mutated HCAs or inflammatory HCAs (inflammatory HCAs can also carry β -catenin mutations) [7]. Most HCAs develop in women without chronic liver disease due to use of oral contraceptives, which demonstrates a relationship between sex hormones and liver tumors [8]. Apart from the histopathologic criteria, several biomarkers have been used to discriminate between dysplastic foci, early HCC and moderately to poorly differentiated HCC. They include the heparin sulfate glypican-3, heat shock protein 70, glutamine synthetase (encoded by a typical β -catenin target gene), CD34 or α -fetoprotein [6,9].

Cell of origin for HCC

Most HCCs contain both, mature hepatocyte-like cancer cells and cells similar to hepatic progenitor cells (HPCs). Based on this observation, the origin of HCC was inferred from HPCs [10]. Chronic liver injury leads to activation of HPCs that have a bipotential differentiation capacity and can regenerate cholangiocytes and hepatocytes. Fate determination is mainly controlled by Notch and Wnt signaling. The ligands that activate these pathways seem to be provided by myofibroblasts (Jagged 1 for Notch activation) or by inflammatory cells (macrophages produce Wnt3a) that are present in cirrhotic livers [11,12]. Fibroblasts and macrophages are also present in the stroma of HCCs [13,14]; Notch and Wnt ligands derived from these cell types could induce fate changes of transformed hepatocytes. Given the close cellular proximity of hepatocytes and cholangiocytes, it is not surprising that some HCCs express both hepatocyte and cholangiocyte markers [15]. These hepatocellular cholangiocarcinomas may arise from HPCs with bipotential hepatocyte–cholangiocyte differentiation capacity or from hepatocytes/cholangiocytes that underwent dedifferentiation and restart expression of bilineal markers [15]. Interestingly, formation of HCCs with stem cell-like characteristics (Alb⁺ and K19⁺) occurred in aged mice with conditional ablation of p53 in hepatocytes [16]. This indicates that loss of p53 can give rise to HCCs with bilineal characteristics.

A different HCC entity is represented by HCCs with stemness-related marker expression. Stemness markers include K19, EpCam, c-Kit and CD133. These HCCs seem to have a higher potential for epithelial–mesenchymal transition [17] and tumor angiogenesis [18], thereby increasing the rate of intrahepatic metastasis. As a consequence, expression of stemness markers is associated with poor prognosis [19]. Stemness markers, such as CD133, are expressed by tumor-initiating cells or cancer stem cells [20]. CD133⁺ cancer stem cells have an enhanced potential to form new tumors in transplanted mice and are more resistant to chemotherapy [1,21]. They could, however, also represent new

targets for chemotherapy directed against the cancer stem cell population [22,23].

Genetic & epigenetic alterations in HCC

Screening of the COSMIC catalog of somatic mutations in cancer identifies *p53*, *ARID1A*, *CTNNB1*, *AXIN1*, *CDKN2A*, *ARID2*, *PIK3CA*, *NFE2L2*, *ATM*, *BRAF*, *PTEN*, *HNFI1A*, *APC*, *COL1A1*, *CSF1R*, *KRAS*, *RBI*, *MET*, *NRAS* and *ERBB2* as frequently mutated genes in HCC [201]. Most of these mutations are driver mutations for hyperactivation of Wnt-, PI3K-, MAPK- and HGF-signaling pathways. Moreover, gene expression and protein activities in HCC are controlled by gene polymorphisms [24], epigenetic changes [25–27] and miRNAs (miRs) [28]. The methylation patterns of distinct genes seem to be robust and have been used for HCC classification [29,30]. Candidate approaches have identified several tumor suppressor genes, such as *RASSF1A*, *CDKN2A/INK4* (p16) and *INK4B* (p15), that are commonly methylated in HCCs. More recently, several genome-wide methylation screens have identified thousands of CpG sites in HCCs that were either hypermethylated or hypomethylated [31–33]. Aberrant methylation and silencing of tumor suppressor genes, such as *CDH1/E-cadherin*, can be induced in HCCs by reactive oxygen species [34] and is molecularly due to aberrant expression or activity of DNA methyltransferases [25].

Several miRs have been identified that are associated with HCC formation and also HCC prestages such as liver fibrosis and hepatitis [28]. A recent study using Illumina Solexa® (Illumina, Inc., CA, USA) sequencing has confined the number of miRs that may be implicated in HCC. Among most abundantly dysregulated miRs in HCCs, when compared with matched non-neoplastic liver tissue, were three miRs with increased expression and ten miRs with decreased expression. The postulated tumor-suppressive role of miR-199a/b-3p was further evaluated *in vitro*. The study demonstrated that miR-199a/b-3p suppressed expression of PAK4, a kinase that activates the MAPK signaling cascade [35]. Other studies have demonstrated tumor-suppressive activities of miR-199b-5p [36,37] and miR-122, the most abundant miR in the liver [35], which are based on interference with Wnt/ β -catenin signaling [38]. Ectopic expression of tumor-suppressing miRs may be a promising option for highly specific miR-based therapies as demonstrated for miR-26a in Myc-induced murine HCCs [39].

