*Minireview*

# Activated STAT Signaling in Human Tumors Provides Novel Molecular Targets for Therapeutic Intervention[1]

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## Abstract

**The signal transducers and activators of transcription (STAT) factors function as downstream effectors of cytokine and growth factor receptor signaling. Compared with normal cells and tissues, constitutively activated STATs have been detected in a wide variety of human cancer cell lines and primary tumors. STATs are activated by tyrosine phosphorylation, which is normally a transient and tightly regulated process. In tumor cells, constitutive activation of STATs is linked to persistent activity of tyrosine kinases, including Src, epidermal growth factor receptor, Janus kinases, Bcr-Abl, and many others. Such oncogenic tyrosine kinases are often activated as a consequence of permanent ligand/receptor engagement in autocrine or paracrine cytokine and growth factor signaling or represent autonomous constitutively active enzymes as a result of genetic alterations found in tumor but not normal cells. Persistent signaling of specific STATs, in particular Stat3 and Stat5, has been demonstrated to directly contribute to oncogenesis by stimulating cell proliferation and preventing apoptosis. STATs participate in oncogenesis through up-regulation of genes encoding apoptosis inhibitors and cell cycle regulators such as Bcl-$x_L$, Mcl-1, cyclins D1/D2, and c-Myc. Inhibition of constitutively active STAT signaling pathways has been shown repeatedly to inhibit tumor cell growth *in vitro* and *in vivo* and provides a novel means for therapeutic intervention in human cancer. In this review, we will: (*a*) explain the mechanisms of STAT activation in normal and malignant signaling; (*b*) summarize recent evidence for the critical role of constitutively activated Stat3 and Stat5 in oncogenesis; (*c*) identify candidate STAT target genes implicated in tumor progression; and (*d*) discuss molecular and pharmacological strategies to interfere with STAT signaling for potential therapeutic intervention in human cancer.**

## Introduction

STATs[3] comprise a family of cytoplasmic transcription factors that transmit signals, usually generated at cell surface receptors, to the nucleus where STATs bind to specific DNA promoter sequences and thereby regulate gene expression (1–5). Since discovery of the first STATs as key mediators of IFN signaling, a total of seven different STAT family members (Stat1, Stat2, Stat3, Stat4, Stat5a, Stat5b, and Stat6) encoded in distinct genes have been identified in mammalian cells (6). Considerable progress in defining normal physiological functions of individual STAT proteins was derived from STAT knockout mice and/or by tissue-specific deletions (7–9). These experiments demonstrate that STAT signaling is critical for normal cellular processes such as embryonic development, organ genesis and function, innate and adaptive immune function, regulation of cell differentiation, growth, and apoptosis (10–19). Besides IFNs, a large number of other cytokines as well as growth factors are now known to trigger STAT activation (20–22).

The activation duration of individual STAT proteins in normal physiological conditions is temporary and usually lasts anywhere from a few minutes to several hours. However, numerous studies have demonstrated constitutive activation of STATs, in particular Stat1, Stat3, and Stat5, in a large number of diverse human tumor cell lines (23–26). As shown in Table 1, elevated activities of these transcription factors are found frequently in a wide variety of human tumors, including blood malignancies (leukemias, lymphomas, and multiple myeloma) as well as solid tissues (such as head and neck, breast, and prostate cancers; Refs. 26–31).[4,5] Studies to date provide strong evidence that aberrant STAT signaling, in particular Stat3 and

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[3] The abbreviations used are: STAT, signal transducers and activators of transcription; SCCHN, squamous cell carcinoma of the head and neck; CML, chronic myelogenous leukemia; JAK, Janus kinase; EGF, epidermal growth factor; IL, interleukin; TGF, transforming growth factor; SH2, Src homology 2; SOCS, suppressor of cytokine signaling; PIAS, protein inhibitors of activated STATs; TAD, transcriptional activation domain; VEGF, vascular endothelial growth factor.

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*Table 1* STAT activation in human cancer cell lines and primary tumors[a]

| | Activated STAT |
|---|---|
| Blood tumors | |
| Multiple myeloma | Stat1, Stat3 |
| Leukemias | |
| HTLV-I-dependent | Stat3, Stat5 |
| Erythroleukemia | Stat1, Stat5 |
| Acute lymphocytic leukemia | Stat1, Stat5 |
| Chronic lymphocytic leukemia | Stat1, Stat3 |
| Acute myelogenous leukemia | Stat1, Stat3, Stat5 |
| Chronic myelogenous leukemia | Stat5 |
| Megakaryotic leukemia | Stat5 |
| Large granular lymphocyte leukemia | Stat3 |
| Lymphomas | |
| EBV-related/Burkitt's | Stat3 |
| Mycosis fungoides | Stat3 |
| HSV saimiri-dependent (T-cell) | Stat3 |
| Cutaneous T-cell lymphoma | Stat3 |
| Hodgkin's disease | Stat3 |
| Solid tumors | |
| Breast cancer | Stat1, Stat3 |
| SCCHN | Stat1, Stat3 |
| Renal cell carcinoma | Stat3 |
| Melanoma | Stat3 |
| Ovarian carcinoma | Stat3 |
| Lung cancer | Stat3 |
| Prostate carcinoma | Stat3 |
| Pancreatic adenocarcinoma | Stat3 |

[a] Based on references cited in Refs. 23, 25, and 26 and our unpublished results.

