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STAT Transcription Factors in Tumor Development and Targeted Therapy of Malignancies

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1. Introduction

The signal transducers and activators of transcription, STAT proteins, were originally discovered in interferon (IFN)-regulated gene transcription in the early 1990's. Since then, a number of cytokines have been recognized to activate various STAT proteins. STATs constitute a family of seven transcription factors, STAT1 α/β , STAT2, STAT3 α/β , STAT4, STAT5A, STAT5B and STAT6, that transduce signals from a variety of extracellular stimuli initiated by different cytokine families that aside from interferons (interferon α , β and γ) include gp130 cytokines, i.e., IL-6, IL-12, IL-23 and γ C cytokines that include IL-2, IL-15 and IL-21 [1].

Although structurally similar, the seven STAT family members possess diverse biological roles and are engaged in numerous processes from embryonic development, organogenesis, cell differentiation to regulation of immune processes. Awareness of their important role in regulation of cell proliferation, differentiation and survival has spurred interest in investigation of their activity in malignant transformation [2]. Evidence has now accumulated that confirms their role in pathogenesis of leukemias and numerous solid tumors [3] (Table1).

Aside from cytokine receptors, STATs are also activated by receptors for growth factors (family of tyrosine kinase receptors) that include receptors for epidermal growth factor - EGFR, platelet-derived growth factor - PDGF, hepatocyte growth factor - HGF and colony-stimulating factor 1- CSF-1 receptors that possess an intrinsic tyrosine kinase activity [4]. These receptors may activate STAT proteins either directly or indirectly by means of JAK kinase proteins. Also, free intracellular enzymes, i.e., non-receptor tyrosine kinases that include oncogenes *src* and *bcr-abl* activate various STATs [5].

Different biological processes regulated by STAT proteins
Embryonic development
Organogenesis and function
Cells proliferation
Cell differentiation, growth and apoptosis
Innate and adoptive immunity
Inflammation
Angiogenesis
Wound healing
Malignant transformation

Table 1. Role of STATs in the organism

Interaction of cytokines and their specific receptors directly activates free intracellular non-receptor enzymes, Janus kinases, and subsequently, latent STAT transcription factors that through the JAK/STAT signaling pathway lead to the expression of numerous genes that regulate important cellular processes. It is of importance that numerous cytokines, growth factors in different cell types activate STAT1, STAT3 and STAT5 and mediate broadly diverse biologic processes that control cell homeostasis. On the other hand, STATs such as STAT4 and STAT6 have a more specific role and they are engaged in T helper cell differentiation and maintenance of equilibrium between Th1 and Th2 immune response [6]. Defects in STAT molecules can lead to serious defects in development and to fetal death indicating the importance of JAK/STAT pathway in normal cell development. Defects in the JAK/STAT signaling pathway are often encountered in primary malignant tumors, as well as in peripheral blood lymphocytes [7,8,9] and STAT3 has been the first to be identified as a potential oncogene [2] (Fig.1).

Given the critical roles of STAT proteins such as activation of pro-inflammatory and anti-proliferative processes by STAT1 and control of cell-cycle progression and apoptosis by STAT3 and STAT5 it has been established in many studies that their dysregulation can contribute to oncogenesis [10] by increasing proliferation and slowing-down apoptosis. In this sense, studies show that STAT3 is activated in a majority of breast and prostate cancers, and that STAT3 inhibition using RNA interference or a dominant negative genotype leads to reduced cell proliferation, survival, and induces wound healing. Further, blocking STAT3 interaction with EGFR using peptide aptamers has been shown to reduce tumor growth. On the other hand, STAT1 has been primarily defined as a tumor suppressor gene and its inactivation was associated with malignant transformation. Initially STAT proteins were extensively studied in leukemias, but later their role in the development of different solid tumors has been shown.

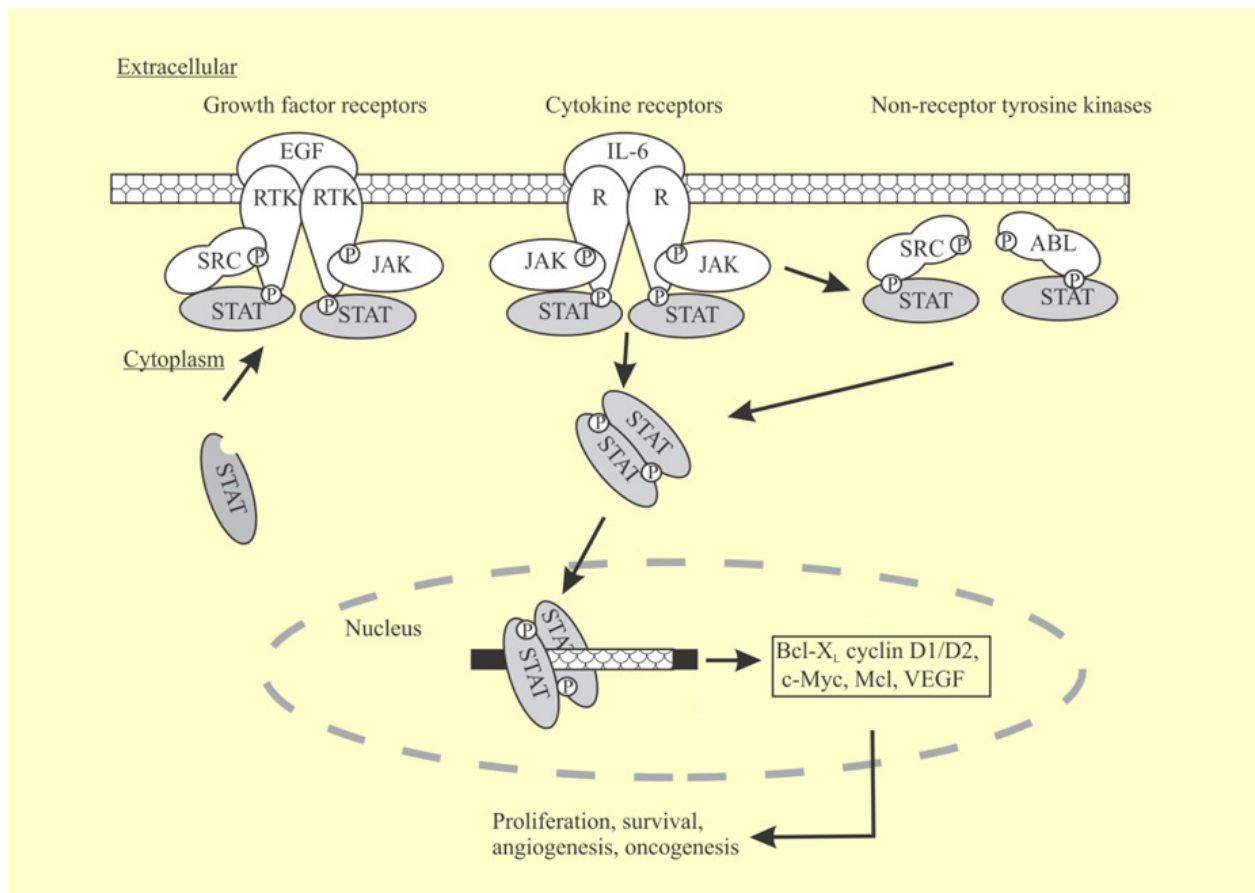


Figure 1. Mechanisms of STAT signaling upon activation of different tyrosine kinase (TK) signaling pathways that can induce activation of STAT proteins. In the case of growth factors like EGF that bind to receptor tyrosine kinases (RTKs), the receptor can directly phosphorylate STATs and/or indirectly induce STAT phosphorylation. Also, cytokines, like IL-6, that bind to cytokine receptors lacking intrinsic TK activity undergo ligand-induced dimerization of the receptor that results in phosphorylation of receptor-associated JAK kinases. JAKs in turn phosphorylate the receptor cytoplasmic tails on tyrosine, providing "docking sites" for recruitment of monomeric STATs. JAKs then phosphorylate the recruited STAT proteins on tyrosine, inducing dimerization, nuclear translocation, and DNA-binding activity. Other non-receptor bound free intracellular enzymes named non-receptor TKs such as SRC family kinases are also involved and can directly induce STAT activation. Once in the nucleus, activated STAT proteins bind to specific DNA sequences in the promoters of genes and induce their expression. In the context of oncogenesis, constitutive activation of TK-STAT signaling pathways induces elevated expression of genes involved in controlling cellular processes such as cell proliferation and survival.

