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Review

Potential role of signal transducer and activator of transcription (STAT)3 signaling pathway in inflammation, survival, proliferation and invasion of hepatocellular carcinoma

Aruljothi Subramaniam [a,b,1], Muthu K. Shanmugam [a,1], Ekambaram Perumal [b], Feng Li [a], Alamelu Nachiyappan [a], Xiaoyun Dai [a], Shivananju Nanjunda Swamy [c], Kwang Seok Ahn [d], Alan Prem Kumar [a,e,f,g], Benny K.H. Tan [a], Kam Man Hui [h,*], Gautam Sethi [a,e,**]

[a] Department of Pharmacology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore 117597
[b] Molecular Toxicology Lab, Department of Biotechnology, Bharathiar University, Coimbatore 641046, Tamil Nadu, India
[c] Department of Biotechnology, Sri Jayachamarajendra college of Engineering, JSS Technical Institutions Campus, Mysore 570006 Karnataka, India
[d] Kyung Hee University, College of Oriental Medicine, Seoul 130-701, Republic of Korea
[e] Cancer Science Institute of Singapore, National University of Singapore, Centre for Translational Medicine, 14 Medical Drive, Singapore 117599
[f] School of Biomedical Sciences, Faculty of Health Sciences, Curtin University, Western Australia
[g] Department of Biological Sciences, University of North Texas, Denton, Texas, USA
[h] Division of Cellular and Molecular Research, Humphrey Oei Institute of Cancer Research, National Cancer Centre, Singapore 169610

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ABSTRACT

Hepatocellular carcinoma (HCC) is one of the most lethal malignancies, and is also the fourth most common cancer worldwide with around 700,000 new cases each year. Currently, first line chemotherapeutic drugs used for HCC include fluorouracil, cisplatin, doxorubicin, paclitaxel and mitomycin, but most of these are non-selective cytotoxic molecules with significant side effects. Sorafenib is the only approved targeted therapy by the U.S. Food and Drug Administration for HCC treatment, but patients suffer from various kinds of adverse effects, including hypertension. The signal-transducer-and-activator-of-transcription 3 (STAT3) protein, one of the members of STATs transcription factor family, has been implicated in signal transduction by different cytokines, growth factors and oncogenes. In normal cells, STAT3 activation is tightly controlled to prevent dysregulated gene transcription, whereas constitutively activated STAT3 plays an important role in tumorigenesis through the upregulation of genes involved in anti-apoptosis, proliferation and angiogenesis. Thus, pharmacologically safe and effective agents that can block STAT3 activation have the potential both for the prevention and treatment of HCC. In the present review, we discuss the possible role of STAT3 signaling cascade and its interacting partners in the initiation of HCC and also analyze the role of various STAT3 regulated genes in HCC progression, inflammation, survival, invasion and angiogenesis.

Contents

* Corresponding author. Tel.: +65 64368337; fax: +65 62263843.
** Corresponding author at: Department of Pharmacology, Yong Loo Lin School of Medicine, Cancer Science Institute of Singapore, National University of Singapore, Singapore 117597. Tel.: +65 65163267; fax: +65 68737690.
E-mail addresses: cmrhkm@nccs.com.sg (K.M. Hui), phcgs@nus.edu.sg (G. Sethi).
[1] Both authors contributed equally to this work.

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1. Introduction

The liver is the central organ for xenobiotic metabolism and executes several key biological functions to maintain homeostasis and health. These activities include production of proteins and hormones, detoxification of foreign chemicals, as well as glucose and lipid metabolism [1]. There are four major types of liver diseases that severely affect liver health [2]. These are (1) liver cirrhosis, (2) fatty liver, (3) virus-induced hepatitis and (4) liver cancer. Hepatitis and liver cancer have been considered as the most serious global public health problems [3–5]. Hepatocellular carcinoma (HCC), accounts for >90% of all primary liver cancers and is the fifth most common and aggressive malignancy worldwide and the third cause of global liver cancer mortality [6–8]. It accounts for approximately one million deaths each year [9] with median survival duration of 7 to 8 months from the time of diagnosis [10]. The major risk factors for HCC development include cirrhosis, hepatitis, infections with chronic hepatitis B (HBV) or C (HCV) viral infections and environmental factors like aflatoxin exposure and alcohol or tobacco consumption [2,11,12]. For example, liver cirrhosis causes functional abnormalities that are characterized by serum albumin level that is lower than 4 g\dl, increased prothrombin time and persistently high serum alanine amino transferase (ALT) levels and predisposes patients to the increased risk of HCC [2]. Similarly, chronic hepatitis caused by HBV or HCV viral infections can result in death of hepatocytes with accompanying inflammatory cell infiltration. Virus-infected hepatocytes can be destroyed by host immune cells or by potential degenerative effects of either HBV or HCV [11,12]. Continuing hepatocyte death triggers compensatory repair and regeneration and eventually leads to severe fibrosis, the major clinical risk factor for the development of HCC [4,5,13].

The HBV is a DNA virus that can integrate into the host genome and is considered a major risk factor for initiation and development of HCC [14]. The *HBx* gene of HBV encodes a viral protein that plays a central role in HBV infection and liver cancer [15–17]. Point mutations in the *HBx* gene leading to exchanges at position 31 with serine to alanine (Ser31Ala), position 130 with lysine to methionine (Lys130Met), and position 131 with valine to isoleucine (Val131Ile) were found to be prevalent in patients with HCC [18,19]. The HBx protein is thought to play a major role in HCC by modifying apoptosis, inhibiting nucleotide excision and repair of damaged cellular DNA, and modulating transcriptional activation of cellular growth regulating genes [20]. Strong epidemiological evidence correlating HCC to HBV infection is demonstrated by the presence of HB surface antigen (HBs Ag) and HB core antibodies (HBc antibodies) in the blood of HCC patients [21]. Hepatocyte transformation may also be indirectly influenced by HBV DNA integration [22]. Integrated HBV DNA is frequently observed in HCC, thereby suggesting that HBV has a direct oncogenic effect through interaction with transformation-associated genes [14]. Similarly HCV is an enveloped single-stranded positive-sense RNA virus, approximately 9.6 kb in length, and encodes a polyprotein of about 3000 amino acids [23] which is processed by viral-encoded and host-encoded enzymes into structural and non-structural proteins. This RNA virus does not integrate into the host genome but likely induces HCC through viral proteins by host–protein interactions or via the proinflammatory response to the virus [14]. Several HCV proteins, including core, NS3, and NS5A, have been shown to induce oxidative stress in cultured cells [24,25]. ROS, which act as second messengers, activate cellular kinases, although the mechanism of this activation remains unclear.

Alongside with etiological risk factors, environmental risk factors have also been reported to contribute to the development of HCC (Fig. 1). Alcoholic liver disease (steatohepatitis) has been shown to be an important risk factor for HCC development [26]. Alcohol either directly initiates HCC after its oxidation to acetaldehyde, which is genotoxic, or indirectly through causing cirrhosis [26,27]. Cigarette smoking is considered as one of the primary sources of exposure to 4-aminobiphenyl in humans. An important report measuring DNA adducts of 4-aminobiphenyl, a hepatic carcinogen showed a significant increase in HCC risk with increasing levels of adducts [27]. Diabetes increases the risk of HCC, as shown by the first population-based study to assess the risk of HCC in diabetic patients [28]. An increased risk of cancer mortality in general has long been associated with obesity [29].

It has been proposed that lipid accumulation in obesity induces a low-grade inflammatory response, which in turn increases IL-6 and TNF expression in adipose tissue and Kupffer cells. IL-6 and TNF are the main mediators in the development of steatohepatitis through activation of the JAK/STAT pathway leading to HCC development [30]. Non-alcoholic fatty liver disease (NAFLD), known as non-alcoholic steatohepatitis, is a risk factor for progressive liver disease [29]. Hepatic iron overload, or hemochromatosis can also lead to cirrhosis and ultimately to HCC. HCC is an important cause of mortality in cirrhotic patients with chronic hemochromatosis [31]. Increased cancer risk in a cohort of 230 patients with hereditary hemochromatosis in comparison to matched control patients with non-iron-related chronic liver disease [31]. It is an autosomal recessive condition with a homozygous C282Y mutation in the HFE gene characterized by excessive iron deposition in hepatocytes due to increased intestinal absorption of iron from normal diet [32]. Thus, while liver disease is the commonest cause of death in patients with hereditary hemochromatosis, 6% of men and 1.5% of women are at absolute risk of liver cancer [31,32]. Aflatoxin is a mycotoxin that commonly contaminates corn, soybeans and peanuts, and is reported to be a cause for hepatocarcinogenesis [33]. High dietary aflatoxin intake has been associated with HCC. In another study from Shanghai, the odds of developing HCC in individuals with HBV and exposure to aflatoxin were 59.4 times that of the normal population [34]. Sex hormones, such as estrogens, progesterone, and oral contraceptives have been shown to increase hepatic tumor development in animals. Moreover, there have been several reports of HCC developing in patients who have been treated with androgenic or anabolic steroids or oral contraceptives [35,36].