Treatment of HCC

HCC is the third most common cancer-related cause of death worldwide and treatment options are still limited. In principle, only five interventions are currently accepted worldwide for treatment of HCC [40]. Resection of solitary tumors; liver transplantation for patients with single tumors <5 cm or three tumors <3 cm (which corresponds to the Milan criteria); radiofrequency ablation of tumors that are not suitable for surgery; transarterial chemoembolization for multinodular tumors without vascular invasion or extrahepatic spread; and treatment with the tyrosine kinase inhibitor sorafenib for advanced HCCs. The latter, however, extends patient survival for only 3 months [41]. Several additional drugs and combination therapies, together with sorafenib, have been tried in clinical trials [40,42]. Most of them have been halted owing to ineffectiveness or toxicity reasons. Clinical trials that are still ongoing examine, for example, the potency of monoclonal antibodies against VEGFR2 (ramucirumab) or mTOR inhibitors (everolimus) in HCC [40]. It is a particular problem that HCCs develop in the context of liver damage and dysfunction. This implicates that drugs used for other types of cancer in patients without liver disease (e.g., sunitinib) display a pronounced toxicity in cirrhotic patients [43]. Moreover, the genetic heterogeneity of HCCs limits treatment options. Cancers can be most effectively treated when they depend on certain driver mutations. A paradigm for such oncogene-addicted cancers is chronic myeloid leukemia (CML). Targeting the Bcr–Abl oncoprotein, which is essential for CML, has improved the prognosis of patients substantially. Unfortunately, such oncogene addictions have not yet been found in HCCs [40,42].

STAT3 activation & its role in tumors

■ JAK/STAT signaling system

STATs are cytoplasmic transcription factors that mediate signal transduction from various growth factors and cytokines to the nucleus [44,45]. STAT proteins are present in vertebrates and invertebrates, but are absent in yeast [46]. The seven STAT proteins (STAT1–4, STAT5a, STAT5b and STAT6) present in mammals share several structural domains such as N-terminal coiled-coil domains, DNA-binding domains, SH2 domains and C-terminal transactivation domains [47]. The latter can be absent in STAT proteins due to alternative splicing [47,48]. The

coiled-coil domains mediate dimerization or tetramerization of STAT proteins and interactions with additional proteins. The DNA-binding domain is not only required for DNA binding, but is also implicated in nuclear translocation of dimeric STAT proteins. The SH2 domain is needed for interaction of STATs with phosphorylated receptor molecules and for dimerization of activated STAT monomers. They confer selectivity of STAT binding to specific cytokine receptors. The transactivation domain contains specific tyrosine (all STATs) and serine residues (not present in STAT2 and STAT6) that are required for STAT transcriptional activation. The tyrosine residues (located at position 705 in STAT3) are more important for STAT activation than the serine residues (located at position 727 in STAT3). However, full transcriptional activation needs phosphorylation at both sites, which has been demonstrated for STAT1 and STAT3 [46].

Activation of STATs occurs when cytokines bind to corresponding cytokine receptors that recruit Janus kinases (JAKs; JAK1, JAK2, JAK3 or TYK2) via their FERM domains [44,46]. This interaction activates the N-terminal kinase domain located in the JAK homology 1 (JH1) region of JAKs (a second JAK homology region, JH2, adjacent to JH1 represents a pseudokinase domain that lacks tyrosine kinase activity). Activated JAKs then phosphorylate tyrosine residues in the intracellular receptor domains thereby creating specific docking site for STAT proteins [44,46]. As long as unphosphorylated STAT proteins are not recruited to receptors, they exist as latent, antiparallel dimers that shuttle continuously between the cytoplasm and the nucleus (STAT1 and STAT2 being exceptions; they accumulate in the nucleus only after phosphorylation). Upon receptor recruitment and activation by JAKs, antiparallel STAT dimers are rearranged to phosphorylated, parallel dimers that accelerates nuclear shuttling [49]. Nuclear STATs bind to IFN- γ activated site (GAS) that are 8–10 bp long and have the consensus sequence 5'-TTN₄₋₆-AA-3'. In addition to JAK/STAT activation, cytokine receptors can also activate the MAP kinase and PI3K cascades [44].

■ Specific cytokines & growth factors activate STAT3

STAT3 is predominantly activated by cytokines that bind a common gp130 receptor subunit that is associated with a cytokine-specific subunit

(e.g., IL-6-mediated STAT3 activation is mediated through IL-6R/gp130 receptors that activate JAK1 or JAK2) [47]. Other STAT3 activating cytokines are IL-10, IL-11, IL-22, oncostatin M, LIF or CNTF [47]. Moreover, STAT3 is activated by receptor tyrosine kinases, such as EGF receptor (EGFR), and nonreceptor tyrosine kinases, such as Src [47]. Negative regulation of STAT activity is achieved by various inhibitory factors and mechanisms. Most important are proteins of the suppressor of cytokine signaling (SOCS) family that consists of eight members (CIS and SOCS1–7) [47]. SOCS proteins are induced by cytokines, thereby establishing a negative feedback loop that keeps STAT activity under control. SOCS members inhibit STAT activity by various molecular mechanisms and ensure that cytokine signaling remains transient [44].

