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STAT3 Signaling in Cancer

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Abstract

In recent years, signal transducers and activators of transcription (STAT) proteins have been recognized as cytoplasmic transcription factors that mediate extracellular signaling to the nucleus controlling fundamental functions, such as cell proliferation, apoptosis, differentiation, immune responses and angiogenesis. Among them, STAT3 is a major player, aberrant activation of which is involved in several diseases, including cancer. Among other upstream regulators, IL-6/Jak signaling can activate STAT3 and its role appears to be critical in various types of cancer. Although STAT3 has been traditionally recognized as amoncogene, more recently the dual role of STAT3 in cancer, either tumor inductive or suppressive, has been appreciated. The importance and differential effect of STAT3 on tyrosine or serine residues are also a matter of continuing debate. Interestingly, recent findings suggesting that STAT3 plays an important role in cancer stem cell regulation have gained significant attention. This review summarizes current literature focusing on the significance of STAT3 in several diseases as well as in cancer. Understanding the complexity of STAT3 function has the potential to elucidate important molecular aspects of cancer with significant therapeutic implications.

Keywords

STAT3, Signaling, Cancer, Dual Role, Tyrosine and Serine Phosphorylation

1. Introduction

STAT proteins (signal transducers and activators of transcription) constitute a large family of transcription factors with a dual role as signal transduction and transcription activators. STATs were first described in 1994 [1] as

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members of interferon (IFN) signaling complex [2] [3]. They are found in the cytoplasm in a latent form and become active in response to stimulation by cytokines and growth factors, hormones and peptides. STAT3 is a significant member of STAT family and has been largely studied, in recent years. This review attempts to elucidate the role of STAT3 molecule signaling in occurrence of diseases including cancer.

2. STATs: The Members of the Family

STAT family consists of 7 members in mammals including STAT-1, STAT-2, STAT-3, STAT-4, STAT-5 a and b, and STAT-6 [4]. STAT proteins comprise 750 to 850 amino acids and some authors classify them according to their functional role into two groups [5] [6]: STAT2, STAT4, and STAT6 represent the first group which is involved in T-cell development and IFN-γ signaling and become activated through several cytokines. The second one consists of STAT1, STAT3, and STAT5, being activated in different tissues through a series of different ligands and considered to be involved in various processes, such as IFN-γ signaling, development of mammary glands, growth hormones response and embryogenesis. This latter group of STATs is supposed to play a crucial role in cancer development by controlling fundamental cellular functions including cell cycle and apoptosis [6].

2.1. STAT1

Darnell *et al.* first proposed that the initial physical role of STAT1 is to mediate the antiviral and immune effects of IFNs. Both STAT1 and STAT2 directly mediate IFN-α and IFN-γ biological effects and play a significant role in the mechanisms that control cell growth and apoptosis [1]. The involvement of STAT1 in cancer is controversial; for example, it is suggested that STAT1 activity enhances breast tumor growth and immune suppression [7], while other evidence indicates the loss of its expression in different types of malignant cells, such as breast cancer, head and neck cancer [8], melanoma, leukemia, and lymphoma [9]-[12].

2.2. STAT2

STAT2 is supposed to be vital in innate immunity and specific viruses are found to target STAT2 to surpass the IFN antiviral response. STAT2 plays also a crucial role in promoting IFN-induced apoptosis, while its transcriptional activity is controversial, potentially acting both as a repressor and activator. Moreover, even though STAT2 forms heterodimers with STAT1, recent data proposed an alternative STAT2 signaling pathway, independent of STAT1 [13].

2.3. STAT3

STAT3 activation in normal conditions drives a well organized gene regulation schedule. After STAT3 is exposed to cytokine stimulation, the molecule can reach a maximum of phosphorylation within the first 15 - 60 minutes, but STAT3 activation gradually decreases in the following hours [6]. STAT3 activation is mediated by the JAK family of tyrosine-kinases, most notably by JAK1 [14]. STAT3 can be activated independent of JAKs by other non-receptor tyrosine kinases, mostly by c-Src kinases [3]. STAT3 is phosphorylated at tyrosine 705 and serine 727 residues with tyrosine phosphorylation being associated in many cases with disease progression and tumorigenic potential [15]. Upon activation, phosphorylated STAT3 molecules form dimers and translocate into the nucleus to regulate transcription of genes, controlling cell survival and proliferation [5] [16]. Moreover, activated STAT3 regulates the expression of anti-apoptotic, pro-proliferative and immune response genes [5] [17].

2.4. STAT4

Several lines of evidence imply that STAT4 expression is exclusively found in myeloid cells (activated monocytes, macrophages, and dendritic cells [18]. STAT4 is mostly activated by IL-12, which in turn regulates tissue inflammation, fibrogenesis and antiviral defense [19]. The binding of IL-12 with its receptor, in $CD4^+$ Th1 cells is followed by phosphorylation of tyrosine 693 and serine 721 of STAT4 [20]. Consequently, the activated STAT4 moves into the nucleus, binds to DNA and enhances the production of inflammatory cytokines such as IFN-γ [21].