2. Role of STAT signaling pathway in the development of HCC

Signal transducer and activator of transcription (STAT) protein was first discovered in 1993 by James Darnell [37]. It can be induced

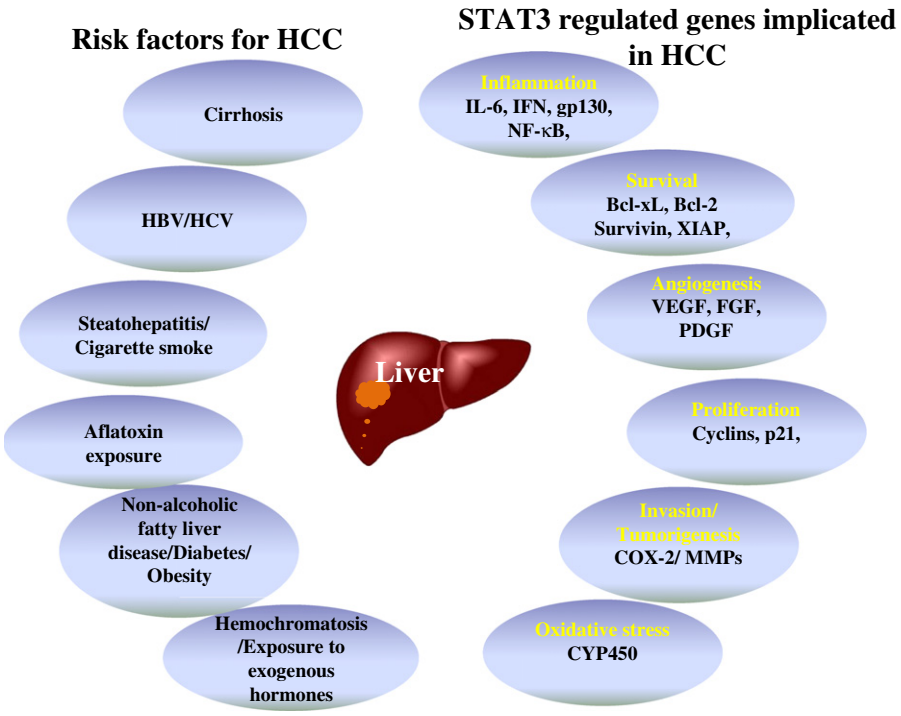

Fig. 1. Abbreviations used: HBV, hepatitis B virus; HCV, hepatitis C virus, IL-6, interleukin 6, IFN, interferon; Bcl-xL, B-cell lymphoma-extra large; Bcl-2, B-cell lymphoma 2; XIAP, X-linked inhibitor of apoptosis protein; VEGF, vascular endothelial growth factor; FGF, fibroblast growth factor; PDGF, platelet derived growth factor; MMP; matrix metalloproteinases; gp130, glycoprotein 130; COX-2, cyclooxygenase 2; CYP, cytochrome.

by signals from the cell membrane directly to the nucleus to activate gene transcription, thus evading the involvement of secondary messengers [37]. STAT proteins have been shown to play pivotal roles in cytokine signaling pathways, which are involved in regulating cell growth and differentiation in systems ranging from *Dictyostelium* to mammals [38]. STAT proteins have also been identified in *Drosophila*, [39,40] *Caenorhabditis elegans* and [41] *Anopheles* [42] but are strikingly absent in yeast [43]. The STAT family comprises seven members: STAT1, STAT2, STAT3, STAT4, STAT5a, STAT5b, and STAT6. They range in size from 750 to 850 amino acids [44], (Fig. 2). The entire STAT family can be divided into two groups, according to their specific functions. The first group is comprised of STAT2, STAT4, and STAT6, which are activated through a small number of cytokines and are engaged in the development of T-cells and IFN-γ signaling. The other group consists of STAT1, STAT3, and STAT5, which are activated in different tissues through a series of ligands and are involved in IFN signaling, development of mammary glands and response to GH, and embryogenesis, respectively. This latter group of STATs plays a key role in controlling cell-cycle progression and apoptosis and thus contributes to oncogenesis [45]. All the STAT members are organized on 3 different chromosomes. In the human genome, STAT1 and STAT4 are clustered on chromosome 2, whereas STAT3, STAT5a, and STAT5b are huddled together on chromosome 17, and STAT2 and STAT6 are assembled on chromosome 12 [44,46].

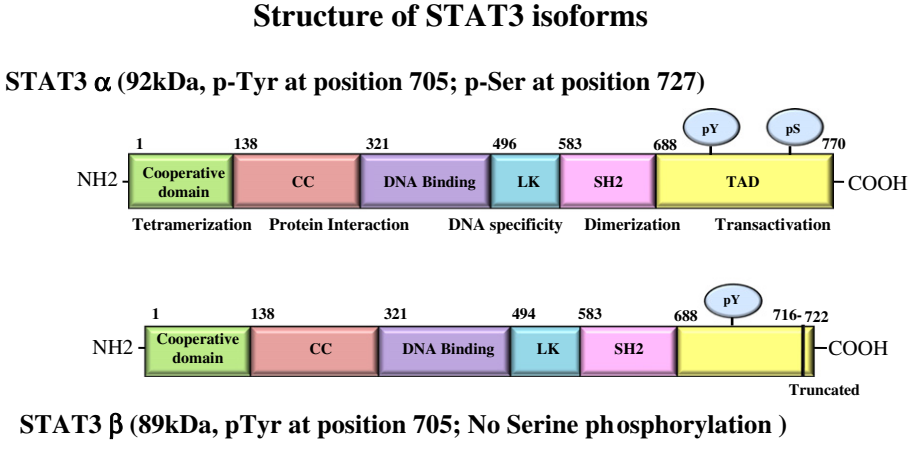

Fig. 2. The N-terminal domain mediates STAT dimer–dimer interaction to form a tetramer. This interaction is essential for stabilizing the binding of dimers to DNA. The DNA binding domain makes physical contact with STAT3-response elements in promoters of target genes and is linked to the Src homology 2(SH2) domain by the linker domain. The phosphorylation of tyrosine residue in the dimerization region mediates the interaction with the SH2 domain of another monomer that stabilizes STAT dimer formation. The STAT3β splice variant contains a COOH- terminal deletion resulting in an altered open reading frame. This truncated isoform still dimerizes and binds to DNA but fails to activate gene expression.

Among other STAT family proteins, STAT3 has received considerable attention during the last decade since it is a convergent point for a number of oncogenic signaling pathways and controls intra-cellular signal transduction pathways of several pro-inflammatory cytokines and growth factors that are implicated in liver damage and repair mechanisms [47–50]. STAT3 was initially identified as an acute phase response factor (APRF), an inducible DNA binding protein that binds to the IL-6 responsive element within the promoters of hepatic acute phase genes [51] and as a DNA binding protein in response to epidermal growth factor [52]. The gene that encodes STAT3 is located on chromosome 17q21. The 92 kDa protein is 770 amino acids long [53]. A splice variant of the mammalian STAT3 with deletion of a 50 nucleotides sequence near the C-terminus, codes for an 80-kDa STAT3 isomer (STAT3b) that functions as a negative regulator of STAT3 [20].