Aside from their role in the development of tumors STAT1,3 and 5 can be considered as molecular markers for early detection of certain types of tumors, as well as prognostic factors for determining tumor aggressiveness and predictors of response to various types of therapy. Novel data also indicate functional interplay between several activated STATs and association of STAT5 with certain well differentiated tumors with favorable prognosis [11]. Based on numerous new data it appears that dysregulation of STAT signaling pathway may serve as a basis for designing novel targeted molecular therapeutic strategies that hold great potential for the treatment of solid tumors and leukemias.

1.1. Structural and functional characteristics of STATs

STATs share structurally and functionally conserved domains that include the amino-terminal domain (NH2), the coiled-coiled domain (CCD), the DNA binding domain (DBD), the linker domain and the SH2/tyrosine activation domain [12]. In contrast, the carboxyl-terminal transcriptional activation domain (TAD) is quite divergent and contributes to STAT specificity (Table 2).

Functionally, the amino-terminal domain of STAT molecules is the oligomerization domain that interacts with other proteins and mediates oligomerization of STAT dimers to form tetramers [13]. The DNA binding domain defines the DNA-binding specificity to tandem GAS elements and each STAT component of the dimer recognizes bases in the most proximal half of GAS and mediates distinct signals for specific ligands.

SH2 domain, located near the C-terminal domain, plays an important role in signaling through its capacity to bind to specific phosphotyrosine motifs and to mediate specific interactions. Consistent with this, it is the most highly conserved STAT domain. The ability of this SH2 domain to recognize specific phosphotyrosine motifs plays an essential role in three STAT signaling events that include recruitment to the phosphorylated cytokine receptor through recognition of specific receptor phosphotyrosine motifs, association with the activating JAKs, as well as STAT homo- or heterodimerization [14].

Domain	Role
NH2-terminal domain	Interacts with other proteins and mediates oligomerization of
Oligomerization domain	STAT dimers to form tetramers
DNA binding domain	Defines the DNA-binding specificity and mediates distinct signals for specific ligands
SH2 domain	Mediates specific interactions between STAT and receptors, STAT and JAK and STAT homo or hetero dimerization
COOH-terminal domain	TAD regulates the transcriptional activity of STATs and
Transcription activation domain (TAD)	provides specificity
Tyrosine residue	Phosphorylation site in the COOH-terminal domain that regulates the DNA-binding activity of all STATs. On phosphorylation mediates STAT dimerization
Serine residue	A second phosphorylation site in the C-terminal domain

Table 2. STAT structure

Close to the SH2 domain the critical tyrosine residue is located that is required for SH-phosphotyrosine interaction and thus STAT activation. This tyrosine residue is then rapidly phosphorylated by the active JAK determining STAT dimerization by binding to the SH2 domain of the reciprocal STAT molecule.

A conserved serine residue in the C-terminal domain of STAT1,3, and 5 is a second phosphorylation site that enhances DNA binding affinity and transcriptional activity [15]. It has been determined that the transcriptional activity of several STATs can be modulated through serine phosphorylation. Serine phosphorylation appears to enhance the transcription of some, but not all target genes. It has been suggested that serine phosphorylation may alter the affinity for other transcriptional regulators like minichromosome maintenance complex component 5 (MCM5) and BRCA1 [12].

C-terminal domain also encodes transcriptional activation domain (TAD) that contributes to STAT specificity and is thought to be involved in communication with transcriptional complexes, to regulate the transcriptional activity of STATs and provide functional specificity. Altered serine phosphorylation site associated with the c-terminal transactivation domain truncation of STAT1 and STAT3 reduces their transcriptional capacity by 20% [16]. Moreover, a c-TAD truncation leads to the α and β isoforms of STAT proteins that are biologically significant and appear to affect the cell's fate [13].

1.2. Mechanism and regulation of STAT protein function

When ligands bind to their receptors they initiate a cascade of intracellular phosphorylation events. However, members of the hematopoietin receptor family possess no catalytic kinase activity. Rather, they rely on members of the JAK family of tyrosine kinases to provide this activity. JAKs are constitutively associated with a proline-rich domain of these receptors [17]. Upon ligand stimulation, receptors undergo the conformational changes that bring JAKs into proximity of each other, enabling activation by trans-phosphorylation [18]. Once activated, JAKs mediate the described signal transduction. Several studies have also suggested that JAKs associate with the receptor tyrosine kinases [12]. The phosphorylated JAKs, in turn, mediate phosphorylation at the specific receptor tyrosine residues, which then serve as docking sites for STATs and other signaling molecules. Once recruited to the receptor, STATs also become phosphorylated by JAKs, on a single tyrosine residue. The position of these tyrosines in STAT molecule is specific for each member of STAT family of proteins, such as Tyr 701 for STAT1, Tyr690 for STAT2, Tyr 705 for STAT3, Tyr 693 for STAT4, Tyr 694 for STAT5, and Tyr 641 for STAT6. Their phosphorylation mediates STAT dimerization which occurs by binding of the SH2 domain of one molecule with the domain containing the phosphotyrosine of another STAT molecule [19], so the resulting dimers are thus stabilized by bivalent bonds. STAT2 is the only STAT representative that does not act as a homodimer, forming instead a complex with STAT1 and p48. As a response to several cytokines, the heterodimers STAT1-2, STAT1-3 STAT5A-5B are formed, while no heterodimers with STAT 4 and STAT6 have been identified [20] (Table 3).

Activated STATs dissociate from the receptor, dimerize, translocate to the nucleus and bind to members of the GAS (gamma activated site) family of enhancers. There are several more recent developments regarding STAT signaling, structural studies, nuclear as well as mitochondrial translocation, gene targeting and newly identified regulatory molecules.

Classical activation of STATs occurs after cytokine binding to cell-surface receptors that initiates a cascade of intracellular phosphorylation events. The phosphorylation of STATs is essential not only for dimerization, but also for the concomitant translocation of the dimers into the nucleus. Binding of STAT1 and STAT5B to importin- α 5, a part of the nucleocytoplasmic transport machinery, has been described [21].

Considering that a second phosphorylation site is serine residue in the c-terminal domain, STATs, in addition to tyrosine phosphorylation can be serine phosphorylated by various serine kinases [22] that regulate and increase STAT1,3 and 5 transcriptional activity. It is of interest that one of the kinases responsible for the phosphorylation of this serine in STAT1 and STAT3, belongs to the MAP kinases family (ERKs, JNK and p38) which emphasizes the important “cross-talk” occurring between the two transductional pathways [23]. Furthermore, there is also evidence of the activity of ERK-independent serine kinases [24], such as the role of protein kinase C (PKC) in serine phosphorylation of STATs [25] and mTOR of the PI3K pathway. The relative contribution of each of these serine kinases to STAT signaling in vivo would depend on cell-type specific expression of kinases [22]. Therefore, STATs can be phosphorylated in great many serine/threonine residues, which may modulate DNA binding and/or their transcriptional activity [26].

One can envision a negative feedback mechanism in which serine phosphorylation of STATs promotes the induction of physiologic inhibitors of STAT signaling, such as those of the suppressor of cytokine signaling (SOCS) family that inhibit at the level of JAKs [27]. Assumingly dual functional role is thus implied for STAT serine phosphorylation events, whereby the same serine kinases can apparently both enhance and repress STAT signaling, the indirect negative effect being due to preferential association of STAT proteins with the serine kinases, precluding interaction with tyrosine kinases [2, 25].

In addition to classical, canonical activation by tyrosine phosphorylation, the noncanonical STAT activation includes, besides serine phosphorylation, other, phosphorylation-independent modifications that regulate their activity. In this sense, it has been shown that following stimulation of cells with IL-1 plus IL-6 unphosphorylated STAT3 affects gene expression in the nucleus through binding to NF- κ B that mediates its nuclear import [28]. Furthermore, the classical IL-6 mediated activation of STAT3 induces tyrosine-phosphorylation of STAT3 and activates many genes, including the STAT3 gene itself that results in STAT3 synthesis that in its unphosphorylated form can induce not only the synthesis of IL-6 but also the expression of other genes such as *RANTES*, *IL-8*, *Met*, and *MRAS*.

Aside from this, the noncanonical STAT activation includes acetylation of lysine 685 in the SH2 STAT domain [29] that occurs in IL-6-induced acute phase reactions [30]. Novel findings indicate that acetylation of STAT3 is an important regulatory modification that influences protein-protein interaction and its transcriptional activity. Moreover, in oncogenesis new data regarding transmembrane glycoprotein CD44 [31], a marker of tumor metastatic phenotype, translocates into the nucleus in association with acetylated STAT3 and by regulating transcription of cyclin D enhances cell proliferation [32] (Fig. 2).

