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Phosphatidyl inositol signaling by BCR/ABL: opportunities for drug development

Abstract The t(9;22) translocation associated with chronic myelogenous leukemia (CML) fuses the c-ABL gene on chromosome 9 with the BCR gene on chromosome 22, resulting in the production of one or more of a family of chimeric oncoproteins, p190, p210, or p230 BCR/ABL. These proteins have activated ABL kinase activity and are located in the cytoplasm of CML cells, predominantly in the cytoskeleton. Recent studies have led to the identification of numerous potential substrates for BCR/ABL, including many proteins that normally function in signal transduction pathways downstream from hematopoietic growth factor receptors. BCR/ABL is autophosphorylated on tyrosine residues and attracts a variety of adapter proteins and other signaling proteins, setting up large signaling complexes that ultimately result in growth, viability, and adhesion signals. Using new in vitro and animal model systems, it is now becoming possible to link specific signaling pathways to biological abnormalities in CML cells. Furthermore, the relative importance of some BCR/ABL-activated pathways is becoming clear. In vivo studies in certain lines of transgenic mice suggest that the antiapoptotic effect of Bcr/Abl is more important than previously thought. Our current studies indicate important roles for phosphoinositide 3-kinase/Akt and for STAT molecules. As a result of these more detailed biochemical analyses of BCR/ABL function, new targets for future drug development have been identified.

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Introduction

Chronic myelogenous leukemia (CML) was the first leukemia identified as being associated with a specific chromosomal translocation, the Philadelphia (Ph) chromosome t(9;22)(q34;q11). The molecular basis of CML has been clarified in elegant detail by Baltimore, Groffen, Grosveld, Heisterkamp, Witte, Canaani, and others (for reviews see 16, 28, 43), and it is now clear that virtually all cases are the result of fusion of the c-ABL tyrosine kinase with BCR, a ubiquitously expressed gene probably involved in regulating p21rac activity. Three translocations have been identified, varying in the amount of the BCR gene fused to ABL, resulting in the production of: p190BCR/ABL, associated with Ph^+ acute lymphoid leukemia (ALL); p210BCR/ABL, associated with CML; and p230BCR/ABL, associated with chronic neutrophilic leukemia (CNL). All forms have elevated tyrosine kinase activity compared to c-ABL, but among the three forms, kinase activity is inversely related to length [24].

CML is characterized by massive expansion of myeloid cells in the marrow and blood, splenomegaly, and a high propensity to convert to acute leukemia. In many respects, the "stable" phase of CML is a preleukemic disorder, progressing to blast phase in a median of 4 years. Therapy with hydroxyurea reduces symptoms, but does not induce cytogenetic remission. Interferon-α induces cytogenetic responses in a significant fraction of patients, but complete, durable, cytogenetic remissions are uncommon. The small molecule tyrosine kinase inhibitor STI571 (Novartis, Basel, Switzerland) has shown remarkable activity in early clinical trials and induces cytogenetic responses in the majority of stable-phase patients and even polymerase chain reaction-negative status in a small number [33]. Allogeneic bone marrow

transplantation (BMT) can cure up to 50% of younger patients who have a human leukocyte antigen (HLA)-matched donor. The impact of STI571 on the duration of the chronic phase is not yet known. In the blast phase, many patients respond well to STI571, although remissions tend to be short. The blast crisis phase is characterized by the emergence of poorly differentiated primitive subclones, which typically have acquired new genetic mutations in addition to the Ph chromosome.

The BCR/ABL oncogene

BCR/ABL is unique to CML, CNL, and Ph^+ ALL, and does not transform most nonhematopoietic cells effectively, even in tissue culture [8]. The tyrosine kinase activity of ABL is critical for transformation, but the actual mechanisms remain to be elucidated. c-ABL is ubiquitously expressed both as a nuclear and as a cytoplasmic protein [22]. Recent studies suggest that nuclear c-Abl is likely to function as a negative regulator of growth and may possibly be involved in the cellular response to DNA damage [18, 52]. In contrast, p210BCR/ABL is exclusively cytoplasmic, with about 70% of the protein associated with the cytoskeleton. Not surprisingly, cytoskeletal proteins are commonly targets for phosphorylation by BCR/ABL, and this is likely to account for some of the adhesion defects characteristic of these cells [2, 15].

