Modulation of Cellular Signaling Pathways: Prospects for Targeted Therapy in Hematological Malignancies

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Abstract
The high remission rates observed in patients with chronic myelogenous leukemia who receive Imatinib mesylate (Gleevec) indicate that targeted therapy for hematological malignancies is achievable. At the same time, progress in cellular biology over the past decade has resulted in a better understanding of the process of malignant transformation, a better classification of subtypes of each disease on the basis of molecular markers, and a better characterization of the molecular targets for drug development. These advances have already spawned the development of such effective agents as monoclonal antibodies and specific enzyme inhibitors. This review attempts to provide a practical introduction to the complex and growing field of targeted therapy in hematological malignancies.

Introduction
Cellular proliferation, differentiation, and death are regulated by a number of extracellular molecules such as cytokines and hormones, as well as intercellular interactions mediated by neighboring cell surface antigens. These effectors mediate gene transcription either directly or indirectly by activating intracellular signaling pathways, which in turn activate appropriate cellular machinery (1). Cell-surface receptors that convert external stimuli into intracellular signals are pivotal in this signaling process. They activate intracellular pathways either through their inherent enzymatic function or as a result of their association with other catalytic proteins. Indeed, most growth factors and cytokines bind these receptors and exert their function through activation, commonly by phosphorylation (1).

Normal hematopoiesis is dependent on intricately regulated signaling cascades that are mediated by cytokines and their receptors. Ordinarily, function of these pathways leads to the generation of appropriate constellation of hematopoietic cells, and their abnormal activation results in neoplastic transformation, impaired apoptosis, and uncontrolled proliferation. Cytokines function in a redundant and pleiotropic manner; different cytokines can exert similar effects on the same cell type, and any particular cytokine can have several differing biological functions (2). This complexity of function is a result of shared receptor subunits as well as overlapping downstream pathways culminating in activation of common transcription factors (3).

The signal transduction cascades involve three major classes of proteins: kinases, adaptor or docking proteins, and transcription factors. Early insights into the cellular signaling pathways came from studies of IFN function (4–6). These experiments led to the identification of a family of nonreceptor TKs (1) called Jaks and their target proteins, Stats, which mediate gene transcription (4, 7). The Jak-Stat pathways are commonly activated during cytokine signaling through phosphorylation of specific tyrosine residues (3). The interaction of a cytokine with its receptor induces its tyrosine phosphorylation and leads to activation of downstream protein TKs including Jaks and Stats. Apart from their catalytic domain, protein TKs contain several other characteristic motifs including the SH2, SH3, and pleckstrin homology domain, which enable them to interact with other signaling molecules and propagate the message (8, 9).

The phosphorylation of serine and threonine residues is integral to the activation of numerous other intracellular proteins that mediate a number of other signaling pathways (10). Cytokine receptors without intrinsic kinase activity transmit their signals primarily through activation of Jak kinases. These receptors as well as those with intrinsic kinase activity, the RTKs, were previously thought to transmit their signals independently of the serine/threonine kinase cascades. More recently, it has been established that both of these pathways interact with serine/threonine kinase cascades such as the Ras/Raf/MEK/ERK (MAPK; Ref. 10). For example, after ligand binding, the β-subunit of IL-3 and GM-CSF receptors are phosphorylated and, through recruitment of adaptor proteins such as Shc, Grb2, and...
Sos, activate the Ras signaling pathway (11). This in turn activates Raf followed by downstream activation of ERK1 and ERK2, and increased expression of transcription factors c-fos and c-jun (12–14). Other members of the MAPK family such as p38, and JNK/SAPK are also activated after phosphorylation of their serine/threonine residues as a result of cytokine/receptor interaction (15–18). Similarly, PI3K associates with the β chain of IL-3 receptor, recruits PKB/AKT by phosphorylation of its serine residues, and transmits cellular survival signals (19–21). Another downstream protein to IL-3 activation is the p70S6 kinase, which also interacts with the β chain and mediates appropriate signals (22, 23). Ultimately, these pathways influence gene transcription through their ability to recruit transcription factors, regulate apoptosis through the phosphorylation of apoptotic proteins, and cause the cell to progress through cell-cycle checkpoints by activation of specific kinases.

Of considerable interest is the description of a number of oncogenes with constitutive kinase activity. These molecules are derived from genes including c-ABL, c-FMS, FLT3, c-KIT, and PDGFRβ, which are normally involved in the regulation of hematopoiesis (24). The kinase activity of the oncogene is constitutively activated by mutations that remove inhibitory domains of the molecule or induce the kinase domain to adopt an activated configuration (24). As a result of such constitutive activation a number of signaling cascades such as the Jak-Stat pathway, the Ras/Raf/MAPK pathway, and the PI3K pathway are activated.