Stat5, participates in the development and progression of human cancers by either preventing apoptosis, inducing cell proliferation, or both (26). Although Stat1 activation is elevated in some tumors and cell lines, the function of this molecule has been associated with growth suppression rather than malignant transformation and thus can be considered a potential tumor suppressor (32).

Targeted disruption of Stat1 results in viable mice displaying a normal phenotype; however, all physiological functions associated with signaling by IFNs, which are the main activators of Stat1, are missing (33, 34). These animals demonstrate compromised innate immunity to viral diseases (33, 34) and spontaneously develop tumors (35, 36). Furthermore, constitutive activation of Stat1 caused by aberrant fibroblast growth factor receptor signaling is associated with growth arrest via the cyclin-dependent kinase inhibitor p21 in chondroblast cells (37). Accordingly, targeting of constitutive Stat1 activation in SCCHN using antisense oligonucleotides or dominant-negative Stat1 constructs had no effect on cell growth (38). In addition, Stat1 has been shown to negatively regulate the expression of c-Myc, a critical regulator of cell cycle progression (39). It has been proposed that the lack of Stat1 could potentially promote tumor cell survival attributable to the loss of an IFN-dependent tumor surveillance system (32, 35).

In contrast to Stat1 function, considerable evidence suggests that constitutive activation of Stat3 and Stat5 actively participates in tumor formation and progression, and many studies have contributed to delineation of the mechanisms underlying persistent, oncogenic STAT signaling in tumor cells (23, 25, 26). In many cases, tyrosine kinases, essential mediators between extracellular stimuli and STAT activation, are known to be constitutively active and thereby continuously phosphorylate and activate STAT molecules (24). Oncogenic tyrosine kinase signaling can either be generated by persistent cytokine and growth factor stimulation, such as IL-6-mediated JAK signaling in multiple myeloma and prostate cancer or TGF-α-mediated EGF receptor signaling in head and neck cancer (31, 38).[5] Alternatively, genetic abnormalities of certain cancer types as seen with the Bcr-Abl fusion protein in CML represent another mechanism of oncogenic tyrosine kinase activation (28, 29). Because uncontrolled activities of many different tyrosine kinases have been long known to participate in oncogenesis, it is not surprising that STATs, which are key mediators of tyrosine kinase signaling, are involved not only in normal physiological processes but also in cancers with aberrantly activated tyrosine kinases.

Inhibition of constitutive Stat3 or Stat5 activation in diverse tumor cell lines, by blocking of tyrosine kinase signaling using small molecular inhibitors, has been repeatedly associated with growth suppression and induction of cell death (30, 31, 40, 41).[4,5] By contrast, normal cells or tumor cells lacking STAT activation are typically more tolerant to the pharmacological doses used in these experiments (30, 31, 40).[4] Furthermore, similar effects are obtained when strategies were applied that interfere directly with STAT signaling, such as dominant-negative STATs or antisense oligonucleotides (30, 31, 38).[5] Collectively, these findings indicate that targeting the constitutive signaling pathways of Stat3 and Stat5 provides a potential novel strategy for therapeutic intervention in human cancer.

## Structure-Function Relationships in the STAT Signaling Pathway

Progress in basic research on STAT signaling pathways has uncovered many of the molecular events involved in the activation of these transcription factors, as illustrated in Fig. 1. Upon binding of cytokines to cognate receptors on the surface of cells, receptors dimerize and thereby activate receptor-associated tyrosine kinases such as JAKs that phosphorylate the receptor cytoplasmic portion (1, 20). Alternatively, receptors with intrinsic tyrosine kinase activity, such as platelet-derived growth factor or EGF receptor, autophosphorylate the receptor cytoplasmic tail (2, 26). In some cases, nonreceptor tyrosine kinases of the Src family also participate in STAT activation (22). Tyrosine-phosphorylated receptors provide docking sites for the recruitment of cytoplasmic monomeric STAT proteins via their SH2 domains (42). Receptor-bound STATs subsequently become tyrosine phosphorylated either by receptor intrinsic or associated tyrosine kinase activities (1, 20, 22, 26). Oncogenic derivatives of nonreceptor tyrosine kinases such as v-Src or Bcr-Abl can phosphorylate STATs independently of receptor engagement (18, 26, 29, 43).