Several domains in p210BCR/ABL have been shown to play a functional role in transformation. BCR has been shown to be important for cytoplasmic localization, multimer formation [27, 30], and activation of the ABL kinase [30]. It has also been shown to have unique activities, including serine/threonine kinase activity and a domain that binds to the ABL SH2 domain in a phosphotyrosine-independent manner [34]. The racGAP domain of BCR is not part of the BCR/ABL fusion protein. Importantly, Y177 of BCR is phosphorylated and serves as a binding site for GRB2 [36], although downstream targets of this pathway are not defined. Mutations of Y177 reduce transformation by BCR/ABL, particularly when there are also mutations of other domains involved in transformation, such as the CRKL binding site [44]. In addition, recent in vivo studies have shown that an intact Y177 is needed for induction of a myeloproliferative syndrome [29].

The SH3 domain of ABL is inhibitory to kinase activity, and deletion of the SH3 domain makes c-ABL into an oncogene, suggesting that one or more cellular protein(s) bind to the SH3 domain and downregulate c-ABL activity. Several potential ABL-SH3 binding proteins have been identified. The SH3 domain is deleted in v-Abl. However, the role of the SH3 domain in BCR/ABL is controversial [47]. Deletion of sequences distal to ABL amino acid 585 does not prevent transformation by an SH3-deleted c-ABL. The ABL SH2 domain binds to cellular phosphoproteins in ABL-transformed cells [25], and is important for transformation in some assays [26]. BCR/ABL and v-Abl are heavily tyrosine phosphorylated, but only some of the sites are known. For example, p210BCR/ABL has 51 tyrosines and systematic mutagenesis of the tyrosines has not been undertaken. The major phosphorylation site within the kinase domain of p190 is important for transformation [35].

Biological effects of BCR/ABL

Unlike most leukemia oncogenes, BCR/ABL does not generally block differentiation [14]. CML progenitor cells are actively cycling, probably more so than normal progenitor cells. Immature hematopoietic cells tend to be partly or completely factor independent. There have been a number of well-documented reports of decreased sensitivity to inhibitory chemokines, but the significance of these observations remains unclear [7]. In addition to mitogenesis and cell cycle effects, several other biological effects are likely to be important (Fig. 1). First, BCR/ABL is antiapoptotic, and the significance of this has probably been underestimated in the past [1]. BCR/ABL has strong antiapoptotic activity in myeloid and lymphoid cells, and recent studies from several groups, including our own, have suggested that this is a primary and important activity of this oncogene [5, 9, 10, 20]. Studies in our laboratory with BCR/ABL-inducible cell lines indicate that viability signaling is a direct and rapid effect of the oncogene [5].

CML cells have altered adhesion to marrow stromal cells and some extracellular matrix proteins, notably fibronectin [2, 15]. The potential significance of this is substantial, as CML cells leave the marrow when immature, circulate in the blood in high numbers, and proliferate actively in tissues such as the spleen and liver, which are not normally hematopoietic in adult humans. This phenomenon is likely to be explained in part by changes in adhesion molecule expression or function in CML progenitor cells, and there is growing evidence that CML cells have altered integrin expression and function [2]. In this situation, the effects of BCR/ABL in cell lines may differ from its effects in primary marrow cells. In virtually all cell lines, BCR/ABL increases adhesion to fibronectin. In trying to explain these differences, our group recently reported that cell lines and primary CML cells adhere normally to fibronectin for short periods (< 30 min), but are hypermotile and much

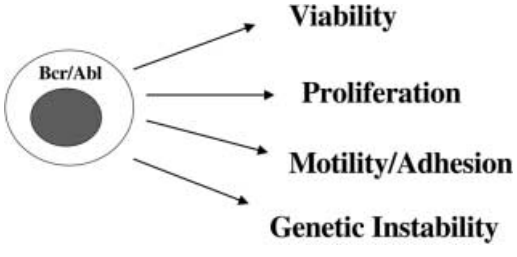

Fig. 1 Biologic effects of BCR/ABL

more likely to come off the plate than normal cells [41]. The result of altering cytoskeletal dynamics is premature release of malignant CML progenitors.