STAT3 can be activated by IL-6, leukemia inhibitory factor (LIF), oncostatin M, and the ciliary neurotrophic factor (CNTF) family of cytokines, which are all known to mediate their signal through the gp130 protein [54–56]. These receptor molecules harbor a common STAT3 docking motif (YxxQ) in their cytoplasmic domain [57]. Indeed, the expression of IL-6, one of the major STAT3 activating cytokines, is elevated in human liver diseases and HCC [58,59]. IL-6 is a key event in tumorigenesis with high levels associated with HCC [60]. One of the normal functions of IL-6 in adult liver is to protect against apoptosis via STAT3 pathway following viral infection or ingestion of chemicals [61,62]. When IL-6 binds to its specific receptor subunit, it can induce dimerization of the gp130 receptor and activation of the gp130-associated Janus kinase (JAK). As in the gp130-deleted animals, no STAT3 DNA binding was found, and activation is likely to be mediated through a gp130-dependent cytokine while in IL-6 null animals, no STAT3 activation was found [63]. IL-6 levels in liver cancer patients are 25-fold higher than in healthy adults [64]. Subsequent studies showed that the levels of IL-6 in SNU-387 and SNU-449 liver cancer cell lines were much higher than those in human hepatocytes [65]. Acute-phase response is impaired in both IL-6 and STAT3-deficient animals. It is most likely that the L−/− STAT3 mice failed to survive because there was a disturbance in the acute-phase response, which is fundamentally dependent on STAT3 activation [66]. Another cytokine, IL-22 induced phosphorylation of STAT3 on a serine residue and has been shown to induce acute phase genes in the liver [67], an effect that has been described to be regulated by IL-6 mainly through STAT3 activation. It has been shown that STAT3 Ser727 phosphorylation is induced upon IL-22 stimulation and is required for maximal transcriptional activation [68].

Recent findings indicate that STAT activation is not mediated exclusively by cytokine receptors that lack intrinsic tyrosine kinase domains. STAT proteins are also triggered by receptor tyrosine kinases such as epidermal growth factor-receptor (EGF-R), PDGF-R [69], and colony stimulating factor-1R (CSF-1R) [70], seven transmembrane G-protein-coupled receptors such as angiotensin II receptor [71] and serotonin 5-HT2A receptor [72] and through the T cell receptor complex [73] and the CD40 receptor [74]. EGF, TGFβ, and PDGF receptors are also capable of directly phosphorylating STAT proteins in the absence of JAK activation, leading to the up-regulation of genes that promote cell proliferation, survival, and cell transformation [75–77].

The malignant transformation of hepatocytes in humans is a multi-step process that occurs through progressive sequential evolution of chronic liver injury, regeneration, fibrosis and cirrhosis, small cell dysplasia, and low-grade and high-grade dysplastic nodules, resulting in the formation of fully developed HCC [78,79]. Different subtypes of preneoplastic and neoplastic liver lesions may exhibit common alterations of some key signal transduction pathways that underlie cell survival and proliferation. Interference with these molecular mechanisms may be essential for HCC prevention and treatment. These may involve over expression of several cytokines (interleukins 1–11) and their receptors, growth factors and their receptors, including vascular endothelial growth factor (VEGF), and platelet-derived growth factor (PDGF) [69]. Transforming growth factor (TGF) and epidermal growth factor (EGF) receptors (also known as ErbB1), fibroblast growth factor (FGF)), and its FGF receptor, hepatocyte growth factor (HGF) receptor (with unchanged or diminished expression of HGF), and insulin-like growth factor (IGF) family members are also thought to play an important role in hepatocarcinogenesis. These ligands and their receptors activate various signal transduction pathways, including the JAK/STAT3 pathway, a critical signaling pathway that supports the proliferation of preneoplastic and neoplastic liver lesions [80].

3. Structure of STAT proteins

STAT proteins exhibit a modular structure with highly defined domains, which include the N-terminal coiled-coil domain, DNA binding domain, a linker, SH2 domain, and a C-terminal transactivation domain [77]. Each of these domains is important for the physiological functions of STAT proteins [81]. The analysis of the crystal structure of an NH2 conserved-terminal domain with ~130 residues show that it is formed of eight α-helices that can form a hook like interface which is involved in protein–protein polar interactions affecting transcription, and thereby enhance the ability of dimerized STATs to bind to DNA [82]. The N-terminal domain is involved in STAT dimerization and also in tertramerization interactions. The tetramerization of STATs contributes to stability of the STAT-DNA binding by means of the interaction with randomly arranged low-affinity STAT binding sites, thus increasing transcriptional activity [83]. Several studies have implicated the N-domain in various protein–protein interactions affecting transcription and it has been suggested that this domain enables dimerized STAT molecules to polymerize and to bind multiple DNA sites that are involved in oncogenic growth signaling pathways [84]. The region of STATs between residues 130 and 315 consists of a four-stranded helical coiled coil domain. This domain associates with a number of potentially important regulatory modifiers, including IRF-9 and STIP1 which are ligand dependent [85]. A sub-region of the C-terminal domain and the coiled-coil domain are necessary for receptor binding and functional recruitment of STAT3 to gp130 upon stimulation with IL-6 [86], (Fig. 2).

The DNA-binding domain forms complexes between STAT proteins and DNA and determines the DNA-binding specificity for each STAT protein [87]. In this domain, a region of beta-sheet structures is connected by unstructured loops. The DNA-binding domain adopts an immunoglobulin-fold structure, and binds to DNA as a dimer. It is also involved in nuclear translocation, probably by maintaining proper conformation for importin binding and to exportin when STAT is dephosphorylated and exiting the nucleus [88]. A linker domain from ~500–575 is α-helical followed by a classical -SH2 domain [89]. Domain SH2, sited in the region between the amino acid residues 600 and 700, is essential for the recruitment of STATs to phosphorylated receptors and for the dimerization between two activated STAT monomers through reciprocal phospho-tyrosine (pTyr)-SH2-domain interactions between monomeric STATs to form dimers [89]. The differences in the STAT SH2 domain bring about selectivity of the STAT protein-binding to the different cytokine receptors [20], which in turn appear to be critical for nuclear localization and DNA-binding activities. Thus, it is critical for the recruitment of STATs to the activated receptor complexes and is also required for the interaction with JAK and Src kinases. It is also possible that this domain participates in other protein–protein interactions that have not yet been fully deciphered [89]. The Src homology 2 (SH2) domain-containing protein tyrosine phosphatase, SHP-2, interacts with many proteins by recognizing the tyrosine-phosphorylated Y (I/V)X(L/V/I) motifs through its amino-terminal SH2 domain [90].

The C-terminal portion of the protein, which functions as the transcriptional activation domain (TAD), is natively unfolded and forms

structure only upon binding with interacting partners and is involved in communication with transcriptional complexes [89]. Immediately downstream of the SH2 domain, in position 705, is a tyrosine residue, which plays a critical role in STAT activation [20]. Three-dimensional structure of the Stat3β homodimer bound to DNA. Phosphorylation of this tyrosine appears to be achieved by growth factor receptors as well as JAK and Src kinases, depending on the nature of the cell type and the ligand/receptor interactions [89]. This has been found to be essential for the activation and dimerization of STATs. A conserved serine in this STAT domain (apart from STAT2 and STAT6, which have no such serine, is a phosphorylation site and regulates STAT transcriptional activity [89]. STAT1 and 3, which have an altered serine, have their transcriptional capacity reduced by 20% [91]. Structural determination of the transactivation domain is essential to understand its binding partners, which may provide crucial insight into the regulation of this domain, and how it interacts with other proteins in the transcriptional process.

4. Structure of JAK family proteins

JAKs phosphorylate STAT proteins when activated by signals from interleukins and other cytokines. The unique structure of the JAKs clearly separates them from other members of the protein tyrosine kinase family [92], (Fig. 3). The most critical feature of these proteins is the presence of two JAK homology (JH) domains, JH1 and JH2, which share extensive homology to tyrosine kinase domains. JH1 domain is a functional tyrosine kinase domain but the JH2 domain, lacks observable tyrosine kinase activity [93,94]. The SH2 domain also contains JH3 and JH4 domains [95]. However, despite of the homology to SH2 sequences, these regions do not directly bind to phosphotyrosine residues [96–98]. The JH6–JH7 domains comprise the FERM domain, and the residues located in the JH7 domain mediate binding to the box 1/proline-rich region of cytokine receptors [93,99,100]. Moreover, this interaction between JAKs and cytokine receptors can regulate receptor localization [101,102]. Specifically, the surface expression of EPO receptors is regulated by the FERM domain of JAK2 [103] and both JAK2 and TYK2 have been shown to inhibit the proteasomal degradation of the thrombopoietin receptor [99]. The selectivity of STAT activation by various ligands is determined mainly by the highly specific interactions between the SH2 domain and the phosphotyrosine residues on each receptor [94]. Although JAK kinases have not been shown to have any substrate specificities, they do have different specific biological functions, as demonstrated *in vivo* by gene targeting studies [104–106].

