Targeting the PI3K-Akt pathway in human cancer: Rationale and promise

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Introduction

A cell needs to overcome a number of failsafe mechanisms in order to become cancerous (Hahn and Weinberg, 2002). The cell must evade apoptosis and senescence programs to survive the withdrawal of the proper growth factors and nutrients (Schmitt, 2003); it must override DNA damage checkpoints and continue proliferating to propagate existing mutations and acquire new mutations (Malumbres and Barbacid, 2001); and it must maintain a high growth rate to keep up with the demands of rapid cell division (Ruggero and Pandolfi, 2003).

Phosphoinositide 3-kinase (PI3K) is a major signaling component downstream of growth factor receptor tyrosine kinases (RTKs) (Cantley, 2002). PI3K catalyzes the production of the lipid second messenger phosphatidylinositol-3,4,5-triphosphate (PIP3) at the cell membrane. PIP3 in turn contributes to the recruitment and activation of a wide range of downstream targets, including the serine-threonine protein kinase Akt (also known as protein kinase B). The PI3K-Akt signaling pathway regulates many normal cellular processes including cell proliferation, survival, growth, and motility—processes that are critical for tumorigenesis. Indeed, The role of this pathway in oncogenesis has been extensively investigated and altered expression or mutation of many components of this pathway have been implicated in human cancer (Vivanco and Sawyers, 2002).

Although both growth factor RTKs and G protein-coupled receptors can activate PI3K signaling, the involvement of the latter in cancer is less clear. Here we will limit the scope of our discussion only to the PI3K-Akt pathway downstream of growth factor RTKs. The molecular pathways downstream of PI3K and Akt have been reviewed in detail recently (Cantley, 2002; Vivanco and Sawyers, 2002). Therefore, we below highlight the contribution of this pathway to the development of cancer and focus on the promising approaches toward targeting this pathway for cancer therapeutics.

Cellular functions of the PI3K-Akt pathway and its dysregulation in human cancer

Growth factor RTKs engage the class-Ia PI3K, which is a heterodimer comprised of the p85 regulatory and p110 catalytic subunits (Cantley, 2002). Specific phospho-tyrosine residues on the activated receptor or on associated adaptor proteins bind the Src-homology 2 (SH2) domains of p85 and recruit the enzyme to the membrane. The small GTPase Ras can also recruit and activate PI3K through direct binding to p110. At the membrane, PI3K phosphorylates phosphatidylinositol-4,5-bisphosphate (PIP2) at the 3′ position on its inositol ring and converts PIP2 to PIP3. Subsequently, PIP3 recruits other downstream molecules—particularly the serine-threonine kinases Akt and PDK1—via binding to their pleckstrin-homology (PH) domains. At the membrane, Akt is partially activated through phosphorylation at threonine 308 in its activation loop by PDK1. Additional phosphorylation at serine 473 in the C terminus of Akt results in its full activation. Akt in turn regulates a wide range of target proteins that control cell proliferation, survival, growth, and other processes (Figure 1). The levels of PIP3 in the cell are strictly regulated and several lipid phosphatases act to rapidly remove it. Of particular interest is the 3′ phosphatase PTEN, which converts PIP3 back to PIP2 and thus shuts off PI3K signaling (Cantley and Neel, 1999).

Aberrant activation of the PI3K-Akt pathway has been widely implicated in many cancers. PI3K was first identified as an enzymatic activity associated with the Rous sarcoma pp60v-src protein and the polyoma middle T antigen that is essential for the transforming activity of these oncogenes (Sugimoto et al., 1984; Whitman et al., 1985, 1988), and *Akt* was also found to be a viral oncogene (Bellacosa et al., 1991). Gene amplification of p110 occurs in some cases of human ovarian cancer, and amplification of *Akt* is found in ovarian, breast, and colon cancer. In addition, activating mutations in p85 have been identified in ovarian and colon cancer (Vivanco and Sawyers, 2002). Most striking is the discovery of PTEN being a major tumor suppressor in humans (Li et al., 1997; Steck et al., 1997). Germline mutation of *PTEN* results in autosomal dominant cancer syndromes such as Cowden's disease. Furthermore, loss-of-function mutations in the *PTEN* gene are extremely common among sporadic glioblastomas, melanomas, prostate cancers, and endometrial carcinomas, and a significant percentage of breast tumors, lung cancers, and lymphomas also bear *PTEN* mutations (reviewed in Cantley and Neel, 1999). Hyperactivation of the PI3K-Akt pathway is therefore often genetically selected during tumorigenesis, and the normal cellular functions regulated by this pathway are recruited to promote proliferation and survival of cancer cells.