Once phosphorylated, STATs dimerize (either as homodimers as seen with all STATs except Stat2, or in some cases as heterodimers, as seen with Stat1/2, Stat1/3, and Stat5a/5b) via reciprocal phosphotyrosine-SH2 domain interactions (6, 44). Within minutes, the dimers translocate to the nucleus, where they interact with other transcriptional modulators bound to

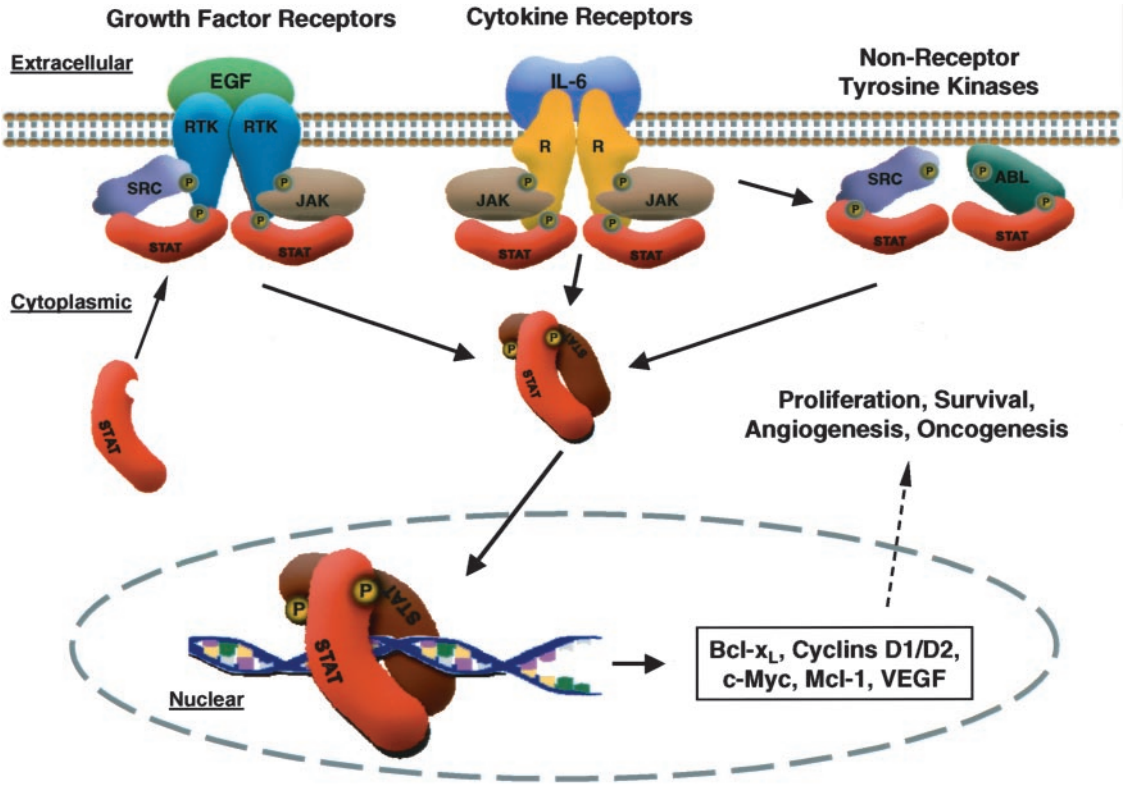


*Fig. 1* Normal and oncogenic STAT signaling pathways. Stimulation of cells with growth factors or cytokines results in dimerization of their cognate receptors and activation of intrinsic receptor tyrosine kinase activity (as shown for the EGF receptor tyrosine kinase, *RTK*) or activation of receptor-associated tyrosine kinases such as JAKs (as shown with the IL-6 cytokine receptor, *R*). Both receptor intrinsic and associated tyrosine kinases can subsequently phosphorylate the receptor cytoplasmic tail to provide docking sites for the recruitment of monomeric, nonphosphorylated STATs via their SH2 domain. Once STATs are recruited to activated tyrosine kinases, they become themselves substrates for tyrosine phosphorylation. Although receptor-associated tyrosine kinases such as JAKs and Src can cooperate in STAT activation by both growth factor and cytokine receptors, oncogenic forms such as Src and Abl can also phosphorylate STATs independently of receptor engagement. Phosphorylation of STAT monomers induces their dimerization via reciprocal phosphotyrosine-SH2 domain interactions and translocation of STATs to the nucleus, where the dimers bind to specific STAT DNA-response elements and directly regulate gene expression. In normal cells, STAT-mediated gene regulation is both transient and tightly regulated, whereas constitutive activation of STATs, in particular Stat3 and Stat5, is associated with permanent changes in the expression of genes that control fundamental cellular processes subverted in oncogenesis. STATs are proposed to participate in oncogenesis through up-regulation of genes encoding apoptosis inhibitors (*Bcl-$x_L$*, *Mcl-1*), cell cycle regulators (cyclins D1/D2, c-Myc), and inducers of angiogenesis (VEGF). See text for more details on the mechanisms of STAT signal transduction.

specific promoter sequences and induce gene expression (2, 6). STAT signaling is assumed to be terminated by dephosphorylation through nuclear tyrosine phosphatases and/or through proteolytic degradation (45).

