Inhibition of the PI3K Pathway: Hope We Can Believe in?

Michiel S. van der Heijden and René Bernards

Abstract

The phosphoinositide 3-kinase (PI3K) pathway is one of the most commonly activated pathways in human cancer and has roles in cell proliferation, apoptosis, protein synthesis, and metabolism. The PI3K pathway can be activated by amplification or activating mutation of upstream receptor tyrosine kinases, and by mutations or deletions downstream in the pathway. Trastuzumab, a monoclonal antibody targeting the human epidermal growth factor receptor 2 (HER2), has been one of the most successful and most widely used targeted therapies. However, many HER2-positive cancers are not sensitive to HER2-based therapies or become resistant during treatment; downstream activation of the pathway is one of the causes of resistance. Because of the common activation of the PI3K pathway in cancer, compounds targeting proteins downstream in the pathway have been developed in recent years. The mammalian target of rapamycin (mTOR) inhibitors everolimus and temsirolimus have been shown to be beneficial in certain cancer types; many other inhibitors of the PI3K pathway are in various stages of clinical development. Ongoing research should clarify which molecular cancer subtypes are most susceptible to specific compounds and explore combinatorial approaches, ultimately leading to individualized patient treatment.

Background

The phosphoinositide 3-kinase (PI3K) pathway is an important signal transduction pathway commonly activated in cancer (see Fig. 1). Because of its prominent role in many cancer types, the PI3K pathway has become the target of many new cancer therapies. There are three PI3K classes, with different structures and characteristics; class I can be further subdivided into class Ia and class Ib. As most of the oncogenic mutations occur in the p110α subunit of class Ia PI3Ks, we will limit ourselves to this class. The PI3K pathway can be activated by upstream receptor tyrosine kinases (RTK), leading to phosphorylation of phosphatidylinositol-4,5-bisphosphate (PIP2) to generate phosphatidylinositol-3,4,5-trisphosphate (PIP3). PIP3 can be dephosphorylated by the phosphatase and tensin homolog (PTEN), which terminates PI3K signaling. The accumulation of PIP3 activates a signaling cascade starting with the phosphorylation (activation) of the protein serine-threonine kinase AKT at threonine 308 by phosphoinositide-dependent kinase 1 (PDK1). Through several downstream proteins, the activation of AKT results in the stimulation of protein translation and cell proliferation, and the inhibition of apoptosis. Through tuberous sclerosis complex and RAS homolog enriched in brain, AKT activates the mammalian target of rapamycin (mTOR), which leads to phosphorylation of its downstream targets, the translation-regulators 4EBP1 and S6K1. mTOR is segregated in at least two complexes: the raptor-containing complex mTORC1 and the rictor-containing mTORC2. Only mTORC1 is sensitive to inhibition by rapamycin, although prolonged rapamycin treatment may partially inhibit mTORC2 activity (1). mTORC1 integrates proliferative signals from the PI3K pathway with an energy-sensing pathway, which is triggered by the availability of ATP and amino acids, and is linked to mTOR by the Peutz-Jeghers tumor-suppressor serine-threonine kinase 11 (also known as LKB1; ref. 2). mTOR residing in the mTORC2 complex is an important effector of pathways regulating cell growth and survival (3); it phosphorylates and activates AKT at the Ser473 position (4), placing the mTOR protein upstream as well as downstream of AKT. Studies of mTOR function in the last decade have added to the complexity of the PI3K/AKT/mTOR circuitry, which does not seem to function as a simple linear pathway (5). A recent study by Vasudevan and colleagues adds to this increasing complexity of the oncogenic effects of PI3K pathway activation, by showing that the extent to which cancers with upstream PI3K pathway activation depend on the phosphorylation of AKT may vary. PTEN-deficient cancer cells and PIK3CA mutant cells with high levels of
PIP3 had high levels of phosphorylated AKT and were dependent on AKT for viability, whereas other PIK3CA mutant cancer cells, especially those with wild-type PTEN and low levels of PIP3 and phospho-AKT, were much less dependent on AKT. Knockdown of serum/glucocorticoid regulated kinase 3 (SGK3) could suppress viability in cancer cells with low phospho-AKT, and knockdown of PDK1 decreased SGK3 phosphorylation. These results suggest that, in the absence of AKT-phosphorylation, mutant PIK3CA exerts its oncogenic effects through a PDK1-SGK3 pathway (6).