The PI3K-Akt pathway is a key regulator of cell survival through multiple downstream targets. The FOXO family of forkhead transcription factors AFX, FKHR, and FKHRL1 are known to mediate apoptosis by activating the transcription of proapoptotic genes such as *FasL* and *Bim* (Burgering and Medema, 2003). Phosphorylation of the FOXO proteins by Akt results in their cytoplasmic retention by interaction with 14-3-3 proteins, thereby sequestering them from their gene targets. Similarly, Akt can phosphorylate the proapoptotic Bcl-2 family member Bad, causing its sequestration from the mitochondrial membrane by 14-3-3 proteins (reviewed in Datta et al., 1999). In addition, under some conditions, Akt can promote cell survival by indirectly activating the pro-survival transcription factor nuclear factor-κB (NF-κB) through the phosphorylation of I-κB kinase (IKK) (Ozes et al., 1999; Romashkova and Makarov,

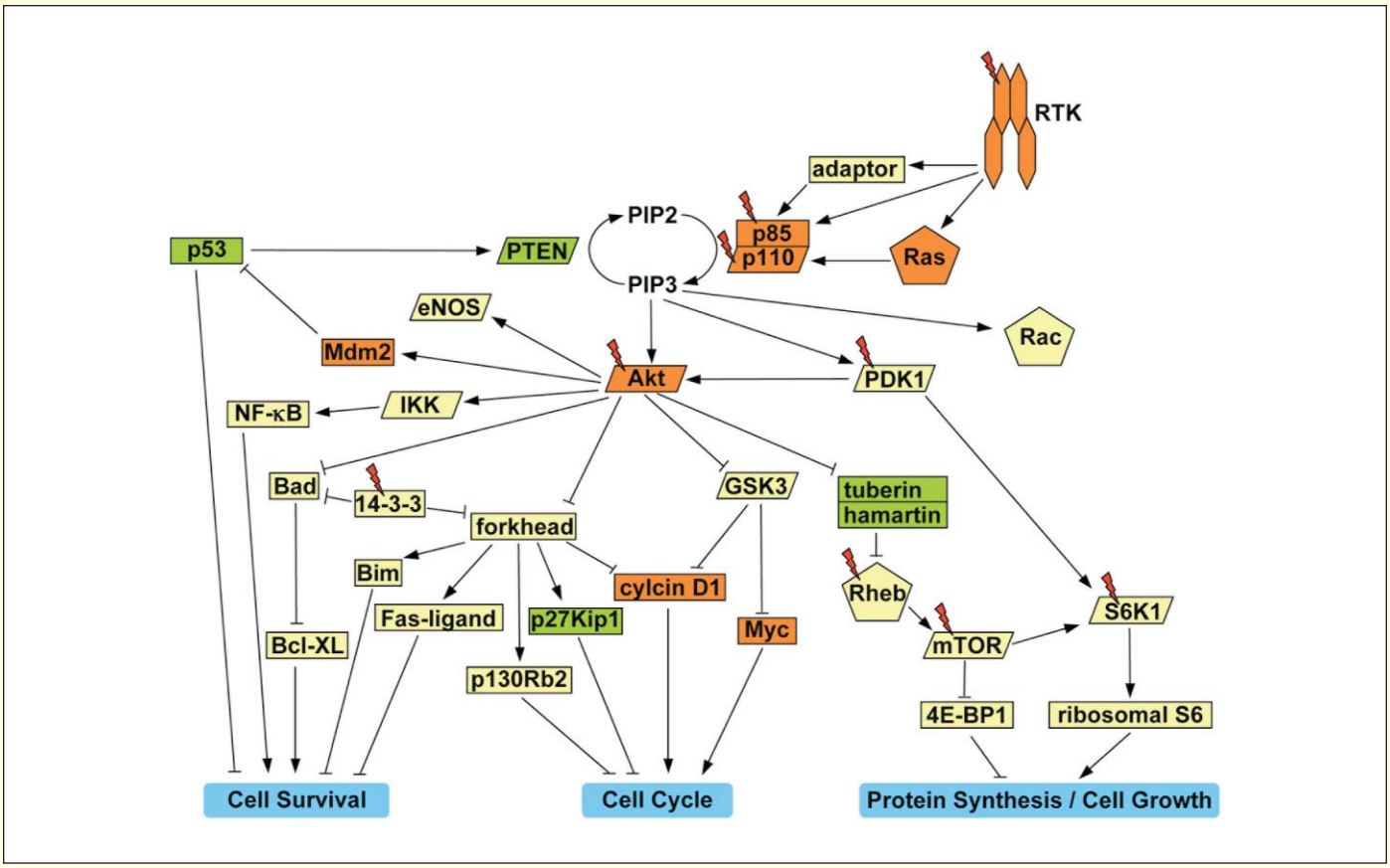

Figure 1. The PI3K-Akt signaling pathway involved in human cancer

Growth factor RTKs recruit class-Ia PI3K, which is a heterodimer of p85 regulatory subunit and p110 catalytic subunit, through direct interaction or adaptor molecules. Ras can also recruit PI3K. At the membrane, PI3K phosphorylates PIP2 and converts it to PIP3, which recruits the serine-threonine protein kinases Akt and PDK1 to the membrane. PDK1 consequently phosphorylates and activates Akt, which in turn regulates a number of downstream targets. Akt promotes cell survival by inhibiting proapoptotic proteins such as Bad, forkhead, and p53, and activating pro-survival proteins