Phosphatidyl inositol signaling and phosphoinositide 3-kinases

Phosphoinositide 3-kinases (PI3Ks) comprise a family of signal transducers that have been linked to the control of proliferation, adhesion, motility, and viability in a variety of cell types [6, 39]. There have been tremendous advances in understanding this field over the past few years, particularly using the insulin receptor as a model, and with the discovery of homologous pathways in organisms such as *Caenorhabditis elegans*, identification of modifiers and downstream targets has proceeded rapidly. PI3Ks phosphorylate the 3′-OH position of the inositol ring of phosphatidyl inositol (PtdIns), generating (in mammalian cells) PtdIns(3)P_1, PtdIns(3,4)P_2, PtdIns(3,5)P_2, and PtdIns(3,4,5)P_3, or PIP_3. In hematopoietic cells, the basal levels of PtdIns(3,4)P_2, PtdIns(3,5)P_2, and PtdIns(3,4,5)P_3 are low, but can rise dramatically after activation of a receptor or oncogene thymidine kinase.

In broad terms, recruitment of PI3K to the cell membrane, or possibly other sites such as the cytoskeleton, is critical to bring the kinase to its substrates. PI3K lipid products, such as PtdIns(3,4,5)P_3, are generated in the membrane, and then serve as signaling intermediates to attract and activate other molecules, which have one of two known motifs that recognize these lipids: a pleckstrin homology (PH) domain, preferentially binding PtdIns(3,4)P_2 or PtdIns(3,4,5)P_3, and a FYVE finger domain, preferentially binding PtdIns(3)P_1 [50]. PH domain molecules activated through this mechanism include PDK1 and related kinases (which then phosphorylate and activate Akt or PKB), Tec kinases, guanine-nucleotide exchange factors (GEFs), or GTPase-activating factors (GAPs) for Ras and Rac.

The FYVE finger domain-containing proteins include a number of important proteins involved in membrane trafficking. Through these steps, PI3K activates parallel cascades of signaling that have remarkable effects on cells, including activation of Akt (survival, growth), Ras (survival, growth, differentiation), Rac (motility, survival), S6kinase (protein synthesis), GSK3 (glucose metabolism), PKC (cell function, proliferation), and others (Fig. 2). PI3K can also be activated by heterotrimeric G-protein-coupled receptors, which are not discussed here. There are important interactions between the Ras and PI3K pathway that go in both directions [13].

The activation of PI3K involves recruitment of the catalytic domain to the membrane through the regulatory subunit, typically due to SH2 binding to a phosphotyrosine motif. In the simplest model, a receptor tyrosine kinase recruits the adapter/catalytic PI3K complex to itself through the SH2 domain recognition of pYXXM motifs in the activated receptor (Fig. 3). However, other intermediate adapter proteins are often involved, and may serve as scaffolds to assemble clusters of other signaling molecules in the same site. For example, the IRS-1 and -2 molecules associated with the insulin receptor and relatives of these adapters, such as GAB1 and GAB2, are needed to activate PI3K in various systems.

PI3Ks have been classified in three groups [11]. Class Ia PI3Ks comprise the "PI3K" activity typically referred to as downstream of receptor tyrosine kinases, and are composed of an adapter, p85, and a 110-kDa catalytic subunit. There are three related catalytic units, p110α, β, and δ, and three p85 isoforms. Class Ib PI3Kγ has a 110-kDa catalytic subunit complexed with a single adapter, p101. PI3Kγ is activated by G-protein-coupled receptors and is associated with colorectal carcinomas in knockout mice. Class II PI3K contains three related catalytic

