Leukemia (2011), 1–16

www.nature.com/leu

REVIEW

Targeting the translational apparatus to improve leukemia therapy: roles of the PI3K/PTEN/Akt/mTOR pathway

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It has become apparent that regulation of protein translation is an important determinant in controlling cell growth and leukemic transformation. The phosphoinositide 3-kinase (PI3K)/phosphatase and tensin homologue deleted on chromosome ten (PTEN)/Akt/mammalian target of rapamycin (mTOR) pathway is often implicated in sensitivity and resistance to therapy. Dysregulated signaling through the PI3K/PTEN/Akt/mTOR pathway is often the result of genetic alterations in critical components in this pathway as well as mutations at upstream growth factor receptors. Furthermore, this pathway is activated by autocrine transformation mechanisms. *PTEN* is a critical tumor suppressor gene and its dysregulation results in the activation of Akt. PTEN is often mutated, silenced and is often haploinsufficient. The mTOR complex1 (mTORC1) regulates the assembly of the eukaryotic initiation factor4F complex, which is critical for the translation of mRNAs that are important for cell growth, prevention of apoptosis and transformation. These mRNAs have long 5′-untranslated regions that are G + C rich, rendering them difficult to translate. Elevated mTORC1 activity promotes the translation of these mRNAs via the phosphorylation of 4E-BP1. mTORC1 is a target of rapamycin and novel active-site inhibitors that directly target the TOR kinase activity. Although rapamycin and novel rapalogs are usually cytostatic and not cytotoxic for leukemic cells, novel inhibitors that target the kinase activities of PI3K and mTOR may prove more effective for leukemia therapy.
Leukemia advance online publication, 25 March 2011;
doi:10.1038/leu.2011.46
Keywords: translation; resistance; therapy; PI3K; sensitivity; mTOR

Introduction

The phosphoinositide 3-kinase (PI3K)/phosphatase and tensin homologue deleted on chromosome ten (PTEN)/Akt/mammalian target of rapamycin (mTOR) signaling pathway plays important roles in the transmission of proliferative signals from membrane-bound receptors. Pathway activation can be caused by mutations in the genes encoding pathway constituents (for example, Ras, PI3K, PTEN, Akt) or in upstream receptors (for example, Kit, Fms, Fms-like tyrosine kinase), or by chromosomal translocations leading to the expression of aberrant fusion proteins (for example, BCR-ABL, TEL-platelet-derived growth factor receptor-β (PDGF-Rβ). Furthermore, many components of this pathway are overexpressed in leukemia. This pathway relays its information through interactions with various other proteins to affect cell growth at multiple levels, by altering the activity of transcription factors to control gene expression, by changing the levels of apoptotic regulators to influence the induction of apoptosis, by modulating the induction of autophagy and finally by regulating protein translation.[1] This review will discuss how this pathway may be aberrantly regulated in leukemia and contribute to therapeutic sensitivity/resistance, drug resistance and in some cases poor prognosis.[1,2] However, increased activation of this pathway can often be correlated with enhanced sensitivity to targeted therapy.[1,2] Inhibition of PI3K, Akt, mTOR and in some cases Ras or Ras homolog enriched in brain (Rheb) may prove useful in leukemia treatment. These observations have propelled the pharmaceutical industry to develop inhibitors that target important components of these pathways.

The PI3K/PTEN/Akt/mTOR signaling pathway consists of a complicated kinases cascade that is regulated by phosphorylation and de-phosphorylation by specific kinases, phosphatases as well as GTP/GDP exchange proteins, adaptor proteins and scaffolding proteins. An overview of this pathway is presented in Figure 1. The sites of intervention of signal-transduction inhibitors and some that have been investigated with regard to leukemia are also shown in this diagram.

Overview of the PI3K/PTEN/Akt/mTOR pathway

PI3K is a heterodimeric protein with an 85-kDa regulatory subunit and a 110-kDa catalytic subunit (*PIK3CA*). PI3K serves to phosphorylate a series of membrane phospholipids, including phosphatidylinositol 4-phosphate (PtdIns(4)P) and phosphatidylinositol 4,5-bisphosphate (PtdIns(4,5)P_2), catalyzing the transfer of ATP-derived phosphate to the D-3 position of the inositol ring of membrane phosphoinositides, thereby forming the second messenger lipid phosphatidylinositol 3,4-bisphosphate (PtdIns(3,4)P_2) and phosphatidylinositol 3,4,5-trisphosphate (PtdIns(3,4,5)P_3).[2] Most often, PI3K is activated via the binding of a ligand to its cognate receptor, whereby p85 associates with phosphorylated tyrosine residues on the receptor via an Src-homology 2 domain. After association with the receptor, the p110 catalytic subunit then transfers phosphate groups to the aforementioned membrane phospholipids.[2] It is these lipids,

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Received 14 January 2011; revised 5 February 2011; accepted 21 February 2011

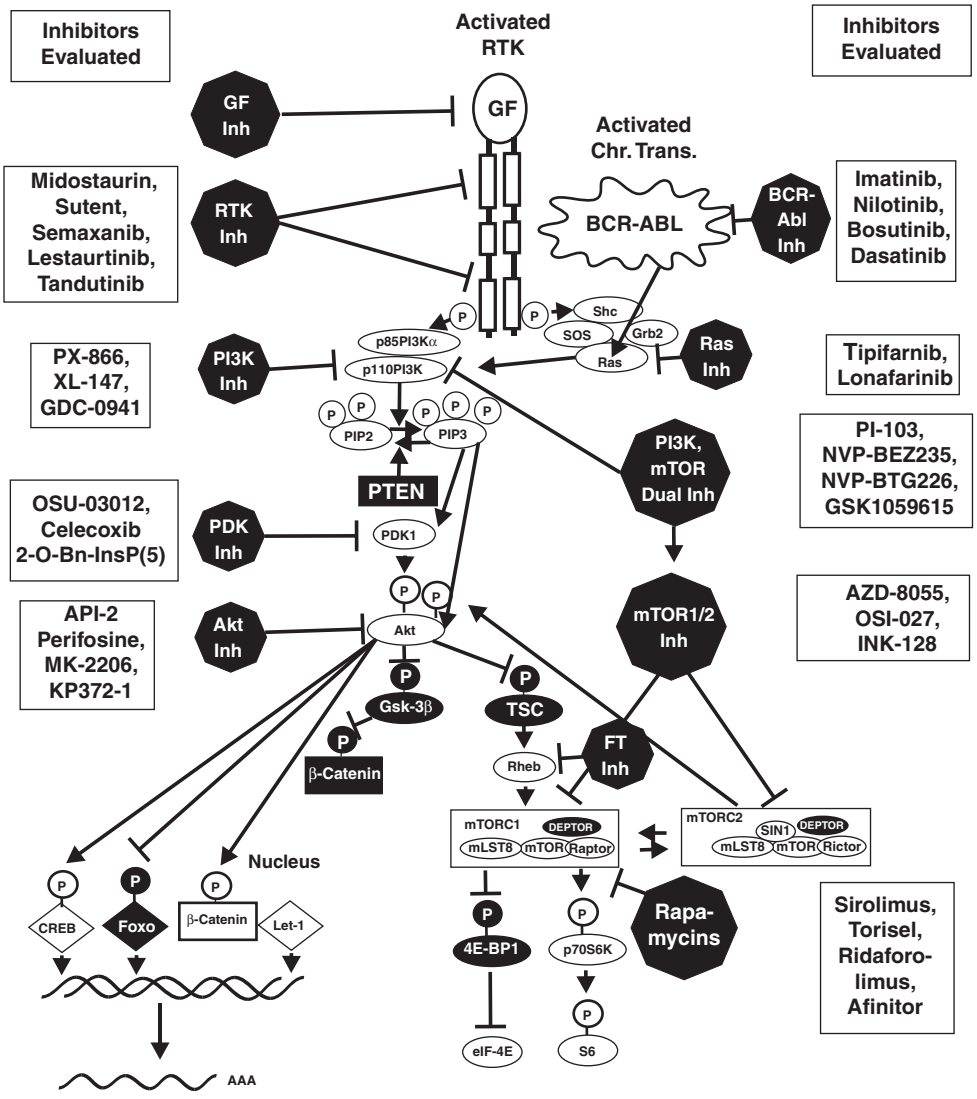

Figure 1 Overview of the PI3K/PTEN/Akt/mTOR pathway and potential sites of therapeutic intervention. The PI3K/PTEN/Akt/mTOR pathway is regulated by Ras, as well as various upstream growth factor receptors. The downstream transcription factors regulated by this pathway are indicated in diamond-shaped outlines. This drawing depicts some of the complicated regulations of this pathway by both positive and negative phosphorylation events, which serve to fine tune this pathway. Molecules depicted in black ovals, squares and triangles are either negative regulators (for example, GSK-3β, PTEN, Foxo, 4E-BP1) or inactivated by phosphorylation (for example, GSK-3β, β-catenin, Foxo, 4E-BP1), whereas those in white ovals are positive regulators of the PI3K/PTEN/Akt/mTOR pathway. Phosphorylation of some molecules by certain kinases (for example, phosphorylation of β-catenin by GSK-3β) results in their proteasomal degradation, whereas phosphorylation of some molecules by certain kinases (for example, β-catenin by Akt) results in their activation (nuclear translocation). This figure also illustrates that Akt can result in the activation of downstream mTORC1, which can subsequently serve as either a negative feedback to inactivate Akt by $p70^{S6K}$ or activate Akt by Rictor. GF = growth factor; GFR = growth factor receptor. Sites where various small molecule inhibitors suppress this pathway are indicated by black octagons. Drugs that have been evaluated to suppress this pathway (many in clinical trials, see below) are indicated in open boxes. Drugs that have been approved to treat cancer patients (not necessarily in leukemia patients) include: Sutent, Celecoxib, Imatinib, Nilotinib, Dasatinib, Sirolimus, Torisel and Afinitor. Drugs in clinical trials to treat cancer patients (not necessarily in leukemia patients) include: Midostaurin, Semaxanib, Lestaurtinib, Tandutinib, PX-866, XL-147, GDC-0941, OSU-03012, Perifosine, MK-2206, Tipifarnib, Lonafarinib, NVP-BEZ235, GSK1059615, AZD-9044, OSI-027, INK-128 and Ridaforolimus.

