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

Doris Germain[1] and David A. Frank[2]

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 NH_2 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 (Tyr^{705}), 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. Alternatively, dimerization of receptors with intrinsic tyrosine kinase activity such as the epidermal growth factor receptor can also directly 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 STAT3C 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 Tyr^{705} retains transcriptional activity

Authors' Affiliations: [1]Division of Hematology/Oncology, Department of Medicine, Mount Sinai School of Medicine, New York, New York and [2]Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, Massachusetts
Received 10/18/06; revised 11/10/06; accepted 12/8/06.
Requests for reprints: David A. Frank, Department of Medical Oncology, Dana-Farber Cancer Institute, 44 Binney Street, Boston, MA 02115. Phone: 617-632-4714; Fax: 617-632-6356; E-mail: david.frank@dfci.harvard.edu.

doi:10.1158/1078-0432.CCR-06-2491

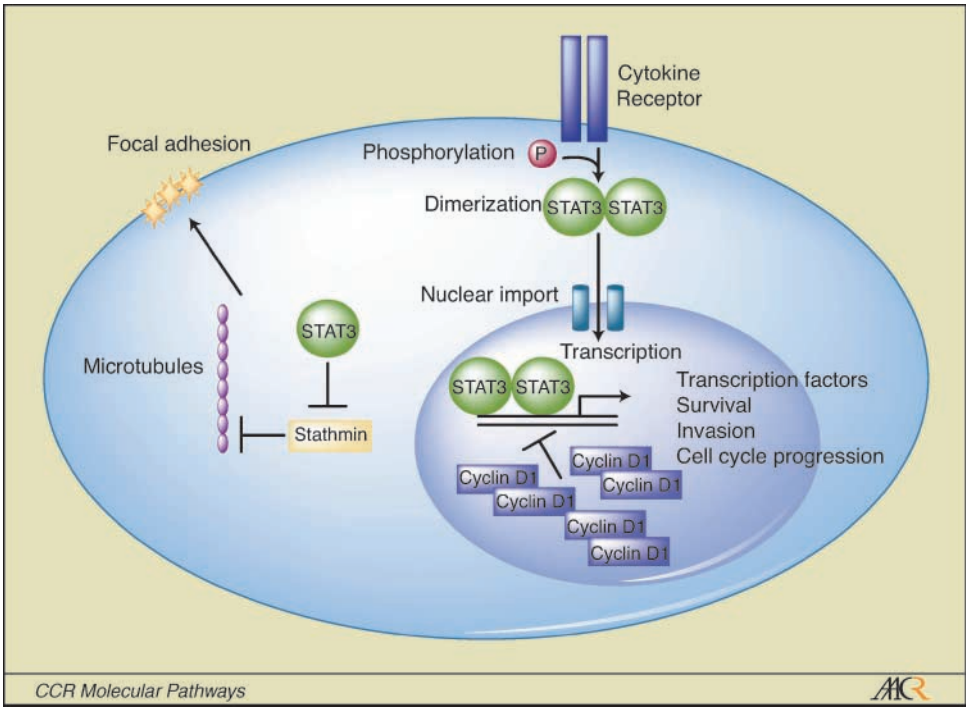

Fig. 1. Cytoplasmic and nuclear functions of STAT3. On receptor activation, STAT3 becomes tyrosine phosphorylated, dimerizes, and translocates to the nucleus where it activates transcription of genes regulating key biological processes. Among these is cyclin D1, which is an important mediator of transformation. However, when overexpressed, cyclin D1 inhibits STAT3 activity and leads to its down-regulation. In addition to its role in the nucleus, STAT3 affects cell migration both by interacting with focal adhesions and by inhibiting stathmin, thereby allowing microtubule polymerization.

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 4 and 6 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 $p130^{CAS}$, 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. More recently, stathmin, a tubulin binding protein, which promotes microtubule depolymerization, was identified as a binding partner of STAT3 (33). Although stathmin did not seem to regulate STAT3 transcriptional activity, unphosphorylated STAT3 was found to prevent stathmin from binding to microtubules, thereby promoting microtubule polymerization and cell migration (33). Thus, STAT3 can affect migration both via its transcriptional regulation of genes involved in cell migration (19) and also through its transcription-independent interactions with focal adhesion molecules and its inhibition of stathmin (33).