5. Src family of kinases (SFK)

Src comprises a family of 9 tyrosine kinases that regulate cellular responses to extra-cellular stimuli [107]. SFKs have a critical role in cell adhesion, invasion, proliferation, survival, and angiogenesis during tumor development [35]. They share similar structure and function. Over expression or high activation of SFKs occurs frequently in tumor tissues and they are central mediators in multiple signaling pathways that are important in oncogenesis [108]. SFKs can interact with tyrosine kinase receptors, such as EGFR and the VEGF receptor. The Src family of cytoplasmic non-receptor protein tyrosine kinases was first discovered in the context of the transforming retroviral oncogene v-src, which is responsible for the potent sarcoma-inducing activity of the Rous sarcoma virus [109]. Src kinase is representative of the non-receptor tyrosine kinases which are present in essentially all metazoan cells, where their regulated activation by diverse growth factor, cytokine, adhesion, and antigen receptors is critical for generating an appropriate cellular response to external stimuli [35,107]. The nine members of the Src family include Src, Lck, Hck, Fyn, Blk, Lyn, Fgr, Yes and Yrk. SFK proteins range in molecular mass from 52 to 62 kDa and share a conserved domain structure consisting of consecutive SH4 domain, unique domain, SH3 domain, SH2–SH3 linker, SH2 domain, SH1 (catalytic domain), and C-terminal negative regulatory region. The SH3 domain contributes to substrate recruitment [110,111] and is critical for the regulation of kinase activity [112–114], (Fig. 4). The SH3 domain can bind to proline rich peptide binding sites and thus is important for protein–protein interactions. The SH2 domain also functions in protein–protein interaction(s) by virtue of its affinity for phosphotyrosine-containing peptide sequences [115,116]. All family members also contain an SH4 membrane-targeting region at their N-terminus, is always myristoylated and sometimes palmitoylated prior to membrane localization [117,118]. The SH4 region is followed by an 'unique' domain, which is the only non-conserved region within the kinase family [119]. It contains 50–70 residues which are divergent among family members and also contain a C-terminal regulatory region [119]. The unique domain is followed by modular SH3 and SH2 domains, a regulatory linker, the catalytic or kinase domain (SH1 domain), and a C-terminal negative regulatory tail.

6. Activation of STAT3

STAT activation by phosphorylation is highly regulated and transient. Unphosphorylated STAT exists predominantly as dimers, with a small fraction as monomers and higher-order complexes, which shuttle continuously between the cytoplasm and nucleus in the latent state [120]. In this "resting" state, there is also a small fraction of STATs that is hypo-phosphorylated, with low level of serine, and threonine phosphorylation. In this latent state, the STAT dimer was postulated to be in the anti-parallel conformation in the cytoplasm as inactive homodimers [121]. STAT signaling is critical for normal cellular processes such as embryonic development, organogenesis and organ function, innate and adaptive immune function, regulation of cell differentiation, growth, and apoptosis [122–127]. In normal cells, STAT3 protein activation is strictly controlled to prevent unscheduled gene regulation, while constitutive activation of STAT3 has been detected in a wide number of human cancer cell lines and primary tumors, such as 50% of HCC, leukemias, lymphomas, multiple myelomas, prostate, gastric, breast, lung, and head and neck cancer [128–134].

Peak STAT3 phosphorylation occurs within 15–60 min of exposure to cytokine, and even in the presence of continuous cytokine,

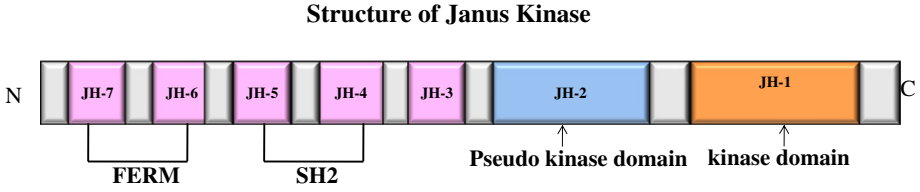

Fig. 3. Structural domains shown here are referred to as JAK homology regions (JH1-JH7). JAKs harbor four functional domains, the FERM domain, the SH2 domain, the pseudotyrosine kinase (TK) domain and a catalytically active TK domain.

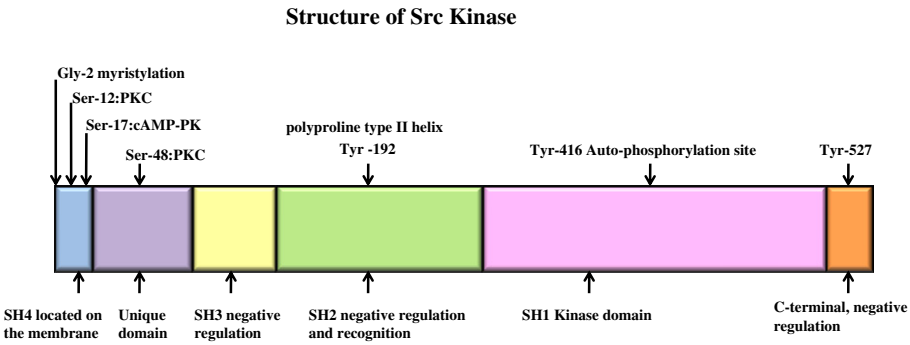

Fig. 4. Src family kinase family members contains a myristylation signal at the N-terminus end that regulate membrane binding followed by a unique domain that varies in different family members. A conserved auto-phosphorylation site (Tyr-416) and negative regulatory tyrosine (Tyr-527) is also unique feature of Src kinases.

STAT3 activation decreases over several hours [135]. This constitutive activation of STAT3 is not due to mutations in STAT3 but occurs due to deregulation of protein tyrosine kinases or constitutive release of growth factors that activate STAT3 [136]. Binding of cytokines or growth factors to their surface receptors resulted in rearrangement of antiparallel unphosphorylated STAT dimers in the cytoplasm as parallel unphosphorylated dimers [120]. The phosphorylation is mediated through the activation of non-receptor protein tyrosine kinases called Janus-like kinase (JAK). JAK1, JAK2, JAK3, and tyrosine protein kinase 2 have been implicated in the activation of STAT3 [137]. In addition, the role of c-Src kinase has been shown in STAT3 phosphorylation [138].

When IL-6 binds to its specific receptor subunit, it can induce dimerization of gp130 receptor and activation of the gp130-associated JAK [52,139]. The critical role of JAKs in cytokine signaling is evident by the inherited immune-deficiencies caused by mutations that prevent receptor-JAK interactions or the kinase activity of the JAKs [38]. JAKs can either bind to intracellular domains of cytokine receptor signaling chains or catalyze ligand-induced phosphorylation of intracellular tyrosine residues on the receptor [140]. The JAKs in turn phosphorylate the specific tyrosines in the intracellular domain of gp130, providing docking sites for the Src homology 2 (SH2) domain of signaling molecules of STAT3. Phosphorylation of STAT3 has been shown to occur both at the tyrosine 705 (Y705) and at the serine 727 (S727) residue on their cytoplasmic tail [141]. Homo- or heterodimerization of STATs are achieved via reciprocal binding of this critical pTyr of one monomer and SH2 domain of the partner dimer [52]. Activated STAT dimers can translocate into the nucleus and bind to specific elements [52]. STAT homodimers bind to members of the IFN-γ activated sequences (GAS) family of enhancers (TTCNNNGAA). Most STAT dimers recognize an 8- to 10-base pair inverted repeat DNA element with a consensus sequence of 5-TT(N)AA-3. The observed variation in the binding affinity of a particular activated STAT dimer for a single target DNA sequence is determined by differences in the nucleotide sequence [140]. Other known STAT modifications include arginine methylation, ubiquitination and sumoylation covalent modifications [142].