In addition to STAT3 functions that depend on canonical activation (Tyr-705 phosphorylation), noncanonical functions have been described [50]. STAT3 autoregulates its own transcription, which results in elevated STAT3 protein levels in cytokine-treated cells. These STAT3 molecules are not Tyr-705-phosphorylated (U-STAT3) and regulate different sets of target genes upon physical interaction with unphosphorylated NF- κ B [51]. Another important noncanonical STAT3 function supports Ras-induced cell transformation and depends on mitochondrial STAT3 localization [52]. This function requires STAT3 phosphorylation at Ser-727 rather than Tyr-705 [52].

■ STAT3 in tumorigenesis

Oncogenic functions of STAT3 signaling have been demonstrated in many mouse tumor models and tumor types. Moreover, it has been demonstrated that expression and activity of STAT3 is enhanced in a wide range of malignancies [53]. STAT3 activation is usually transient in non-transformed cells, even in the continuous presence of cytokines. However, cancer cells have developed a positive feed forward loop for persistent activation of STAT3 that depends on SphK1, sphingosine-1-phosphate and the S1PR1 [54,55]. The protumorigenic activity of STAT3 may be due to several molecular mechanisms. Protumorigenic STAT3 target genes have been implicated in tumor cell proliferation, survival, angiogenesis and invasiveness (Figure 1). Moreover, STAT3 is an antagonist of the closely related STAT1 transcription factor that acts as a tumor suppressor [56]. STAT1 is frequently downregulated in many

tumor types and mediates proapoptotic and antiproliferative activities of interferons (IFNs) [56]. Interestingly, ablation of STAT1 switches the proapoptotic and antiproliferative activities of IFNs to survival and proliferation signals in various cell types. This functional switch is at least in part due to aberrant activation of STAT3 by IFN signals that occurs in the absence of STAT1 [56]. By contrast, ablation of STAT3 leads to aberrant activation of STAT1 through gp130-inducing signals. As a consequence, STAT3-deficient cells respond to IL-6 with substantial STAT1 activation resulting in IL-6-mediated activation of IFN-responsive genes [56]. Several pathological conditions seem to be associated with unbalanced activation of STAT1 and STAT3 [56]. The proposed molecular mechanisms that underlie the reciprocal effects of STAT1 and STAT3 activation include competition for common cytokine receptors, actions of SOCS proteins and mutual inactivation of STATs by sequestering in STAT1:STAT3 heterodimer complexes [44].

However, the pro-oncogenic role of STAT3 in cancer cells has been challenged by several reports that claim antioncogenic functions. *In vitro* studies have shown that constitutively active STAT3 can suppress c-Myc-induced transformation of p53-deficient fibroblasts [57]. Moreover, glioblastomas displayed malignant progression when STAT3 and PTEN were concurrently ablated [58]. Similarly, intestinal tumors of *Apc^{Min/+}* mice with concomitant deficiency in STAT3 showed increased progression from adenomas to invasive carcinomas, which was accompanied by nuclear β -catenin accumulation [59]. This is caused by GSK3 β -mediated degradation of SNAI, which prevents intestinal epithelial to mesenchymal transition [60]. Finally, thyroid cancer formation was enhanced when STAT3 was ablated in thyrocytes, which was due to increased aerobic glycolysis [61].

Apart from these tumor cell autonomous oncogenic and antioncogenic functions, STAT3 links inflammation and cancer development via several non-cell-autonomous activities (Figure 1). In established tumors, activation of STAT3 is usually not confined to tumor cells, but occurs also in inflammatory cells and cells of the tumor stroma. This dual activation is required to establish paracrine tumor–stroma interactions that promote tumorigenesis. Many cytokines, chemokines and growth factors, such as IL-6 or IL-1 β , are produced by tumor cells in a STAT3-dependent manner [62]. These factors, in turn,

