6 Possible Involvement of Signal Transducer and Activator of Transcription-3 (STAT3) Signaling Pathway in the Initiation and Progression of Hepatocellular Carcinoma

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Abstract

Hepatocellular carcinoma (HCC) is the most common type of liver cancer and the third leading cause of cancer death worldwide, with 75 % of cases occurring in Southeast Asian countries like China, Hong Kong, Taiwan, Singapore, Korea, and Japan. The etiology of HCC is likely to involve interactions between multiple risk factors. The most commonly reported risk factors are nonspecific cirrhosis (21 %), followed by alcohol-induced liver disease (16 %), HCV infection (10 %), and HBV infection (5 %). In addition, obesity and type II diabetes are also suspected to increase the risk of acquiring liver cancer. Persistent activation of signal transducers and activators of transcription-3 (STAT3) is frequently observed several human cancers and transformed cell lines including HCC. The significance of constitutively STAT3 in HCC is due to its induction of several tumorigenic genes that substantially contribute to the initiation and progression of the malignancy. These include antiapoptotic proteins like Bcl-2, Bcl-xL,

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Mcl-1, XIAP, and survivin. Examples of other STAT3-regulated oncogenic genes include c-Myc and cyclin D1, which regulates cell proliferation; matrix metalloproteinase-9 which mediates cellular invasion; and vascular endothelial growth factor, which controls angiogenesis. Thus, novel agents that can suppress constitutive and/or inducible activation of STAT3 have the potential for HCC therapy. In this chapter, we discuss in detail the potential role of STAT3 signaling cascade both in HCC initiation and progression and also various therapeutic strategies employed to block aberrant activation of this proinflammatory transcription factor in HCC.

Keywords
HCC • STAT3 • Proliferation • Apoptosis • Angiogenesis

Abbreviations

Bad	Bcl2 associated death promoter protein
Bax	Bcl-2-associated X protein
Bcl2	B-cell lymphoma 2
Bcl-xL	B-cell lymphoma extra large
Bid	BH3 interacting-domain death agonist
c-myc	Myelocytomatosis cellular oncogene
CSF-1R	Colony-stimulating factor-1R
EGF	Epidermal growth factor
G-CSF	Granulocyte colony-stimulating factor
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HGF	Hepatocyte growth factor
IGF	Insulin-like growth factor
IL	Interleukin
IFN-γ	Interferon-gamma
JAK	Janus kinase
MMPs	Matrix metalloproteases
NAFLD	Nonalcoholic fatty liver disease
NASH	Nonalcoholic steatohepatitis
NF-κB	Nuclear factor kappa B
PDGF	Platelet-derived growth factor
PTPase	Protein tyrosine phosphatase
ROS	Reactive oxygen species
SOCS	Suppressor of cytokine signaling
STAT3	Signal transducer and activator of transcription 3
TGF	Transforming growth factor
VEGF	Vascular endothelial growth factor

6.1 Introduction: Risk Factors Associated with Initiation and Development of HCC

Hepatocellular carcinoma (HCC) is the fifth most prevalent cancer worldwide (Ferlay et al. 2010) and possibly the most common malignant tumor found among men (Dominguez-Malagon and Gaytan-Graham 2001; Subramaniam et al. 2013). Due to its late presentation, aggressiveness, and limited response to therapy, HCC has become the third most deadly cancer and causes approximately one million deaths annually (Ferlay et al. 2010; Carr et al. 2010). While considered a rare form of cancer in many western countries, HCC is endemic in East and Southeast Asia where over three-quarters of liver cancer-caused deaths occur (Ferlay et al. 2010). Infections with chronic hepatitis B (HBV)/hepatitis C virus (HCV) and associated liver cirrhosis/hepatitis have been attributed to more than 80 % of the cases of HCC (Lau and Lai 2008). For example, chronic hepatitis caused by HBV/HCV can cause significant damage to hepatocytes and adversely affect their normal functioning (Subramaniam et al. 2013; Nakamoto and Kaneko 2003) (Fig. 6.1).

In addition, various environmental risk factors also including aflatoxin B1 exposure, alcohol over-abuse, and cigarette smoking have been reported to contribute to the development of HCC (Subramaniam et al. 2013; Abdel-Hamid 2009). For example, an increased risk of mortality in

