Targeting Signal-Transducer-and-Activator-of-Transcription-3 for Prevention and Therapy of Cancer

Modern Target but Ancient Solution

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ABSTRACT: Recent evidence indicates a convergence of molecular targets for both prevention and therapy of cancer. Signal-transducer-and-activator-of-transcription-3 (STAT3), a member of a family of six different transcription factors, is closely linked with tumorigenesis. Its role in cancer is indicated by numerous avenues of evidence, including the following: STAT3 is constitutively active in tumor cells; STAT3 is activated by growth factors (e.g., EGF, TGF-α, IL-6, hepatocyte growth factor) and oncogenic kinases (e.g., Src); STAT3 regulates the expression of genes that mediate proliferation (e.g., c-myc and cyclin D1), suppress apoptosis (e.g., Bcl-xL and survivin), or promote angiogenesis (e.g., VEGF); STAT3 activation has been linked with chemoresistance and radioresistance; and chemopreventive agents have been shown to suppress STAT3 activation. Thus inhibitors of STAT3 activation have potential for both prevention and therapy of cancer. Besides small peptides and oligonucleotides, numerous small molecules have been identified as blockers of STAT3 activation, including synthetic molecules (e.g., AG 490, decoy peptides, and oligonucleotides) and plant polyphenols (e.g., curcumin, resveratrol, flavopiridol, indirubin, magnolol, piceatannol, parthenolide, EGCG, and cucurbitacin). This article discusses these aspects of STAT3 in more detail.

KEYWORDS: STAT3; interleukin-6; cancer; chemoresistance

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INTRODUCTION

Signal-transducer-and-activator-of-transcription (STAT) is a family of six different transcription factors, first discovered in 1993 by James Darnell, which play major roles in cytokine signaling. A typical STAT protein consists of a coiled-coil domain, a DNA-binding domain, a linker, an SH2 domain, and a transactivation domain (TAD) (Fig. 1). The TAD contains tyrosine and serine phosphorylation sites that are needed for the activation of STAT. Because it plays a major role in tumorigenesis, STAT3 is the focus of the remaining discussion.

STAT3 was discovered independently and simultaneously by two different groups in 1994. It was initially regarded as an acute-phase response factor activated by interleukin-6 (IL-6), leukemia inhibitory factor, oncostatin M, and the ciliary neurotrophic factor (CNTF) family of cytokines, all known to mediate their signal through the gp130 protein. Engagement of cell-surface cytokine receptors activates the janus kinase (JAK) family of protein kinases, which in turn phosphorylates and activates latent cytoplasmic STAT3 protein to an active dimer capable of translocating to the nucleus and inducing transcription of specific target genes (Fig. 1). Although four different members of

![FIGURE 1. Signaling pathway leading to STAT3 activation.](image-url)
the JAK family have been described (JAK1, JAK2, JAK3, and TYK2). JAK2 is one of the major mediators of STAT3 phosphorylation. Several other kinases have been implicated in the phosphorylation of STAT3, including members of the Src family (hck, src), Erb B1, Erb B2, anaplastic lymphoma kinase, protein kinase C (PKC)-δ, c-fes, gp130, and epithelial growth factor (EGF) receptor.\(^5\)–\(^11\) Although both serine and tyrosine phosphorylation of STAT3 are needed for full activation, evidence indicates that STAT3 contains a single serine phosphorylation site at position 727, which has no effect on its ability to bind to DNA.\(^12\) Various studies also indicate that ERKs,\(^13\) JNK,\(^14\) p38 mitogen-activated protein kinase,\(^15\) and PKC\(^\text{a}\) participate in serine phosphorylation of STAT3.