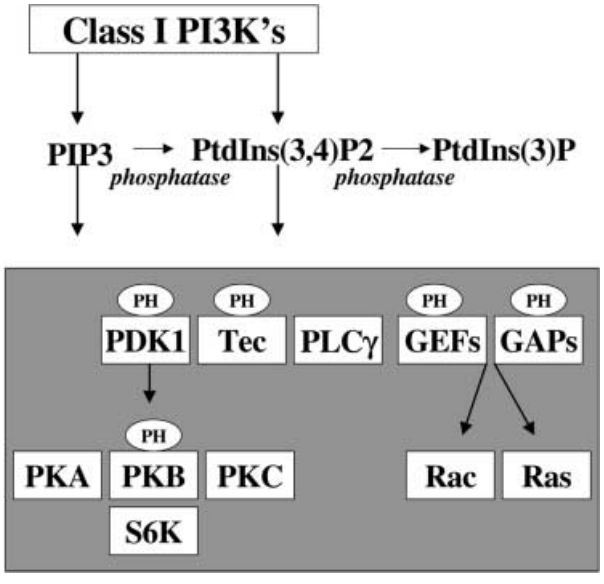

Fig. 2 Phosphatidyl inositol signaling and phosphoinositide 3-kinase

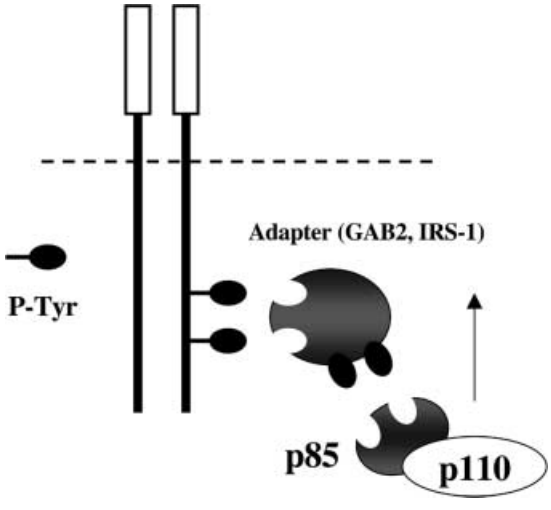

Fig. 3 Activation of PI3K

units, PI3K-C2α, β, and δ, and may be activated by receptor tyrosine kinases. Class III PI3K generates much of the PtdIns(3)P in cells, but does not appear to respond to cellular activation.

BCR/ABL and PI3K

High PI3K activity is associated with several tyrosine kinase oncogenes, and is required for transformation by BCR/ABL both in cell lines and in murine models [45]. This pathway is potentially a target for drug development in myeloproliferative syndromes, and a thorough understanding of the relationship of BCR/ABL to phosphoinositide signaling is important. It is not yet clear how BCR/ABL activates PI3K, except that one or more adapter molecules is likely involved since p85 does not bind directly to BCR/ABL [3]. Our preliminary data suggest that one of the adapters is CBL (Fig. 4).

PI3K targets: PDK1 and AKT

The discovery of the PH domain was a major breakthrough, explaining how lipid signaling molecules connect back to the kinases, phosphatases, GTPases, and other enzymes that mediate much of the signaling within the cytoplasm. Although there are a number of known pathways activated downstream from PI3K, the AKT/PKB kinase clearly plays a central role [49]. AKT was discovered originally by several groups because of its homology to v-Akt (a retroviral oncogene that causes thymomas in AKR mice). AKT is an S/T kinase with a PH domain that specifically binds the lipid products of PI3K. Only class I PI3Ks generate lipids that bind to AKT [49]. The AKT PH domain is believed to bind PtdIns(3,4,5)P_3 and PtdIns(3,4)P_2, but the affinity for PtdIns(3,4,5)P_3 may be higher [21]. Since PtdIns(3,4,5)P_3 is used as a substrate to generate PtdIns(3,4)P_2, 5′-phosphatases such as SHIP may play a critical role in determining the ratio of these two key PtdIns.

The recruitment of AKT to the membrane is not sufficient for its activation, however, as it must first be phosphorylated on Thr308 and Ser473. The responsible kinase has been identified as PDK1 [49]. Thus a current model of AKT activation involves recruitment of both AKT and PDK1 to the membrane, phosphorylation of AKT, and then phosphorylation of downstream targets. AKT has been reported to be important for BCR/ABL transformation [46].

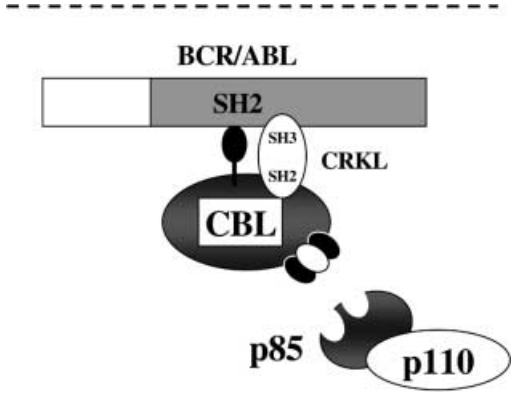

Fig. 4 A potential target for drug development in myeloproliferative syndromes

Downstream from AKT, signaling becomes both interesting and complicated (but critically important for understanding BCR/ABL). There are many identified possible targets for AKT potentially related to viability and cell cycle regulation, including Forkhead (FH) transcription factors, Bad, Caspase-9, and IκB [21]. Others, including Raf, IRS-1, GSK3, etc., are potentially of equal importance, and may deserve more attention in the future [21].

