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Review Article

STAT Signaling in the Pathogenesis and Treatment of Cancer

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Abstract

Exceptional advances have been made recently in our understanding of the signaling pathways that control cellular growth, differentiation, and survival. These processes are regulated by extracellular stimuli such as cytokines, cell–cell interactions, and cell–matrix interactions, which trigger a series of intracellular events culminating in the modulation of specific genes. STATs are a highly homologous group of transcription factors that are activated by various pathways and regulate many of the genes controlling cellular function. STATs are activated by tyrosine phosphorylation and modulated by serine phosphorylation, placing them at a convergence point for numerous intracellular signaling pathways. Given the importance of STATs in the control of normal physiologic processes, it is not surprising that inappropriate activation of these proteins has been found in human malignancies. A number of distinct mechanisms have been elucidated by which STATs are activated inappropriately, including autocrine or paracrine stimulation of normal receptors and increased activity of tyrosine kinases through enhanced expression, mutations, or the presence of activating proteins. Furthermore, inappropriate STAT serine phosphorylation has been found in several tumors as well. The increased understanding of signaling pathways in tumors can be translated into therapeutic strategies that have the potential to be more selective and less toxic than current anti-cancer treatments. Approaches which may be effective include the development of antagonists of receptors that can trigger STAT activation, inhibitors of the tyrosine and serine kinases that phosphorylate and activate STATs, agents that decrease STAT levels or inhibit their recruitment to kinases, and molecules that can prevent the binding of STATs to target DNA sequences. Thus, elucidation of cellular and biochemical processes in tumors has enhanced our understanding of the pathogenesis of malignancies and may provide the basis for significant advances in therapy.

Introduction

At the most basic level, cancer represents an abnormal accumulation of cells that arise from a selective advantage in one of three processes: (*1*) an enhanced ability to grow; (*2*) a defect in undergoing cell death; or (*3*) a loss of the capacity to differentiate. Each of these processes is normally initiated by extracellular stimuli such as soluble factors, cell–cell interactions, or cell–matrix interactions, although the actual phenotypic program is determined by the pattern of genes that are activated or repressed. A variety of signaling cascades are present in the cell that link events at the cell membrane with programs of gene expression in the nucleus. In recent years a number of lines of evidence have suggested that path-

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ways that lead to the activation of a group of transcription factors termed STATs may play a particularly important role in the biology of both hematopoietic and nonhematopoietic tumors.

STAT Signaling Pathways

Two approaches can be used to dissect the intracellular events that connect the engagement of a cell surface receptor with the modulation of transcription in the nucleus. The first is to study the receptor, and to discern modifications, such as phosphorylation, that occur following binding by a ligand. Through biochemical and genetic techniques, one can then move step-wise to elucidate proteins that are activated, ultimately arriving at the transcription factors that directly modulate gene expression. The second approach is to start in the nucleus with genes known to be activated by a specific stimulus. One can then define DNA sequences in the promoter of the gene that confer sensitivity to the stimulus. The transcription factors that bind to these DNA sequences can be identified, and the kinases or other enzymes that modulate the transcription factors can then be isolated. In this way, one can work from the inside of the cell outward to discern the pathway controlling new gene transcription. In the last several years, a number of pioneering experiments examining the signal transduction pathways utilized by interferons (IFNs) has employed both of these approaches to define a signaling cascade termed the STAT pathway, which has become important in the understanding of both normal and malignant cell growth.

In the late 1980s, a number of genes were identified whose transcription was induced by IFN-α or IFN-γ. Specific DNA elements, termed the IFN stimulation response element (ISRE) for IFN-α (1) and the gamma (IFN) activated site (GAS) for IFN-γ (2), were identified which mediate responsiveness to each IFN. Using ISRE and GAS DNA sequences as probes, proteins that bound to these sites were isolated and their genes identified (3). The proteins activated in response to IFN-α and IFN-γ are in the cytoplasm under basal conditions, though following stimulation with an IFN, they rapidly translocate to the nucleus and bind to their cognate DNA sequences. It was subsequently shown that the activation of these factors requires tyrosine phosphorylation (4).

These elegant studies defining the activation of IFN-responsive transcription factors were complemented by experiments performed at about the same time on events occurring at the cell membrane. Although it was known that the IFN receptors become tyrosine phosphorylated following stimulation with their ligand, and that inhibitors of tyrosine kinases block the action of IFNs, in contrast to polypeptide growth factor receptors [such as the receptors for epidermal growth factor (EGF), platelet-derived growth factor (PDGF), and insulin], the IFN receptors themselves lack tyrosine kinase activity. To determine how the IFN receptors activate tyrosine kinases, a series of mutants that lacked the ability to respond to IFNs was employed. Genes were transfected into these cell lines, and the restoration of sensitivity to IFNs was examined. These experiments revealed that three members of a recently described family of cytoplasmic tyrosine kinases, termed Jaks, associated with the cytoplasmic domains of these receptors and could restore their signaling ability (5–9). As a result of these experiments, a unified model for interferon signaling was proposed that is now known to mediate signals generated by a multitude of cytokines (Fig. 1). When a cytokine binds to its receptor, it causes the receptor chains to aggregate, bringing the associated Jak family kinases into juxtaposition. This activates the Jak kinases which mediate the subsequent tyrosine phosphorylation of the Jaks and the cytokine receptor chains. The highly tyrosine phosphorylated receptor–kinase complex then serves as a docking site for proteins, such as STATs, which possess src-homology-2 (SH2) domains that allow binding to specific tyrosine-phosphorylated amino acid sequences (10,11). The STATs recruited in this way become phosphorylated on unique tyrosine residues necessary for activation (12), then dissociate from the receptor–kinase complex and dimerize via reciprocal phosphotyrosine–SH2 interactions (13). The STAT dimers translocate to the nucleus where they bind to the DNA sequences necessary to mediate gene activation in response to the cytokine (14). The actual induction of transcription appears to require interactions between STATs and the p300/CREB binding protein (CBP) family of co-activators (15–18), and requires their histone acetyltransferase activity (19).

The activation of STATs is both rapid and transient. Following IFN stimulation, STAT1 becomes maximally tyrosine phosphorylated in 15 to 30 min, and returns to its basal unphosphorylated state in 1 to 2 hr. The de-activation of STATs may occur through dephosphorylation