specifically PtdIns(3,4,5)P_3, that attract a series of kinases to the plasma membrane, thereby initiating the signaling cascade.[2]

The genes encoding the PI3K catalytic and regulatory chains are members of multi-gene families. Historically, the p110α isoform has been the best-characterized catalytic subunit; however, it has been recently shown that the p110δ isoform may play a larger role in leukemogenesis[3–5] and tumor surveillance than expected previously. The activation of the PI3Kδ isoform does not appear to be due to genetic mutations.[6] Importantly, PI3Kδ specific inhibitors (for example, IC87114) have been developed.[7]

Downstream of PI3K is the primary effector molecule of the PI3K signaling cascade, Akt/protein kinase B.[8–11] Akt contains an amino-terminal pleckstrin homology domain that serves to

target the protein to the membrane for activation.[10] Within its central region, Akt has a large kinase domain that is flanked on the carboxy terminus by hydrophobic and proline-rich regions.[11] Akt is activated via phosphorylation of two residues: T308 and S473.

Phosphoinositide-dependent kinase 1 (PDK1) is responsible for phosphorylation of T308.[12] Akt is also phosphorylated on S473 by the mTOR complex 2 referred to as mTOR complex2 (mTORC2)[2] (see Figure 1). Therefore, phosphorylation of Akt is somewhat complicated as it is phosphorylated by a complex that lies downstream of activated Akt itself.[2] Once activated, Akt leaves the cell membrane to phosphorylate intracellular substrates.

After activation, Akt is also able to translocate to the nucleus,[2] in which it affects the activity of a number of transcriptional regulators. CREB,[13] E2F,[14] nuclear factor κ from B cells via inhibitor κ B protein kinase α (Iκ-Kα)[15] and the forkhead transcription factors.[16]

These are all either direct or indirect substrates of Akt and each can promote either cellular proliferation or survival. Aside from transcription factors, Akt is able to target a number of other molecules to affect the survival state of the cell, including: inhibitor kappa B protein kinase α (this is an example of molecules that are activated by Akt phosphorylation), the pro-apoptotic molecule Bcl-2-associated death promoter (BAD)[17] and glycogen-synthase kinase-3β (GSK-3β)[18] (these are examples of molecules that are activated by Akt phosphorylation). When these targets are phosphorylated by Akt, they may either be activated (inhibitor kappa B protein kinase α) or inactivated (BAD and GSK-3β), but the end result is to promote survival of the cell.

Negative regulation of the PI3K pathway is accomplished primarily through the action of the PTEN tumor suppressor protein. We will discuss the regulation of PTEN expression in more detail than other signaling molecules as *PTEN* is an important tumor suppressor gene, which is frequently inactivated in human cancer and its inactivation has also been shown to have important roles in the generation of leukemia stem cells (see below). *PTEN* encodes a lipid and protein phosphatase whose primary lipid substrate is PtdIns(3,4,5)P_3.[2,19–22] The purported protein substrate(s) of PTEN are more varied and controversial, including focal adhesion kinase, the Shc exchange protein and the transcriptional regulators ETS-2, CREB and Sp1 and the platelet-derived growth factor receptor as well as others.[23,24] PTEN has been recently shown to have nuclear activities and it may serve to dephosphorylate some transcription factors such as ETS-2, Sp1, CREB and others.

PTEN has four primary structural domains. On the amino terminus is the lipid and protein phosphatase domain, which is flanked to the C2 domain that is responsible for lipid binding and membrane localization. Next are two protein sequences rich in proline (P), glutamic acid (E), serine (S) and threonine (T) domains that regulate protein stability. Lastly, PTEN has a PDZ domain, which helps facilitate protein–protein interactions. PDZ is an acronym derived from the first three proteins determined to have this domain (post-synaptic density protein (PSD95), *Drosophila* disc large tumor suppressor (DlgA) and zonula occludens-1 protein (zo-1). Mutations within the phosphatase domain have been reported to nullify the endogenous function of PTEN.[21,22,25,26] Thus, PTEN is an enticing therapeutic target for activation as it is frequently inactivated in many human cancers through point mutations as well as other means (for example, promoter hypermethylation, gene deletion) and its inactivation results in elevated Akt activity and abnormal growth regulation.[21,22,27,28] Moreover, PTEN can be inactivated by phosphorylation and oxidation in human cancer and which results in elevated Akt activity and abnormal growth regulation.[29] Some cells become therapy resistant by inactivation of PTEN.[30] Thus, reactivating PTEN could potentially be very useful in those tumors driven by PTEN inactivation.

Subtle reductions in PTEN levels, without entire homologous gene deletion or silencing, can lead to cancer.[31,32] Many previous studies that have looked at PTEN protein levels by western blot analysis and assumed that the cells were 'wild type' for PTEN are probably misleading. Maintaining adequate PTEN levels are probably critical to prevent neoplastic transformation and invasiveness. Recently, it has been determined that there are microRNAs (miRNAs) (for example, the miR-106b∼25 miRNA cluster) that regulate PTEN expression.[33] These and other miRNAs (see below) are detected at enhanced levels in some transformed and drug-resistant cells. A critical concept concerning miRNA is the imperfect base pairing with their target mRNA, which allows a single miRNA to target more than one mRNA. This leniency in specificity allows a single miRNA to target a class of mRNAs, which may all be involved in some crucial aspect of growth regulation. For example, miR-19 suppresses PTEN, protein phosphatase PP2A, Bim and AMP-activated kinase.[34]

Furthermore, there may be more than one miRNA binding site on a particular mRNA. Thus in certain scenarios, the mRNA may have a single miRNA bound, whereas in other growth situations where additional miRNAs are expressed, there may be multiple miRNAs directed to the mRNA, totally extinguishing the function of the mRNA. The genes encoding miRNAs can suffer genomic alterations in cancer, which can lead to their aberrant expression in human cancers.[35,36] This can result in the silencing of tumor suppressor genes such as PTEN. Some miRNA arise from miRNA precursors possessing a single hairpin structure (for example, miR∼21), whereas other miRNAs are contained within a poly-cistronic structure (for example, miR∼17–92). There are multiple miRNAs that can target PTEN. These include: miR∼17–92, miR∼19a, miR∼21, miR∼26a, miR-106b∼25, miR1∼155, miR1∼214, miR1∼216a and miR1∼217. The miR-106b∼25 cluster has also been discovered to be a PTEN-targeting intron, which cooperates with the *mini chromosomal maintenance-7* (*MCM7*) gene in the transformation of prostate and other cells.[33] *MCM7* is one of six members of the MCM family. This is a family of DNA helicases that are essential for initiation of DNA replication. *MCM7* expression is often increased in human cancers, sometimes due to genomic amplification. c-Myc is frequently involved in chromosomal translocations in lymphoma cells and overexpressed in other cancer types.[37,38] *MCM7* transcription is regulated by both c-Myc and N-Myc.[39–41] Thus, these miRNAs targeting PTEN will most likely be shown to have roles in therapy resistance in leukemia.