FH transcription factors

The FH transcription factors are a family of DNA binding factors that have been shown to be phosphorylated directly by AKT in mammalian cells after a link was established genetically in *C. elegans* [31]. In quiescent cells, FH factors (FKHR, FKHRL1, and AFX are discussed here) regulate transcription of genes that are prodeath, such as Fas ligand. Akt phosphorylates FH factors at specific sites, following which they are rapidly exported from the nucleus and sequestered by binding to cytoplasmic 14-3-3 proteins. Genetic studies suggest that FH factors play a key role in PI3K/AKT signaling, but the most important target genes are not yet known [19]. Bad is a well-known prodeath molecule that heterodimerizes with antiapoptotic molecules Bcl-2 and Bcl-X. When phosphorylated by AKT on Ser136, Bad can no longer heterodimerize, thus promoting viability. However, Bad is not found in many cell types, and is only part of the picture of viability signaling downstream of AKT.

Caspase 9 is an essential member of the caspase cascade signaling to apoptosis. Procaspase 9 is a mitochondrial membrane protein that is cleaved after cytochrome *c* is released, and then functions with caspase 8 to activate procaspase 3,7. It is a target of AKT and is reported to be inhibited by phosphorylation [4]. The role of caspase 9 in CML is unknown. IκB sequesters NF-κB in the cytoplasm until it is phosphorylated by an IκB kinase, IKK. IKK has been reported to be phosphorylated and activated by AKT, thus resulting in translocation of NF-κB to the nucleus, where it activates transcription of antiapoptotic proteins and others [40]. NF-κB has been shown to play an important role in BCR/ABL transformation, but the mechanism of its activation has not been explained.

Thus it is a reasonable hypothesis that activation of Akt through PI3K acts on a number of downstream factors to promote viability. Some potential Akt targets are of more interest because of their potential as targets for drug discovery. For example, in addition to PI3K and Akt themselves, IKK would be of interest.

PI3K targets not mediated through Akt

Rac, Rho, and Cdc42 comprise a family of small GTP binding proteins known to be critical for signaling to the cytoskeleton. In preliminary studies, we have shown that BCR/ABL activates motility of leukemic cells, and that this activity requires PI3K [41]. In BCR/ABL-transformed cells, Rac is important for transformation, and is downstream from PI3K, since an activated Rac mutant can restore motility blocked by LY294002 [41]. The mechanism of activation of Rac in BCR/ABL-transformed cells is not known [48].

SHIP is a family of 110–145 kDa SH2-containing inositol 5-phosphatases generated by alternative splicing and proteolytic cleavage [37, 38, 51]. There are two related SHIP genes, SHIP1 and SHIP2. Although both are 5′-phosphatases, they may have slightly different specificity and functions. SHIP1 was observed originally as a 145 kDa tyrosine phosphoprotein coprecipitating with SHC, SHP2, an Fc receptor, or the B cell receptor after activation [51]. SHIP1 is expressed predominantly in the hematopoietic system and the testis, while SHIP2 is more widely expressed. SHIP1 dephosphorylates the 5′ position only in lipids in which the 3′ position is also phosphorylated, suggesting that SHIP functions in the PI3K pathway, in much the same way as PTEN (PTEN is a 3′ phosphatase) [23].

SHIP is of considerable interest in understanding myeloproliferative syndromes for two reasons. First, a SHIP1 knockout mouse develops a significant myeloproliferative disorder early in life [17], and second, SHIP protein levels are directly and significantly downregulated by BCR/ABL through an unknown mechanism [42]. Taken together, these results suggest that SHIP may play a significant role in BCR/ABL transformation, possibly by modulating PI3K signaling.

Other tyrosine kinase oncogenes that cause myeloproliferative syndromes and AML

Although BCR/ABL is the prototypic tyrosine kinase leukemia oncogene, an increasing number of other kinases have also been implicated. The ets family member TEL is involved in a number of kinase fusions and may serve to dimerize (and thus activate) kinases [12]. Its partners include platelet-derived growth factor receptor, ABL, ARG, and JAK2. Furthermore, activating mutations in c-KIT have been described, along with more common internal duplications in FLT3 [32].

