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REVIEW ARTICLE

Targeting Signal Transducer and Activator of Transcription Signaling Pathway in Leukemias

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ABSTRACT

Signal transducer and activator of transcription (STAT) proteins comprise a seven-member family of latent cytoplasmic transcription factors that are activated through tyrosine phosphorylation by a variety of cytokines and growth factors. Aberrant activation of STATs accompanies malignant cellular transformation with resultant leukemogenesis. Constitutive activation of STATs has been demonstrated in various leukemias. A better understanding of the mechanisms of dysregulation of the STAT pathway and understanding of the cause and effect relationship in leukemogenesis may serve as a basis for designing novel therapeutic strategies directed against STATs. Mechanisms of STAT activation, the potential role of STAT signaling in leukemogenesis, and recent advances in drug discovery targeting the STAT pathway are the focus of this review.

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INTRODUCTION

Signal transducer and activator of transcription (STAT) proteins are a family of cytoplasmic transcription factors involved in cytokine, hormone, and growth factor signal transduction to mediate a variety of biologic processes including cellular growth, differentiation, and apoptosis (Fig 1).[1] Seven members of the STAT family have been identified: STAT1, STAT2, STAT3, STAT4, STAT5a, STAT5b, and STAT6. The exact chromosomal localizations of the STAT genes in humans were identified during the sequencing of the human genome.[2] Several domains are conserved in all STAT family members (Table 1; Fig 2).[3,4]

STAT ISOFORMS

STAT isoforms lacking parts of the carboxy (COOH)–terminal domain (STATβ) have a competitive dominant negative (DN) effect counteracting the full-length isoform, STATα.[5-7] The transcriptional activities of the different isoforms are distinct, suggesting that the balance of these isoforms regulates gene activation, leading to distinct biologic responses (Table 2). Truncated STATβ that lacks the tyrosine residues at the 699 to 705 position can still be recruited to tyrosine phosphorylated receptor proteins via the remaining SH2 domain, but STAT signaling terminates.

STATβ isoforms are generated by alternative mRNA splicing[5] or proteolytic processing.[6,7] The characterization of this proteolytic activity revealed a serine endopeptidase capable of cleaving both STAT3 and STAT5, but not STAT6.[7] A recent provocative study claimed cathepsin G as STAT5 protease and argued that COOH-terminally truncated STAT5 was in fact an artifact generated during in vitro sample preparation with no in vivo significance.[8] Further studies are needed to clarify this controversy.

REGULATION OF STAT SIGNALING

Transcriptional activity of the STAT proteins is tightly regulated by endogenous inhibitory molecules and post-translational modification mechanisms for appropriate physiologic cellular functions including ubiquitination, ISGylation, sumoylation, methylation, and acetylation.[9-11] Increasing evidence suggests that loss of function or methylation silencing of these negative regulators is likely involved in chronic constitutive activation of STATs.

The suppressor of cytokine signaling (SOCS) family of proteins (SOCS1 to SOCS7 and cytokine-inducible SH2-containing protein [CIS]) downregulates STAT signaling as a classic negative feedback loop.[9,12] COOH-terminal domain SOCS box is responsible for the recruitment of the ubiquitin-transferase complex. SOCS1 directly binds to tyrosine phosphorylated Janus family tyrosine kinases (JAKs) to inhibit catalytic activity.[12] In contrast, the SH2 domains of SOCS2 and SOCS3 proteins bind to phosphotyrosine residues of the activated cytokine receptors. Additionally, SOCS proteins induce

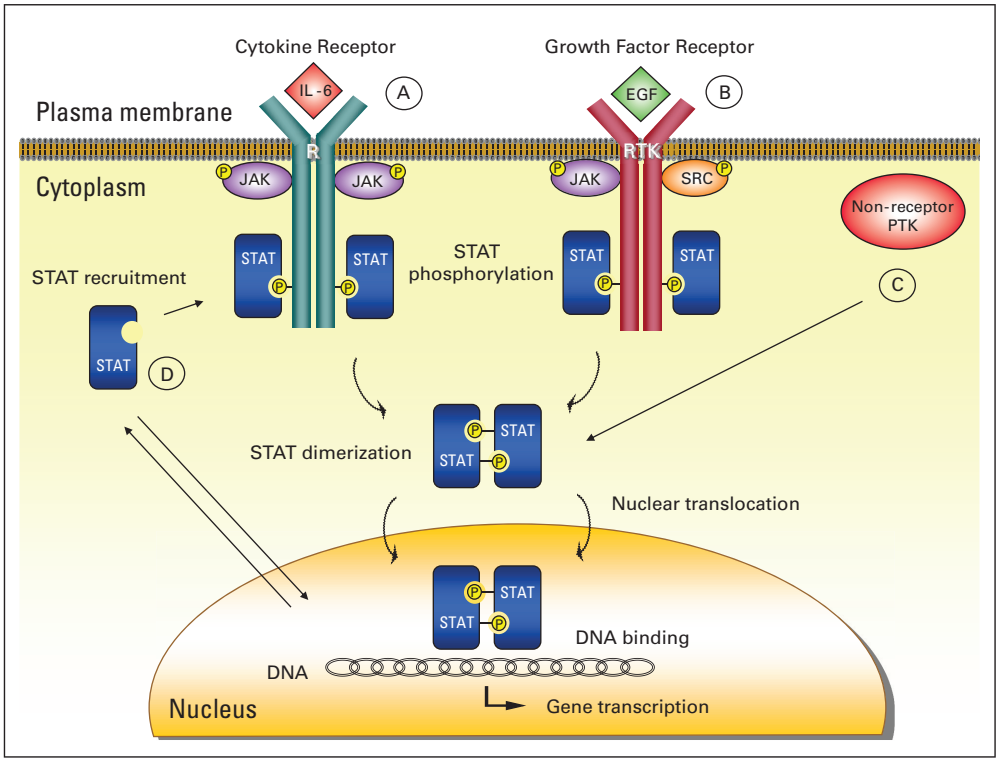

Fig 1. Signal transducer and activator of transcription (STAT) proteins are activated by receptor and nonreceptor tyrosine kinases through several mechanisms. (A) The receptor-associated Janus family tyrosine kinases (JAKs) are activated on cytokine-receptor binding through cross-phosphorylation in the classical pathway. Activated JAKs phosphorylate tyrosine residues on the receptor (R), which become docking elements for cytoplasmic STAT proteins. STATs are subsequently phosphorylated on a single tyrosine residue in the carboxy (COOH) -terminal portion and form homo- or heterodimers through reciprocal interaction between the phosphotyrosine of one STAT and the Schmidt-Ruppin A-2 viral oncogene homolog (avian) (SRC) -homology-2 (SH2) domain of another. Dimerized STATs shift into the nucleus via importins to induce target gene transcription by binding to specific regulatory elements. (B) Receptors with intrinsic tyrosine kinase activities (RTK), including platelet-derived growth factor receptor (PDGFR), epidermal growth factor receptor (EGFR), and FMS (formerly McDonough feline sarcoma viral oncogene homolog) -related tyrosine kinase 3 (FLT3), may directly activate STATs without involvement of JAKs. (C) STATs can be phosphorylated by constitutively active nonreceptor protein tyrosine kinases (PTKs), such as SRC and BCR-ABL. (D) Unphosphorylated STATs can independently enter the nucleus to mediate gene transcription possibly by acting as a transcriptional coregulator to bind DNA. IL-6, interleukin-6; P, phosphorus.