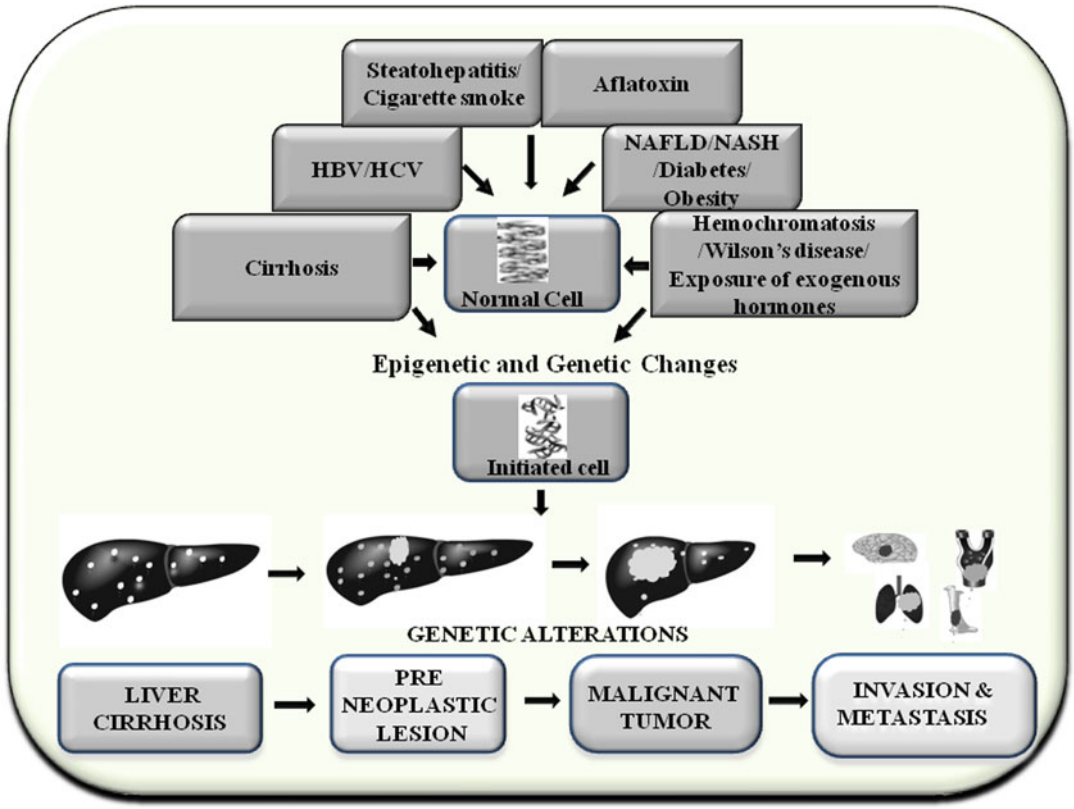

Fig. 6.1 A multi step cascade for HCC initiation and development

HCC patients has been closely associated with obesity. It has been found that obesity can induce an inflammatory response, which in turn may increase levels of proinflammatory cytokines [interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) expression] in adipose tissue and Kupffer cells (Subramaniam et al. 2013; Toffanin et al. 2010).

6.2 Role of STAT3 Signaling Pathway in the Initiation of HCC

Signal transducer and activator of transcription (STATs) were initially discovered in 1993 by James Darnell and can be activated by diverse stimuli to activate gene transcription (Shuai et al. 1993). STAT proteins have been in particular shown to play a critical role in cytokine signaling cascades that regulate various cell growth and differentiation signal transduction (Subramaniam et al. 2013). The STAT family consists of seven members; these are STAT1, STAT2, STAT3, STAT4, STAT5a, STAT5b, and STAT6 that can be further classified into two groups, according to their biological functions. The first group comprising of STAT2, STAT4, and STAT6 is reported to actively participate interferon-gamma (IFN-γ) signaling and T cell maturation. On the other hand, the second group consisting of STAT1, STAT3, and STAT5 is involved in development of mammary glands, embryogenesis, as well as oncogenesis (Subramaniam et al. 2013; He and Karin 2011).

Among various STAT family proteins, STAT3 has gained significant attention as it has been found to be an important regulator of distinct signal transduction pathways involved in liver damage and repair mechanisms (Subramaniam et al.