Also, many more posttranslational STAT modifications such as isgylation [33], sumoylation [34] and ubiquitination [35] are being explored in STAT-dependent tumor formation and metastasis. These noncanonical pathways include the many roles of nontyrosine phosphorylated STATs, which alter their stability, dimerization, nuclear localization, transcriptional activation function, and association with histone acetyltransferases (HAT), and histone deacetylases (HDAC) [36] (Fig. 2).

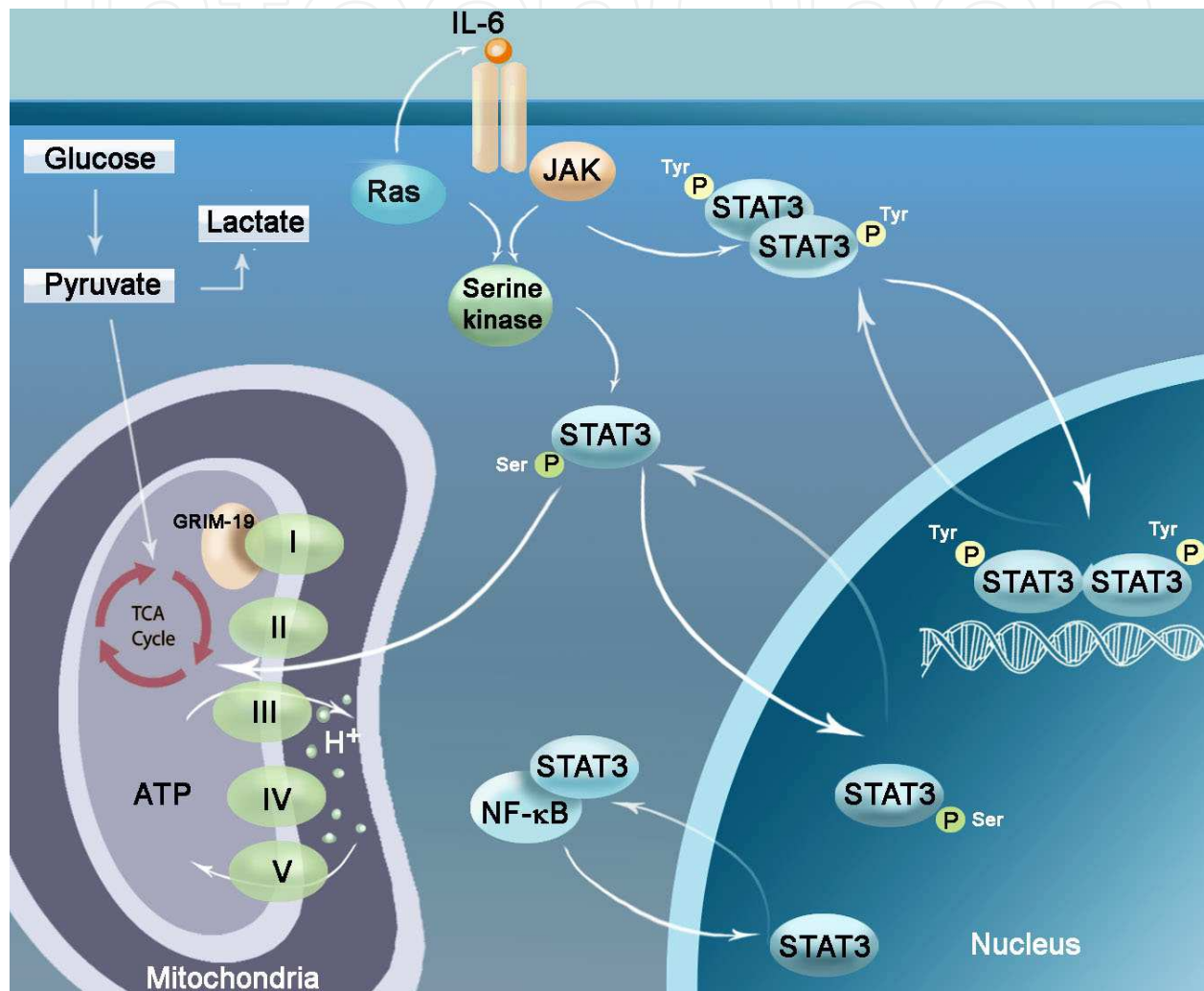


Figure 2. Different signaling pathways initiated by phosphorylation of STAT3 on tyrosine or serine residues. STAT3 is constitutively imported into and exported from the nucleus independent of its phosphorylation status. Oncogenic Ras can stimulate the autocrine production of IL-6, and the resulting phosphorylation of STAT3 Tyr705 promotes dimerization and the ability to bind specific DNA target sequences. STAT3 can also be phosphorylated on Ser727 and can mediate nuclear import of the NF-κB transcription factor. Serine phosphorylated STAT3 stimulates the electron transport chain in mitochondria and augments transformation by oncogenic Ras.

The duration of STATs activation is a temporary process, thus within hours the activating signals decay and the STATs are exported back to the cytoplasm. Negative nuclear regulators of STATs are nuclear tyrosine phosphatases that induce STAT dephosphorylation

in the nucleus important for its export back to the cytoplasm. There is evidence that a specific nuclear tyrosine phosphatase (TC45), is a phosphatase relevant for STAT1 and STAT3 [37]. In addition, it has been reported that cells lacking this enzyme retain tyrosine phosphorylated STAT1 for much longer than normal cells, and overexpression of TC45 leads to dephosphorylation of STAT5 [38]. However, TC45 has also been implicated in regulating cytoplasmic dephosphorylation of JAK1 and JAK3 [39].

Recently, the negative activity on STAT protein of a group of nuclear proteins termed “proteins that inhibit activated STATs” (PIAS) has been discovered. Studies in cultured mammalian cells indicated that PIAS1 and PIAS3 interact only with tyrosine-phosphorylated STAT1 and STAT3, respectively [40]. PIAS prevents their binding to DNA, especially of STAT1, or it speeds-up their degradation in the proteasome.

Besides nuclear, other phosphatases in the cytoplasm also represent negative STAT regulators, they include phosphatases such as SH2-containing phosphatase-1 (SH1), SH2, and protein-tyrosine-phosphatase-1B (PTP1B) implicated as cytoplasmic regulators of JAKs or STATs’ phosphorylation [38].

The activity of STAT proteins is also regulated by the inhibitors of the suppressors of the cytokine signal (SOCS) family, responsible for modulating the JAK-STAT pathway by acting on the JAK kinases. These cytokine-induced SOCS proteins are recruited to active receptor complexes to cause inhibition, and can also cause protein turnover of the receptor through a process of proteolytic degradation ubiquitine-proteasome mediated [41]. As SOCS belong to the family of target STAT genes they constitute with them a classical negative feedback mechanism [12] that can negatively regulate their own phosphorylation state [42]. Several members of this family have been identified, SOCS1,2,3,4,5,6 and 7. These regulatory proteins have an indirect negative effect on STATs by inhibiting their activating enzymes, especially Janus kinases (JAK1, JAK2, JAK3 and Tyk2), as well as, upstream receptors for growth factors [43]. Considering their negative regulatory role, SOCS proteins represent an important intracellular mechanism for limiting the potentially adverse effects of cytokines in immune reactions [44].

Aside from these mechanisms, mutations that augment the function of their activators or decreases the function of their inhibitors may lead to STAT hyperactivity and their engagement in malignant transformation.

Moreover, due to alternate splicing of STAT gene the short forms of STATs, i.e., inactive STAT β form, can potentially act as dominant-negative protein and by competitive inhibition occupy DNA as non-functional protein without transcriptional capability or by binding to wild-type STATs form [45] competitive inhibition, prevent binding of the STAT α isoform and transcription of target genes. Aside from that, the truncated STAT γ isoform of this molecule that is created by proteolysis, also competitively inhibits transcription mediated by the active α form (Table 3).

