

ABL Oncogenes and Phosphoinositide 3-Kinase: Mechanism of Activation and Downstream Effectors

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Abstract

The *BCR-ABL* oncogene is responsible for most cases of chronic myelogenous leukemia and some acute lymphoblastic leukemias. The fusion protein encoded by *BCR-ABL* possesses an aberrantly regulated tyrosine kinase activity. Imatinib mesylate (Gleevec, STI-571) is an inhibitor of ABL tyrosine kinase activity that has been remarkably effective in slowing disease progression in patients with chronic phase chronic myelogenous leukemia, but the emergence of imatinib resistance underscores the need for additional therapies. Targeting signaling pathways activated by BCR-ABL is a promising approach for drug development. The study of signaling components downstream of BCR-ABL and the related murine oncogene *v-Abl* has revealed a complex web of signals that promote cell division and survival. Of these, activation of phosphoinositide 3-kinase (PI3K) has emerged as one of the essential signaling mechanisms in ABL leukemogenesis. This review describes molecular mechanisms by which PI3K is activated and the downstream PI3K effectors that propagate the signal to promote myeloid and lymphoid transformation. Of particular recent interest is the mammalian target of rapamycin, a PI3K-regulated kinase that regulates protein synthesis and contributes to leukemogenesis. (Cancer Res 2005; 65(6): 2047-53)

Introduction

The *BCR-ABL* oncogene results from a translocation (9;22) that fuses sequences from the *BCR* gene with the *ABL* gene. This translocation, which creates the Philadelphia chromosome, is found in most human patients with chronic myelogenous leukemia (CML) and a fraction of those with acute lymphoblastic leukemia (1-3). A virally encoded form of ABL (*v-Abl*) causes pre-B-cell leukemias in mice (4). Cellular ABL, a tightly regulated tyrosine kinase, regulates important cellular processes, including actin reorganization, differentiation, and apoptosis following DNA damage, and is an important component in T-cell receptor signaling (5-8). In contrast, BCR-ABL and *v-Abl* are aberrantly localized in cells and their kinase activity is increased. Imatinib mesylate (Gleevec, STI-571) is a selective tyrosine kinase inhibitor that has been proven to be a powerful treatment for leukemias caused by *BCR-ABL*. Nevertheless, many patients exhibit primary or acquired resistance to imatinib and the long-term efficacy of treatment in sensitive patients has still to be determined (9). Further study of signaling pathways that

contribute to the transformed phenotype of cells transformed by *ABL* oncogenes will therefore be valuable in identifying new targets for therapeutic intervention.

Extensive research has uncovered some of the complex signaling cascades that result from aberrant activation of the ABL tyrosine kinase. Major downstream targets include Ras, signal transducers and activators of transcription, c-Jun NH₂-terminal kinase, and phosphoinositide 3-kinase (PI3K; refs. 10-12). The PI3K signaling pathway is deregulated in a large fraction of human cancers and is considered an attractive target for the development of novel chemotherapeutic agents (13). It has been known for some time that the PI3K pathway contributes to transformation by BCR-ABL. Moreover, it was reported recently that PI3K inhibitors synergize with imatinib by greatly increasing apoptosis of CML chronic phase and blast crisis patient cells (14). However, the mechanism of activation of PI3K and the role of specific downstream effectors has only been analyzed recently in detail. The most commonly used PI3K inhibitors have pleiotropic effects and are likely to be too toxic for clinical use (15). Therefore, identifying the PI3K isoforms and downstream targets critical for BCR-ABL⁺ leukemias could lead to the development of more useful inhibitors. In this review, we highlight recent advances in the understanding of the mechanisms by which *ABL* oncogenes activate PI3K, the specific PI3K isoforms involved, and the downstream effectors that are critical for mitogenic and antiapoptotic function of this pathway.