2.5. STAT5 (A and B)

Janus kinases can also phosphorylate STAT5 proteins when the latter bind to the phospho-tyrosine residues in their receptors. STAT5 is phosporylated at Y694 and Y699 residues for STAT5A and STAT5B, respectively, and these phosphorylations are essential for the steady formation of STAT5 dimers through their SH2 domains. Both STAT5 isoforms are activated by the same set of cytokines, nevertheless some cytokines selectively activate either STAT5A or STAT5B (e.g. prolactin activates STAT5A) [22]. Upon activation, STAT5 dimers move into the nucleus and bind to specific DNA sequences, mainly concerning IFN-γ [23]. Moreover, interactions between STAT5 N-terminal domains lead to stable tetramer formation of Stat5 [24] [25], which is critical for cytokine regulation and immune responses [26]. Aberrant STAT5 signaling is often associated with leukemogenesis and other cancers [27] [28].

2.6. STAT6

The seventh member of STAT family, STAT6, regulates gene expression in several cell types, a crucial function for maintaining the balance between host immune defense and allergic inflammatory responses [19]. STAT6 gene regulation in response to IL-4/IL-13 stimulation varies and depends on the cell type [29] [30]. For example STAT6 promotes IgE chain and CD23 gene expression in B cells, while in T cells enhances Th2 differentiation genes gata3 and crth2 [31]-[34]. Moreover, Lawrence *et al.* reported that stimulation of macrophages by IL-4/IL-13 induces STAT6-dependent activation and transcription of arginase 1, Retnla, and Chi3L3 in mouse and indolamine 2,3-dioxygenase (IDO) in humans [31]. STAT6 not only acts as a transcriptional activator by regulating Th2 development, but also suppresses gene expression through steric hindrance of binding by other transcription factors [29]. This repressing activity leads to subsequent side effects and probably plays a significant role in Th2 cell programming [35].

3. Role of STAT3 in Diseases

Normally, STAT3 functional role contributes to controlled gene regulation. Nevertheless, STAT3 aberrant activation has been involved not only in oncogenesis but also in several other types of disease. For example, previous studies suggested that Stat3 plays a crucial role in the pathogenesis of diabetic nephropathy [36] and is involved in cytokine- and nutrient-induced insulin resistance [37]. Moreover, excessive STAT3 signaling leads to development of skeletal muscle insulin resistance in type 2 diabetes [37].

Aberrant IL-6/STAT3 signaling has been also studied in endometriosis, which is an estrogen-dependent inflammatory disease. Kim *et al.* found higher levels of phospho-STAT3 and HIF1α (Hypoxia-inducible factor 1-alpha, a downstream substrate of STAT3) in the endometrium of patients with endometriosis compared with healthy women and proposed that consistent activation of STAT3 contributes in the pathogenesis of endometriosis [38].

The JAK/STAT signaling pathway is found to be active in a variety of renal diseases and it is proposed to contribute in the pathophysiology of renal fibrosis. Matsui *et al*. referred that inhibition of the JAK/STAT signaling pathway, especially JAK2 and STAT3, appeared to diminish renal fibrosis and protected renal activity [39]. As a result Tanq *et al.* [40] demonstrated that fluorofenidone (FD), a novel pyridone agent, exerts an antifibrotic effect through inhibiting STAT3 tyrosine phosphorylation in the JAK2/STAT3 pathway, consequently adding a new therapeutic strategy in renal fibrosis.

STAT3 disease involvement has been also linked to dyregulations of IL-6 signaling. Several studies suggested that IL6 trans-signaling (complex of IL6 and soluble IL6 receptor) played a pathogenic role in lung airway smooth muscle diseases [41]. Classical IL6 as well as IL6 trans-signaling in human airway smooth muscle involve activation of Stat3, but IL6 trans-signaling has been shown to specifically contribute to asthma pathogenesis and can be considered as a potential modifier of airway inflammation and remodeling [41].

Furthermore, it has been shown that high levels of IL10 are found in serum and tissues of patients with systemic lupus erythematosus (SLE). Hedrich *et al.* reported that Stat3 and Stat5 regulate trans-activation and epigenetic remodeling of IL10 by interacting with the histone acetyltransferase p300. Specifically, the activation of Stat3 in T cells from SLE patients led to enhanced recruitment to regulatory regions and competitive replacement of Stat5, leading to enhanced IL-10 expression [42]. As a result, Wang *et al.* suggested that Natura-alpha (a novel STAT3-Y705 inhibitor) could be used as a potential SLE inhibitor [43].

In addition STAT3 plays a significant role in some autoimmune disorders including inflammatory bowel disease (IBD). Several lines of evidence suggested that STAT3 activation posseses a dual and contradictory role between innate and acquired immune responses in colitis [44]. STAT3-mediated activation of acquired immune responses contributed in pathogenesis of colitis by supporting the survival of pathogenic T cells, while STAT3-mediated activation of innate responses suppressed the pathogenesis of colitis [44]. More recent data suggested that several interleukins, like IL-6, IL-11, and IL-22, are highly expressed in IBD cases, and that consequent activation of JAK/STAT3 pathway can ameliorate disease and protect the epithelial lining cells [45].

