Molecular Pathways

Targeting the PI3K/AKT Pathway for the Treatment of Prostate Cancer
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Abstract
Despite recent advances in our understanding of the biological basis of prostate cancer, the management of the disease, especially in the castration-resistant phase, remains a significant challenge. Deregulation of the phosphatidylinositol 3-kinase pathway is increasingly implicated in prostate carcinogenesis. In this review, we detail the role of this pathway in the pathogenesis of prostate cancer and the rapidly evolving therapeutic implications of targeting it. In particular, we highlight the importance of the appropriate selection of agents and combinations, and the critical role of predictive and pharmacodynamic biomarkers.

Background
Prostate cancer is a heterogenous disease whose underlying pathogenic mechanisms are being increasingly elucidated. Androgen deprivation therapy with Luteinizing hormone-releasing hormone (LHRH) analogs or orchidectomy is usually initially effective at controlling metastatic disease, but patients inevitably progress from an androgen-sensitive to a castration-resistant phenotype. Effective treatment at this stage has been largely limited to docetaxel chemotherapy (1). Improved understanding of the molecular events underlying prostate carcinogenesis and castration resistance is vital to improving outcome. There is now incontrovertible evidence that androgen receptor (AR) signaling continues to play a critical role in many patients with castration-resistant disease. The phosphatidylinositol 3-kinase (PI3K) pathway has also been implicated in prostate carcinogenesis and castration resistance, although its precise function remains to be fully elucidated.

The PI3Ks are enzymes that are primarily involved in the phosphorylation of membrane inositol lipids, mediating cellular signal transduction (2). Both receptor tyrosine kinases (RTKs) and non-RTKs result in the activation of PI3K, which generates the second messenger Phosphatidylinositol (3,5)-bisphosphate (PIP3) from Phosphatidylinositol 4,5-bisphosphate (PIP2; see Fig. 1). This recruits pleckstrin homology (PH) domain-containing proteins to the cell membrane, including the AKT/PKB kinases, driving their conformational change and resulting in their phosphorylation by the constitutively active phosphoinositide-dependent kinase 1 (PDK1) at Thr 473 (3) and by PDK2 [mammalian target of rapamycin complex 2 (mTORC2)] at Ser 417 (4). Activated AKT translocates to the cytoplasm and nucleus and activates downstream targets involved in survival, proliferation, cell cycle progression, growth, migration, and angiogenesis. AKT is negatively regulated by the tumor suppressor PTEN (phosphatase and tensin homolog deleted on chromosome 10), which dephosphorylates PIP3. AKT mediates the phosphorylation and activation of mTOR complex 1 (mTORC1), a serine/threonine kinase that plays critical roles in the regulation of protein translation and synthesis, angiogenesis, and cell cycle progression.

Deregulation of the pathway can occur through a range of processes. The most important known mechanisms are listed as follows:

1. Gain of function oncogenic mutations of PIK3CA, the p110α catalytic subunit, have been reported in many malignancies, including ovary, breast, and colorectal cancer (5).
2. Loss of function of the tumor suppressor PTEN through gene deletion, mutation, microRNA expression, or epigenetic silencing (2, 6–8).
3. Amplification or mutation of AKT/PKB isoforms (9).
4. Upstream activation through RTK signaling, e.g., ERBB or IGF1R.
5. Loss of the tumor suppressor FBXW7 has recently been shown to correlate with increased sensitivity of tumor cell lines to treatment with PI3K pathway inhibitors (10) and appears to have a reciprocal relationship with PTEN loss.

A full description of the pathway and its underlying complexities is beyond the scope of this article, and the reader is directed to a number of excellent recent reviews (11–14). Recent advances in PI3K pathway biology and its relevance to this disease will be discussed.