Despite functional differences of individual STAT proteins, crystallographic studies of the cores of Stat1 and Stat3 and the $NH_2$ terminus of Stat4, as well as sequence comparisons and deletion mutagenesis experiments, revealed common STAT structural features (44, 46). The basic molecular topology of STAT proteins is illustrated in Fig. 2 using Stat3 as the prototype. The $NH_2$-terminal portion of STATs provides protein-protein interaction sites and is required for dimer-dimer interactions to form tetrameric STAT molecules. Tetramerization of STATs contributes to stabilized DNA-binding activity on weak promoters (47, 48). The adjacent coiled-coil domain is another protein-protein interaction site that provides potential contacts for transcription factors and other regulatory proteins (2). The DNA-binding domain in the center of the molecule determines DNA sequence specificity of individual STATs (44, 46). A linker region that participates in DNA-binding leads to the COOH-terminal SH2 domain, which is required for the recruitment of STATs to phosphorylated receptors and for reciprocal SH2-phosphotyrosine interactions between monomeric STATs to form dimers (49). The critical tyrosine (Y) residue required for SH2-phosphotyrosine interaction and thus STAT activation is located near the SH2 domain.

The TAD at the COOH-terminal end of the molecule is involved in communication with transcription complexes and, in case of Stat1 and Stat3, contains a serine (S) phosphorylation site that enhances transcriptional activity (50). The primary amino acid sequence of the TAD domain varies between individual STATs but is essential for STAT function as shown by deletion experiments. TAD-deficient STATs still bind to DNA but usually function as dominant-negative counterparts of the full-length forms (51, 52). Such truncated forms, for example Stat3β (51), have been identified as naturally occurring splice

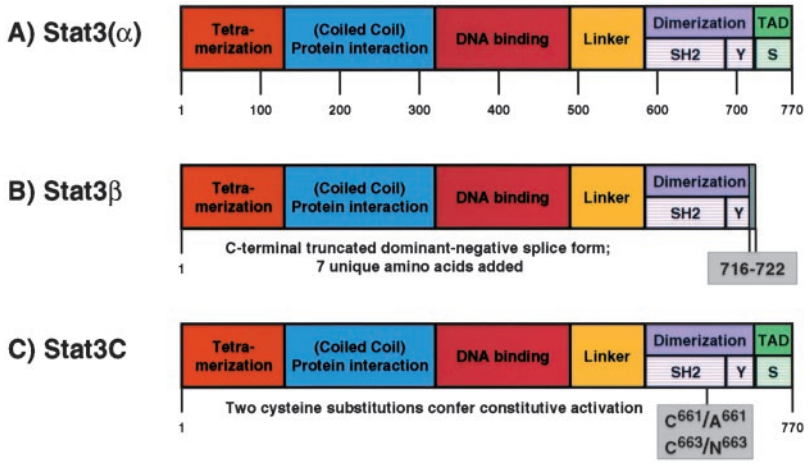


*Fig. 2* Molecular topology of STAT proteins. Schematic structure of STAT proteins as defined by sequence comparisons, biochemical assays, and mutagenesis experiments, illustrated using Stat3 as the prototype. *A*, the domain structure of full-length Stat3 protein (designated either Stat3 or Stat3α). *B*, as compared with Stat3α, the Stat3β splice variant has an COOH-terminal deletion, resulting in an altered open reading frame with the addition of seven new amino acids downstream of the deletion, and loss of the TAD domain. This naturally occurring truncated isoform still dimerizes and binds to DNA but fails to activate gene expression. *C*, substitution of two cysteine residues in Stat3α generates Stat3C, a mutant that dimerizes spontaneously (through Cys-Cys disulfide bonds) independently of tyrosine phosphorylation and is therefore permanently activated. Color scheme: identical domains are in same colors; unique sequences in Stat3β and Stat3C are indicated in *gray*. *Y*, critical phosphotyrosine in all STATs (amino acid 705 in Stat3); *S*, critical phosphoserine in some STATs (amino acid 727 in Stat3). See text for more details on the STAT functional domains.

variants, but their normal physiological functions are not yet clear. Dominant-negative STATs as well as activated mutants of STATs, such as Stat3C which is constitutively activated without tyrosine phosphorylation (53), have been used to unravel the role of STATs in malignant transformation.