Phosphoinositide 3-Kinase Structure and Function

PI3K is a lipid kinase that phosphorylates phosphatidylinositol or its derivatives on the 3-hydroxyl of the inositol head group. The PI3K signaling pathway plays an integral role in many normal cellular processes, including survival, proliferation, differentiation, metabolism, and motility, in a variety of cell types. PI3K enzymes in cells are classified according to structure, substrate selectivity, and mechanism of regulation (16). The class that is activated downstream of tyrosine kinases, denoted class I_A PI3K, are heterodimers consisting of catalytic and regulatory subunits (Fig. 1A). There are three genes that code for the 110-kDa catalytic subunits (p110 α , p110 β , and p110 δ) and three genes that code for regulatory subunits (p85 α , p85 β , and p55 γ). Additional forms of the regulatory subunit (p50 α and p55 α) are produced by alternative transcripts from the *Pik3r1* gene encoding p85 α . Modular domains within both subunits mediate the association of the heterodimer with membrane-associated signaling complexes that form following tyrosine kinase activation. Activated class I_A PI3K converts phosphatidylinositol-4,5-bisphosphate to phosphatidylinositol-3,4,5-trisphosphate (PIP₃; Fig. 1B), an important second messenger (17). PIP₃ and other PI3K lipid products on the inner leaflet

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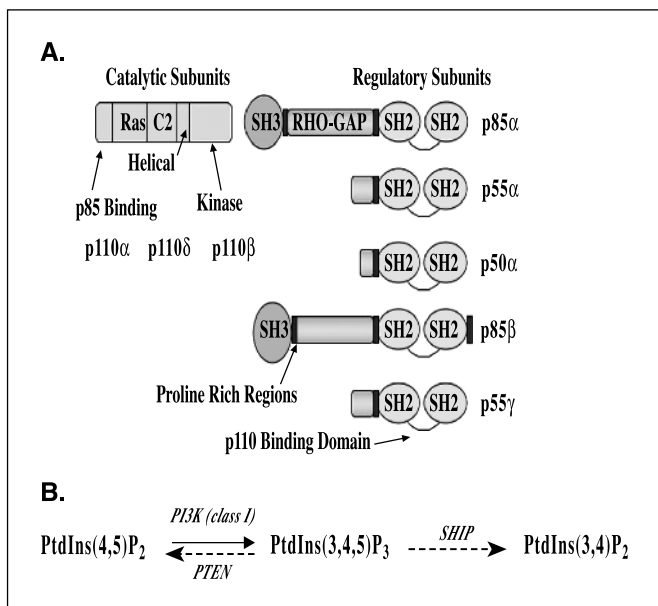


Figure 1. A, schematic diagram of domain structure of class I_A PI3K catalytic and regulatory subunit isoforms. B, diagram of lipid synthesis and degradation pathways.

of the membrane create docking sites for proteins that contain pleckstrin homology domains. An example relevant to this review is the recruitment of two pleckstrin homology domain-containing serine/threonine kinases, phosphoinositide-dependent kinase-1 (PDK-1) and Akt (also known as protein kinase B). Colocalization and PIP₃ binding allows PDK-1 to phosphorylate and activate Akt (18). PI3K signaling is opposed by phosphatase and tensin homologue deleted on chromosome 10 (PTEN; Fig. 1B), which removes the 3-phosphate from PIP₃ (17). The phosphatase SH2-containing inositol-5-phosphatase (SHIP) removes the 5-phosphate to produce phosphatidylinositol-3,4-bisphosphate (ref. 17; Fig. 1B), which may have different signaling functions.

Mechanisms of Phosphoinositide 3-Kinase Activation by ABL Oncogenes

It has been known for years that PIP₃ accumulates in cells transformed by ABL oncogenes (19, 20). The finding that global PI3K inhibitors (wortmannin and LY294002) block both myeloid and lymphoid transformation supports the functional relevance of the PI3K signal (21, 22). However, a continuing controversy in ABL signaling has been the mechanism of PI3K activation. PI3K proteins and enzyme activity can be detected in ABL immunoprecipitates, which led to the initial assumption that PI3K activation occurred mainly from a direct association of ABL with class I_A PI3K. Within the ABL sequence is a YXXM motif that when phosphorylated corresponds to the optimal binding motif for the SH2 domains of class I_A regulatory subunits (20). However, mutation of this motif does not abrogate PI3K activation, leading to the conclusion that PI3K activation is mediated primarily by associations with other tyrosine-phosphorylated proteins that are recruited to ABL (20).

Understanding the complex interplay of signaling proteins downstream of ABL oncogenes has been complicated by effects of cellular context, which differ among primary myeloid or lymphoid transformants, ABL-transformed cell lines, and the different BCR-ABL isoforms and v-Abl. The development of faithful and reproducible mouse models of BCR-ABL-induced leukemia has allowed hypotheses to be tested rigorously using mouse genetics, in some cases contradicting conclusions from cell lines (23). These mouse leukemia models have begun to define critical signaling components for PI3K pathway activation in myeloid versus lymphoid cells and downstream of different forms of ABL. For example, the SH2 domain within p210-BCR-ABL was shown to be dispensable for PI3K activation (24).