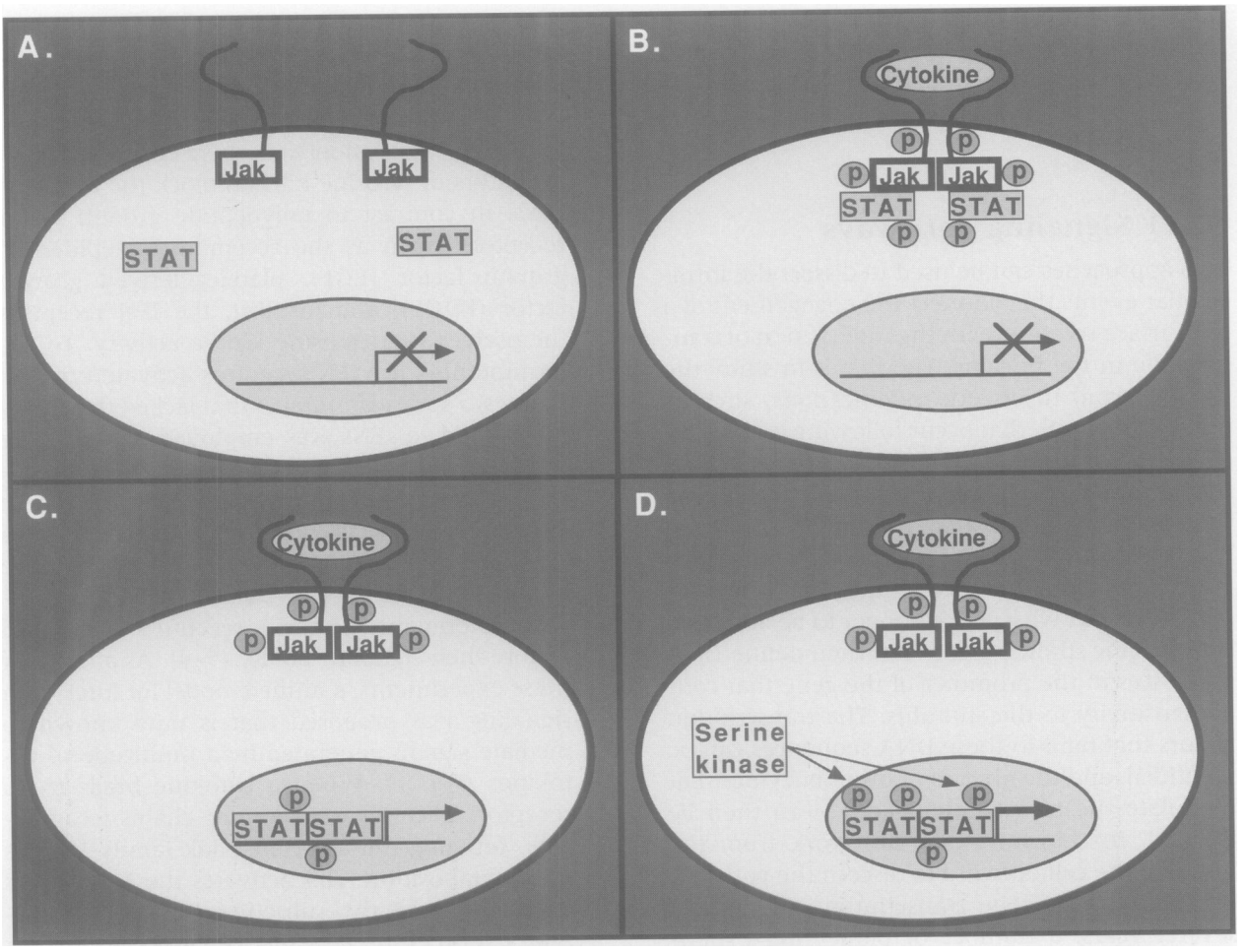

Fig. 1. Cytokine-induced STAT activation. (A) STATs are latent transcription factors found in the cytoplasm of cells. (B) When a cytokine interacts with its cell surface receptor, it induces receptor dimerization, bringing into juxtaposition associated Jak family tyrosine kinases. The Jaks become activated, leading to the tyrosine phosphorylation of themselves and the associated receptor chains. STATs are recruited to the activated kinase–receptor complex, where they become phosphorylated on unique tyrosine residues. (C) Once tyrosine is phosphorylated, the STATs dimerize, translocate to the nucleus, and bind to specific DNA sequences in the regulatory regions of target genes where they can modulate transcription. (D) STATs can also be phosphorylated on specific serine residues. Although this is not sufficient to activate the STATs, it leads to an enhancement of the transcriptional response mediated by tyrosine-phosphorylated STATs.

(20,21) and/or proteolysis (22–24). In addition, cytokines can induce inhibitors of Jak family kinases or of the STATs themselves, and this may contribute to the transient nature of STAT activation under physiologic conditions (25–30).

Although this signaling cascade was initially elucidated in the context of IFNs, it rapidly became clear that many hematopoietic cytokines whose receptors lacked intrinsic tyrosine kinase activity could interact with Jak family members and transduce signals by virtue of STAT activation. Such cytokines include interleukin-2 (IL-2) (31–35), IL-3 (36), IL-4 (37,38), IL-6 (39,40), IL-12 (41), leukemia inhibitory factor (42–46), erythropoietin (36), thrombopoietin (47–49), colony-stimulating factor-1 (CSF-1) (50,51), granulocyte-CSF (G-CSF) (52,53), and granulocyte-macrophage-CSF (GM-CSF) (36). In addition, receptors for other soluble factors were also found to activate Jaks and STATs, such as growth hormone (54,55), oncostatin M (56), prolactin (57–59), ciliary neurotrophic factor (60), tumor necrosis factor (TNF) (61), and angiotensin II (62,63). Given the importance of the Jaks in mediating the tyrosine phosphorylation of STATs, this pathway was initially referred to as the Jak-STAT pathway. However, it is clear that other kinases can participate in the phosphory-

lation of STATs, including src family kinases (64), Tec family kinases (65,66), and the EGF, insulin, and PDGF receptors (50,51,67–72). In addition, other stimuli can induce STAT activation such as engagement of the antigen receptor on B cells (73), or of CD2 on T cells (74). STAT activation in response to these stimuli is delayed, suggesting that the mechanism for phosphorylation is distinct from that mediated by cytokines. STAT phosphorylation is also enhanced by reactive oxygen species, which may contribute to the phosphorylation of STATs triggered by physiologic cytokines and growth factors (75,76). Thus, STATs are activated by a large variety of stimuli mediated by a number of tyrosine kinases.

The hypothesis that STATs occupy a convergence point for a variety of cellular pathways was enhanced by the finding that STATs could also be phosphorylated on distinct serine residues (77–79). Although serine phosphorylation is not sufficient to lead to STAT activation, the phosphorylation of specific serine residues enhances the transcriptional activation that occurs following tyrosine phosphorylation. The kinases mediating the serine phosphorylation of STATs are still being elucidated, although there is evidence that JNK (80), p38 (80,81), MAP kinase (82), protein kinase C, and protein kinase A (83) might all be able to lead to phosphorylation of these sites.

Six STAT family members have been identified, of which one, STAT5, is encoded by two highly related genes (84–88). All STATs share structural similarities including a unique tyrosine residue toward the carboxy terminus that is required for activation, a serine residue distal to the critical tyrosine that also can be phosphorylated, a phosphotyrosine-binding SH2 domain, and a DNA-binding domain. STATs are activated individually or in combination in response to a wide variety of factors. STAT1, STAT3, and STAT5 are each activated by a large number of cytokines; STAT2, STAT4, and STAT6 are activated by relatively few of them. An important area of research centers on determining how the spectrum of STATs activated in response to a given stimulus leads to a unique transcriptional response.

In addition to forming dimers and binding DNA directly, evidence is mounting that STATs can interact with other families of transcription factors. STAT1-STAT2 heterodimers formed in response to treatment with IFN-α associate with a non-STAT DNA-binding protein termed p48, a member of the IFN regulatory factor-1 family (3,89). Subsequently, various STAT family members have been found to associate with c-*jun* (90), C/EBPα (91,92), SP1 (93), and the glucocorticoid receptor (94). These studies suggest that STATs may have important effects acting both independently and in concert with other transcription factors.

Cellular Functions Modulated by STATs

Proliferation

Given that STATs integrate signals from a variety of pathways, it would be expected that they regulate genes important for critical cellular functions such as growth, differentiation, and survival. In fact, there is evidence that STAT binding sites are located within the promoter regions of genes that affect each of these processes. Protooncogenes such as c-*fos*, which may play an important role in the progression of the cell cycle, can be induced by STAT proteins through the so-called sis-inducible element (67,82,95–99), although maximal induction of c-*fos* may require concomitant activation of the *ras* pathway as well (71,95,99). IL-3-induced proliferation is at least partly dependent on STAT5, as dominant inhibitory forms of this STAT decrease the mitogenic effect of IL-3 (99). Similarly, STAT3 is activated during EGF-induced mitogenesis and appears to be necessary for cellular proliferation (100). Furthermore, the increased synthesis of STAT1 induced by IFN-γ can potentiate the mitogenic actions of growth factors such as EGF and PDGF (101). By contrast, STAT1 may also mediate growth arrest via induction of the cyclin-dependent kinase inhibitor p21 (102). Thus, although STAT activation is necessary for proliferation in many cell types, it can be associated with growth inhibition as well.

Differentiation

In addition to modulating cellular proliferation, STATs can mediate transcriptional events associated with the differentiated functions of cells. For example, STAT5, originally described as "mammary gland factor," mediates the transcriptional activation of milk proteins in response to prolactin (57,58,103). STAT6, activated in response to IL-4, regulates expression of cell surface proteins such as major histocompatibility (MHC) class II antigens and is involved in immunoglobulin class switching and Th2 differentiation (104–106). That STAT-mediated modulation of MHC class II

expression is important to host immunity is indicated by the finding that cytomegalovirus (CMV) may escape immune clearance by interfering with STAT-mediated up-regulation of MHC class II molecules (107). Complementing the actions of STAT6, STAT4 plays an important role in the Th1 differentiation pathway for T lymphocytes (108,109). In addition to being associated with cell growth, STAT3 activation has been found to be critical for differentiation of astrocytes (110), keratinocytes (111), and myeloid cells (112), and plays an important role in mediating the formation of epithelial tubules in response to hepatocyte growth factor (113). Thus, STAT activation can be an important approach to overcome blocks in differentiation in malignant cells.