Pseudo-PTEN and other oncogenes decoys for miRNAs

The genome of higher eukaryotes contains many pseudogenes.[42] For years the biological functions of pseudogenes have been obscure, but recently it was shown that some have functions as decoys for miRNAs.[43] The pseudo-PTEN gene (PTENP1) can serve as a decoy to target the binding of miRNAs specific for PTEN. Thus, the PTEN pseudogene can serve as a tumor suppressor gene. The PTEN pseudogene, like PTEN, is selectively lost in human cancer. The PTEN pseudogene is biologically active as it serves to regulate the levels of PTEN by enticing the binding of miRNAs, which would normally

depress PTEN expression. This decoy activity permits normal PTEN mRNAs to be translated into functional PTEN proteins. The 3′-untranslated region (3′-UTR) of the PTEN pseudogene has tumor suppressive activity. This was determined by overexpression studies followed by soft-agar cloning experiments. Importantly, the tumor suppressor effects of the PTEN pseudogene were determined to be dependent on functional DICER activity, indicating that the 3′-UTR of the PTEN pseudogene requires mature miRNAs to exert its 'decoy' activity towards PTEN. Likewise, siRNAs directed to the PTEN pseudogene enhanced cellular proliferation. Actually the 3′-UTR of the PTEN pseudogene is a more potent growth suppressor than the 3′-UTR of the *PTEN* gene. The authors showed that the 3′-UTR of the PTEN pseudogene could affect other genes including $p21^{Cip\text{-}1}$. Thus as expected, the miRNAs can bind more than one target, and in this particular case, these miRNAs bind target mRNAs associated with growth suppression and they are bound by the 3′-UTR of the PTEN pseudogene.[43]

The 3′-UTR of PTEN can also derepress PTEN pseudogene expression, which will serve as feedback to enhance PTEN and other tumor suppressors. Suppression of the PTEN pseudogene will likely be shown to have effects on leukemia. Other oncogenes such as *KRAS* also have pseudogenes (for example, *KRAS1P*). The 3′-UTR of *KRAS1P* increased KRAS mRNA and protein expression and accelerated cell growth of prostate cancer cells. Overall, these results indicate a novel mechanism of growth regulation, pseudogenes serving as decoys for miRNAs. These results also indicate that genomic and proteomic approaches should also include analysis of the levels of pseudogene expression. In terms of lymphoid leukemia, this is also relevant, as there are many pseudoimmunoglobulins and T-cell receptor genes or gene segments.

PTEN mRNA expression can be regulated by diverse mechanisms. These include promoter hypermethylation, transcription factors and miRNAs. Regulation of PTEN mRNA has been shown to play critical roles in many different types of cancers. PTEN transcription is also regulated by histone acetylation/deacetylation. The SALL4 transcription factor is involved in the maintenance of pluripotency and self-renewal of embryonic stem cells.[44–46] There are numerous transcription factors that bind the PTEN promoter region to regulate transcription. Various transcription factors can have positive (for example, Egr-1, Atf2, p53, CBP/p300, PPARγ, CBF-1) or negative effects (for example, nuclear factor kappa from B cells, Snail1, Bmi-1, c-Jun, p53, PPARγ, CBF-1) on PTEN transcription. The Egr-1 transcription factor normally stimulates PTEN transcription. CBF-1 can either negatively or positively regulate PTEN transcription, depending on which other transcription factors it is associated with. The interactions between p53 and PTEN are complex. High p53 levels promote PTEN proteasomal degradation.[47] However, PTEN can also regulate p53 levels and activity.[48] Regulation of PTEN and p53 levels is critical for the prevention of leukemia. Often it may be more advantageous for the tumor to maintain heterozygosity of these genes rather than total homologous deletion.[49]

There are many mechanisms by which the PTEN protein can be modified, which will alter its activity. When PTEN is polyubiquitinated, it can be targeted for destruction through the proteasome pathway. The NEDD4-1 and XIAP ubiquitin E3 ligases ubiquitinate PTEN.[50–52]

PTEN phosphatase activity is also regulated by oxidization. At the center of PTEN's phosphatase catalytic site is a cysteine nucleophile, which is subject to oxidization.[53,54] PTEN oxidization occurs in response to reactive oxygen species (ROS), which can be generated by many different sources including chemotherapeutic drugs used to treat leukemia patients. PTEN oxidization has been proposed to contribute to the development of T-cell acute lymphoblastic leukemia (T-ALL), which displays abnormally high levels of ROS.[27,55]

The peroxidase peroxiredoxin1 (Prdx1) gene product regulates PTEN in response to ROS.[56] In Prdx1-deficient mouse fibroblasts and mammary epithelial cells, Akt is hyperactive. Prdx1 was shown to be essential for the lipid phosphatase activity of PTEN and Prdx1 binding to PTEN is essential for protecting PTEN from oxidation-induced inactivation. The ability of Prdx to suppress Ras- or Erb2-induced tumors was determined to be mediated by PTEN. In summary, there are a growing number of proteins that have been shown to interact and either inhibit or suppress PTEN function. Proteins that activate PTEN function include: PICT1, RAK and Prdx1. Proteins that inhibit PTEN function include: NEDD4-1, P-Rex2 (phosphatidylinositol 3,4,5-triphosphate RAC exchanger 2a) and SIPL1 (Shank interacting protein-like 1). All of these proteins have effects on transformation and most likely will influence therapy sensitivity/resistance.

PTEN is also regulated by protein phosphorylation. PTEN is constitutively phosphorylated at a series of serine (S) and threonine (T) residues at the C terminus (S380/T382/T383/S385). These phosphorylated residues serve to lock PTEN in a stable closed conformation, which reduces both its membrane localization and lipid phosphatase activity.[57,58] There are multiple kinases that phosphorylate PTEN, including casein kinase 2, and GSK-3β, which may occur at other residues including T366 and S370.[55,59,60] Proteins such as PICT1 and RAK were initially identified as proteins that bind PTEN. These proteins may promote PTEN phosphorylation. These proteins may also be considered tumor suppressor proteins, as decreasing their expression enhances malignant transformation. RAK is a tyrosine kinase and may phosphorylate PTEN directly at Y336.[61] The function of these proteins may be lost in certain tumors and inhibition of their expression has been associated with tumorigenicity.[62,63]

Other proteins interact with PTEN and inhibit its activity. P-Rex2 and SIPL1 bind to PTEN directly and inhibit its lipid phosphatase activity.[64,65] Both of these proteins can stimulate cell growth, at least in part, through their effects on PTEN. P-Rex2a inhibits PTEN lipid phosphatase activity and stimulates the PI3K pathway in the presence of PTEN, whereas knockdown of SIPL1 reduces cell growth in the presence and absence of PTEN, indicating that SIPL1 has other targets besides PTEN. P-Rex2a is a component of the PI3K/PTEN/Akt signaling pathway and antagonizes PTEN activity. It is a guanine nucleotide exchange factor for the RAC guanosine triphosphate binding protein and critical for RAC-mediated formation of ROS in response to the activation of certain receptors.

Mutations that result in partial protein truncation will affect PTEN function, even if present in a heterozygotic state. Diverse mechanisms of regulation may affect the half-life of the PTEN protein as well as its activity and substrates. PTEN may have different roles depending on where in the cell it is localized. For example, when PTEN is nuclear localized, its effects are very diverse and independent of Akt. PTEN has been shown to interact with many proteins in the nucleus including MSP58, which is a proto-oncogene and can transform cells in PTEN-deficient mice.[66] Clearly, the nuclear activities of PTEN are diverse. Continued elucidation of the roles of PTEN in the nucleus will help us to unravel the various functions of this essential tumor suppressor gene. It has been proposed that both casein kinase 2 and γ-secretase inhibitors (which alter Notch

cleavage and activation) could be of therapeutic value in T-ALL owing to their capability of affecting PTEN regulation.[55]

Another negative regulator of the PI3K pathway is the pleckstrin homology domain leucine-rich repeat protein phosphatase. Pleckstrin homology domain leucine-rich repeat protein phosphatase dephosphorylates S473 on Akt, which induces apoptosis and inhibits tumor growth.[67] Two other phosphatases, *S*rc-*h*omology 2 domain-containing *i*nositol 5′*p*hosphatase (SHIP)-1 and SHIP-2, remove the 5-phosphate from PtdIns(3,4,5)P_3 to produce PtdIns(3,4)P_2.[68,69] Mutations in these phosphatases, which eliminate their activity, can lead to tumor progression. Consequently, the genes encoding these phosphatases are referred to as antioncogenes or tumor suppressor genes.

Downstream targets of Akt regulating mTOR activity

Next, we will discuss some of the important downstream targets of Akt that can also contribute to abnormal cellular growth and are important therapeutic targets. Some of these targets and how they interact with the PI3K/PTEN/Akt/mTOR pathways are indicated in Figure 2. Also presented in this figure are the sites of action of mTOR inhibitors, some of which have been evaluated in leukemia.[70,71]

Akt inhibits tuberous sclerosis 2 (TSC2 or hamartin) function through direct phosphorylation.[72] TSC2 is a GTPase-activating protein that functions in association with the putative TSC1 (or tuberin) to inactivate the small G-protein Rheb.[73]

TSC2 phosphorylation by Akt represses the GTPase-activating protein activity of the TSC1/TSC2 complex, allowing Rheb to accumulate in a GTP-bound state. Rheb-GTP then activates, through a mechanism not yet fully elucidated, the protein kinase activity of mTOR when complexed with the Raptor (Regulatory-associated protein of mTOR) adaptor protein and mLST8, a member of the Lethal-with-Sec-Thirteen gene family, first identified in yeast.[2] DEPTOR is also a component of this complex. DEPTOR is an mTOR inhibitor that is overexpressed in multiple myeloma cells and is essential for their survival.[74] The mTOR/Raptor/mLST8/DEPTOR complex (mTORC1) is sensitive to rapamycin and, importantly, inhibits Akt via a negative feedback loop, which involves, at least in part, $p70^{S6K}$.[75] This is due to the negative effects that $p70^{S6K}$ has on insulin receptor substrate-1 (ref. 75) (see Figure 2).

