The disruption of STAT signaling blocks neoplastic transformation, thus making inhibitors of STAT proteins a potential novel molecular approach to treat human cancer.

**Background:** Through specific activation of gene expression, the family of proteins known as signal transducers and activators of transcription (STATs) converts extracellular stimuli into diverse biological responses. Beyond the normal signaling functions of STATs, recent evidence indicates that aberrant activation of STATs contributes to neoplastic transformation.

**Methods:** Current literature pertaining to the role of STAT proteins in oncogenesis is presented. Also, the rationale for developing novel approaches to disrupt STAT signaling is discussed, and the potential of STATs as anticancer targets in treating human cancer is reviewed.

**Results:** The discovery that certain oncoproteins constitutively activate specific STATs, coupled with observations that elevated STAT activity occurs frequently in a spectrum of human tumors, establishes a direct link between STAT activation and neoplastic transformation. Significantly, abrogation of STAT signaling blocks oncogenesis in model in vitro and in vivo systems. These results make STATs attractive targets for rational design of small molecule inhibitors and gene therapy approaches to disrupt STAT signaling.

**Conclusions:** As a result of genetic, biochemical, and crystallographic analyses, the functional domains of STAT proteins have been well characterized. Based on these data, selective inhibitors of STAT function can be designed. Because disrupting STAT signaling has proven effective in blocking neoplastic transformation, it is proposed that STAT proteins represent promising targets for development of novel molecular therapeutics to treat human cancer.

**Introduction**

Signal transduction (by growth factors, for example) is classically thought to employ a series of second messengers or intermediaries that act sequentially to relay extracellular stimuli to the nucleus. In contrast, studies of interferon (IFN)-dependent gene expression have led to the discovery of novel pathways that signal directly from the cell surface to the nucleus. Essential mediators of signaling in these direct pathways are the signal transducers and activators of transcription (STATs). STAT proteins comprise a family of transcription factors that become activated by tyrosine kinases in the cytoplasm and then migrate to the nucleus where they directly regulate gene expression. Thus, STATs perform a dual function with respect to signal transduction and gene regulation, thereby obviating the need for additional intermediaries.

**Structure-Function Relationships in STAT Proteins**

Seven mammalian STAT family members (Stat1 through Stat6, with Stat5a and Stat5b representing distinct genes) have been molecularly cloned and share common structural elements. Fig 1 is a generalized diagram depicting the location of important structural motifs common to most STAT family members. These domains and their associated functions have been elucidated based on biochemical and molecular studies. Each STAT molecule contains an Src-homology 2 (SH2) domain, a common protein-protein interaction domain among signaling proteins. Monomeric, inactive STAT proteins associate with each other to form active dimers through a key phosphotyrosine (pY) residue, which binds to the SH2 domain of another STAT monomer. Furthermore, such reciprocal SH2-pY interactions are critical for STAT functions, including nuclear transport and DNA binding. Thus, the activating event in STAT signaling is tyrosine phosphorylation. The DNA-binding domain resides in the N-terminal portion of the STAT molecule. Located within the C-terminal portion is the transactivation domain, which contains a serine residue, the phosphorylation of which is required for maximal transcriptional activity. Due to the structure-function relationships inherent in STAT activation, these structural domains pose excellent targets for the design and development of small molecule inhibitors that disrupt STAT signaling.

**Fig 1.** — Generic structure of a STAT protein illustrating common functional domain elements shared by STAT family members. The sites of tyrosine (Y) and serine (S) phosphorylation are shown. SH2 = Src-homology 2 domain, N = amino terminus, C = carboxyl terminus.
The following sequence of events illustrates the prevailing model of the role of STATs in normal signaling (Fig 2). This signal cascade initiates when cytokines (such as IFNs and members of the interleukin [IL] family) or growth factors (epidermal growth factor and platelet-derived growth factor, for example) bind to their cognate cell surface receptors. Growth factor receptors possess intrinsic tyrosine kinase activity and phosphorylate STATs directly, thereby activating STAT signaling. In contrast, cytokine receptors lack intrinsic kinase activity and must recruit members of the Janus kinase (JAK) family of cytoplasmic tyrosine kinases to activate STATs. Depending on which STAT family members are activated, STATs may associate as homodimers or heterodimers and then translocate to the nucleus. The activated STAT dimers then bind to specific DNA-response elements in promoters and induce expression of target genes.