Positive regulation of STATs	Effects
Canonical regulation of STATs	
Phosphorylation of tyrosine	STAT1 - Tyr 701 STAT2 - Tyr690 STAT3 - Tyr 705 STAT4 - Tyr 693 STAT5 - Tyr 694 STAT6 - Tyr 641
Noncanonical regulation of STATs	
Phosphorylation of serine	STAT3 - Ser727 STAT4 - Ser721 STAT5 - Ser725/730
Unphosphorylated STAT	IL-6 gene dependant expression IL-6 mediated acute phase reactions
NF κ B	Nuclear import of CD44
Acetylation	
Isgylation	
Sumoylation	
Genetic regulation	
Mutations	
Hypermorphic allele of STAT3	Increased transcription
Epigenetic regulation	
Histone acetyl transferase (HAT)	
Negative regulation of STATs	
Negative cytoplasmic regulators	
Tyrosine phosphatase (SHP1,2)	Dephosphorylation
Protein-tyrosine-phosphatase-1B	
Suppressors of cytokine signals (SOCS1-7)	Inhibit JAK degrade receptors
Proteases	STAT inactive forms (β and γ)
Negative nuclear regulators	
Nuclear tyrosine phosphatase	Dephosphorylation
Proteins that inhibit activated STATs (PIAS1-3)	Inhibits STAT1-3 DNA binding Proteasome degradation
DNA methyltransferase (DNMT)	Decreased transcription
Ubiquitination	Degradation

Table 3. Regulation of STAT activity

2. STAT proteins in carcinogenesis

Aside from their essential role in mediating the effect of cytokines, it has been shown that STATs can have a significant role in tumor development and they are being considered as potential oncogenes. In normal cells, the activation of STAT proteins is transient, ranging from between a few minutes to a few hours. However, in a large group of different tumors constitutive activation of STAT family, especially STAT3 and STAT5 members, as well as the loss of

STAT1 signaling, has been detected [3, 46]. Novel results indicate that STAT proteins regulate numerous pathways that participate in oncogenesis, such as cell cycle progression, apoptosis, angiogenesis, tumor invasiveness, metastasis, and immune response evasion. Based on this STAT proteins have become significant target molecules in novel therapeutic approaches in oncology as blocking of these molecules, directly or indirectly, may arrest the malignant process [47].

Gough et al. [48] provide evidence that STAT3 has joined a set of transcription factors that in mitochondria exhibit noncanonical roles independent of classical STAT3-mediated transcription in the nucleus. In this sense, mitochondria have become important in cancer research because they regulate proapoptotic and antiapoptotic factors.

It is also of importance that according to their general principle of action STAT proteins may be divided into two groups that differ greatly. The group that comprises STAT2, STAT4 and STAT6 is activated by a limited number of cytokines and it is engaged in T cell development and the effect of interferons, while the other group that is comprised of STAT1, STAT3 and STAT5 is activated in numerous tissues and cell types by great many cytokines, different hormones and growth factors and aside from mediating immune reactions, regulates many important general processes such as cell proliferation, differentiation and survival in embryogenesis, as well as breast development [49]. In that sense, this second group of STAT proteins is of importance in malignant transformation. Aside from that, earlier results indicated that active STAT1 protein has tumor-suppressor characteristics as it down-regulates cell proliferation and induces apoptosis, so that its decreased activity is associated with numerous neoplasias. On the other hand, it has been shown for STAT3 and STAT5 that they are proto-oncogenes that activate oncogenes, *c-myc*, *cyklin D* and antiapoptotic Bcl-xL protein, facilitate passage through G1/S check-point and in that sense, aside from down-regulating apoptosis, enhance cell proliferation and transformation [12].

It has been shown that STAT3 is frequently activated in hematological and epithelial malignancies. Constitutive activation of STAT3 leads to proliferation of tumor cells and prevents apoptosis, down-regulates the production of numerous proinflammatory cytokines and chemokines and leads to secretion of factors that prevent dendritic cell (DC) maturation that suppresses adaptive antitumor immunity establishment. Aside from the disturbance of the JAK/STAT signaling pathway in primary tumors, a similar finding is frequently found in peripheral blood lymphocytes of patients with malignancies [3].

2.1. Constitutively activated STATs affect tumor microenvironment

It is known that invasive tumors need to modulate gene expression in a manner that impairs the activity of innate and adaptive immunity in immune surveillance [50, 51]. STAT3 positive tumors achieve this by preventing the production of proinflammatory cytokines, i.e., “danger signals”. Activation of the transcription factor STAT3 in the tumor and adjacent immune cells, including tumor associated macrophages (TAMs), T regulatory cells (Treg

cells), DCs, Th1 cells, Th2 cells, B regulatory cells (Bregs), myeloid derived suppressor cells (MDSCs), Th17 cells, as well as, normal epithelial cells, lead to production of cytokines IL-1 β , IL-6, IL-10, IL-17, as well as VEGF creating a feedback loop that promotes tumor growth, angiogenesis, evasion of immune surveillance and metastasis [52].

It has been shown that especially tumor produced IL-6 through JAKs/STAT3 signaling has an important role in modulating the tumor-associated immune microenvironment. IL-6 has pleiotropic functions by activating numerous cell types expressing membrane-bound gp130 IL-6 receptor, i.e., classical IL-6 signaling, as well as, by soluble form of the IL-6 receptor (sIL-6 receptor) that after binding IL-6 and interaction with gp130 in the form of IL-6 *trans*-signaling modulates a broad spectrum of target cells including epithelial cells, neutrophils, macrophages, and T cells [53]. Upregulated STAT3 in TAM has been shown to enhance the expression of IL-23 that leads to the expansion of Tregs, while conversely, transcriptionally repressing IL-12 that supports proinflammatory cytokines and antitumor immune reactions within the tumor milieu [54]. Also, tumor-evoked Bregs express activated STAT3 and induce TGF β conversion of Tregs from resting T cells [55] (Fig.3). Therefore, the production

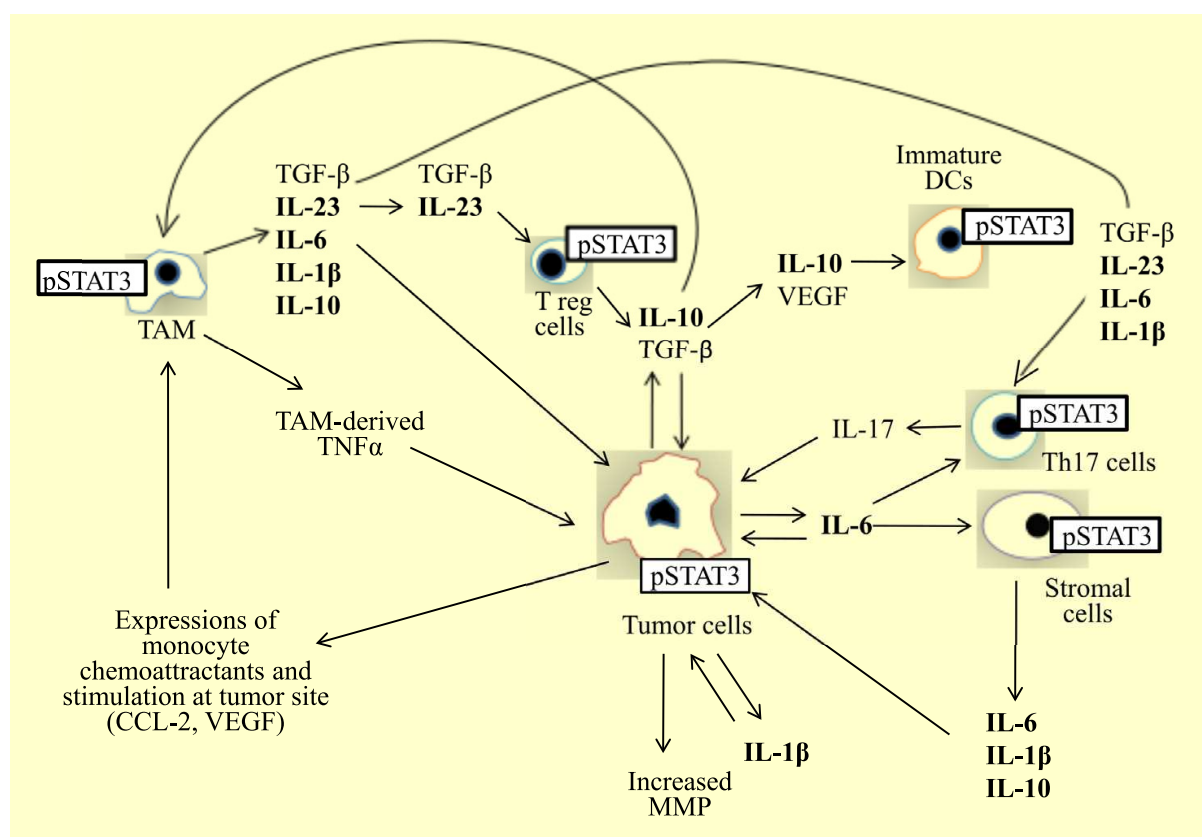


Figure 3. Interaction between tumor cells and tumor microenvironment mediated by cytokines. Tumor cells and different immune cells including TAMs, Treg cells, DC, Th17 cells, and non-tumor (normal epithelial) cells undergo STAT3 activation under the effect of various cytokines, and in turn produce more cytokines forming a feedback loop. STAT3 also regulates cell proliferation, cell cycle progression, apoptosis, angiogenesis together with immune evasion. Inhibition of STAT signaling could eliminate tumor cells while exerting minimal effect on the normal cells. Preclinical models have validated STAT3 as a target for cancer therapy, although only indirect JAK inhibitors have advanced to clinical trials (Cytokines that induce STAT3 activation are written in bold letters).

and release of various survival factors, including IL-6 as a major activator of STAT3, also serve to block apoptosis in cells during the inflammatory process, keeping them alive in very toxic environments. Unfortunately, at the same time these same pathways serve to maintain cells progressing towards neoplastic growth, protecting them from cellular apoptotic deletion and chemotherapeutic drugs.