Apoptosis

STATs may play a role both in facilitating apoptosis and preventing it, depending on the system. In human fibroblasts STAT1 is required for the constitutive expression of several caspases, proteases required for executing the cell death pathway (114). Up-regulation of Fas and FasL, molecules that can initiate the apoptotic cascade, is mediated by STAT1 in response to IFN-γ (115). STAT3 appears to mediate apoptosis in Jurkat T cells following ligation of MHC class I molecules (116). On the other hand, STAT1 activation is associated with the activation of the anti-apoptotic protein bcl-x in colorectal carcinoma cells (117) and in cardiac myocytes (118). Thus, STATs may mediate opposing effects on survival in different cell types, perhaps reflecting the fact that the same physiologic stimulus may lead to survival in some cells and apoptosis in others.

STATs in the Pathogenesis of Malignancy

If STATs are involved in the physiologic regulation of processes such as survival, growth, and differentiation, then it would be expected that derangements in STAT signaling could lead to the development of malignancies. Studies in *Drosophila*, which express a Jak homolog (119) and a STAT homolog (120,121), indicate that gain-of-function mutations affecting the Jak can lead to a form of leukemia (122,123). Over the last several years, evidence has accumulated indicating that, by a variety of mechanisms, inappropriate activation of STATs may play a role in human malignancy as well (Fig. 2). This evidence developed initially from studies on cells taken from patients with hematologic malignancies. Abnormal STAT activation was subsequently found in patients with epithelial and mesenchymal tumors, suggesting that STAT activation might be a common pathway for neoplastic cell growth. Finally, mechanistic studies have begun to shed light on the kinases that mediate STAT phosphorylation in tumors and have confirmed that this activation is necessary for malignant cell growth.

STAT Activation in Hematologic Malignancies

ACUTE LEUKEMIAS. The first evidence that inappropriate activation of STATs might be playing a role in human cancer came from studies on leukemic cells taken directly from patients. Using electrophoretic mobility shift assays to detect tyrosine phosphorylated STATs, constitutive activation of STAT5 and STAT1 was found in acute lymphoblastic leukemia (ALL) cells and of STAT1, STAT3, and STAT5 in acute myelogenous leukemia (AML) cells (124–126). These studies provided direct evidence that in contrast to normal cells, leukemic cells from untreated patients contain activated STAT transcription factors that could be driving their abnormal growth.

CHRONIC MYELOGENOUS LEUKEMIA AND BCR-ABL. Chronic myelogenous leukemia (CML) has long been known to be characterized cytogenetically by the presence of the Philadelphia chromosome, a product of a reciprocal translocation between chromosomes 9 and 22 (127,128). This translocation results in the generation of a fusion protein termed Bcr-Abl, a highly active tyrosine kinase that can transform hematopoietic cells in vitro and in vivo (129–132). Whereas many hematopoietic cell lines require exogenous cytokines such as IL-3 or GM-CSF for survival and proliferation, the introduction of Bcr-Abl into these cells relieves the requirement for cytokines and leads to growth factor independence (133). Since IL-3 and GM-CSF exert at least some of their effects by activating STAT transcription factors, the possibility was considered that Bcr-Abl caused factor-independent cell growth and by extension, cellular transformation, through the activation of STATs. In fact, Bcr-Abl transformation of hematopoietic cells leads to the tyrosine phosphorylation of STAT1 and STAT5 in both model systems and in cells of patients with CML

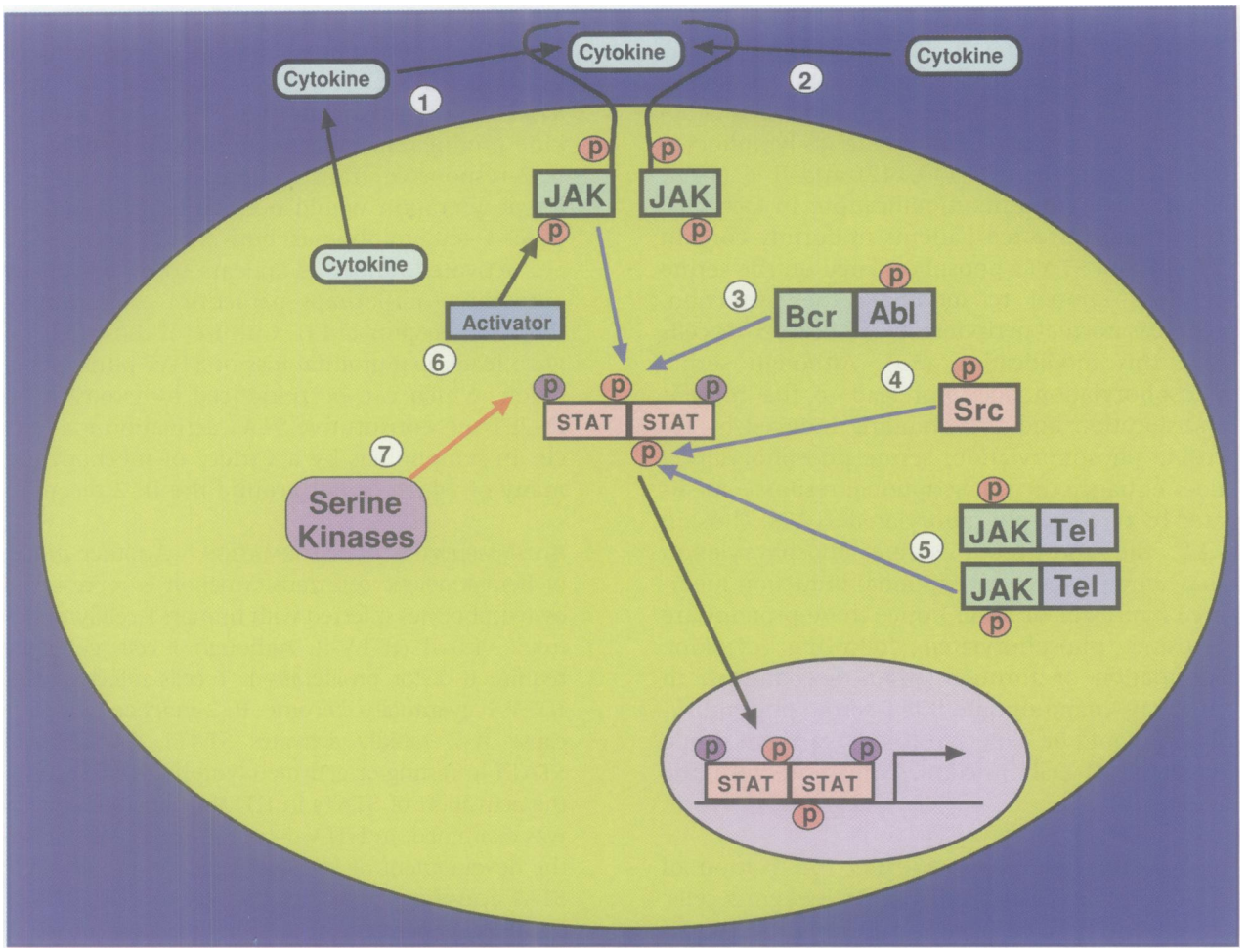

Fig. 2. Mechanisms for STAT activation in cancer. A variety of pathways have been described leading to STAT activation in tumor cells, including (1) autocrine or (2) paracrine activation of cytokine receptors, (3) oncoprotein kinases for which STATs are not physiological substrates (such as Bcr-Abl), (4) mutated cellular tyrosine kinases (such as Src), (5) chimeric oncoproteins which activate kinases that normally phosphorylate STATs (such as Tel-Jak), (6) proteins that can activate a receptor independent of ligand (such as in HTLV-I transformation), and (7) activated serine kinases that do not activate the STATs per se, but may contribute to inappropriate responses to physiologic signals.

(134–138). These cells do not display any evidence of Jak activation, suggesting that the tyrosine phosphorylation of the STATs is mediated directly by the Bcr-Abl kinase. Bcr-Abl has two predominant forms, one of 210 kDa associated with CML, and a 190 kDa form that is associated with ALL (139). The reason that these isoforms are associated with distinct hematologic malignancies is unclear, but there are both qualitative (136) and quantitative (135) differences in STAT phosphorylation mediated by these proteins that may underlie their different biological effects. Recent evidence has suggested that the introduction of a dominant interfering form of STAT5, which inhibits STAT5 function, can block Bcr-Abl–mediated cellular transformation (140). This has provided the first direct evidence that cellular transformation by Bcr-Abl requires the activation of specific STATs.

