Discovery and Optimization of Inhibitors of STAT3 Activation for the Treatment of Squamous Cell Carcinoma of the Head and Neck

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Squamous Cell Carcinoma of the Head and Neck (SCCHN)

- Squamous Cell Carcinoma of the Head and Neck (SCCHN) is the sixth most common form of cancer and accounts for ~500,000 new cancer cases per year worldwide.

- Traditional therapies, including surgery, radiation therapy, and chemotherapy are able to eradicate head and neck cancer in only 50% of cases.

- Treatments incorporating radiation or conventional chemotherapy drugs, such as cisplatin, may result in a host of negative side effects, some permanent.

- As a result, there has been continuing investigation into potential alternative and less toxic therapies for head and neck cancer, with the aim of achieving a more favorable clinical outcome while reducing treatment morbidity.

- Patient-derived primary SCCHN cells and SCCHN cell lines have been shown to overexpress a number of key signaling proteins that contribute to the enhanced growth and survival properties of these cells.

- SCCHN cells commonly exhibit overexpression and/or hyperactivation of EGFR, signal transducer and activator of transcription 3 (STAT3), STAT3 is a key downstream target of EGFR.
Signal Transducers and Activators of Transcription

Crystal structure of STAT3 dimer bound to DNA

- Seven members:
  - STAT1 (α/β), STAT2, STAT3 (α/β/γ), STAT4, STAT5a, STAT5b & STAT6
  - Cytoplasmic transcription factors regulating cytokine gene expression
  - STATs are activated via the tyrosine phosphorylation cascade after ligand binding and stimulation of the Cytokine Receptor–Kinase complex and Growth Factor-Receptor complex
    - EGF (Epidermal Growth Factor), FGF (Fibroblast Growth Factor), PDGF (Platelet-Derived Growth Factor), IL-6 (Interleukin-6), OSM (Oncostatin-M), CSF1R (Colony Stimulating Factor-1 Receptor), c-kit, Insulin receptor, c-Met and GPCRs (G-Protein Coupled Receptors), etc.

Conserved tyrosine residue- Y701, Y705 or Y695

What is STAT3

- STAT3 is one of seven members of the signal transducer and activator of transcription (STAT) family of proteins whose function is to relay signals from the cell surface receptors to the nucleus and initiate transcription.

- STAT3 plays a vital role in regulating cell growth and survival. In response to growth factor and cytokine stimulation, STAT3 is phosphorylated, dimerizes, and translocates into the nucleus to up-regulate transcription of a wide spectrum of genes.

- STAT3 is a key tumor promoting transcription factor, constitutively activated in many types of cancer including SCCHN, prostate, breast and colorectal cancers.

- Activated STAT3 promotes tumor cell proliferation/survival and tumor metastasis, while suppressing the anti-tumor immune response. Inhibition of STAT3 signaling has been shown to inhibit tumor growth in vitro and in vivo.
The Opposing Roles of STAT3 & STAT1 in Cancer

- STAT1 has significant sequence and functional similarity to STAT3, however, whereas STAT3 is oncogenic, STAT1 is associated with tumor suppression.

- STAT1 can be activated by various ligands including interferon-alpha, interferon-gamma, EGF, PDGF and IL-6.

- Activated STAT1 plays a critical role in promoting an effective anti-tumor immune response.

- STAT3 inhibitors identified through target-based screening strategies (e.g. SH2-targeting molecules) often exhibit poor cell permeability, efficacy, selectivity (vs STAT1) and/or stability, and have not progressed into the clinic.

- STAT3 pathway-specificity (i.e. without STAT1 signaling inhibition) is highly critical when developing anticancer agents designed to block STAT3 activation.

- The ultimate goal is to discover and develop selective inhibitors of the STAT3 pathway for the treatment of SCCHN tumors.
The Opposing Roles of STAT3 & STAT1 in Cancer

- STAT3 is a proto-oncogene
- STAT1 is a tumor suppressor

Fig. (1). STAT proteins are activated by receptor and non-receptor tyrosine kinases via several mechanisms. (A) Receptor-associated JAKs are activated upon cytokine receptor binding, through the classic cross-phosphorylation pathway. These activated JAKs phosphorylate Y residues on the receptor, which thereby become docking elements for cytoplasmic STAT proteins. STATs are subsequently phosphorylated on a single Y residue in the C-terminal portion of the receptor, and then form homo- or heterodimers through reciprocal interaction between the pY of one STAT and the Src-SH2 domain of another. Dimerized STATs are carried into the nucleus via importins, where they then induce target gene transcription by binding to specific regulatory elements. (B) Receptors with intrinsic tyrosine kinase activities, including platelet-derived growth factor receptor (PDGFR), epidermal growth factor receptor (EGFR), and FMS-related (formerly McDonough feline sarcoma viral oncogene homologue) tyrosine kinase 3 (FLT3), may directly activate STATs without JAK involvement. (C) STATs can be phosphorylated by constitutively active non-receptor protein tyrosine kinases (PTKs), such as Src and breakpoint cluster region/Abelson tyrosine kinase (Bcr/Abl). (D) Unphosphorylated STATs can independently enter the nucleus to mediate gene transcription, possibly by acting as transcriptional co-regulators in DNA binding.

activated STAT3. Cellular transformation by the viral oncogene v-src requires growth and survival, promotes angiogenesis, metastasis, and immune responses. Multiple lines of evidence place STAT3 at a central node in the regulation of a variety of critical functions, including cell differentiation, proliferation, apoptosis, and 6) that relay signals from activated cytokine and growth factor receptors in the plasma membrane to the nucleus, where they regulate gene transcription and 5) that contain responsive genes involved in the regulation of a variety of critical functions.