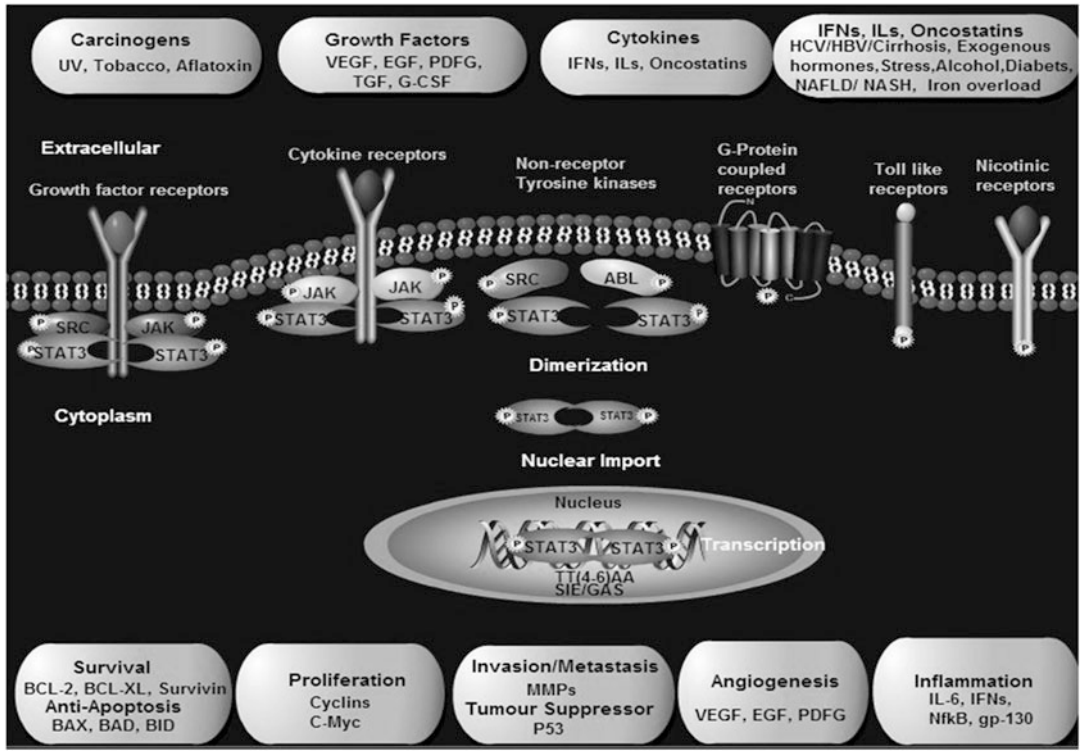

Fig. 6.2 STAT3 activation cascade involved in HCC progression

2013; Strain 1998; Taub 2003). STAT3 was initially demonstrated to be an acute-phase response factor that can bind to the IL-6 responsive element (Wegenka et al. 1993) and subsequently as a DNA-binding protein in response to epidermal growth factor stimulation (Zhong et al. 1994). STAT3 can be induced by various cytokines such as IL-6, leukemia inhibitory factor (LIF), oncostatin M, and ciliary neurotrophic factor (CNTF) that transmit their signals through the gp130 protein (Akira et al. 1994; Hibi et al. 1990; Hirano et al. 1997). Interestingly, the expression of IL-6 is elevated in various liver ailments and HCC (Subramaniam et al. 2013; Trikha et al. 2003; Naugler et al. 2007), and even IL-22-induced STAT3 phosphorylation on Ser727 residue can induce acute-phase genes in the liver (Dumoutier et al. 2000). In addition, STAT proteins can also be substantially stimulated by receptor tyrosine kinases such as epidermal growth factor receptor (EGFR), platelet-derived growth factor (PDGF-R), transforming growth factor (TGF), and colony stimulating factor-1R (CSF-1R) and seven-transmembrane G-protein-coupled receptors such as angiotensin II receptors (Karras et al. 1997). In fact, EGF, TGF-β, and PDGF receptors can even directly activate STAT3 proteins leading to enhanced proliferation and transformation (Subramaniam et al. 2013; Levy and Darnell 2002) (Fig. 6.2). The possible role of STAT3 in different aspects of liver tumorigenesis including transformation, inflammation, antiapoptosis, angiogenesis, cell cycle progression, and cellular invasion is discussed in detail below.

6.2.1 Oncogenic Transformation

Persistent activation of STAT3 is involved in several critical biological processes including growth, survival, invasion, and angiogenesis, all of which promote HCC initiation and progression (Turkson et al. 1998; Subramaniam et al. 2013). The first evidence related to the involvement

