Targeting the Cytoplasmic and Nuclear Functions of Signal Transducers and Activators of Transcription 3 for Cancer Therapy

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Abstract

Signal transducers and activators of transcription (STAT) are a highly conserved family of transcription factors that are activated by phosphorylation in the cytoplasm, after which they translocate to the nucleus to regulate gene expression. Among the seven STATs, STAT3 is of particular interest due to its constitutive phosphorylation in a large proportion of human cancers and its ability to induce neoplastic transformation. Inhibition of STAT3 can reverse tumor growth in experimental systems while having few effects in normal cells. These findings have implicated STAT3 as a potentially important target for therapeutic intervention. In addition to its well-described role as a transcription factor, STAT3 has been found recently to have important effects in the cytoplasm. Collectively, these functions of STAT3 directly contribute to tumorigenesis, invasion, and metastasis. Given the potential importance of STAT3 as a target for cancer therapy, molecules have been developed that can block STAT3 function at a variety of steps. These drugs show promise as anticancer agents in model systems of a variety of common human cancers. Thus, elucidating the functions of STAT3 and developing agents to inhibit this protein remain important scientific and clinical challenges.

Background

Activation of signal transducers and activators of transcription 3. Signal transducers and activators of transcription 3 (STAT3) is a transcription factor that was originally identified as a mediator of the acute phase of the inflammatory response triggered by interleukin-6 (1–3). However, it is now known that STAT3 is activated in response to the binding of a large number of cytokines, hormones, and growth factors to their receptors as well as by activation of intracellular kinases (4, 5). STAT3 shares its general structure with the other STAT members and includes a STAT dimerization domain at its NH₂ terminus, a coiled-coil domain involved in protein-protein interactions, a central DNA binding domain, a Src homology (SH2) domain, a conserved tyrosine residue at position 705 (Tyr705), and a COOH terminus encoding the transcription activation domain (4, 5). Binding of a ligand to its receptor triggers dimerization of the cytoplasmic domain of the receptor and the juxtaposition and activation of associated Janus tyrosine kinases (Janus-activated kinases 1, 2, and 3, or Tyk2), which subsequently phosphorylate STAT3 on its conserved tyrosine residue. STAT3 phosphorylation promotes its dissociation from the receptor and its homodimerization, after which the dimer translocates to the nucleus where it regulates transcription (4, 5).

Nuclear import of STAT3. The presence of STAT3 in the nucleus and the cytoplasm under basal conditions suggests a constant shuttling of STAT3 between the two cellular compartments. Unlike other STATs, such as STAT1 and STAT2, which accumulate in the nucleus only following their phosphorylation, STAT3 can enter the nucleus independently of its phosphorylation. The mechanism underlying these differences relates to the involvement of distinct importins used by STATs for their nuclear import. For instance, the phosphorylation of the nuclear localization signal of STAT1 is a prerequisite for its interaction with importin α-5 and subsequent nuclear import (6, 7). In contrast, STAT3 binds constitutively to importin α-3 and α-6 (8).

Although the shuttling of STAT3 in and out of the nucleus seems independent of its phosphorylation (8), the strict requirement for phosphorylation for the transcriptional activation of STAT3 remains a controversial topic. Initially, the finding that introducing cysteine residues into the COOH terminus of STAT3 (generating the STAT3C mutant) is sufficient to promote the constitutive activation of STAT3 led to the hypothesis that these cysteines promote the formation of disulfide bonds between unphosphorylated monomers. This suggested that although phosphorylation could trigger dimerization, it was not necessary for activation (9). However, because mutation of the critical tyrosine residues within STAT3 abolished its transcriptional activity, it was suggested that STAT3 may be phosphorylated and dephosphorylated at a low rate in the absence of cytokine and that introducing cysteine residues simply acts to trap spontaneously phosphorylated dimers (10). Conversely, another study reported that STAT3 with a mutation of Tyr705 retains transcriptional activity.
but affects a distinct subset of genes compared with wild-type STAT3 (11).