In order for cells to respond to their microenvironments, extracellular stimuli must be received and transmitted to the nucleus such that specific genetic programs become activated, resulting in cell-type-specific biological responses. Regulation of specific cellular responses to extracellular stimuli is primarily determined by integration of the various components involved in the signal transduction pathway. There are several mechanisms by which cells modulate STAT signaling. For example, JAK family members associate selectively with specific cytokine receptor superfamily members. Thus, depending on ligand and cell type, multiple STAT family members may become activated. Since STAT proteins homodimerize or heterodimerize, the level of signaling diversity increases. In addition, the temporal duration of STAT activation is another potential mechanism by which to modulate the response. In normal signaling, activation of STATs occurs rapidly; however, the induction is transient. Finally, activation of parallel signaling pathways, such as mitogen-activated protein (MAP) kinases, also contributes to the complexity of signal transduction.

### Aberrant STAT Activation in Neoplastic Transformation

Since STAT proteins regulate normal mitogenic responses, researchers have begun to investigate whether deregulated activation of STATs contributes directly to cellular transformation. In contrast to normal signaling, aberrant receptor activation or protein tyrosine kinase (PTK) activity induces constitutive STAT activation in oncogenesis. The first genetic evidence implicating aberrant STAT activation in the development of neoplasias was derived from studies of signal transduction in fruit flies. A Drosophila JAK homolog with a lethal gain-of-function mutation that results in hyperactive JAK kinase activity causes leukemia-like defects to develop. Dominant suppressors of this phenotype map to loss-of-function mutations in the Drosophila homolog of a mammalian STAT gene. Thus, these studies suggest that deregulated JAK kinase activity, resulting in constitutive activation of a Drosophila STAT, directly leads to the formation of hematopoietic malignancies.

In mammalian cells, the original report demonstrating that Stat3 DNA binding is constitutively activated in stably transformed fibroblast cells linked activation of the oncogenic Src tyrosine kinase to activation of one STAT family member, Stat3. In these studies, a good correlation was observed between activation of Stat3 and oncogenic transformation by Src. This observation, which was confirmed independently by other investigators, raised the possibility that other diverse oncogenes of the receptor or nonreceptor PTK family may also activate STATs during oncogenic transformation. This prediction has been borne out in numerous studies by many laboratories, and Table 1 lists the viral and cellular oncogenes that activate specific STAT family members.

### Table 1. STAT Activation by Various Oncogenes

<table>
<thead>
<tr>
<th>Oncogene</th>
<th>Activated STATs</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibroblasts</td>
<td></td>
<td></td>
</tr>
<tr>
<td>v-Src</td>
<td>Stat3</td>
<td>16, 17</td>
</tr>
<tr>
<td>c-src&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Stat3</td>
<td>16, 17</td>
</tr>
<tr>
<td>v-Fps</td>
<td>Stat3</td>
<td>21</td>
</tr>
<tr>
<td>v-Bis</td>
<td>Stat3</td>
<td>21</td>
</tr>
<tr>
<td>Polyomavirus middle T antigen</td>
<td>Stat3</td>
<td>21</td>
</tr>
<tr>
<td>SV40 large T antigen</td>
<td>Stat3</td>
<td>21</td>
</tr>
<tr>
<td>v-Ras</td>
<td>Stat3</td>
<td>27</td>
</tr>
<tr>
<td>v-Raf</td>
<td>Stat3</td>
<td>27</td>
</tr>
<tr>
<td>v-Fos</td>
<td>Stat3</td>
<td>27</td>
</tr>
<tr>
<td>Insulin-like growth factor 1</td>
<td>Stat3</td>
<td>27</td>
</tr>
<tr>
<td>receptor</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chicken embryo</td>
<td></td>
<td></td>
</tr>
<tr>
<td>fibroblasts</td>
<td>c-Eyk&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Stat1, Stat3</td>
</tr>
<tr>
<td>v-Eyk</td>
<td>Stat1, Stat3</td>
<td>19, 26</td>
</tr>
<tr>
<td>Pre-B lymphocytes</td>
<td>v-Abl</td>
<td>Stat1, Stat5</td>
</tr>
<tr>
<td>Gallbladder</td>
<td>v-Src</td>
<td>Stat3</td>
</tr>
</tbody>
</table>