STAT SERINE PHOSPHORYLATION IN CHRONIC LYMPHOCYTIC LEUKEMIA (CLL). Given that cytokine-driven cell growth is associated with STAT tyrosine phosphorylation, it is not surprising that constitutive STAT tyrosine phosphorylation is seen in rapidly growing leukemias. However, the form of leukemia most common in the Western world, CLL, is characterized by the gradual accumulation of relatively differentiated B lymphocytes (141). As such, it might be expected that activation of signaling pathways in CLL may be more subtle than the full activation seen in these

other malignancies. In fact, no constitutive tyrosine phosphorylation of STATs is found in CLL cells (83). Thus, the possibility was considered that STAT1 and STAT3, transcription factors activated by cytokines important in lymphocyte function such as IL-2 (33,142) and IL-6 (143), might have a distinct modification. In fact, CLL cells from untreated patients uniformly contain STAT1 and STAT3 phosphorylated on the serine residues known to modulate their function, whereas normal peripheral blood or $CD5^+$ B cells lack this modification (83). Although serine phosphorylation does not lead to the nuclear translocation and DNA binding induced by tyrosine phosphorylation, serine phosphorylation does enhance the transcriptional response mediated by tyrosine-phosphorylated STATs. Thus, in CLL, the constitutive serine phosphorylation may amplify the transcriptional induction mediated by STAT1 or STAT3 once these proteins are tyrosine phosphorylated following cytokine stimulation or through other mechanisms. In this way, inappropriate STAT serine phosphorylation would be expected to have a more subtle effect on B cell function, consistent with the biology of CLL.

STAT ACTIVATION IN LYMPHOMAS. Activation of STAT proteins is not restricted to leukemia cells. A cell line derived from a patient with mycosis fungoides, a form of cutaneous T cell lymphoma, contains STAT3, which is constitutively activated (144). In these cells, the slowly migrating form of STAT3, which is phosphorylated on ser-727 (78,83), is tyrosine phosphorylated as well. This raises the possibility that the dual phosphorylation of STAT3 on both tyrosine and serine residues is an important part of cellular transformation. In the mycosis fungoides cell lines, STAT3 associates with Jak3 and tyk2, and the Jak kinase inhibitor AG490 inhibits STAT3 tyrosine phosphorylation and the growth of these cells (144). These results suggest that inappropriate activation of endogenous Jak family kinases may contribute to STAT activation in mycosis fungoides. However, this Jak activation in the absence of growth factors does not completely recapitulate cytokine-induced signaling. In nonmalignant cell lines and primary cells, IL-2 induces the tyrosine phosphorylation of STAT1 and STAT5 in addition to STAT3. Although STAT5 is not phosphorylated in mycosis fungoides cells under basal conditions, the addition of IL-2 leads to the inducible phosphorylation of STAT5. As such, only part of the IL-2-induced signaling pathway is activated in these cells constitutively. It is possible that of the various biological effects induced by IL-2, STAT3 mediates proliferation, and STAT5 may mediate another effect such as cytokine production. In a transformed cell, only the IL-2-responsive transcription factors necessary for proliferation would need to be activated. In other T cell lymphomas, both STAT3 and STAT5 are activated, and this appears to be driven by autocrine (or perhaps paracrine) activation of the IL-2 receptor (145). Culture of these cells in vitro leads to a gradual loss of STAT phosphorylation, which can be reinduced by exposure to IL-2. Thus, constitutive STAT activation may occur in lymphomas by a variety of mechanisms, many of which center around the IL-2 receptor.

HTLV-I-MEDIATED TRANSFORMATION. Another model of hematopoietic cell transformation is represented by lymphocytes infected with human T cell lymphotropic virus I (HTLV-I). Although T cells generally require IL-2 for proliferation, T cells infected with HTLV-I eventually become IL-2-independent. Because IL-2 rapidly activates STAT1, STAT3, and STAT5 in resting or activated lymphocytes (33,142), the activation of STATs in HTLV-I-transformed cells was examined. In HTLV-1-transformed cells prior to the development of IL-2-independent growth, little STAT activation can be detected. By contrast, following development of the IL-2-independent phase of growth, prominent activation of STAT3 and STAT5 is seen (146). These findings are not restricted to in vitro models of HTLV-1 transformation, as primary leukemic cells from 8 of 12 HTLV-I-seropositive patients with adult T cell leukemia/lymphoma (ATLL) displayed constitutive activation of STAT1, STAT3, and/or STAT5 (147). In Bcr-Abl–transformed cells, there is no evidence for Jak activation, and it is proposed that the STATs become tyrosine phosphorylated directly by the Bcr-Abl kinase (134,136). By contrast, in patients with HTLV-I-associated ATLL, Jak kinases are chronically activated (147,148), and in IL-2-independent T cells transformed by HTLV-I in vitro, Jak1 and Jak3 are activated (146). Although the mechanism for Jak activation in HTLV-I-transformed cells is unknown, it is likely that a protein encoded by the virus can interact with components of the IL-2 receptor to recapitulate the IL-2 signaling pathway. It is also interesting that IFN-β, which normally exerts an anti-proliferative effect on T cells, does not inhibit the growth of HTLV-I-infected T cells (149). IFN-β induces the phosphorylation of STAT1 in both infected and uninfected cells, but in HTLV-I-infected cells a greater proportion of the phosphorylated STAT1 is the truncated STAT1β form, which

may be transcriptionally inactive. It remains to be determined whether this is the mechanism by which HTLV-I infected cells escape IFN-mediated suppression of growth.

STAT ACTIVATION IN MULTIPLE MYELOMA. That inappropriate STAT activation might play a role in multiple myeloma was suggested by the finding that both IL-6 and the IL-6 receptor are expressed by myeloma cells. IL-6, which activates STAT1 and STAT3, promotes the growth and survival of myeloma and other B cell tumors (150–153), and animals that lack IL-6 cannot support the development of these malignancies (154). The importance of STAT3 in these cancers was demonstrated by the finding that IL-6-independent B cell tumors contain constitutively activated STAT3 (155), and STAT3 is activated in the bone marrow of patients with multiple myeloma but not in normal individuals (156). The majority of STAT3 activation in myeloma cells is due to an IL-6 autocrine loop, as blocking the IL-6 receptor leads to a loss of most, but not all, STAT3 phosphorylation. This may reflect the activation of STAT3 by an independent pathway, activation of the IL-6 receptor intracellularly, or incomplete blockade of the IL-6 receptor. IL-6-mediated STAT3 activation can also be blocked by an inhibitor of Jak family kinases or by the introduction of an inhibitory form of STAT3. Inhibiting STAT3 by any of these approaches sensitizes myeloma cells to both spontaneous and fas-mediated apoptosis, perhaps through down-regulation of the anti-apoptotic protein bcl-xl (156). IL-6 can also act as a growth factor in hairy cell leukemia (157), and elevated serum levels of IL-6 confer a poor prognosis in patients with diffuse large-cell lymphoma (158). Thus, cytokines such as IL-6 which can act by autocrine, paracrine, and endocrine routes, may contribute to the development of a variety of human tumors.

STAT Activation in Nonhematologic Tumors

Extensive evidence suggests that STAT activation is important in the pathogenesis of hematologic malignancies such as leukemias and lymphomas. However, recent studies have shown that STAT activation may play an important role in the function of nonhematopoietic cells as well. As noted, transformation of fibroblasts by a number of mechanisms is associated with STAT3 activation. In addition, many of the cytokines that signal through STATs have receptors on mesenchymal and epithelial cells, and widely expressed polypeptide growth factor receptors, such as those for insulin, EGF, and PDGF, can activate STATs. Analogous to the findings in hematopoietic cells, it would be expected that inappropriate activation of STATs in nonhematopoietic tissue could lead to tumorigenesis.