Unlike other STATs, such as STAT1 and STAT2, which accumulate in the nucleus only after phosphorylation, STAT3 can enter the nucleus independent of its phosphorylation. Activated STATs shuttle more rapidly than non-activated ones [143,144]. Direct interaction of unphosphorylated STATs with the nuclear pore proteins (nucleoporins) Nup153 and Nup214 allows carrier-independent nuclear translocation. Nuclear translocation of activated STATs is mediated by the karyopherin family of transport proteins called importins or exportins depending on their movement direction [121]. Cytokine-induced nuclear import involves binding to importin and following inactivation, the nuclear export of STATs involves a CRM1 (chromosome region maintenance 1)/exportin1-dependent process [88]. Specific sequence motifs on the surface of STATs, known as nuclear localization signals and nuclear export signals, allow STAT-importin and STAT-exportin interactions [88]. Additionally, specific adaptor molecules, the importin family, are involved in STAT–importin interaction. Distinct importin subtypes determine trafficking of different STATs. STAT activation with nuclear accumulation terminates within minutes [145,146]. Recently, nucleocytoplasmic shuttling of STAT3 was shown by fluorescence localization, after photobleaching (FLAP), to be a dynamic process that involves constitutive shuttling of unstimulated STAT3 in the absence of cytokine stimulation [144]. Both import and export signals contribute to the balanced shuttling and IL-6 induction reduces the nuclear export signal, resulting in nuclear accumulation of STAT3. Nuclear STAT3 can then bind to specific promoter elements on DNA and activate target gene transcription [139]. Extensive research in the past decades have shown that STATs can control cell growth and differentiation and unraveled many structural features, regulatory mechanisms and functions, and are involved in the regulation of the cells of the immune system and in development of organs and tissues. Most importantly STATs are activated in pathological states such as inflammation and cancer.

7. Functions of STAT3

The functions of STAT3 have been more difficult to define from knockout mice studies as the embryos die early in embryogenesis [147]. In fact, loss of STAT3 is lethal even to embryonic stem cells [148] indicating a key role for STAT3 in cell growth and/or survival. Also, by 7.5 days post-coitum, STAT3 mRNA is substantially expressed in the extra embryonic visceral endoderm, which is the principal site of nutrient exchange between the maternal and embryonic environments [148,149]. STAT3 signaling also seems to play important roles in several liver functions. Conditional knock-out of STAT3 expression partially impairs liver regeneration [150] whereas tissue-specific knock-out of hepatic STAT3 was found to affect glucose homeostasis and induction of insulin resistance [151]. In addition to being associated with cell growth, STAT3 activation has been found to be critical for differentiation of keratinocytes [152], and myeloid cells [153], and plays an important role in mediating the formation of epithelial tubules in response to hepatocyte growth factor [154]. Selective loss of STAT3 in keratinocytes results in impaired wound healing, and skin-specific STAT3-transgenic mice develop psoriasis [155]. *In-vitro* studies on keratinocytes have shown that STAT3 plays an important role in the migration of epidermal cells, and is essential for skin renovation [156].

STAT3 is also involved in the involution of the post-lactating mammary gland [157]. This is an apoptotic process involving the epithelial cells and results from an increased level of the insulin-like growth factor binding protein-5 (IGFBP5) [87,91,158]. Selective targeting of the STAT3b isoform was reported and mice exhibit diminished recovery

from endotoxic shock and hyper-responsiveness of some endotoxin-inducible genes in liver. This is the first *in vivo* evidence that STAT isoforms have essential *in vivo* functions [159]. Whether other STATs have similar cytoplasmic function is yet to be determined. In addition, the functions of STATs in other cytosolic compartments are obscure. The non-canonical regulation and function of non-phosphorylated STATs is certainly an exciting new area of research interest.

8. Negative regulators of STAT3

In normal cells, the extent and duration of STAT activation is controlled by a variety of mechanisms. These include feedback inhibition of the JAK/STAT pathway by suppressor of cytokine signaling proteins (SOCS) 1 and 3 or cytokine-inducible SH2-containing (CIS) protein through inhibition and/or degradation of JAKs, dephosphorylation of the receptor complex or nuclear STAT dimers by protein tyrosine phosphatases (PTPases) and interaction of activated STATs with inhibitory molecules from the protein inhibitors of activated STAT (PIAS) family [160–162]. Other physiological negative protein modulators of the STAT3 signaling pathway also involving negative regulation include the JAK binding protein (JAB) and STAT-induced STAT inhibitor [163].

Cytokine-inducible SH2-containing (CIS) protein, the first member of the SOCS family, was originally identified in 1995 as a cytokine-inducible early gene [164]. The most intensively studied group of negative regulators is the SOCS proteins [165]. Because of their Src homology 2 domains, this family of eight cytokine-inducible proteins (SOCS1–SOCS7 and CIS) binds phosphorylated receptors and/or JAKs and thereby interferes with signaling. The second member of the SOCS family, SOCS-1 (or JAK-binding protein, JAB; STAT-induced STAT inhibitor-1, SSI-1) was independently identified in 1997 by three different groups [166–168]. JAB was identified using a yeast two-hybrid system as a protein that bound the catalytic domain of JAK2 [167]. SSI-1 was identified as a STAT-inducible inhibitor using a monoclonal antibody to the sequence motif of the STAT3 SH2 domain [168]. SOCS-1, -2, and -3 were cloned as inhibitors of IL-6 signaling using the murine monocytic leukemic M1 cell line [166].

SOCS, also known as STAT-induced STAT inhibitor (SSI) protein family comprises several members including SOCS1, SOCS2 and SOCS3 which are encoded by genes located in 16p13.13, 12q, 17q25.3, respectively [164,169]. SOCS proteins have a variable NH2-terminal domain, a central SH2 domain, and a COOH-terminal domain, termed SOCS-box motif [170–172]. The SOCS box is a sequence of 40 amino acids that is conserved throughout the SOCS family. The SOCS box is thought to influence the stability of SOCS proteins [173–175]. The SOCS box appears to mediate interactions with Elongins B and C, which may target proteins for proteasomal degradation [175]. SOCS proteins are induced by cytokines and act in a negative feedback loop to inhibit the receptor. The SH2 domain of SOCS proteins is able to interact with a specific phosphorylated tyrosine residue in the kinase inhibitory region of JAK molecules with high affinity, resulting in the inhibition of STAT phosphorylation. Another domain in SOCS proteins interacts with elongins B and C, and couples the SH2 domain-binding proteins to the ubiquitin–proteasome pathway [175–177]. COOH-terminal domain SOCS box is responsible for the recruitment of the ubiquitin transferase complex. SOCS1 inhibits JAK activation through its N-terminal kinase inhibitory region (KIR) by direct binding to the activation loop of JAKs, while SOCS3 inhibits JAK kinase by binding to the cytokine receptor through its SH2 domain [174,176].

Among the eight members of the SOCS family, SOCS-1 and SOCS-3 appear to be relevant to several aspects of hepatic pathobiology. For example, SOCS-3 is up-regulated 40-fold 2 h after partial hepatectomy in mice [178]. It has been reported that SOCS-1 and SOCS-3, negative regulators of the JAK2-STAT signaling pathway, are silenced by methylation in human hepatoma cell lines and HCC tissues, which leads to constitutive activation of STAT3 in these cells [179,180]. Downregulation of the SOCS-1 may be a crucial event in the hepato-carcinogenic process leading to formation of HCC. Additionally, the results of several studies have shown that forced expression of SOCS1 prevents liver injury [181] and inhibits the abnormal growth of HCC cells [179,182].

The protein inhibitors of activated STATs (PIAS) family of proteins are a negative regulator of STAT-mediated gene transcription [183,184]. The four family members, including alternatively spliced isoforms, have various names that reflect how they were identified: PIAS1 (Gu-binding protein (GBP), PIAS3, PIAS3ß (potassium channel–associated protein (KchaP), PIASxa (androgen receptor–interacting protein 3 (ARIP3), PIASxß (Msx-interacting zinc finger protein 1 (Miz1) and PIASy. A *Drosophila* PIAS homolog, dPIAS (or Zimp), that negatively regulates the JAK-STAT pathway also exists [183]. PIAS proteins contain a SAP (SAF-A/B, Acinus and PIAS) domain, a ring-finger domain and C-terminal serine/threonine rich domain. Although PIAS proteins inhibit STAT mediated gene activation, they inhibit distinct STAT proteins by different modes. For example, PIAS3 inhibits STAT3 and STAT5, whereas PIAS1 blocks STAT1-dependent signaling [185] and directly inhibits STAT-DNA binding activity and recruits other transcriptional co-repressors such as histone deacetylases (HDACs). Furthermore, they have small ubiquitin related modifier (SUMO) E3 ligase activity [183]. Upon cytokine stimulation, PIAS-1 and PIAS-3 bind activated STAT1 and -3, respectively, and prevent their ability to bind to DNA [184].