With better characterization of aberrant signaling through the cell surface receptors and their downstream pathways in neoplastic cells, current research is exploring ways to reverse such dysregulated stimuli (25). Here, we will briefly review the role of cellular signaling pathways in normal cellular processes, neoplastic transformation, and development of hematological malignancies. We then explore the possible ways that their modulation can lead to clinically meaningful benefits.

**Jak-Stat Signaling Pathways**

Hematopoietic cell proliferation and differentiation is regulated by a number of soluble polypeptides such as IFNs, interleukins, and colony-stimulatory factors known collectively as cytokines (26). Cytokines bind to their cognate receptors and mediate downstream effects. A cytokine receptor consists of a unique ligand binding subunit as well as a signal transducing subunit, which may be structurally similar to the other cytokine receptors (27–29). Dimerization of cytokine receptor subunits, phosphorylate them, and in doing so create docking sites on the receptors for binding of SH2-containing proteins (33, 34). In general, Jaks consist of several domains (JH1-JH7) of which the functional significance has been characterized by mutational analysis and include a TK domain (27, 33, 35, 36). The precise functions of JH2-JH7 domains are under current investigation (3). Jaks are able to associate with the cytokine receptors as well as with each other (37, 38). Dimerization/oligomerization of cytokine receptor subunits as a result of ligand binding leads to juxtaposition of Jaks (3). This results in transphosphorylation and activation of their kinase activity and the phosphorylation of downstream signaling proteins such as Stats, Src-kinases, and adaptors such as Src, Grb2, and Cbl (Fig. 1; Refs. 3, 39, 40).

Unlike growth factor receptors (RTKs), cytokine receptors do not possess a cytoplasmic kinase domain, and most cytokines transmit their signal by recruiting other TKs (3). Dimerization of the cytoplasmic component of the cytokine receptor as a result of ligand binding is the initial step in the initiation of cellular signaling (31). The dimerized subunits then associate with intracellular TKs such as members of the Src or Jak families of kinases, and the signal is propagated (Fig. 1). Different Src family members are associated with different receptors and phosphorylate distinct but overlapping sets of downstream target molecules. For example, Lck, Lyn, and Fyn can be activated by IL-2 (29, 32).

The Jak family of kinases comprises four known relatively large proteins (Jak1, Jak2, Jak3, and Tyk2) that can bind cytokine receptor subunits, phosphorylate them, and in doing so create docking sites on the receptors for binding of SH2-containing proteins (33, 34). In general, Jaks consist of several domains (JH1-JH7) of which the functional significance has been characterized by mutational analysis and include a TK domain (JH1; Refs. 3, 33, 35, 36). The precise functions of JH2-JH7 domains are under current investigation (3). Jaks are able to associate with the cytokine receptors as well as with each other (37, 38). Dimerization/oligomerization of cytokine receptor subunits as a result of ligand binding leads to juxtaposition of Jaks (3). This results in transphosphorylation and activation of their kinase activity and the phosphorylation of downstream signaling proteins such as Stats, Src-kinases, and adaptors such as Src, Grb2, and Cbl (Fig. 1; Refs. 3, 39, 40).

Abnormalities of Jak function have been associated with a number of disorders (34, 41). For example, chromosomal translocations resulting in TEL-JAK2 constructs lead to the constitutive activation of Stat5, IL-3-independent cellular proliferation, and leukemogenesis (42, 43). The translocation t(9;12)(p24;p13) results in the fusion of the kinase catalytic
region of JAK2 with the transcription factor TEL generating the constitutively active TEL-JAK2. Similarly, infection with oncogenic viruses such as human T-cell lymphotropic virus, type I, and Abelson murine leukemia viruses results in enhanced TK activity of Jaks, possibly accounting for their leukemogenic potential (44, 45).

The Stat transcription factors are coded by six known mammalian genes and include 10 different Stat proteins including different isomers of Stats 1, 3, 4, and 5 (3, 46). Like other transcription factors Stats have a well-defined structure including a DNA-binding domain, a conserved NH$_2$-terminal domain, a COOH-terminal transactivation domain, and SH2 and SH3 domains (3). Their activation through tyrosine phosphorylation results in their dimerization and translocation into the nucleus where they activate specific genes (6, 47–49).