BREAST CARCINOMA. The observation that prolactin, a hormone with major effects on mammary growth and differentiation, activates STAT5, raised the possibility that inappropriate activation of STATs might be a component of the pathogenesis of breast cancer (57,58,103,159). Using nuclear extracts derived from human breast carcinomas and normal mammary tissue, it was found that activated STATs, principally STAT1 and STAT3, were present in the malignant tissue (160). Furthermore, in five of nine breast cancer cell lines, but not in normal mammary epithelium, STAT3 was found to be constitutively activated (161). Since EGF receptor overexpression has been found in breast and other carcinomas, one hypothesis is that the constitutive activation of STAT3 is mediated by the EGF receptor pathway. In fact, in a cell line derived from a minimally invasive breast carcinoma, constitutive STAT3 activation is driven by the autocrine stimulation of an EGF-like molecule (162). However, in other mammary cell lines, an inhibitor of the EGF receptor kinase does not eliminate STAT3 activation, suggesting that a distinct tyrosine kinase is involved in this process (161). In another model of mammary carcinogenesis, overexpression of ornithine decarboxylase (ODC), the rate-limiting enzyme in polyamine biosynthesis, contributes to cell transformation. Overexpression of ODC leads to activation of STAT3, bypassing the need for EGF (163). Thus STAT3 activation may be a common event in mammary transformation induced by diverse stimuli.

OTHER NONHEMATOLOGIC MALIGNANCIES. Recent evidence has suggested that STAT3 is activated in primary squamous carcinomas of the head and neck (164). Such activation is also found in the normal mucosa of these patients, but not in the mucosa of controls, suggesting that it may be an early change in the transformation of these cells. STAT3 appears to be activated through the EGF receptor and may mediate the increased survival of these cells conferred by EGF family members (165). Similarly, STAT5 has been shown to be

activated in squamous carcinoma cells and appears to promote the growth of these cells (166).

Activation of STAT3 has also been found in cell lines derived from human prostate cancer and ovarian cancer (167). Although Jak activation is not found in these cell lines, activation of various src family members is observed, and these kinases may be mediating the phosphorylation of STAT3. Using both microscopic and biochemical techniques, it has been shown that STAT1 and STAT3 are activated in atypical nevi, lesions that are precursors to melanoma (168). Thus abundant evidence suggests that inappropriate STAT activation is present in epithelial and mesenchymal tumors.

AUTOCRINE LOOPS IN NONHEMATOLOGIC MALIGNANCIES. Although IL-6 is often viewed as a hematopoietic cytokine, it can affect the biology of epithelial cells as well. Increased expression of both IL-6 and the IL-6 receptor has been seen in colorectal carcinoma, suggesting that an IL-6 autocrine loop may play a role in the pathogenesis of this disease (169). IL-6 can activate STAT1 in colorectal carcinoma cells in vitro (117). While IL-6 does not affect the growth or differentiation of these cells, it does promote their survival, and in this way may contribute to the genesis of colorectal tumors. The presence of an IL-6-mediated autocrine loop may have relevance to the prevention of these neoplasms as well. It has been suggested that people who consume a diet high in fiber have a decreased risk of colorectal cancer (170–173). Butyrate, a short-chain fatty acid found in the bowel lumen of people who consume a high-fiber diet, down-regulates expression of the IL-6 receptor in vitro, and in this way breaks the IL-6 autocrine loop (117). Whether this mechanism is important in the development of colon malignancies remains to be determined, but it is a potentially attractive target for developing chemoprotective agents for these cancers. IL-6 can affect the biology of other epithelial malignancies as well. For example, in renal cell carcinoma (174) and prostate cancer (175) IL-6 decreases the sensitivity to chemotherapeutic agents. Thus, cytokine-driven STAT activation may play a major role in the biology of both hematologic and nonhematologic malignancies.

Mechanisms for STAT Kinase Activation in Cancer: Formation of Kinase Fusion Proteins

An important question that arises from these findings is the identification of the kinase(s) catalyzing STAT phosphorylation in tumors. As in the case of Bcr-Abl, recent molecular and genetic evidence has indicated that fusion proteins formed as a result of chromosomal translocations can lead to activation of this pathway. Chromosomal translocations between the short arms of chromosome 9 and chromosome 12 have been described in hematologic malignancies, most commonly, childhood ALL (176–178). Subsequently, it has been shown that as a result of this translocation, Jak2 (on chromosome 9) becomes fused with a member of the Ets family of transcription factors (on chromosome 12) (179,180). Ets transcription factors form complexes physiologically through a specific oligomerization domain (181). The fusion proteins contain the kinase domain of Jak2 and the oligomerization domain of the transcription factor. This results in oligomerization of the Jak2 kinase, which recapitulates the activation of Jaks by cytokine-induced dimerization, and leads to constitutive kinase activity (180,182). Since Jak-mediated STAT phosphorylation normally requires the presence of a cytokine receptor, it might not follow that independent Jak activation in the cytoplasm would induce STAT phosphorylation. Nonetheless, in hematopoietic cell lines, the introduction of Tel/Jak2 results in the activation of STAT1, STAT3, and STAT5, and cytokine-independent growth, and in animal models Tel/Jak2 fusions can induce myeloproliferative disorders (183).

Other examples of fusions between kinases and transcription factors have been described, including those of Tel/Abl (184,185), NPM/ALK (186), and ZNF198/FGFR1 (187). In several leukemias, fusions have been found between the PDGF receptor (PDGFR) and proteins that can mediate dimerization (188–190). The PDGF receptor is one of the polypeptide receptor tyrosine kinases that can induce STAT activation (191,192). In Tel/PDGFR fusions, analogous to Tel/Jak2, the PDGFβR is activated by dimerization mediated by the oligomerization domain of the transcription factor Tel (193,194). Hematopoietic cells transformed with TEL/PDGFβR become growth factor independent, and display constitutive activation of STAT family members (195), providing further evidence that the forced constitutive activation of STAT family members may be critical to the pathogenesis of these leukemias.

Mechanisms for STAT Kinase Activation in Cancer: Activated Cellular Tyrosine Kinases

Kinases that phosphorylate STATs under physiological conditions, such as Jaks and growth factor receptors, can be activated by mutations to induce STAT phosphorylation continuously. Kinases that may not normally phosphorylate STATs can also become activated through mutations to phosphorylate STATs in models of neoplasia and in human cancer (196). With physiologic stimuli, STAT activation is a transient event. Two key unanswered questions related to STAT signaling in cancer are the following: Why are the mechanisms that normally turn off STAT activation not functioning in these cells? Also, what is the difference in gene induction between continuously activated and transiently activated STATs?

ABL. Given that c-abl is a nuclear protein, it is unlikely that it is a physiologic STAT kinase. Nonetheless, the altered activity and subcellular localization of Bcr-Abl clearly allows abl to activate STATs. Pre–B cells transformed with the oncogenic tyrosine kinase v-abl also show constitutive STAT activation (197). In contrast to Bcr-Abl-mediated transformation, v-abl associates with Jak1 and Jak3, both of which are activated in these cells. Several lines of evidence suggest that the ability of v-abl to induce the activation of Jak1 is critical for its ability to transform hematopoietic cells (198). Thus, transforming tyrosine kinases may lead to STAT activation by direct phosphorylation, or indirectly by activating Jak family members.

SRC. In contrast to abl, which is unlikely to be a physiologic STAT kinase, src may be involved in mediating some cytokine-induced STAT phosphorylation. Evidence from a model system in which STAT3 phosphorylation is induced by IL-3 indicates that activation of c-src rather than Jak2 is critical for STAT3 activation (64). This suggests that src family kinases may be important in mediating STAT activation in response to cytokines under physiologic conditions. It is clear that mutated forms of src and related kinases can lead to STAT activation and cellular transformation. In models of fibroblast transformation, many tyrosine kinases have been found to lead to STAT3 phosphorylation, including v-src and v-fps, polyoma middle T antigen (which activates several non-receptor tyrosine kinases), and v-sis, which activates the PDGF receptor (161,199–201). STAT3 activation is not common to all forms of fibroblast transformation, as SV40 large T antigen, v-raf, and v-ras, all of which operate through other mechanisms, fail to lead to STAT3 activation (161).