Various alterations in human cancer can lead to inappropriate activation of the PI3K pathway, often leading to "oncogene addiction": a condition in which the cancer cell is dependent on specific oncogenic pathway signaling for survival and growth. An important mechanism of oncogenic PI3K pathway activation occurs through hyperactivation of upstream RTKs, such as the human epidermal growth factor receptor 2 (HER2) and the epidermal growth factor receptor (EGFR). Both HER2 and EGFR have been targeted successfully by antibodies and small molecule inhibitors. HER2-positive cancers seem to be addicted to PI3K activation, as inhibition of the downstream PI3K pathway can lead to substantial apoptosis. PI3K pathway activation is an important mediator of EGFR activation, however, PI3K pathway inhibition is insufficient for full inhibition of cells with oncogenic EGFR activation. Simultaneous inhibition of the MEK/ERK and the PI3K pathways does lead to apoptosis at similar levels as seen with EGFR inhibitors in EGFR-dependent cells, suggesting that both pathways are important in these cells (7).

The first identified genetic mechanism of PI3K pathway activation was the loss of PTEN function by mutation or deletion, leading to accumulation of the PI3K product PIP3. Germline mutations in the PTEN gene can cause Cowden's cancer predisposition syndrome. Somatic alterations of the PTEN gene occur in a diverse range of human cancers, including endometrial, brain, and prostate cancers (8, 9). In mice, heterozygous Pten loss leads...
to neoplasia in several tissues, including endometrium, liver, prostate, gastrointestinal tract, thyroid, and thymus (10). In mouse models of prostate cancer, the degree of \textit{Pten} loss (by heterozygous mutation or targeted deletion) is associated with the incidence, latency, and invasiveness of neoplasia (11).

In 2004, the \textit{PIK3CA} gene, encoding the p110\textalpha{} subunit of class IA PI3K, was found to contain activating mutations in 74 (32%) of 199 colorectal cancers (12). The \textit{PIK3CA} gene was later found to be commonly mutated in other cancer types with a frequency of approximately 25% in breast and endometrial cancers (13); mutations have also been detected in lung and cervical cancer. Recently, two studies reported mutations in the p85\alpha{} regulatory subunit of PI3K (14, 15). In these two studies, \textit{PIK3R1} mutations and other activating PI3K pathway alterations were mutually exclusive and occurred in 50% of glioblastomas (14, 15). In these two studies, \textit{PIK3R1} mutations and other activating PI3K pathway alterations were mutually exclusive and occurred in 50% of glioblastomas. A study by Jaiswal and colleagues found \textit{PIK3R1} mutations in 9 of 108 colon cancers. Interestingly, in colon cancer \textit{PIK3R1} mutations often co-occurred with mutations of the \textit{PIK3CA} gene (16). Mutation or amplification of the AKT1 gene (17, 18), amplification of the AKT2 gene (19), and \textit{PIK3CA} amplification (20) can also cause activation of the PI3K pathway.

Although oncogenic activation of KRAS is believed to exert its functions mainly through the MAP kinase pathway, it is becoming increasingly clear that the PI3K pathway is an important effector of mutant KRAS as well. RAS can bind and activate PI3K (21), and mice carrying a \textit{PIK3CA} mutation that blocks the interaction with RAS become resistant to RAS oncogene-induced tumorigenesis (22).

In a recent report by Peterson and colleagues, an intriguing mechanism of PI3K pathway activation was shown to be present in a subset of multiple myeloma, especially in those myelomas containing translocations involving the transcription factors c-MAF or MAFB. Overexpression of DEPTOR, an inhibitor of both mTORC1 and mTORC2, was associated with hyperactivation of AKT, presumably as a result of the inhibition of the negative feedback loop from mTORC1, through IRS1, to PI3K (23). The exact role of DEPTOR in activation of the PI3K pathway still needs to be established, as many cancer cells seem to have decreased DEPTOR expression, which is associated with increased mTORC1 activity.

**Clinical-Translational Advances**

**HER2-targeted treatment**

Trastuzumab has become the standard of care in HER2-positive breast cancer patients in the (neo-) adjuvant and palliative setting. However, a significant portion of HER2-overexpressing breast cancer patients fail to respond to trastuzumab-based therapy: only 15 to 20% of patients respond to trastuzumab monotherapy (24). In addition, cardiotoxicity occurs in a small percentage of patients, especially if trastuzumab is given in combination with anthracyclines (25). It is therefore imperative that biomarkers to select patients for trastuzumab-based therapy are identified and developed in the clinical setting. In a study by Berns and colleagues, a large-scale RNA-interference screen for genes involved in trastuzumab resistance identified the \textit{PTEN} gene as a modulator of drug sensitivity. Oncogenic \textit{PIK3CA} mutants also conferred drug resistance in \textit{vivo} and activation of the PI3K pathway was associated with poor prognosis in a cohort of breast cancer patients treated with trastuzumab (26).