Phosphatases are reported to be involved in regulating JAKs and STATs. Numerous PTPs have been implicated in STAT3 signaling including SHP1, SH-PTP2, TC-PTP, PTEN, PTP-1D, CD45, PTP-e, LMW, and PTP [186]. Cytosolic and membrane-bound phosphatases inhibit JAKs, whereas nuclear phosphatases terminate STAT signaling. Three types of PTPs have been shown to negatively regulate JAK–STAT pathways. The first phosphatases that demonstrated a regulatory role in this pathway are the SH2-containing phosphatases. SHP1 is implicated in the negative regulation of JAK/STAT signaling pathways [187] and it has been found that loss of SHP1 may contribute to the activation of JAK or STAT proteins in cancer [188]. The second phosphatase that negatively regulates JAK–STAT signaling is the transmembrane PTPase CD45, which is highly expressed in all hematopoietic lineages at all stages of development and is a key regulator of antigen receptor signaling in T and B cells [90]. Although Src family kinases were identified as primary molecular targets for CD45, targeted disruption of the CD45 gene leads to enhanced cytokine and IFN receptor-mediated activation of JAKs and STAT proteins. Two PTPases, SHP-1 and SHP-2, as well as a protein serine/threonine phosphatase, PP2A, are also strongly implicated in STAT signaling, including STAT1, STAT3 and STAT5 [188]. This suggests that loss of SHP1 is linked to constitutive high levels of activated STAT3. However, it is not clear whether or not the upregulation of SOCS-1, CIS, and PIAS3 can act as a compensatory mechanism to constitutively active STAT3.

9. Role of STAT3 in oncogenic transformation

Constitutive activation of STAT3 is involved in many cellular processes including cell growth, survival, metastasis, angiogenesis, and immune suppression, all of which favor HCC initiation and progression [189]. A critical role for STAT3 in malignant transformation was first proposed after initial studies showed that STAT3 is constitutively activated during v-Src transformation. STAT3 signaling is required for oncogenic transformation by *v-Src* [108,190–192]. Blocking of STAT3 DNA binding with antisense oligonucleotides or a dominant-negative STAT3 protein, further established the critical role of STAT3 in oncogenesis [128,193,194]. In all cases, inhibition of persistent STAT3 signaling suppressed the transformed phenotype and tumor progression. Constitutive activation of STAT3 has been frequently detected in clinical incidences of HCC and in more than 50% of human HCC cell lines but not

in normal or non-transformed liver cells [179,180,195]. For example, STAT3 was found to be over expressed in proteome microarray analysis of primary HCC [196]. Its phosphorylation was highly positive in immunohistochemical analysis of HCC biopsies [197] while increased STAT3 DNA binding activity was observed in chemically-induced HCC [198]. This is surprising, since rapid activation of the STAT3 transcriptional complex has been reported in the regenerating liver following partial hepatectomy [199]. Also, STAT3 antisense oligonucleotide has been reported to significantly reduce the amount of STAT3 protein and inhibit cell proliferation and tumorigenic growth of several human HCC cell lines transplanted into mice [195]. Activating mutations in the gene encoding the gp130 signaling subunit of IL-6 receptor family members have been identified in benign hepatic adenomas [200]. Elevated STAT3 and p705 STAT3 expression in HCC tissue has also been reported [120]. Recent data also indicate an association of pSTAT3 with the histological grade of HCC tissue from 67 patients [201]. When anti-cancer small molecule S3I-201 was administered at a dose of 5 mg/kg every other day to xenografts of the human HCC cell line HUH-7, it was found that S3I-201 inhibited STAT3 tyrosine phosphorylation and tumor growth [6]. The amelioration of the malignant behaviors of HCCLM3 following orthotopic implantation, included impeded migration, hampered neovascularization, inhibited local metastasis, and reduced lung metastasis, indicating that STAT3 also mediates the metastatic potential of HCCLM3, a highly malignant variant of HCC [195].

STAT3 can also act as a stem cell renewal factor, and hyperactive STAT3 signaling results in enhanced liver progenitor cell proliferation [202]. In addition, over-expression of a constitutively active form of STAT3 in immortalized rat or mouse fibroblasts induced tumors in nude mice [203]. Owing to its role in modulating stem cell survival, proliferation and transformation, STAT3 is thought to be critical for cancer stem cell survival in some tissues [204]. Studies from STAT3-deficient mice (STAT3Δhep) were found to exhibit more than a 6-fold reduction in HCC load relative to STAT3F/F mice [46]. Furthermore, tumors in STAT3 mice were smaller, suggesting that STAT3 may play a role in HCC cell proliferation and/or survival. Deletion of STAT3 in cultured STAT3F/F dih cells, accomplished by infecting the cells with a Cre-expressing adenovirus, resulted in cell death, suggesting that activated STAT3 is required for the survival of HCC cells. Although cells that are completely STAT3-deficient cannot survive, cells with a partial reduction of STAT3 expression, accomplished by shRNA transduction are viable, but exhibit a senescent phenotype and fail to form subcutaneous tumors upon transplantation [205].

One can conclude from the above reports that STAT3 indeed plays a major role in HCC initiation and development. As compelling data continue to accumulate, STAT3 has become an attractive molecular target both for the prevention and treatment of HCC and various pharmacological inhibitors derived from synthetic and natural sources have been employed to target aberrant STAT3 activation in HCC (Table 1). While safety remains a point of concern, given the fact that STAT3-null mice are embryonically lethal, tissue-specific STAT3 deletion experiments have indicated that STAT3 may not be essential for the survival of normal differentiated cells. These results provide further evidence that it may be safe to target STAT3 for HCC therapy [206].

10. Role of STAT3 in inflammation

Various published studies indicate the potential role of HCC as a pro-inflammatory transcription factor in HCC and other malignancies [207]. STAT3 was initially discovered as an acute-phase response protein, thus suggesting its casual link to inflammation [51]. IL-6 is one of the major mediators of inflammation and primarily exerts its effects through the activation of the STAT3 pathway [52]. Also, in various tumors, STAT3 can directly interact with nuclear factor NF-κB family member RELA, trapping it in the nucleus and thereby contributing to constitutive NF-κB activation in cancer [208]. STAT3 has also been shown to increase NF-κB activity in cancer cells while persistent activation of STAT3 in tumors, especially in immune cells of the tumor microenvironment, is dependent on NF-κB. This reciprocal relationship with RELA stems from the fact that several cytokines and growth factors encoded by RELA target genes such as IL-6 are STAT3 activators in HCC [206].

Another elegant study by Nadiminty et al. showed that active but not latent STAT3 induces p100 processing to p52 through the activation of IKK and that the subsequent phosphorylation of p100 indicates the diverse targets of STAT3 and may show the use of multiple pathways by cancer cells to survive and escape therapy [209]. The processing of p100 to p52 is a tightly controlled event in many cells and tissues. Constitutive processing of p100 protein resulting in the over expression of p52 leads to lymphocyte hyperplasia and transformation [179]. The p65 subunit of NF-κB has been shown to interact with STAT3 [205,210]. Also, some of the target genes for NF-κB and STAT3 overlap and in addition, the two transcription factors are engaged in both positive and negative cross-talk [210]. In mouse DEN model, DEN-induced hepatocyte death results in release of IL-1α which in turn can activate NF-κB signaling in Kupffer cells, and produce a panel of cytokines and growth factors, including IL-6 [211]. IL-6 released by Kupffer cells activates STAT3 in hepatocytes and STAT3-activated genes are critical for compensatory hepatocyte proliferation and liver tumorigenesis [60,205].