A key question that arises in studying neoplastic transformation, especially that induced by a potent tyrosine kinase, is whether the STAT activation seen is integral to the physiologic changes that occur or merely reflects the presence of an activated kinase with a broad substrate range. In the case of src, strong evidence suggests that STAT3 activation is critical for transformation. Wild-type STAT3 enhances the transforming potential of v-src, and dominant interfering forms of STAT3 inhibit src-mediated transformation (202). STAT3β, a naturally occurring splice variant of STAT3, lacks the transactivation domain and the site of serine phosphorylation, and it also functions in a dominant inhibitory capacity (203). Introduction of STAT3β blocks STAT3-mediated gene activation driven by src (204). Furthermore, STAT3β inhibits src-mediated cellular transformation, but not that induced by activated ras, providing additional evidence that STAT activation is central to the mechanism of cellular transformation mediated by some, but not all, dominant transforming oncogenes. These experiments suggest that STAT3 is not necessary for fibroblast transformation, but represents one pathway by which these cells can become malignant. src associates with STAT3 and can phosphorylate this protein in vitro (200,201). However, in contrast to Bcr-Abl, src may also act through Jak kinases. src-transformed cells have been reported to display activation of Jak1 and, to a lesser extent, Jak2 (205), although this finding may be cell type dependent (201). Since the Jaks themselves are activated through tyrosine phosphorylation, it is possible that src-mediated STAT activation can occur either directly or through the activation of the Jaks. Although STAT1 and STAT5 are also phosphorylated in src-transformed cells, these proteins do not coprecipitate with src (201). Thus, distinct mechanisms may govern the phosphorylation and activation of various STAT proteins in src-transformed cells.

LCK. Another src family member is the Lck tyrosine kinase, which is important for T cell development and function. In a mouse T cell lymphoma characterized by Lck overexpression, STAT3 and STAT5, as well as Jak1 and Jak2, are constitutively activated (206). Lck-induced

STAT3 activation also seems to be important in transformation mediated by the DNA tumor virus herpesvirus saimiri (207,208). The transforming tyrosine kinase–interacting protein Tip-484, which is encoded by the virus, directly interacts with and activates Lck, and leads to the phosphorylation of STAT1 and STAT3. Thus the Lck tyrosine kinase, which is critical for normal T cell activation, can be co-opted in the process of cellular transformation either directly, by overexpression, or indirectly, through a protein that enhances its kinase activity.

EYK. c-Eyk encodes a receptor tyrosine kinase, which can become mutated to form a dominant transforming oncogene. The activated Eyk tyrosine kinase associates with STAT1, STAT3, and Jak1, and leads to the constitutive activation of these STATs (209). Although STAT3 activation is critical for src-mediated transformation, in the case of Eyk, cellular transformation correlates more closely with the activation of STAT1 rather than STAT3 (210). Thus, inappropriate STAT tyrosine phosphorylation can occur in transformed cells as a result of a mutation involving a kinase that may not normally phosphorylate STATs (such as abl) or kinases that do phosphorylate STATs under physiologic conditions (such as Jaks and PDGFβR), or by the inappropriate activation of apparently normal kinases (as with HTLV-I transformation).

Activation of Specific STATs in Tumors

Among the STAT family members involved in experimental and primary human tumors, STAT1, STAT3, and STAT5 appear to be inappropriately phosphorylated most often. This may reflect the fact that these proteins are activated by the widest array of cytokines and may have relatively broad effects in promoting cell growth and/or survival. The three STAT family members that do not play a major role in tumors appear to have more limited and defined actions. STAT2 is activated in response to IFN-α, but generally not in response to other stimuli. Since IFN-α appears to have largely a growth-inhibitory effect, it is not surprising that activation of STAT2 has not been defined as an abnormality in tumor cells. STAT4 and STAT6 are activated by a small number of cytokines, and they appear to play a role largely in regulating the function and differentiation of T lymphocytes and natural killer (NK) cells (35,106,108,109,211,212). Thus, the absence of abnormalities of these transcription factors in malignancy is to be expected.

Anti-Cancer Therapy Targeting STAT Transcription Factors

General Considerations

Given the wide array of tumors in which inappropriate tyrosine or serine phosphorylation of STATs is found, inhibition of STAT signaling is an attractive approach for anti-cancer therapy. In selecting targets for therapeutic intervention, several requirements must be met: (*1*) the target must be expressed or regulated differently in malignant cells compared to normal cells; (*2*) it must be amenable to manipulation that can reverse the abnormality; and (*3*) its modulation in normal cells must not cause undue toxicity. Recent evidence suggests that STATs fulfill each of these criteria. They are clearly activated inappropriately in tumor cells, compared to normal cells, and a variety of approaches can be taken to inhibit their function. However, the most difficult aspect of cancer therapeutics is the issue of selectivity, specifically how to inhibit or kill tumor cells while leaving normal cells unscathed. Although STATs mediate important processes in a variety of cells, it does not follow that inhibition of STAT function will induce toxicity in normal tissue. Both quantitative and qualitative data provide insight into the effects of STAT inhibition. From a quantitative standpoint, the magnitude of STAT activation is considerably greater in models of neoplastic cell growth compared to normal cell growth. Using an IL-2-dependent cell line such as NKL (213), it can be shown that prominent tyrosine phosphorylation of STAT1 and STAT5 occurs after the cells are starved, then stimulated briefly with IL-2 (214). If, however, the cells are allowed to grow continuously in the presence of IL-2, the magnitude of activation of these STATs is greatly reduced (Fig. 3). This may reflect the transient nature of cytokine-induced STAT activation and additional factors such as cell cycle asynchrony. By contrast, the magnitude of STAT activation in tumor systems is usually comparable to that seen in the starvation–stimulation model of STAT activation, which is many-fold higher than that found under physiological growth conditions. It may be that in malignant cells high levels of phosphorylated STATs are required, perhaps to overcome compensatory regulatory pathways. Thus, even partial inhibition of STAT activation might be

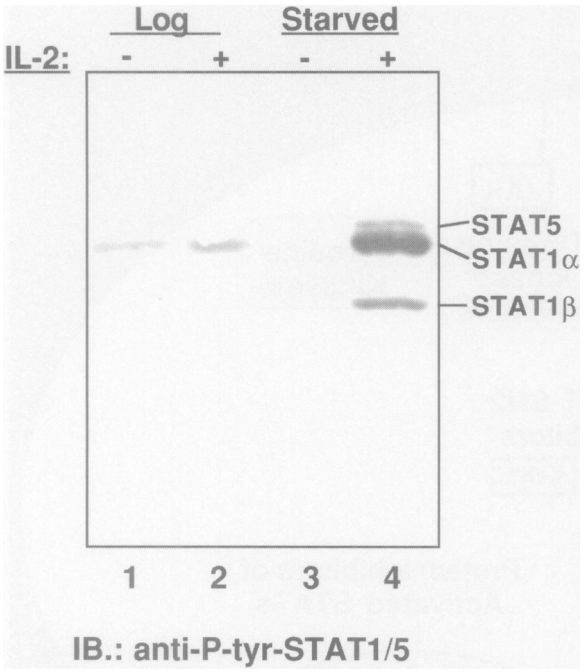

Fig. 3. The magnitude of STAT activation varies with physiologic conditions. IL-2-dependent NKL cells were cultured in IL-2 containing growth medium (lanes 1 and 3) or were starved of IL-2 for 16 hr (lanes 3 and 4). Cells were left untreated (lanes 1 and 3) or were stimulated with supplemental IL-2 (lanes 2 and 4). Tyrosine-phosphorylated STAT1 and STAT5 were determined by performing a Western blot with an antibody that recognizes the activated, tyrosine-phosphorylated form of both proteins. IL-2 treatment of starved cells, which mimics the signaling response in transformed cells, leads to a much greater level of STAT1 and STAT5 activation than that seen in cells growing under physiologic conditions.

sufficient to inhibit tumor cell growth without causing toxicity in normal cells.

A second argument for the feasibility of targeting STATs in anti-cancer therapy makes use of findings from animals in which specific STATs have been eliminated by gene targeting. For example, STAT5 has been found to be activated inappropriately in a number of leukemias and lymphomas. Animals that do not express STAT5a and STAT5b, despite defects with growth hormone and prolactin signaling, are viable and relatively healthy (215). Thus, although STAT5 is involved in mediating the effects of a number of cytokines, there appears to be sufficient redundancy to allow normal processes to occur in its absence. Animals that lack STAT1 also develop normally. However, these animals manifest a defect in cell-mediated immunity, suggesting that targeting of STAT1 may lead to immunosuppression as a side effect. STAT1 null mice may also have an increased susceptibility to malignancies, perhaps through a defect in immune surveillance (216). However, it is unclear whether a similar mechanism is active in humans, as suppression of cell-mediated immunity is generally not associated with epithelial malignancies. The loss of STAT4 (108,109) or STAT6 (106,211,212) affects fairly defined immune functions, but does not lead to widespread abnormalities. One difficulty in interpreting experiments performed in knockout animals is that a defect may result from the absence of a protein during a developmental phase, while the protein itself may be dispensable in adult tissue. In addition to STAT1 and STAT5, the other STAT found to be activated commonly in malignancy is STAT3. Animals that lack this protein die early during embryogenesis (217), making it difficult to predict how inhibition of STAT3 might affect the physiology of an animal in which developmental processes have been completed. Nonetheless, it appears that targeting the activation of STAT proteins in malignancy need not induce intolerable toxicity to normal tissue.