One of the most critical pathways for PI3K activation in BCR-ABL-expressing cells is mediated by Y177 in the BCR portion and the adapter proteins Grb2 and Gab2 (ref. 25; Fig. 2A). Y177 is an autophosphorylation site for BCR-ABL and can also be phosphorylated by Hck, a Src family kinase (26). Grb2 binds through its SH2 domain to pY177 and creates a scaffold for Gab2 to bind via its SH3 domain to Grb2 (25). BCR-ABL or an associated kinase

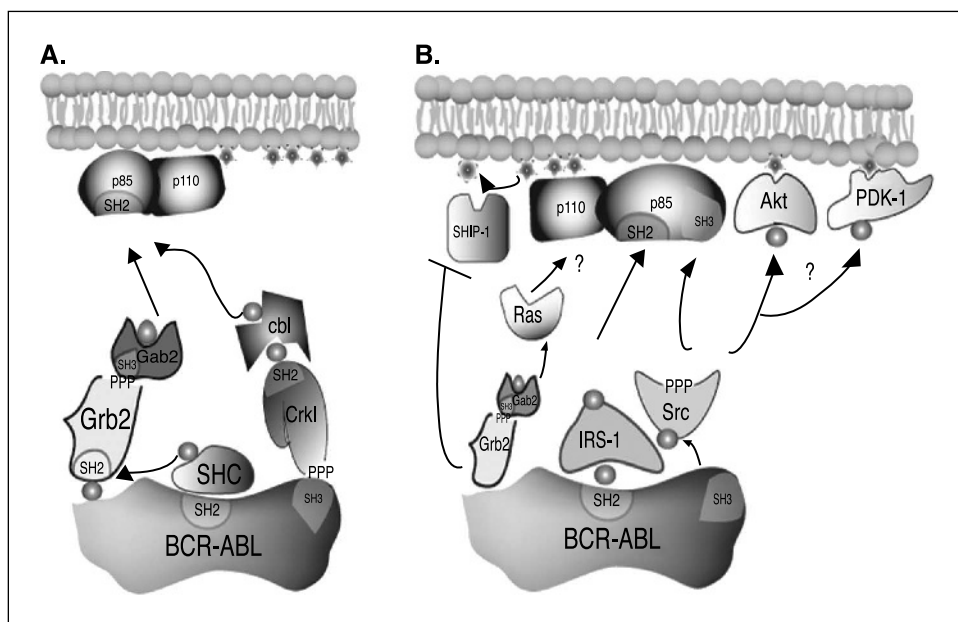


Figure 2. Potential mechanisms of PI3K activation by BCR-ABL. A, the best-characterized pathways include a Grb2-Gab2 complex and a Crkl-c-Cbl complex. Grb2-Gab2 can be recruited directly to BCR-ABL after phosphorylation of Y177 or indirectly via Shc. B, other possible mechanisms include Src family kinases, IRS-1 and Ras. Down-regulation of SHIP1 phosphatase also contributes to increased PI3K signaling. In this figure and in Fig. 3, the lipid products of PI3K are shown as star shapes in the inner leaflet of the membrane and phosphorylations are depicted by ovals.

phosphorylates Gab2 on tyrosines within its YXXM motifs, which then act as docking sites for the SH2 domains of class I_A regulatory subunits. The importance of Y177 phosphorylation depends on cell context. Initial studies using BCR-ABL variants in which Y177 was mutated to phenylalanine (Y177F) showed either normal or decreased transformation and downstream signaling depending on the cellular system (21, 25, 27). Moreover, in mouse leukemia models, the Y177F mutation suppresses the development of CML-like myeloproliferative disease, whereas B lymphoid transformation is only delayed and some T-cell leukemias (T-cell acute lymphoblastic leukemia) emerge (28–30). Similarly, loss of Gab2 abolishes myeloid transformation by wild-type BCR-ABL (p210) but only partially attenuates lymphoid transformation (25). Loss of Gab2 prevents myeloid leukemogenesis *in vivo* (25), but the effect on lymphoid leukemogenesis has not been reported. Notably, Gab2^{-/-} lymphoid transformants exhibit decreased survival and migration *in vitro*, two responses that require PI3K in many cell types.