The mechanism by which Rheb-GTP activates mTORC1 requires Rheb farnesylation and can be blocked by farnesyl transferase inhibitors. It has been proposed that Rheb-GTP would relieve the inhibitory function of FKBP38 (another component of mTORC1) on mTOR, thus leading to mTORC1 activation.[76–81] However, more recent investigations did not confirm these findings.[76–81]

Nevertheless, Akt also phosphorylates proline-rich Akt-substrate-40 (PRAS40), an inhibitor of mTORC1, and by doing so, it prevents the ability of PRAS40 to suppress mTORC1 signaling (recently reviewed in refs.[2,79–81]). Thus, this could be yet another mechanism by which Akt activates mTORC1. Moreover, PRAS40 is a substrate of mTORC1 itself, and it has been shown that mTORC1-mediated phosphorylation of PRAS40 facilitates the removal of its inhibition on downstream signaling of mTORC1.[2,79–81]

The relationship between Akt and mTOR is further complicated by the existence of mTORC2, which in some cell types, displays rapamycin-insensitive activity.[76] Akt and mTOR are linked to each other via positive and negative regulatory circuits, which restrain their simultaneous hyperactivation through a mechanism involving $p70^{S6K}$ and PI3K. Assuming that equilibrium exists between these two complexes, when the mTORC1 complex is formed, it could antagonize the formation of the mTORC2 complex and reduce Akt activity.[73–75] Thus, at least in principle, inhibition of the mTORC1 complex could result in Akt hyperactivation. This is one problem associated with therapeutic approaches using rapamycin that blocks some actions of mTORC1, but not all.

mTOR is a 289-kDa S/T kinase. It regulates translation in response to nutrients and growth factors by phosphorylating components of the protein synthesis machinery, including $p70^{S6K}$ and eukaryotic initiation factor (eIF)4E binding protein-1, the latter resulting in release of the eIF4E, allowing eIF4E to bind to the 5′ cap structure in the mRNA and participate in the assembly of a translational initiation complex (eIF4F), which also consists of an RNA helicase (eIF4A) and a large scaffolding protein (eIF4G) implicated in recruiting the 43S preinitiation complex (see Figure 2). Other factors such as eIF4B and eIF4H aid in the recruitment of ribosomes to the complex by stimulating the helicase activity of eIF4A.[2,79–81] Poly A binding protein may also be involved in assembly to the complex. 4E binding protein-1 acts to inhibit the assembly of the translation complex. The eIF4F complex is necessary for the translation of mRNAs containing long 5′-UTRs, which are highly structured and have a high G + C content. These mRNAs are considered 'weak mRNAs'. These weak mRNAs often encode genes involved in oncogenesis and survival such as c-Myc, Mcl-1, cyclin-D, vascular endothelial growth factor and survivin.

$p70^{S6K}$ phosphorylates the 40S ribosomal protein, S6, leading to active translation of mRNAs.[77,78] Integration of a variety of signals (mitogens, growth factors, hormones) by mTORC1 assures cell cycle entry only if nutrients and energy are sufficient for cell duplication.[77,78] By regulating protein synthesis, $p70^{S6K}$ and 4E-BP1 also control cell growth and hypertrophy, which are important processes for neoplastic progression. Hence, targeting the mTORC1 pathway could have many effects on the regulation of cellular growth.

mTORC1 also controls the translation of hypoxia-inducible transcription factor-1α (HIF-1α) mRNA. HIF-1α is the oxygen-sensitive component of the HIF-1 transcription factor complex. HIF-1α upregulation leads to increased expression of angiogenic factors such as vascular endothelial growth factor and PDGF.[82] Moreover, HIF-1α regulates the glycolytic pathway by controlling the expression of glucose-sensing molecules, including Glut 1 and Glut 3 (ref. 83). Rheb, mTOR, eIF4E and HIF-1α will be further discussed below as they all can modulate drug sensitivity.

Rationale for targeting the PI3K/PTEN/Akt/mTOR pathway to improve leukemia therapy

Effective targeting of signal-transduction pathways activated by mutations and gene amplification may be an appropriate approach to limit leukemia growth and drug resistance. The PI3K/PTEN/Akt/mTOR pathway can be activated by mutations/amplifications of upstream growth factor receptors. The abnormal production of growth factors (for example, insulin-like growth factor-1) can frequently result in receptor activation, which in turns activates PI3K/PTEN/Akt/mTOR signaling. An illustration of some of the receptors, exchange factors, kinases and phosphatases that are mutated/amplified in leukemia and how they may impact the PI3K/PTEN/Akt/mTOR cascade is presented in Figure 3.

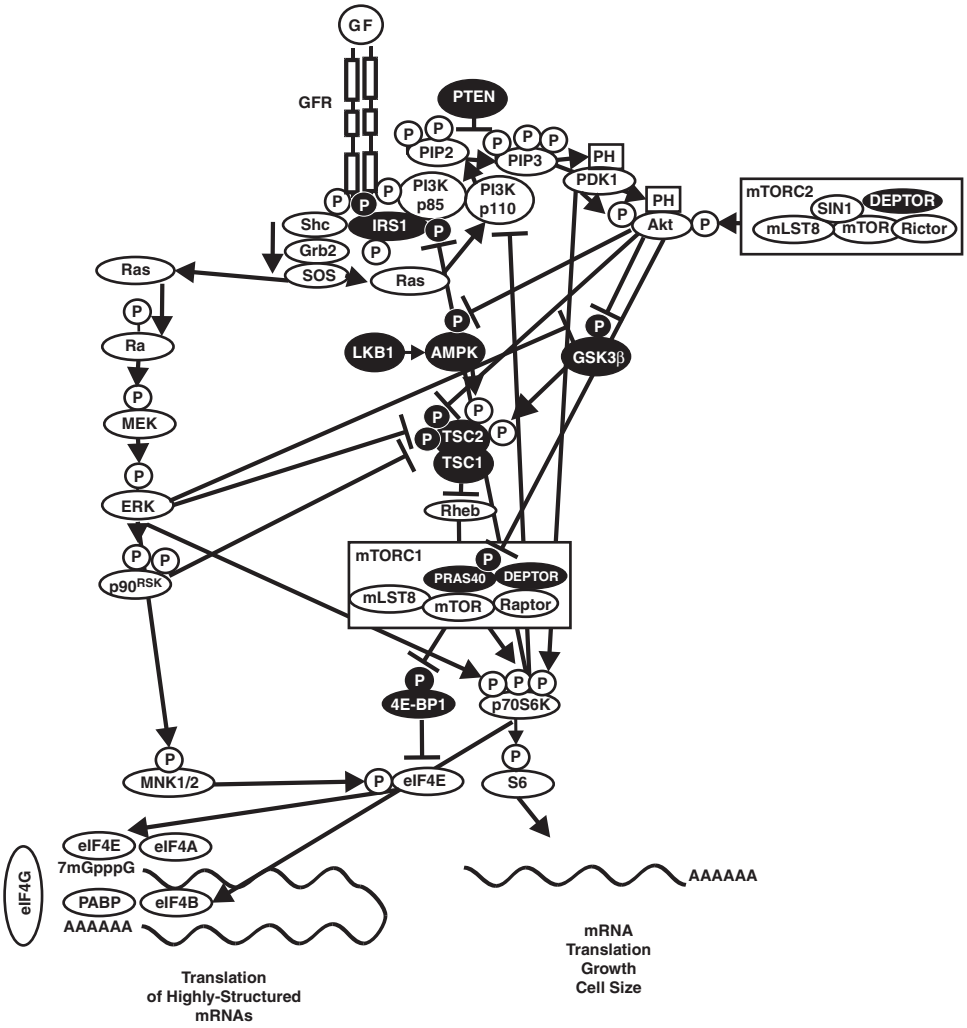

Figure 2 Interactions between PI3K/PTEN/Akt/mTOR pathways that result in the regulation of protein translation. The PI3K/PTEN/Akt/mTOR pathway can affect protein translation by complex interactions regulating the mTORC1 and mTORC2 complexes. Molecules depicted in black ovals are either negative regulators or inactivated by phosphorylation, whereas those in white ovals are positive regulators of the PI3K/PTEN/Akt/mTOR or Ras/Raf/MEK/ERK pathways. GF stimulation results in GFR activation, which can activate the pathway. GF = growth factor; GFR = growth factor receptor. Akt can phosphorylate and inhibit the effects of GSK-3β, TSC2 and PRAS-40, which result in mTORC1 activation. ERK and PDK1 can phosphorylate $p90^{Rsk1}$, which in turn can phosphorylate and inhibit TSC2. Rapamycin targets mTORC1 and inhibits its activity and also results in inhibition of downstream $p70^{S6K}$. The effects of rapamycin are complex, as long-term administration of rapamycin may prevent mTOR from associating with mTORC2, and hence full activation of Akt is prevented. However, rapamycin treatment may result in the activation of PI3K, by inhibiting the effects of $p70^{S6K}$ on insulin receptor substrate-1 phosphorylation, which results in PI3K and Akt activation. Also, rapamycin treatment may result in the activation of ERK in some cells, presumably by inhibition of the $p70^{S6K}$-mediated inhibition of insulin receptor substrate-1. These latter two effects of rapamycin could have positive effects on cell growth. Energy deprivation will result in the activation of *s*erine/*t*hreonine *k*inase 11 (STK11 a.k.a. LKB1) and AMP kinase (AMPK), which can result in TSC2 activation and subsequent suppression of mTORC1. In contrast, Akt can phosphorylate and inhibit the activity of AMPK. Inhibition of PDK1 activity can also result in the activation of mTORC1, presumably by suppression of p70S6K, and hence inhibition of insulin receptor substrate-1 effects on PI3K activity. The PTEN, TSC1, TSC2 and LKB1 tumor suppressor genes all converge on the mTORC1 complex to regulate protein translation. Thus, the Ras/Raf/MEK/ERK and PI3K/PTEN/Akt/mTOR pathways can finely tune protein translation and cell growth by regulating mTORC1. Rapamycin can have diverse effects on these processes.