Jak proteins activate a number of intracellular signaling proteins, among which Stats are the best defined (46, 50). Binding of a cytokine to its receptor rapidly induces tyrosine phosphorylation of the cytoplasmic domains of the receptor by activated Jak kinases, thus providing a docking site for Stat proteins, which are then phosphorylated. This phosphorylation of Stats leads to their homo- or heterodimerization and translocation to the nucleus, followed by DNA binding and gene activation (Fig. 1; Refs. 51, 52). The specificity for Stat phosphorylation is determined by the receptor docking sites and not the Jak kinases (53, 54). Also, different Stat proteins have different DNA-binding affinities, resulting in activation of specific genes. Stats also interact with other transcription factors such as the p300/cyclic AMP-responsive element binding protein family of coactivators to activate genes (55, 56). The transcriptional activity of Stats may also be regulated by the phosphorylation of their serine and threonine residues, although the implications of such regulation are not known (7, 57).

Stats mediate diverse and sometimes opposite cellular events affecting growth, differentiation, and apoptosis (58, 59). For example, Stats can mediate both growth arrest and cellular proliferation. Specifically, Stat1 mediates the growth-inhibitory effects of IFN-γ through the induction of the CDKI p21$^{\text{waf1}}$, whereas Stat5 mediates proliferative effects of IL-3 and GM-CSF (60, 61). Similarly, phosphorylation of Stat3 can result both in IL-6- and IL-10-induced growth arrest, and in GM-CSF- and IL-3-induced proliferation (61–63). Stats also modulate cellular differentiation and apoptosis. Reconstitution of Stat1 in Stat1-null U3A cells (which do not respond to TNF-α) restores basal caspase expression and renders them sensitive to TNF-induced apoptosis (64). Conversely, Stat3 and Stat5 mediate the anti-apoptotic effects of IL-6 and IL-2, respectively (65, 66). Stat1 activates the caspase cascade through up-regulation of Fas and FasL expression in response to IFN-γ (67). The exact mechanisms underlying these diverse effects are being elucidated.

An important property of cellular signaling pathways is that their activation is both rapid and transient. This is because of effective mechanisms of inactivation. In the Jak-Stat system, proteasome-mediated degradation, tyrosine dephosphorylation, and inhibition by various inhibitory proteins are responsible for this process (4). The ubiquitin-proteasome pathway governs the degradation of many intracellular proteins including activated Stats, and effective inhibitors of this system have shown promising early results in clinical trials (68, 69). The cytokine-activated Jak-Stat pathways are also inhibited by tyrosine dephosphorylation mediated by cytoplasmic phosphatases such as SHP-1 (70, 71). SHP-1-deficient mice demonstrate multiple hematopoietic abnormalities, including hyperproliferation and abnormal activation of granulocytes and macrophages in the lungs and in the skin (72). SOCS and PIAS are other important inhibitors of the activated Jaks and Stats (70, 73). Recent studies have established that SOCS are negative regulators of cytokines, and there is ample evidence suggesting the importance of Stats in the induction of SOCS expression, thereby constituting a negative feedback mechanism (74–76). The role of these inhibitory proteins in the pathogenesis of neoplastic transformation is also becoming clearer (77).

**RTK and Serine/Threonine Signaling Pathways**

RTKs are membrane-bound enzymes with an extracellular ligand-binding domain, a transmembrane domain, and a highly conserved intracellular domain that mediates the activation, through tyrosine phosphorylation, of a number of downstream signaling proteins (78–80). These enzymes are activated by ligand binding, by cell-cell interactions via cell adhesion molecules, and by stimulation of G-protein coupled receptors (81). Phosphorylated tyrosine residues in specific domains of these receptors serve as high-affinity docking sites for SH2-containing adaptor and effector proteins (82). RTKs include diverse molecules, which are considered as members of several distinct classes: class I including epidermal growth factor receptor; class II including insulin-like growth factor-1 receptor; class III including PDGFR, macrophage colony-stimulating factor (FMS-R or CSF-1R), stem cell factor receptor (KIT), and FLT3R; and class IV including FGFR (79, 83, 84). The importance of these receptors in malignant transformation and the possibility of modulating them as therapeutic targets are subjects of intense research. The recent reports of constitutive activation of FLT3R resulting in stimulation of multiple signaling pathways and leading to malignant transformation has been of significant interest in leukemia research (85, 86). Such constitutive activation of this receptor has been reported in >30% of patients with AML and results from two well-described molecular events. Internal tandem duplication mutations of FLT3R gene occur at exons 11 and 12 of the gene that code for the juxtamembrane domain of receptor (87–90). More recently, point mutations of codon 835 of FLT3R receptor gene, located in the activation loop of its TK domain, have been reported in 7% of patients with AML (87, 91). Inhibitors of such aberrant activation are undergoing clinical evaluation (92, 93).