ubiquitin-mediated proteasome-dependent degradation of the STATs. Finally, CIS inhibits STAT activation by competing with STATs for phosphotyrosine binding sites on the cytoplasmic portion of the cytokine receptors.

Protein tyrosine phosphatases (PTPs) neutralize the effects of kinases to dephosphorylate active JAKs/STATs in both the cytoplasm and the nucleus.[9,13,14] Members of PTPs include SH2-containing phosphatase (SHP) -1, SHP-2, CD45, T-cell PTP (TCPTP), PTP1B, and serine/threonine phosphatase PP2A. Discovery of how these PTPs confer specificity in dephosphorylation of various STAT family members will be a huge step forward in understanding STAT-mediated leukemogenesis.

Table 1. STATs Structure

Domain	Function
Oligomerization domain	Mediates oligomerization of STAT dimers to form tetramers and interactions with other proteins
DNA binding domain	Mediates distinct signals for specific ligands to define the DNA-binding specificity
SH2 domain	Mediates specific interactions between STAT-receptor, STAT-JAK, and STAT-STAT
COOH-terminal domain	Regulates the transcriptional activity of STATs and provides functional specificity
Tyrosine residue	Phosphorylation site in the COOH-terminal domain approximately 700 residues from the NH_2 terminus that regulates the DNA-binding activity
Serine residue	A second phosphorylation site in the COOH-terminal domain except STAT2 and STAT6

Abbreviation: STAT, signal transducer and activator of transcription; JAK, Janus family tyrosine kinases; COOH, carboxy; NH_2, N-terminal.

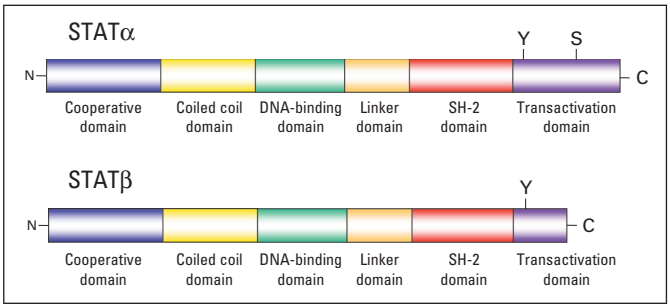

Fig 2. Structure and functional domains of signal transducer and activator of transcription (STAT) molecules. Shown on the top is the full-length STATα. Below is the COOH-terminal (C) transactivation domain truncation resulting in STATβ isoforms. The following two phosphorylation sites in the COOH domain exist: a tyrosine phosphorylation site (Y) that controls dimerization yielding the DNA-binding activity of the STATs, and a serine phosphorylation residue (S) that further modulates the transcriptional activity of STATs.

Table 2. STAT3 Isoforms

Isoform	Description	Molecular Weight (kDa)
STAT3α	Full length	92
STAT3β	Truncated COOH-terminal transactivation domain; functionally distinct, either dominant negative or altered binding	83
STAT3γ	Missing tyrosine residue; able to bind to the remaining SH-2 domain, but functionally inactive	72
STAT3δ	Unknown	64

Abbreviation: STAT, signal transducer and activator of transcription; COOH, carboxy; SH-2, Schmidt-Ruppin A-2 viral oncogene homolog–homology 2.

The protein inhibitors of activated STATs (PIAS) family of proteins (PIAS1, PIAS3, PIASx, and PIASy) is a negative regulator of STAT-mediated gene transcription.[15,16] PIAS1 and PIASy interact with STAT1, PIAS3 interacts with STAT3 and STAT5, and PIASx interacts with STAT4.[16] PIAS proteins inhibit STAT-DNA binding activity and recruit other transcriptional corepressors such as histone deacetylases (HDACs). Furthermore, they have small ubiquitin-related modifier (SUMO) E3 ligase activity.[15] Consequently, transcriptional activity of STATs is inhibited by SUMO conjugation.

The ubiquitin-proteasome degradation pathway represents another negative feedback mechanism.[17] Ubiquitylation involves sequential engagement of ubiquitin-activating enzyme (E1), ubiquitin-conjugating enzyme (E2), and ubiquitin ligase (E3). The SOCS box of SOCS proteins associates with elongins B and C in addition to cullin-2 to form ubiquitin E3 ligase complex. Consequently, the JAK and STAT substrate proteins are selectively targeted to the 26S proteasome for degradation. STAT-interacting LIM protein (SLIM) is a nuclear ubiquitin E3 ligase that contains a PDZ (acronym for the following three proteins: postsynaptic density protein, *Drosophila* disc large tumor suppressor, and zonula occludens-1 protein) domain and a LIM (representing LIN11, Isl1, and MEC-3 proteins) domain.[18] SLIM induces ubiquitination and proteasomal degradation of STAT1 and STAT4. The significance of SLIM in human diseases warrants further investigation.

SUMO and interferon-stimulated gene 15 (ISG15) proteins are members of the ubiquitin-like proteins family.[19,20] Sumoylation and ISGylation pathways are analogous to ubiquitin conjugation with differences in the enzymes involved. However, unlike ubiquitination and sumoylation, protein ISGylation provides a positive feedback for JAK-STAT signaling.[19] The exact roles and relevance of sumoylation and ISGylation in the regulation of STAT signal transduction remain to be fully elucidated.