Control of apoptotic regulatory molecules by the PI3K/PTEN/Akt/mTOR pathway and regulation of leukemia

This pathway regulates the activity of many proteins involved in apoptosis. Deregulated expression of apoptotic regulatory molecules can lead to leukemia. Akt phosphorylates transcription factors that influence the transcription of the Bcl-2 family of genes as well as other important genes involved in the regulation of apoptosis.[1,2,79,81] Furthermore, increased expression of Bcl-2 and Bcl-X_L and decreased expression of Bax is observed in some drug-resistant leukemias.[84–87]

Many of the effects of the PI3K/PTEN/Akt/mTOR pathway on apoptosis are mediated by Akt phosphorylation of important apoptotic effecter molecules (for example, Bcl-2, Mcl-1, BAD, Bim, Foxo, caspase-9 and many others).[88,89] PTEN can also regulate CREB activity, which can alter its ability to regulate the transcription of certain genes.[90] This has been shown to regulate chemotherapeutic drug sensitivity.[91] Bcl-2 expression can be regulated by activated CREB,[92] and the regulation of Bcl-2 by Akt and CREB is implicated in drug resistance.[93] Thus, mutations that eliminate PTEN activity could contribute to therapy resistance by deregulated CREB activity, leading to altered

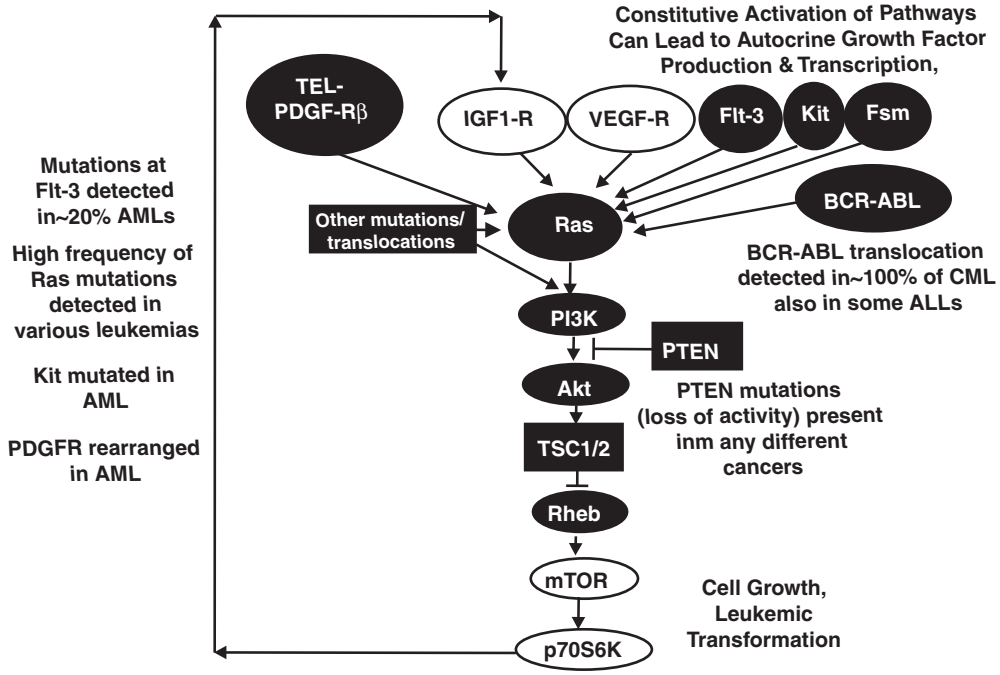

Figure 3 Dysregulated expression of upstream receptors and kinases can result in the activation of the PI3K/PTEN/Akt/mTOR pathway. Mutations have been detected in *FLT3*, *KIT*, *PDGF r*eceptor (*PDGFR*), *PIK3CA*, *PTEN* and *RAS*. The *BCR-ABL* chromosomal translocation is present in virtually all chronic myeloid leukemias and some ALLs. Molecules depicted in black ovals or squares are mutated in leukemia, whereas those in white ovals are not frequently mutated in leukemia. Many of these mutations and chromosomal translocations result in the activation of the PI3K/PTEN/Akt/mTOR cascades. These pathways can also be activated by autocrine growth stimulation, the genetic basis of which is frequently unknown.

Bcl-2 family gene expression. Originally most scientists believed that the effects of PTEN mutations would be primarily mediated by enhanced Akt activation, which would phosphorylate proteins such as CREB and result in their enhanced activity. However, more recently, it has been proposed that the PTEN protein phosphatase activity is also important in regulating CREB phosphorylation in the nucleus.

Aberrant regulation of apoptosis is critically implicated in leukemia as well as many other diseases (for example, inflammation, autoimmune diseases). Therefore, controlling the activity of the PI3K/PTEN/Akt/mTOR pathway has been a keen pharmaceutical objective for many years. The activity of many important components in apoptotic cascades could be sensitive to both inhibitors that target this pathway.

Akt regulates the apoptotic response to a variety of stimuli by its ability to interact with a number of important players in the apoptotic process. Akt can directly phosphorylate BAD on S136 (ref. 94,95), causing its inactivation and inability to interact with antiapoptotic members of the Bcl-2 family of proteins (Bcl-2, Bcl-X_L).[96,97] Activated Akt can inhibit the release of cytochrome *c* from the mitochondria, which is a potent activator of the apoptotic caspase cascade.[97] Certain Bcl-2 inhibitors (Abt-737) may sensitize chronic lymphocytic leukemia and chronic myeloid leukemia cells to chemotherapy, implicating Bcl-2 family members that are sensitive to Abl-737 (for example, Bcl-2 and Bcl-XL, but not Mcl-1) in their drug resistance.[98,99] Bcl-2 inhibitors sensitize B lymphoma cells to rituximab, a chimeric monoclonal antibody that targets CD20 (ref. 100). Bcl-2 inhibitors also sensitize various lymphoid malignancies to proteasome inhibitors.[101] Resistance to fludarabine may also be mediated by other Bcl-2 family members such as increased expression of Mcl-1, which is associated with a poor prognosis.[102,103] Development of specific Mcl-1 inhibitors is in progress based on the crystal structure of Mcl-1.[104,105] However, Mcl-1 confers less protection against chemotherapy than Bcl-2. This has been determined with heavy-chain *e*nhancer μ region-Bcl-2 (Eμ-Bcl-2) and Eμ-Mcl-1 mice[106] (see below for more details on studies with Eμ transgenic mice). This may be due to the effects of mTORC1 as well as extracellular-regulated kinase (ERK).

An important molecule downstream of the PI3K/PTEN/Akt/mTOR cascade is the BH3 domain containing antiapoptotic protein Bim. Bim's activity is regulated by phosphorylation at different residues by Akt, ERK and c-Jun N-terminal kinase.[107–109] When Bim is phosphorylated by Akt and ERK, it is targeted for proteasomal degradation and also inhibits Bim's interaction with Bax, a death executioner protein. In contrast, when Bim is phosphorylated by c-Jun N-terminal kinase, it has enhanced pro-apoptotic activity.[110] Bim is also transcriptionally regulated by Foxo-3a (a transcriptional factor), which is also a target of Akt. Normally when Akt is active, it phosphorylates and inhibits the activity of Foxo-3a. Upon cytokine withdrawal of hematopoietic cells, Foxo-3a is not phosphorylated and enters the nucleus and stimulates the transcription of genes such as Bim and others, which results in apoptosis.[111]

Bim levels can also be regulated by epigenetic silencing. This may be important in the sensitivity of ALL cells to glucocorticoids.[112] The glucocorticoid resistance observed in the xenografts and patient biopsies in this study correlated with decreased histone H3 acetylation. The authors showed that the histone deacetylase inhibitor vorinostat relieved Bim repression and also exerted antileukemic effects when combined with dexamethasone *in vitro* and *in vivo*. These studies suggest a new approach to overcome glucocorticoid resistance and improve therapy for high-risk pediatric ALL patients.