Nevertheless, the nuclear translocation of STAT3 in the absence of phosphorylation raises the question as to whether unphosphorylated STAT3 has a role in the nucleus. The finding that the nuclear tyrosine kinase breast tumor kinase phosphorylates STAT3 (12) raises the possibility that such nuclear rather than cytoplasmic phosphorylation of STAT3 acts as a rapid activation mechanism and may contribute to its oncogenic effect.

STAT3 transcriptional targets. Several individual targets of STAT3 have been identified, including bcl-2, bcl-XL, mcl-1, and cyclin D1 (13–17), reflecting the role of STAT3 in promoting survival and cell cycle progression. However, genome-wide analysis of the effects of STAT3 activation reveal a potentially more complex transcriptional network. A large number of genes that are up-regulated early following STAT3 activation are transcription factors themselves including junB, egr1, KLF4, bcl-6, and NFIL3 (18), suggesting that STAT3 can regulate broad programs of gene expression. Interestingly, some of the previously reported STAT3 targets such as cyclin D1 were not up-regulated during this time frame. This observation suggests that STAT3 may act upstream of several transcription programs such that different targets may be activated or repressed at distinct time points following STAT3 activation. Such interplay of transcription factors and the potentially transient activation of specific targets may significantly complicate the identification of key targets. Despite this potential difficulty, microarray analysis of STAT3-mediated transcriptional changes after longer periods of activation identified several genes involved in several important pathways implicated in oncogenesis. Of particular interest was the identification of a large number of genes involved in cell migration and invasion (19). The validation of such targets represents an important new avenue of research on a relatively under-explored aspect of STAT3 biology. In addition, a recent study indicated that cyclin D1 may represent such a critical target because elimination of cyclin D1 abolished STAT3-mediated transformation and anchorage-independent growth (20). However, other studies indicated that the transcriptional activation of cyclin D1 by STAT3 may be more complex and initiate a negative feedback loop for the inactivation of STAT3.

Cyclin D1 as a negative regulator of STAT3. A key step in expanding our understanding of the functions of STAT3 was the observation by Olivier Croquet’s group of the direct binding of cyclin D1 and STAT3 (21). In recent years, it has become clear that in addition to its role in the regulation of cell cycle progression via the activation of cyclin-dependent kinases 4 and 6, cyclin D1 regulates several transcription factors (22). The transcriptional effects of cyclin D1 are mediated indirectly by acting either as a coactivator or corepressor depending on the specific transcription factors. Such activities of cyclin D1 are independent of cyclin-dependent kinases because mutations abolishing the binding of cyclin D1 to the cyclin-dependent kinases have no effect on its transcriptional effects. Although cyclin D1 activates some transcription factors such as the estrogen receptor, binding of cyclin D1 to STAT3 was found to repress its activity (21). As STAT3 positively regulates its own transcription via STAT3 binding sites within its promoter, one predicted consequence of cyclin D1–mediated repression of STAT3 function is a down-regulation of STAT3 expression. This prediction is supported by four independent observations. First, overexpression of cyclin D1 leads to a reduction in STAT3 levels in HepG2 cells (21); second, in breast cancer cells engineered to overexpress cyclin D1, STAT3 levels are reduced both with constitutive and inducible expression of cyclin D1 (23); third, in multiple myeloma, a mutually exclusive expression of cyclin D1 and STAT3 is observed (24); and finally, in a microarray analysis of a variety of different tumor types, a negative correlation between the two markers is seen (23).
These findings contrast with a recent report of a direct correlation between cyclin D1 and STAT3 (20). However, one potential explanation is that although STAT3 can induce moderate levels of cyclin D1, in tumor samples where cyclin D1 is overexpressed due to gene amplification or other genetic alteration, the abnormally high level of cyclin D1 activates a negative feedback loop to repress STAT3. Therefore, the level at which cyclin D1 is expressed is likely to be important in mediating the effect, and this observation offers a potential explanation for the beneficial effect of cyclin D1 overexpression on breast cancer survival (25–29).