Finally, it is recognized that STATs are not the only transcription factors that may be responsible for malignant cell growth, and neoplastic transformation likely requires abnormalities in more than one pathway. Nonetheless, direct inhibition of STATs may be sufficient to restrict the growth or survival of tumor cells. Furthermore, many of the cellular abnormalities that induce STAT activation, such as the formation of the Bcr-Abl oncoprotein, lead to the activation of other signaling pathways. Thus, targeting molecules upstream of STATs may have multiple beneficial effects in inhibiting tumor cell growth.

Targeting of Receptors

Given our detailed understanding of STAT activation in malignancies, a number of therapeutic strategies can be devised to target this pathway at one or more points (Fig. 4). One mechanism leading to inappropriate STAT activation is the autocrine or paracrine activation of cytokine receptors. As noted earlier, IL-6 plays a role in such pathways in both hematologic and nonhematologic malignancies. If the malignant nature of cells is being driven, even in part, by such stimulation, then the development of antagonists

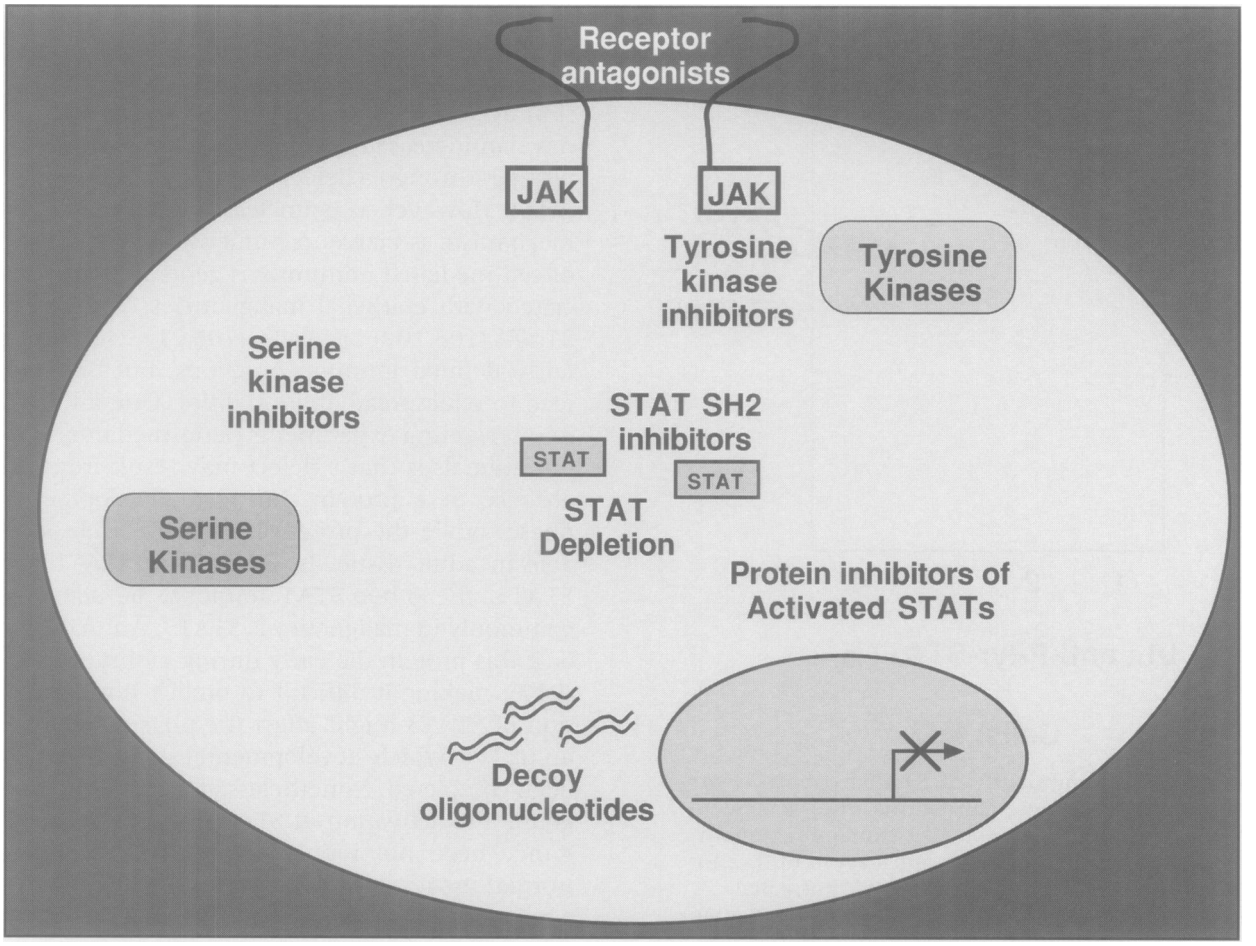

Fig. 4. Strategies for STAT inhibition in tumor cells. STAT-mediated cell growth and survival can be inhibited through the use of receptor antagonists, inhibitors of endogenous or mutated tyrosine kinases, STAT SH2 inhibitors (which would block both STAT recruitment to a tyrosine kinase and STAT dimerization), STAT depletion through anti-sense or pharmacologic approaches, protein inhibitors of activated STATs which can be introduced genetically or activated through biologic therapies, decoy oligonucleotides (which can compete for STAT binding to the promoters of target genes), and serine kinase inhibitors.

that can block these loops might be effective. Both natural product IL-6 receptor antagonists (218) and genetically modified IL-6 variants, so-called super-antagonists (219,220), have been developed, and evidence in multiple myeloma suggests that IL-6 antagonists inhibit cell growth and make tumor cells more susceptible to cell death (156). Complete absence of IL-6 is compatible with normal existence in mice (221), and antibodies to IL-6 have been used in patients (222), demonstrating that this approach is clinically feasible.

Since IL-6 autocrine loops may play a role in the genesis of colorectal neoplasms, inhibitors of the IL-6 receptor may be useful in the treatment or prevention of colon tumors (117). For example, an enteric coated form of an IL-6 antagonist could block IL-6 autocrine loops within the lumen of the colon, while causing no systemic effects. Butyrate, which may mediate the protective effect of a high-fiber diet in preventing colon cancers, appears to work by down-regulating the IL-6 receptor and disrupting this autocrine loop. Similarly, aspirin and other nonsteroidal anti-inflammatory agents that lower the risk of colon cancer also specifically interfere with IL-6 signaling in these cells (H. Yuan, S. Mahajan, D. Frank, unpublished observations). Thus, inhibition of IL-6-induced STAT1 activation might be an effective assay to screen compounds that could prevent colorectal cancer.

Finally, in T cell lymphomas there is evi-

dence that STAT phosphorylation mediated through autocrine (or paracrine) activation of the IL-2 receptor may be central to the pathophysiology of these tumors (145). These studies indicate that targeting cytokine receptors may be a useful intervention for a variety of hematologic and nonhematologic malignancies.

Targeting of Kinases

Since phosphorylation is required for STAT activation, the inhibition of kinases is an attractive strategy for disrupting STAT function. This can include the tyrosine kinases that are necessary for dimerization, nuclear localization, and DNA binding of STATs and/or the serine kinases that can amplify the transcriptional response mediated by a STAT. Evidence has already accrued suggesting that such a strategy may be beneficial. In ALL, Jak2 activation and STAT activation has been found. A small-molecule inhibitor of Jak2, AG490, specifically decreases Jak2 tyrosine kinase activity and inhibits the growth of ALL cells in a mouse model (223). Normal hematopoiesis is unaffected by this drug. AG490 also inhibits both the spontaneous and IL-2-driven growth of mycosis fungoides cells, a T cell malignancy characterized by constitutively activated STAT3 (144). Similarly, small-molecule inhibitors of growth factor receptor tyrosine kinases and of the abl kinase block the growth of cells transformed by Bcr-Abl, Tel-Abl, and Tel-PDGFR (224–230). Such agents may be effective in malignancies by inhibiting the activation of STATs and potentially other pathways as well. In addition, endogenous Jak inhibitors, which likely serve as part of a homeostatic mechanism to limit the signaling response triggered by cytokine stimulation, are inducibly expressed in normal cells (25–30). The introduction of such proteins or the genes encoding them into tumor cells could potentially have anti-cancer activity.