Moreover, experimental evidence showed that several inflammatory cytokines, such as IL-1β, tumor necrosis factor alpha and IL-6, are highly expressed in patients with rheumatoid arthritis (RA), leading to direct or indirect activation of STAT3, which in turn enhances expression of IL-6 family cytokines and promotes sustained inflammation and joint destruction [46]. In addition, Gao *et al.* found that there is an interaction between HIF1α, STAT3 and Notch-1 signaling in the regulation of pro-inflammatory pathway in RA, which implied a role for targeting STAT3 in treatment of RA [47].

STAT3 regulation has also been involved in the pathophysiology of behavior. Several lines of evidence suggested a relationship between changes in the immune system, mostly in IL6 expression, and depression [48]. Kong *et al*. examined the existence of an IL6-induced modulation of serotonergic neurotransmission through the STAT3 signaling pathway which may enhance the role of IL6 in depression. Indeed, they found that IL6 directly regulated Serotonin Transporter protein levels (SERT) and consequently affected serotonin reuptake, thus proposing that IL6 could be connected to depression through a potent STAT3-dependent regulation model of SERT [48].

Moreover, JAK/STAT3 pathway has been connected with Alzheimer's and Huntington's diseases [49]. Experimental evidence suggested that astrocyte reactivity is a hallmark of neurodegenerative diseases and that JAK/STAT3 pathway correlates with reactive astrocytes in models of acute injury. Ben Haim *et al*. examined astrocyte reactivity in progressive pathological conditions such as Alzheimer's and Huntington's disease and showed that JAK/STAT3 pathway is a common inducer of astrocyte reactivity thus adding novel information to the pathogenesis of neurodegenerative diseases [49] (**Figure 1**).

4. Dual Role of STAT3 in Cancer

4.1. STAT3 Tumorigenic Role

Inappropriate STAT3 activation, mainly due to persistent tyrosine 705 phosphorylation signaling, has been convincingly shown to contribute to oncogenesis and to promote the acquisition of a malignant phenotype [50]-[53]. Consistent activation of STAT3 in cancer transfers signals from cytokines and growth factors [54] and stimulates specific target genes such as Fos, Cyclin-D, CDC25A, c-Myc or Pim1 that induce cell proliferation, suppress apoptotic genes (Fas) [55] and up-regulate antiapoptotic genes including BCL2 (B-Cell CLL/Lymphoma-2), BCLXL and Beta2-Macroglobulin [56].

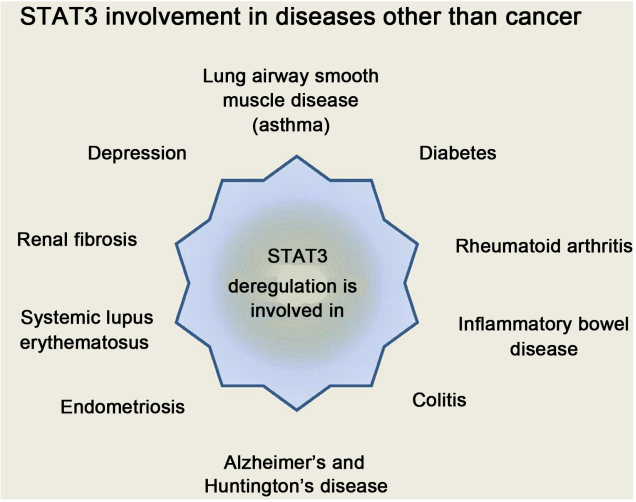

Figure 1. STAT3 involvement in diseases other than cancer.

Persistent STAT3 activation has been described in various types of solid and hematological cancers and targeting STAT3 expression could be a useful strategy for cancer therapies in the future [57]. For instance, activation of STAT3 by Src kinase has been shown to be essential in prostate and ovarian cancer [58]. Moreover, enhanced expression of BRCA1 gene (breast cancer susceptible gene 1), which has been associated with breast, ovarian and prostate cancer, induced constitutive phosphorylation of STAT3 at serine and tyrosine residues in prostate cancer cell lines, also interacting with the upstream activators JAK1 and JAK2 [59]. In addition, STAT3 constitutive activation, mostly associated with aberrant TGF-*α*/EGFR signaling, contributed to HNSCC development and growth [53] [60]. Further, activated STAT3 expression was found to significantly correlate with extent of tumor invasion, lymph node metastasis and tumor grade in colorectal cancer [61].

IL-6 signaling through STAT3 transcriptional activity is the main pathway involved in the growth and differentiation of B cells in plasma cells malignancies [62] [63] and STAT3 is constitutively active in mononuclear cells of bone marrow in patients with multiple myeloma [64]. STAT3 and STAT5 are also activated in T-cell lymphotrophic virus type I (HTLV-I-related adult T-cell) lymphoma [65]. Activated STAT3 was detected in cell lines of Hodgkin disease (HD), and constitutive phosphorylation of STAT3 and STAT6 is identified in Reed-Sternberg cells of patients with Hodgkin disease [66]. Finally, STAT3 overexpression is associated with more aggressive disease in acute myeloid leukemia [67].