of STAT3 in transformation came to light initially after studies showed that STAT3 is constitutively activated during transformation induced by oncogene v-Src. Several subsequent studies also reemphasized the important finding that STAT3 signaling is indeed required for oncogenic transformation by v-Src (Cao et al. 1996; Chaturvedi et al. 1997; Bromberg et al. 1998). Deregulated STAT3 activation has been consistently observed in HCC clinical samples and cell lines but not in non-transformed liver cells (Subramaniam et al. 2013; Yoshikawa et al. 2001; Niwa et al. 2005; Li et al. 2006). On the contrary, in a recent study, Schneller and colleagues elucidated the role of STAT3 in Ras-dependent HCC progression in the presence and absence of p19 (ARF)/p14 (ARF). They found that constitutive active STAT3 is tumor suppressive in Ras-transformed p19 (ARF−/−) hepatocytes, whereas the expression of STAT3 lacking Tyr (705) phosphorylation (U-Stat3) can enhance tumor formation. Accordingly, Ras-transformed STAT3 (Δhc)/p19 (ARF−/−) hepatocytes showed increased tumor growth, compared to those expressing STAT3, demonstrating a tumor-suppressor activity of STAT3 in cells lacking p19 (ARF) (Schneller et al. 2011). Moreover, Wu et al. found in another study that phosphorylated STAT3 expression in monocyte was significantly correlated to advanced clinical stage of HCC and a poor prognosis. They also noticed that pharmacological STAT3 inhibitor, NSC 74859, significantly suppressed tumor growth in mice with diethylnitrosamine (DEN)-induced HCC. Interestingly, NSC 74859 treatment also attenuated cancer-associated inflammation in DEN-induced HCC model (Wu et al. 2011). Moreover, Chen and coworkers evaluated the efficacy of combination therapy using cetuximab and NSC 74859 (a novel STAT3 inhibitor) in EGFR and STAT3 overexpressing hepatoma cells and found that NSC 74859 potentiated the antiproliferative effect of cetuximab in all three cell lines. siRNA knockdown of STAT3 increased the sensitivity of these cell lines to cetuximab, whereas STAT3 overexpression antagonized these effects (Chen et al. 2012a). Also, it has been reported that even multitargeted tyrosine kinase inhibitor sorafenib can inhibit growth and metastasis of HCC in part by blocking the MEK/ERK/STAT3 and PI3K/Akt/STAT3 signaling pathways, but independent of JAK2 and phosphatase shatterproof 2 (SHP2) activation (Pfitzner et al. 2004). All these above-cited reports and also the findings of our recently published review article (Subramaniam et al. 2013) clearly establish that the aberrant activation of STAT3 indeed plays a pivotal role in both HCC initiation and development. This is further supported by the fact that various novel STAT3 inhibitors have been identified in recent years that can suppress proliferation and induce apoptosis in various HCC cell lines and mouse models (Table 6.1).

6.2.2 Inflammation

Several reports indicate the potential role of HCC as a proinflammatory transcription factor in HCC and other liver diseases (Subramaniam et al. 2013; Pfitzner et al. 2004). STAT3 was initially discovered as an acute-phase response protein, thus suggesting its possible connection to inflammation (Wegenka et al. 1993). IL-6 is one of the key regulators of inflammation and predominantly exerts its biological effects through the activation of the STAT3 pathway (Zhong et al. 1994). Liang et al. recently tested the effect of IL-6 family cytokines Golgi phosphoprotein (GP73) mRNA and/or protein levels in human hepatoblastoma HepG2 cells. They found that levels of GP73 mRNA and protein were upregulated in HepG2 cells following treatment with either proinflammatory cytokine IL-6 or the related cytokine oncostatin M (OSM). Induction required the shared receptor subunit gp130 and correlated with increased tyrosine phosphorylation of STAT3. ELISA measurement of GP73 and IL-6 levels in the sera of patients with premalignant liver disease revealed a significant correlation between circulating levels of the two proteins. OSM levels were also elevated six- to sevenfold in sera from patients

Table 6.1 Reported STAT3 blockers in HCC cell lines and mouse models

Natural/synthetic inhibitors	Mechanism of inhibition	*Cell lines/mouse models*	References
Celecoxib	Inhibited JAK2 phosphorylation	Hep3B, HepG2, Huh-7, SNU-387, and SNU-449	Liu et al. (2011a)
Parthenolide along with TRAIL	Suppressed activation of JAK proteins	HepG2, Hep3B, and SK-Hep1	Carlisi et al. (2011)
Galiellalactone – a fungal metabolite from the ascomycete *Galiella rufa*	Exerted STAT3 inhibitory effect by covalently modifying a cysteine residue in the STAT3 DNA-binding domain	HepG2	Lavecchia et al. (2011)
XZH-5 small molecule	Reduced constitutive STAT3 phosphorylation at Tyr705 and the expression of STAT3- regulated genes	Hep3B, HepG2, Huh-7, SNU-387, and SNU-449	Liu et al. (2011b)
Sorafenib SC-1-synthetic molecule	Caused SHP-1-dependent STAT3 inactivation	HCC cell lines (PLC5, Huh-7, Hep3B, and Sk-Hep1)/nude mice with Huh xenografts	Tai et al. (2011)
Sorafenib with TRAIL	Upregulated SHP-1 activity	PLC5, Huh-7, Hep3B, and Sk-Hep1/nude mice with PLC5 xenografts	Chen et al. (2010)
3-[3,4-Dihydroxy-phenyl]-acrylic acid 2-[3,4-dihydroxy-phenyl]-ethyl ester (CADPE)	Inhibited both IL-6-mediated STAT3 activation and recruitment of STAT3 to the cyclin D1 promoter	Huh-7	Won et al. (2010)
FLLL32	JAK/STAT inhibitor suppressed STAT3 phosphorylation, STAT3 DNA-binding activity, and STAT3-regulated gene products	SNU-449, SNU-398, HEP3B, and SNU387	Lin et al. (2010) and Liu et al. (2010b)
LLL12	Inhibited IL-6-induced STAT3 phosphorylation	Hep3B, SNU-387, SNU-398, SNU-449	Liu et al. (2010a)
NSC-74859	Abrogated STAT3 activation	HepG2, PLC/PRF/5, Huh-7, SNU-398, SNU-449, SNU-182 and SNU-475, Huh-7 in nude mice	Lin et al. (2009)
ENMD-1198	Inhibited STAT3 phosphorylation	Huh-7 and HepG2	Moser et al. (2008)
Decoy ODN	Caused abrogation of STAT3-mediated cell cycle and antiapoptotic genes	HepG2, H7402, and PLC/PRF/5	Sun et al. (2008)
AG490	Janus kinase 2-specificinhibitor	Huh-1, Huh-7, HepG2 and Hep3B cells, Huh-7 tumors in athymic mice	Kusaba et al. (2007)