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 tyrphostin 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 stathmin 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 β_4 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

1. Akira S, Nishio Y, Inoue M, et al. Molecular cloning of APRF, a novel IFN-stimulated gene factor 3 p91-related transcription factor involved in the gp130-mediated signaling pathway. Cell 1994;77:63–71.
2. Lutticken C, Wegenka UM, Yuan J, et al. Association of transcription factor APRF and protein kinase Jak1 with the interleukin-6 signal transducer gp130. Science 1994;263:89–92.
3. Wegenka UM, Buschmann J, Lutticken C, Heinrich PC, Horn F. Acute-phase response factor, a nuclear factor binding to acute-phase response elements, is rapidly activated by interleukin-6 at the posttranslational level. Mol Cell Biol 1993;13:276–88.
4. Darnell JE, Jr. Transcription factors as targets for cancer therapy. Nat Rev Cancer 2002;2:740–9.
5. Yu H, Jove R. The STATs of cancer—new molecular targets come of age. Nat Rev Cancer 2004;4:97–105.
6. Sekimoto T, Imamoto N, Nakajima K, Hirano T, Yoneda Y. Extracellular signal-dependent nuclear import of Stat1 is mediated by nuclear pore-targeting complex formation with NPI-1, but not Rch1. EMBO J 1997;16:7067–77.
7. Sekimoto T, Nakajima K, Tachibana T, Hirano T, Yoneda Y. Interferon-γ-dependent nuclear import of Stat1 is mediated by the GTPase activity of Ran/TC4. J Biol Chem 1996;271:31017–20.
8. Liu L, McBride KM, Reich NC. STAT3 nuclear import is independent of tyrosine phosphorylation and mediated by importin-α3. Proc Natl Acad Sci U S A 2005;102:8150–5.
9. Bromberg JF, Wrzeszczynska MH, Devgan G, et al. Stat3 as an oncogene. Cell 1999;98:295–303.
10. Liddle FJ, Alvarez JV, Poli V, Frank DA. Tyrosine phosphorylation is required for functional activation of disulfide-containing constitutively active STAT mutants. Biochemistry 2006;45:5599–605.
11. Yang J, Chatterjee-Kishore M, Staugaitis SM, et al. Novel roles of unphosphorylated STAT3 in oncogenesis and transcriptional regulation. Cancer Res 2005;65:939–47.
12. Liu L, Gao Y, Qiu H, Miller WT, Poli V, Reich NC. Identification of STAT3 as a specific substrate of breast tumor kinase. Oncogene 2006;25:4904–12.
13. Puthier D, Bataille R, Amiot M. IL-6 up-regulates mcl-1 in human myeloma cells through JAK / STAT rather than ras / MAP kinase pathway. Eur J Immunol 1999;29:3945–50.
14. Karni R, Jove R, Levitzki A. Inhibition of pp60c-Src reduces Bcl-XL expression and reverses the transformed phenotype of cells overexpressing EGF and HER-2 receptors. Oncogene 1999;18:4654–62.
15. Ivanov VN, Bhoumik A, Krasilnikov M, et al. Cooperation between STAT3 and c-jun suppresses Fas transcription. Mol Cell 2001;7:517–28.
16. Fukada T, Hibi M, Yamanaka Y, et al. Two signals are necessary for cell proliferation induced by a cytokine receptor gp130: involvement of STAT3 in anti-apoptosis. Immunity 1996;5:449–60.
17. Sinibaldi D, Wharton W, Turkson J, Bowman T, Pledger WJ, Jove R. Induction of p21WAF1/CIP1 and cyclin D1 expression by the Src oncoprotein in mouse fibroblasts: role of activated STAT3 signaling. Oncogene 2000;19:5419–27.
18. Alvarez JV, Febbo PG, Ramaswamy S, Loda M, Richardson A, Frank DA. Identification of a genetic signature of activated signal transducer and activator of transcription 3 in human tumors. Cancer Res 2005;65:5054–62.
19. Dauer DJ, Ferraro B, Song L, et al. Stat3 regulates genes common to both wound healing and cancer. Oncogene 2005;24:3397–408.
20. Leslie K, Lang C, Devgan G, et al. Cyclin D1 is transcriptionally regulated by and required for transformation by activated signal transducer and activator of transcription 3. Cancer Res 2006;66:2544–52.
21. Bienvenu F, Gascan H, Coqueret O. Cyclin D1 represses STAT3 activation through a Cdk4-independent mechanism. J Biol Chem 2001;276:16840–7.
22. Bernards R. CDK-independent activities of D type cyclins. Biochim Biophys Acta 1999;1424:M17–22.
23. Ishii Y, Pirkmaier A, Alvarez JV, et al. Cyclin D1 overexpression and response to bortezomib treatment in a breast cancer model. J Natl Cancer Inst 2006;98:1238–47.
24. Quintanilla-Martinez L, Kremer M, Specht K, et al. Analysis of signal transducer and activator of transcription 3 (Stat 3) pathway in multiple myeloma: Stat 3 activation and cyclin D1 dysregulation are mutually exclusive events. Am J Pathol 2003;162:1449–61.