<sup>a</sup> The relationship of c-src to the polyomavirus middle T antigen has not been determined.

<sup>b</sup> This table lists the viral and cellular oncogenes that activate specific STAT family members.
Significantly, recent reports provide direct evidence that constitutive STAT activation has a causal role in oncogenesis. Constitutive Stat3 DNA-binding activity induced by the Src oncoprotein results in stimulation of Stat3-dependent gene expression. Moreover, interference with Stat3 signaling by co-expression of dominant-negative forms of the Stat3 protein blocks the transforming ability of Src. In contrast, co-expression of dominant-negative Stat3 together with the Ras oncoprotein, which does not activate Stat3, does not block Ras-induced transformation. The combined results of these studies demonstrate that activation of STAT signaling is one pathway required for cellular transformation by specific classes of oncoproteins with PTK activity. STAT proteins presumably contribute to oncogenesis by eliciting permanent changes in the genetic program required for the initiation or maintenance of transformation.

Activation of STAT Signaling in Human Cancer

Overexpression and/or elevated kinase activity of Src, epidermal growth factor receptor, and other PTKs is associated with various human cancers. As a consequence, a growing body of evidence indicates that abnormal STAT signaling in response to hyperactive PTK activity is frequently detected in human tumors in association with the progression of oncogenesis (Table 2). In particular, increased levels of Src and epidermal growth factor receptor or their associated kinase activities correlate with carcinoma of the breast. In surveys of normal breast epithelial or breast carcinoma cell lines, studies reveal that Stat3 is activated with high frequency in the carcinoma cell lines but not in the cell lines derived from normal epithelium. In addition, elevated Stat3 activity has been detected in primary breast tumors and lymphomas. Stat3 is frequently activated in both multiple myeloma cell lines and tumors derived from patient bone marrows.

Table 2. — STAT Activation in Human Tumors and Cell Lines

<table>
<thead>
<tr>
<th>Tumor Type</th>
<th>Activated STATs</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast cancer (tumors)</td>
<td>Stat1, Stat3</td>
<td>35</td>
</tr>
<tr>
<td>Breast cancer (cell lines)</td>
<td>Stat3</td>
<td>21, 34</td>
</tr>
<tr>
<td>Head and neck cancer (cell lines)</td>
<td>Stat1, Stat3</td>
<td>36</td>
</tr>
<tr>
<td>Multiple myeloma (tumors and cell lines)</td>
<td>Stat1, Stat3</td>
<td>49</td>
</tr>
<tr>
<td>Leukemia (tumors and cell lines)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HTLV-1-dependent</td>
<td>Stat3, Stat5</td>
<td>41</td>
</tr>
<tr>
<td>Erythroleukemia</td>
<td>Stat1, Stat5</td>
<td>28</td>
</tr>
<tr>
<td>Acute lymphocytic leukemia</td>
<td>Stat1, Stat5</td>
<td>38, 42</td>
</tr>
<tr>
<td>Acute myelocytic leukemia</td>
<td>Stat1, Stat3, Stat5</td>
<td>37-39, 42</td>
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<tr>
<td>Chronic myelocytic leukemia</td>
<td>Stat5</td>
<td>24, 37</td>
</tr>
<tr>
<td>Megakaryocytic leukemia</td>
<td>Stat5</td>
<td>40</td>
</tr>
<tr>
<td>Lymphoma (tumors and cell lines)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EBV-related Burkitt’s</td>
<td>Stat1, Stat3</td>
<td>42</td>
</tr>
<tr>
<td>Mycosis fungoides</td>
<td>Stat3</td>
<td>45</td>
</tr>
<tr>
<td>Herpesvirus saimiri-dependent T cell</td>
<td>Stat1, Stat3</td>
<td>43, 44</td>
</tr>
<tr>
<td>LSTRA cell line (T cell)</td>
<td>Stat3, Stat5</td>
<td>47</td>
</tr>
<tr>
<td>Cutaneous T cell lymphoma</td>
<td>Stat3, Stat5</td>
<td>46, 48</td>
</tr>
</tbody>
</table>