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Clinical-Translational Advances

**PI3K and prostate cancer.** Despite reduced androgen levels, it is now widely accepted that AR signaling commonly remains important in castration-resistant prostate cancer (CRPC; ref. 15). Several mechanisms have been proposed as to how prostate cancer cells grow in the presence of reduced or absent circulating ligand: (a) a hypersensitive AR, due to altered AR expression or AR complex stoichiometry, activated by low androgen levels synthesized de novo by the tumor itself (intracrine synthesis), or from an extragonadal source such as the adrenal glands (16); (b) AR promiscuity due to mutations rendering it capable of activation by alternative ligands (17); (c) altered transcriptional AR activity as a result of altered coactivator and corepressor expression (18); or (d) ligand-independent, even constitutive, activation or hypersensitization of the AR...
by other pathways, such as PI3K/AKT signaling (19). This review aims to address the role of the PI3K pathway in prostate cancer through these mechanisms.

Somatic mutation of PI3K pathway genes and mechanisms of PTEN loss in human prostate cancer. The incidence of activating PI3K mutations in early and advanced prostate cancer remains to be elucidated, although data from the Sanger Institute Collaboration indicate that approximately 30% of patients with CRPC harbor p110α mutations.1 The importance of loss of function of PTEN is better described in both localized and metastatic prostate cancers and includes homozygous deletions, loss of heterozygosity (LOH), and inactivating mutations (see Fig. 1; 7, 8, 20–24). However, the reported frequency and mode of inactivation at different stages of prostate cancer vary. Homozygous deletions of PTEN have been detected in up to 15% of locally confined cancers and up to 30% of metastases (7, 8, 21–24). Heterozygous loss has been reported in 13% of locally confined cancers and up to 39% of metastases (7, 8, 21–23). A recent study of radical prostatectomies by fluorescent in situ hybridization (FISH) suggested that 63% of tumors had heterozygous loss, a much higher proportion than seen in any previous study of locally confined cancer (24). PTEN point mutations have been described in up to 16% of primary prostate cancers (22, 25, 26) and in 20%-30% of metastases (7, 21, 22). Mutations have been reported to be of the truncation and missense types (21, 22). Immunohistochemical studies indicate a loss of PTEN expression in 20%-27% of primary tumors (27) and in 79% of CRPC samples (28), although additional studies are warranted due to concerns regarding antibody validation (27). In these studies, PTEN loss correlated with advanced stage and high Gleason grade (29).

PI3K and AR signaling and progression to CRPC. Reports suggest that PI3K signaling may play a critical role, allowing prostatic cancer systems to maintain continued proliferation in low-androgen environments (30). Functional loss of PTEN is associated with increased AKT-1 phosphorylation, higher Gleason grade, advanced stage, and poor prognosis (31), predicting disease recurrence after primary treatment (32). The generation of transgenic mouse models that recapitulate features of the disease has advanced understanding of this pathway (19), and studies of knockout mice with targeted deletion of prostate-specific PTEN (PTEN−/−) have revealed prostate intraepithelial neoplasia (PIN) formation, invasive adenocarcinoma progressing to metastatic disease, an initial response to androgen ablation therapy, and eventual tumor growth despite castration (33). These data are in keeping with PI3K signaling inducing continued AR gain of function despite reduced steroid ligand levels (30), possibly through activation by posttranslational modification, increased coactivator activity (e.g., Wnt/β-catenin), or reduced corepressor activity (Fig. 1). Another transgenic model expressing intraprostatic AKT-1 resulted in a highly penetrant PIN phenotype; this was reversed by the mTOR inhibitor Everolimus (RAD001, Novartis; 20, 34). Moreover, mice with inactivation of one allele of PTEN with loss of p27Kip1 have accelerated spontaneous neoplastic transformation and develop prostate cancer (35).

Importance of p110β in prostate cancer. Recent reports suggest a critical role for p110β in prostate cancer. Both p85α and p110β appear to be essential for androgen-induced AR transactivation because they are required for cell proliferation and tumor growth (36). These PI3K isoforms may regulate AR-DNA interactions or the assembly of the AR-based transcriptional complex (37). Conditional knockout mice studies have also specifically evaluated the impact of deletion of p110α in the presence of PTEN loss in prostatic epithelium. Prostates had a normal appearance in the absence of p110α alone and had universal high-grade PIN in the anterior lobe by 12 weeks in the absence of PTEN alone; crucially, ablation of p110β prevented the tumorigenesis caused by PTEN loss (38). PTEN loss led to increased AKT phosphorylation in the prostate; additional ablation of p110β diminished phospho-AKT levels (38). These changes were p110α specific because p110α knockout did not abrogate tumor formation or AKT phosphorylation, supporting the evaluation of p110α inhibitors in prostate cancer treatment.