While genetic deletion of the STAT3 gene is lethal to mouse embryos,\(^16\) selective loss of STAT3 in keratinocytes results in impaired wound healing,\(^17\) and skin-specific STAT3-transgenic mice develop psoriasis.\(^18\) Increasing evidence also indicates that STAT3 mediates its tumorigenic effects not alone but through its cross-talk with various other transcription factors. These include PPAR-γ,\(^19\) β-catenin,\(^20\) nuclear factor-κB (NF-κB),\(^21,22\) hypoxia-inducible factor 1-α,\(^23\) c-myc,\(^24\) c-fos,\(^25\) c-jun,\(^26\) glucocorticoid receptors,\(^27\) and estrogen receptors.\(^28\)

**STAT3 IS ACTIVATED BY GROWTH FACTORS**

Various growth factors that have been linked to tumor cell proliferation have been found to activate STAT3 (TABLE 1).\(^29\)–\(^55\) EGF signaling, which has been linked with proliferation of almost 30% of all tumor cells, has been shown to mediate its effect through activation of STAT3.\(^3\) Similarly, IL-6, which has been linked with proliferation of multiple myeloma, renal cell carcinoma, prostate cancer, and other cancers, has been shown to mediate its effect through activation of STAT3.\(^3\) The activation of STAT3 by both EGF and IL-6 involves tyrosine phosphorylation of STAT3 at position 705. Moreover, several other cytokines have been shown to activate STAT3. These include growth hormone,\(^56\) transforming growth factor-α (TGF-α),\(^57\) oncostatin M,\(^4\) thrombopoietin,\(^58\) platelet-derived growth factor (PDGF),\(^59\) IL-5,\(^60\) IL-6,\(^3\) IL-9,\(^61\) IL-10,\(^62,63\) IL-12,\(^64\) IL-22,\(^65\) and leptin.\(^66\) Certain chemokines, such as macrophage inflammatory protein 1α and RANTES (regulated upon activation of normal T cell expressed and secreted) also have been shown to activate STAT3.\(^67\) Whether all these cytokines stimulate STAT3 through activation of JAK2 is not fully understood. Besides growth factors and cytokines, other factors that activate STAT3 include oxidative stress,\(^68\) tobacco chewing,\(^69\) hepatitis C virus,\(^70,71\) ultraviolet B,\(^72\) lipopolysaccharide,\(^73\) osmotic shock,\(^74\) and progestins.\(^75\)

**STAT3 IS CONSTITUTIVELY ACTIVE IN TUMOR CELLS**

The role of STAT3 in cancer is supported through numerous lines of evidence. First, all Src-transformed cell lines have persistently activated STAT3,
<table>
<thead>
<tr>
<th>Constitutive STAT3</th>
<th>Activators</th>
<th>Genes</th>
<th>Kinases</th>
<th>Inhibitors</th>
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**TABLE 1. Tumors that express constitutively active STAT3, activators of STAT3, genes regulated by STAT3, and inhibitors of STAT3**
### TABLE 1. Continued