Recently, the role of STAT signaling as it relates to the pathogenesis of multiple myeloma has been elucidated. Malignant progression of multiple myeloma depends on the IL-6 signaling pathway for the growth and survival of myeloma cells. Previous studies have correlated elevated levels of the antiapoptotic regulatory protein, Bcl-xL, with IL-6 signaling in myeloma. Results from this recent study demonstrate that constitutive activation of Stat3 signaling, an important component of the IL-6 pathway, directly contributes to the induction of Bcl-xL gene expression. Moreover, interfering with Stat3 activation by blocking components of the IL-6 signaling pathway inhibits Bcl-xL expression and leads to apoptosis. Thus, constitutive activation of Stat3 signaling by IL-6 induces the expression of the Bcl-xL gene through Stat3-dependent gene regulation and thereby prevents apoptosis. These results demonstrate that Stat3 activation is required for promotion of tumor cell survival and directly contributes to the malignant progression of multiple myeloma by allowing accumulation of long-lived plasma cells.
Rationale Behind Targeting STAT Signaling for Drug Discovery

The implication of the above studies is that aberrant STAT signaling contributes to a permanent alternation in the genetic program of cells that ultimately results in malignant progression. Disruption of Stat3 function using a dominant-negative Stat3 protein blocks transformation of fibroblasts by the Src oncoprotein. Consistent with the results of these studies, growth and survival of multiple myeloma requires Stat3-dependent signaling. Since STAT proteins are involved in regulating fundamental biological processes, including apoptosis and cell proliferation, disruption of STAT signal transduction is a novel approach to block malignant progression in a wide variety of human tumors that depend on activation of STATs for tumorigenesis.

Although the STAT family is highly structurally conserved, there are distinct differences in both primary sequence and function. Targeted disruption of the Stat1, Stat4, Stat5a, Stat5b, and Stat6 genes in mice demonstrates tissue specificity with respect to function for each family member. In the case of Stat2 and Stat3, homozygous deletion of the gene encoding either protein is embryonic lethal. These results demonstrate that while the STAT family members share common structural features, they do not substitute for each other functionally. The nonredundant role of STAT family members is due in large part to the diversity of STAT signaling discussed above. The specificity imparted by ligand/receptor signaling results in divergent signaling pathways depending on the profile of activated STAT proteins. Thus, the lack of functional overlap among the STAT family members is an important criterion for development of drugs that specifically disrupt a particular STAT signaling pathway.

A critical test that must be met in order for STATs to be candidates for therapeutic intervention is whether loss of function of the target molecule is generally cytotoxic. Specifically, the results of disrupting Stat3 signaling in normal mouse fibroblasts demonstrate that inhibition of Stat3 activation is not deleterious to normal cell growth. Thus, normal cellular functions may not be grossly impaired by blocking Stat3 signaling, perhaps due in part to low levels of residual Stat3 signaling being sufficient for sustaining normal biological processes. One possible explanation for the sensitivity of transformed cells compared to normal cells is that tumor cells may have become irreversibly dependent on STAT signaling to sustain their growth and survival, while normal cells may be able to use alternative pathways to compensate for loss of STAT signaling.