It is of importance that activation of STAT3 within tumors is heterogeneous and it has been found that pSTAT3 are highest on the leading edge of tumors and that this is associated with stromal, immune, and endothelial cells. This follows from IL-6 from cancer-associated fibroblasts or myeloid cells that in a feedback loop induces autocrine production of IL-6 and pSTAT3 expression in tumor cells, thus also leading to heterogeneous levels of pSTAT3 [56].

Therefore tumor STAT3 activity can mediate tumor immune evasion and induce tolerance rather than immunity by blocking both the production and sensing of inflammatory signals by components of the innate and adaptive immune systems that have been recently defined as “extrinsic tumor suppressors” [57].

Regarding tumor microenvironment, in physiological conditions the activation of STAT3 is of paramount importance during tissue remodeling in the process of „wound healing“ [58]. As tumor growth also includes tissue damage, the dysregulation of STAT3 in the context of tumor microenvironment has a detrimental effect that instead of wound healing leads to further tissue destruction, together with evasion of immune response.

2.2. STATs support oncogene-dependent cellular transformation

Oncogenes can only transform cells that have been immortalized by carcinogens or other oncogenes exemplifying the paradigm of multistep carcinogenesis. In this sense, mammal cells transformed by oncogenic *src* show constitutively active STAT3 and negative-dominant forms of STAT3 block the transforming ability of *src*, demonstrating a close correlation between STAT3 activation and the oncogenic transformation by this oncogene [59].

Moreover, recent studies have shown that constitutive activation of STAT3 in human breast cancer cells correlates with EGFR family kinase signaling and also with aberrant JAK and Src activity [60]. In addition to Src, many other transforming tyrosine kinases, such as Eyk, Ros and Lck, constitutively activate STAT3 in the context of oncogenesis. Another example of tumorigenic stimuli known to activate STAT proteins is Abl that may constitutively activate STAT3 and STAT5, whereas the fusion protein, Bcr-Abl, may activate them in the absence of constitutive JAK activation, showing that the presence of the JAK kinases is not always essential for STAT activation [2] (Table 4).

In addition to its previously characterized nuclear roles, transformation specific function for mitochondrial STAT3 has now been shown. Although previous data implicated a Ras-STAT3 axis in transformation, those cases were in the context of activated tyrosine kinases, such as NPM-ALK [61], RET [62], or autocrine cytokine signaling requiring STAT3 function in the nucleus. However, it has now been shown that for cellular transformation and anchorage-independent growth induced by activated H-, N- or K-Ras, STAT3

phosphorylated on Serine727 and expressed exclusively in mitochondria was required. In contrast, recent findings also show that mitochondrially restricted STAT3 did not support *src*-driven anchorage-independent growth, consistent with former data that *src* requires nuclear functions of STAT3 [63].

Cell type	Oncogene	Activated STATs
Fibroblasts	v-Src	STAT3
	c-Src	STAT3
	v-Sis	STAT3
	v-Ras	
	v-Raf	
	IGF-1 receptor	STAT3
Myeloid	v-Src	STAT1, STAT3, STAT5
T cell	Lck	STAT3, STAT5
Mammary/Lung epithelial	v-Src	STAT3
Gallbladder adenocarcinoma	v-Src	STAT3
Pre-B lymphocytes	v-Abl	STAT1, STAT5
Erythroleukemia/blast cells/ basophils/mast cells	Bcr-Abl	STAT1, STAT5
Primary bone marrow	Bcr-Abl	STAT5

Table 4. STAT activation by oncogenes

Mitochondrial STAT3 contributes to Ras-dependent cellular transformation by augmenting electron transport chain activity, particularly that of complexes II and V, accompanied by energy production to favor cytoplasmatic glycolysis that represents a hallmark of cancer formulated in the 1950's by Warburg [64]. Additional analyses are required to understand the connections between glycolysis and oxidative phosphorylation affected by STAT3 in the presence or absence of oncogenic Ras.

STAT3 apparently enters mitochondria associated with GRIM-19 that was identified as a subunit of the mitochondrial complex I and Ser727 appears to be needed for their interaction [65].

Therefore, the “metabolic shift” important for tumor growth mediated by mitochondrial STAT3 may reflect exploitation of a normal function and in this sense mitochondrial STAT3 function could provide a new target for therapeutic approaches to cancer [65].

2.3. Anti-oncogenic and oncogenic characteristics of STAT1

STAT1 has been considered to be an anti-oncogene, i.e., tumor-suppressor protein that blocks proliferation and induces apoptosis [66]. Moreover, it has been shown that its dysfunction leads to the loss of immune surveillance [67]. Loss of STAT1 supports angiogenesis and metastasis of tumors.

It has been established that STAT1, the first STAT to be discovered, is required for signaling by the IFNs which in addition to their role in innate immunity, serve as potent inhibitors of proliferation and promoters of apoptosis. The involvement of STAT1 in growth arrest and apoptosis in many cell types may be explained by its capacity to induce caspase and p21 expression [68] and reduce c-myc expression. Although, normally, high p21 expression is associated with cell growth arrest, p21 increase has also been observed in some human neoplasias. This contradiction has been explained by Bowman et al. (2000) [2] with the fact that p21 is also responsible for the correct association of the cyclin D1/CDK cyclin complex, and thus its increase may be necessary for cell-cycle progression. Interestingly, in mammary cells p21 upregulation by STAT1 appears to involve BRCA1, which is often lost in familial and other forms of breast cancer. Effective STAT1-BRCA1 binding is mediated by serine phosphorylation of STAT1. More recently besides its role as tumor suppressor, new evidence has shown that STAT1 can be activated in some malignancies such as breast, lung, head and neck cancer and brain tumors [46]. In this sense, STAT1 tyrosine 701 phosphorylation increase was demonstrated in human breast tumor cells with elevated levels of HER-2/Neu as well as in cell lines transfected with HER-2/Neu gene [70]. However, it is of interest that breast cancer patients with higher levels of phosphorylated and DNA-bound STAT1 show better prognosis and live longer.

Besides increased STAT activation, high expression of the unphosphorylated form of STAT1 was also found in cancer cells. Moreover, it has been also shown that recurrent tumors express higher levels of unphosphorylated STAT1 compared to the original tumors [72], as well as cancer cells resistant to ionizing radiation and anticancer agents [73]. Recently, functions of some STAT1-induced genes in cancer cells have been investigated, and some have been shown to have pro-metastatic, pro-proliferative, or antiapoptotic properties [74]. In this sense it has been found in melanoma cells that high levels of STAT1 expression inhibits caspase 3/7 activation in response to doxorubicin which contributes to patients' resistance to this chemotherapeutic agent [75]. It has also been shown by Khodarev et al. (2007) [76] that ectopically increased expression of STAT1 can induce a radiation-resistant phenotype.

Both type I and type II IFNs increase STAT1 expression in many cell types, including normal fibroblasts and mammary epithelial cells, and the newly synthesized STAT1 protein persists for many days after IFN stimulation in unphosphorylated form [77]. Certain types of human tumors are unresponsive to IFNs due to defects in the STAT1 activation pathway.

Contrary to these findings, recent data states that the expression level of STAT1 does not influence the response to IFN adjuvant therapy in cancer [72] and that the overexpression of STAT1 in recurrent tumors might be caused by IFN treatment. In these tumor cells the found increase in STAT1 level does not result in enhanced anticancer effects of STAT1 as many IFN-induced pro-apoptotic and antiproliferative proteins as APO2L/TRAIL and IRF1 [78] are not upregulated in resistant cells. This strongly indicates that IFN signaling is not responsible for STAT1 upregulation in cancer cells. It has also been found that high level of unphosphorylated STAT1 in tumors protects cancer cells from DNA damage [79].