4.1.1. STAT3 Overactivation

Persistent activation of STAT3 in cancer is a consequence of alterations that either overactivate this molecule or deactivate negative regulators of STAT3. Aberrant expression of various oncogenic protein tyrosine kinases (PTKs), including Src, can drive STAT3 overactivation in cancer cells [50]. Indeed, Src induces STAT3 activation, which in turn controls genes whose expression is required for the tumorigenic cellular transformation [68]-[70]. Furthermore, NPM-ALK is a constitutively active tyrosine kinase which has been proved to activate STAT3 in ALK-positive anaplastic large cell lymphoma [50]. Moreover, certain mutations in the kinase domain of epidermal growth factor receptor (EGFR) result in excess production of IL6 and subsequent STAT3 activation in lung cancer and glioblastoma cells [71] [72].

STAT3 is a transcription factor which enhances the expression of many cytokines including IL-6 and IL-10. These STAT3-stimulating cytokines are often found in tumors [73], in addition to those produced from inflammatory cells as a response to the tumor progression (IL-6, IL-10, IL-11, IL-21, IL-23, leukemia inhibitory factor and oncostatin) [73]. This autocrine or paracrine stimulatory pathway leads to further activation of STAT3. Stimulation of STAT3 could also occur as a result of a positive feedback loop. For example, the activation of STAT3 by v-src leads to activation of NF-*κ*B, which in turn and under certain conditions can induce IL-6 production and consequently STAT3 feedback activation [74]. In another feedback model, STAT3 has been rported to promote the expression of a G protein-coupled receptor for the lysophospholipid sphingosine-1-phosphate (sphingosine-1-phosphate receptor-1), which in turn induces STAT3 activation by increasing the expression of IL-6 and enhancing JAK2 tyrosine kinase activity [75].

Furthermore, somatic mutations in STAT3 have been diagnosed in several malignancies, including hepatocellular adenomas, T-cell large granular lymphocytic leukemia (T-cell LGL), chronic lymphoproliferative disorders of natural killer cells (CLPD-NKs), diffuse large B-cell lymphoma, and $CD30^+$ T-cell lymphomas [76]-[80].

4.1.2. Disruption of STAT3 Negative Regulation

STAT3 persistent activation exists when a disruption in negative regulations of STAT3 occurs. Suppressors of cytokine signaling (SOCS) and protein tyrosine phosphatases (PTPs) are known to control STAT3 homeostasis of phosphorylation [81]-[83]. Experimental data suggested that loss of SOCS3 by genetic disruptions induced STAT3 activation and increased proliferation, survival and motility in several cancer cells [50].

SOCS1 can also be suppressed by aberrant gene methylation and this condition has been shown to result to persistent STAT3 activation in several types of cancer [84]-[87]. Similarly SHP-1, a member of tyrosine phosphatase family can be deregulated after epigenetic alterations, notably in hematologic malignancies [86] [88] [89]. Disruption of SHP-1 has been proposed to correlate with constitutive activation of STAT3 in cancer types including ALK-positive anaplastic large cell lymphoma, chronic myeloid leukemia and multiple myeloma.

Finally, protein inhibitors of activated STAT3 (PIAS) reduce DNA-binding of STAT3 and consequently intervene to gene transcription. An example of PIAS3 dysfanction is found in glioblastoma where reduced PIAS3 expression correlated with increased levels of STAT3 activation and cell proliferation [90].

4.2. STAT3 Tumor Suppressive Role

The oncogenic role of STAT3 is widely described in various types of cancer and mounting evidence suggests that aberrant STAT3 signaling contributes to malignancy through mechanisms that alter normal STAT3 activation. Nevertheless, a smaller number of recent investigations imply a tumor suppressive role of STAT3, which contradicts its well known oncogenic function. Noteworthy is that the vast majority of these studies involve experiments with mice xenografts and generally mice models.

Using siRNA techniques in astrocytes derived from conditional knockout mice, De la Iglesia *et al*. demonstrated a tumor suppressive effect of STAT3 in the absence of PTEN expression in glioblastoma. On the other hand, they observed an oncogenic STAT3 effect following co-expression of EGFRvIII and the interaction between these molecules in the nuclei of glioblastoma cells [91].

Furthermore, Musteanu *et al.* [92] and Lee *et al.* [93] used an adenomatous polyposis coli (Min/+) (multiple intestinal neoplasia gene) model of colorectal cancer and reported that loss of Stat3 induced tumor development at later stages, promoted invasion, and significantly reduced the lifespan of Stat3 (DeltaIEC) Apc (Min/+) mice [92]. In contrast, deletion of STAT3 in the intestinal epithelial cells reduced the multiplicity of early adenoma formation.