Introduction

Breast and prostate tissue immortalized fibroblasts and normal epithelial cells derived from cancers characterized by a constitutively activated form of STAT3 is sufficient to transform tumors, validating STAT3 as an anticancer target (Table 1) as well as transcriptional profiles that are consistent with STAT3-regulated gene expression (Table 1) and 6) that show little sequence similarity with STAT3, has also been implicated in oncogenesis that constitutively activated STAT5, which shows little sequence similarity with STAT3, has also been implicated in oncogenesis (12, 17, 20). In the cancer context, activated STAT3 is oncogenic, whereas activated STAT1 behaves as a tumor suppressor. Therefore, the selective inhibition of STAT3 might be well tolerated and Regulation

Upstream/Downstream Abnormalities of STAT3 Signaling

Table 1. STAT3 in the Context of Various Cancers: Validation as an Anticancer Target

<table>
<thead>
<tr>
<th>Cancers Characterized by Elevated STAT3 Expression or Activity</th>
<th>Poor Prognosis Linked to High STAT3 Levels</th>
<th>Xenograft Models Responsive to Inhibition of STAT3</th>
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<tbody>
<tr>
<td>Leukemia</td>
<td>Renal cell carcinoma</td>
<td>Head and neck squamous cell carcinoma</td>
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<tr>
<td>Lymphomas</td>
<td>Colorectal cancer</td>
<td>Glioblastoma</td>
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<td>Multiple myeloma</td>
<td>Ovarian carcinoma</td>
<td>Myeloproliferative neoplasms</td>
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<td>Breast cancer</td>
<td>Gastric carcinoma</td>
<td>Renal cell carcinoma</td>
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<td>Prostate carcinoma</td>
<td>Intestinal-type gastric adenocarcinoma</td>
<td>Breast cancer</td>
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<tr>
<td>Lung cancer (non-small-cell)</td>
<td>Cervical squamous-cell carcinoma</td>
<td>Lung adenocarcinoma</td>
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<td>Renal cell carcinoma lung cancer</td>
<td>Osteosarcoma</td>
<td>Acute lymphoblastic leukemia</td>
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<td>Hepatocellular carcinoma</td>
<td>Epithelial ovarian carcinoma</td>
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<td>Cholangiocarcinoma</td>
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<td>Ovarian carcinoma</td>
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<td>Pancreatic adenocarcinoma</td>
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<td>Melanoma</td>
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<td>Head and neck squamous cell carcinoma</td>
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Elevated EGFR expression
Constitutively activated EGFR-RTK
Overexpression of SFKs
Hyperactivated JAKs
Elevated TGFα/IL-6

Inhibitors of IL-6 receptor complex pSTAT3 Activation: Mechanisms of Action

- Indirectly block the upstream molecules of Stat3 signaling pathway
- Antagonists of IL6 binding to IL6Rα
- Antagonists of IL6-IL6Rα recruitment of gp130
- Inhibitors of IL6 –IL6Rα-gp130 complex activation
- JAK Inhibitors
- Directly target Stat3 protein, disrupt Stat3 phosphorylation, dimerization, nuclear translocation, and/or DNA binding
- Recruitment of STAT3 in Cytoplasm to pY of activated IL6-IL6Rα-gp130 complex
Current Status of STAT3 Inhibitors

- **Direct target Stat3 protein**

- **STA-21**: inhibits luciferase activity at 20 µM; inhibit STAT3 dimerization, nuclear translocation, STAT3-DNA binding in MDA-MB-435s cells at 20 or 30 µM; inhibit Bcl-X<sub>L</sub> and cyclin D1 in MDA-MB-468 breast carcinoma cells.

- **S31-201**: selectively inhibits STAT3 DNA binding (STAT3:STAT3 IC<sub>50</sub> = 86 µM, STAT1:STAT3 IC<sub>50</sub> = 160 µM, STAT1:STAT1 IC<sub>50</sub> > 300 µM) in EGF-stimulated mouse fibroblasts NIH 3T3/hEGFR.

- **Stattic**: inhibits luciferase activity at IC<sub>50</sub> = 5.1 µM; inhibit STAT3 DNA binding at 10 µM in the nuclear extracts form EGF-stimulated cells; selectively inhibit IL-6 induced pSTAT3 over IFN-γ induced pSTAT1 in HepG2 liver carcinoma cells.
Current Status of STAT3 Inhibitors

- \textit{indirectly block the upstream molecules of Stat3 signaling pathway}

- \textbf{Curcumin}: inhibits JAK2, Src and Erb2, and epidermal growth factor receptor; no selectivity between pSTAT3 and pSTAT1; inhibition of pSTAT3 is reversible.

- \textbf{Capsaicin}: preferentially inhibited constitutive Stat3 phosphorylation in multiple myeloma cells through the inhibition of JAK1 and c-Src.

- \textbf{Emodin}: inhibited Stat3 phosphorylation by targeting JAK2.

The goal of the STAT3 project is to identify small molecules that selectively inhibit STAT3 over STAT1 signaling and that can be developed into clinical compounds for the treatment of squamous cell carcinoma of the head and neck.

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