such as NF-κB. Akt regulates cell cycle progression by indirectly stabilizing cyclin D1 and Myc. Akt also stimulates protein synthesis and cell growth by activating the mTOR pathway through inhibition of the tuberin/hamartin complex. Known oncogenes are colored orange and tumor suppressors green; red flashes indicate promising targets for therapeutic intervention. Other signaling pathways mediated by class-Ia PI3K (such as glucose homeostasis downstream of the insulin receptor) are omitted for clarity. See text for further details.

1999). Finally, there may exist a reciprocal regulation between the PI3K-Akt pathway and the tumor suppressor protein p53 (Trotman and Pandolfi, 2003): PTEN is a transcriptional target of p53, while under some conditions, Akt can promote p53 degradation by phosphorylating and activating its negative regulator Mdm2 (Mayo and Donner, 2001; Zhou et al., 2001). Evasion from apoptotic stimuli is a prerequisite for tumor formation. Using a mouse lymphoma model, it was found that the loss of p53 circumvents normal apoptotic pathways (reviewed in Schmitt, 2003). It is likely that for the same reason, hyperactivation of the PI3K-Akt pathway is selected for in many tumors as a mechanism of overcoming apoptotic stimuli. This possibility is supported by the recent finding that *PTEN* and *p53* mutations are rarely found together in the same breast tumor (Kurose et al., 2002). A plausible explanation is that the loss of either *PTEN* or *p53* would reduce the selective pressure for loss of the other since either mutation would enable unregulated cell survival.