Many intracellular signaling proteins bind the phosphoryrosine on the activated RTKs. These proteins include GTPase activating protein, PI3K, Grb2, and Src-like tyrosine-kinases (1, 10, 78). The activation of these proteins by serine/threonine phosphorylation in turn activates a number of downstream signaling cascades that lead to gene transcription (10).

Although knowledge of the Jak-Stat pathway has been instrumental in understanding cytokine signaling (94), the importance of signaling cascades that involve the activation of serine/threonine kinases is increasingly apparent (10). The serine/threonine MAPKs, which include the Ras-Raf-MEK-ERK pathway, the p38 family of kinases, and the JNK (SAPK)
family, are activated by upstream signals and mediate effects on inflammation, cell growth, cell cycle progression, cell differentiation, and apoptosis (95). The Ras family of proteins belongs to the large superfamily of GTPases that localize to the inner surface of the plasma membrane (1, 96). Ras proteins play a pivotal role in a number of signaling pathways mediated by RTKs and other receptors. Ligand binding to these receptors initiates the autophosphorylation of specific tyrosine residues in their cytoplasmic domain and creates phosphoryrosyl-binding sites for adapter proteins such as Shc and Grb2, which in turn recruit guanine nucleotide exchange factors and thereby initiate Ras activation (97, 98).

Once induced, Ras activates Raf serine/threonine kinase, which then phosphorylates MAPK kinases (otherwise known as MEKs; Refs. 10, 97, 99). These in turn activate MAPKs (or ERKs; Refs. 100, 101), which in turn move to the nucleus where they phosphorylate and activate nuclear transcription factors such as Elk-1 (102). ERKs were initially described as a novel family of protein kinases that, when activated, produced proliferative stimuli (103). ERKs can also activate other kinases such as RSKs (also known as MAPK-activated protein kinases), which are involved in cell-cycle regulation and apoptosis (104). ERK-activated RSK kinase catalyzes the proapoptotic protein Bad and suppresses Bad-mediated apoptosis (105). Similarly, the Ras-Raf-MEK-ERK cascade modulates cellular proliferation by regulating the activity of several proteins, including cell-cycle regulators (e.g., cyclin D1, p21waf1/cip1, p27kip1, and cdc25A) and transcription factors (e.g., c-Myc; Ref. 106).

The G1/S cell cycle checkpoint is a critical point determining the commitment of cells to growth arrest or proliferation. During this stage cells are responsive to cytokines (107). Regulatory proteins p21waf1/cip1 and p27kip1 are of particular importance in this transition, which is controlled by both positive and negative regulators. Distinct Rb-E2F repressor complexes suppress the transcription of genes required for progression of various phases of cell cycle. For example, Rb-E2F1 complex suppresses the progression through G1 (108). During this progression from G1 to S-phase cyclin/CDKs are sequentially activated, which then inactivate suppressor complexes such as Rb-E2F1 (109). Cyclin/CDK activity results in Rb phosphorylation and its dissociation from E2F1 leading to activation of genes necessary for S phase (110). Activity of a number of these cyclin/CDKs as well their inhibitors such as p21waf1/cip1 is modulated by cytokine-mediated signals through their phosphorylation.

The p38 family of MAPKs is involved in various cellular processes such as inflammation, cell cycle progression, and cell death (111, 112). The four different p38 isoforms (α, β, γ, and δ) are activated by two MEK isoforms (113). Originally, the p38 kinase pathway was reported to have a critical role in the generation of signals in response to stress stimuli. However, its role in cytokine signaling and regulation of the Jak-Stat pathway has been elucidated recently (114). In particular, from the standpoint of leukemogenesis, it modulates the growth-inhibitory effect of type I IFNs in BCR-ABL-expressing cells as well as normal hematopoietic progenitors (115, 116).

The third group of MAPKs includes the JNK (otherwise known as SAPK; Ref. 95). The four different JNK kinases have a similar role to p38 kinases in cellular function and are activated by specific MAPK kinases (MEKs) in response to inflammatory cytokines such as TNF-α, and other stress stimuli such as reactive oxygen species, heat, and withdrawal of growth factors (117). The MEKK1/JNK signaling increases p53 stability and transcriptional activation, and MEKK1/JNK potentiates the ability of p53 to initiate apoptosis (118).

Normal functioning of MAPK-mediated signaling necessitates its efficient inactivation (95). A number of dual-specificity MAPK phosphatases serve to dephosphorylate and, hence, inactivate MAPKs (119–121). Similarly, protein phosphatases PP1 and PP2 dephosphorylate and inactivate a number of phosphoproteins including components of the MAPK pathway (122, 123).

