

4. STAT PROTEINS AND CANCER

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Introduction to Cellular Signal Transduction

Normal signal transduction is characterized by the regulated biochemical transmission of extracellular stimuli from cell surface receptors to genes in the nucleus. Transmission of the signal proceeds via the specific and sequential activation of a series of second messengers or intermediaries. Ultimately, this sequence of events leads to the induction of unique genetic programs that dictate the biological response to the extracellular stimuli. Regulation of normal signaling is required for development, mitogenesis, and programmed cell death (apoptosis) as well as specialized functions of differentiated cells. In contrast, deregulation or uncoupling of signal transduction pathways is increasingly recognized as being responsible for a growing number of pathological conditions, including human malignancies.

In order to provide therapeutic intervention in treatment of cancer based on targeting aberrant signaling, the molecular mechanisms

underlying the disease must be determined. Recent studies point to a novel family of signaling molecules, known as signal transducers and activators of transcription (STATs), which directly contributes to the progression of a wide variety of human malignancies. This review focuses predominantly on one STAT family member, Stat3, and discusses the contribution of aberrant Stat3 signaling to oncogenesis. In addition, the rationale behind designing small molecule inhibitors to disrupt STAT function is discussed.

Structure-Function Relationships in STAT Proteins

Studies of interferon (IFN)-dependent gene expression have led to the discovery of STAT proteins as signaling molecules with dual functions.¹ STAT proteins not only transmit a signal from the cell surface to the nucleus but also directly participate in gene regulation.² Seven mammalian STAT family members (Stat1-Stat6, with Stat5a and Stat5b representing distinct genes) have been molecularly

cloned and share common structural elements.² Certain key amino acid residues and domains are essential for the function of STAT proteins (Fig 1). The amino-terminal half of STATs contains the DNA-binding domain and a region that mediates cooperative binding among STAT proteins, while the carboxyl-terminus half contains the dimerization region and the transcriptional activation (transactivation) domain. Within the dimerization region, there is a key tyrosine (Y) residue and an Src-homology 2 (SH2) domain, a common structural motif among signaling molecules that mediates protein-protein interactions by binding directly to phosphotyrosine. Phosphorylation of the tyrosine residue activates STAT proteins by inducing dimerization through reciprocal phosphotyrosine-SH2 interactions between two STAT monomers. Phosphorylation of a serine (S) residue in the transactivation domain is required for full transcriptional activity of some STAT family members.²

The following description and Fig 2 illustrate the role of STATs in normal signal transduction.^{1,2} Sig-

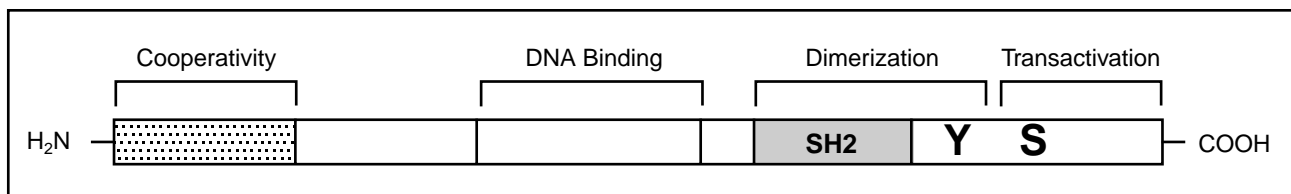


Fig 1. — Generic structure of a STAT protein illustrating common functional domain elements shared by STAT family members. The sites of tyrosine (Y) and serine (S) phosphorylation are shown. SH2 = Src-homology 2 domain, H₂N = amino terminus, COOH = carboxyl terminus.

naling initiates when ligands such as cytokines or growth factors bind to and activate their cognate cell surface receptors. Activated growth factor receptors with intrinsic protein-tyrosine kinase (PTK) activity may directly phosphorylate STAT monomers on tyrosine. In the case of cytokine receptors, which lack intrinsic PTK activity, they can recruit members of the Janus kinase (JAK) family of cytoplasmic tyrosine kinases as intermediaries to phosphorylate STATs. The phosphorylated STAT monomers dimerize when the phosphotyrosine of one molecule binds to the SH2 domain of another molecule, and then the dimers migrate to the nucleus. The activated STAT dimers bind to specific DNA-

response elements through interaction of the STAT DNA-binding domain with specific nucleotide sequences in the promoters of target genes and thereby induce gene expression (Fig 2).