There are also few reports in literature that have analyzed the potential cross talk between STAT3 and Wnt/β-catenin signaling pathways in HCC. Wang et al. by using Hepatitis B virus X (HBx) transgenic mice and a 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC)-induced liver injury model, the relationship between HBx expression and tumorigenicity of hepatic progenitor cells (HPCs) was analyzed. All HBx transgenic mice developed liver tumors characterized by histological features of both HCC and cholangiocarcinoma after 7 months of DDC feeding. They also found higher titers of circulating IL-6, activities of IL-6/STAT3, and Wnt/β-catenin signaling pathways in HBx transgenic mice, suggesting HBx may induce intrinsic changes in HPCs by way of the above signaling cascades that may enable HPCs with tumorigenicity potential [212]. In another study, while evaluating the role of Wnt/β-catenin signaling cascade in HCC development, Wang and coworkers found that transfection with β-catenin siRNA in HepG2 cells effectively knocked down β-catenin mRNA and protein expression levels and also suppressed tumor cell proliferation. Flow cytometry assay showed that tumor cells were arrested at the G0/G1 phase of the cell cycles. However, there no change was observed between the expression of STAT3 and the HSP27 protein following transfection [213]. Also, recent studies have indicated that a number of signaling cascades including STAT3 and Wnt/β-catenin pathways may play an important role in the development of hepatic cancer stem cells [214]. For example, in order to understand the role of Oct4 in HCC and the relationship among Oct4 and Wnt/β-catenin and TGF-β signal pathways, Yuan and coworkers detected the expression of Oct4, Nanog, Sox2, STAT3 as well as the genes in Wnt/β-catenin, and TGF-β families in HCC cell lines and in tumor specimens from HCC patients. They observed that both HCC cell lines and HCC samples from patients express more than one key modulator in embryonic development such as Oct4, Nanog, Sox2, and STAT3 together with the genes in Wnt/β-catenin and TGF-β families. Knocking down Oct4 reduced the expression of TGF-β family genes Wnt/β-catenin family genes, as well as STAT3 [215]. Overall, the exact crosstalk between STAT3 and Wnt/β-catenin signaling cascades in HCC requires further investigation.

11. Role of STAT3 in regulation of apoptosis

Constitutively activated STAT3 is found to participate in oncogenesis of the liver through up-regulation of STAT3-targeted genes

Table 1
Selective list of pharmacological STAT3 inhibitors in HCC.

Natural/synthetic Inhibitors	Mechanism of inhibition	Cell lines	Reference
Celecoxib	Inhibits JAK2 phosphorylation	Hep3B, HepG2, HUH-7, SNU-387 and SNU-449	[254]
Parthenolide along with TRAIL	Inhibits activation of JAK proteins	HepG2, Hep3B and SK-Hep1	[272]
Galiellalactone	STAT3 inhibitory effect by covalently modifying a cysteine residue in the STAT3 DNA-binding domain	HepG2	[273]
XZH-5	Reduced constitutive STAT3 phosphorylation at Tyr705 and the expression of STAT3-regulated genes.	Hep3B, HepG2, HUH-7, SNU-387 and SNU-449	[255]
SD-1029	Inhibits JAK/STAT3 pathway	Hep3B, SNU398, SNU-387, HepG2 and HUH-7	[274]
Sorafenib	SHP-1-dependent STAT3 inactivation.	PLC/PRF5, HUH-7, Hep3B and SK-Hep1	[275]
Soreafenib in combination with TRAIL	Upregulates SHP-1 activity	PLC/PRF5, HUH-7, Hep3B and Sk-Hep1	[276]
CADPE	Inhibits both IL-6-mediated STAT3 activation and recruitment of STAT3 to the cyclin D1 promoter.	HUH-7	[277]
FLL32	JAK/STAT inhibitor inhibits STAT3 phosphorylation, DNA binding activities, and STAT3-regulated gene products.	SNU-449, SNU-398, HEp3B and SNU387	[256]
Diosgenin	Induces the expression of Src homology 2 phosphatase 2 (SH-PTP2) that correlate with down-regulation of constitutive STAT3 activation	HepG2, C3A	[259]
Butein	Inhibits activation of upstream kinases c-Src, JAK2 and induces the expression of SHP-1	HepG2, SNU-387, HCCLM3 and PLC/PRF5	[261]
γ-tocotrienol	Increases the expression of SHP-1 in HCC cells	HepG2 and HUH-7	[262]
β-escin	Inhibits activation of upstream kinases c-Src, JAK1, and JAK2.	HepG2, HUH-7, PLC/PRF5	[260]
NSC 74859	TGF-β signaling	HepG2, PLC/PRF/5, HUH-7, SNU-398,SNU-449, SNU-182 and SNU-475	[6]
17-Hydroxy-jolkinolide B (HJB)	HJB reacts with cysteine residues of JAKs to form covalent bonds that inactivate JAKs.	HepG2	[278]
ENMD-1198	Inhibits STAT3 phosphorylation	HUH-7 and HepG2	[257]
AG490	Janus kinase 2 specific inhibitor	HUH-1, HUH-7, HepG2 and Hep3B	[224]
IL-6-RFP	A high affinity cytokine-binding protein	HepG2	[279]
YC-1	Inhibits STAT3 activity by enhancing the polyubiquitination of p-STAT3(705) induced by cisplatin	HepG2, Hep3B and PLC/PRF/5	[280]
Atiprimod	Suppresses STAT3-mediated through the inhibition of activation of upstream kinases c-Src, JAK1 and JAK2	HUH-7 and HepG2	[281]
Antisense oligonucleotide	Suppression of phosphorylated STAT3 reduced its DNA-binding activity	HCCLM3, SNU423, HUH-7 and HCCLM3	[195]
Stattic	Inhibit SH2 domain, STAT3 dimerization and DNA binding.	HepG2	[271]
Luteolin	Accelerated ubiquitin-dependent degradation in the Tyr705-phosphorylated STAT3	HepG2, HLF and HAK-1B	[282]
Statins	Statins reduced IL-6-induced serine phosphorylation of STAT3.	Hep3B	[283]
2-(1-chloropropenyl)-4, 5-dihydroxy-cyclopent-2-enone	Suppresses IL-6-dependent pathway by inhibiting the tyrosine phosphorylation of STAT3 as well as the serine phosphorylation of the STAT3 by direct inhibition of JAK.	HepG2	[270]

encoding apoptosis inhibitors, e.g., *Bcl-2*, *Bcl-xL*, and *survivin*, *Mcl-1*, *XIAP*, and subsequently inhibiting pro-apoptotic molecules such as Bax, Bad and Bid [81,189]. For example, STAT3 activation can support tumor cell survival through up-regulating the expression of the Bcl-xL. In fact, Bcl-xL was the first antiapoptotic factor shown to be regulated by STAT3 [216,217]. *Mcl-1* is another anti-apoptotic gene of the Bcl-2 family that is a target of both STAT3 and STAT5. Blocking either of these STAT proteins in human tumor cells has been shown to downregulate Mcl-1 expression and can induces apoptosis [218].

Of all inhibitor of apoptosis (IAP), X-linked inhibitor of apoptosis (XIAP) is a principal inhibitor of apoptosis through its ability to inhibit caspase-3 and caspase-7, particularly in HCC cells. XIAP is constitutively expressed in all HCC cell lines and in approximately 70% of HCC tissue [219], whereas little or no expression is seen in chronic hepatitis or cirrhotic tissue [220]. Among the members of the IAP family, survivin is particularly highly expressed in various types of human cancers, including HCC [132,221]. Survivin is expressed at high levels in HCC and is regulated by STAT3 [222,223]. *In vitro* and *in vivo* investigations have also revealed that the application of STAT3 decoy ODN of the sequence of 5′CATTTCCCGTAAATC-3′ can significantly block STAT3-dependent transcription of such genes as *cyclin D1, c-Myc, Bcl-xL*, and *survivin*, leading to reduced proliferation and induction of apoptosis in HCC cells [224].