**Cytoplasmic role of STAT3.** Although most attention has been focused on the transcriptional function of STAT3, the recent discovery of cytoplasmic partners of STAT3 has raised the possibility of unsuspected new roles for this protein in the cytoplasm (Fig. 1). One of the first clues was the observation that p130<sup>CAS</sup>, an adaptor protein that localizes to focal adhesion sites and which assembles with focal adhesion kinase and its partner paxillin, was hyperphosphorylated in STAT3-deficient keratinocytes. Further, phosphorylated STAT3 was found to localize to the migrating protrusions and focal adhesions in migrating cells (30–32). These observations therefore suggested a transcription-independent function of STAT3 in regulating cell migration. Moreover, stathmin, a protein that regulates microtubule depolymerization, was identified as a binding partner of STAT3 (33). Although stathmin did not seem to regulate STAT3 transcription, was identified as a binding partner of STAT3 (33). Thus, STAT3 can affect migration both via its transcriptional regulation and in nontransformed cells has little effect (41). This may reflect the redundancy of signaling pathways under normal conditions, but the requirement for certain key pathways to be amenable to pharmacologic manipulation that might not have been predicted in advance. Screening strategies have been useful in identifying potent and specific STAT3 inhibitors from a variety of chemical sources including natural products (58, 59).

The finding that amplified cyclin D1 can inhibit STAT3 expression represents an additional therapeutic opportunity. It was reported recently that stabilization of cyclin D1 in cyclin D1–overexpressing breast cancer cells using the proteasome inhibitor bortezomib led to a complete inhibition of STAT3 expression (23). Bortezomib-induced apoptosis requires calcium release from the endoplasmic reticulum and is limited by bcl-XL, a transcriptional target of STAT3. Thus, the inhibition of STAT3 in cyclin D1–overexpressing cells led to an increased sensitivity to bortezomib (23). This observation raises the possibility that bortezomib, a drug that has been approved by the Food and Drug Administration for the treatment of multiple myeloma (60), may also act indirectly as a STAT3 inhibitor in cancer cells that are characterized by cyclin D1 overexpression.

**Clinical-Translational Implications**

**STAT3 activation in cancer.** Reflecting the fact that STAT3 regulates genes involved in survival, proliferation, self-renewal, and invasion, it is not surprising that constitutive activation of STAT3 is a common finding in a wide spectrum of human cancers (34). For example, histochemical evidence of nuclear tyrosine-phosphorylated STAT3 can be found in roughly 75% of primary breast and prostate cancers (18). Furthermore, activation of STAT3 is associated with decreased survival in leukemias and other tumors, suggesting that STAT3 significantly alters the biology of these cells (35). Inhibition of STAT3 in model systems has been shown to decrease the survival and proliferation of tumor cells with activated STAT3 (36–40). This supports the contention that STAT3 is directly contributing to the pathogenesis of these tumors, rather than serving only as a marker of tumorigenicity. Despite the central role played by STAT3 in normal cellular physiology, loss of function of STAT3 in nontransformed cells has little effect (41). This may reflect the redundancy of signaling pathways under normal conditions, but the requirement for certain key pathways to be activated in malignant cells (42).

**Therapeutic approaches to targeting STAT3.** The large body of data validating STAT3 as a target for cancer therapy, and the tolerance of normal cells to the loss of STAT3 function, has driven the effort to identify molecules able to inhibit STAT3 (43–45). However, this approach faces several challenges because STAT3 does not have enzymatic activity and, unlike the estrogen receptor, it does not naturally bind to small ligands. Nevertheless, several therapeutic approaches, using both rational design and screening of chemical libraries, have been pursued. The multiple steps involved in STAT3 activation afford several possible targets, including inhibition of Janus-activated kinases and other kinases, dimerization, nuclear translocation, DNA binding, coactivator recruitment, and STAT3 expression. For example, there is increasing evidence that inhibition of STAT3 tyrosine phosphorylation can be a useful anticancer strategy. Inhibition of Janus-activated kinases using the tyrophostin AG490 (46–49) or interference with STAT3 binding to epidermal growth factor receptor using peptides (50) has been shown to inhibit the growth of epithelial and hematologic tumors. The latter mechanism seems particularly appealing because activating mutations of epidermal growth factor receptor in lung cancer lead to neoplastic transformation that is STAT3 dependent (51).