Potential targets for drug development

There is abundant evidence that PI3K is important for BCR/ABL transformation [45, 46], and to the extent studied, it is also felt to be required for transformation by other tyrosine kinase oncogenes. In preliminary studies in BCR/ABL-transformed cells in vitro, we have shown that an inhibitor of the PI3K pathway, LY294002, can be added to STI571 with significant efficacy. Since STI571 has impressive antiviability effects on BCR/ABL-transformed cells, it appears reasonable that another drug that inhibits the viability of leukemia cells may have additive effects. Where are the best targets in this pathway? Even if one considers only the kinases in the PI3K pathway, many potential sites stand out. First, PI3K itself may be an excellent target if high-affinity kinase inhibitors can be developed. AKT would also be a target. Finally, IKK could be considered, since NF-κB appears important in CML. Overall, further dissection of this pathway in CML and in other leukemias and a better appreciation of the critical viability signaling pathways in general are likely to reveal excellent targets for future leukemia chemotherapy.

References

1. Bedi A, Zehnbauer BA, Barber JP, Sharkis SJ, Jones RJ (1994) Inhibition of apoptosis by BCR-ABL in chronic myeloid leukemia. Blood 83:2038
2. Bhatia R, Wayner EA, McGlave PB, Verfaillie CM (1994) Interferon-alpha restores normal adhesion of chronic myelogenous leukemia hematopoietic progenitors to bone marrow stroma by correcting impaired beta 1 integrin receptor function. J Clin Invest 94:384
3. Calabretta B, Skorski T (1996) BCR/ABL regulation of PI-3 kinase activity. Leuk Lymphoma 23:473
4. Cardone MH, Roy N, Stennicke HR, Salvesen GS, Franke TF, Stanbridge E, Frisch S, Reed JC (1998) Regulation of cell death protease caspase-9 by phosphorylation. Science 282:1318
5. Carlesso N, Griffin JD, Druker BJ (1994) Use of a temperature sensitive mutant to define the biological effects of the p210bcr/abl tyrosine kinase on proliferation of a factor-dependent murine myeloid cell line. Oncogene 9:149
6. Carpenter CL, Cantley LC (1996) Phosphoinositide 3-kinase and the regulation of cell growth. Biochim Biophys Acta 1288:M11
7. Cashman JD, Eaves CJ, Sarris AH, Eaves AC (1998) MCP-1, not MIP-1alpha, is the endogenous chemokine that cooperates with TGF-beta to inhibit the cycling of primitive normal but not leukemic (CML) progenitors in long-term human marrow cultures. Blood 92:2338
8. Daley GQ, McLaughlin J, Witte ON, Baltimore D (1987) The CML-specific p210bcr/abl protein, unlike v-abl, does not transform NIH/3T3 fibroblasts. Science 237:532
9. Druker BJ, Tamura S, Buchdunger E, Ohno S, Segal GM, Fanning S, Zimmermann J, Lydon NB (1996) Effects of a selective inhibitor of the Abl tyrosine kinase on the growth of Bcr-Abl positive cells. Nat Med 2:561
10. Evans CA, Owen-Lynch PJ, Whetton AD, Dive C (1993) Activation of the Abelson tyrosine kinase activity is associated with suppression of apoptosis in hemopoietic cells. Cancer Res 53:1735
11. Fruman DA, Meyers RE, Cantley LC (1998) Phosphoinositide kinases. Annu Rev Biochem 67:481
12. Gilliland DG (1998) Molecular genetics of human leukemia. Leukemia [Suppl] 12:7
13. Gire V, Marshall C, Wynford-Thomas D (2000) PI-3-kinase is an essential anti-apoptotic effector in the proliferative response of primary human epithelial cells to mutant RAS. Oncogene 19:2269
14. Gishizky ML, Witte ON (1992) BCR/ABL enhances growth of multipotent progenitor cells but does not block their differentiation potential in vitro. Curr Topics Microbiol Immunol 182:65