Biochemical experiments in BaF3, a murine lymphoid cell line, expressing BCR-ABL-Y177F and in Gab2^{-/-} myeloid cells and lymphoblasts have established that the Y177-Gab2 axis mediates a substantial fraction of PI3K activation (25). However, in addition to association of the Grb2-Gab2 complex with pY177, this complex might also be recruited to the ABL portion via a third adapter protein, Shc (refs. 31, 32; Fig. 2A). Because v-Abl lacks BCR sequences, this might be one route by which v-Abl recruits Gab2 and promotes PI3K activation. However, v-Abl transformation is not affected by the absence of Gab2.¹ Residual Akt activation was observed in Gab2^{-/-} cells expressing BCR-ABL, and addition of PI3K inhibitors further enhanced apoptosis and cell cycle arrest (25). The related Gab1 and Gab3 proteins may be expressed and compensated to some degree. However, Gab-independent routes to PI3K activation might also exist downstream of BCR-ABL. These pathways might play a more significant role in lymphoid cell transformation based on the partial retention of B-cell leukemia in the absence of Gab2 and the selective B lymphoid transformation by v-Abl, which is Gab2 independent.

One possible Gab2-independent mechanism of PI3K activation involves the adapter proteins Crkl and c-Cbl (Fig. 2A). Each of these is highly tyrosine phosphorylated in manner dependent on ABL kinase activity (33, 34). The SH3 domain of Crkl mediates its association with ABL, and subsequent Crkl phosphorylation provides a SH2 docking site for c-Cbl (34). Phosphorylation of c-Cbl at a YXXM motif generates a docking site for class I_A PI3K regulatory subunits, and PI3K protein and enzyme activity can be detected in c-Cbl immunoprecipitates from BCR-ABL⁺ cells (34). The c-Cbl association with BCR-ABL depends in part on pY177 and Grb2, suggesting that reduced PI3K activation in Y177F mutants is not solely the result of impaired Gab2 recruitment (35). Crkl-overexpressing transgenic mice show enhanced sensitivity to p190-BCR-ABL-induced lymphoid leukemia (36). However, other genetic experiments have cast doubt on the importance of the Crkl/c-Cbl complex for cell transformation. Crkl deletion does not impair lymphoid transformation by p190-BCR-ABL (37), and bone marrow cells lacking c-Cbl can mediate efficient CML-like disease following transduction of either p210 or p190 forms of BCR-ABL (38). Akt activation seems

to be unaltered in B lymphoid transformants lacking c-Cbl (38). It seems likely that residual PI3K signaling via Gab2 and other mechanisms compensates for the absence of the Crkl/c-Cbl complex.

Alternative signaling components that might contribute to PI3K activation downstream of ABL oncogenes include Src, Ras, and insulin receptor substrate-1 (IRS-1; Fig. 2B). The role of the Src family of non-receptor tyrosine kinases in ABL signaling has received increasing attention. Several dual Src and ABL inhibitors have been designed and one was shown to overcome the resistance from imatinib in cells that express BCR-ABL mutants (39). However, cells carrying one of the BCR-ABL mutants remained resistant to the dual kinase inhibitor, indicating that ABL remains the primary target that must be blocked to prevent disease (39). On the other hand, a distinct Src inhibitor (CGP76030) was shown to suppress Akt activation in ABL-transformed cells without inhibiting ABL tyrosine kinase activity (40, 41). Cells from mice lacking three Src family kinases (Lyn/Hck/Fgr) are selectively resistant to lymphoid transformation by BCR-ABL, with myeloid leukemia unaffected (42). This suggests that Src family kinases might act selectively in lymphoid cells as an additional factor for activating PI3K signaling independently of Grb2-Gab2. Data from non-ABL-dependent cell systems suggest that Src kinases can promote PI3K pathway activation by (a) PI3K recruitment to the membrane, as the SH3 domains of Src kinases bind to proline-rich motifs within p85 α and p85 β , and (b) phosphorylating tyrosines on adapter proteins, some of which could be distinct from those targeted by ABL kinase activity. Finally, Src activation might influence Akt activation independent of PIP₃ production by directly phosphorylating Akt as well as PDK-1 (43, 44).