(continued)

Table 6.1 (continued)

Natural/synthetic inhibitors	Mechanism of inhibition	*Cell lines/mouse models*	References
IL-6 receptor fusion protein (IL-6-RFP)	A high-affinity cytokine-binding protein	HepG2	Metz et al. (2007)
YC-1	Inhibited STAT3 activity by enhancing the polyubiquitination of p-STAT3 (705) induced by cisplatin	HepG2, Hep3, and PLC	Lau et al. (2007)
Atiprimod	Suppressed STAT3 mediated through the inhibition of activation of upstream kinases c-Src, JAK1, and JAK2	Huh-7, HepG2, HepG2.2.15, and HepG2	Choudhari et al. (2007)
2′-*O*-methoxyethylribose-modified phosphorothioate antisense oligonucleotide (ASO)	Caused suppression of phosphorylated STAT3 and reduced its DNA-binding activity	HCCLM3, SNU423, Huh7, HCCLM3 nude mouse model	Li et al. (2006)
Stattic (non-peptide small molecule)	Inhibited SH2 domain, STAT3 dimerization, and DNA binding	HepG2	Schust et al. (2006)
Statins	Reduced IL-6-induced serine phosphorylation of transcription factorSTAT3	Hep3B	Arnaud et al. (2005)
SOCS-1 (peptide inhibitor)	(SOCS-1; also known as JAB and SSI-1) switched cytokine signaling "off" by means of its direct interaction with JAK	Human HCC lines SNU-182, SNU-423, SNU-387, SNU-398, SNU-449, SNU-475, and PLC/PRF/5	Yoshikawa et al. (2001)
Cyclopentenones, 2-(1-chloropropenyl)-4,5-dihydroxycyclopent-2-enone (CPDHC)	Suppressed IL-6and IL-6-dependent pathway by inhibiting the tyrosine phosphorylation of the STAT3 and STAT1 as well as the serine phosphorylation of the STAT3 by direct inhibition of JAK	HepG2	Weidler et al. (2000)
Celastrol	Abrogated JAK/STAT pathway and induced apoptosis of HCC cells in vitro and in vivo	C3A, HepG2, Hep3B, PLC/PRF5, and Huh-7	Rajendran et al. (2012)
β-Escin	Inhibited activation of upstream kinases c-Src, JAK1, and JAK2	HepG2, Huh-7, PLC/PRF5, wild, and STAT3 KO mice fibroblasts	Tan et al. (2010)
γ-Tocotrienol	Increased the expression of SHP-1 in HCC cells	HepG2, Huh-7 xenografts in nude mice	Rajendran et al. (2011a)
Butein	Inhibited activation of upstream kinases c-Src and JAK2 induced the expression of SHP-1	HepG2, SNU-387, HCCLM3, and PLC/PRF5/HCCLM3 nude mouse models	Rajendran et al. (2011b)

(continued)

Table 6.1 (continued)

Natural/synthetic inhibitors	Mechanism of inhibition	*Cell lines/mouse models*	References
Diosgenin	Induced the expression of Src homology 2 phosphatase 2 (SH-PTP2) that correlated with downregulation of constitutive STAT3 activation	HepG2, C3A	Li et al. (2010)
Luteolin	Accelerated ubiquitin-dependent degradation in the Tyr705-phosphorylated STAT3	HepG2, HLF, and HAK-1B	Selvendiran et al. (2006)
Cucurbitacin B	Inhibited STAT3 phosphorylation	HepG2 cells and mouse model	Zhang et al. (2009)
17-Hydroxy-jolkinolide B (HJB)	Reacted with cysteine residues of JAKs to form covalent bonds that inactivate JAKs	HepG2	Wang et al. (2009)