## Activators of Normal and Constitutive STAT Signaling

Targeted disruption of Stat3 revealed that it is essential for early embryonic development, because mice lacking Stat3 die before birth (54). Tissue-specific gene deletions have demonstrated a critical role of Stat3 in the regulation of epithelial cell apoptosis, involution in the postlactating mammary gland, skin remodeling, keratinocyte migration, macrophage inactivation, and down-regulation of inflammatory cytokines in T-helper cell responses (55–57). Furthermore, Stat3 has been correlated with positive regulation of cell growth and is highly activated in cancer cells (26, 53, 58). It has been deduced from many different studies investigating STAT signaling (using established cell lines, primary cell cultures, and animal models as well as clinical tumor samples with matched adjacent normal tissues), that constitutively activated Stat3 and Stat5 actively participate in tumor development and progression (2, 23, 25, 26).

A critical role for Stat3 in malignant transformation was first proposed after initial studies showed that Stat3 is constitutively activated during v-Src transformation (59). More recent studies have confirmed this view and have demonstrated that Stat3 signaling is required for oncogenic transformation by v-Src (60–63). Blocking of Stat3 DNA binding with antisense oligonucleotides or a dominant-negative Stat3 protein, Stat3β, further established the critical role of Stat3 in oncogenesis (27, 30, 38).[5] In all cases, inhibition of persistent Stat3 signaling suppressed the transformed phenotype. Genetic evidence for the intrinsic oncogenic potential of Stat3 derives from a constitutively active mutant of Stat3, designated Stat3C (53). The Stat3C protein is sufficient for mediating certain aspects of cellular transformation of rodent fibroblast cells when stably expressed, and cells transformed by Stat3C have the ability to form tumors in nude mice (53). In addition to v-Src, many other transforming tyrosine kinases, such as v-Eyk, v-Ros, v-Fps, Etk/BMX, and Lck, constitutively activate Stat3 in the context of oncogenesis (26).

Because many different cytokines are known to activate STATs (20), it is not surprising that constitutive activation of Stat3 is observed downstream of aberrant cytokine signaling derived from either autocrine or paracrine sources. In the context of cytokines, IL-6 signaling through the gp130 receptor subunit is particularly relevant in multiple myeloma and prostate cancer because IL-6-mediated activation of Stat3 has a key role in preventing apoptosis and stimulating growth in cancer cells derived from these tumors (31, 64).[5] Stat3 activation is also linked to a number of receptors with intrinsic tyrosine kinase activities that are independent of the cytokine receptor gp130 subunit (24, 26). With regard to growth factor receptor signaling, the role of Stat3 in oncogenesis is well investigated in SCCHN and breast cancer. Human SCCHN cells, but not normal mucosal epithelial cells, typically overexpress both the TGF-α and its cognate receptor, EGF receptor (27, 38). It has been demonstrated that TGF-α/EGF receptor-mediated growth of transformed epithelial cells is dependent on the activation of Stat3 (38). Furthermore, interrupting Stat3 signaling by either antisense oligonucleotides or dominant-negative Stat3 protein abrogates TGF-α-induced growth of these cells (38). As seen in SCCHN, Stat3 is constitutively activated in human breast carcinoma cell lines but not in normal breast epithelial cells (58).

Similar to SCCHN, constitutive activation of Stat3 in human breast cancer cells correlates with EGF receptor family kinase signaling and also with aberrant JAK and c-Src activity; and blocking of Stat3 signaling with Stat3β results in apoptosis of breast cancer cells (30, 58).

Stat5 is represented by two highly homologous genes encoding Stat5a and Stat5b (14, 65). These are activated in response to a variety of cytokines and growth factors, including granulocyte/macrophage-colony-stimulating factor, growth hormone, prolactin, EGF, as well as by oncogenic tyrosine kinases such as Bcr-Abl. Although these two STAT proteins share considerable functional overlap, gene-disruption experiments have revealed that Stat5a and Stat5b are functionally not redundant (66–68). Stat5a knockout experiments have demonstrated that the product of this gene mediates prolactin signaling along with mammary gland development (67, 69, 70), whereas disruption of Stat5b abrogates sexually dimorphic liver gene regulation and is associated with loss of male characteristic body growth rates (66). Stat5a/b double knockouts show (in addition to impaired breast development) defects in hematopoiesis (68, 71, 72). Activated Stat5 has also been shown to promote cell cycle progression in T cells (72).

Similar to Stat3, Stat5 was subsequently demonstrated to be involved in proliferation and inhibition of apoptosis in cancer cells. Furthermore, a mutated, constitutively activated form of Stat5 was shown to be sufficient to induce certain properties of transformed cells (73). Constitutive activation of Stat5 is present in a variety of blood-derived malignancies including CML (29, 74). A genetic alteration that generates a chimeric protein between Bcr and the Abl tyrosine kinase is present in virtually all CML patients (75). The Bcr-Abl fusion protein is constitutively activated in CML patients and is essential for malignant progression of this type of cancer (76, 77). Moreover, Stat5 has been shown to be a major effector of Bcr-Abl signaling and is associated with Bcr-Abl-mediated transformation (28, 29, 78, 79). It has been demonstrated that constitutively active Stat5 is essential for Bcr-Abl-induced transformation and that dominant-negative Stat5 abrogates cellular transformation induced by Bcr-Abl (78–80).[4]