These observations suggest that increased levels of unphosphorylated STAT1 might participate in oncogenesis as well as resistance to cell death by inducing target genes that increase proliferation, decrease cell death, or increase repair of DNA damage. Increased DNA damage in cancer is due to oncogene-induced damage, chromosome instability, and other causes that are intrinsic to tumorigenesis. Therefore, evolving cancer cells must learn to resist the consequences of DNA damage, avoiding normal cellular responses such as cell cycle arrest or apoptosis, thus relying on support mechanisms that are characteristic for the tumor “stress phenotype”. A working hypothesis that is now being formulated is that the increase in STAT1 expression in cancers is due to processes intrinsic to tumorigenesis [77].

2.4. Oncogenic characteristics of STAT3 and STAT5

Although STAT3 was originally identified as an acute phase response factor that is activated after stimulation by interleukin-6 (IL-6) [65], the biological functions of STAT3 are diverse, in part stemming from the activation of STAT3 by a wide range of cytokines, growth factors, as well as oncogenes. Among its many effects, it is now known to promote oncogenesis, while a hypermorphic allele of STAT3 can function as an oncogene [10].

It is established that the basic role of STAT3 in tumors is the prevention of apoptosis that is achieved by increased expression of antiapoptotic molecule, Bcl-2, or by affecting cell cycle progression by increased expression of c-myc and cyclin D1 engaged in the transition through G1/S check point. This is a characteristic of tumor cell lines with deleted STAT3 gene (STAT3 ^{-/-}) where the lack of STAT3 activity leads to the appearance of apoptosis due to an increase in the level of caspases, and a decrease in the level of Bcl-2, while down-regulated proliferation follows from decreased level of cycline D i c-myc oncogenes.

In contrast to normal cells, in which STAT tyrosine phosphorylation occurs transiently, it has been determined that STATs 1, 3, and 5 are persistently tyrosine phosphorylated in most malignancies (particularly STAT3) [2, 46]. The mechanisms by which STAT3 is persistently or constitutively tyrosine phosphorylated in cancers include increased production of cytokines and cytokine receptors, which is initiated by tumor cells in an autocrine, and by tumor microenvironment in a paracrine manner, by a decrease in the expression of the SOCS proteins through gene promoter methylation, as well as loss of tyrosine phosphatase activity [11].

Most of the described oncogenic functions of STAT3 depend on the phosphorylation status of Tyr705, however, another role of STAT3 is independent of tyrosine phosphorylation, as unphosphorylated STAT3 can also affect gene expression in the nucleus, one mechanism is through binding to NF-κB and mediating its nuclear import [80].

STAT3 has been directly linked to human cancer as it is required for cell transformation by the *src* oncogene [81], as well as in promoting cellular transformation by the *H-ras* oncogene. This function, which is dependent on the noncanonical serine phosphorylation of STAT3, takes place in mitochondria.

Unlike another member of STAT family, STAT1, that is imported in the nucleus only in phosphorylated form, STAT3 dynamically shuttles in and out of the nucleus independent of

its tyrosine phosphorylation status [82, 83]. Nuclear import requires binding of STAT3 to an importin- α -importin- β dimer. On the other hand, mitochondrial import could be mediated in several ways, including by association with the cytosolic chaperones, heat shock proteins (Hsp70, Hsp90) [84] or associated with GRIM-19, a subunit of mitochondrial complex I of the electron transport chain [85] engaged in cell death processes in mitochondria that when overexpressed inhibits the activity of STAT3 by direct binding [86].

In light of this finding and the fact that STAT3 function has been linked to cancer, Gough et al. (2009) [48] evaluated the contribution of STAT3 to Ras oncogenic transformation. Ras protooncogenes become constitutively active oncogenes with the acquisition of specific point mutations [87], which stabilize Ras binding to guanosine 5'-triphosphate (GTP), thus allowing Ras in its GTP-bound state to stimulate numerous downstream effectors. However, Ras oncogenes can only transform cells that have been immortalized by carcinogens or other oncogenes, in the classical multistep carcinogenesis. Some of the signaling molecules activated in response to Ras can impact the STAT3 transcription factor. For example, mitogen-activated protein kinases (MAPKs) can phosphorylate STAT3 on Ser727 and downstream activation of the NF- κ B transcription factor induces autocrine IL-6 production canonical tyrosine phosphorylation of STAT3 [88].

Cancer cells tend to have reduced oxidative phosphorylation in mitochondria, and have increased glycolysis in the cytoplasm leading to lactate production [89]. STAT3, in spite of its role in cellular transformation and cancer, promotes oxidative phosphorylation in mitochondria. New findings show that Ser727 phosphorylation of STAT3 contributed to oxidative phosphorylation in mitochondria. The effect of STAT3 on oxidative phosphorylation in mitochondria was investigated by comparing enzyme activity in STAT3^{+/+} to STAT3^{-/-} cells [48]. Wegrzyn et al. (2009) [90] showed that STAT3^{+/+} cells had comparatively greater activity of electron transport complex I and complex II but no difference in the activities of complex III or complex VI. Comparing Ras-transformed STAT3^{+/+} and STAT3^{-/-} cells revealed that, the presence of STAT3 increased activities of electron transport complex II and V. Analogous to cells that lack oncogenic Ras [90], STAT3 appears to stoke the powerhouse, i.e., mitochondria.

Unexpectedly, STAT3-expressing cells also had decreased mitochondrial membrane potential and increased lactate dehydrogenase production, indicating a shift to cytoplasmic glycolysis. Additional analyses are required to understand the complex connections between glycolysis and oxidative phosphorylation affected by STAT3 in the presence or absence of oncogenic Ras.

Originally, STAT5 was originally identified as a specific transcription factor that mediates the effects of prolactin [91]. STAT5A and STAT5B forms are 96% conserved at the protein level but they differ in their C terminal domain as STAT5A has 20 and STAT5B 8 unique amino acids in the C-terminus [92]. However, STAT5A transmits predominantly the signals initiated by the prolactin receptor, while STAT5B mediates the biological effects of growth hormone.

The most important role of STAT5A and STAT5B is in lymphoid, myeloid and erythroid cell development and function as they are activated by multiple cytokines, including IL-2, IL-3, IL-5, IL-7, IL-9, IL-15, GM-CSF and erythropoietin [93]. STAT5B serine 193 is a novel cytokine induced phospho-regulatory site that is constitutively activated in primary hematopoietic malignancies [94]. Following cytokine stimulation, human STAT5A and STAT5B are phosphorylated by JAK1, JAK2 or Tyk on the conserved tyrosine residues 694 and 699, respectively, which allows for their dissociation from the receptor complex, formation of hetero- or homo-dimers, and nuclear translocation to bind specific elements in the promoter of target genes and activate transcription [95]. While tyrosine phosphorylation is a part of activation signal, the serine 726 on STAT5A and 731 on STAT5B phosphorylation may abrogate the transcriptional activity of STAT5A/B [96].

In addition to the physiological role of STAT5 in hematopoietic cell development, dysregulation of the STAT5 signaling pathway plays a role in oncogenesis and leukemogenesis [97]. Specifically, STAT5 has been shown to be constitutively activated in several forms of lymphoid, myeloid and erythroid leukemia [98-100]. Persistent activation of STAT5 was found to be a result of deregulated cytokine signaling [101] or the presence of oncogenic tyrosine kinases. STAT5 proteins can activate many oncogenic tyrosine kinases, including Bcr-Abl, mutated forms of Flt-3 and Kit, and the JAK2 V617F mutant [102-104]. In acute promyelocytic leukemia (APL) beside the most common PML-RAR α chromosomal translocation, RAR α gene can be fused with STAT5B forming a fusion protein that blocks myeloid differentiation [105].

The most probable molecular mechanism by which STAT5 promotes tumorigenesis is upregulation of cyclin D and c-myc expression which promotes progression from the G1 to the S-phase of the cell cycle [2]. Aside from stimulating proliferation, STAT5 inhibits apoptosis by inducing the expression of anti-apoptotic Bcl-x_L protein and promotes survival of tumor cells [106].

In addition to several types of leukemia and hematopoietic disorders [8], active STAT5A/B is also frequently detected in solid tumors, such as prostate cancer, breast cancer, uterine cancer, squamous cell carcinoma of the head and neck [107].

STAT5A/B controls viability and growth of prostate and breast cancer. The expression of nuclear, active STAT5A/B is often associated with high grade prostate cancer, predicts early disease recurrence and promotes metastatic dissemination. In prostate cancer, active STAT5A/B signaling pathway increases transcriptional activity of androgen receptors. Androgen receptor, in turn, increases transcriptional activity of STAT5A/B. STAT5A/B potentially contributes to castration resistant growth of prostate cancer [108]. The molecular mechanisms underlying constitutive activation of STAT5 in primary and recurrent human prostate cancers are currently unclear, and may involve the autocrine prolactine-JAK2 pathway [109], Src kinases, or Rho GTPases.