Bim protein levels are also regulated by stromal cell interactions. When leukemia cells are attached to stroma and the β1-integrin is activated, there is suppression of Bim expression and apoptosis is inhibited and drug resistance is increased. Bim is targeted to the proteasome and degraded.[113] Proteasomal inhibitors suppressed Bim degradation and rendered the cells sensitive to therapy. The authors of this study suggested that β1-integrin-mediated stromal interactions with leukemia cells and subsequent Bim protein degradation may contribute to minimal residual disease. The tumor microenvironment can regulate drug resistance, perhaps due to the presence of cytokines that stimulate antiapoptotic Bcl-2 and other factors that prevent apoptosis.[114–116]

Interactions between BCR-ABL and Bim in leukemia

BCR-ABL-transformed hematopoietic precursor cells have low levels of Bim and are refractory to the induction of apoptosis after cytokine withdrawal.[117,118] BCR-ABL induces ERK activation, and thus Bim is not targeted to the proteasome and is active. Therefore, suppression of ERK by either imatinib or dasatinib results in the suppression of Bim phosphorylation. Thus, these drugs are proposed to exert some of their inhibitory effects by induction or enhancement of Bim activity. Knockdown of Bim by siRNA abrogates imatinib-induced cell death in chronic myeloid leukemia cells.[119] Imatinib induces Foxo-3a in BCR-ABL-transformed cells, which subsequently stimulates Bim transcription and apoptosis.[120] Imatinib induces the transcription of Bim and Bmf and also augments the post-translational modification favoring apoptosis via Bim and BAD. Bcl-2 inhibitors can overcome resistance to imatinib owing to decreases in Bim and BAD levels.[119]

PI3K/PTEN/Akt/mTOR, drug resistance and leukemia therapy

The PI3K/Akt pathway can regulate molecules involved in drug resistance such as the Mdr-1/PgP molecule. Targeting Akt can reduce the drug resistance of the cells.[121–125] In some cell types, Akt can inactivate ERK, which leads to altered responses to chemotherapeutic drugs.[125]

Akt can phosphorylate and inactivate the transcription factor Foxo-3a. High levels of phosphorylated Foxo-3a have been shown to be an adverse prognostic indicator in acute lymphoblastic leukemias (AML).[126,127]

Foxo-3 is capable of upregulating Fas ligand and Bim, two very important molecules that are potent inducers of apoptosis; however, when inactivated by Akt, Foxo-3 is localized to the cytosol where it is unable to augment expression of these genes.[29,64] Mutant receptors such as Fms-like tyrosine kinase may induce Foxo-3a inactivation, which can lead to resistance to Fms-like tyrosine kinase inhibitors and result in a poor prognosis.[128,129] A diagram illustrating some of the effects of the PI3K/PTEN/Akt/mTOR pathway on apoptosis and drug resistance is presented in Figure 4.

Recently, it has been shown that Foxo-3a is inactive in AML cells and is localized in the cytoplasm. Treatment of AMLs with MEK and PI3K/Akt inhibitors did not result in the nuclear translocation of Foxo-3a, in which it could potentially induce cell cycle inhibitory and apoptotic genes. In contrast, it was shown that IκK controlled Foxo-3A expression. Treatment of cells with the IκK-specific inhibitor NEMO resulted in the nuclear translocation of Foxo-3a and induced the expression of $p21^{Cip-1}$ and Fas.[130]

Foxo-3a can induce the expression of *PIK3CA* gene which can contribute to drug resistance of erythroleukemia K562 cells.[131] In this case, the unphosphorylated Foxo-3a pool is sufficient to drive the transcription of *PIK3CA* even though Akt will be subsequently activated, which will result in some phosphorylated Foxo-3a. This group pointed out that normally Foxo-3a activity would be transient and Akt phosphorylation would result in inactivation of Foxo-3a; however, in the drug resistant lines, this homeostatic mechanism was disrupted and the active Foxo-3a was able to induce *PIK3CA* transcription. Additional studies by this same group showed that doxorubicin can induce Foxo-3a expression, which subsequently stimulated Mdr-1 expression in this same model system.[132] There are other mechanisms of regulation of Foxo-3a besides Akt.[100] c-Jun N-terminal kinase, which is induced by doxorubicin treatment, can phosphorylate Foxo-3a on residues that are dominant to those residues on Foxo-3a phosphorylated by Akt.[133] Furthermore, the nicotinamide adenine dinucleotide-dependent deacetylase Sirt1 regulates Foxo-3a expression and drug resistance.[134–136] These and other studies indicate the complex feedback loops involved in drug resistance.[137] Sometimes inactivation of Foxo-3a mediated by Akt phosphorylation is associated with drug resistance and a poor prognosis, whereas in other scenarios, Foxo-3a can induce the expression of genes such as *ABCB1* (that encodes Mdr-1) and *PIK3CA* and lead to drug resistance.[138] Thus in some cases, activation or inactivation of a protein such as Foxo-3a is associated with drug resistance and response to therapy, which may vary depending on the particular cell type or treatment conditions.

mTOR and leukemia therapy

mTOR is an important molecule that is being targeted in many different cancer types.[139] The constitutive action of the mTORC1 pathway has been detected in over 90% of primary AML samples tested in some surveys.[140] In many AMLs, mTORC1 activation may be dependent on an autocrine insulin-like growth factor-1/insulin-like growth factor-1 receptor loop.[141,142]

It is emerging that protein synthesis in some AMLs is resistant to rapamycin and that other targeting approaches should be developed.[140] Indeed, it has been shown that the 4E-BP1 protein was phosphorylated indirectly by events mediated by the Pim-2 (proviral integration Moloney murine leukemia virus) serine/threonine on S65, which contributed to the rapamycin resistance of the cells. This phosphorylation event prevented the assembly of the eIF4F complex. In subsequent studies, they showed the effectiveness of inhibitors that directly target the kinase activity of mTOR. Thus, targeting mTOR more globally is becoming an important therapeutic option in AML therapy.[139,143–147]

Studies by other investigators performing high-throughput screening assays have identified 4EGI-1, which is a 4E-BP1 mimetic and a potent inhibitor of the interactions between eIF4E and eIF4G. The inhibitor 4EGI-1 has been shown in some systems to inhibit the growth of BCR-ABL-transformed Ba/F3 hematopoietic cells, but not the parental interleukin-3-dependent cells line (Ba/F3).[148] The specificity of the inhibitor 4EGI-1 was also examined on AML blasts and normal $CD34^+$ hematopoietic precursor cells and it was shown that the inhibitor 4EGI-1 induced apoptosis in the AML blasts, but had much less effects on the differentiation and survival of normal $CD34^+$ cells.[140]

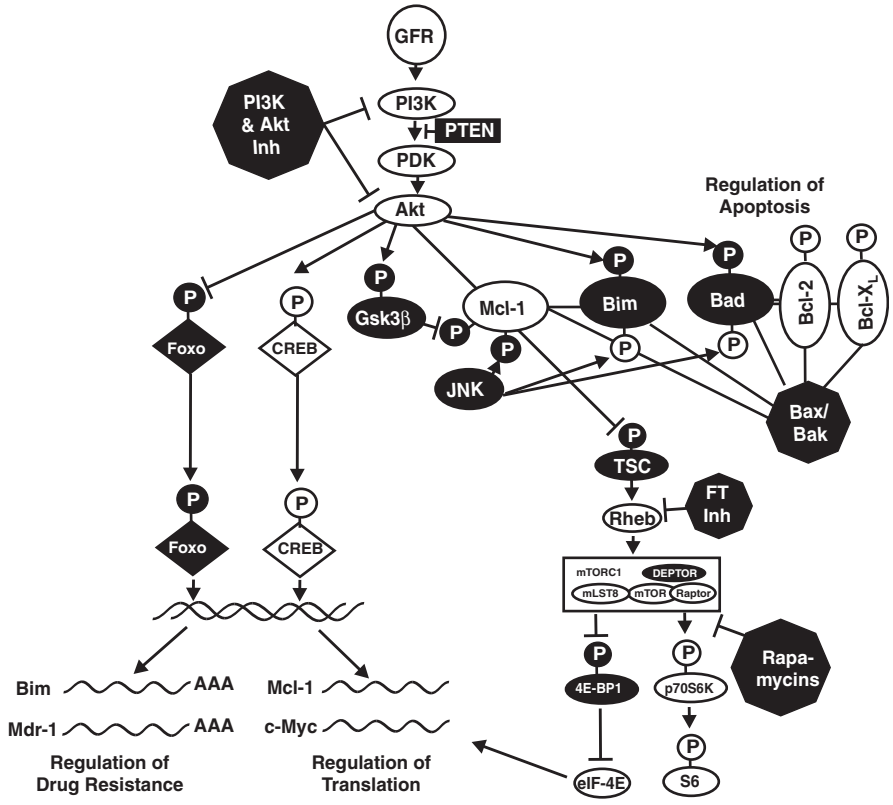

Figure 4 Effects of targeting PI3K/PTEN/Akt/mTOR pathways on the regulation of apoptosis and drug resistance. The PI3K/PTEN/Akt/mTOR pathways are critically involved in the regulation of many proteins involved in apoptosis and drug resistance. Molecules depicted in black ovals, octagons and triangles are either negative regulators or inactivated by phosphorylation, whereas those in white ovals or triangles are positive regulators of the PI3K/PTEN/Akt/mTOR pathway. Inhibitors targeting these pathways can have significant effects on the induction of apoptosis and drug resistance. Inhibitors are depicted in black octagons with white lettering. Inhibitors may alter post-translational phosphorylation of apoptotic molecules or the translation of important molecules involved in apoptosis, cell survival and drug resistance.