ERG translocations. Arguably the single most important breakthrough in prostate cancer biology has been the identification of the oncogenic ETS gene rearrangements that were initially reported to fuse untranslated sequences of TMPRSS2 with TMPRSS2-ERG in androgen-regulated gene-with ETS-family transcription factor genes (ERG or a truncated form of ETV1; ref. 39). Further studies have identified multiple other, less common, rearrangements with multiple promoters and ETS genes, including ETV4 and ETV5 (40). Overall, these ETS gene rearrangements are thought to occur in 50%-70% of prostate cancers, although it is probable that other androgen-driven oncogenes will be identified. The clinical significance of these translocations is under intense scrutiny; some studies indicate that the presence of TMPRSS2-ERG predicts a poorer prognosis, especially in tumors with a duplication of the TMPRSS2-ERG fusion (41). Recent data have shown that loss of PTEN cooperates with TMPRSS2-ERG activation in prostate cancer oncogenesis. Transgenic mouse expressing TMPRSS2-ERG in the prostate were developed and showed failure to develop PIN or invasive prostate cancer (42). However, when these were crossed either with PTEN+/− mice, or prostate-specific AKT transgenic mice, PIN but not invasive cancer developed. In another strain of transgenic mouse overexpressing ERG, both PIN and invasive cancer developed only when crossed with PTEN deficient mice (43). In addition, this study showed that prostate cancer specimens containing the TMPRSS2-ERG rearrangement (~40%) are significantly enriched for PTEN loss. These data implicate PTEN loss and ERG rearrangements as associated events that act in tandem to promote prostate cancer progression, potentially by inducing transcription of downstream checkpoint genes involved in promoting cell proliferation, senescence, and survival. In addition, it is therefore imperative that ERG-targeted therapies are developed and that these are rationally combined with inhibitors of the PI3K pathway.

Upstream chemokines. Studies indicate that p110β is not primarily regulated by tyrosine kinase receptors, but by G-protein-coupled receptors. Chemokines have been implicated in prostate carcinogenesis, by impacting cancer cell proliferation, survival, adhesion, and invasion (44) through their activation of G-protein-coupled transmembrane-spanning receptors activating downstream p110β signaling (45). Chemokines such as CCL2 (monocyte chemoattractant protein; MCP1) have also been reported to stimulate monocyte/macrophage migration into tumors, potentially fueling tumor growth, and to play a role in angiogenesis. This PI3K signaling downstream of
CCL2 in prostate cancer cells has been implicated in the inhibition of autophagic death (46). It is envisioned that targeting such chemokine signaling could impact prostate cancer cell survival.

How Do We Best Assess Deregulation of the PI3K/AKT Pathway and Select Patients Likely to Benefit from Drugs Targeting This Pathway?

The challenge of intrapatient prostate cancer cell heterogeneity. Prostate cancer is clinically a heterogeneous disease, and gene expression profiling describes at least three subtypes of prostate cancer (47). Molecular intrapatient tumor heterogeneity has also been reported, suggesting multiple and different cell clones in the same patient. In an analysis of 50 metastatic tissues from 19 patients with fatal prostate cancer, a significant degree of mutational heterogeneity in PTEN was found among different metastatic sites within the same patient (7). In another report based on 45 patients with localized disease, radical prostatectomy samples were evaluated by immunohistochemistry for the expression and phosphorylation levels of AKT and its relevant downstream targets, including GSK3-B, mTOR, FKHRL1, and 4E-BP1, and also showed intrapatient heterogeneity (48). Overall, this complicates patient selection for the rational use of inhibitors of this pathway. Further evaluation of the importance of this heterogeneity is indicated.