Aberrant STAT Signaling in Neoplastic Transformation

As discussed above, precise regulation of signal transduction is required to control normal biological processes. In contrast, deregulation of signaling pathways potentially results in loss of growth control and resistance to apoptosis. Thus, a breakdown in signaling has the potential to derail normal biological responses and drive a cell

toward neoplastic transformation. From studies of targeted disruption of STAT family members in mice, STAT proteins participate in diverse biological processes such as cell differentiation, proliferation, and apoptosis.³ Thus, deregulated STAT signaling due to aberrant activation of PTKs has the potential to interfere with normal biological responses and contribute to oncogenesis.

The first report in mammalian cells that linked a specific oncoprotein to STAT activation demonstrated that Stat3 DNA binding is constitutively activated in rodent fibroblast cells transformed by the Src oncoprotein, which is a nonreceptor PTK.⁴ Recent reports provide direct evidence that constitutive Stat3 activation induced by the Src oncoprotein results in stimulation of Stat3-dependent gene expression.^{5,7} Moreover, abrogation of Stat3 signaling by coexpression of a dominant-negative form of Stat3, which interferes with endogenous Stat3 function, blocks the transforming ability of Src.^{5,7} Subsequent studies have shown that other diverse oncoproteins of the receptor or nonreceptor PTK families, including Bcr-Abl, activate STATs during oncogenic transformation.^{8,16} The combined findings of these studies suggest that constitutive activation of STAT signaling by specific classes of oncoproteins with PTK activity contributes to oncogenesis by eliciting permanent alterations in cells' genetic programs.

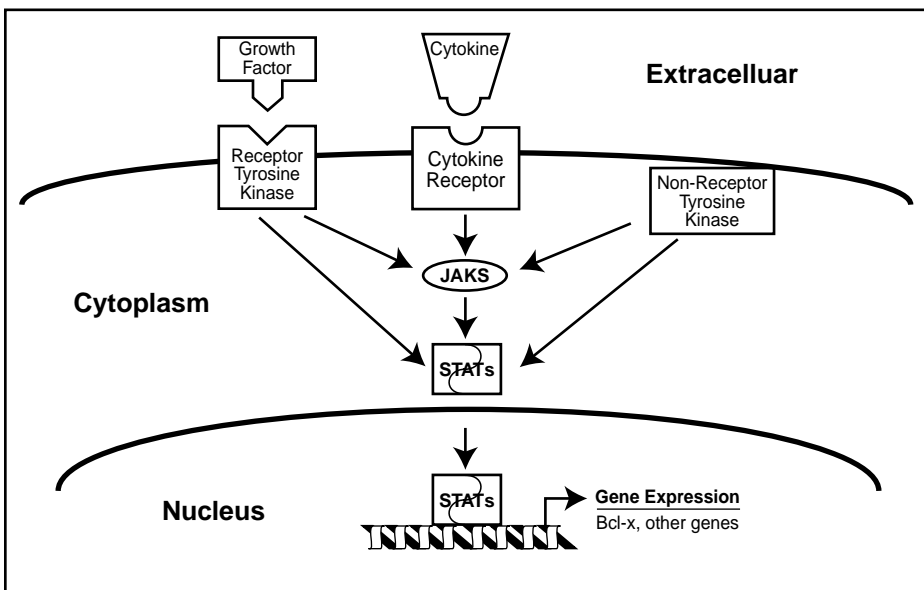


Fig 2. — Signal transduction pathways leading to STAT activation. Stimulation with growth factors or cytokines at the cell surface results in receptor activation and subsequent tyrosine phosphorylation of STATs. Phosphorylation of STATs induces dimerization and translocation to the nucleus, where STAT dimers bind to specific STAT response elements and directly regulate gene expression. In contrast to normal signaling, oncogenic PTKs constitutively activate STATs, leading to deregulated expression of STAT-dependent genes. In some cases, but not all, JAK family tyrosine kinases are known to have a role in STAT activation.

Overexpression and/or elevated protein kinase activity of epidermal growth factor receptor, Src, and other PTKs is associated with the progression of numerous 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).¹⁷ 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.^{13,18} In addition, elevated Stat3 activity has been detected in primary breast tumors¹⁹ as well as head and neck squamous cell carcinoma.²⁰ STAT activation also correlates with the progression of diverse hematopoietic malignancies (Table), such as various leukemias^{10,15,21-26} and lymphomas.²⁶⁻³² Furthermore, Stat3 is frequently activated in both multiple myeloma cell lines and tumors derived from patient bone marrows.³³

The first genetic evidence that Stat3 possesses oncogenic potential in the absence of upstream PTK activating events was demonstrated recently by generation of a constitutively activated form of Stat3, Stat3-C.³⁴ As a result of a specific amino acid modification in the SH2 domain, enforced dimerization of

Stat3-C molecules results in nuclear translocation and induction of Stat3-specific gene expression. Moreover, these studies demonstrate that Stat3-C induces colonies in soft agar and tumors in nude mice. Thus, the combined results of this study and those above point to a pivotal role for aberrant STAT activation in oncogenesis.