Inhibition of Bcr-Abl (and thus Stat5) by a selective inhibitor of Bcr-Abl activity suppressed cell proliferation and induced apoptosis in the Bcr-Abl-positive/Stat5-positive CML cell line K562.[4] This inhibitor had no effect on either a Bcr-Abl-negative/Stat5-postive or a Bcr-Abl/Stat5 double-negative myeloid cell line,[4] indicating that Stat5-mediated signaling leading to growth and survival is dependent on Bcr-Abl. Furthermore, disruption of Stat5 signaling in K562 cells with dominant-negative Stat5 blocks colony formation in soft-agar, suggesting that Stat5 has an essential role in Bcr-Abl-mediated transformation (79). These experiments demonstrate preferential inhibition of CML tumor cells harboring both Stat5 and its upstream activator Bcr-Abl. Other tumorigenic stimuli known to activate Stat5 include v-Abl (transformation of pre-B lymphocytes) and HTLV-1 (81, 82). In addition, mutationally activated forms of Stat5 have been shown to possess transforming properties, providing genetic evidence for the oncogenic potential of Stat5 (73, 83). Thus, Stat3 and Stat5 are the STAT family members most strongly associated with human cancer.

## STAT-regulated Genes in Malignant Transformation

Consistent with their critical roles in oncogenesis, subsequent studies have demonstrated that activation of Stat3 and Stat5 signaling regulates the expression of numerous genes involved in growth control and survival (2, 26). Many studies have shown that the antiapoptotic gene encoding Bcl-$x_L$ protein is a downstream target of Stat3 and Stat5 (31, 53, 68, 84, 85). Blocking of Stat3 activity using Stat3β suppressed IL-6 induced expression of Bcl-$x_L$ in multiple myeloma cells, resulting in a sensitization of these cells to Fas-mediated apoptosis (31). Similarly, disrupting Stat3 signaling in head and neck squamous cell carcinoma inhibits Bcl-$x_L$ expression accompanied by induction of apoptosis (84). Furthermore, inhibition of Src kinase activity leads to diminished phosphorylation of Stat3, followed by a decrease in Bcl-$x_L$ expression as well as induction of cell death (86). Activated Stat5 can confer resistance to apoptosis in Bcr-Abl-positive CML cells, in part by up-regulation of Bcl-$x_L$ (68).[4] Mcl-1, another antiapoptotic protein, was shown to be regulated by Stat3 in leukemic large granular lymphocytes (41) and by Stat5 in CML cells.[4] Mcl-1 also represents a survival factor for hematopoietic cells (87) and contributes to STAT-dependent granulocyte/macrophage-colony stimulating factor delayed apoptosis in human neutrophils (88).

The cell cycle control gene c-*Myc* has been shown to be induced in response to Stat3 signaling in v-Src-transformed NIH3T3 fibroblasts as well as through Stat5 activation (89, 90). Stat3-mediated c-Myc expression is required for v-Src-induced oncogenesis and platelet-derived growth factor-induced mitogenesis (90). In addition, growth inhibition and induction of apoptosis (associated with inhibition of Bcr-Abl/Stat5 signaling using a Bcr-Abl selective inhibitor), correlates well with down-regulation of c-Myc.[4] On the other hand, Stat1 can negatively regulate the expression of the c-Myc promoter in response to IFN-γ, consistent with its role as a mediator of growth suppression (39). Another example of critical cell cycle control genes regulated by Stat3 and Stat5 are cyclins. Constitutive activation of Stat3 signaling is associated with up-regulation of cyclin D1 in mouse fibroblasts, whereas the cyclins D1/D2 have been demonstrated to be a target of the Stat5 protein (91, 92).[4] It can be deduced from the above that the constitutive activation of Stat3 and Stat5 is associated with permanent changes in expression of genes that control fundamental cellular processes involved in oncogenesis.

## STAT Signaling Is Modulated by STAT-interacting Molecules

Many tumor cells simultaneously possess more than one permanently activated STAT family member (Table 1) and determining how the signaling of individual STATs is regulated and which of these STATs participate in the regulation of growth and survival in these cells is an area of current research. Because Stat1, Stat3, and Stat5 are often activated by the same ligand and/or intracellular tyrosine kinase, it has been suggested that cytoplasmic and nuclear proteins interact with common and unique elements in individual STATs to modulate STAT-specific responses. A number of modulators of STAT signaling pathways have been described, and more are likely to be discovered (93, 94).