Other approaches to targeting the mTORC1/mTORC2 complexes are being developed. The diabetes drug metformin activates LKB1/AMP kinase/TSC2, which in turn inhibits mTOR activity. This results in dephosphorylation of 4E-BP1, which in turn inhibits eIF4F assembly. This inhibition suppresses the translation of the 'oncogenic' mRNAs.[149]

The antiviral drug ribavirin can block the binding of eIF4E to mRNA.[150,151] Thus, the effects of this compound were examined in some patients with relapsed M4/M5 AML who were no longer eligible for chemotherapy. Although the number of patients in this preliminary trial were low ($n=11$), one complete and two partial responders were observed.[152]

The Eμ-Myc mouse lymphoma model has been used to evaluate the roles of many of the important genes in the PI3K/PTEN/Akt/mTOR pathway in their abilities to interact and enhance lymphomagenesis and result in sensitivity to targeted therapies. Elevated active Akt (Akt-Myr$^+$) expression accelerated Eμ-Myc-induced lymphomagenesis and promoted chemotherapeutic drug resistance and sensitivity to rapamycin in Eμ-Myc mice.[77] Some of the experiments were performed by retroviral transfer of the various genes (Akt-Myr$^+$, Rheb, eIF4E) into hematopoietic stem cells (HSC) from the Eμ-Myc mice, followed by adoptive transfer into irradiated recipients. Other experiments were performed by genetic crosses (for example, between Eμ-Myc and PTEN$^{+/-}$ or TSC2$^{+/-}$ mice). In both types of experiments, the effects of often mutants (dominant negative or inactive) and other genes in the pathway were also examined. Many of the genes (for example, Akt-Myr$^+$, PTEN, eIF4E, TSC2, Rheb and Bcl-2) were observed to complement Eμ-Myc and are believed to suppress the pronounced apoptosis induced by Eμ-Myc. With the exception of Bcl-2, all of the above-mentioned complementing genes affect protein translation. Increased c-Myc expression can induce apoptosis, but prevent cellular senescence. In contrast, enhanced Akt, eIF4E, TSC2, Rheb and of course Bcl-2 expression can prevent the induction of apoptosis. This is believed to be a reason for the cooperation between the oncogenes, prevention of apoptosis and cellular senescence. Although a pronounced acceleration in lymphomagenesis or loss of heterozygosity was not observed in PTEN$^{+/-}$Eμ-Myc mice, the resulting tumors were resistant to doxorubicin and sensitive to rapamycin.[153]

mTORC1 is also involved in drug resistance. Rapamycin was initially shown to inhibit insulin-like growth factor-1 signaling in rhabdomyosarcoma cells.[154] Rapamycin resistance in these cells was postulated to be due to low levels of the 4E-BP1, which normally serves to sequester eIF4B and prevents its effects on protein translation. Rapamycin resistance has also been linked to increases in eIF4E expression, decreases in 4E-BP1 expression and enhanced 4E-BP1 phosphorylation.[155,156] Rapamycin may sensitize some cells to chemotherapy by inhibition of p21^{Cip-1} translation.[157]

PI3K/PTEN/Akt/mTORC1/eIF4E translation initiation/elongation and leukemia therapy

Recently, it has become apparent that the PI3K/PTEN/Akt/mTORC1 pathway can also influence leukemia therapy by

controlling translation initiation. Deregulation of Akt/PTEN expression in various cancers has been shown to make the cells or tumors resistant to chemotherapy, yet hyper-responsive to the mTOR inhibitor rapamycin.[30,153,158,159]

Many 5′-UTR on mRNAs involved in cell survival are G + C rich and highly structured and can affect leukemic transformation. To translate these survival mRNAs efficiently requires the assembly to a translation complex, which binds to the 5′-UTR.[160] eIF4E is a component of the eIF4F complex, which stimulates ribosome recruitment. This translation complex is comprised of eIF4E, the RNA helicase eIF4A and its activator eIF4B, which serve to unwind the 5′-UTR. Other proteins are also involved, eIF4G serves as a scaffold necessary for assembly of the complex and eIF2α is a negative regulator that can block translation if it is phosphorylated by protein kinase R. eIF4E is the critical rate-limiting factor in the formation of the complex and its effects on translation of the survival mRNAs with their unique 5′-UTRs. Ectopic overexpression of eIF4E can transform certain cells in culture and cooperate with Eμ-Myc in the induction of lymphomatogenesis and induced chemotherapeutic drug resistance.[77] However, overexpression of eIF4E, which is downstream of mTORC1, does not confer sensitivity to the mTORC1 inhibitor rapamycin; in fact, the tumors are rapamycin resistant. Furthermore, overexpression of eIF4E in cancers that were previously rapamycin sensitive made them rapamycin resistant.[161]

The ability of eIF4E to enter the eIF4F complex is controlled by 4E-BP1, which are regulated by mTORC1 phosphorylation. Upon phosphorylation of 4E-BP1 by mTORC1, eIF4E disassociates from the 4E-BP1 (and others) and eIF4E can enter the eIF4F complex and stimulate transcription of the survival mRNAs with the unique 5′-UTRs. Recently, it has been shown that cyclopenta[*b*]benzofuran flavagines such as silvestrol can modulate eIF4A activity and inhibit translation initiation. Silvestrol and related compounds may be able to enhance chemosensitivity in cancers (for example, lymphomas), which grow in response to deregulated PTEN and downstream eIF4E.[162]

TSC2/Rheb/mTORC1 translation initiation/elongation and leukemia

Another gene that can synergize with Eμ-Myc to induce drug-resistant, rapamycin-sensitive lymphomas is TSC2 (ref. 163) As stated previously, the tumor suppressor TSC2 lies above mTORC1 in this pathway. One of the deregulated targets in the TSC2- (loss of heterozygosity)/Eμ-Myc lymphomas is Mcl-1 (ref. 164). Mcl-1 expression is induced and the mRNAs encoding Mcl-1 are translated by the increased activity of the mTORC1-eIF4E complex. This is a site where there is convergence of the PI3K/PTEN/Akt/mTOR and Raf/MEK/ERK pathways as Mcl-1 expression is regulated by eIF4E, which is in turn regulated by MNK1/2 phosphorylation. MNK1/2 is phosphorylated by ERK1/2. Mcl-1 can counteract some of the pro-apoptotic events induced by c-Myc in the Eμ-Myc mice and result in lymphomas.[161,163,164]

As mentioned before the Rheb GTPase is an activator of mTORC1. Rheb is highly expressed in some human lymphomas[31,165–167] and other cancers.[168] Rheb activity is dependent on farnesylation. The effects of enhanced expression of Rheb on the induction of lymphomas in the Eμ-Myc mouse system were examined. Introduction of Rheb into the Eμ-Myc mouse model enhanced the development of lymphomas. These lymphomas were more resistant to chemotherapeutic drugs such as doxorubicin. Rheb expression induced cellular senescence and treatment with rapamycin prevented the induction of senescence. In contrast, c-Myc expression blocked the induction of senescence. However, the combination of c-Myc expression and Rheb prevented the induction of senescence induced by Rheb and the apoptosis induced by c-Myc, which may explain the increased incidence of lymphomas in the Rheb/Eμ-Myc mice than in Eμ-Myc mice.[161,163,164]

This group has shown that the abilities of Ras, Akt and Rheb to activate cellular senescence are dependent, at least in part, on their ability to activate mTORC1. This effect may be owing to eIF4B activation as eIF4B also induces cellular senescence. MEK inhibitors also inhibit cellular senescence in some systems.[169] Thus, in some scenarios mTOR and MEK inhibitors may promote cell growth by preventing cellular senescence.[169,170]

Similar to previous experiments performed with the introduction of eIF4E into Eμ-Myc mice, the Rheb-Eμ-Myc mice also expressed high levels of the antiapoptotic Mcl-1 protein. Lymphomas that overexpress Rheb are sensitive to both farnesyltransferase inhibitors and rapamycin.[170] The effects of farnesyltransferase inhibitors on PTEN-deficient tumor cells are dependent on functional Rheb activity. In summary, in some cases, Rheb can be an oncogenic regulator of mTORC1 and eIF4E and Rheb is a direct target of farnesyltransferase inhibitors in cancer.

mTORC1-HIF drug resistance

HIF-1α has been proposed to be intimately involved in drug resistance by regulating the expression of genes involved with energy metabolism.[171] HIF-1α overexpression can induce chemotherapeutic drug resistance.[172] Suppression of HIF-1α can also influence the sensitivity to angiostatin, a naturally occurring inhibitor of angiogenesis.[173] This may occur in part by suppressing glucose transporter-1 and vascular endothelial growth factor expression,[173] although other investigators have observed that suppression of HIF-1α will also affect the expression of Bcl-2 family members such as Bid.[174] Thus, targeting mTOR expression would influence the expression of HIF-1α and may prevent drug resistance.