Cytoplasmic and nuclear STAT-interacting proteins, such as STAT3-interacting protein (StIP1 = ELP2, elongation protein), minichromosome maintenance 5 protein (MCM5), *N*-c-myelocytomatosis viral oncogene homolog (MYC) –interacting protein (Nmi), and p300/CBP (cyclic AMP–responsive element-binding protein [CREB] –binding protein), have also been described to regulate transcriptional activation.[21,22]

STAT NUCLEOCYTOPLASMIC SHUTTLING

After ligand stimulation, cytoplasmic latent STATs rapidly accumulate in the nucleus by crossing through nuclear pore complexes.[23] Most of the nuclear translocation is phosphorylation dependent, whereas some STAT proteins shuttle between the nucleus and cytoplasm independent of phosphorylation.[24-28] Activated STATs shuttle more rapidly than nonactivated ones.[24,28] Direct interaction of unphosphorylated STATs with the nuclear pore proteins (nucleoporins) Nup153 and Nup214 allows carrier-independent nuclear translocation.[25] Nuclear translocation of activated STATs is mediated by the karyopherin-β family of transport proteins called importins or exportins depending on their moving direction.[23] Specific sequence motifs on the surface of the STATs, known as nuclear localization signals and nuclear export signals, allow STAT-importin and STAT-exportin interactions. Specific adaptor molecules, the importin-α family, are involved in STAT–importin-β interaction. Distinct importin-α subtypes determine trafficking of different STATs.[26] STAT activation with nuclear accumulation terminates within minutes.[29] STATs dephosphorylated by nuclear phosphatases, such as TC45, are actively exported back to the cytoplasm by binding to chromosome region maintenance 1 (CRM1; also called exportin-1) protein. Interestingly, nuclear export of truncated STATβ isoforms was shown to be reduced with resultant prolonged nuclear retention, mainly as a result of the accumulation of unphosphorylated proteins.[27,30] Nuclear export differences determine the cytokine sensitivity of STAT isoforms. Inhibition of nuclear export breaks up the STAT reactivation cycle and results in reduced STAT phosphorylation and gene induction with consequent apoptosis.

STATS IN CANCER: EMPHASIS ON LEUKEMIC TRANSFORMATION

Dysregulation of the STAT signaling pathway plays a role in oncogenesis and leukemogenesis.[31,32] As a proof of concept, inhibition of aberrant STAT activity has been repeatedly shown to result in arrest of tumor development and apoptosis. Constitutive STAT activation is associated with malignant transformation induced by various oncoproteins, such as sarcoma (Schmidt-Ruppin A-2) viral oncogene homolog (avian) (v-SRC), breakpoint cluster region (BCR) -abelson (ABL), and epidermal growth factor receptor (EGFR) tyrosine kinases.[33-41] The candidate target genes regulated by the STAT pathways, such as *cyclin D1/D2*, *MYC*, B-cell chronic lymphocytic leukemia (CLL)/lymphoma (*Bcl-x*), and myeloid cell leukemia sequence-1 (*Mcl-1*), seem to contribute to oncogenesis through the control of cell cycle progression and/or the prevention of apoptosis.[35-39,41-43] In addition, recombinantly altering the transactivation domain of STAT3 was shown to induce constitutive activation, leading to malignant transformation in the absence of tyrosine phosphorylation.[42] Importantly, activated STAT3 was reported to mediate p53 inhibition by binding to the p53 promoter.[44] This finding is significant because of the recent demonstration that activated STAT3 regulates murine thymoma viral oncogene homolog (*AKT*) gene expression,[45] a crucial downstream target of phosphatidylinositol 3-kinase (PI3K), which is constitutively active in acute myeloid leukemia (AML) and regulates survival and chemotherapy resistance via nuclear factor-κB, mitogen-activated protein kinase (MAPK), and the p53 pathways (Fig 3).[46] Similarly, a constitutively active STAT5 mutant was shown to form a complex with PI3K and the scaffolding adapter Gab2, resulting in AKT activation in myeloid leukemias.[47] Moreover, a direct cross talk between the STAT and the MAPK pathways has been demonstrated as

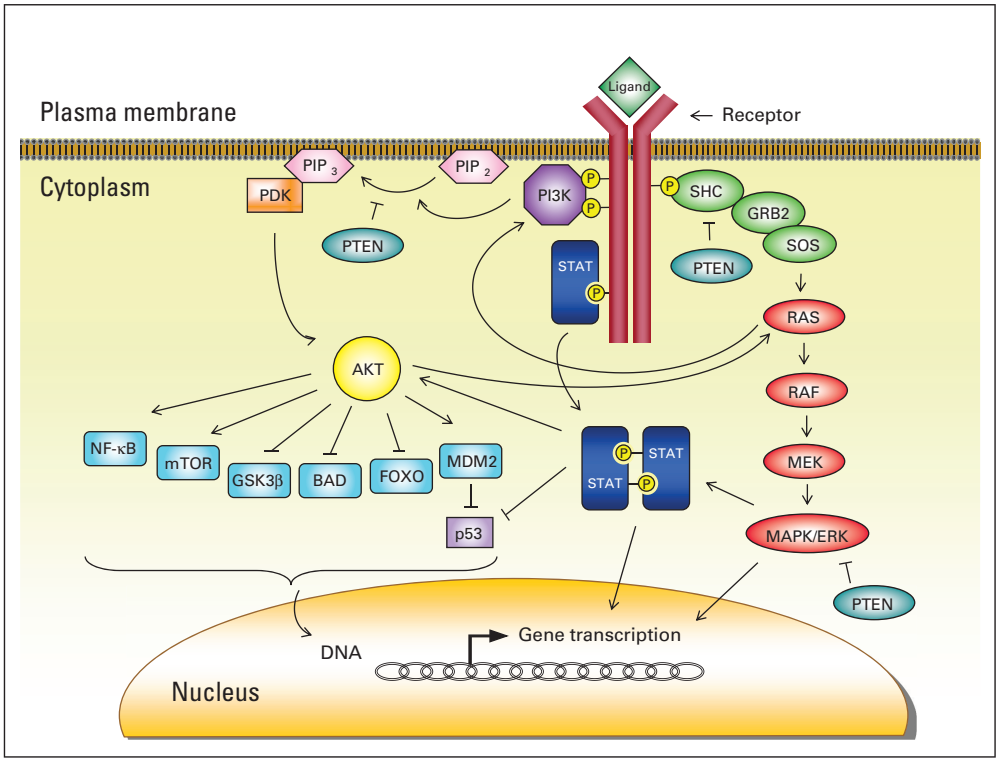

Fig 3. Phosphatidylinositol 3-kinase (PI3K) and mitogen-activated protein kinase (MAPK) signaling pathways. Ligand binding to a receptor tyrosine kinase induces PI3K phosphorylation, which in turn converts phosphatidylinositol (4,5)-bisphosphate (PIP_2) to phosphatidylinositol (3,4,5)-trisphosphate (PIP_3). Phosphatidylinositol-dependent kinase (PDK) is then recruited and activated, which subsequently phosphorylates murine thymoma viral oncogene homolog (AKT). Activated AKT controls fundamental cellular processes such as cell cycle regulation, angiogenesis, survival, and apoptosis via its target molecules. However, the MAPK pathway is initiated by interaction of SRC homology 2 domain containing transforming protein (SHC) with growth factor receptor–bound protein 2 (GRB2) and son of sevenless (SOS), resulting in activation of RAS and downstream MAPK cascade. BAD, Bcl-2 antagonist of cell death; ERK, extracellular signal-regulated kinase; FOXO, forkhead transcription factor; GSK3β, glycogen synthase kinase-3β; MDM2, murine double minute; MEK, mitogen-activated ERK kinase; mTOR, mammalian target of rapamycin; NF-κB, nuclear factor-κB; PTEN, phosphatase and tensin homolog deleted on chromosome 10; STAT, signal transducer and activator of transcription.