The SOCS protein family comprises a group of cytokine-inducible genes that were discovered to suppress STAT signaling by binding to and inhibiting JAKs (95, 96). Some of these proteins are transcriptionally regulated by STATs themselves, suggesting that STATs can negatively regulate their own phosphorylation state. SOCS-3, which is known to inhibit Stat3 and Stat5 activation, has been shown to be critical in the negative regulation of fetal liver hematopoiesis (97). The kinase activity of the Tel-JAK2 fusion protein is associated with leukemia and known to activate Stat5 (36). SOCS-1 has been demonstrated to block Tel-JAK2-mediated transformation of hematopoietic cells (98). Recently, a deletion on chromosome 16p that contains SOCS-1 was found in 48% of primary hepatocellular carcinomas, raising the possibility that inactivation of this gene may participate in hepatocarcinogenesis (99). It can therefore be speculated that negative regulators of STAT signaling might play important roles in the control of tumor incidence and/or progression.

The PIAS represent another group of proteins that normally serve to decrease DNA activation by blocking of STAT DNA-binding activity (100, 101). The $NH_2$-terminal region of the PIAS proteins contains a conserved motif, LXXLL, which is also present in a number of other nuclear receptor coregulators (102). The PIAS-STAT interaction seems to be dependent on cytokine stimulation, a finding that is consistent with the ligand-dependent interaction of other LXXLL motif-containing nuclear receptor coregulators. Reports have demonstrated that overexpression of PIAS1 and PIAS3, specific nuclear inhibitors of Stat1 and Stat3, respectively, suppress gene transcription mediated by these STATs (100, 101). A PIAS1 mutant lacking the Stat1 interaction region is defective in suppressing Stat1-mediated gene activation (103). Members of the PIAS family have also been suggested to function in androgen and estrogen signaling (104, 105). A critical role of PIAS3 in IL-6-induced Stat3 activation has been demonstrated in multiple myeloma cells. In these cells, IL-6-induced activation of Stat3 is blocked by pretreatment of myeloma cells with estrogen (104). It has been shown that myeloma cells up-regulate PIAS3 synthesis upon estrogen receptor stimulation and that PIAS3 binds to and blocks Stat3 DNA-binding activity (104). Given the importance of IL-6 in multiple myeloma, the estrogen receptor-PIAS3-Stat3 interaction might play a critical role in the progression of this disease and may have significant therapeutic implications.

Several STAT proteins, including Stat1, Stat3, and Stat5, contain a critical serine residue near the COOH terminus that provides another phosphorylation site within the STAT molecule (Fig. 2). Serine phosphorylation has been shown to be required for maximal transcriptional activation of STATs. Cell lines mutated in the critical residue in Stat1 retain only 20% of the activity of the wild-type Stat1 and are defective in response to IFN (50). Candidate serine kinases for the phosphorylation of STATs include the various mitogen-activated protein kinase family members (106, 107). The significance of STAT serine phosphorylation is not completely understood. However, the finding that STATs cross-talk with members of other pathways, such as mitogen-activated protein kinase, indicates that STATs are embedded in complex signaling networks.

Besides serine kinases, many other (co)activators of STAT signaling have been identified thus far, including the histone acetyl transferases p300/CBP, the transcription factors c-Jun, Sp1, and glucocorticoid receptor, the coactivators Nmi and MCM, the nuclear translocation protein NPI-1, and p48 (2, 93). The physiological importance of the above-mentioned modulators of STAT signaling remains to be determined. Because deregulation of STAT activity has been repeatedly implicated in initiation and progression of human cancer, STAT-interacting proteins might play a significant role in the regulation of STAT activity in cancer formation and progression.

## STAT Signaling and Therapeutic Intervention

Increased knowledge of the molecular mechanisms underlying cancer initiation and progression has led to the identification of signaling pathways that have gone awry in tumor cells and thus provide new molecular targets for therapeutic intervention in cancer. Fundamental basic research has contributed not only to the identification of particular oncogenes involved in cancer development but also to the delineation of whole signaling pathways. This knowledge of signaling pathways has proven to be of even broader benefit for the development of cancer drugs. Targeting of molecules embedded in such pathways but not identical with the causal oncogene is especially applicable when the causal trigger is difficult to inhibit (108).

In this context, we and others have carried out the critical proof-of-principle experiments in cell culture and animal models establishing the Stat3 and Stat5 signaling pathways as valid molecular targets for therapeutic intervention in a variety of human cancers (2, 23, 26, 27). Inhibition of STAT signaling has repeatedly been demonstrated to result in growth inhibition and induction of apoptosis in tumor cells harboring constitutive activation of Stat3 or Stat5 (2, 25, 26, 29). Furthermore, studies using normal mouse fibroblasts demonstrate that disrupting Stat3 signaling causes growth arrest but not apoptosis (90, 109), suggesting that blocking Stat3 signaling may not be grossly toxic. One possible explanation for the increased sensitivity of transformed cells to apoptosis compared with normal cells is that tumor cells may have become irreversibly dependent on STAT signaling to sustain their survival. The observed dependence of certain tumors but not normal cells on constitutive STAT activation for survival has wide implications for cancer therapy, offering the potential for preferential tumor cell killing.