Other signaling pathways such as those mediated by PI3K, AKT (also known as PKB), and protein kinase C are also controlled by serine/threonine phosphorylation (Fig. 2; Ref. 10). PI3K consists of two subunits, the p85 regulatory subunit and the p110 catalytic subunit (124, 125). The p85 subunit binds to the cytokine receptor as a consequence of ligand-receptor interaction and receptor autophosphorylation (126). As a result, phosphatidylinositol-dependent kinases and their downstream substrate AKT/PKB are recruited to the membrane (127). PI3K-AKT pathway activates several downstream targets including p70 RSK, forkhead transcription factors, and NFκB (128–130). The serine/threonine kinase AKT is an important component of the cell survival machinery (10, 130–133). Its activation via the PI3K pathway leads to a number of events (10, 131, 134, 135). For example, the phosphorylation of the cytosolic protein IκB by AKT releases NFκB from its association with IκB. NFκB then moves into the nucleus, where it induces a number of genes involved in cell survival (131). Meanwhile, the inhibitory protein IκB is degraded by the proteasome (136). AKT also phosphorylates the proapoptotic protein Bad, which leads to higher levels of free antiapoptotic Bcl-xL and thereby inhibits the cell-death protease caspase-9 (134). The tumor suppressor gene PTEN codes for a phosphatase that acts by removing a phosphate group from the 3 position of the inositol ring of the PIP3,4,5 phospholipids located at the cellular membrane. This prevents the proximation of AKT and phosphatidylinositol-dependent kinases, and prevents AKT activation (137–140). Several lines of evidence including studies of PTEN knockout mice support the role of PTEN as a tumor suppressor gene (141). Serine/threonine kinases, in general, also influence the activity of other antiapoptotic proteins of the Bcl-2 family (10, 135, 142). In the normal cell cycle, Bcl-2 is phosphorylated on its serine/threonine residues at several points during the G2 to M phase transition (10, 143).

PKC, another important signaling enzyme, phosphorylates specific serine or threonine residues on target proteins in different ways (Fig. 2). For example, PKC is a potent activator of Raf-1, which activates the MAPK cascade. This leads to phosphorylation of IκB, release of NFκB, translocation of NFκB into the nucleus, and gene transcription as described above (144–146). PKC also regulates cytokine signals through its effects on the Jak-Stat pathway in some myeloid progenitor cell lines (147). The significant role of PKC in phosphorylation and activation of Raf has led to its targeting for inhibition of the Raf-Ras-MEK-ERK pathway (95, 148). For example, stauro-
porine analogs UCN-O1 and CGP 41251 are being examined as inhibitors of PKC and MAPK signaling (149, 150). Various signaling pathways interact resulting in their modulation at several levels. For example, PI3K interacts with and enhances Raf-Ras-MEK-ERK pathway (151–153). Other serine/threonine phosphorylation pathways modulate cytokine signals through the Jak-Stat pathway (10, 154). In addition to being tyrosine phosphorylated, several Stats (Stat1α, Stat3, and Stat4) are serine phosphorylated by ERKs at conserved serine residues (155). In fact, during cytokine signaling, Jak and Raf kinases carry on an intricate cross-talk (10).

**Therapeutic Implications**

Signaling pathways may be particularly attractive targets in cancer therapy because they may be inappropriately activated in malignant cells. However, several factors must be carefully considered while developing agents that modulate these pathways. One is the possible toxicity of such therapy. Because these pathways are activated to a significantly greater degree in malignant cells than normal cells, their partial inhibition may be sufficient to interfere with malignant cell growth without causing significant toxicity (25). Therefore, despite the pivotal role of these pathways in normal cellular function, their inhibition may not be toxic to normal cells.

Another point of consideration in designing inhibitors is the specificity of the target pathway and the selectivity of its inhibitors. Adding to this challenge is the fact that these pathways are part of a complex network of interconnecting cascades resulting in a certain degree of redundancy and overlap.

Diseases induced by specific oncogenic alterations leading to constitutive activation of pivotal molecules in the pathways may provide us with such specificity and selectivity. A number of translocations occurring in hematological malignancies are known to result in fusion genes with enhanced kinase activity (24). The oncoprotein Bcr-Abl results from a translocation between chromosomes 9 and 22, and occurs in CML and ALL. Its transforming activity is the product of activation of a number of signaling molecules such as Ras, Raf, PI3K, JNK/SAPK, Crkl, and Stat5 (156–160). As a result of their activation, a number of pathways, which inhibit apoptosis and promote cell survival, are induced (24). Bcr-Abl is constitutively phosphorylated on a number of tyrosine residues, allowing the docking of adaptor proteins such as Cbl, Crkl, Shc, and Grb2, which recruit the Ras signaling pathway, the p85 regulatory subunit of PI3K, the focal adhesion proteins paxillin and talin, and a number of other signaling pathways (159, 161–163). The specific Bcr-Abl inhibitor Imatinib mesylate has proven to be very effective in suppressing these pathways in vitro and in patients with CML and ALL (164–170).