25. Jirstrom K, Ringberg A, Ferno M, Anagnostaki L, Landberg G. Tissue microarray analyses of G_1/S-regulatory proteins in ductal carcinoma *in situ* of the breast indicate that low cyclin D1 is associated with local recurrence. Br J Cancer 2003;89:1920–6.
26. Pelosio P, Barbareschi M, Bonoldi E, et al. Clinical significance of cyclin D1 expression in patients with node-positive breast carcinoma treated with adjuvant therapy. Ann Oncol 1996;7:695–703.
27. Gillett C, Smith P, Gregory W, et al. Cyclin D1 and prognosis in human breast cancer. Int J Cancer 1996;69:92–9.
28. Nielsen NH, Emdin SO, Cajander J, Landberg G. Deregulation of cyclin E and D1 in breast cancer is associated with inactivation of the retinoblastoma protein. Oncogene 1997;14:295–304.
29. Porter PL, Lund MJ, Lin MG, et al. Racial differences in the expression of cell cycle-regulatory proteins in breast carcinoma. Cancer 2004;100:2533–42.
30. Jia Z, Barbier L, Stuart H, et al. Tumor cell pseudopodial protrusions. Localized signaling domains coordinating cytoskeleton remodeling, cell adhesion, glycolysis, RNA translocation, and protein translation. J Biol Chem 2005;280:30564–73.
31. Silver DL, Naora H, Liu J, Cheng W, Montell DJ. Activated signal transducer and activator of transcription (STAT) 3: localization in focal adhesions and function in ovarian cancer cell motility. Cancer Res 2004;64:3550–8.
32. Kira M, Sano S, Takagi S, Yoshikawa K, Takeda J, Itami S. STAT3 deficiency in keratinocytes leads to compromised cell migration through hyperphosphorylation of p130(cas). J Biol Chem 2002;277:12931–6.
33. Ng DC, Lin BH, Lim CP, et al. Stat3 regulates microtubules by antagonizing the depolymerization activity of stathmin. J Cell Biol 2006;172:245–57.
34. Frank DA. STAT signaling in cancer: insights into pathogenesis and treatment strategies. Cancer Treat Res 2003;115:267–91.
35. Benekli M, Xia Z, Donohue KA, et al. Constitutive activity of signal transducer and activator of transcription 3 protein in acute myeloid leukemia blasts is associated with short disease-free survival. Blood 2002;99:252–7.
36. Alas S, Bonavida B. Rituximab inactivates signal transducer and activation of transcription 3 (STAT3) activity in B-non-Hodgkin's lymphoma through inhibition of the interleukin 10 autocrine/paracrine loop and results in down-regulation of Bcl-2 and sensitization to cytotoxic drugs. Cancer Res 2001;61:5137–44.
37. Alas S, Bonavida B. Inhibition of constitutive STAT3 activity sensitizes resistant non-Hodgkin's lymphoma and multiple myeloma to chemotherapeutic drug-mediated apoptosis. Clin Cancer Res 2003;9:316–26.
38. Xi S, Gooding WE, Grandis JR. *In vivo* antitumor efficacy of STAT3 blockade using a transcription factor decoy approach: implications for cancer therapy. Oncogene 2005;24:970–9.
39. Rivat C, De Wever O, Bruyneel E, Mareel M, Gespach C, Attoub S. Disruption of STAT3 signaling leads to tumor cell invasion through alterations of homotypic cell-cell adhesion complexes. Oncogene 2004;23:3317–27.
40. Gao L, Zhang L, Hu J, et al. Down-regulation of signal transducer and activator of transcription 3 expression using vector-based small interfering RNAs suppresses growth of human prostate tumor *in vivo*. Clin Cancer Res 2005;11:6333–41.
41. Garcia R, Yu C-L, Hudnall A, et al. Constitutive activation of Stat3 in fibroblasts transformed by diverse oncoproteins and in breast carcinoma cells. Cell Growth Differ 1997;8:1267–76.
42. Weinstein IB. Addiction to oncogenes—the Achilles heal of cancer. Science 2002;297:63–4.
43. Frank DA. STAT inhibition in the treatment of cancer: transcription factors as targets for molecular therapy. Curr Cancer Ther Rev 2006;2:57–65.
44. Darnell JE. Validating Stat3 in cancer therapy. Nat Med 2005;11:595–6.
45. Desrivieres S, Kunz C, Barash I, Vafaizadeh V, Borghouts C, Groner B. The biological functions of the versatile transcription factors STAT3 and STAT5 and new strategies for their targeted inhibition. J Mammary Gland Biol Neoplasia 2006;11:75–87.
46. Nielsen M, Kaltoft K, Nordahl M, et al. Constitutive activation of a slowly migrating isoform of Stat3 in mycosis fungoides: tyrphostin AG490 inhibits Stat3 activation and growth of mycosis fungoides tumor cell lines. Proc Natl Acad Sci U S A 1997;94:6764–9.