activate STAT3 in stromal cells, which also start to produce STAT3-activating cytokines, thereby establishing a positive feedback loop between tumor cells and cells in the tumor microenvironment [63]. Further amplification of this feedback loop is achieved by additional cytokines such as IL-17. The latter can promote IL-6 production by HCC cells that depends on AKT and STAT3 activation [64]. The tumor-promoting function of STAT3 in the tumor microenvironment is mediated through several molecular and cellular mechanisms (Figure 1). STAT3 blocks maturation of dendritic cells and inhibits expression of immune-stimulatory molecules, such as IL-12 and IFNs in T-cells [65,66]. Alternatively, expression of immune-suppressive factors, such as IL-10 and IL-23, is induced [65,66]. These processes limit the immune surveillance capacities of inflammatory cells in the tumor stroma. Moreover, STAT3 induces production of CCL2, CXCL2, IL-1 β , VEGF, basic FGF and MMP-9 in myeloid-derived suppressor cells and tumor-associated macrophages, thereby promoting angiogenesis and invasiveness. This is partly due to activation of HIF1 α expression, which is a direct target of STAT3 [67]. There is also an extensive cross-talk between STAT3 and NF- κ B, another crucial transcription factor that links inflammation and cancer [68]. The major subunit of the NF- κ B complex, RelA (p65), interacts directly with STAT3 [69]. Consequently, STAT3 and NF- κ B coregulate several target genes. STAT3 is required for maintenance of constitutive NF- κ B activity in tumor cells and hematopoietic stromal cells. Activated (Tyr-705- and Ser-727-phosphorylated) STAT3 can promote p300-dependent acetylation of RelA, leading to prolonged nuclear localization and constitutive NF- κ B activation [69]. Similarly, STAT3/p300-induced acetylation is responsible for processing of the NF- κ B subunit p100 [70]. In addition to acetylation, U-STAT3 promotes nuclear translocation of unphosphorylated NF- κ B through a piggyback mechanism. The U-STAT3/U-NF- κ B complex recognized responsive elements in a set of target genes, such as *RANTES*, *MET* and *M-RAS*, that is different from canonic STAT3 targets [51].

STAT3 in HCC

■ Hepatic STAT3 functions

IL-6 and IL-22 are considered to be major cytokines that activate STAT3 in hepatocytes and hepatoprotective activities of IL-6/STAT3

Figure 1. Schemes of oncogenic STAT3 functions in hepatocellular carcinoma tumor cells and in the tumor stroma. (A) STAT3 is activated by several cytokines and growth factors in hepatocellular carcinoma tumor cells. Inhibition of negative regulators SHP1/2 and SOCS proteins by NF-κB- and methylation-dependent mechanisms further augments STAT3 activity. STAT3 promotes hepatocellular carcinoma cell survival and proliferation via direct regulation of target genes and via establishment of an epigenetic circuit that amplifies IL-6 signaling through miRNA-induced downregulation of HNF4α. Moreover, STAT3 interferes with STAT1 activity, thereby preventing STAT1-mediated apoptosis and cell cycle arrest. The autoregulatory activity of STAT3 leads to accumulation of unphosphorylated U-STAT3 protein in hepatocellular carcinoma tumor cells that associate with unphosphorylated NF-κB and activate additional protumorigenic target genes. U-STAT3 also enters the mitochondria and synergistically supports cell transformation with activated Ras. **(B)** Cytokines, produced by tumor cells, establish a reciprocal STAT3 activation loop between tumor cells and stromal cells. This activation loop is supported by NF-κB activity in tumor cells and stromal cells, which is partially sustained by a STAT3/p300-dependent mechanism. Moreover, sphingosine signaling through SphK1, S1P, S1PR1 and JAK2 contributes to persistent STAT3 activation in tumor cells and cells of the tumor microenvironment. Activation of STAT3 exerts various protumorigenic effects in stromal cells. STAT3 blocks maturation of dendritic cells and activation of T cells, thereby reducing anticancer immune surveillance. Moreover, activation of STAT3 in MDSCs and TAMs leads to upregulation of several target genes implicated in angiogenesis and metastasis. This is facilitated by STAT3-induced M2 polarization of TAMs. These stromal STAT3-dependent mechanisms were identified in various tumors, but may also operate in HCCs.

DC: Dendritic cell; ER: Endoplasmic reticulum; HCC: Hepatocellular carcinoma; MDSC: Myeloid-derived suppressor cell; ROS: Reactive oxygen species; T: T cell; TAM: Tumor-associated macrophage.

or IL-22/STAT3 signaling pathways have been demonstrated in several liver injury models (Table 1) [71–76]. IL-6, released from Kupffer cells or inflammatory cells, stimulates hepatocytes to produce a variety of acute-phase proteins in

a STAT3-dependent manner. Consequently, IL-6-deficient mice are susceptible to liver injury induced by ethanol, concanavalinA, acetaminophen, bile acids and bile duct ligation, which underlines hepatoprotective IL-6