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<th>Constitutive STAT3</th>
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<th>Genes</th>
<th>Kinases</th>
<th>Inhibitors</th>
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<td>EKB569&lt;sup&gt;172&lt;/sup&gt;</td>
<td>Magnolol&lt;sup&gt;130&lt;/sup&gt;</td>
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STAT, signal-transducer-and-activator-of-transcription; CLL, chronic lymphocytic leukemia; CML, chronic myeloid leukemia; AML, acute myelogenous leukemia; MCL, mantle cell lymphoma; SCCHN, squamous cell carcinoma of the head and neck; HTLV, human T cell lymphotropic virus; EBV, Epstein–Barr virus; Nelfinavir, HIV-1 protease inhibitor; R115777, farnesyl transferase inhibitor; AG490 and piceatannol, tyrosine kinase inhibitors; PIAS, protein inhibitor of activated STAT3; GQ-ODN, G-quartet oligonucleotides; SOCS, suppressor of cytokine signaling; GRIM, gene associated with retinoid-IFN-induced mortality; EGCG, epigallocatechin-3-gallate; SSI, STAT-induced STAT inhibitor; PTPεC, protein tyrosine phosphatase εC; DN, dominant negative; EKb-569, EGF-R inhibitor; DIF-1, differentiation-inducing factor-1; JAB, SH2-domain-containing protein; IL, interleukin; TNF, tumor necrosis factor; MDA, melanoma differentiation antigen; MCP, monocyte chemoattractant protein; GCSF, granulocyte colony-stimulating factor; LIF, leukemia inhibitory factor; OSM, oncostatin M; IFN, interferon; MIP, macrophage inflammatory protein; RANTES, regulated upon activation, normal T cell expressed and secreted; HB-EGF, heparin-binding epidermal growth factor; LPS, lipopolysaccharide; VEGF, vascular endothelial growth factor; MMP, matrix metalloproteinase; HSP, heat shock protein; hTERT, human telomerase reverse transcriptase; thr, thyrotropin-releasing hormone; ATL, adult T cell leukemia/lymphoma; SLF, steel factor; HCV, hepatitis C virus.
and dominant-negative STAT3 blocks transformation.\textsuperscript{76–79} Second, STAT3-C, a constitutively active mutant dimerized by cysteine–cysteine bridges instead of pTyr-SH2 interaction, can transform cultured cells so that they form tumors when injected into mice.\textsuperscript{77} Indeed, STAT3 functions in fibroblast development to resist apoptosis.\textsuperscript{80} Third, constitutive activation of STAT3 has been reported in large number of tumors, including breast cancer,\textsuperscript{31} prostate cancer,\textsuperscript{44} head and neck squamous cell carcinoma,\textsuperscript{42} multiple myeloma,\textsuperscript{37} lymphomas and leukemia,\textsuperscript{81} brain tumor,\textsuperscript{47} colon cancer,\textsuperscript{54} Ewing sarcoma,\textsuperscript{55} gastric cancer,\textsuperscript{52} esophageal cancer,\textsuperscript{51} ovarian cancer,\textsuperscript{38} nasopharyngeal cancer,\textsuperscript{50} and pancreatic cancer.\textsuperscript{45}

Why STAT3 is constitutively active in tumor cells is not fully understood. Because no mutations in STAT3 have been reported that results in persistent activation, the only putative mechanisms to account for the constitutive activity of STAT3 are dysregulation of signaling molecules or mutation or deletions in the protein that negatively regulates STAT3 (e.g., protein inhibitor of activated STAT3 or suppressor of cytokine signaling [SOCS]).\textsuperscript{82} For instance SOCS-1, a negative regulator of cytokine signaling is frequently silenced by methylation in various tumors.\textsuperscript{82–86} Both receptor tyrosine kinases and nonreceptor tyrosine kinases have been linked with activation of STAT3. Besser \textit{et al.} found that a single amino acid substitution in the v-Eyk intracellular domain results in activation of STAT3 and enhances cellular transformation.\textsuperscript{87} Yu \textit{et al.} showed that enhanced cells transformed by the Src oncoprotein have constitutively active STAT3.\textsuperscript{78} Besides Src, inducers of STAT3 activation include a nonreceptor tyrosine kinase, v-Fps; polyoma virus middle T antigen, which activates Src family kinases; and v-Sis, which acts as a ligand for the PDGF receptor.\textsuperscript{88} Additional nonreceptor tyrosine kinases include c-Fes,\textsuperscript{11} Lck,\textsuperscript{89} Ras/Rac1-mediated p38, and c-Jun N-terminal kinase.\textsuperscript{15} STAT3 activation also is regulated by protein tyrosine phosphatases.\textsuperscript{90}