Clinical trials, such as ALTTO and NEO-ALTTO, may prospectively validate the use of downstream activation of the PI3K pathway as a biomarker for trastuzumab sensitivity (27). If validated, one could envision that breast cancer patients with HER2 overexpression would be tested for PI3K pathway activation and subsequently treated with either trastuzumab-based therapies or, in the case of downstream PI3K activation, a regimen including a PI3K or mTOR inhibitor, or perhaps with HER2-targeting antibodies covalently coupled to a toxic conjugate, like for instance trastuzumab-MCC-DM1 (28). Because of the dependency on PI3K pathway activation in many cancers, often caused by pathway activation downstream of HER2, numerous new anticancer drugs targeting proteins in the PI3K pathway have been developed (29).

**PI3K inhibitors**

Wortmannin and LY294002 were the first drugs to target PI3K family members. Because of their toxicity in animal studies, a range of new PI3K inhibitors has been developed. Some of these drugs only target specific PI3K isoforms, whereas other inhibitors have a broader spectrum including both p110\alpha{} and mTOR. Several PI3K inhibitors are in clinical development: phase I-II trials testing the dual PI3K/mTOR inhibitors BEZ235, BGT226 and XL765, and the PI3K inhibitor XL147 seem to be the most advanced. Some of these drugs are also tested in combination with erlotinib or trastuzumab (http://clinicaltrials.gov).

It is currently not known if clinically meaningful differences in the response to PI3K inhibitors can be expected between cancers with different mechanisms of PI3K pathway activation. In a recent study by Brachmann and colleagues, the PI3K/mTOR dual inhibitor NVP-BEZ235 selectively induced cell death only in cancer cells having \textit{HER2} amplification and/or an activating \textit{PIK3CA} mutation, but not in cells with \textit{PTEN} loss or an activating \textit{KRAS} mutation (30). Likewise, a higher dose of NVP-BEZ235 was necessary to abrogate AKT phosphorylation in BT474 breast cancer cells with stable \textit{PTEN} knockdown as compared with BT474 cells expressing mutant forms of \textit{PIK3CA} (31). Most of the oncogenic effects of PI3K stimulation by upstream RTKs or RAS are dependent on p110\alpha{} as cells deficient in the p110\alpha{} isoform are resistant to oncogenic transformation induced by a variety of oncogenic RTKs (32). Some \textit{PTEN}-deficient cancers, on the other hand, may be mostly
dependent on the p110β isoform (33, 34). Currently, most PI3K inhibitors target all isoforms; drugs aimed specifically at the appropriate PI3K isoform or at the mutated p110α subunit would potentially lack side effects caused by inhibition of the other isoforms.

**AKT inhibitors**

As AKT is the most important downstream target of PI3K, several inhibitors of AKT have been developed. Perifosine is a phosphatidylinositol analog and has been tested in several phase I and II trials; it seems to have little activity as a single agent. MK2206, an allosteric inhibitor of AKT1 and 2, is being clinically tested in combination with various chemotherapy regimens, HER2 inhibitors, and with the MEK-inhibitor AZD6244. The combination of a downstream PI3K pathway inhibitor with a MEK-inhibitor is particularly intriguing as it has become increasingly clear that the PI3K pathway and the MAPK pathways are interconnected and, in many cancer cells, when either pathway is inhibited, the other pathway becomes more active.

**mTOR Inhibitors**

mTOR inhibitors can be divided into two classes: the rapalogs, of which rapamycin is the prototype, and the small molecule mTOR inhibitors. Three rapalogs are under investigation in clinical cancer trials: the rapamycin-prodrug temsirolimus and the rapamycin-analogs everolimus and deforolimus. Rapalogs form a complex with FK506 binding protein 12 (FKBP12), binding and inhibiting mTORC1. As a class of agents, rapalogs seem to have activity against a wide range of malignancies, including mantle cell lymphoma, sarcoma, and renal cancer (35). A multicenter phase III clinical trial compared temsirolimus to interferon alpha (the standard treatment) and to a combination of temsirolimus and interferon in patients with advanced renal cell carcinoma. Temsirolimus showed significantly better progression-free survival and overall survival than interferon alone (36). A phase 2 study of temsirolimus found modest activity in heavily pretreated patients with locally advanced or metastatic breast cancer; 9.2% of patients had an objective response (37). Although several encouraging results have been achieved in the treatment of cancer patients with rapalogs, survival in this setting has not been shown in most cancer patients so far. A major reason for this lack of effectivity could be the existence of a negative feedback loop from mTORC1, through S6K1, to upstream PI3K pathway activity (5): mTORC1 inhibition by rapamycin may stimulate AKT through increased levels of insulin receptor substrate-1 and higher insulin-like growth factor activity (38–40). A phase I study of patients with PTEN deficient glioblastomas found activation of AKT after treatment with rapamycin in a subset of patients, presumably due to loss of negative feedback inhibition. Activation of AKT after rapamycin treatment was associated with shorter time-to-progression (41). This feedback loop between mTORC1 and AKT presents a potential therapeutic problem as inhibition of this aspect of mTOR’s functions could promote cell survival. For these reasons, compounds that can directly inhibit mTOR kinase activity in both complexes have been developed, most notably AZD8055 and OSI-027, and are now being tested in phase I-II trials.