Role of STAT Activation in Neoplastic Progression

Because an increasing body of evidence implicates aberrant STAT activation in the progression of malignant disease, researchers have endeavored to determine the role of STATs in oncogenesis. Recent progress has been made in elucidating the mechanism of constitutive Stat3 signaling in the patho-

genesis of the human blood malignancy, multiple myeloma.³³ Progression of multiple myeloma is dependent on signaling by the cytokine interleukin-6 (IL-6) and is characterized by the expansion of long-lived plasma cells. IL-6-dependent accumulation of these cells is due to elevated levels of a key regulatory protein, Bcl-x_L, a member of the Bcl-2 family of proteins, which function to prevent apoptosis. 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-x_L gene expression.³³ Moreover, interference with Stat3 signaling, resulting in inhibition of Bcl-x_L expression, induces cell death. Thus, constitutive activation of Stat3 signaling, in response to IL-6, promotes tumor

STAT Activation in Human Tumors and Cell Lines

Tumor Type	Activated STATs	References
Breast cancer (tumors)	Stat1, Stat3	19
Breast cancer (cell lines)	Stat3	13, 18
Head and neck cancer (cell lines)	Stat1, Stat3	20
Multiple myeloma (tumors and cell lines)	Stat1, Stat3	33
Leukemia (tumors and cell lines):		
HTLV-1-dependent	Stat3, Stat5	25
Erythroleukemia	Stat1, Stat5	10
Acute lymphocytic leukemia	Stat1, Stat5	22, 26
Acute myelocytic leukemia	Stat1, Stat3, Stat5	21-23, 26
Chronic myelocytic leukemia	Stat5	15, 21
Megakaryocytic leukemia	Stat5	24
Lymphoma (tumors and cell lines):		
EBV-related Burkitt's lymphoma	Stat1, Stat3	26
Mycosis fungoides	Stat3	29
Herpesvirus saimiri-dependent T cell	Stat1, Stat3	27, 28
LSTRA cell line (T cell)	Stat3, Stat5	31
Cutaneous T cell lymphoma	Stat3, Stat5	30, 32

cell survival and malignant progression of multiple myeloma by directly inducing expression of a key apoptosis regulatory protein.³³ Another potentially important consequence of the antiapoptotic activity of Stat3 is that it may confer resistance to chemotherapy and radiation therapy, both of which rely on apoptotic mechanisms to kill tumor cells.

STATs as Targets for Cancer Drug Discovery

The implication of the above studies is that aberrant STAT signaling contributes to a permanent alteration in the genetic program of cells that ultimately results in malignant progression, most likely through increased proliferation and cell survival. Significantly, disruption of STAT function has proven effective in blocking neoplastic transformation in model in vitro systems.^{5,7,20,33} Thus, STAT proteins represent attractive targets for development of small molecule inhibitors to disrupt STAT signaling in cancer cells.

Several approaches can be taken to design inhibitors of STAT activation. One approach involves identification of upstream STAT activators and design of pharmacologic inhibitors that specifically disrupt their function. Examples of such upstream targets are cytokines and PTKs (Fig 2). However, a more direct approach is the rational design of small molecules that selectively target STAT proteins and disrupt STAT signaling. Detailed struc-

ture-function relationships of STAT proteins have been elucidated based on genetic, biochemical, and crystallographic analyses, facilitating the design of compounds that specifically disrupt STAT function. For example, one obvious structural element against which to design selective inhibitors of STAT function is the phosphotyrosine-SH2 interaction domain (Fig 1). Compounds that interfere with STAT functions such as dimerization, DNA-binding, or transcriptional activation can be screened using high-throughput assays based on inhibition of STAT biochemical properties. In addition, the efficacy and cytotoxicity of these compounds can be evaluated utilizing cultured human tumor cells and in vivo animal models. Preliminary results utilizing normal mouse fibroblasts⁷ demonstrate that disrupting Stat3 signaling is not deleterious to normal cell growth. Thus, normal cellular functions may not be grossly impaired by blocking Stat3 signaling. One possible explanation for the sensitivity of transformed cells compared with 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.

Conclusions and Future Directions

Escalating numbers of studies associating aberrant activation of STATs with neoplastic transformation point to this signaling pathway as having considerable

promise for therapeutic intervention. In particular, based on recent studies in the multiple myeloma model system, disruption of STAT signaling not only may directly kill tumor cells but also may sensitize them to apoptosis by chemotherapeutic agents.³³ Thus, development of selective inhibitors of STAT function for use in combination with conventional treatments may improve the chemotherapy response in tumors that are resistant to anticancer drugs. Additional 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.

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