part of their involvement in oncogenic transformation.[48,49] Finally, a particularly intriguing role for constitutive STAT3 activity in the up-regulation of vascular endothelial growth factor expression and tumor angiogenesis was demonstrated.[45,50] In summary, STAT3 seems to be the central transcription factor for many signaling pathways.

CONSTITUTIVE STAT ACTIVITY IN LEUKEMIAS

STAT activation in leukemic cell lines[51,52] and blasts from AML and acute lymphoblastic leukemia (ALL) patients has been demonstrated (Table 3).[49,51,53-59] STAT3 activity is most prevalent in AML, whereas STAT5 activity is more common in ALL. Autocrine/paracrine stimulation of the JAK-STAT pathway by hematopoietic cytokines, such as interleukin (IL) -6, might cause constitutive STAT activity.[60] However, the role of IL-6–induced STAT3 activation in leukemogenesis remains controversial because of the antiproliferative effects of IL-6 in AML.[61] The clinical significance of constitutive STAT3 activity was demonstrated in AML patients. Disease-free survival (DFS) was significantly shorter in patients with constitutive STAT3 activity compared with patients without STAT3 activity.[59] In a subgroup analysis, patients with both constitutive STAT3 activity and the truncated STAT3β isoform had the shortest DFS and shorter overall survival compared with all other patients. This was the first demonstration of the clinical prognostic significance for STAT proteins in any malignancy.

Constitutive serine, but not tyrosine, phosphorylation of STAT1 and STAT3 in CLL cells was demonstrated using specific antibodies against the phosphorylated Ser-727 residue.[62] However, the significance of this finding in CLL pathobiology remains undetermined.

STAT ACTIVATION BY LEUKEMOGENIC FUSION PROTEINS AND TYROSINE KINASES

Aberrant STAT activation may be associated with leukemic transformation by various oncoproteins. Leukemic fusion proteins with protein tyrosine kinase (PTK) activity have been shown to activate STATs without the need for receptor activation.

BCR-ABL

The BCR-ABL chimeric protein is a constitutively activated tyrosine kinase that causes growth factor–independent proliferation and transformation of hematopoietic cells in chronic myeloid leukemia (CML), ALL, and rarely AML.[33,34] The Philadelphia chromosome (Ph) is generated by reciprocal translocation of chromosomes 9 and 22, t(9;22)(q34;q11). As a result, two different fusion proteins, $p190^{BCR\text{-}ABL}$ (190 kDa) and $p210^{BCR\text{-}ABL}$ (210 kDa), are produced depending on the breakpoint site on the *BCR* gene. $p210^{BCR\text{-}ABL}$ is the characteristic feature of CML, whereas both p190 and p210 are involved in ALL and AML. Constitutive STAT5 and/or STAT1 activity was demonstrated in BCR-ABL–

Table 3. Constitutive STAT Activity in AML Blasts

Study	STAT1		STAT3		STAT5		ERK		*FLT3*	
	No. of Patients/ Total No.	%	No. of Patients/ Total No.	%	No. of Patients/ Total No.	%	No. of Patients/ Total No.	%	No. of Patients/ Total No.	%
Hayakawa et al[49]	—	—	—	—	10/10	100	10/10	100	10/10*	100*
Aronica et al[53]	10/20	50	—	—	—	—	—	—	—	—
Gouilleux-Gruart et al[54]†	1/5	20	5/5	100	2/5	40	—	—	—	—
Weber-Nordt et al[55]‡	10/14	71	10/14	71	1/14	7	—	—	—	—
Hayakawa et al[56]	—	—	17/23	74	40/50	80	50/50	100§	—	—
Xia et al[57]	—	—	10/36	28	8/36	22	—	—	—	—
Birkenkamp et al[58]	—	—	—	—	18/26	69	—	—	12/17	71
Benekli et al[59]	—	—	28/63	44	11/50	22	—	—	—	—

Abbreviations: STAT, signal transducer and activator of transcription; AML, acute myeloid leukemia; ERK, extracellular signal-regulated kinase.
*All samples had previously known tandem duplication of *FLT3* gene.
†In addition to AML, constitutive STAT1 and STAT5 activities were demonstrated in peripheral blood samples of one and three of three acute lymphoblastic leukemia (ALL) patients, respectively.
‡In addition to AML, constitutive STAT1 activity was found in one (4%) of 24 patients with ALL, and STAT5 activity was found in 15 (63%) of 24 patients.
§All samples had previously known constitutive mitogen-activated protein kinase activity.

positive cell lines, peripheral-blood samples from CML patients, and hematopoietic cell lines transfected in vitro with *BCR-ABL*, leading to malignant transformation.[33-39] STAT activation was stronger in cells transformed by the $p190^{BCR\text{-}ABL}$ isoform, in contrast to the $p210^{BCR\text{-}ABL}$ isoform,[33,34] suggesting a decisive role for the magnitude of STAT phosphorylation on the biologic effects of BCR-ABL. Finally, DN STAT5 isoforms were shown to inhibit $p210^{BCR\text{-}ABL}$-dependent STAT5 phosphorylation, with subsequent inhibition of cell growth confirming the central role for STAT proteins in BCR-ABL signaling.[36]

Possible downstream targets of BCR-ABL and STAT5 activity involve genes regulating apoptosis. BCR-ABL increases the expression of the antiapoptotic Bcl-x_L protein via STAT5 phosphorylation in IL-3–dependent cell lines.[36-39] Interestingly, the BCR-ABL-tyrosine kinase inhibitor imatinib mesylate was shown to induce apoptosis by suppressing STAT5 binding to the *Bcl-x* promoter and downregulating Bcl-x_L expression in BCR-ABL–expressing cell lines and $CD34^+$ cells from CML patients.[38,39]

Interestingly, a study by Sexl et al[63] suggested that there may not be a definitive requirement for STAT5 and that redundant pathways (yet undiscovered) independent of STAT5 may be involved in BCR-ABL–mediated transformation.