In breast cancer, the role of STAT5A/B is more complex. In rodent model systems STAT5A/B may promote malignant transformation and enhance growth of breast tumors [110], while in

contrast, STAT5A/B activation in established human breast cancer positively correlates with tumor differentiation [111], prevents metastatic dissemination, and predicts favorable clinical outcome [112] of node-negative breast cancer. In addition, active STAT5A/B, induced by Akt-1, positively correlated with mammary epithelial cell differentiation and possibly a better response to endocrine therapy [113]. Collectively, these studies suggest a dual role for STAT5A/B in the mammary gland as an initiator of tumor formation, as well as a promoter of differentiations of established tumors.

2.5. STAT dysfunction associated with different malignancies

In addition to individual roles of each STAT, they may be coactivated in cancers. In this sense, STATs 1, 3, and 5 are simultaneously tyrosine phosphorylated in a number of human cancers including breast, lung, and head and neck tumors (Table 5). The presence of pSTAT5 in addition to pSTAT3 in head and neck tumors can enhance tumor growth and invasion and may contribute to resistance to EGFR inhibitors and chemotherapy [114].

The functional interplay between activated STAT3 and STAT5 has also been described in breast cancers. Considering that STAT3 is included in breast development in association with EGFR, it has been shown on breast cancer cell lines and primary tumors that EGFR mutations, as well as the activity of *src* proto-oncogene, lead to hyperactivity and STAT3 oncogenic properties [115]. JAK/STAT3 signaling pathway is required for growth of CD44⁺CD23⁻ breast cancer stem cells in tumors [116]. It has been shown that STAT1 blocking by EGFR in this tumor, unlike inhibition of STAT3, does not show any influence on cell proliferation [117].

Activated STAT3 and IL-6 are preferentially found in triple-negative breast cancers or in high-grade tumors and are associated with poor response to chemotherapy [118]. In human tumors, however, the presence of pSTAT5 is found predominantly in well-differentiated estrogen receptor (ER)-positive tumors and is associated with favorable prognosis. Furthermore, the presence of pSTAT5 is a predictive factor for endocrine therapy response and strong prognostic molecular marker in ER-positive breast cancer. Tumors expressing both activated STAT3 and STAT5 were more likely to be ER positive and human EGFR2 negative and of a lower stage.

Aside from the detected STAT dysregulation in tumors, more recent data report STAT status in peripheral blood lymphocytes (PBL). Results of an investigation of STATs in PBL of patients with breast cancer indicates constitutive, as well as stage-dependent, decrease in STAT1, STAT3, STAT5 expression and impaired induction of these proteins by Th1 cytokines [119]. The commonly found dysfunction of NK cells in breast cancer patients [120-122] is probably the consequence of cytokine dysbalance due to the prevalence of immunosuppressive cytokines such as IL-10 and TGFβ [123], as well as tumor-produced inhibitory factors [124]. This finding is in concordance with the only previous study published for breast cancer patients [125] and also with several other investigations showing STAT dysregulation in PBL of melanoma and renal cell carcinoma patients [126,127]. Moreover, we showed that breast cancer patients' T and NK cell subsets have lower pSTAT1

level that could be a biomarker of decreased NK cell cytotoxicity and IFN γ production associated with progression of this disease [120, 128, 129].

Constitutively active STAT3 present in breast cancer and many human solid tumors, is associated with immunosuppression of the host immune response. STAT3 expression promotes the production of IL-1 β , IL-6, IL-10, TGF β and VEGF by tumor cells [130] leading to STAT3 activation in immune cells and in turn production of more cytokines forming a feedback loop. These cytokines also inhibit dendritic cell maturation, exerting a pro-tumor response. In this sense, evaluation of STATs in PBL is of importance in predicting the possibility of immunomodulatory and antitumor effect of immunotherapy with cytokines in patients with malignancies.

Constitutive activation of STATs has been detected in human head and neck squamous carcinoma cells [131]. In these cells, activation of STATs is dependent on TGF α induced activation of EGFR and studies utilizing antisense oligonucleotides have demonstrated that STAT3 mediates oncogenic growth of these cells. Activation of STATs in non-small cell lung carcinoma (NSCLC) increased production of TGF α by activating EGFR tyrosine kinase [132] induces downstream STAT3 activation and engages it in the pathogenesis of this malignancy. EGFR constitutive activation of STATs has also been detected in prostate, renal cell, lung, ovarian, and pancreatic cancers, as well as melanomas.

In addition, activation of *src* also occurs with elevated frequency during progression of human breast, ovarian and prostate cancer, and EGFR and Src have been shown to cooperate in human breast cancer [133]. Aside from that, it is of importance that in prostate cancer cell lines the role of BRCA1 gene has been shown in forming of hyperactive STAT3 [134]. When castration resistant disease develops in androgen receptor (AR) positive prostate cancer, these tumors often express higher levels of AR, possibly through activated STAT3, which can transcriptionally regulate AR. Thus, combining antiandrogens with anti-STAT3 drugs should be considered, rather than with chemotherapy in hormone-refractory metastatic prostate cancer [11]. Also, in B16 mouse melanoma cell line hyperactive STAT3 has also been detected [135] (Table 5).

STAT hyperactivity has been demonstrated in lymphomas and leukemias. In acute myeloid leukemia (AML), characterized by the presence of immature myeloid cells in the bone marrow, STAT3 and STAT5 hyperactivity has been found. This may follow from an overproduction of hematopoietic cytokines by tumor cells [136]. An increased level of STAT3 β isoform in leukemic blasts in the bone marrow has been found in patients with this leukemia that have an overall shorter time of survival [137]. It is presumed that STAT5 in AML is activated by mutations in the *flt-3* gene. It has also been shown that hyperactive STAT3 induces increased production of VEGF in bone marrow of acute and chronic leukemia. This is in accord with the common finding of increased blood vessel density in bone marrow in these malignancies [138]. Constitutive activation of STATs 1 and 5 has been additionally detected in acute myelogenous leukemia (AML) and chronic myelogenous leukemia (CML) cells possessing the activated Bcr-Abl tyrosine kinase [139]. Moreover, T cell leukemia that arise in HIV infections, as well as Hodgkin's disease, express active STAT3.

Tumor type	Activated STAT proteins
Solid tumors	
Breast cancer	STAT1,STAT3, STAT5
Head and neck cancer	STAT1,STAT3, STAT5
Melanoma	STAT3
Lung cancer	STAT3,STAT5
Ovarian cancer	STAT3
Pancreatic cancer	STAT3
Prostate cancer	STAT3,STAT5
Hematological malignancies	
Acute myelogenous leukemia	STAT1,STAT3
HTLV-1 dependent leukemia	STAT3,STAT5
Multiple myeloma	STAT1,STAT3, STAT5
Acute lymphoblastic leukemia	STAT5
LGL leukemia	STAT3
Chronic myelogenous leukemia	STAT5
Lymphomas	
Cutaneous T cell lymphoma	STAT3
EBV-related and Burkitt's lymphoma	STAT3
B-cell non-Hodgkin's lymphoma	STAT3
Anaplastic LGL lymphoma	STAT3

Table 5. Activated STAT proteins found in various solid and hematologic tumors

The constitutive activation of STAT3 is more striking than STAT5 in ALK+ anaplastic large T-cell lymphoma (ALCL). In Sezary Syndrome, a leukaemic form of cutaneous T cell lymphoma (CTCL), the JAK3-STAT3 pathway is constitutively activated, while STAT5 activation is inducible [140]. In APL, aside from characteristic RAR α - PML chimeric fusion protein, the novel translocation resulting in STAT5B - RAR α is considered to be responsible for the lack of response to ATRA-mediated prodifferentiation therapy [141]. Moreover, inadequate activity of STAT4 leads to T helper 2 (Th2) cytokine (IL-4, IL-5 and IL-10) production and prevents adequate antitumor immune response.

3. STATs as therapeutic targets

As malignant tumors are now treated, aside from standard chemo and radiation therapy, by novel therapeutic approaches based on tumor molecular profile, therapy of different tumors now includes agents for specific targeted therapy designed to neutralize pathogenic mutations, a goal that is complex and in development. For this reason, novel therapy has extended to transcription factors, such as STATs, and agents have been designed that directly or indirectly block oncogenic STAT3 and STAT5 activity.

Following extensive cell-based screening systems for these agents in different normal, gene modified and malignant cell lines, as well as studies in experimental animals, it has been established that oncogenic STATs may be inhibited in a direct manner. One of the means is by decreasing STAT gene expression by antisense oligonucleotides (DNA and RNA) or by blocking STAT3 and STAT5 activity by small inhibitory molecules and peptide analogues. These STAT inhibitory agents have been most commonly designed to target the domains responsible for STAT dimerization, i.e., the N-terminus domain and the Src homology (SH2) domain, as well as the DNA-binding domain that makes physical contact with the STAT-responsive elements in the promoters of target genes [142] (Figure 4).