with either cirrhosis or HCC relative to controls without liver disease. Although there was an association between levels of GP73 and OSM in serum from people with liver cirrhosis, there was not a statistically significant correlation in HCC, thereby suggesting that the role of the proinflammatory cytokines in determining circulating levels may be complex (Liang et al. 2012). Furthermore, in various tumors, STAT3 can directly interact with nuclear factor NF-κB family member RELA (p65), keeping it localized in the nucleus and thereby contributing to constitutive NF-κB activation in cancer (Lee et al. 2009). Also, in a recent study, Mano and coworkers examined STAT3 activation, cytokine expression, and infiltration of tumor-associated macrophages in resected HCCs as well as the alteration of cell growth and migration by cytokine stimulation in HCC cell lines. They observed that in HCC specimens, the pSTAT3-positive group showed high levels of α-fetoprotein, large tumor size, frequent intrahepatic metastasis, high Ki-67 and Bcl-xL, poor prognosis, and high recurrence rate (Mano et al. 2013). Overall, their findings clearly indicate that STAT3 activation was correlated with aggressive behavior of HCC and may be mediated via tumor-associated macrophage.

6.2.3 Regulation of Apoptosis

STAT3 hyperactivation can also lead to increased transcription of various STAT3-regulated cell survival genes, e.g., Bcl-2, Bcl-xL, and survivin, Mcl-1, and XIAP, and thereby inhibiting pro-apoptotic proteins such as Bax, Bad, and Bid (Subramaniam et al. 2013; Al Zaid Siddiquee and Turkson 2008; Germain and Frank 2007). For example, Chen and coworkers reported that sorafenib can augment the antitumor effect of recombinant tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) in resistant HCC. They found that STAT3 played a significant role in mediating TRAIL sensitization and showed that sorafenib downregulated phospho-STAT3 (pSTAT3) and subsequently reduced the expression levels of STAT3-related proteins (Mcl-1, survivin, and cyclin D1) in a dose- and time-dependent manner in TRAIL-treated HCC cells. Knockdown of STAT3 by RNA interference overcame apoptotic resistance to TRAIL in HCC cells, and ectopic expression of STAT3 in HCC cells abolished the TRAIL-sensitizing effect of sorafenib (Chen et al. 2010). Moreover, Liu et al. reported that IL-6 promoted survival of human liver cancer cells through activating STAT3 in response to doxorubicin

treatment. Neutralizing IL-6 with anti-IL-6 antibody decreased survival of SNU-449 cells in response to doxorubicin. Also, targeting STAT3 with STAT3 siRNA reduced the protection of IL-6 against doxorubicin-induced apoptosis, indicating that STAT3 signaling contributed to the antiapoptotic effect of IL-6. They also observed that LLL12, a STAT3 small molecule inhibitor, can block IL-6-induced STAT3 phosphorylation, resulting in the attenuation of the antiapoptotic activity of IL-6. Overall, these results demonstrated that targeting STAT3 signaling could interrupt the antiapoptotic function of IL-6 in HCC cells (Liu et al. 2010a). Furthermore, Peroukides and colleagues studied by immunohistochemistry the protein expression of survivin in relation to cyclin D1, p-STAT3, beta-catenin, E-cadherin, and p-Akt in 69 cases of HCC and adjacent liver cirrhosis. Survivin was expressed in 63/69 (91.3 %) cases of HCC and in 40/47 (85.1 %) cases of liver cirrhosis. Survivin localization in HCC was exclusively nuclear, while intense cytoplasmic and low nuclear expression of survivin was observed in cases of cirrhosis. Survivin expression in HCC correlated significantly with low-grade tumors and expression of cyclin D1 and p-STAT3. Expression of survivin in liver cirrhosis correlated with downregulation of E-cadherin expression. Overall, they noticed a clear association of nuclear survivin with well-differentiated HCC, as well as with the expression of the cell cycle regulator cyclin D1 (Peroukides et al. 2010). Interestingly, Chen and coworkers recently reported that a novel obatoclax derivative, SC-2001, can induce apoptosis through SHP-1-dependent STAT3 inactivation in HCC cells (Chen et al. 2012b).