Although the platelet-derived growth factors, PDGFRα and PDGFRβ have significant homology, only the PDGFRβ has been implicated in hematological cancers where a significant number of patients with chronic myelomonocytic leukemia have t(5;12)(q33;p13) generating the fusion protein TEL-PDGFRβ (171, 172). As a result of ligand-independent dimerization and autophosphorylation of the PDGF β-subunit, the TK is constitutively active. PDGF is able to stimulate the growth of primitive hematopoietic, erythroid, and megakaryocytic precursors, and TEL-PDGFRβ can confer cytokine-independent growth to Ba/F3 cells (173). Imatinib mesylate inhibits the kinase PDGFRβ and has been shown anecdotally to be effective in patients with this translocation (174).

Similarly, the chimeric gene NPM-ALK is produced as a result of translocation t(2;5)(p23;q35; Refs. 175, 176). Fusion of NH₂-terminal domain of NPM to the cytoplasmic region of the ALK TK receptor results in constitutive activation of its catalytic domain (177, 178). NPM-ALK associates with a number of adaptor proteins such as Grb2 and Crkl, and results in the activation of the downstream signaling proteins such as PI3K, AKT, and Stat5 (179–182). Therefore, design of specific inhibi-
Cellular Signaling Pathways as Therapeutic Targets

The TK modulated pathways such as the Jak-stat cascade can be targeted at several steps along the way (Fig. 3). Because a number of cytokines and growth hormones play an important role in the suppression of apoptosis in the malignant clone in hematological cancers (e.g., IL-6 in myeloma; IL-2 in some T-cell lymphomas; and IL-1, IL-3, and GM-CSF in AML; Refs. 25, 184), inhibition of the cytokines and their receptors is a plausible therapeutic strategy (185, 186). Furthermore, where there is constitutive activation of the receptor leading to neoplastic change, selective inhibition of kinase activity of the receptor is likely to be of benefit. For example, inhibition of FLT3-R TK activity is selectively cytotoxic to AML blasts harboring the appropriate activating mutations (92, 187). Activating mutations of FLT3-R including the internal tandem duplication mutation and mutation of the TK domain occur in >30% of patients with AML, confer adverse prognosis, and can be targeted by selective inhibitors (92, 188, 189). Clinical trials examining the efficacy and safety of such inhibitors are currently under way. Intracellular activators of STATS such as Jaks and Src are also likely targets (164–166). For example, the Jak2 inhibitor AG490 inhibited the growth of ALL cells in a mouse model without affecting normal hematopoiesis (190). AG490 also acts by inhibiting Stat function, and induces apoptosis in Sezary cells and myeloma cells (191, 192). Furthermore, numerous other cellular TKs have been identified that likely have roles in neoplastic cell survival and proliferation, and a number of TK inhibitors are already being explored for their effects on signaling cascades. In general, two classes of TK inhibitors are being developed: inhibitors of the ATP binding site and inhibitors of the substrate binding sites (164, 193, 194).

There are other ways to interrupt Stat signaling. One is the specific inhibition of the Stat SH2 domain, because it is essential for the recruitment of Stats to the receptor subunit and for Stat dimerization (25). Antisense oligonucleotides directed at Stats (195), modulation of the activity of natural Stat inhibitors such as SOCS and PIAS, and inhibition of the binding of activated Stat dimers to their DNA targets (25, 196, 197) are other mechanisms currently under investigation.

A better understanding of the effects of current antineoplastic agents on these pathways may also elucidate their mechanism of action and lead to the development of more effective and less toxic agents. For example, fludarabine causes a profound and specific loss of Stat1 (198), which may be at least partly responsible for its antineoplastic properties as well as its immunosuppressive properties, which is similar to Stat1 knockout mice.

Multiprotein complexes, which can modify the structure of chromatin and influence gene regulation, are often aberrant in hematological malignancies. Although their interactions with various signaling pathways have not been clearly defined, they merit specific mention as they are subject of intense research. Such complexes include proteins such as HDAC, histone acetyltransferase, and DNA methyltransferases. In a significant proportion of patients with AML-M2, the translocation t(8;21)(q22;q22) results in the fusion of the DNA-binding domain of AML1 with ETO, which interacts with corepressor complexes such HDAC and repress cellular differentiation (199, 200). Similarly, t(12;21)(p13; q22) in childhood ALL results in recruitment of the repressor N-CoR by the fusion protein TEL-AML1 (201, 202). Inhibitors of HDACs and DNA methyltransferase such as butyrate, trichostatin A, 5-azacytidine, and 2′-deoxy-azacytidine are currently under investigation in clinical trials (203, 204).