IRS-1 and related family members possess several YXXM motifs and are major mediators of PI3K activation downstream of receptors for insulin and related growth factors. IRS-1 also participates in cytokine receptor signaling and is phosphorylated in cells expressing BCR-ABL (45). PI3K activity associates with IRS-1 in the Ph⁺ K562 cell line in an imatinib-sensitive manner (45). The role of IRS-1 in transformation of primary myeloid or lymphoid cells has not been tested.

Activation of Ras G proteins is required for transformation by ABL oncogenes. GTP-bound Ras activates several signaling pathways, most notably the Raf-Mek-Erk kinase cascade. Ras-GTP also can contribute to PI3K activation by binding a site within class I catalytic subunits (refs. 46, 47; Fig. 1A). It is worth noting that Ras activation is also partially dependent on the Grb2-Gab2 pathway, as Erk activation is impaired in Gab2-deficient transformants (25). However, whether Ras activation contributes to PI3K signaling in ABL-transformed cells is not known.

A working model (Fig. 2) is that ABL oncogenes promote the assembly of multiprotein complexes that provide partially redundant inputs to activation of PI3K and other critical downstream signaling molecules. To date, the Gab2 adapter protein is the only component shown genetically to be essential for optimal PI3K activation in primary (murine bone marrow-derived) transformants. It should also be noted that ABL oncogenes can promote PI3K pathway activity not only by increasing the synthesis of PIP₃ but also by opposing its degradation. The main evidence for this is the finding that BCR-ABL expression in BaF3 cells suppresses the expression of SHIP1 phosphatase, an enzyme that converts PIP₃ to phosphatidylinositol-3,4-bisphosphate (ref. 48; Fig. 2B). Interestingly, mice lacking SHIP1 develop a lethal CML-like myeloproliferative disease (49). One report suggested that SHIP1 expression

¹ B. Neel, personal communication.

and function differs during distinct stages of disease, acting to promote expansion of early CML cells but suppressing progression in late-stage CML (50).

It is not yet clear which PI3K isoforms are essential for ABL transformation in different cell contexts. Hematopoietic cells express all three class I_A PI3K catalytic isoforms as well as the class I_B isoform (p110 γ) that is activated by G protein-coupled receptors (51). Gene-targeted mice lacking the individual PI3K catalytic isoforms have been developed but thus far have not been used in studies of ABL transformation. Information from such studies will be timely, considering that various pharmaceutical companies are developing PI3K inhibitors that are isoform specific (15). Mouse models have revealed some redundancy in the function of class I_A regulatory isoforms in ABL transformation. The *Pik3r1* gene, encoding the predominant isoforms p85 α and its smaller variants p55 α and p50 α , is dispensable for B lymphoid transformation by *v-Abl* but contributes to transformation efficiency by the p190 form of *BCR-ABL* (22). Pre-B cells transformed by *ABL* oncogenes also express p85 β , but this isoform is also dispensable (22). There seems to be less redundancy in human CML cells, in which specific down-regulation of p85 α by antisense oligonucleotides strongly suppresses Ph⁺ colony formation (52).

Akt, a Major Downstream Effector of Phosphoinositide 3-Kinase

Proteins recruited to the membrane by PI3K lipid products are often termed PI3K effectors. A large number of PI3K effectors have been identified, and most seem to be differentially activated depending on the cell type and receptor or oncogene that initiates the signal. The PI3K effector most closely associated with cell transformation is Akt. Activated Akt has many substrates that regulate cell cycle, growth, metabolism, and survival (see below; refs. 17, 53, 54). Like other members of the AGC kinase superfamily (named initially for cyclic AMP-dependent and cyclic guanosine 3', 5'-monophosphate-dependent protein kinases and protein kinase C), Akt activation requires phosphorylation of two sites in the kinase domain, one in the "T-loop" and the other in a hydrophobic motif near the COOH terminus. In Akt, binding of the pleckstrin homology domain to PIP₃ changes the conformation of the protein to allow its phosphorylation of the T-loop by PDK-1 (Fig. 3) and phosphorylation of the hydrophobic motif by either autophosphorylation or a second kinase (termed PDK-2) whose identity remains controversial (55, 56).

Expression of dominant-negative (kinase-inactive) Akt greatly diminishes BCR-ABL-dependent myeloid colony formation and leukemogenesis (21). However, kinase-inactive Akt may sequester PIP₃ and block multiple PI3K effectors. In addition, evidence is emerging that Akt activation downstream of ABL oncogenes varies depending on cell context (22, 57). Despite these caveats, an important function for Akt is supported by the finding that constitutively active Akt can complement defects in cells expressing a transformation-deficient variant of BCR-ABL (21). Studies of a murine cell line model of *v-Abl*-dependent survival have also suggested a role for Akt in this system (58).