functions [47,77]. Targeted disruption of IL-6 in mice also resulted in delayed liver regeneration after partial hepatectomy, although controversial data have also been published [47]. Most of these IL-6 functions have been linked to STAT3 activation. Loss of STAT3 in hepatocytes aggravates CCl₄, Fas, bile acid and ischemic reperfusion-induced liver injury [47]. 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 3,5-diethoxycarbonyl-1,4-dihydrocollidine and loss of the *Mdr2* gene as a genetic model for sclerosing cholangitis [77,78]. *Mdr2*^{-/-} mice suffer from cholestasis-induced liver injury, which does not affect their lifespan, but leads to liver fibrosis. Liver fibrosis is strongly aggravated in IL-6-deficient and STAT3-deficient *Mdr2*^{-/-} mice indicating that IL-6/gp130/STAT3 signaling protects from cholestatic liver injury in sclerosing cholangitis [77]. Moreover, mice lacking gp130 in hepatocytes and cholangiocytes (gp130^{hepa}) were more sensitive to 3,5-diethoxycarbonyl-1,4-dihydrocollidine feeding (a mouse model of hepatic liver injury resembling human sclerosing cholangitis) resulting in severe liver fibrosis and collagen deposition [78]. The hepatoprotective activity of gp130 signaling was attributed to gp130-mediated STAT activation using specific gp130 knockin mutant alleles that lack either the region for STAT1/3 activation (gp130^{ΔhepaSTAT}) or carry a Y757F mutation that impedes activation of the Ras/MAPK pathway (gp130^{ΔhepaRas}) [78].

In addition to hepatoprotection and hepatocyte proliferation, STAT3 regulates metabolic liver functions. STAT3 suppresses the expression of genes that control gluconeogenesis via SIRTUIN 1, a fasting-activated longevity protein [79]. 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 [47].

Apart from hepatocytes, several STAT3 functions in nonparenchymal liver cells have been identified. In cholangiocytes, expression of cholangioprotective factors TFF3 or small proline-rich proteins (SPRR2A, SPRR2B, SPRRE and SPRRI) is stimulated by IL-6/gp130/STAT3 [47]. This mechanism protects cholangiocytes

from bile-acid-induced injury and triggers proper bile duct wound healing responses. In hepatic stellate cells (HSCs), STAT3 is activated by the adipokine leptin, which acts as profibrogenic factor in the liver [47]. HSCs predominantly express the ObRb leptin receptor that signals through Jak2 [80]. Leptin is not expressed by quiescent HSC but is induced in activated HSCs. Therefore, leptin may establish an autocrine activation loop in activated HSC that leads to increased STAT3 activity and expression of extracellular matrix components such as α2 (I) collagen [47]. In hepatic endothelial cells, STAT3 activation protects from apoptotic cell death during alcoholic liver injury [81]. Kupffer cells are implicated in hepatic inflammation since they are a major source for proinflammatory cytokines IL-6 and TNF-α. STAT3 limits excessive cytokine production by Kupffer cells, thereby controlling liver inflammation [82]. This has been demonstrated in several liver injury models and may be due to the aforementioned antagonism between STAT1 and STAT3 [82–86]. STAT1 is a known proinflammatory factor in liver diseases [82]. In fact, STAT1 is upregulated in STAT3-deficient Kupffer cells, which aggravates hepatic inflammation in concanavalinA and partial hepatectomy mouse models [85,86].

■ Oncogenic STAT3 functions in HCC

STAT3 is traditionally considered to be an oncogene in HCC (Figure 1). Interference with STAT3 activity promotes apoptosis and impairs proliferation of several hepatoma cell lines *in vitro* and in transplantation models [87]. Expression studies using human HCC tissue samples and tumor-associated macrophages demonstrated a general increase in STAT3 expression. Nuclear Tyr-705-phosphorylated STAT3 was present in 60% of HCC cases independent of the tumor etiology and correlated with tumor progression and poor prognosis [88]. Interestingly, there was also a significant correlation between STAT3 activity in myeloid cells of the HCC tumor stroma and poor prognosis [89]. Coculture experiments have demonstrated that IL-6/STAT3 signaling in myeloid cells promotes proliferation of HepG2 or Huh-7 cells *in vitro* [89]. These data underline the importance of concomitant STAT3 activation in the stromal compartment of HCCs. Despite increased activation, no oncogenic STAT3 or STAT3-activating JAK mutations seem to exist in HCCs. However, approximately 60% of inflammatory HCAs carry activating mutations

Table 1. Hepatoprotective activities of STAT3 identified in mouse models and published during the last 5 years.