47. Burke WM, Jin X, Lin HJ, et al. Inhibition of constitutively active Stat3 suppresses growth of human ovarian and breast cancer cells. Oncogene 2001;20:7925–34.

48. De Vos J, Jourdan M, Tarte K, Jasmin C, Klein B. JAK2 tyrosine kinase inhibitor tyrphostin AG490 downregulates the mitogen-activated protein kinase (MAPK) and signal transducer and activator of transcription (STAT) pathways and induces apoptosis in myeloma cells. Br J Haematol 2000;109:823–8.

49. Nefedova Y, Nagaraj S, Rosenbauer A, Muro-Cacho C, Sebti SM, Gabrilovich DI. Regulation of dendritic cell differentiation and antitumor immune response in cancer by pharmacologic-selective inhibition of the Janus-activated kinase 2/signal transducers and activators of transcription 3 pathway. Cancer Res 2005;65:9525–35.

50. Buerger C, Nagel-Wolfrum K, Kunz C, et al. Sequence-specific peptide aptamers, interacting with the intracellular domain of the epidermal growth factor receptor, interfere with Stat3 activation and inhibit the growth of tumor cells. J Biol Chem 2003;278:37610–21.

51. Alvarez JV, Greulich H, Sellers WR, Meyerson M, Frank DA. Signal transducer and activator of transcription 3 is required for the oncogenic effects of non-small-cell lung cancer-associated mutations of the epidermal growth factor receptor. Cancer Res 2006;66:3162–8.

52. Jing N, Li Y, Xiong W, Sha W, Jing L, Tweardy DJ. G-quartet oligonucleotides: a new class of signal transducer and activator of transcription 3 inhibitors that suppress growth of prostate and breast tumors through induction of apoptosis. Cancer Res 2004;64:6603–9.

53. Real PJ, Sierra A, de Juan A, Segovia JC, Lopez-Vega JM, Fernandez-Luna JL. Resistance to chemotherapy via Stat3-dependent overexpression of Bcl-2 in metastatic breast cancer cells. Oncogene 2002;21:7611–8.

54. Battle TE, Frank DA. STAT1 mediates differentiation of chronic lymphocytic leukemia cells in response to bryostatin 1. Blood 2003;102:3016–24.

55. Barton BE, MurphyTF, Shu P, Huang HF, Meyenhofen M, Barton A. Novel single-stranded oligonucleotides that inhibit signal transducer and activator of transcription 3 induce apoptosis *in vitro* and *in vivo* in prostate cancer cell lines. Mol Cancer Res 2004;3:1183–91.

56. Turkson J, zhang S, Mora LB, Burns A, Sebti S, Jove R. A novel platinum compound inhibits constitutive Stat3 signaling and induces cell cycle arrest and apoptosis of malignant cells. J Biol Chem 2005;280:32979–88.

57. Lynch R, Etchin J, Battle TE, Frank DA. A small molecule enhancer of STAT1-dependent gene activation potentiates the anti-proliferative effects of interferon-g in breast cancer cells. Under review 2006.

58. Turkson J, Zhang S, Palmer J, et al. Inhibition of constitutive signal transducer and activator of transcription 3 activation by novel platinum complexes with potent antitumor activity. Mol Cancer Ther 2004;3:1533–42.

59. Blaskovich MA, Sun J, Cantor A, Turkson J, Jove R, Sebti SM. Discovery of JSI-124 (cucurbitacin I), a selective Janus kinase/signal transducer and activator of transcription 3 signaling pathway inhibitor with potent antitumor activity against human and murine cancer cells in mice. Cancer Res 2003;63:1270–9.

60. Bross PF, Kane R, Farrell AT, et al. Approval summary for bortezomib for injection in the treatment of multiple myeloma. Clin Cancer Res 2004;10:3954–64.

61. Guo W, Pylayeva Y, Pepe A, et al. β_4 Integrin amplifies ErbB2 signaling to promote mammary tumorigenesis. Cell 2006;126:489–502.