6.2.4 Cell Cycle Progression

It has been documented that the expression of cyclin D1, which can associate with cdk4 or cdk6 and controls progression from G1 to S phase, is elevated in STAT3-C expressing cells (Bromberg et al. 1999). Also, several studies have shown that dysregulated expression of cell cycle-related proteins, such as cyclin D1, cyclin-dependent kinase 4 (Cdk4), cyclin E, cyclin A, p16, and p27, may significantly contribute to both HCC initiation and progression (Subramaniam et al. 2013; Matsuda and Ichida 2006). Guo and coworkers recently showed that $p27^{-/-}$ mice display increased proliferation and decreased apoptosis of tumor cells, accompanied by an increase in the serum inflammatory cytokines IL-6 and TNF-α. Furthermore, they observed that the increased number and STAT3 phosphorylation status of infiltrated inflammatory cells was accompanied by increased IL-6 and TNF-α mRNA levels in tumor and normal liver tissue in the $p27^{-/-}$ mice. Overall, their data demonstrated that the loss of p27 promotes carcinogen-induced HCC genesis and progression via the elevation of inflammatory cytokines and the augmented activation of STAT3 signaling in tumor cells and infiltrated inflammatory cells (Guo et al. 2013). On the contrary, Hu et al. found that low doses of NSC 78459 (a novel STAT3 inhibitor) had little effect on HCC cell proliferation but efficiently inhibited STAT3 activation. Huh-7, Hep3B, and HepG2 cells, with epithelial phenotypes, displayed significantly enhanced doxorubicin cytotoxicity following cotreatment with NSC 74859, whereas mesenchymal SNU-449 cells did not show significant enhancement. NSC 74859 inhibited STAT3 activity and suppressed doxorubicin-induced epithelial-mesenchymal transition (EMT) in epithelial HCC cells. siRNA-mediated STAT3 knockdown resulted in EMT inhibition, which led to attenuation of NSC 74859-mediated chemosensitivity. Collectively, their data indicated that STAT3 deactivation and associated EMT attenuation contribute to the synergistic antitumor effects of combined NSC 74859/doxorubicin therapy (Hu et al. 2012).

6.2.5 Angiogenesis

A large number of studies have implicated the critical role of STAT3 in the process of angiogenesis that facilitates formation of new blood vessels from existing ones to supply nutrients to tumor cells (Subramaniam et al. 2013; Folkman 1990). Ji and colleagues found that angiotensin II (Ang II) can upregulate angiogenic factors

production such as vascular endothelial growth factor (VEGF), angiopoietin-2 (Ang-2), and Tie-2 in HCC (MHCC97H) cells in a time- and concentration-dependent manner. Moreover, Ang II-induced JAK2 and STAT3 phosphorylation was significantly suppressed by losartan but not PD123319. Further, STAT3 phosphorylation and SOCS3 expression induced by Ang II were evidently impaired by AG490. More importantly, SOCS3 siRNA remarkably reinforced Ang II-induced VEGF, Ang-2, and Tie-2 generation in MHCC97H cells (Ji et al. 2012). Additionally, it has been also noticed that the cross-talk pathway between AngII and the EGFR mediated by EGF-like ligands cleaved by a disintegrin and metalloprotease is involved in the proliferation and invasion activities of several HCC cell lines (Itabashi et al. 2008). Moreover, aberrant VEGF expression is considered to be an important clinical feature in HCC and may correlate with HCC tumor invasion and metastasis (Subramaniam et al. 2013; El-Assal et al. 1998). In another recent study, Jia et al. tested the effect of a combination therapy consisting of endostatin (a powerful angiogenesis inhibitor) and STAT3-specific small interfering RNA, using a DNA vector delivered by attenuated *S. typhimurium*, on an orthotopic HCC model in C57BL/6 mice. Although antitumor effects were observed with either single therapeutic treatment, the combination therapy provided superior antitumor effects. Correlated with this finding, the combination treatment resulted in significant alteration of STAT3 and endostatin levels and that of the downstream gene VEGF, decreased cell proliferation, induced cell apoptosis, and inhibited angiogenesis (Jia et al. 2012). Also, silencing of STAT3 expression by RNA interference has been reported to significantly inhibit expression of STAT3 mRNA and protein and suppress the growth of human HCC in tumor-bearing nude mice through the downregulation of survivin, VEGF, and c-myc and upregulation of p53 and caspase-3 expression (Li et al. 2009). Interestingly, Lang and coworkers also found that the dual inhibition of Raf and VEGFR2 reduces growth and vascularization of HCC in a subcutaneous tumor model (Lang et al. 2008).

6.2.6 Cellular Invasion

Several studies have shown that STAT3 is intimately linked to the process of tumor invasion in HCC (Subramaniam et al. 2013). STAT3 activation can modulate the expression of matrix metalloproteinases MMP-1, MMP-2, and MMP-9 which in turn can mediate tumor migration and invasion (Subramaniam et al. 2013; Xie et al. 2006). Yan and colleagues recently identified the presence of mesenchymal stem cells (MSCs) in HCC tissues. They demonstrated that liver cancer-associated MSCs (LC-MSCs) significantly enhanced tumor growth in vivo and promoted tumor sphere formation in vitro. LC-MSCs also promoted HCC metastasis in an orthotopic liver transplantation model. cDNA microarray analysis showed that S100A4 expression was significantly higher in LC-MSCs compared with liver normal MSCs (LN-MSCs) from adjacent cancer-free tissues and that S100A4 secreted from LC-MSCs can promote HCC cell proliferation and invasion. They also noticed that S100A4 promoted the expression of miR-155, which mediates the downregulation of suppressor of cytokine signaling 1 (SOCS1), leading to the subsequent activation of STAT3 signaling. This promoted the expression of MMP9, which resulted in increased tumor invasiveness (Yan et al. 2013). Lin et al. noticed significantly greater STAT3 and tyrosine-phosphorylated STAT3 in human HCC tissues than in human normal liver. Further, in HCC cells with loss of response to TGF-beta, NSC 74859, a STAT3-specific inhibitor, markedly suppresses growth. In contrast, CD133 (+) status did not affect the response to STAT3 inhibition: both CD133 (+) Huh-7 cells and CD133 (−) Huh-7 cells are equally sensitive to NSC 74859 treatment and STAT3 inhibition. Thus, the TGF-beta/beta2 spectrin (beta2SP) pathway may reflect a more functional "stem/progenitor" state than CD133. Overall, their findings indicate that inhibiting interleukin 6 (IL6)/STAT3 in HCCs with inactivation of the TGF-beta/beta2SP pathway may be an effective approach in management of HCCs (Lin et al. 2009).