Based on a study using different carcinogens in a drug-induced liver carcinogenesis investigation of conditional STAT3 knockout mice, it seems that the role of STAT3 depends on the type of the used carcinogen. Specifically, in hepatocytes with STAT3 expression compared to hepatocytes with STAT3 ablation, induction by chronic carbon tetrachloride hepatocytes resulted in less tumor formation, while induction by diethylnitrosamine led to significantly higher tumor formation [94].

The former studies found that STAT3 can have both tumor suppressive or promoting role at the same cancer cells, indicating that the function of STAT3 may depend on the genetic or biochemical background of the cells. In addition, Wang *et al.* postulated that STAT3 role depends on tumor stage suggesting that, as hepatic cancer cells have developed, STAT3 is likely to promote tumor growth [94].

Moreover, Schneller *et al.* examined the role of Stat3 in Ras-dependent hepatocellular carcinoma progression in the presence and absence of p19 (ARF)/p14 (ARF) and suggested that constitutive active Stat3 played an onco-suppressive role, while expression of Stat3 lacking Tyr (705) induced tumor progression [95].

Finally, some immunohistochemical studies have also correlated STAT3 expression with better clinical outcome and prognosis. For example, Pectasides *et al.* studied a cohort of 102 patients with HNSCC and found that high nuclear expression levels of STAT3 correlated with a favorable clinical outcome [96]. In addition Ettl *et al.* examined a cohort of 286 salivary gland carcinomas and indicated that patients with strong nuclear pSTAT3 expression had a better clinical outcome compared with specimens exhibiting moderate or weak nuclear staining. Further, decreased lymph node and distant metastases were correlated with strong pSTAT3 nuclear staining in low histologic grade cases [97].

Similarly, Sato *et al*. reported that breast cancer patients with positive nuclear pSTAT3 (tyr) staining tended to have better survival, although not reaching statistical significance; in addition, patients with low grade, but not with high grade, who were positive for nuclear pStat3 (tyr) appeared to have significantly prolonged overall survival [98].

5. Role of Il6/STAT3 Signaling in Cancer

Interleukin-6 (IL-6) is a cytokine secreted by T-cells and macrophages and is involved in immune and inflammatory responses [99]. IL-6 signaling launches with ligand binding to its receptor IL-6R and a common receptor subunit gp130. By this interaction, a hexameric receptor complex of two IL-6, IL-6R, and gp130 hetero-trimers is formed [100]. This signaling pathway is triggered during early immune responses, consequently stimulating the expression of various acute-phase proteins, and is referred to as classical signaling of IL-6 [101]. In addition, IL-6 can also bind to an existing soluble type of the IL-6 receptor (sIL-6R) and form a complex that interacts with gp130. This type of IL-6 signaling, called IL-6 trans-signaling, differs from classical IL-6 signaling and plays a significant role in the function of several cells including neutrophils, macrophages, epithelial cells and T cells [102]. Both IL-6 signaling pathways trigger responses including activation of JAK (Janus Kinase) kinases. JAKs are cytoplasmic tyrosine kinases which in mammalians consitute a protein family of four members. JAK1, JAK2 and TYK2 are expressed in several cells, while JAK3 is found only in cells of the hematopoietic system [103]. JAKs and especially JAK1 is involved in the activation of STAT3 via phosphorylation of a specific tyrosine re-

sidue [14] [104]. As previously described, when STAT3 becomes phosphorylated, it forms dimers and moves from the cytoplasm to the nucleus stimulating transcription of STAT3 target genes, including cyclin D1, Bcl-xL, c-myc, Mcl1 and vascular endothelial growth factor (VEGF) [104] (**Figure 2**).

IL-6 has been demonstrated to be implicated in several malignancies including prostate [105], breast [106], lung cancer [107], and oral SCC [108], by regulating critical cellular activities such as proliferation [105] [107] (asangari, lin2), apoptosis [105], and invasion [106] (lin1). For example, Chang *et al.* has demonstrated that IL-6 induced transient increase of STAT3 tyrosine phosphorylation in a dose-dependent manner, which in turn resulted in neuroendocrine dedifferentiation and cell proliferation in non-small cell lung cancer cells [109]. Moreover, Wan *et al.* observed that cancer stem cells played a role in progression and recurrence of hepatocellular carcinoma after therapy and that tumor-associated macrophages (TAMs) expression was associated with poor outcomes. They suggested that TAMs produce IL-6 and contributed to expansion of human hepatocellular carcinoma stem cells via STAT3 signaling [110].

Patel *et al.* documented that, in colorectal cancer cells, colonic inflammation through IL-6 signaling can result in metabolic changes of epithelial cells by controlling expression of cytochrome enzymes including CYP2E1 and CYP1B1, through transcriptional and epigenetic mechanisms. Specifically CYP2E1 overexpression, as a result of STAT3 pathway, enhanced activation of dietary carcinogens and DNA damage, thus promoting colorectal carcinogenesis [111]. Similarly, modulation of the IL-6/JAK/STAT3 signaling pathway has been proposed as a potential therapeutic approach to treat patients with colorectal cancer [104].