Finally, serine phosphorylation of STATs may be important to the biology of CLL (83), cutaneous T cell lymphomas (144), and other malignancies. Although serine phosphorylation does not activate STATs per se, by amplifying physiologic signals received by a cell, it may alter the growth or survival characteristics of a cell sufficiently to cause a tumor. This may be particularly important in relatively low-grade cancers like CLL. Thus, identification of the kinases that mediate STAT serine phosphorylation and the subsequent development of their inhibitors may be an important therapeutic strategy.

Targeting STAT Activation

The mechanisms by which STATs become activated and transduce signals suggest several possible strategies for targeting STATs themselves. STATs become phosphorylated when they are recruited to an activated tyrosine kinase through their SH2 domain (10,11). In addition, the STAT SH2 domain is essential for STAT dimerization, which occurs through reciprocal interactions between the phosphorylated tyrosine of one STAT and the SH2 domain of its dimerization partner (13,14). Thus, small-molecule inhibitors of SH2 domains could interrupt STAT signaling at two points of the pathway: recruitment to an activated kinase and dimerization. With advances in delineating the structural requirements for SH2 interactions, the development of relatively specific inhibitors of this site is feasible.

A second method to inhibit STAT signaling directly is to reduce the concentration of a STAT within a cell. One approach is the use of anti-sense oligonucleotides to directly reduce STAT production. The half-life of a STAT protein is relatively short, less than 24 hr for STAT1 (unpublished observations), making depletion of STATs through anti-sense an appealing and feasible strategy. Such an approach has been used to decrease STAT1 levels in human cells in vitro, with a concomitant reduction in the mitogenic response to growth factors such as EGF and PDGF (101). In addition, recent evidence suggests that drugs currently used as anti-cancer agents might work at least in part through the depletion of STAT1. For example, the purine analog fludarabine is an effective agent in CLL. Although fludarabine was hypothesized to act through incorporation into DNA, relatively few CLL cells are traversing the cell cycle at any given time, making such a mechanism unlikely. Given that inappropriate STAT serine phosphorylation is a hallmark of CLL (83), it was hypothesized that fludarabine might work by interfering with STAT signaling. In fact, fludarabine leads to a pronounced and specific loss of STAT1 (231). This may underlie both the anti-neoplastic actions of fludarabine and the immunosuppression that accompanies the use of this drug, which is similar to that seen in STAT1-deficient animals. These findings strengthen the hypothesis that STAT inhibition is a potentially important target in cancer therapy.

Targeting STAT DNA Binding

Even if STATs become phosphorylated within a cell, they cannot exert their biological effect until they bind to specific DNA sequences in the promoters of target genes. Thus, inhibiting the ability of an activated STAT dimer to bind to its target DNA is an effective strategy to inhibit STAT-mediated transcriptional activation. Several approaches can be envisioned. The first involves inhibiting the translocation of activated STATs from the cytoplasm, where they are phosphorylated, to the nucleus, where they exert their effects. However, the mechanisms that regulate the process of nuclear localization are poorly understood, and thus its inhibition would be a distant goal. More practically, it is conceivable that the ability of an activated STAT to bind to a target sequence in a promoter region can be inhibited. One approach involves the development of small-molecule inhibitors that could interact with the DNA binding sites of activated STATs, thereby preventing the binding to a promoter sequence. A second approach, which could be developed more easily, involves the introduction into the cell of short stretches of double-stranded DNA which mimics the target STAT binding sequence. These "decoy oligonucleotides" would be present in great molar excess over the endogenous sequences within promoter regions. When a STAT becomes activated, it would bind to the decoy oligonucleotide, precluding its interaction with the target gene promoters. Such a strategy could diminish the ability of STATs to activate genes critical for neoplastic cell growth, and might be particularly useful in combination with inhibitors of STAT phosphorylation.

Dominant Inhibitory STATs

STATs can also be inhibited by dominant inhibitory forms. Such STATs, which lack a DNA binding domain or a transactivation domain, can still from dimers with endogenous STATs. However, the dimer is functionally inactive, and thus the mutant STAT can suppress STAT function in a cell in which it is expressed (99,203,232). Although potentially cumbersome to use clinically, this approach has been useful in demonstrating the importance for STAT signaling in a variety of systems and can inhibit malignant cell growth in in vitro models (140,202,204).

STAT Modulation by Biological and Physical Agents

Biological agents in current clinical use may act by modulating STAT function. For example, IFN-α can decrease the rate of recurrence of malignant melanoma after the primary tumor has been removed (233). Although melanoma precursor lesions demonstrate constitutive activation of STAT1 and STAT3, when patients are treated with systemic IFN-α, the DNA binding of these STATs is lost (168). Although IFN-α itself can induce STAT activation acutely in melanoma cells (234), chronic systemic administration may decrease constitutive STAT activation through an independent mechanism. One attractive hypothesis is that the chronic presence of a stimulus for STAT activation induces inhibitors of activated STATs as part of a homeostatic feedback process to limit STAT-mediated transcription (25–30). Thus, chronic systemic IFN-α may be able to suppress the function of STATs that have been activated by another pathway. However, an added complexity to the role of STAT signaling in melanoma is the finding that melanoma cell lines that become resistant to the cytostatic effects of IFNs show a loss of IFN-activated proteins, most commonly STAT1 (235). Similarly, in cutaneous T cell lymphoma, resistance to the growth-inhibitory effects of IFN-α is associated with the loss of STAT1 (236). These results suggest that on the one hand, STAT1 activation is present in melanoma precursors and decreases with IFN-α treatment, but that on the other hand, the loss of sensitivity to IFN correlates with a loss of STAT1. The resolution of this paradox may depend on one or more differences between the systems, such as the use of atypical nevi versus melanomas, or the use of primary human tissue versus cell lines. In addition, other modifications that affect STAT activity, such as the concomitant presence of activated STAT3 or the state of phosphorylation of ser-727 of STAT1, may also explain these differences. This is not an inconsequential issue, as activation of STAT1 can lead to growth arrest in response to cytokines other than the IFNs (237). For example, in A431 cells, EGF leads to STAT1 activation and growth inhibition (232). The introduction of a dominant interfering form of STAT1 abrogates the growth-suppressive effects of EGF, indicating that STAT1 mediates this inhibition. Thus, a key unanswered question in the role of STATs in malignant cell physiology concerns this discrepancy between growth-stimulatory and growth-suppressive effects.

STAT activation can be used therapeutically for the induction of cellular differentiation, which may underlie the actions of another class of biological agents, the retinoids. Among this class of compounds, all-*trans* retinoic acid (ATRA) has potent abilities to induce the differentiation of acute promyelocytic leukemia cells (238). Among the effects of ATRA is the upregulation of STAT1 and STAT2, the two STATs activated in response to IFN-α. In addition, STAT1 becomes tyrosine phosphorylated for prolonged periods following ATRA treatment, and ATRA potentiates the growth-inhibitory effect of IFN-α (239–243). These studies lend further support to the importance of STAT modulation in the mechanism of anti-tumor activity mediated by biological agents.

The inhibition of STAT activation can also occur through nonpharmacologic means. Physical and biological agents that induce immune suppression may act by blocking cytokine-mediated STAT activation. For example, UV light (244) and the adenoviral protein E1A (245) can each prevent the activation of STAT1 induced by IFN-γ. These findings suggest that modulation of STAT activity is an important target for altering cellular behavior and can be achieved through a variety of modalities.

Summary

Extensive work in the last several years has highlighted the importance of the STAT family of transcription factors in mediating the actions of cytokines and growth factors. Because these molecules transduce physiologic signals regulating growth, differentiation, and apoptosis, it is not surprising that inappropriate STAT activation has been found in a wide range of human tumors. The goal now is to translate this enhanced understanding of the molecular pathogenesis of malignancies into effective strategies to treat cancer with more specificity and less toxicity. Reagents developed for this purpose may be useful not only in the treatment of malignancies but also in further dissecting the role that STAT signaling plays in normal and neoplastic cellular physiology.

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