15. Gordon MY, Dowding CR, Riley GP, Goldman JM, Greaves MF (1987) Altered adhesive interactions with marrow stroma of haematopoietic progenitor cells in chronic myeloid leukaemia. Nature 328:342
16. Groffen J, Heisterkamp N (1997) The chimeric BCR-ABL gene. Baillieres Clin Haematol 10:187
17. Helgason CD, Damen JE, Rosten P, Grewal R, Sorensen P, Chappel SM, Borowski A, Jirik F, Krystal G, Humphries RK (1998) Targeted disruption of SHIP leads to hemopoietic perturbations, lung pathology, and a shortened life span. Genes Dev 12:1610
18. Kharbanda S, Bharti A, Pei D, Wang J, Pandey P, Ren R, Weichselbaum R, Walsh CT, Kufe D (1996) The stress response to ionizing radiation involves c-Abl-dependent phosphorylation of SHPTP1. Proc Natl Acad Sci USA 93:6898
19. Kops GJ, Burgering BM (1999) Forkhead transcription factors: new insights into protein kinase B (c-akt) signaling. J Mol Med 77:656
20. Laneuville P, Timm M, Hudson AT (1994) Bcr/abl expression in 32D cl3(G) cells inhibits apoptosis induced by protein tyrosine kinase inhibitors. Cancer Res 54:1360
21. Leevers SJ, Vanhaesebroeck B, Waterfield MD (1999) Signalling through phosphoinositide 3-kinases: the lipids take center stage. Curr Opin Cell Biol 11:219
22. Lewis JM, Baskaran R, Taagepera S, Schwartz MA, Wang JY (1996) Integrin regulation of c-Abl tyrosine kinase activity and cytoplasmic-nuclear transport. Proc Natl Acad Sci USA 93:15174
23. Liu Q, Sasaki T, Kozieradzki I, Wakeham A, Itie A, Dumont DJ, Penninger JM (1999) SHIP is a negative regulator of growth factor receptor-mediated PKB/Akt activation and myeloid cell survival. Genes Dev 13:786
24. Lugo TG, Pendergast AM, Muller AJ, Witte ON (1990) Tyrosine kinase activity and transformation potency of bcr-abl oncogene products. Science 247:1079
25. Mayer BJ, Jackson PK, Baltimore D (1991) The noncatalytic src homology region 2 segment of abl tyrosine kinase binds to tyrosine-phosphorylated cellular proteins with high affinity. Proc Natl Acad Sci USA 88:627
26. Mayer BJ, Jackson PK, Van Etten RA, Baltimore D (1992) Point mutations in the abl SH2 domain coordinately impair phosphotyrosine binding in vitro and transforming activity in vivo. Mol Cell Biol 12:609
27. McWhirter JR, Galasso DL, Wang JY (1993) A coiled-coil oligomerization domain of Bcr is essential for the transforming function of Bcr-Abl oncoproteins. Mol Cell Biol 13:7587
28. Melo JV (1997) BCR-ABL gene variants. Baillieres Clin Haematol 10:203
29. Million RP, Van Etten RA (2000) The Grb2 binding site is required for the induction of chronic myeloid leukemia-like disease in mice by the Bcr/Abl tyrosine kinase. Blood 96:664
30. Muller AJ, Young JC, Pendergast AM, Pondel M, Landau NR, Littman DR, Witte ON (1991) BCR first exon sequences specifically activate the BCR/ABL tyrosine kinase oncogene of Philadelphia chromosome-positive human leukemias. Mol Cell Biol 11:1785
31. Murakami S, Tedesco PM, Cypser JR, Johnson TE (2000) Molecular genetic mechanisms of life span manipulation in *Caenorhabditis elegans*. Ann N Y Acad Sci 908:40
32. Nakao M, Yokota S, Iwai T, Kaneko H, Horiike S, Kashima K, Sonoda Y, Fujimoto T, Misawa S (1996) Internal tandem duplication of the flt3 gene found in acute myeloid leukemia. Leukemia 10:1911
33. O'Dwyer ME, Druker BJ (2000) Status of BCR/ABL tyrosine kinase inhibitors in chronic myelogenous leukemia. Curr Opin Oncol 12:594
34. Pendergast AM, Muller AJ, Havlik MH, Maru Y, Witte ON (1991) BCR sequences essential for transformation by the BCR-ABL oncogene bind to the ABL SH2 regulatory domain in a non-phosphotyrosine-dependent manner. Cell 66:161