Relevance of STAT Activation to Chemotherapy Response

One of the goals in the treatment and prevention of cancer is to minimize the toxic effects of the chemotherapeutic regimen while simultaneously eradicating the tumor cells. Many types of tumors, particularly aggressive cancers, are initially refractory to chemotherapy or eventually become resistant to the therapies. One of the mechanisms of tumor cell killing by anticancer agents involves programmed cell death (apoptosis). Early studies have indicated that elevated Bcl-xL expression induces resistance to some chemotherapeutic drugs that use apoptosis pathways for tumor cell killing. As discussed above, myeloma tumor cells with constitutively activated Stat3 signaling and elevated Bcl-xL expression are resistant to apoptosis and hence are predicted to be resistant to chemotherapeutic drugs that utilize apoptosis pathways.

Minimizing the side effects of chemotherapy while maximizing the antitumor activity has been difficult to achieve. Thus, one potential advantage to disrupting STAT signaling in tumors is that inactivation of STATs may sensitize the STAT-dependent cells to chemotherapeutic agents. At the same time, the undesirable side effects of more aggressive anticancer treatments may be avoided if sensitization allows for lower doses of these potent agents to be administered. Because blocking STAT signaling inhibits Bcl-xL expression and induces apoptosis in myeloma cells, therapeutic strategies that disrupt STATs may confer sensitivity to chemotherapeutic drugs. Thus, development of selective inhibitors of STAT activation for use in combination therapy with more conventional chemotherapeutic agents appears to be a promising area in the field of novel anticancer therapeutics.

Targeting STATs by Gene Therapy

While gene therapy approaches to cancer treatment are still in relatively early stages of development, gene therapy offers a powerful experimental tool to establish "proof of principle" that a particular molecular pathway is a valid target for cancer treatment. Stat3 is an excellent example of the power of this approach. The studies summarized above point to a critical role for activated Stat3 signaling in human cancer, and they suggest that Stat3 is a novel molecular target for cancer therapies. To evaluate Stat3 as a potential target for cancer therapy, recent studies have used gene therapy approaches to block Stat3 signaling in a mouse model of melanoma. Using a mouse melanoma cell line containing constitutively activated Stat3 to induce tumors in syngeneic mice, vector DNA encoding a dominant-negative form of Stat3 was delivered intratumorally by electroporation. Results show significant inhibition of tumor growth and tumor regression as a result of the gene therapy. This block in tumorigenesis is associated with massive apoptosis of the melanoma tumor cells in vivo. These findings are consistent with the earlier observations that blocking Stat3 signaling induces apoptosis in human myeloma tumor cells in vitro.

These gene therapy studies demonstrate that blocking Stat3 signaling induces potent antitumor activity in vivo, and they provide evidence that Stat3 is a promising target for therapy of human cancers harboring activated Stat3. Based on other studies demonstrating antitumor effects of cytokine-based genetic immunotherapy, it is likely that combination gene therapy with antitumor cytokines and Stat3 dominant-negatives will have more potent activity than either approach alone. These Stat3 gene therapy studies establish "proof of principle" that Stat3 is a valid molecular target for cancer therapy, not only by genetic approaches, but also by small molecule inhibitors of Stat3.

Methods for Screening Compounds That Disrupt STAT Signaling

Detailed elucidation of the structure-function relationships of STAT proteins will facilitate the rational design of molecules capable of disrupting the critical functions of STAT proteins. Augmenting the research goal of designing such molecules is the recent determination of the crystal structures of Stat1 and Stat3 bound to their DNA consensus sequences. The requirement of tyrosine phosphorylation for STAT dimerization and activation offers tyrosine kinases and SH2 signaling to adversely affect the outcomes of these fundamental biological processes and thereby contribute to oncogenesis. In recent years, a multitude of studies associating aberrant activation of STATs with neoplastic transformation point to this signaling pathway as having considerable promise for therapeutic intervention.