STAT3-protective function	Involved liver cell types	STAT3-activating cytokine(s)	Involved genes	Ref.
Alcoholic liver injury	Hepatocytes	IL-22	<i>FATP, MTI/II and LCN2</i>	[71]
Alcoholic liver injury	Endothelial cells	IL-6	ND	[81]
Alcoholic liver injury	Kupffer cells, hepatocytes	IL-6	<i>FASN, SREBP-1, ACC1, SCD1, CPT1 and PPAR-α</i>	[84]
Alcoholic and nonalcoholic steatohepatitis	Hepatocytes	IL-6, IL-10	<i>FASN, SREBP-1 and ACC1</i>	[74]
CCL4-induced liver inflammation	Kupffer cells, hepatocytes	IL-6, OSM, IL-10	<i>TNF-α, IFN-γ, Mip1α, MCP1, IL-1β, ICAM1, VCAM1, MIP2 and C5</i>	[83]
CCL4-induced liver fibrosis	Stellate cells	IL-22	<i>p53, p21 and SOCS3</i>	[100]
Cholestatic liver injury	Hepatocytes, cholangiocytes	IL-6	<i>EGFR, IGF-1 and bile acid biosynthesis genes</i>	[77]
Cholestatic liver injury	Hepatocytes, cholangiocytes	IL-6	Acute phase response genes	[78]
Cholestatic and hepatotoxic liver injury and fibrosis	Kupffer cells, stellate cells	IL-17, IL-22	<i>TGF-β1, IL-6, TNF-α, IL-1β, α-SMA, Col1-α1(I), MMP3 and TIMP1</i>	[73]
ConA-induced T-cell mediated hepatitis	Kupffer cells	IL-22	<i>IFN-γ and IL-17</i>	[85]
D-Galactosamine/LPS-induced acute liver failure	Hepatocytes	IL-22	<i>bcl-XL, HO-1 and Ref-1</i>	[75]
Steatohepatitis	Hepatocytes	IL-6	<i>SAA, SOCS3, FASN, SREBP-1, TNF-α and ADIPOQ</i>	[72]
Steatosis	Hepatocytes	IL-22	<i>SREBP-1, PPAR-γ, PPAR-α, LXR-α, LXR-β, ChREBP, FASN, SCD1, ACC1, ACLY, GPAM, DGAT2, ELOVL6 and HMGCR</i>	[76]

For a comprehensive overview regarding hepatoprotective STAT3 functions reported before 2008 see [52].
ConA: ConcanavalinA; LPS: Lipopolysaccharide; ND: Not defined.

in the *IL-6st* gene that encodes the gp130 component present in the gp130/IL6R complex [90]. The activating mutations correspond to small deletions in gp130 that activates IL-6 signaling in the absence of ligand. As a consequence, the transcription factor *STAT3* is constitutively activated, which is considered to drive oncogenesis. Mutations in *IL-6st* have also been identified in HCCs, albeit at low frequency. They are always accompanied by β -catenin mutations [90].

The function of *STAT3* was directly evaluated in diethylnitrosamine (DEN)-treated mice with conditional inactivation of *STAT3* in hepatocytes. These studies provided controversial results with two studies suggesting an oncogenic activity [88,91] and one study suggesting an antioncogenic activity [92]. However, the latter study also demonstrated an oncogenic function of *STAT3* that depends on SHP2, a negative regulator of *STAT3* activity. The proteins tyrosine phosphatases SHP1/SHP2 seem to modulate *STAT3* activity in HCCs. Hepatocytes lacking IKK β had an increased potential to form HCCs, an observation that was initially quite surprising since IKK β -deficiency reduced the activity of NF- κ B. However, IKK β -deficiency also induced accumulation of reactive oxygen species

and interfered with SHP1/SHP2 activity resulting in *STAT3* induction and promotion of HCC formation [88]. Consistently, hepatocyte-specific deletion of SHP2 resulted in persistent *STAT3* activation, which is required for DEN-induced HCC promotion [92]. SHP2 is positively regulated by GADD45G. Downregulation of GADD45G, as observed in HCC and liver cancer cell lines, led to reduced SHP2 activity [93]. This may result in increased JAK2, TYK2 and *STAT3* activation, which prevent cellular senescence of liver cancer cells [93]. Apart from SHP proteins, additional negative regulators of *STAT3* have been described as tumor suppressors in HCC. For example, genetic ablation of SOCS3, which led to enhanced *STAT3* activity, promoted formation of DEN-induced HCCs [94].

The oncogenic function of canonically activated *STAT3* in HCC is underlined by a multitude of studies that demonstrated pro-oncogenic functions of *STAT3*-inducing cytokines IL-6 and IL-22. IL-6 levels are elevated in human patients with liver disease and HCC [95]. Data obtained in mice suggest that obesity-driven HCC development, associated with enhanced production of tumor-promoting cytokines IL-6 and TNF- α , is dependent on *STAT3*

activation [96,97]. Consistently, IL-6-deficient male mice displayed reduced DEN-induced HCC formation when compared with IL-6-proficient controls [98]. Interestingly, IL-6 deficiency abolished the gender-specific difference in DEN-induced HCC formation (female mice are less susceptible) [98]. This indicates that IL-6-signaling is responsible for gender disparity in HCC formation [98]. However, IL-6-independent mechanisms for sexual dimorphism that implicate FOXA transcription factors have also been reported [99]. The second major cytokine that activates STAT3 in the liver is IL-22. Similar to IL-6, several hepatoprotective activities of IL-22/STAT3 signaling have been described [71,75,76,100]. IL-22 expression is elevated in tumor-infiltrating lymphocytes of HCCs and HCC tumor cells expressed high levels of IL-22 receptors [101]. Moreover, DEN-induced HCC formation was reduced in IL-22-deficient mice when compared with IL-22-proficient controls [101]. Together, these data support the concept that *STAT3* is a *bona fide* oncogene in HCC [102].