Some potentially important Akt substrates are described in the subsections that follow and illustrated in Fig. 3.

Forkhead Box, Subgroup O. Members of the forkhead box, subgroup O (FOXO) family of transcription factors have diverse roles in the regulation of cellular metabolism, stress resistance, proliferation, and survival (59). The four members of this family

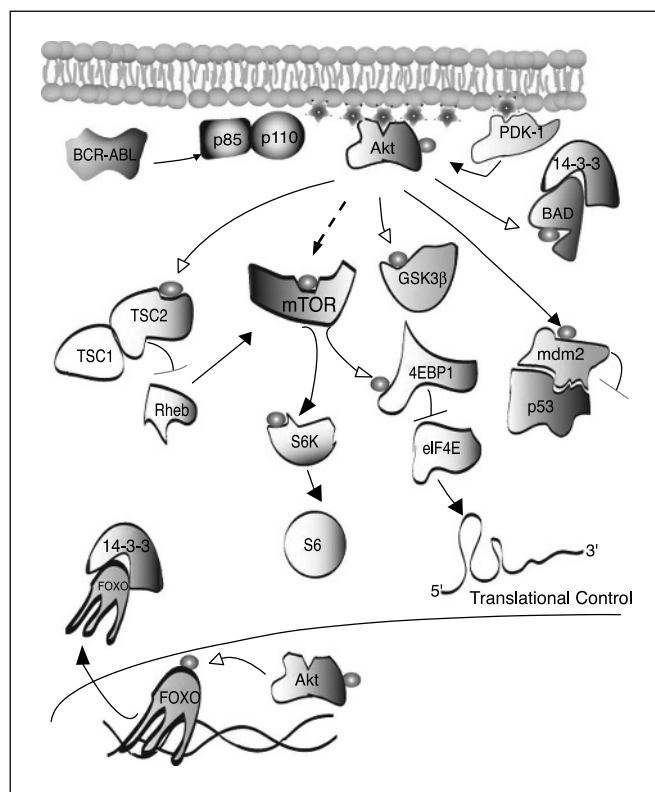


Figure 3. Akt phosphorylates many substrates in ABL-transformed cells to promote cell growth, proliferation, and survival. *Closed arrowheads*, interactions/phosphorylations that increase the function of the target protein; *open arrowheads*, phosphorylation events that decrease the function of the target; *T-shaped lines*, other inhibitory events; *dashed line*, the role of direct mTOR phosphorylation by Akt is uncertain.

(FoxO1, FoxO3a, FoxO4, and FoxO6) possess Akt phosphorylation sites that are conserved through evolution. Phosphorylation of these sites by Akt following PI3K activation directs the cytoplasmic sequestration and/or degradation of FOXO target genes (60, 61). In hematopoietic cells, forced expression of FOXO mutants that cannot be inactivated by Akt causes cell cycle arrest and apoptosis mediated in part by up-regulation of the FOXO target genes *p27^{KIP}*, *Bim*, and *TRAIL* (60, 61). FOXO proteins are phosphorylated in a manner dependent on both ABL and PI3K in human CML cell lines, murine pre-B cells transformed with BCR-ABL or *v-Abl*, or murine BaF3 cells expressing BCR-ABL (22, 62, 63). Expression of Akt-independent variants of FOXO proteins causes apoptosis in the murine cell systems but only cell cycle arrest in the human blast crisis CML cell line KCL22. Indeed, a dominant-negative FOXO variant actually enhances the efficacy of imatinib in killing KCL22 cells, suggesting that activation of FOXO in these cells promotes survival along with cycle arrest (63). Despite these differences among cell systems, the data in aggregate provide strong evidence that PI3K-dependent FOXO inactivation contributes to leukemogenesis by ABL oncogenes.

BAD. BAD is a Bcl-2 family member that confers survival signals by inhibiting the prosurvival family members Bcl-2 and Bcl-x_L. In some cell types, Akt promotes survival by phosphorylation and inactivation of BAD (17, 64). However, there are other kinases that can phosphorylate BAD (54). Furthermore, in cell lines expressing BCR-ABL, BAD phosphorylation is not required for PI3K-dependent survival (65).