\section*{STAT3 REGULATES EXPRESSION OF GENES INVOLVED IN TUMORIGENESIS}

STAT3 is one of the major mediators of tumorigenesis.\textsuperscript{91,92} The oncogenic significance of activated STAT3 molecules is due to their effects on numerous parameters of the development and progression of malignancy, such as apoptosis, cell proliferation, angiogenesis, and immune system evasion.\textsuperscript{79,93,94} Constitutively active STAT3 has been implicated in the induction of resistance to apoptosis,\textsuperscript{37} possibly through the expression of Bcl-x\textsubscript{L},\textsuperscript{95} and cyclin D1.\textsuperscript{42} Its role in tumorigenesis is mediated through the expression of various genes that suppress apoptosis, mediate proliferation, invasion, and angiogenesis. These include Mcl-1,\textsuperscript{40,96} Bcl-x\textsubscript{L},\textsuperscript{95} and survivin,\textsuperscript{97} all of which suppress apoptosis; c-myc\textsuperscript{98} and cyclin D1,\textsuperscript{42} which mediate cell proliferation; matrix metalloproteinase-9,\textsuperscript{99} which mediates cellular invasion; and vascular
endothelial growth factor (VEGF), which mediates angiogenesis. Other genes that have been shown to be regulated by STAT3 include p21, SOCS-3, receptor activator of NF-κB ligand (RANKL), tumor necrosis factor (TNF), MyD 88, interferon-regulatory factor 1, c-fos, β-macroglobulin, antichymotrypsin, and angiotensinogen, which also have been linked with tumorigenesis.

STAT3 ACTIVATION INHIBITS APOPTOSIS

Numerous reports suggest that activation of STAT3 suppresses apoptosis. For instance, Shen et al. showed that constitutively activated STAT3 protects fibroblasts from serum withdrawal and ultraviolet-induced apoptosis and antagonizes the proapoptotic effects of activated STAT1. Thus constitutively active STAT3 can contribute to oncogenesis by protecting cancer cells from apoptosis. This implies that suppression of STAT3 activation could facilitate apoptosis.

INHIBITORS OF STAT3 HAVE POTENTIAL IN PREVENTION AND THERAPY OF CANCER

Because of the critical role of STAT3 in tumorigenesis as reviewed here, inhibitors of STAT3 have potential in both prevention and treatment of cancer. Perhaps one of the best-known inhibitors of STAT3 activation is AG490, which inhibits the activation of JAK2. Other blockers of STAT3 include small peptides, oligonucleotides, and small molecules. Turkson et al. identified phosphotyrosyl peptides that block STAT3-mediated DNA-binding activity, gene regulation, and cell transformation. Various small molecules that block STAT3 include 15-PGJ2, platinum complex, ethanol, sodium salicylate, retinoic acid, atiprimod, PS-341, and statins.

Several plant polyphenols have been identified that can suppress STAT3 activation. These include curcumin, resveratrol, curcurbitacin, indirubin, piceatannol, parthenolide, flavopiridol, magnolol, and epigallocatechin-3-gallate. How these agents suppress STAT3 activation is not fully understood. Curcumin, a well-established chemopreventive agent, has been shown to inhibit JAK2, Src, Erb2, and EGFR, all of which are implicated in STAT3 activation. Furthermore, curcumin has been shown to downregulate the expression of Bcl-xL, cyclin D1, VEGF, and TNF all of which are known to be regulated by STAT3. A recent study by Kim et al. has shown that curcumin phosphorylates SHP-2, which in turn associates with JAK1 and JAK2, thus inhibiting initiation of the JAK–STAT pathway. Thus, pharmacologically safe and effective therapeutic agents that can block constitutive or inducible activation of STAT3 have potential for efficacy in
treatment of cancer. Given that growing evidence implicates a number of important STAT3 target genes in the formation of tumors, it seems logical to conclude that inhibition of STAT3 through pharmacological blockage of upstream molecules, such as Src and JAK may reduce tumor formation.

**CONCLUSION**

The major hallmarks of cancer include deregulated cell growth, tumor cell invasion, angiogenesis, and metastasis. This review clearly shows that STAT3 can regulate all these different phases of tumorigenesis. Suppression of STAT3 in certain tumor cell models has led to expression of proinflammatory cytokines and chemokines by tumor cells that are needed for the innate immune response against the tumor cells. Because molecular targets in the prevention of cancer do not differ from those in the treatment of cancer, STAT3 is an ideal target for both prevention and treatment of cancer. Thus small molecules that can suppress STAT3 activation and are pharmacologically safe have potential for suppression of tumorigenesis.

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