6.3 Link Between Oxidative Stress and STAT3 Activation

Several reports in literature also indicate a critical link between oxidative stress and STAT3 activation in various human malignancies, including HCC (Wang et al. 2011; Toyokuni et al. 1995). For example, a study by Kamata and coworkers showed that inactivation of IKK-β in HCC cells or hepatocytes favors the accumulation of ROS which oxidize the catalytic cysteine of various protein tyrosine phosphatases (PTPs) (Kamata et al. 2005), including SHP1 and SHP2 [the phosphatases that dephosphorylate STAT3 and JAK2] (Valentino and Pierre 2006). Oxidation of SHP1 and SHP2 results in loss of their catalytic activity and accumulation of phosphorylated and activated JAK2 and STAT3, which stimulate the proliferation and tumorigenic growth of NF-κB-deficient HCC (He et al. 2010). Sustained oxidative stress is continuously maintained in tumor cells (Toyokuni et al. 1995). Interestingly, many HCC risk factors, including HCV infection and hepatosteatosis, cause oxidative stress (El-Serag and Rudolph 2007; Parekh and Anania 2007; Wang and Weinman 2006), and just like JNK, STAT3 can also be activated in response to ROS accumulation (He et al. 2010). STAT3 was required for the activation of several immediate-early genes at the gene expression level, including c-*fos* and *jun*B. These two genes are the most strongly affected immediate-early genes in IL-6/livers, and their expression is likely to be directly regulated by STAT3 because their full transactivation requires the STAT-binding elements in their promoters (Wagner et al. 1990; Coffer et al. 1995). In addition, a positive correlation between c-jun and STAT3 was observed in the HCC progression (Zhang et al. 1999), c-jun being the first discovered nuclear proto-oncogene (Maki et al. 1987). The c-Jun interaction does not occur with STAT1. Furthermore, there are a number of enhancer elements that contain c-Jun and STAT3 sites. The transcription factor c-Jun was found to interact with activated STAT3, and STAT3 supplemented the transcriptional activation capacity of c-Jun in a transfection assay (Schaefer et al. 1995). These results suggest that STAT3/JAKs signaling cascade may also contribute to malignant transformation of hepatocytes besides Ras/Raf/ MAPK signaling pathway in HCC (Feng et al. 2001). Also, Machida et al. reported that HCV infection can cause production of ROS and lead to the reduction of mitochondrial transmembrane potential (Delta Psi(m)) in HCV-infected cell cultures. Furthermore, an inhibitor of ROS production, antioxidant *N*-acetyl-L-cysteine (NAC), or an inhibitor of nitric oxide (NO) prevented the alterations Delta Psi(m). The HCV-induced DSB was also abolished by a combination of NO and ROS inhibitors. These findings indicated that the mitochondrial damage and DSBs in HCV-infected cells were mediated by both NO and ROS (Machida et al. 2006).

6.4 Conclusion and Perspectives

This chapter clearly indicates that STAT3 activation plays a major role in both HCC initiation and development, and thereby the abrogation of STAT3 activation using novel pharmacological inhibitors can form the basis of future HCC therapy. Interestingly, a number of strategies, including the use of antisense oligonucleotide targeting STAT3, synthetic drugs (including AG490, YC-1, ENMD-1198, LLL12, NSC-74859, XZH-5, sorafenib, and celecoxib), small molecules derived from natural sources (diosgenin, β-escin, butein, celastrol, γ-tocotrienol, garcinol, honokiol, emodin, ursolic acid, capsaicin, resveratrol, curcumin), and gene therapy techniques have been reported to suppress STAT3 signaling cascade in different HCC cell lines and mouse models. An important issue related to the safety of these blockers still remains to be addressed as inhibition of STAT3 in normal tissues may have detrimental effects. Hence, to further develop STAT3 pharmacological blockers for potential clinical application, complete toxicological and pharmacokinetics analysis in HCC mouse models should be carried out in future studies.

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Conflict of Interest The authors declare that they have no conflict of interest.

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