Targeting of tyrosine kinase activity upstream of STAT pathways has drawn special attention because of the recent development of tyrosine kinase-selective inhibitors (108, 110). For example, inhibitors of JAK family kinases block Stat3 activation and survival of human myeloma cells, breast cancer cells, and prostate cancer cells (30, 31).[5] Furthermore, Src family kinase inhibitors have been shown to block Stat3 activation and induce apoptosis of breast cancer cells (30), and EGF receptor inhibitors block Stat3 activation and survival of prostate cancer cells.[5] In the case of CML, a Bcr-Abl tyrosine kinase inhibitor blocks Stat5 signaling accompanied by growth inhibition and apoptosis.[4] Although tyrosine kinase inhibitors in general often compete with ATP for the binding site, these inhibitors appear to have sufficient biochemical specificity for their target (110). However, one potential drawback of tyrosine kinase inhibitors is that they block multiple downstream signal-

ing pathways in addition to STAT proteins, increasing the likelihood of undesirable toxicity.

More specific inhibitors of STAT signaling include Stat3 antisense oligonucleotides, which block the growth and survival of SCCHN cells, leukemic large granular lymphocytes, and prostate cancer cells (38, 41).[5] Protein inhibitors of constitutive Stat3 signaling also have been proven to be of great value in suppressing cancer cell growth *in vitro* and *in vivo* (26, 27, 109). For example, gene therapy of a mouse model of melanoma using the dominant-negative Stat3 variant, Stat3β, showed inhibition of tumor growth and tumor regression (109). Although in this approach only about 10–15% of the tumor cells were transfected *in vivo*, the Stat3β-induced antitumor effect was associated with massive apoptosis of melanoma cells, indicative of a potent bystander effect. This bystander effect is mediated in part by the release of soluble factors that are capable of inducing apoptosis and cell cycle arrest of nontransfected tumor cells (111). In addition, recent studies indicate that constitutive Stat3 signaling induces VEGF expression and tumor angiogenesis,[6] suggesting that suppression of angiogenesis may further contribute to the therapeutic efficacy of Stat3 inhibitors.

Targeting STAT proteins for therapeutic intervention in cancer remains to be fully explored. In addition to the development of tyrosine kinase inhibitors, antisense STAT oligonucleotides, and dominant-negative STAT proteins, it will be important to consider alternative strategies for targeting of constitutive STAT signaling (23, 112). Such strategies could potentially include: (*a*) development of receptor-ligand interaction antagonists, such as cytokine antagonists and receptor-neutralizing antibodies; (*b*) alteration of STAT interacting proteins, such as the PIAS and SOCS family members; (*c*) inhibition of STAT-activating serine kinases; (*d*) activation of STAT-specific phosphatases; (*e*) targeting of STAT-regulated genes involved in malignant progression; and (*f*) development of small molecule inhibitors that interfere with STAT dimerization and/or DNA binding. With regard to the latter, recent progress has been made in design of short peptides that effectively block Stat3 dimerization and DNA-binding activity both *in vitro* and *in vivo* (113). Importantly, these peptides inhibit cell transformation mediated by activated Stat3 and provide the basis for development of peptidomimetics with drug-like features (113).

## Conclusions and Perspectives

Cancer often arises from activation of any one of a very large number of different oncogenic tyrosine kinases. STATs have been shown to provide a point of convergence for tyrosine kinase signaling and thus themselves represent promising targets for the development of cancer drugs. One of the attractive features of STAT proteins for cancer therapy is that there are only two molecular targets, Stat3 and Stat5, as opposed to a multitude of tyrosine kinases. Moreover, in many cases it has been demonstrated that blocking Stat3 or Stat5 function alone is sufficient to inhibit tumor cell growth and induce apoptosis. In addition, it is likely that resistance to any single therapy such as tyrosine kinase inhibitors will develop in cancer patients, and alternative therapies will be required for more effective cancer treatment. For drug development, molecular assays that are designed to specifically measure activated Stat3 and Stat5 DNA-binding or gene-regulatory activities can be applied for drug refinement through structure-activity relationship studies. In the clinical setting, immunohistochemical assays for detection of activated phosphotyrosine-forms of Stat3 and Stat5 can provide convenient molecular markers for monitoring the efficacy of inhibitors of STAT signaling in biopsies from cancer patients. Gene expression profiling by microarray technology is expected to reveal a molecular signature of STAT-regulated genes that may have diagnostic as well as prognostic applications. In summary, STAT proteins not only provide a potential mechanism of action for tyrosine kinase inhibitors but also represent new molecular targets for novel cancer therapy approaches. Momentum for developing inhibitors of STAT proteins, particularly Stat3 and Stat5, for molecular-targeted cancer therapy is accelerating as the importance of these proteins in human cancer is becoming increasingly evident.

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