Several STAT3 target genes have been implicated in hepatocellular tumor initiation, angiogenesis and progression to invasive carcinomas (Figure 1). Apart from this direct regulation of oncogenic target genes, STAT3 may promote HCC formation by alternative mechanisms. One mechanism is based on the aforementioned antagonistic activity between STAT1 and STAT3. STAT1 is mainly activated by IFNs and displays opposing roles to STAT3 in hepatic physiology and HCC. STAT1 mediates the suppressive effect of poly I:C treatment on liver regeneration after partial hepatectomy via regulation of IRF-1 and p21. Disruption of IFN- γ expression, the IFN- γ receptor or STAT1 abolished liver damage in concanavalinA- and lipopolysaccharide/galactosamine-induced liver injury. By contrast, a recent report has demonstrated that loss of IFN- γ enhances liver damage and DEN-induced tumor load in *Fxr*^{-/-} mice [103,104] suggesting a hepatoprotective activity of IFN- γ -signaling in this condition. Aggravated liver damage was accompanied by several molecular changes that promote HCC formation including activation of STAT3 [104], which might be due to impaired IFN- γ /STAT1 signaling. Recently, polymorphisms in the *STAT1* gene have been identified that were associated with an increased HCC risk [105]. However, DEN-induced HCC formation was not impaired in STAT1-deficient mice and a possible negative

correlation between STAT1 and STAT3 activity in HCC awaits further confirmation [91].

It has recently been demonstrated that STAT3 can promote hepatocarcinogenesis via establishment of an epigenetic circuit that involves several miRs [106]. A key event in this mechanism is downregulation of HNF4 α , a hepatocyte differentiation factor and suppressor of HCC formation. This is achieved through miR-24 and miR-629, which are induced by IL-6/STAT3-signaling. As a consequence, HNF4 α -regulated expression of another miR, miR-124, is switched off. Since miR-124 negatively regulates expression of IL-6R, this epigenetic circuit leads to permanent suppression of HNF4 α through an amplified IL-6/STAT3/miR-24/miR-629 feedback loop [106,107]. Other miRs are directly implicated in the regulation of STAT3 expression in HCC. miR-26a suppresses STAT3 expression in HCC cells and downregulation of miR-26a in human HCCs correlates with poor prognosis [108].

■ Antioncogenic STAT3 functions in HCC

In opposition to the oncogenic role of *STAT3* described above, recent reports demonstrated antioncogenic functions of STAT3 in HCC development (Figure 2). STAT3-deficient hepatocytes, immortalized by additional deletion of the cyclin-dependent kinase inhibitor p19^{ARF} (the mouse homolog of human p14^{ARF}) and transformed by Ras, displayed enhanced tumor formation in xenotransplants, suggesting tumor-suppressive functions of STAT3 [109]. By contrast, STAT3 promoted HCC development in the presence of p19^{ARF}, indicating that p19^{ARF} is a critical modulator of oncogenic and antioncogenic STAT3 functions in liver cancer. Consistently, DEN-induced STAT3 wild-type HCCs showed augmented p19^{ARF} levels whereas STAT3-deficient HCCs exhibited reduced p19^{ARF} expression [88]. These correlations were confirmed in human HCC cell lines that underscore the modulator function of p19^{ARF}/p14^{ARF} in STAT3-induced HCC formation [109]. At the molecular level, a hypothetical binding partner of p19^{ARF}/p14^{ARF} called ARF-X, which controls tumor-promoting versus tumor-suppressive STAT3 activities, has been proposed [109]. ARF-X may interfere with oncogenic and promote tumor-suppressive functions of canonically activated STAT3. Therefore, p19^{ARF}-deficient HCCs might benefit from reduced STAT3 activity. NF- κ B has been shown to interfere with STAT3 activation in HCC tumor cells [88] and p19^{ARF} is a negative regulator

Figure 2. Scheme of antioncogenic STAT3 functions in hepatocellular carcinoma.

STAT3 can act as a tumor suppressor in two different ways. First, STAT3 activates hepatoprotective target genes that ameliorate chronic liver injury and prevent formation of hepatocellular carcinoma. However, the same genes might act oncogenic in established HCCs. Second, a putative p19^{ARF}-sequestered factor termed ARF-X is released in HCCs that have lost the p19^{ARF} tumor suppressor. ARF-X forms a complex with U-STAT3 that acts pro-oncogenic. However, ARF-X may also interfere with the oncogenic activity of canonically activated STAT3 and switches it to a tumor-suppressive activity. This model is based on transplantation experiments with HCC cells that express either a constitutive active caSTAT3 or a nonphosphorylable U-STAT3 variant. Loss of p19^{ARF} also leads to enhanced NF-κB activation, which is a negative regulator of STAT3. Therefore, loss of p19^{ARF} indirectly interferes with STAT3 activation via NF-κB. This further promotes oncogenesis since canonically activated STAT3 acts tumor suppressive in p19^{ARF}-deficient HCCs.