**Future Directions**

Sensitivity to targeted therapy is often determined by alterations downstream of the target: PI3K activation likely confers resistance to HER2 inhibition, and oncogenic KRAS mutations are a cause of resistance to EGFR inhibition. As activation of the PI3K pathway usually occurs upstream in the pathway, resistance caused by downstream genetic alterations is less likely to be present at the initiation of therapy targeted at PI3K or mTOR. However, the PI3K pathway is divergent in its downstream effects on (cancer) cell function as it has several downstream targets. Other molecular pathways may influence the downstream PI3K targets and could influence the sensitivity to inhibition of members of the PI3K pathway. Also, it is currently not known which pathways are primarily responsible for the antiproliferative and apoptotic effects of PI3K pathway inhibition; alterations in these downstream pathways could cause resistance in response to PI3K or mTOR inhibition.

The assessment of PI3K pathway activation in cancer specimen remains an important hurdle for biomarker-based treatment with PI3K pathway inhibitors. In the ideal situation, one test should be able to assay activation of the pathway and should preferably distinguish upstream (RTK activation) from downstream (PTEN insufficiency, PIK3CA mutation) activation, which would guide the decision between RTK-targeting agents and downstream inhibitors. Unfortunately such a test is not available yet; attempts to find an expression signature for PI3K pathway activation so far have failed and the detection of PTEN loss in cancer tissue remains problematic. The development of reverse phase protein arrays that can assess the phosphorylation pattern of biologically relevant pathways could provide an alternative way of PI3K pathway analysis (42, 43).

Despite some exciting successful examples, meaningful clinical progress for drugs targeting a single kinase or a single molecular pathway has been rare, especially in the cancers responsible for the largest proportion of cancer deaths. Even though some cancers seem to be addicted to signaling in one molecular pathway, inhibition of such a pathway is often not enough for a meaningful clinical response. An important concept for the improvement of targeted therapies is synthetic lethality (44): whereas the inhibition of a pathway induces only a modest toxicity, inhibition of this pathway in the context of a specific genetic defect or in combination with inhibition of another molecular pathway, is far more effective.
lethal. An example of such a synthetic lethal interaction could be the combination of PI3K inhibitors with inhibitors of the MEK/ERK pathway in KRAS-driven lung cancers (45). Unbiased functional genetic screens could help to identify these lethal interactions and could provide biomarkers of resistance to targeted therapies, as has been shown by the identification of PTEN loss or PIK3CA mutations as markers for resistance to HER2-targeted therapy (26). These approaches will lead to the rational use of targeted therapies in patients with tumors that have the appropriate molecular profiles.

Disclosure of Potential Conflicts of Interest

R. Bernards, commercial research grant, AstraZeneca. M.S. van der Heijden declared no potential conflict of interest.

Grant Support

Grants from the Centre of Biomedical Genetics, the Cancer Genomics Centre, the Netherlands Organization for Scientific Research (NWO), and the Dutch Cancer Society.

Received 03/22/2010; accepted 03/24/2010; published OnlineFirst 04/16/2010.

References

19. Cheng JQ, Godwin AK, Bellacosa A, et al. AKT2, a putative onco-


Inhibition of the PI3K Pathway: Hope We Can Believe in?

Michiel S. van der Heijden and René Bernards


Updated version
Access the most recent version of this article at:
doi:10.1158/1078-0432.CCR-09-3004

Cited articles
This article cites 45 articles, 19 of which you can access for free at:
http://clincancerres.aacrjournals.org/content/16/12/3094.full.html#ref-list-1

Citing articles
This article has been cited by 6 HighWire-hosted articles. Access the articles at:
/content/16/12/3094.full.html#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.