The PI3K-Akt pathway, in parallel to the Ras/MAPK pathway, contributes to the regulation of cell cycle progression, particularly at the G1/S transition. The kinase GSK3 phosphorylates and promotes the degradation of cyclin D1 and Myc, two proteins that drive S phase entry. Akt phosphorylates and inhibits GSK3, thereby contributing to the stabilization of cyclin D1 and Myc (Diehl et al., 1998; Sears et al., 2000). The FOXO transcription factors repress cyclin D1 expression while enhancing expression of the cell cycle inhibitors p27kip1 and p130Rb2 (reviewed in Burgering and Medema, 2003). Akt-mediated phosphorylation of the FOXO proteins therefore promotes cyclin D1 expression and prevents the expression of cell cycle inhibitors. Although the roles of the FOXO proteins and GSK3 in human cancer are unclear, tumor cells often show cyclin D1 or Myc overexpression and/or reduced levels of p27kip1—alterations that confer a proliferative advantage (Barnes and Gillett, 1998; Pelengaris et al., 2002; Sherr and Roberts, 1999). Further investigation is required to establish a direct correlation between hyperactivation of the PI3K-Akt pathway and the dysregulation of these cell cycle proteins in tumors. For example, a high percentage of breast tumors are characterized by overexpression of the HER2/Neu receptor, an RTK that potently activates PI3K, by high levels of cyclin D1, a downstream effector of

Akt, and/or by the loss of PTEN, a negative regulator of the PI3K pathway (Barnes and Gillett, 1998; Vivanco and Sawyers, 2002).

Coordination between cell cycle progression and cell growth (i.e., an increase in cell size) is critical for cell proliferation. Cell growth is controlled, in large part, through the regulation of protein synthesis, and dysregulation of protein synthesis is likely to contribute to the abnormally high growth rate observed in tumor cells (Ruggero and Pandolfi, 2003). In response to both nutrient availability and mitogenic growth factors, the mammalian target of rapamycin (mTOR) controls cell growth by activating the 70 kDa ribosomal S6 kinase (S6K1) and inhibiting the elongation-initiation factor 4E binding protein-1 (4E-BP1), two events which stimulate protein translation (Fingar et al., 2002). In response to growth factors, the PI3K-Akt pathway activates mTOR, at least in part, through a recently defined pathway involving Akt's phosphorylation and inhibition of the tuberous sclerosis complex-2 (*TSC2*) gene product tuberin (reviewed in Manning and Cantley, 2003). Tuberin has recently been shown by several groups to be a GTPase-activating protein (e.g., Garami et al., 2003), and therefore inhibitor, of the Ras-like small G protein Rheb, which is an activator of mTOR. *TSC2* is one of two genes found mutated in the tuberous sclerosis complex disease (the other one being *TSC1*, which encodes the protein hamartin, an obligatory binding partner of tuberin). The disease is characterized by the formation of hamartomas in a wide variety of tissues. Interestingly, one of the most common tumor types in TSC is subependymal giant cell astrocytomas within the brain, which contain abnormally large cells of astrocyte origin (Gomez et al., 1999), demonstrating the contribution of uncontrolled cell growth to the formation of some tumor types. Furthermore, this PI3K-Akt-TSC-mTOR branch is emerging as a critical contributor to the oncogenic properties of the PI3K-Akt pathway (see below; rapamycin suppressing PTEN tumors).

PI3K may contribute to other aspects of tumorigenesis in both Akt-dependent and -independent manners. PIP3 regulates the small GTPase Rac by activating a subset of its GTP-GDP exchange factors (Welch et al., 2003). Since Rac controls rearrangement of the actin cytoskeleton and cell motility and is important for Ras-mediated transformation, PI3K might activate both Rac- and Ras-dependent invasion events that contribute to metastasis. In addition, Akt has been reported to phosphorylate and activate endothelial nitric oxide synthase (eNOS) (Fulton et al., 1999). As nitric oxide is an important regulator of endothelial cells and overexpression of eNOS has been associated with tumors (Xu et al., 2002), the PI3K-Akt pathway may also contribute to tumor angiogenesis.