HCC: Hepatocellular carcinoma.

of NF-κB [110]. Consequently, loss of p19^{ARF} might lead to enhanced NF-κB activity, which interferes with STAT3 activation. This molecular mechanism could abolish the tumor-suppressive activity of canonically activated STAT3 in p19^{ARF}-deficient HCCs [102,109]. In contrast to p19^{ARF}, absence of the cyclin-dependent kinase inhibitor p27^{Kip1} synergistically cooperated with STAT3 activation in HCC development [111]. This suggests that loss of distinct cyclin-dependent kinase inhibitors promotes tumor-suppressive STAT3 activities, whereas loss of others promotes

oncogenic activities. The precise role of STAT3 in HCC formation may also depend on the etiology since oncogenic STAT3 functions were demonstrated in DEN-induced HCCs whereas tumor-suppressive activities were shown for HCCs that developed after CCl₄-induced chronic liver damage and fibrosis [91]. Loss of STAT3 and concomitant prosurvival signals may enhance CCl₄-induced chronic liver damage, leading to elevated levels of inflammatory cytokines, compensatory proliferation of hepatocytes and increased HCC formation [112]. In conclusion, these data emphasize an emerging concept that STAT3 is able to act as a tumor suppressor in HCC, depending on the genetic context (i.e. loss of tumor suppressor genes) and HCC etiology.

■ STAT3 dichotomy in HCC therapy

Several reports have proposed clinical trials for HCC based on application of low molecular weight compounds for specific inhibition of JAK/STAT3 signaling, either as monotherapies or in combination with sorafenib [87,88]. Drugs against STAT3 that are currently in clinical trials for lymphoid malignancies and solid tumors include pyrimethamine, OPB-31121, OPB-51602, WP1066 or compound 53. In addition, a Phase I clinical trial with AZD9150 for patients with advanced HCC has been initiated [113,202]. Several other small molecular compounds and peptide inhibitors that affect STAT3 phosphorylation, dimerization, nuclear translocation or DNA binding are currently under development [113]. Sorafenib targets the Raf/MAPK pathway downstream of several receptor tyrosine kinases, such as VEGFR-2, VEGFR-3, PDGFRβ, Flt-3, Ret and Kit. Moreover, sorafenib decreases STAT3 activation in a JAK2-independent but MAPK- and AKT-dependent manner, which may be crucial for its therapeutic effect on HCC [114]. However, oncogenic versus antioncogenic functions of STAT3 demand a more complex view on the therapeutic impingement and several aspects have to be considered. First, conceivably, STAT3 acts as an oncogene in established HCC, but has a tumor-suppressive function at early stages that is based on hepatoprotective activities in the environment of hepatic inflammation, fibrosis and cirrhosis [91,112]. This would implicate that interference with STAT3 activation could be beneficial for patients with advanced HCC stages but may facilitate formation of secondary HCCs in patients with liver cirrhosis. Notably,

HCCs are quite heterogeneous, representing different states of neoplastic transformation at the same time. Second, expression of p14^{ARF} in HCC is critical for the oncogenic function of STAT3, which is corroborated by upregulation of p19^{ARF} in DEN-induced STAT3-dependent HCC [109]. In agreement with these data, experimental anti-STAT3 intervention was more efficient in human hepatoma cell lines Huh7 and Snu475 that express p14^{ARF}, than in p14^{ARF}-negative HCC cell lines HepG2 and PLC/PRF/5 [87,88]. This underlines the need for careful stratification of HCC patients by molecular profiling in order to predict the efficacy or even adverse effects of anti-STAT3 therapy. Third, it may be very challenging to specifically interfere with STAT3 activity in tumor cells without touching the tumor stroma. Therefore, possible pro- and anti-oncogenic functions of STAT3 in the stromal compartment of HCCs should be better characterized.

Conclusion & future perspective

Despite considerable knowledge regarding molecular events leading to HCC, clinicians have to face the daunting fact that treatment options are still unsatisfactory. A major challenge is the identification of oncogene addictions in HCC. Assuming that these addictions exist and cannot be bypassed easily by alternative pathways, they would be attractive targets for anticancer therapies. Potential HCC driver candidates should be evaluated for tumor-cell autonomous and stromal functions. This is of particular importance for therapeutic strategies implicating STAT3. Mainly oncogenic functions of STAT3 in the

stroma have been reported and blocking STAT3 in HCC tumor cells and stroma could have a synergistic anticancer effect. However, it remains to be shown to what degree HCCs are addicted to STAT3 signaling. STAT3 exerts its protumorigenic function partly through mechanisms that are also implicated in hepatoprotection. Consequently, interference with STAT3 could have side effects and reduce the survival potential of hepatocytes in cirrhotic patients. This would further aggravate hepatic damage and foster development of secondary HCCs. Moreover, the modulating effects of certain tumor-suppressor protein ablations that may attribute tumor-suppressive functions to STAT3 should be considered. Therefore, interference with JAK/STAT3 signaling may be a promising strategy for HCC treatment, but requires further characterization of possible adverse effects and personalized medicine approaches.

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