35. Pendergast AM, Gishizky ML, Havlik MH, Witte ON (1993) SH1 domain autophosphorylation of P210 BCR/ABL is required for transformation but not growth factor independence. Mol Cell Biol 13:1728
36. Pendergast AM, Quilliam LA, Cripe LD, Bassing CH, Dai Z, Li N, Batzer A, Rabun KM, Der CJ, Schlessinger J, Gishizky ML (1993) BCR-ABL-induced oncogenesis is mediated by direct interaction with the SH2 domain of the GRB-2 adaptor protein. Cell 75:175
37. Pesesse X, Deleu S, De Smedt F, Drayer L, Erneux C (1997) Identification of a second SH2-domain-containing protein closely related to the phosphatidylinositol polyphosphate 5-phosphatase SHIP. Biochem Biophys Res Commun 239:697
38. Pesesse X, Moreau C, Drayer AL, Woscholski R, Parker P, Erneux C (1998) The SH2 domain containing inositol 5-phosphatase SHIP2 displays phosphatidylinositol 3,4,5-trisphosphate and inositol 1,3,4,5-tetrakisphosphate 5-phosphatase activity. FEBS Lett 437:301
39. Rameh LE, Cantley LC (1999) The role of phosphoinositide 3-kinase lipid products in cell function. J Biol Chem 274:8347
40. Romashkova JA, Makarov SS (1999) NF-κB is a target of AKT in antiapoptotic PGDF signalling. Nature 401:86
41. Salgia R, Li JL, Ewaniuk DS, Pear W, Pisick E, Burky SA, Ernst T, Sattler M, Chen LB, Griffin JD (1997) BCR/ABL induces multiple abnormalities of cytoskeletal function. J Clin Invest 100:46
42. Sattler M, Verma S, Byrne CH, Shrikhande G, Winkler T, Algate PA, Rohrschneider LR, Griffin JD (1999) BCR/ABL directly inhibits expression of SHIP, an SH2-containing polyinositol-5-phosphatase involved in the regulation of hematopoiesis. Mol Cell Biol 19:7473
43. Sawyers CL (1999) Chronic myeloid leukemia. N Engl J Med 340:1330
44. Senechal K, Halpern J, Sawyers CL (1996) The CRKL adaptor protein transforms fibroblasts and functions in transformation by the BCR-ABL oncogene. J Biol Chem 271:23255
45. Skorski T, Kanakaraj P, Nieborowska-Skorska M, Ratajczak MZ, Wen SC, Zon G, Gewirtz AM, Perussia B, Calabretta B (1995) Phosphatidylinositol-3 kinase activity is regulated by BCR/ABL and is required for the growth of Philadelphia chromosome-positive cells. Blood 86:726
46. Skorski T, Bellacosa A, Nieborowska-Skorska M, Majewski M, Martinez R, Choi JK, Trotta R, Wlodarski P, Perrotti D, Chan TO, Wasik MA, Tsichlis PN, Calabretta B (1997) Transformation of hematopoietic cells by BCR/ABL requires activation of a PI-3k/Akt-dependent pathway. EMBO J 16:6151
47. Skorski T, Nieborowska-Skorska M, Wlodarski P, Wasik M, Trotta R, Kanakaraj P, Salomoni P, Antonyak M, Martinez R, Majewski M, Wong A, Perussia B, Calabretta B (1998) The SH3 domain contributes to BCR/ABL-dependent leukemogenesis in vivo: role in adhesion, invasion, and homing. Blood 91:406
48. Skorski T, Wlodarski P, Daheron L, Salomoni P, Nieborowska-Skorska M, Majewski M, Wasik M, Calabretta B (1998) BCR/ABL-mediated leukemogenesis requires the activity of the small GTP-binding protein Rac. Proc Natl Acad Sci USA 95:11858
49. Vanhaesebroeck B, Alessi DR (2000) The PI3K-PDKI connection: more than just a road to PKB. Biochem J 346:561
50. Vanhaesebroeck B, Waterfield MD (1999) Signaling by distinct classes of phosphoinositide 3-kinases. Exp Cell Res 253:239
51. Ware MD, Rosten P, Damen JE, Liu L, Humphries RK, Krystal G (1996) Cloning and characterization of human SHIP, the 145-kD inositol 5-phosphatase that associates with SHC after cytokine stimulation. Blood 88:2833
52. Yuan ZM, Huang Y, Whang Y, Sawyers C, Weichselbaum R, Kharbanda S, Kufe D (1996) Role for c-Abl tyrosine kinase in growth arrest response to DNA damage. Nature 382:272