PI3K-specific inhibitors are used in clinical trials for breast cancer treatment against tumors harboring PIK3CA mutations with conflicting results suggesting that some tumors may show resistance to PI3K inhibitors. Based on these observations, Yang *et al.* found that the existence of an IL6-STAT3 pathway contributes to resistance to PI3K inhibitors by effectively triggering epithelial-mesenchymal transition and expanding cancer stem cell population in human breast cancer cells [112].

In another study Zheng *et al.* observed that Gankyrin, a small protein with seven ankyrin-repeat domains, expressed in various cancers including hepatocellular carcinoma, colorectal cancer and pancreatic cancer, induced tumor growth and metastasis via IL-6/STAT3 signaling pathway in human cholangiocarcinoma [113].

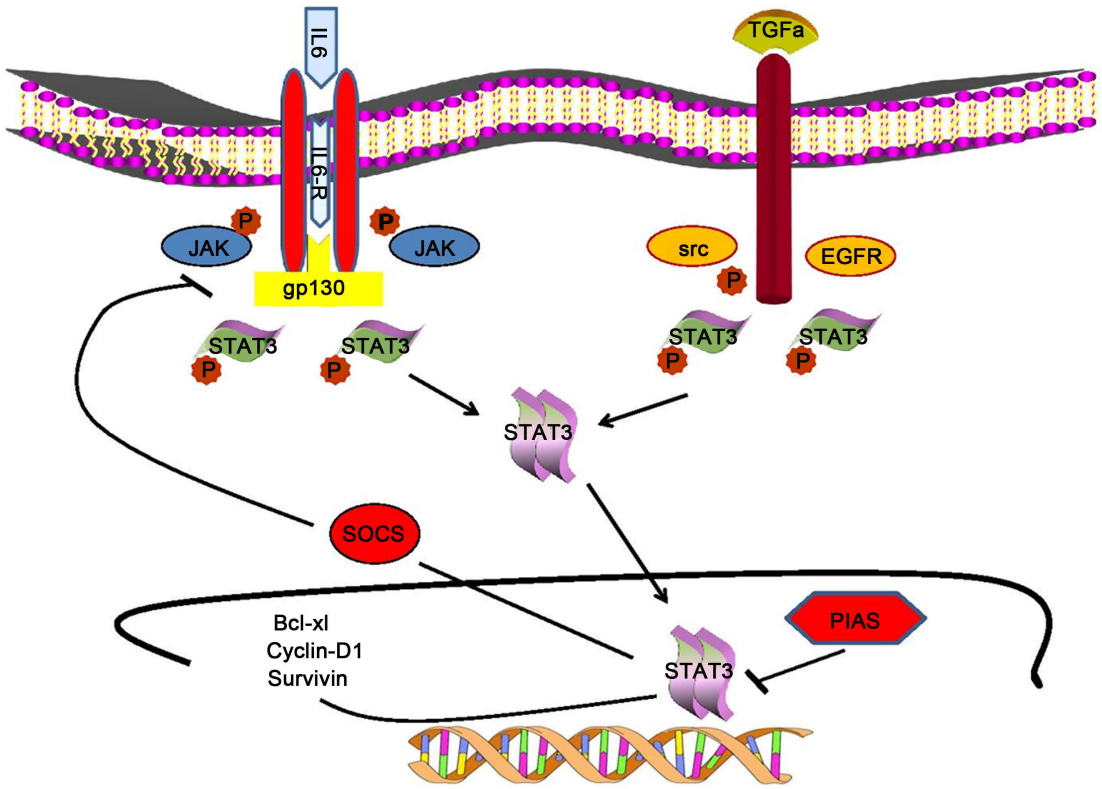

Figure 2. STAT3 activation and signaling.

Furthermore, Liu *et al*. showed that the IL6-Stat3-AR (androgen receptor) cascade is a significant regulator of enzalutamide (androgen receptor antagonist drug) resistance in prostate cancer. This study also demonstrated that the drug Niclosamide could target IL6-Stat3-AR pathway and consequently overcome enzalutamide resistance, resulting in inhibition of migration and invasion in advanced prostate cancer [114]. Finally, Cheng *et al.* have also proposed salivary IL6 and IL-8 as potential biomarkers for oral SCC [108].

6. Role of STAT3 Serine Phosphorylation

As descried before, numerous investigations have demonstrated that overexpression of STAT3 is responsible for various oncogenic processes, such as solid tumor progression, pathological angiogenesis [115], and promotion of cell growth and transformation [116]. As alluded previously, it is also widely known that the oncogenic potential of STAT3 depends mainly on the phosphorylation status of Tyr705 [117].

In contrast, STAT3 serine phosphorylation may also arise as a result of growth factor and cytokine stimulation, but the role of this activation remains controversial [117]. Several studies suggested that serine activation could drive to both stimulating and inhibitory effects on gene transcription [118]-[122], while others postulated that Ser-727 phosphorylation may inhibit Tyr-705 phosphorylation or, quite the opposite, result in further STAT activation [123].