Conclusions

STATs participate in regulating normal cellular processes, converting stimuli from cytokines and growth factors into appropriate biological responses. To accomplish this, STATs regulate specific genetic programs that coordinate the cellular effectors mediating these biological outcomes. Indeed, STATs have been reported to participate in the regulation of development, cell proliferation, differentiation, and apoptosis in addition to specialized cellular functions. Therefore, there exists the potential for aberrant STAT signaling to adversely affect the outcomes of these fundamental biological processes and thereby contribute to oncogenesis. In recent years, a multitude of studies associating aberrant activation of STATs with neoplastic transformation point to this signaling pathway as having considerable promise for therapeutic intervention.
Future Directions

The advances made in treating human neoplasias have formerly relied on development of cytotoxic agents that would, in the best-case scenario, eradicate the tumor before healthy cells succumb to the effects of chemotherapy. New approaches in drug discovery and design are moving toward developing antioncogenic compounds that will result in remission or complete regression of the disease with decreased toxicity. These agents are designed to attack cancer cells at their molecular “Achilles heel.” In other words, research is being devoted to developing chemotherapeutic agents that target specific molecular pathways essential for cancer cell survival and proliferation but that are less essential for normal cellular functions. Disruption of STAT signaling holds the potential for effecting this type of favorable outcome.

A large percentage of cancers fall into the category of sporadic rather than inherited types. Discovery of the molecular mechanisms responsible for the initiation and progression of these sporadic forms of human cancer is ultimately required in order for anticancer treatment to be safer and more effective. Efforts are underway to investigate the mechanisms by which aberrant STAT activation influences the progression of neoplastic transformation. Clinically important benefits from the discovery of the contribution of STAT activation to oncogenesis include development of new diagnostic and prognostic assays based on the molecular STAT profile of tumors. Furthermore, because STAT activation has been shown to be required for oncogenic transformation, discovery and development of novel inhibitors of STAT signaling hold significant promise for providing more effective treatment for a wide variety of cancers at various stages of malignant progression.

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References


Chai SK, Nichols GL, Rothman P. Constitutive activation of JAKs and STATs in BCR-Ab1-expressing cell lines and peripheral blood cells derived from leukemic patients. J Immunol. 1997;159:4720-4728.


**Glossary**

**Apoptosis**: the cellular process, also known as programmed cell death, in which the cell undergoes a series of molecular events leading to morphological changes such as DNA fragmentation, chromatin condensation, nuclear envelope breakdown, and cell shrinkage.

**Bcl-x**: a member of the Bcl-2 (B-cell lymphoma) family of proteins involved in regulating the response of the cell to apoptosis; Bcl-x prevents programmed cell death.

**DNA consensus sequence**: a specific nucleotide motif found in the promoters of genes to which a transcription factor binds through interaction of the protein's DNA-binding domain with the nucleotide sequence.

**Dominant-negative protein**: a protein that has been genetically altered so that when expressed in a cell interferes with the function of the endogenous wild-type protein.

**Interleukin 6 (IL-6)**: cytokine involved in regulating growth, survival, and function of cells.

**Janus kinase (JAK)**: a member of a closely related family of nonreceptor tyrosine kinases that transfers a phosphate moiety to tyrosine on recipient proteins.

**Phosphotyrosine**: modification of the tyrosine amino acid residue in which a phosphate group has been transferred to the hydroxyl group.

**Promoter**: region of gene preceding the protein coding sequence that contains nucleotide sequence elements to which transcription factors bind and regulate gene expression.

**Protein tyrosine kinase (PTK)**: signal transduction molecule possessing an enzymatic function that transfers phosphate moieties to tyrosine on recipient proteins and thereby modulates the activity of the target protein.

**Signal transduction and activator of transcription (STAT)**: member of a family of proteins that, when activated by PTKs in the cytoplasm, migrate to the nucleus and activate gene transcription.

**Src homology 2 domain (SH2)**: a specific protein structural motif among signaling molecules that recognizes and binds to phosphotyrosine moieties, creating sites of protein-protein interaction.

**Src tyrosine kinase (Src)**: a member of a closely related family of nonreceptor tyrosine kinases that participate in signal transduction by phosphorylating downstream effectors; the src gene is the first viral oncogene and was identified in Rous sarcoma virus.
Syngeneic mice: mice derived from a genetically identical background.

Transcriptional activation: the induction of gene expression via the interaction of regulatory proteins with the promoter elements of target genes.

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