12. Role of STAT3 in cell cycle progression

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 [203]. Previous studies have also indicated a role for STAT3 in the G1 to S phase transition, mediated by the gp130 receptor subunit. Since STAT3-C possesses oncogenic potential, it is possible that STAT3-mediated transcriptional regulation of key components of cell cycle control contributes to malignant progression by promoting inappropriate cell cycle traversal [120]. Interestingly, at the peak of S phase, which is approximately 40 h post-hepatectomy in mouse livers, the percentage of hepatocytes undergoing DNA synthesis is 5-fold lower in IL-6 −/− livers than IL-6 +/+ livers, whereas the difference between the Alb + and Alb- STAT3 *fl/fl* livers is smaller at about 3-fold [150]. Thus the regulation of cyclin D1 expression is critical for the proliferation and differentiation of hepatocytes. Recent studies have indicated that inappropriate expression of cell cycle-related proteins, such as cyclin D1, cyclin-dependent kinase 4 (Cdk4), cyclin E, cyclin A, p16 and p27, as one of the major factors contributing to HCC initiation and development [225–227]. Moreover, Cressman et al. found that IL-6-deficient mice exhibit defects in STAT3 activation and in cyclin D1 induction after partial hepatectomy [63]. Therefore, it is likely that STAT3 can act as a potential negative regulator of cyclin D1 transcription during fetal liver development, whereas it positively regulates cyclin D1 expression in hepatoma cells and at the initial phase of liver regeneration. These findings clearly indicate that *cyclin D1* gene is an important target of STAT3 in hepatocytes and that its regulation by STAT3 varies, depending on the cell stage, *i.e.* proliferation or differentiation.

13. Role of STAT3 in angiogenesis

Angiogenesis, considered as one of the ten hallmarks of cancer is required not only for tumor growth at primary sites but also for

continued tumor growth at metastatic sites [228]. During organogenesis, all cells in a tissue must reside within close proximity of a capillary [229]. Similarly, most tumors cannot sustain their growth unless they are supplied with oxygen and nutrients from newly formed blood vessels, and a crucial role of activated oncogene products in stimulating angiogenesis has been established [230]. The most potent angiogenesis-inducing signal identified so far is vascular endothelial growth factor (VEGF) [231–235]. Compared with their normal counterparts, cancer cells produce increased levels of VEGF, which binds to transmembrane receptor tyrosine kinases of endothelial cells [230]. This activates endothelial-cell migration and proliferation, which is necessary for the formation of new blood vessels [230,231]. Activated STAT3 affects tumor angiogenesis by regulating the expression of multiple pro-angiogenic molecules in tumor cells and by participating in the signal transduction of angiogenic molecule receptors in tumor endothelial cells. Hepatocellular cancer is notable for its highly aggressive behavior, hypervascularity, portal and hepatic vein invasion and metastasis [236–238]. Aberrant VEGF expression is a prominent clinical feature in HCC and may correlate with HCC tumor invasion and metastasis [239]. It has been observed that STAT3 can also regulate the expression of other angiogenic molecules, such as basic FGF (bFGF) [133] which participates in angiogenesis by inducing the migration, proliferation, and differentiation of endothelial cells and by regulating VEGF expression in tumor cells in an autocrine and paracrine fashion [240]. It is widely believed that the ability of PDGFs to induce liver fibrosis and neoplastic cell transformation is closely associated with the transcriptional induction of TGFβ, an essential mediator of fibrogenesis [241,242]. TGFβ and PDGFs act through activation of STAT3 [75,76] leading to the upregulation of genes promoting cell proliferation, survival, and cell transformation [77]. The role of TGFβ and PDGF pathways in the induction of liver fibrosis and cirrhosis, and putative contributing events to the neoplastic transformation of hepatocytes [243–246] is well established. In addition, STAT3 also regulates the transcription of VEGF indirectly by controlling the expression of hypoxia-inducible factor (HIF)-1, a key inducible transcription factor for the VEGF gene [84].

14. Role of STAT3 in cellular invasion

Recent studies have linked STAT3 to metastatic progression of liver cancer [195]. Contribution of STAT3 to metastatic progression of liver cancers occurs through a variety of molecular mechanisms [53]. For example, STAT3 activation regulates the expression of matrix metalloproteinases MMP-2 and MMP-1, which then mediate tumor invasion and metastasis [133]. Moreover, a number of studies using mouse embryo fibroblasts as the model system established STAT3 as a component of the Rho GTPase-signaling cascade and an effector of cell migration via regulation of actin cytoskeleton [247–250]. Recent results from gene profiling analysis indicate that the expression signature of primary HCC is very similar to that of its corresponding metastases, suggesting that transcriptional changes which control metastatic progression are initiated in the primary tumors [251]. STAT3 however, is also known to upregulate tissue inhibitors of metalloproteinase TIMP-1, a cytokine known to block metalloproteinases and decrease invasiveness in certain cancer cell types [252]. STAT3 also controls the expression of the MUC1 gene, which can mediate tumor invasion [253]. Interestingly, the malignant development of HCCLM3 tumors orthotopically implanted in athymic mice prior was effectively inhibited, including inhibition of tumor growth, local transmission, and lung metastasis, resulting in significantly prolonged survival time upon treatment with STAT3 antisense oligonucleotide [195]. Thus, it is clear that STAT3 signaling plays a key role in HCC invasion and metastasis, and that targeting STAT3 may have therapeutic benefit for patients with primary or recurrent HCC.

15. Pharmacological inhibition of STAT3 activation pathway in HCC

Numerous studies as described above have validated the critical role of aberrant STAT3 activity in malignant transformation and tumor progression in HCC [65,254–256]. Since constitutive activation of STAT3 has also been reported in a number of hematological neoplasias, as well as in solid tumors other than HCC, STAT3 protein has emerged as a promising molecular target for the treatment of cancer [257]. Natural agents, peptides, platinum compounds and other small molecules have been used to inhibit STAT3 activity in various tumor models including HCC [258]. Our group has identified number of STAT3 inhibitors including diosgenin, β-escin, γ-tocotrienol, butein, honokiol, and celastrol that can suppress growth and induce apoptosis in diverse HCC cell lines [259–264]. In addition, Chen and coworkers recently reported that a novel obatoclax derivative, SC-2001, can induce apoptosis in hepatocellular carcinoma cells through SHP-1-dependent STAT3 inactivation [265]. Sorafenib has already been reported to inhibit both the growth and metastasis of HCC by blocking STAT3 activation [266].

Moreover, another multikinase inhibitor Dovitinib has been found to induce apoptosis and overcome sorafenib resistance in HCC through SHP-1-mediated inhibition of STAT3 [267]. Additionally, STAT3 inhibitor NSC74859 has been found to be greatly effective in HCC with disrupted TGF-β signaling [6]. Also, oligonucleotide-based decoys of the STAT3 DNA-binding sequence have already entered clinical trials [268].

Furthermore, as discussed above, tumorigenesis induced by IL-6 has also been linked to constitutive or aberrant activation of STAT3 in various cancers, including HCC [65,81,256,269]. Thus, molecules which inhibit IL-6 or GP-130 receptor may act as good target for inhibition of STAT3 mediated tumorigenesis [65]. The necessity of an intact SH2 domain for optimal STAT3 activation also makes it a rational target for the disruption of STAT3 signaling [221]. Targeting the SH2 domain would uncouple STAT3 from the growth and survival signaling pathways and is a reasonable approach for the development of anticancer agents [270,271]. Also, targeting the upstream molecules of STAT3 such as JAKs and Src kinases can also be used as a strategy to block STAT3 activation [254,271]. However, ideal STAT3 inhibitors should not affect activation of other STAT proteins and should exhibit minimal side effects. Various natural and synthetic inhibitors of STAT3 targeting aberrant proliferation in HCC are described in Table 1.

16. Conclusions

A variety of animal models have been used to study the role of STAT3 signaling cascades in HCC development. In addition, most of our mechanistic understanding of STAT3 pathway in HCC comes from studies using cell type-specific knockout mice. STAT3 in these mice is knocked out only in specific cell types and remains functional in most other cell types. Thus, the results obtained may not precisely predict the effect of inhibitors that interfere with the activity of this transcription factors in cells that remain unaffected in knockout mice. Hence, the knowledge gained about STAT3 in HCC will depend on solutions to these potential problems. The current literature clearly indicates that STAT3 activation plays a major role in oncogenesis and that the suppression of STAT3 activation will pave the way for more effective treatment of HCC in near future. However, appropriate human studies are required to validate the promising results obtained in mice and move the STAT3 inhibitors from the bench side to the bed for the treatment of HCC patients.

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