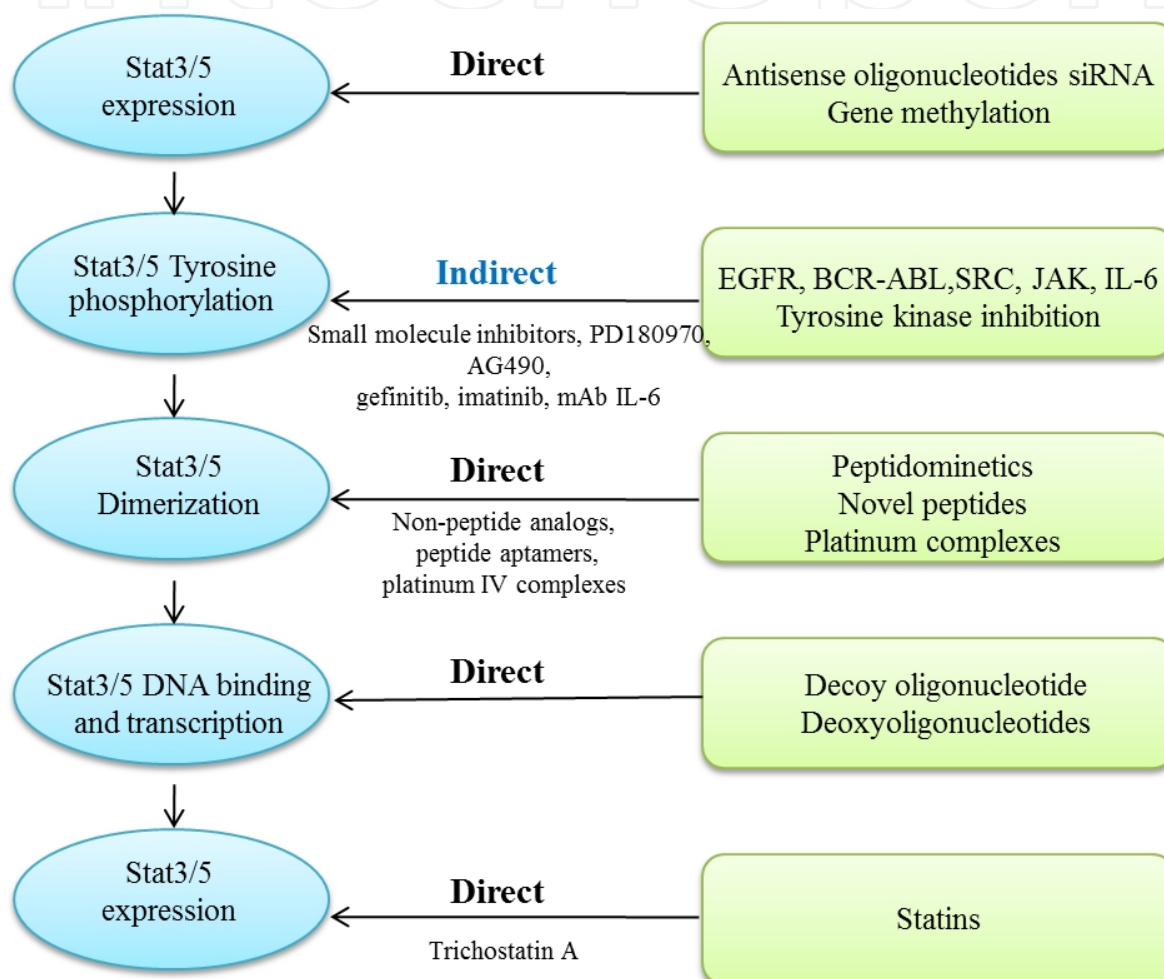


Figure 4. Available approaches and strategies to target STAT signaling pathways. These approaches target directly or indirectly STAT signaling in tumors and include interfering with STAT3 and/or STAT5 expression, phosphorylation, degradation, inhibition of receptor and non-receptor tyrosine kinases, direct interaction with STAT proteins intended to disrupt dimerization, and finally approaches to inhibit DNA-binding activity and gene transcription. These strategies should lead to a decrease in STAT signaling activity and even lower their level to normal values.

On the other hand, hyperactive STAT molecules can also be inhibited indirectly by inhibiting up-stream, either receptor or non-receptor tyrosine kinases that drive tyrosine phosphorylation and activate STATs leading to their hyperactive state [143]. In this sense,

aside from JAK enzyme inhibitors, in use are also inhibitors of *src* oncogene and inhibitors of EGFR enzymatic activity, including tyrosine kinase inhibitor gefitinib, and imatinib, an inhibitor of *bcr-abl* oncogene characteristic for CML, as well as passive immunotherapy with antibody for IL-6 or its receptor [47].

JAK enzyme inhibitors, such as tyrphostine AG490, have been shown in clinical trials to be effective in the therapy of multiple myeloma and other hematological malignancies and solid tumors with aberrant activation of the JAK-STAT signaling pathway [144]. Other agents of this type, including ruxolitinib, by showing promising results in phase III clinical trials for myelofibrosis provide a basis for their study in solid tumors such as prostate cancer. In addition to improved outcome, many JAK inhibitors have been found to be tolerable with no adverse impact on the quality of life of patients possibly due to redundancies in signaling downstream of cytokine receptors, with STATs being only a part of the signaling network.

Considering both the crosstalk between STAT and other signaling pathways and activation of other pathways by STAT inhibiting agents, such as activation of Erk MAPK kinases during pimozone STAT5 inhibitor therapy, therapeutic modalities may include STAT inhibitors in combination with MEK inhibitors, an approach defined as complementary signaling pathway inhibition [145]. Although STAT inhibitors may decrease expression of pro-survival genes, this may not be sufficient to induce apoptosis, but may merely lower the threshold for apoptosis. In this sense, a STAT inhibitor may reduce resistance to cytotoxic agents or ionizing radiation and may best be used in combination with standard therapies.

Other indirect methods for inhibition include modulation of the activity of STAT molecule by using their natural negative regulators. Thus, the activity of these signaling molecules is suppressed by increased protease activity, especially for hyperactive STAT5, induction of nuclear and cytoplasmic STAT inhibitory proteins, SOCS and PIAS, respectively, or up-regulation of tyrosine-phosphatases that dephosphorylate them [146]. Application of statins, as trichostatin A, leads to inhibition of enzyme histone deacetylase (HDAC) that by decreasing STAT transcriptional activity promotes apoptosis of malignant cells, whereas direct binding of statins to STATs leads to their covalent modification and enhanced degradation [147].

In this sense, different approaches in the context of modern targeted therapy of malignancies by decreasing expression, phosphorylation, dimerization or DNA binding of STATs can decrease the activity of these important signaling molecules or down-regulate them to almost normal level. Considering that inhibition of STAT3 and STAT5 leads to growth arrest and selective apoptosis of tumor cells, sparing benign cells, this approach may be of importance not only in the therapy, but also in chemoprevention of tumors. These aspects of molecular targeted therapy of cancer patients need to be validated in additional, properly designed clinical trials.

4. Conclusion

As STAT proteins are involved in regulating fundamental biological processes, including apoptosis and cell proliferation that are known to be dysregulated in tumors, it is not

surprising to frequently find defects in STAT signaling pathways in malignancies. In the past few years advances have been made in understanding molecular mechanisms that are responsible for STAT protein dysregulation in different malignant diseases. The critical role of constitutively active STAT3 and STAT5 in tumorigenesis has now been definitely established. Aside from that, STAT1, 3 and 5 can be considered as molecular markers for early detection of certain tumors, as well as prognostic parameters for evaluation of tumor aggressiveness and response to various types of therapies.

Obtained data that associate these molecules with tumor development support the use of STATs as molecular targets in the therapy and chemoprevention of malignancies. Inhibition of oncogenic STATs represents a comprehensive approach in tumor therapy that leads to decreased cell proliferation, survival, angiogenesis and evasion of immune response. Blocking of constitutively active STATs in tumors allows the destruction of tumor cells with minimal effect on normal cells. It is of importance that this type of molecular therapy that inhibits hyperactive STATs can potentiate response to chemo or radiation therapy and may have great potential in the therapy of solid tumors and leukemia. The efficacy of STAT inhibitors in oncological therapy remains still to be evaluated in numerous ongoing and future clinical trials.

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