TEL-JAK2

In some patients with T-cell ALL, pre–B-cell ALL, and atypical CML, t(9;12)(p24;p13) results in the fusion of the 3′ functional JH1 kinase domain of JAK2 to the 5′ pointed domain of translocated erythroblastosis (ETS) leukemia (TEL), a member of the ETS transcription factor family.[64,65] The TEL-JAK2 fusion protein induces STAT1, STAT3, and STAT5 activation with subsequent cytokine-independent proliferation in the IL-3-dependent Ba/F3 pre–B-cell line.[64,65] In addition, TEL-JAK2 transgenic mice develop T-cell leukemia with constitutive STAT5 and STAT1 activity.[66] Finally, activation of STAT5 was demonstrated to cause myelo- and lymphoproliferative diseases by TEL-JAK2 in a murine bone marrow transplantation model.[67] These data indicate a cardinal role for STAT5 activity in TEL-JAK2–induced growth factor–independent hematopoietic transformation.

TEL-PDGFβR

TEL-platelet-derived growth factor β receptor (PDGFβR) tyrosine kinase fusion protein results from t(5;12)(q33;p13) in chronic myelomonocytic leukemia.[68,69] Constitutive STAT1[68] and STAT5[65] activities were observed in Ba/F3 cell lines transfected with *TEL-PDGFβR*. Transformation by *TEL-PDGFβR* causes hyperphosphorylation of STAT5 on tyrosine residues.[69] However, full transformation required engagement of a combination of signaling intermediates, PI3K and phospholipase C-γ, as well as activation of STAT5, suggesting that constitutive activation of STAT5 by itself may not be sufficient for transformation in this model.

FLT3

FMS (formerly McDonough feline sarcoma viral oncogene homolog) -related tyrosine kinase 3 (FLT3) is a member of the class III receptor tyrosine kinase family expressed on hematopoietic progenitor cells.[70] Its ligand promotes clonal expansion of stem cells. Activating FLT3 mutations, either involving internal tandem duplications (ITDs) or point mutations in the activating loop (tyrosine kinase domain), are observed in approximately 30% of AML patients and are associated with poorer prognosis. Mutated FLT3 by either mechanism is constitutively activated, leading to dual constitutive STAT5 and MAPK activation with factor-independent proliferation.[49,71,72] Furthermore, STAT5 target genes such as *CIS*, *Pim-2*, and cyclin-dependent kinase (CDK) inhibitor *p21* were highly induced by FLT3-ITD.[72,73] Moreover, constitutive STAT5 activity was shown to be associated with spontaneous phosphorylation of mutated FLT3 in primary blasts from AML patients.[49,58,72] Recently, activation of STAT5 by FLT3-ITD has been shown to be direct and independent of JAK and nonreceptor PTKs, such as SRC.[74] Interestingly, Pallis et al[75] suggested that phosphorylated STAT5 was a general feature of AML, not specifically associated with mutated FLT3, even though the FLT3 ligand was repeatedly shown to activate the PI3K/AKT and/or MAPK pathways rather than the STAT5 pathway to transmit proliferative signals in cells expressing the wild-type FLT3 (FLT3-WT).[49,72] These data collectively indicate diversity and differential activation of pathways involved in FLT3 signaling.

Non-PTK Fusion Proteins

STAT proteins are also directly incorporated in non-PTK leukemic fusion proteins. Acute promyelocytic leukemia (APL) is characterized by reciprocal translocations between the retinoic acid receptor α (*RAR*α) gene and five different partner genes including *STAT5b*.[76] An interstitial deletion within chromosome 17 gives rise to the STAT5b-RARα fusion protein, which blocks myeloid differentiation through its interaction with a corepressor complex containing HDAC activity.[77-79] Additionally, APL fusion proteins, including STAT5b-RARα, were shown to enhance STAT3 transcriptional activity through a mechanism involving interaction of HDAC and nuclear coactivators.[76,77] The APL fusion proteins act to shift the balance between the corepressor and the coactivator associated with STAT3. All-*trans*-retinoic acid (ATRA) reverses this balance to switch off STAT3 activation.[76] However, STAT1/STAT2 activation may be one of the mechanisms of ATRA-induced granulocytic differentiation. ATRA was shown to induce expression of the interferon (IFN)-stimulated transcription factors STAT1, STAT2, and IFN regulatory factor-1 (IRF-1) during myeloid cell differentiation.[80,81] These results indicate involvement of an aberrant regulation of the STAT signal transduction pathway in APL.

ROLE OF STAT ISOFORMS IN LEUKEMOGENESIS

STATβ isoforms have been observed in several AML-derived cell lines and myeloblasts from AML patients.[5,7,57,59,82] The demonstration of constitutive STAT3 activation with resultant neoplastic transformation in genetically engineered COOH-terminal STAT3 mutants was the first suggestion that the COOH-terminal transactivation domain might play a causative role in oncogenesis.[42] In this context, STAT3β isoforms with distinct transcriptional activities and intracellular dynamics were proposed to play a role in leukemogenesis. Recent demonstration of reduced nuclear export and prolonged intranuclear accumulation of STAT1β and STAT3β mapping to their COOH-terminal end is particularly intriguing.[27,30] It is likely that truncated STATβ forms have a more versatile multifaceted role in hematopoietic transformation, rather than a simple opposition to STAT function. This would lend further explanation(s) to our findings that constitutive STAT3β activity in leukemic cells identified a group of patients with shorter DFS and overall survival.[59] Furthermore, truncated STAT proteins were demonstrated to be prevalent at relapse of AML, suggesting that STATβ isoform expression, rather than the level of constitutive activity, may be involved in disease progression.[82] Aberrant constitutive activation of the STATβ proteins likely has important implications in the pathophysiology of AML, albeit with many unanswered questions.

TREATMENT STRATEGIES TARGETING STAT PROTEINS

Tampering with STAT signaling emerges as an attractive target to inhibit oncogenesis and leukemogenesis.[83] Treatment strategies including inactivation of upstream STAT activators as well as direct targeting of STAT molecules and downstream effector end proteins are applicable to both hematologic and nonhematologic malignancies (Fig 4; Table 4). Because neoplastic cells are dependent on constitutive STAT activation (oncogene addiction phenomenon), targeting STATs causes preferential cancer cell killing with minimal effects on normal cells.