Mouse cancer models of the PI3K-Akt pathway

Mouse cancer models have been invaluable in understanding the process of tumorigenesis: they allow defined tests of tumor genetics, close analysis of tumor pathology, and validation of therapeutic targets. A number of mouse models have been established to investigate the role of the PI3K-Akt pathway in cancer (Vivanco and Sawyers, 2002). Targeted deletion of *PTEN* recapitulates many of the ramifications of *PTEN* loss in human cancers: mice heterozygous for *PTEN* develop tumors in lymphoid, thyroid, breast, endometrial, prostate, and other tissues (e.g., Di Cristofano et al., 1998). Tissue-specific deletion of *PTEN* in T cells causes aggressive lymphomas, and mammary gland deletion of *PTEN* causes breast tumors (reviewed in Kishimoto et al., 2003). The consequences of mutating downstream components of the PI3K-Akt pathway have also been examined in mice. For instance, mice heterozygous for *TSC2* develop kidney and lung adenomas analogous to those seen in the human TSC disease (Onda et al., 1999). Interestingly, tissue-specific deletion of either *PTEN* or *TSC1* in the brain results in similar phenotypes, including larger brain, increased cell size, and astrogliosis, arguing the importance of cell growth regulation in the tumorigenesis of this organ (Kishimoto et al., 2003; Uhlmann et al., 2002). Furthermore, expression of activated Akt in the murine prostate results in prostate intraepithelial neoplasia with gene expression profiles resembling that of human prostate cancer, highlighting the role of the PI3K-Akt pathway in prostatic tumors (Majumder et al., 2003).

Mouse models have been useful in determining the cooperativity among different oncogenes and tumor suppressors. For example, it has been shown that the expression of either activated Ras or activated Akt alone is not sufficient to cause glioblastoma in mice, while their coexpression results in aggressive glioblastoma multiforme (Holland et al., 2000). Furthermore, mice that are doubly heterozygous for *PTEN* and *p27kip1* develop prostate cancer with 100% penetrance, a phenotype much more severe than mice heterozygous for either gene alone. Interestingly, unlike the single heterozygous mice, loss of heterozygocity of neither *PTEN* nor *p27kip1* is seen in these tumors, suggesting that the haploinsufficiency of two tumor suppressors may be sufficient to allow tumor formation (Di Cristofano et al., 2001). Mouse genetic studies also offer the opportunity for drug target validation. For example, in mice, loss of cyclin D1 completely protects against breast tumors elicited by *MMTV-neu* overexpression (Yu et al., 2001). Analogously, it would be interesting to test whether targeted deletion of *PI3K* or *Akt* would protect *PTEN* heterozygous mice from developing tumors. It has been shown that deletion of *Akt-1* from *PTEN*$^{-/-}$ ES cells partially reverses the enhanced cell survival and proliferation phenotype resulting from PTEN deletion (Stiles et al., 2002). Such genetic analysis would help in verifying drug targets in tumors with a given genetic lesion.

Finally, mouse tumor models are ideal for evaluating experimental therapeutic agents in vivo. For example, both the PI3K inhibitor LY294002 and the mTOR inhibitor CCI-779 have been shown to be effective in vivo at reducing tumor growth in mouse cancer models (see below). Such in vivo validation is critical in the preclinical development of candidate drugs.

Therapeutic approaches of targeting the PI3K-Akt pathway in cancer

Components of the PI3K-Akt pathway present promising targets for therapeutic intervention for several reasons. First, this pathway serves to inhibit many "tumor suppressor-like" proteins such as the FOXO transcription factors, Bad, GSK3, and the tuberin/hamartin complex that negatively regulate cell survival, proliferation, and growth. Blocking this pathway could therefore simultaneously inhibit the proliferation of tumor cells and sensitize them toward apoptosis. Second, many components in the PI3K-Akt pathway are kinases, one of the most "druggable" classes of intracellular targets, and are ideal for the development of small molecule inhibitors. Third, as hyperactivation of the PI3K-Akt pathway is found in a wide range of tumors, drugs inhibiting this pathway are likely to have broad applications for treating different types of cancer.

Since cancer is a group of heterogeneous diseases, the

Table 1. Promising therapeutic targets in the PI3K-Akt pathway

Target	Strategy
PI3K	The p110 catalytic site inhibitors LY294002 and wortmannin are successful research tools. Agents could also be developed to disrupt the binding of the p85 SH2 domains to RTKs, or to disrupt Ras-p110 interaction.
Akt and PDK1	UCN-01 is a good PDK1 inhibitor, though it lacks specificity. Novel phosphatidylinositol analogs that disrupt Akt PH domain-PIP3 interaction are effective at blocking Akt activation. Two other experimental agents, SR13668[a] and deguelin (Chun et al., 2003) also inhibit Akt through as yet undefined mechanisms.
mTOR	Several rapamycin analogs such as CCI-779 and RAD001, which specifically inhibit mTOR, are being developed clinically as cancer therapeutics.