The most common translocation in APL, t(15;17)(q22; q11.2), results in the fusion of RARα with the PML genes, with the resulting fusion protein retaining the functional domains of both (205, 206). The fusion protein recruits nuclear receptor corepressors SMRT and N-CoR, which together with HDACs keep the chromatin in the closed configuration and suppress/inactivate retinoid receptor target genes in myeloid development (207, 208). In patients with PML-RARα, pharmacological doses of ATRA can convert the fusion protein from a dominant-negative inhibitor of retinoid-regulated transcription to an activator (209). Signaling pathways that interact with retinoid-mediated transcription may be modulated to potentiate ATRA-induced differentiation (210). ATRA may also exert effects on upstream signaling pathways. For example, ATRA up-regulates Stat1 and Stat2 activity in the IFN-α signaling pathway, thereby potentiating the growth-inhibitory effects of IFN-α (211–213). Another novel therapeutic agent in APL, arsenic trioxide, affects a number of cellular signaling pathways, leading to induction of apoptosis and differentiation (214–217). Therapeutic effects of arsenic trioxide in APL are partly related to its degradation of PML-RARα fusion protein through targeting PML to proteasomal degradation by Sumolation (218). However, arsenic trioxide has a number of other effects such as activation of JNK kinases; translocation of PKC from cytosol to plasma membrane to mediate downstream signaling; enhancement of CDKIs, p21<sup>Waf1/Cip1</sup>, and p27<sup>Kip1</sup>; and inhibition of IκB kinase and NFκB (214).

Monoclonal antibodies such as rituximab (anti-CD20) and alemtuzumab (anti-CD52) are increasingly used in treating hematological malignancies (219–221). Their exact mechanism of
B cells (222). Rituximab has also been shown to initiate signal transduction events that induce an elevation of CD20 by rituximab in the presence of FcR-expressing cells. These may function by activating the caspase cascade through blockade of the IAPs, inhibition of the NFκB pathway (using IκB kinase inhibitors or proteasome inhibitors), and inhibition of the PI3K pathway (using kinase inhibitors; Refs. 69, 134, 248). IAPs including the XIAP can block the process of programmed cell death through their interaction with members of the caspase family of cysteine proteases (249). The human family of caspases includes 11 members involved in processing of inflammatory cytokines as well as execution of apoptosis (250). Dysregulation of caspase cascade has been implicated in human neoplasia, and IAPs, the natural inhibitors of caspases, are pivotal in this regulatory process (250, 251). XIAP, the most potent member of the IAP family blocks both the initiator caspase-9, and the effector caspases-3 and -7 (252). An association between XIAP levels and survival has been reported in AML (253). Down-regulation of XIAP protein by adenoviral antisense vector results in sensitization to radiation-induced cell death (254).

The ubiquitin-proteasome pathway is the central pathway for degradation of intracellular proteins (255, 256). Targeting of proteases to proteasome is through covalent attachment of a poly-ubiquitin chain. This system is specific allowing up-regulation of degradation of certain proteins without affecting the proteolysis of other substrates; this enables the proteasome to function as a regulator of metabolic pathways (256). Therefore, different classes of proteasome inhibitors can be used to differentially affect cellular levels of oncogenic proteins (256). Important substrates for proteasome degradation include cyclins and CKDs; transcription factors such as p53, NFκB, c-Myc, c-fos, and c-Jun; a number of apoptosis family of proteins; IAPs; and some caspases (256–262). PS-341 is a specific and potent inhibitor of proteasome (263). It induces apoptosis and overcomes resistance in a number of cell lines as well as primary cells from patients with chronic lymphocytic leukemia (264–268). Its in vitro activity in myeloma has led to its ongoing examination in clinical trials (269–271).