Receptor Inhibition

Blocking autocrine and paracrine activation loops of cytokine receptors with receptor antagonists or monoclonal antibodies targeting receptors might prove beneficial in the treatment of leukemias. As a proof of principle, inhibition of constitutive STAT3 activity has been demonstrated by the anti-CD20 (a transmembrane B-cell antigen) chimeric antibody rituximab in non-Hodgkin's lymphomas.[84] Similarly, EGFR-directed antibodies were suggested to downregulate STAT3 signaling.[40] However, this strategy remains to be elucidated in leukemias.

Tyrosine Kinase Inhibition

Tyrosine kinase inhibitors have become the focus of intensive investigation in disrupting STAT activation. Selective blockage of JAK2 activity by a tryphostin family tyrosine kinase inhibitor, AG490, was shown to impede in vitro and in vivo growth of leukemic cells.[85] Furthermore, AG490 was shown to promote apoptosis by suppressing STAT3-mediated antiapoptotic Bcl-x_L expression in U266 myeloma cells[83] and Mcl-1 expression in large granular lymphocyte leukemia.[43] Cucurbitacin I (JSI-124) is another selective JAK kinase inhibitor identified in the National Cancer Institute Diversity Set.[86] This compound was reported to suppress STAT3 activation in various cancer cell lines, resulting in inhibition of STAT3-mediated gene transcription. Recently, another cucurbitacin derivative, cucurbitacin Q, was shown to suppress the growth of STAT3-transformed tumors in nude mice xenograft models.[87]

The BCR-ABL tyrosine kinase inhibitor imatinib mesylate inhibits the growth of cells expressing BCR-ABL, TEL-ABL, and TEL-PDGFβR fusion proteins,[88] which are all known to transmit signals through the STAT5 pathway. As direct supporting evidence, blockade of BCR-ABL kinase activity by imatinib mesylate was demonstrated to induce apoptosis of BCR-ABL–positive cell lines and $CD34^+$ cells from CML patients by suppressing the STAT5-dependent expression of Bcl-x_L.[38,39] Currently, imatinib mesylate is approved by the US Food and Drug Administration as first-line treatment for CML and BCR-ABL–positive ALL.

FLT3 tyrosine kinase inhibitors are under investigation for the treatment of FLT3-positive AML. Small-molecule FLT3 inhibitors, such as AG1296, CEP701, GTP14564, PKC412, and SU5614, have been shown to inhibit STAT5 activation, leading to growth arrest and apoptosis through downregulation of the STAT5 target genes (*Bcl-x_L* and *p21*) in cells expressing FLT3 mutations.[58,71,72,89] Cells harboring FLT3–tyrosine kinase domain are more sensitive in vitro to these inhibitors compared with FLT3-ITD cells.[72] Moreover, cotreatment with PKC412/GTP14564 and 17-allylamino-demethoxygeldanamycin, an inhibitor of the heat shock protein 90 (Hsp90), was synergistically effective with associated downregulation of phosphorylated STAT5.[89] Preliminary results of the phase I and II studies suggested that PKC412 had hematologic activity in patients with relapsed/refractory AML and myelodysplastic syndrome expressing FLT mutations; however, the responses were brief.[90-92]

Indirubin is a Chinese herbal medicine used for the treatment of CML that serves as a CDK inhibitor resulting in cell cycle arrest.[93] Derivatives of indirubin were shown to directly block SRC kinase