Another approach to inhibiting STAT3 function has been to inhibit dimerization. One method to do this uses G-quartet oligonucleotides, which form complex tertiary structures (52). These molecules can inhibit the growth of squamous cell carcinoma cells in vitro and in mouse models. Reflecting the fact that many STAT3 target genes promote survival and resistance to cytotoxic therapies (37, 53), G-quartet oligonucleotides show prominent activity when combined with chemotherapeutic agents.

Strategies have also been used to inhibit the binding of STAT dimers to their cognate DNA sequences. Both double-stranded and single-stranded oligonucleotides can be used as decoys to compete the binding of STATs to DNA (54, 55). Such single-stranded competitors have proven effective in vitro and in vivo against prostate cancer cells with activated STAT3 but not cells lacking this characteristic.

A complementary approach involves the use of high-throughput platforms in which large chemical libraries can be screened. Particularly useful are cell-based systems in which STAT phosphorylation or function can be assessed rapidly (56, 57). This approach allows the identification of active compounds, from which mechanistic information can then be determined. Such a “chemical biology” approach can uncover targets amenable to pharmacologic manipulation that might not have been predicted in advance. Screening strategies have been useful in identifying potent and specific STAT3 inhibitors from a variety of chemical sources including natural products (58, 59).

The finding that amplified cyclin D1 can inhibit STAT3 expression represents an additional therapeutic opportunity. It was reported recently that stabilization of cyclin D1 in cyclin D1–overexpressing breast cancer cells using the proteasome inhibitor bortezomib led to a complete inhibition of STAT3 expression (23). Bortezomib-induced apoptosis requires calcium release from the endoplasmic reticulum and is limited by bcl-XL, a transcriptional target of STAT3. Thus, the inhibition of STAT3 in cyclin D1–overexpressing cells led to an increased sensitivity to bortezomib (23). This observation raises the possibility that bortezomib, a drug that has been approved by the Food and Drug Administration for the treatment of multiple myeloma (60), may also act indirectly as a STAT3 inhibitor in cancer cells that are characterized by cyclin D1 overexpression.
Finally, targeting cytoplasmic functions of STAT3 may also be a useful strategy. For example, the discovery of statin as a binding partner of STAT3 raises the possibility that drugs such as paclitaxel, which stabilizes microtubules, may also act as a STAT3 inhibitor by blocking its cytoplasmic function. In fact, there is evidence that the combination of inhibitors of the transcriptional functions of STAT3 and paclitaxel may have enhanced therapeutic effects (52). In addition, the recent finding that activated ErbB2 forms a complex with βγ integrin and activates STAT3 raises the possibility that STAT3 inhibitors may also find application in the treatment of ErbB2-positive breast cancers (61). Therefore, combining inhibitors of the cytoplasmic and nuclear functions of STAT3 may represent a synergistic approach.

**Conclusion**

STAT3 is a transcription factor that integrates signals from a variety of extracellular stimuli and kinase pathways and regulates genes involved in many key cellular processes. In addition, its effects mediated through interactions with other proteins allows it to control cellular function on multiple levels. The inappropriate activation of STAT3 in cancer contributes directly to malignant tumor behavior but also provides an opportunity for therapeutic intervention. In view of these exciting new findings and the vast number of potential clinical applications, the discovery of STAT3 inhibitors remains an important goal.

**References**


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