[a]Dr. L. Jong (ling.jong@sri.com), SRI International, personal communication.
Examples are chosen to illustrate different strategies for drug development, see text for further details. We expect this list of targets to expand significantly in the near future as several programs aimed at targeting this pathway are underway.

choice of therapeutics thus depends on the particular molecular signature of a given tumor. Successful identification of tumors with a hyperactive PI3K-Akt pathway is a prerequisite to the targeting of this pathway. The loss of PTEN can be directly assessed by either Western blot or immunohistochemistry of primary tumor samples (McMenamin et al., 1999). The advent of high-quality phosphospecific antibodies has made it possible to measure the activation of various components in the PI3K-Akt pathway in primary tumors. Immunohistochemistry using antibodies against phosphorylated Akt serine 473, which closely parallels Akt activation, has revealed high levels of Akt phosphorylation in aggressive prostate tumors (Malik et al., 2002). It has also been demonstrated that glioblastomas can be classified based on their reactivities toward phosphospecific antibodies against various components in the PI3K-Akt pathway (Choe et al., 2003). This ability to assess signaling in situ is useful for evaluating the effectiveness of therapeutics, and such feedback should help physicians to quickly and rationally identify the most effective treatment.

Inhibitors against RTKs have been an active area of drug development (Shawver et al., 2002). As hyperactivation of the PI3K-Akt pathway is critical for tumor cell transformation and resistance to chemotherapeutic agents (Knuefermann et al., 2003; Skorski et al., 1997), it is likely that RTK inibitors such as Herceptin (which blocks the Erb2/HER2 receptor) and Gleevec (which inhibits the BCR-Abl, c-KIT, and PDGF-R tyrosine kinases) achieve their anti-tumor effects, at least in part, by shutting off upstream signaling to the PI3K-Akt pathway. The success of RTK inhibitors, however, could be hampered by activating mutations or gene amplifications affecting downstream signaling components (such as p85, p110, and Akt) or by the loss of negative regulators (such as PTEN). Indeed, it has been recently shown that loss of PTEN in tumor cells can confer resistance to Iressa (an epidermal growth factor receptor RTK inhibitor) by setting a high threshold of Akt activation (Bianco et al., 2003).

The most direct approach to inhibit the PI3K-Akt pathway would be to target PI3K itself (Table 1). The PI3K inhibitors LY294002 and wortmannin, both targeting the catalytic site of p110, have been extensively used as research tools. In a mouse xenograft model of ovarian tumors, LY294002 has been show to inhibit tumor growth in vivo (Hu et al., 2002). Since the PI3K catalytic domain is highly conserved among PI3K family members, it is not surprising that neither compound discriminates among the various isoforms of PI3K. The recent elucidation of the p110 catalytic subunit crystal structure (reviewed in Djordjevic and Driscoll, 2002) should aid the development of more isoform-specific inhibitors that will spare other PI3K isoforms (such as the class-Ib PI3K downstream of G protein-coupled receptors) and thus affect fewer cellular processes. An alternative and potentially more selective approach is to block the phosphotyrosine binding of the p85 SH2 domains, hence preventing the recruitment and activation of class-Ia PI3K by RTKs. The p85 SH2 domains recognize a short phosphotyrosine motif (Nolte et al., 1996), and small peptidomimetics of this motif have been shown to be effective inhibitors of these SH2 domains (Eaton et al., 1998). Similarly, inhibitors that disrupt the Ras-p110 interaction may also be useful at blocking PI3K activation (Djordjevic and Driscoll, 2002).