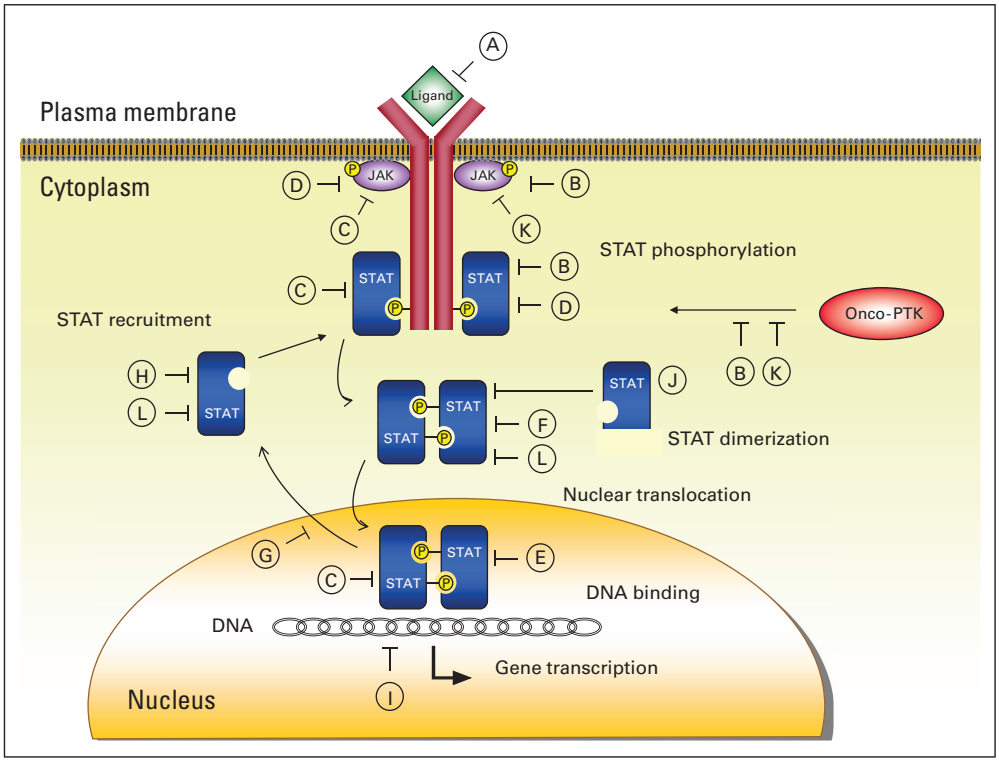

Fig 4. Signal transducer and activator of transcription (STAT) targeting strategies. (A) Cytokine receptor antagonists or receptor-directed monoclonal antibodies. Blocking activation of growth factor/cytokine receptors with monoclonal antibodies might be beneficial.[40,84] (B) Tyrosine kinase inhibitors. Inhibition of upstream tyrosine kinases, such as Janus family tyrosine kinase (JAK), breakpoint cluster region-abelson tyrosine kinase, FMS-related tyrosine kinase 3, and sarcoma (Schmidt-Ruppin A-2) viral oncogene homolog (avian), with resultant downregulation of STATs has proven to be the most effective STAT-targeting strategy.[38,39,43,58,71,72,83,85-93] (C) Phosphotyrosine phosphatases,[9,13,14] (D) suppressors of cytokine signaling proteins,[9,12] and (E) protein inhibitors of activated STATs[9,16] are negative regulators of STAT activity. Activating these molecules represents another promising therapeutic approach.[10,95] (F) Peptidomimetic inhibitors of STAT dimerization directly and selectively target STATs.[96,97] (G) Targeting STAT nucleocytoplasmic shuttling.[28] (H) Antisense oligodeoxynucleotides and small interfering RNA molecules cause selective STAT mRNA inhibition.[98-100] (I) Disruption of STAT-DNA binding by G-quartets and decoy oligonucleotides.[98,101,102] (J) Dominant-negative STAT_isoforms inhibit STAT signaling pathway and tumor growth.[7,57,59,82,83] (K) Arsenic trioxide indirectly decreases STAT activation by direct protein tyrosine kinase (PTK) inhibition.[94,103-107] (L) Novel platinum (IV) compounds directly bind to STAT molecule and block DNA-binding activity.[108,109] P, phosphorus; Onco-PTK, oncogenic PTK.

activity and constitutive STAT3 signaling in human breast and prostate cancer cells, resulting in apoptosis through downregulation of the antiapoptotic proteins Mcl-1 and survivin.[93] Another CDK inhibitor, roscovitine, was shown to cause apoptosis of human T-cell leukemia virus-1–transformed MT-2 T cells by inhibiting STAT5 phosphorylation.[94] It was suggested that roscovitine inhibited the interaction of STAT5 with PDGFRα receptor rather than JAK-dependent STAT5 activation. The exact mechanism of action of these CDK inhibitors in the blockade of STAT activation deserves further study.

Activating STAT Inhibitors

Pharmacologic modulation of STAT signaling by STAT-interacting proteins has been suggested as another promising therapeutic approach, although practical accomplishment is still elusive.[9] PTPs negatively regulate JAKs and STATs by dephosphorylation of kinases.[9,13,14] Specific customized molecules that induce phosphatase activities to dephosphorylate activated STATs may have potential as therapeutic agents. As a proof of principle, PP2A activating agents such as 2-amino-2-[2-(4-octylphenyl)ethyl]-1,3-propanediol hydrochloride (FTY720) and forskolin were shown to downregulate BCR-ABL and STAT5 and subsequently suppress leukemogenesis in both in vitro and in vivo CML and Ph-positive ALL models.[95]

The SOCS and PIAS families of proteins are negative regulators of STAT signaling.[9,12,16] Their clinical significance has been put forward by the demonstration of sustained STAT activity as a result of epigenetic silencing by hypermethylation.[10] Literature reports are conflicting regarding *SOCS1* methylation in AML. Although two groups[110,111] observed *SOCS1* methylation resulting in transcriptional silencing, two other groups[112,113] failed to detect any *SOCS1* methylation in AML. Interestingly, hypermethylation of *SHP-1* and *PIASy* were also reported variably in samples from AML patients.[112-114] The reason for these inconsistencies might be the area of the gene promoter studied. Regardless, further work on this subject is warranted, especially in view of the rapidly developing field of treatment with demethylating agents in AML.

Peptidomimetic Inhibitors of STAT Dimerization

Disruption of the STAT3 dimerization by the SH2 domain–binding phosphotyrosyl peptide PY*LKTK and its tripeptide derivatives PY*L and AY*L was demonstrated to block STAT3-mediated DNA binding activity, gene regulation, and cell transformation in vitro and in vivo.[96] On the basis of these tripeptide derivatives, more potent specific peptidomimetics (ISS610) were generated and shown

Table 4. STAT Targeting Strategies

Molecule	Strategy	Mechanism	Examples	References
Receptor antagonists, monoclonal antibodies	Blockade of cytokine/growth factor binding to the receptor	Indirect	Anti-CD20, anti-EGFR antibody	40,84
Tyrosine kinase (JAK, BCR-ABL, TEL-ABL, TEL-PDGFβR, FLT3, SRC) inhibitors	Inhibition of upstream tyrosine phosphorylation	Indirect	AG490, cucurbitacin, imatinib mesylate, AG1296, CEP701, GTP14564, PKC412, SU5614, indirubin	38,39,43,58,71,72,83,85-93
PTP activators	Activating negative regulators of STAT	Indirect	FTY720, forskolin	9,13,14,95
SOCS protein demethylators	Activating negative regulators of STAT	Indirect	Demethylating agents	9,10,12
PIAS demethylators	Activating negative regulators of STAT	Indirect	Demethylating agents	9,10,16
Small-molecule peptidomimetics	Disruption of STAT dimerization	Direct	PY*LKTK, PY*L, AY*L, ISS 610	96,97
STAT nuclear export (exportin-1) inhibitors	Blockade of STAT nucleocytoplasmic shuttling	Indirect	Ratjadone A	28
Antisense oligodeoxynucleotides, siRNA molecules	Selective STAT mRNA inhibition	Direct	ISIS 345794, siRNAs	98-100
G-quartets, decoy oligonucleotides	Disruption of STAT-DNA binding and transcription	Direct	G-quartets, decoy oligonucleotides	98,101,102
STATβ isoforms	Dominant-negative STAT inhibition	Direct	STATβ isoforms	7,57,59,82,83
Arsenic trioxide	PTK inhibition	Indirect	Arsenic trioxide	94,103-107
Chemotherapy	Direct irreversible interaction with STAT to block DNA-binding activity	Direct	Novel platinum (IV) compounds	108,109

Abbreviations: STAT, signal transducer and activator of transcription; EGFR, epidermal growth factor receptor; JAK, Janus family tyrosine kinase; BCR-ABL, breakpoint cluster region-abelson; TEL-ABL, translocated erythroblastosis (ets) leukemia-abelson; TEL-PDGFβR, translocated erythroblastosis (ets) leukemia–platelet-derived growth factor β receptor; FLT3, FMS-related tyrosine kinase 3; SRC, sarcoma (Schmidt-Ruppin A-2) viral oncogene homolog (avian); PTP, phosphotyrosine phosphatase; SOCS, suppressors of cytokine signaling; PIAS, protein inhibitors of activated STATs; siRNA, small interfering RNA; PTK, protein tyrosine kinase.

to inhibit cell growth.[97] Because STAT proteins are directly and selectively targeted, fewer nonspecific adverse effects are theoretically expected than with other strategies that block upstream STAT signaling.