Mdm2. The tumor suppressor p53 promotes apoptosis in response to cellular stress. Akt can contribute to p53 inactivation by phosphorylation of the p53 regulatory protein Mdm2 (66, 67). Two groups have reported that BCR-ABL suppresses p53 by enhancing Mdm2 expression in a manner dependent on Abl kinase activity (68, 69). However, a role for Akt-dependent Mdm2 phosphorylation in ABL-transformed cells has not been established.

Glycogen Synthase Kinase 3 β . Glycogen synthase kinase 3 β (GSK3 β) is a serine/threonine kinase that is constitutively phosphorylated in transformed cells in an ABL/PI3K-dependent pathway (70, 71). Activated GSK3 β phosphorylates β -catenin and cyclin D1, which leads to their degradation (72). Akt-mediated phosphorylation maintains GSK3 β in an inactive state (73). Detection of phospho-GSK3 β has been used as surrogate readout for Akt activity in numerous studies, including some measuring PI3K pathway activity downstream of ABL oncogenes (22, 25). The β -catenin pathway is activated and important in the self-renewal of granulocyte-macrophage progenitor cells that may provide the source for the Ph⁺ leukemia cells (74). However, a functional role for GSK3 β inactivation downstream of PI3K in ABL-transformed cells has not been established.

Tuberous Sclerosis-2 and the Mammalian Target of Rapamycin Pathway. The mammalian target of rapamycin (mTOR) is both a nutrient sensor and an important downstream target of PI3K/Akt signaling in response to growth factors and oncoproteins (75, 76). mTOR activation leads to coordinated changes in cellular protein translation that are required for cell growth (size increase) and cycle progression. Originally studied mainly in the context of lymphocyte activation, the mTOR pathway has been emerging as an attractive drug target for multiple cancers that exhibit excess PI3K signaling, such as ovarian and breast cancers (77, 78). Recently, rapamycin was found to enhance the antileukemia effects of imatinib *in vitro* and *in vivo* (79, 80) even in cells expressing imatinib-resistant BCR-ABL variants. These and other findings have spurred increased interest in the mechanisms of translational control by oncogenes and the role of the mTOR pathway in tumorigenesis (81, 82).

The mechanism by which ABL oncogenes activate mTOR in hematopoietic cells has not been defined but seems to be PI3K dependent (79) and is thus presumed to follow the paradigm established in other cell types (reviewed in refs. 75, 76). Although Akt can directly phosphorylate mTOR, the major route by which Akt regulates mTOR is indirect through inactivation of the protein tuberous sclerosis-2 (TSC2). Together with TSC1, TSC2 forms a complex that suppresses mTOR activity in the absence of growth factor/oncogene signals. Activated Akt phosphorylates TSC2 and blocks the ability of TSC1/TSC2 to inhibit the small G protein Rheb. Released from this inhibition, Rheb activates mTOR.

mTOR is a serine/threonine kinase whose targets include the ribosomal S6 kinases (S6K1 and S6K2) and the translation inhibitor protein 4EBP-1 (75, 76). mTOR-mediated phosphorylation of 4EBP-1 blocks its ability to inhibit eIF4E. The released eIF4E protein binds to the 5'-cap structure of mRNAs to allow a general increase in cap-dependent translation efficiency. Many kinases regulate S6K activation, but the ability of S6K to phosphorylate the S6 protein of 40S ribosomes is absolutely dependent on a rapamycin-sensitive phosphorylation of the hydrophobic motif. Of note, S6K enzyme activation also requires phosphorylation in the activation loop by PDK-1; however, unlike

Akt phosphorylation by PDK-1, this phosphorylation of S6K is independent of PI3K lipid products. S6K enzyme activation promotes cell growth and proliferation by a mechanism that remains unclear (83).

Phosphorylation of 4EBP-1 as well as S6K and its target S6 are blocked in BCR-ABL-expressing cells treated with imatinib or rapamycin (79, 80). Further work is necessary to distinguish how S6K activation, versus 4EBP-1 inactivation, contribute to ABL transformation. In addition, it is worth investigating whether specific mRNAs are regulated by ABL oncogenes in a rapamycin-sensitive manner. Expression of several cell cycle-promoting proteins in BCR-ABL⁺ cells, including *c-myc* and cyclin D family members, is rapidly down-regulated by imatinib treatment (84–86). The mechanism of regulation is not fully clear for *c-myc*, but cyclin D2 is mainly regulated transcriptionally, whereas cyclin D3 is controlled post-transcriptionally (84–86). A more global approach to this question would be the use of microarrays to probe target cDNA prepared from polysomal mRNA, a method that has proven useful for uncovering PI3K/Akt-dependent target mRNAs in cancer cells (81).