Cell-cycle proteins such as the CDKs and their inhibitors (CDKIs), which modulate cell-cycle progression in response to appropriate signals, are also possible targets. Orderly cell division requires a tight control of cyclins and CDKs, particularly at the G1/S checkpoint (272). Complexes of Rb family of tumor suppressors with E2F transcription factors inhibit the transcription of several genes involved in the S phase of cell cycle both by active repression as well as through the recruitment of HDACs (273, 274). Phosphorylation of Rb by cyclin/CDK complexes leads to release of E2Fs and transactivation of target genes by binding of E2Fs to E2F response elements (275). The...
activity of cyclin D:CDK4/6 and cyclin E:CDK2 complexes is regulated by CDKIs of the INK4 and KIP families such as p16ink4a, p15ink4b, p18ink4c, p19ink4d, p21waf1/cip1, p27kip1, and p57kip2, which act as inhibitors of G1/S transition. Disrupted activity of a number of G1 checkpoint genes/proteins has been implicated in carcinogenesis (275). Decreased Rb expression, and a negative correlation between Rb expression and survival have been reported in ALL (276). Deletions and mutations of Rb have been reported in a number of hematological malignancies (276). Genes for p16ink4a and p15ink4b are suppressed in various hematological cancers mainly by deletion and by hypermethylation of their promoters (277, 278). The role of inactivation of p16ink4a, p15ink4b, p18ink4c, p19ink4d, p21waf1/cip1, p27kip1, and p57kip2, which act as inhibitors of G1/S transition. Disrupted activity of a number of G1 checkpoint genes/proteins has been implicated in carcinogenesis (275). Decreased Rb expression, and a negative correlation between Rb expression and survival have been reported in ALL (276). Deletions and mutations of Rb have been reported in a number of hematological malignancies (276). Genes for p16ink4a and p15ink4b are suppressed in various hematological cancers mainly by deletion and by hypermethylation of their promoters (277, 278). The role of inactivation of p16ink4a, p15ink4b, p18ink4c, p19ink4d, p21waf1/cip1, p27kip1, and p57kip2, which act as inhibitors of G1/S transition. Disrupted activity of a number of G1 checkpoint genes/proteins has been implicated in carcinogenesis (275).

Concluding Remarks

Over the past decade extensive research has elucidated the role of cellular signaling pathways in cellular growth, differentiation, and apoptosis. The disruption of these pathways, on the other hand, has been shown to promote the neoplastic transformation of cells. This knowledge has led to the development of a number of molecules that in preclinical and clinical studies have been shown to be capable of reversing the neoplastic process. Our current challenge is to further understand the complexity of these processes and devise specific agents that target the neoplastic cells whereas sparing normal tissues. This would truly realize the concept of targeted therapy in treating hematological malignancies and solid tumors alike.

References

10. Purvalanol Cdc2 inhibition
11. Rapamycin P70S6K inhibition
12. Suramin, SU5416 VEGFR and bFGFR
13. Wortmannin, LY294002 PI3K inhibition
14. Geldanamycin, radicicol Ras FT inhibition
15. AG490 Imatinib mesylate

Table 1 Targets for drug development

<table>
<thead>
<tr>
<th>Target</th>
<th>Prototype drug</th>
<th>Mode of action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tyrosine and serine/threonine</td>
<td>Rituxan, Campath-1H</td>
<td>Ligand-binding inhibition</td>
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<tr>
<td>kinases</td>
<td>Genistein</td>
<td>FLT3R kinase inhibition</td>
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<tr>
<td></td>
<td>Limonene, perillyl alcohol, R115777, SCH</td>
<td>Non-specific kinase</td>
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<tr>
<td></td>
<td>66336, L778123, BMS214662</td>
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<tr>
<td></td>
<td>Geldanamycin, radicicol</td>
<td>Ras FT inhibitiona</td>
</tr>
<tr>
<td></td>
<td>Wortmannin, LY294002</td>
<td></td>
</tr>
<tr>
<td>Cell-cycle proteins</td>
<td>Rapamycin</td>
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</tr>
<tr>
<td></td>
<td>Flavopiridol, Olomoucine, UCN-01</td>
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<tr>
<td></td>
<td>Purvalanol</td>
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<tr>
<td>Apoptosis-related proteins</td>
<td>Staurosporine, UCN-O1, CGP41251, PKC412</td>
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<td>BAYstatin</td>
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<td>PS341</td>
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<tr>
<td>Angiogenesis-related proteins</td>
<td>Suramin, SU5416</td>
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<td>Cell life span targets</td>
<td>Anthraquinones</td>
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<tr>
<td>Transcription factors (Myc,</td>
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<tr>
<td>Ets, Fos, Jun, Rel, Myb)</td>
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</table>

[a] Ras FT, Ras farnesyl transferase; MDM2, mouse double minute-2; PKC, protein kinase C; VEGFR, vascular endothelial growth factor receptor; bFGFR, basic fibroblast growth factor receptor.


546 Cellular Signaling Pathways as Therapeutic Targets


Modulation of Cellular Signaling Pathways: Prospects for Targeted Therapy in Hematological Malignancies

Farhad Ravandi, Moshe Talpaz and Zeev Estrov