Inhibition of STAT Nucleocytoplasmic Shuttling

Targeting STAT nuclear traffic is another promising strategy of developing cancer drugs.[28] This is especially important for STAT3 because of its prominent nuclear presence independent of its phosphorylation. Specific structures determining STAT3 nuclear translocation should be targeted to avoid excessive toxicity as a result of complete block of nucleocytoplasmic shuttling.

mRNA Inhibition

Targeting STATs using single-stranded antisense oligonucleotides (ODNs) with a sequence complementary to the mRNA target has been proven to be effective in reducing intracellular STAT levels with resultant loss of function in in vitro and preclinical models.[98] However, modulation of STAT3 expression in every cell is expected in the setting of systemic application of ODN. Preclinical studies of a potent second-generation antisense ODN, ISIS 345794, which selectively inhibits STAT3, are currently ongoing, and phase I trials are expected to begin in the near future.[99] Similar to antisense ODNs, RNA interference phenomenon using STAT3-specific small interfering RNAs for mRNA inhibition was demonstrated to block STAT3-mediated prostate cancer cell growth in vitro.[100]

Targeting STAT3 DNA Binding

Using a nonantisense mechanism, guanosine-rich ODNs that form intramolecular G-quartet structures were developed to inhibit STAT3-DNA binding with a decrease in Bcl-2, Bcl-x_L, and Mcl-1 and resultant tumor cell apoptosis in prostate and breast tumor xenografts.[101]

Decoy ODNs

Intracellular delivery of short double-stranded DNA pieces (decoy ODNs) carrying the consensus STAT-binding sequences has been shown to prevent STAT3 binding to the STAT3 response element within the c-*FOS* (FBJ murine osteosarcoma viral oncogene homolog) promoter and to interfere with head and neck squamous carcinoma growth in vitro.[98] Furthermore, the use of a STAT1 decoy has been reported to inhibit bryostatin 1–induced differentiation of CLL cells with decreased immunoglobulin M production and CD22 expression.[102] Modulation of endogenous gene transcription by introducing STAT decoy ODNs emerges as a novel approach to disrupt STAT activation, but it demands a firm understanding of the causative role of STATs in specific leukemia cases.

Targeting STAT3β

DN STAT isoforms have been shown to inhibit STAT signaling pathways with resultant loss of function in in vitro and in vivo tumor models.[83] However, constitutively active truncated STAT3β isoforms have been suggested to be involved in leukemic transformation[7,57,59,82] based on the reports that the COOH-terminal transactivation domain of STATs plays a causative role in oncogenesis.[42] A novel serine-dependent proteolytic activity is responsible for the truncation of the STAT3 COOH-terminal domain in human AML blasts.[7] Characterization and cloning of the proteolytic activity with subsequent design of custom-made targeted therapies might hold promise for AML treatment.

Arsenic trioxide

Arsenic trioxide (ATO), which is used as a second-line treatment for relapsed/refractory APL, induces apoptosis of non-APL AML cells in vitro.[115] We have demonstrated that ATO indirectly decreases activation of STAT1, STAT3, and STAT5 proteins by direct inhibition of various PTKs in a dose-dependent manner.[103] This effect was specific to the STAT pathway without any effect on the MAPK pathway. Furthermore, we have recently shown that ATO synergizes with Hsp90 inhibitors to downregulate constitutive STAT3 activity in an AML cell line model.[104] Interestingly, Hayashi et al[105] showed that ATO abrogates IL-6–induced phosphorylation of STAT3 in MM.1S myeloma cells via inhibition of JAK1 and JAK2 activity without affecting MAPK and PI3K pathways. Likewise, Cheng et al[106] demonstrated inhibition of STAT3 activity in HepG2 hepatoma cells as a result of direct JAK1 and JAK2 suppression by sodium arsenite, independent of the MAPK pathway. STAT5 activity was also shown to be inhibited by ATO through undisclosed mechanisms, resulting in apoptosis of leukemic MT-2 cells.[94] These results are encouraging, but a phase II study of ATO alone failed to show any survival advantage in relapsed/refractory elderly AML patients.[107] Phase I and III clinical trials to study the in vivo effects of ATO on the STAT pathway in AML patients are ongoing.

Chemotherapy and Biologic Therapy

Pharmacologic modulation of STAT activity after chemotherapy and biologic therapy has been well-documented.[108,109,116-118] The novel alkylator compounds of the platinum (IV) family, CPA-1, CPA-7, platinum tetrachloride, and IS3 295, were shown to disrupt STAT3 activity, block neoplastic proliferation, and induce apoptosis in solid tumor cell lines and animal models.[108,109] Moreover, blockade of STAT3 by IS3 295 also suppressed the expression of STAT3-induced genes *Bcl-x* and *cyclin D1*.[109] It seems that this compound interacts directly with the DNA-binding domain of STAT3, both the inactive monomer and the activated dimer, and irreversibly blocks the binding of activated STAT3 to its consensus DNA response element. Similarly, the antimetabolite fludarabine was shown to cause specific depletion of STAT1 mRNA and protein in CLL cells.[117] Additionally, STAT proteins were suggested to be involved in ATRA-induced growth inhibition and myeloid differentiation of APL cells.[81] In myeloid leukemia cell lines, ATRA was shown to activate STAT1, STAT2, p48, and IRF-1 expression as essential molecules in IFN-α signal transduction.[80,81] Moreover, chronic systemic administration of IFN-α has been reported to cause loss of constitutively active STAT1 and STAT3 DNA-binding abilities in precursor melanoma lesions, with associated STAT3 dephosphorylation.[118] The cross talk between retinoic acid and IFN signaling suggests a potentially useful synergistic combination in the treatment of leukemias.

CONCLUSION AND FUTURE DIRECTIONS

Dysregulated STAT signaling is associated with precipitous cellular proliferation, disturbed differentiation, and arrested apoptosis, which are the hallmarks of leukemogenesis. Constitutive activation of STAT3 and the presence of COOH-terminally truncated STAT3β isoform have been demonstrated to be correlated with poor clinical outcome in AML. However, it is still unclear whether constitutive STAT activity itself is the cause or the result of a transforming process. Although blocking the STAT pathway has been shown to be sufficient to inhibit malignant transformation, the STAT pathway is probably not the only transcription pathway involved in leukemogenesis.

Identification of STAT-regulated genes by gene expression profiling may provide important insights into the role of STATs in the development of leukemias. Understanding the molecular and biologic mechanisms of how aberrant STAT signaling is involved in cellular transformation is of paramount importance for the development of tailored therapeutic approaches to interrupt STAT signaling. Important developments in drug discovery targeting STATs have been made in recent years, and clinical interest is rapidly growing to construct more selective and efficacious agents.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

AUTHOR CONTRIBUTIONS

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