Kinase inhibitors that target either Akt or PDK1 would also be effective at turning off this pathway. Due to the high similarity of the catalytic domains among AGC family kinases (which include Akt and PDK1), first-generation inhibitors thus far lack specificity (Davies et al., 2000). High-resolution crystal structures of the Akt and PDK1 kinase domains should aid in the development of more selective inhibitors (Komander et al., 2003; Yang et al., 2002). Another potentially powerful strategy for inhibiting Akt is to disrupt the binding of its PH domain to PIP3 (Thomas et al., 2002), thereby preventing its membrane translocation and activation by PDK1. Novel analogs of the PIP3 phosphoinositide ring have been shown to be effective Akt inhibitors in cell culture (Kozikowski et al., 2003). Such inhibitors are also likely to block other PIP3 binding PH domains such as the PH domain of PDK1.

Because only a subset of the cellular processes regulated by the PI3K-Akt pathway are involved in tumorigenesis, the choice of drug targets must take into account the adverse effects resulting from the inhibition of other PI3K-Akt-dependent cellular processes. For example, insulin's effects on metabolism are mediated through the PI3K-Akt pathway (Saltiel and Kahn, 2001): inhibitors of PI3K or Akt are therefore likely to perturb glucose homeostasis. It would be desirable, therefore, to target components of branches further downstream in the PI3K-Akt pathway, such as mTOR, Bad, and the FOXO proteins that are more exclusively involved in cell growth, survival, and proliferation. For example, the binding of 14-3-3 proteins to phosphorylated Bad or the FOXO proteins could potentially be disrupted by peptidomimetics or small molecules. Such inhibitors would preserve the activity of these proapoptotic proteins downstream of Akt, and thus sensitize tumors toward apoptosis.

Recently, much attention has been focused on targeting mTOR in cancer treatment. Originally discovered as an immunosuppressant, the mTOR inhibitor rapamycin and its analogs (such as CCI-779) have been shown to inhibit the proliferation of some tumor cells in vitro, as well as to reduce tumor growth in vivo in *PTEN* heterozygous mice and in mice carrying xenografted human tumors (Neshat et al., 2001; Dudkin et al.,

2001; Podsypanina et al., 2001). Several rapamycin analogs have already been approved for use in transplant patients as immunosuppressants (Saunders et al., 2001), and some are currently in clinical trials as cancer therapeutics (Table 1). Other components of the PI3K-Akt-TSC-mTOR branch, such as Rheb and S6K1, may also be exploited as drug targets in the future.

As the PI3K-Akt pathway is involved in the survival, growth, and proliferation of normal cells, a reasonable therapeutic index would depend on tumors being more sensitive to inhibitors of this pathway than normal tissues. The prevalence of a hyperactivated PI3K-Akt pathway in human cancers indicates this may indeed be the case, and a partial inhibition of this pathway might be sufficient to inhibit tumor growth while sparing normal cells. The success of Herceptin and Gleevec serve as encouraging precedents: in both cases, the therapeutic index is high because the tumor cells being treated are much more dependent on the activation of the respective signaling pathways than are normal cells. In addition, maximum efficacy and minimum side effects might result from combining PI3K-Akt pathway inhibitors with cancer therapeutics having other mechanisms of action, as demonstrated in a mouse tumor model with the combination of LY294002 and paclitaxel (Hu et al., 2002). Finally, tumors of different origin that arise from hyperactivation of the PI3K-Akt pathway are likely be differentially sensitive to the inhibition of different downstream targets. The effectiveness of targeting a specific branch of this pathway will depend on, for example, which Akt substrates are phosphorylated in the given tumor and could also depend on the differentiation state of the tumor cells. The selective use of different PI3K-Akt pathway inhibitors based on such knowledge should further improve their therapeutic index.

Perspective

The last two decades have seen the PI3K-Akt signaling pathway being firmly established as a critical contributor toward tumorigenesis. The elucidation of the role of this pathway in cell growth, survival, and proliferation has shed light on why regulation of this pathway is so often altered within tumors. Components of the PI3K-Akt pathway have emerged as promising new targets for the development of cancer therapeutics, and first-generation inhibitors of this pathway have been shown to be effective at reducing tumor cell growth both in vitro and in vivo. The continuing efforts to develop specific, high-affinity inhibitors against the PI3K-Akt pathway will surely yield new therapeutics to treat human cancer.

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