An important characteristic of BCR-ABL-transformed cells is an increase of metabolism and the concomitant boost in reactive oxygen species (ROS; ref. 87). Higher levels of ROS may provide a positive feedback by opposing the action of tyrosine phosphatases that oppose ABL kinases as well as lipid phosphatases, including PTEN, that regulate PI3K pathway activity (88, 89). Rapamycin greatly reduces ROS production in BCR-ABL-transformed cells (90), identifying mTOR as a central mediator of this cellular response. This provides a potential explanation for the ability of rapamycin to sensitize imatinib-resistant cells (i.e., the blockade of positive feedback that would normally occur through ROS production).

Combined treatment with imatinib and rapamycin increases the latency of leukemia disease in a mouse model of CML (80). Thus, targeting the mTOR pathway for treatment of Ph⁺ leukemias is an attractive option. Rapamycin is currently approved for clinical use as an immunosuppressant, and rapamycin analogues are being tested for combination therapy of cancers that have activated PI3K signaling.

Other Akt Substrates. Caspase-9 was identified as a Akt substrate whose phosphorylation reduces apoptosis following cytochrome *c* release (91). However, caspase-9 does not seem to be phosphorylated in BCR-ABL-expressing cells and the Akt phosphorylation site in the human protein is not conserved in mice, a species whose cells are susceptible to the antiapoptotic effects of ABL oncogenes (92, 93).

Activation of the nuclear factor- κ B (NF- κ B) transcription factor is required for BCR-ABL transformation (94). Interestingly, I κ B kinase can be phosphorylated and activated by Akt in some cellular contexts, leading to NF- κ B-dependent transcription of antiapoptotic genes. However, I κ B kinase activity is not deregulated in primary acute lymphoblastic leukemia cells or in BaF3 cells expressing BCR-ABL or v-Abl (95, 96). The mechanism of NF- κ B activation in ABL-transformed cells seems to involve Ras-dependent phosphorylation and nuclear translocation of the p65/RelA component (94, 96).

Other Phosphoinositide 3-Kinase Effectors. Although Akt coordinates multiple leukemogenic pathways downstream of PI3K in ABL-transformed cells, other PI3K effectors might also contribute to the transformed phenotype. PI3K signaling can profoundly influence cell adhesion and motility through activation

of small G proteins and other cytoskeletal regulatory proteins (17). Of relevance, overexpression of SHIP1 phosphatase reduces motility in BCR-ABL-transformed cells (97). It should also be noted that SGK enzymes (serum- and glucocorticoid-induced kinases), which are AGC family members closely related to Akt, can phosphorylate many of the same target proteins as Akt (e.g., FOXO and GSK3 β) in a PI3K-dependent manner (98).

Conclusions

With the success of imatinib, the promise of molecular based therapy is finally emerging as the new standard in treating malignancies. In Ph⁺-positive leukemias, like other cancers, the cellular web of complex signaling networks is altered to promote cell proliferation, survival, and motility. In each specific cancer, several major oncogenic pathways are used and targeting multiple arms can command a more prolonged and favorable outcome. BCR-ABL⁺ leukemias are an attractive battleground in which to test this approach due to the defined nature of the genetic lesion (at least during chronic phase) and the availability of suitable mouse models. With regard to the PI3K pathway, several points of intervention could be tested. One approach would be to use

novel PI3K inhibitors that are selective for individual isoforms (or subgroups) and therefore less toxic. Targeting the mTOR pathway is also emerging as a feasible approach. Specific Akt inhibitors could also prove useful. Novel Akt inhibitors were identified recently in a screen for compounds that promote nuclear localization of FOXO proteins (99). The same screen identified a set of compounds that maintain nuclear FOXO without affecting Akt and might therefore be less toxic. There is increasing hope that patients with primary or acquired resistance to imatinib alone will benefit from improved ABL kinase inhibitors (39) combined with agents that block aspects of PI3K signaling and other oncogenic pathways, such as Ras (100).

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