The negative impact of Ser727 residue phosphorylation on STAT3 activity has been proposed in several studies indicating that Ser727 phosphorylation downregulates STAT3 tyrosine phosphorylation and causes alterations in nuclear translocation and transcriptional activity [122]. Moreover, it has been suggested that Ser727 phosphorylation either inhibits tyrosine activation or increases tyrosine dephosphorylation [124]. Mandal *et al.* found that when Stat3 Ser727 phosphorylation was reduced, tumorigenicity of glioma cells was increased probably through a CK2-PP2A (casein kinase 2—Protein phosphatase 2A) pathway followed by conversely increased STAT3 tyrosine phosphorylation [15]. Moreover, Venkatasubbarao *et al.* [125] and Wakahara *et al.* [126] proposed that phospho-Ser727 regulated the direction of STAT3 activity by enhancing either tyrosine dephosphorylation or phosphorylation, mainly through TC45 phosphatase. An Erk1/2-STAT3 crosstalk in oral SCC has been also described by Gkouveris *et al.*, who demonstrated that ERK1/2 inhibition is followed by increases in STAT3 serine phosphorylation and increases in tyrosine phosphorylation, while ERK1/2 induction had the opposite effects [53].

On the other hand, other researchers have proposed that serine phosphorylation correlated with increased nuclear translocation and enhanced transcriptional activity [120] [127]. Moreover, MEK-ERK signaling has been shown to drive Ras-induced phosphorylation of STAT3 on Ser727 and mitochondrial STAT3 is a crucial substrate of the Ras-MEK-ERK pathway during cellular transformation [128]. In addition, Zhang *et al.* suggested that Ser727 phosphorylation status mediated the behavior of a variety of tumors, also demonstrating that Ser727 phospshorylation of mitochondrial Stat3 is required for Ras-mediated transformation of MEFs (mouse embryo fibroblasts) [116]. In addition, it has been shown that Ser727 phosphorylation may correlate with the growth and transformation of other malignancies, such as chronic lymphocytic leukemia, prostate and breast cancer [129]-[131]. Lee *et al.* reported that YB-1 (y box binding protein) prevented the apoptosis of breast cancer cells by AKT/mTOR signaling, resulting in STAT3 serine phosphorylation [132]. Hazan-Halevy *et al.* suggested that constitutive STAT3 Ser727 phosphorylation played a crucial role in chronic lymphocytic leukemia (CLL) and targeting serine phosphorylation could be used as a novel therapeutic strategy [120].

Furthermore, Waitkus *et al*. described that activation of epidermal growth factor receptor (EGFR) and protease-activated receptor 1 (PAR-1) leads to Both Thr714 and Ser727 STAT3 phosphorylation and consequently in a STAT3-dependent gene induction in endothelial cells and found that this double phosphorylated STAT3 complex is highly expressed compared to tyrosine-STAT3 levels in clear-cell renal-cell carcinoma [115].

In another study, Miyakoshi *et al.* revealed that MAPK activation through FBS treatment of mouse hepatic carcinoma cells enhanced STAT3 phosphorylation in Ser727 and increased STAT3 nuclear translocation and cell proliferation [133]. Finally, Sakaguchi *et al.* showed that constitutive Ser727 phosphorylation in melanoma cells, partially mediated by the B-Raf-MEK-Erk1/2 pathway, affected cell survival and nuclear translocation of STAT3 [134].

7. STAT3 Activation in Cancer Stem Cells

Aberrant regulation and transformation of stem cells into cancer stem cells (CSCs) are found to correlate with

cancer development, metastasis and drug resistance [135]. It is suggested that a critical event which causes these cellular alterations is the existence of high Reactive oxygen species (*ROS*), Reactive nitrogen species (*RNS*) (ROS/RNS) and Lipoma-preferred partner (LPPs) levels in the cellular microenvironment as a result of chronic systemic or local inflammation [136]. In turn, the presence of high ROS/RNS expression levels is postulated to lead to DNA damage and oncogene activation [136]. Tumor inflammation can exist in cases of chronic trauma or infections (viruses or parasitic infections), chemical carcinogens, or autoimmune disorders [137]. Considering the fact that STAT3 is well known to play a significant role in tumor inflammatory environment, it is plausible that STAT3 activation is involved in CSCs regulation [136].

STAT3 is suggested to promote prostate tumorigenesis and high tyrosine phosphorylated STAT3 levels correlate with higher Gleason score and pathologic stage of the disease [138] [139]. In contrast, inhibition of STAT3 signaling appears to exert antitumor effects in patient-derived PCa xenograft models [140]. Noteworthy is that STAT3 activation by IL-6 [141] or stress factors like ROS [142] results in enhanced self-renewal and tumor-propagating capacity of prostate CSCs [143]. Moreover, Hossain *et al*. found that glioma-associated-human mesenchymal stem cells (GA-hMSCs) enhance tumorigenic activity of glioma stem cells (GSCs) by inducing their proliferation and self-renewal through the IL-6/gp130/STAT3 pathway [144].