Suppressing leukemia stem cell growth: targeting the PI3K/PTEN/Akt/mTOR pathway

An area of intense interest in cancer biology is the cancer stem cell, more appropriately referred to as the cancer initiating cell (CIC). The PI3K/PTEN/Akt/mTOR pathway, and specifically PTEN levels, may be critical for the development of CICs.[175,176] The concept that the PI3K/PTEN/Akt/mTOR pathway serves as a therapeutic target in CICs is beginning to emerge. CICs have unique properties as they can be both quiescent and also resistant to chemotherapeutic- and hormonal-based drugs. Their inherent chemoresistance may be due to their increased expression of proteins involved in drug transport (Mdr-1 and others). However, under certain conditions, they resume proliferation and hence could be potentially susceptible to PI3K, Akt or mTOR inhibitors.

The *PTEN* gene exerts effects on CICs, especially in hematopoietic, breast and prostate cells. In conditional *PTEN* knockout mice, upon inactivation of *PTEN*, there is a transient increase in HSCs followed by a myeloproliferative disease, and the mice subsequently develop leukemia after 4–6 weeks.[175] If the mice are treated with rapamycin, the myeloproliferative

disorder and leukemia are prevented. The preleukemic cells that arise after conditional *PTEN* deletion by themselves are not able to induce leukemia upon transfer into severe combined immunodeficiency disease-recipient mice, but if the leukemic cells were derived from the *PTEN*-conditional mice that had already developed overt leukemia, they were able to transfer leukemia to the severe combined immunodeficiency disease-recipient mice (thus by definition they are CICs), which could be prevented by rapamycin treatment. Also, the normal HSCs from the *PTEN*-conditional knockout mice could repopulate the hematopoietic cell component of irradiated mice treated with rapamycin, indicating that it is possible to selectively eliminate preleukemic cells before the onset of an overt leukemia. However, rapamycin treatment does not eradicate the PTEN-deficient leukemia CICs after overt leukemia onset. Consistent with these results, Guo and co-workers,[177] using a murine model of PTEN-null T-ALL, have documented that long-term rapamycin treatment of preleukemic PTEN-null mice prevented CIC formation and halted T-ALL development. In contrast, rapamycin did not inhibit mTOR signaling in the c-Kit^{mid}/CD3^{+}/Lin^{-} population already enriched for CICs and could not eliminate these cells.

The efficacy of targeting PI3K/PTEN/Akt/mTOR signaling to eliminate putative CICs in human T-ALL has been documented by two recent studies from our group, in which we used NVP-BEZ235 (a dual PI3K/mTOR inhibitor)[178] and triciribine[179] (an Akt inhibitor) for targeting the side-population of T-ALL cell lines and primary cells from T-ALL patients. The side-population, which overexpresses ABCG2 and other ABC family plasma membrane transporters like Mdr-1, is thought to be enriched in leukemic CICs. This has been shown in human AML[180] and in a mice model of adult T-cell leukemia/lymphoma.[181] Moreover, recently it has been shown that active site mTOR inhibitors could induce apoptosis and decrease glucocorticoid resistance in a T-ALL pediatric patient cell subset (CD34^{+}/CD7^{-}/CD4^{-}), which is enriched in putative leukemic CICs.[182] Intriguingly, rapamycin was not as effective as the active-site mTOR inhibitors.[183]

PTEN plays critical roles in regulating cell cycle progression in HSC and other cells. PTEN influences the decision of whether the cells remain in quiescence (G_0) or enter G_1 and also controls the subsequent speed of proliferation.[184] Importantly, PTEN influences hematopoietic differentiation and PTEN deficiency blocks differentiation of B lymphoid stage, and which results in an increase in the presence of myeloid and T cells, but a decrease in cells of the B lineage.[184]

Additional studies have identified some of the genes that may interact with *PTEN* deletion to influence leukemogenesis.[185] The authors showed that *PTEN* deletion induced the expression of p16^{Ink4a} and p53 in the HSC and p19^{Arf} and p53 in other hematopoietic cells. Functional p53 suppressed leukemogenesis in *PTEN*-deficient mice and also promoted HSC depletion. Although p16^{Ink4a} also stimulated HSC depletion, it had a limited role in suppressing leukemia development. In contrast, p19^{Arf} strongly suppressed leukemia development, but did not decrease HSC levels. The authors showed that in the *PTEN*-mutant mice, secondary mutations inhibited the tumor suppressor response. Therefore in the leukemic clones that arose in these *PTEN*-deficient mice, mTOR activation depleted HSC by a tumor suppressor response, which was inhibited by secondary mutations. These authors did not observe a role for ROS in the depletion of HSCs in *PTEN*-deficient mice. In contrast, other studies have suggested roles for ROS in depletion of *TSC1*-deficient HSCs.[186] In these studies, TSC-1 depletion causes mTOR activation, exit from quiescence, increased proliferation, but leads to exhaustion of HSCs without the development of leukemia.

Other genes in this pathway also have been shown to have effects on HSC depletion. *TSC1*-deficient mice results in increased PI3K/PTEN/Akt/mTOR signaling, which leads initially to increased HSC cell cycling and mobilization, which were followed by progressive depletion and defective long-term repopulation.[187] These authors also showed that TSC1 regulation of HSC mobilization is effected by mTORC1-independent mechanisms and that one of the genes involved is the actin-bundling protein FSCN1 (fascin). Fascin is believed to be an important mTORC1-independent mediator of the effects of TSC1 on the regulation of HSC mobilization. FSCN1 plays important roles in controlling cytoskeleton organization and motility and is upregulated in many human cancers. Fascin promotes cell migration and invasion.[188] TSC1 regulation of fascin may have important roles in the dissemination of leukemia CIC. This is an interesting observation as in solid tumor CICs a frequent observation is the formation of sphere-like cells (for example, mammospheres) and masses. Foxo is also an important regulator of HSC homeostasis. Foxo is essential for long-term HSC regeneration. Foxo regulates quiescence and survival in response to oxidative stress.[189,190]

Another important gene involved in HSC and leukemia CICs is promyelocytic leukemia (PML). The *PML* gene is involved in the t(15:17) chromosomal translocation found in acute promyelocytic leukemia. Targeting of PML also leads to increased HSC cycling and to mTOR-mediated HSC depletion.[191–193] PML is highly expressed in HSCs and decreases as the HSC differentiate. PML loss or lower expression predicts a better prognosis in chronic myeloid leukemia.[192,193] PML is a negative regulator of mTOR[193] and is important in HSC maintenance. PML$^{-/-}$ HSC lack long-term repopulating capacity, which is likely due to defective maintenance of quiescence. Arsenic trioxide (As_2O_3) selectively targets PML for degradation. As_2O_3 specifically targets KSL (c-Kit^{+}, Sca-1^{+}, Lin^{-}) cells and reduced their colony-forming ability, but did not affect KSL cells isolated from PML$^{-/-}$ mice. As_2O_3 reduced PML levels in leukemic CICs and decreased the number of quiescent CIC in the absence of the induction of apoptosis.[191–193] Consistent with these results, the long-term maintenance of the CIC was dramatically suppressed. The authors examined the effects of cytosine arabinoside and As_2O_3 on leukemia CICs. They hypothesized that interventions that increase the cycling of quiescence CIC might enhance their death by chemotherapeutic agents. The combined treatment resulted in eradication of the CICs even after 4 weeks after termination of treatment. These studies indicate the potential therapeutic approaches of combining PML-reducing drugs and chemotherapeutic drugs for certain leukemia therapies.

Conclusions

The PI3K/PTEN/Akt/mTOR pathway exerts a dominant role in controlling cellular proliferation. Dysregulation of this pathway occurs frequent in human cancer by mutations in upstream receptors on intrinsic components of the pathways. Many of these mutations result in abnormal gene expression, either by altering gene transcription or protein translation. Although it has been known for the past 10 years that increased Akt activity can result in the phosphorylation of apoptotic regulatory proteins, which in most cases inhibits their pro-apoptotic effects or alter the activity of transcription factors such as Foxo-3a, a more recently recognized consequences of dysregulation of this pathway are the effects on protein translation. Genes that have

5′-UTR regions, which are difficult to translate such as Mcl-1 and others, are translated with higher efficiency in cells with dysregulated mTORC1 and downstream eIF4E activity. Thus, a therapeutic opportunity is opened up to potentially target these activities. Dysregulation of this pathway may also occur in leukemia stem cells (CICs) also indicating a novel approach to treat leukemia. Combination with cytotoxic drugs such as As_2O_3 and others may be required to eliminate the leukemia CIC. The further development of drugs that target the PI3K/PTEN/Akt/mTOR pathway may result in enhanced leukemia therapy.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

This work was supported in part by grants from Fondazione del Monte di Bologna e Ravenna, MinSan 2008 'Molecular therapy in pediatric sarcomas and leukemias against insulin-like growth factor-1 receptor system', PRIN 2008 and FIRB 2010 (RBAP10447J) to AMM.

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