High levels of aldehyde dehydrogenase (ALDH), a detoxifying enzyme mostly expressed in progenitor and stem cells, in endometrial cancer patients are associated with relatively lower survival rates compared to patients with low levels of ALDH. Recently, van der Zee *et al*. reported that endometrial cancer cells with high levels of ALDH activity, accompanied by upregulation of IL-6 receptor subunits, exhibited CSC activities. Notably, inhibition of the IL-6 receptor and its downstream effectors JAK1 and STAT3 dramatically reduced tumor cell growth [145].

Recently, Islam *et al.* examined the role of RhoC (a pro-metastatic oncogene) in CSCs formation in HNSCC cell lines. ShRNA inhibition of RhoC resulted in lower expression of ALDH and CD44 stem cell markers, while STAT3 serine and tyrosine levels were significantly downregulated in those RhoC-depleted HNSCC cell lines. The authors concluded that over activation of IL6/STAT3 pathway, mainly regulated by RhoC, controls CSC functions [146].

Furthermore, Won *et al.* indicated that high levels of CSC marker CD133 correlate with tumor growth and poor prognosis in hepatocellular carcinoma (HCC) and reported that STAT3 activation via IL-6 stimulation increased protein levels of CD133 and promoted cancer progression. In contrast, silencing of CD133 resulted in cell cycle arrest and tumor suppression by causing downregulation of cytokine-related genes, including TACC1, ACF7 and CKAP5. Also, treatment with sorafenib and nifuroxazide inhibited STAT3 activation and CD133 expression leading to reduced HCC xenograft formation [147].

Moreover, Chen *et al*. investigated the effect of the neuroleptic drug pimozide in HCC cells or stem-like cells and found that treatment with pimozide resulted in reduced STAT3 activity, mainly manifested by both lower luciferase activity and expression of STAT3 target genes. Furthermore, IL6-induced tumorigenic effect in stem-like cells was decreased after pimozide treatment [148].

In a recent investigation, Zhao *et al*. showed that vascular endothelial growth factor-A (VEGF), promotes breast and lung CSC self-renewal via VEGFR-2/JAK2/STAT3 binding, resulting in STAT3 activation and ensuing upregulation of Myc and Sox2. These novel findings support the notion that, in addition to angiogesis, VEGF drives tumor-initiating CSC self-renewal through VEGFR-2/STAT3 signaling [149]. Finally, Thakur *et al.* studied the effect of Shikonin (Shk) on breast cancer and investigated the existence of a possible anti-CSC role. Treatment of cells with Shk drove to lower levels of various epithelial to mesenchymal transition (EMT) and CSC associated markers, accompanied by inhibition of STAT3, FAK (Focal adhesion kinase) and Src, proposing a tumor suppressive effect in breast cancer [135].

8. Conclusions

In summary, a plethora of studies indicate that deregulation of STAT3 pathway is involved in various diseases, including many cancer types, revealing the significance of retaining normal STAT3 signaling for cellular stability.

STAT3 constitutive activation has been shown to contribute to tumor development and progression, while IL-6/JAK pathway plays a crucial role in aberrant STAT3 signaling cascades. In addition, the role of STAT3 serine phosphorylation, as supporting or opposing the effects of tyrosine phosphorylation in the mechanisms of

cancer pathology, needs further elucidation. More intriguing is the recent evidence demonstrating divergent roles of STAT3 in cancer biology, which may on certain occasions function as a potent tumor suppressor. Even grater enthusiasm has been generated by the latest discoveries implicating STAT3 in the regulation of cancer stem cells in various types of malignancies.

Understanding the complexity of STAT3 activation, as well as the significance of this signaling pathway in cancer, holds great promise for the development of new therapeutic strategies in several disorders, including cancer.

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Abbreviations

ALDH: Aldehyde dehydrogenase
AR: Androgen receptor
BRCA1: Breast cancer susceptible gene 1
CK2-PP2A: Casein kinase 2—protein phosphatase 2A
CLL: Chronic lymphocytic leukemia
CSCs: Cancer stem cells
EGFR: Epidermal growth factor receptor
EMT: Epithelial to mesenchymal transition
FD: Fluorofenidone
GSCs: Glioma stem cells
HCC: Hepatocellular carcinoma
HIF1α: Hypoxia-inducible factor 1-alpha
HNSCC: Head and neck squamous cell carcinoma
IBD: Inflammatory bowel disease
IFN: Interferon
JAK: Janus kinase
LPPs: Lipoma-preferred partner
MEFs: Mouse embryo fibroblasts
PAR-1: Protease-activated receptor 1
PIAS: Protein inhibitors of activated STAT
PTKs: Protein tyrosine kinases
PTPs: Protein tyrosine phosphatases
RA: Rheumatoid arthritis
RNS: Reactive nitrogen species
ROS: Reactive oxygen species
SERT: Serotonin transporter
Shk: Shikonin
SLE: Systemic lupus erythematosus
SOCS: Suppressors of cytokine signaling
STAT: Signal transducers and activators of transcription
TAMs: Tumor-associated macrophages
VEGF: Vascular endothelial growth factor
YB-1: y box binding protein