Predictive biomarkers. A composite of laboratory techniques assessing pathway components is likely to be required to comprehensively assess pathway overactivity. The clinical success of agents targeting the PI3K/AKT pathway could be maximized by prospectively identifying patients harboring molecular abnormalities in this pathway who may have a higher likelihood of responding. It is envisioned that analytically validated and clinically qualified biomarkers may need to be developed in tandem with inhibitors of this pathway. Acquiring tumor tissue to perform these analyses is desirable, but, depending on tumor site, this can be impractical and sometimes unsafe. For example, in prostate cancer, a prostate biopsy is a relatively invasive procedure, and acquiring bone and lymph node metastasis is difficult owing to inaccessibility and the invasiveness of acquisition procedures (Fig. 2A).

Circulating tumor cells (CTCs) hold promise as a tool for the analysis of potential predictive biomarkers. They have been shown to be present in patients with many different tumor...
types, but they are especially common in advanced prostate cancer (49). In a recent trial of a monoclonal antibody to IGF-1R, IGF-IR-positive CTCs could be detected and were seen to decrease after antibody therapy (50). Molecular characterization of CTCs by FISH, mutation analyses by sequencing, and protein expression by immunofluorescence hold much promise for monitoring efficacy and tailoring personalized therapy (Fig. 2B4).

The main current techniques for determining activation status of the pathway are immunohistochemistry and immunofluorescence. There are advantages and disadvantages to each method. Immunohistochemistry can localize pathway proteins intracellularly and distinguish tumor from stroma, but it is, at best, semiquantitative (Fig. 2C2 and C3). Immunofluorescence may be more quantitative and, for the evaluation of PTEN loss, has many advantages because this protein can be lost at a genomic level, by mutation or by epigenetic silencing (23). In addition, FISH for PTEN loss correlates with outcome, whereas, to date, PTEN immunohistochemistry alone did not (51). The latter may, however, have been due to poor antibody specificity and validation. Overall, nonetheless, acquiring a comprehensive assessment of pathway activation will likely necessitate combining different assessment techniques.

Reversible phosphorylation of serine/threonine residues is central to signaling in the PI3K pathway. Developments in the field of phosphoproteomics have been fueled by the need to simultaneously monitor many different phosphoproteins within signaling networks of interest (52). A recent application of this technology is the development of phosphospecific antibodies that specifically recognize the consensus substrate-phosphorylated motif of a given protein kinase. Phosphopeptide arrays have been constructed to assess cellular signaling events. In a prostate cancer study, laser-capture-microdissected cells were collected from normal prostate epithelium and invasive cancer to assess alterations in cell signaling in prostate cancer progression by using such technology (53).

**Novel Inhibitors of the Pathway**

**PI3K Inhibitors.** Potent and isoform-selective PI3K inhibitors with improved pharmacologic properties (54–57) are now entering clinical trials (4). Phase I studies of the oral PI3K pathway inhibitors XL147 (Exelixis), BEZ235 (Novartis), and GDC-0941 (Genentech) are currently in progress (58). Preliminary results from these studies suggest that these agents are well tolerated and have favorable pharmacokinetic-pharmacodynamic (PK-PD) profiles. Emerging data indicate that isoform selectivity may be important to maximize therapeutic benefit and minimize toxicity, although concerns remain about tumor cell redundancy between different isoforms. Studies also indicate that, in mutated PI3K with an oncogenic p110α charge-reversal mutation in the helical domain (E545K), inhibitory interactions are abrogated, resulting in constitutive PI3K activation (59). The p110α E545K mutant may therefore be susceptible to highly specific compounds that bind to its unique helical domain surface, sparing wild-type PI3K and reducing the likelihood of unwanted toxicity. Increasing knowledge of the structural biology of these mutated proteins may impact the development of the next generation of isoform-specific inhibitors (60).

**AKT inhibitors.** The importance of the individual AKT isoforms in prostate cancer has yet to be fully elucidated, although it has been suggested that AKT-1 isoform expression may be a prognostic marker for biochemical recurrence depending on its localization (61). There are several classes of AKT inhibitors currently in development, including isoform-selective AKT catalytic-domain inhibitors and inhibitors of the PI3K domain. Of the latter class, an alkylphospholipid, perifosine, has undergone a Phase II clinical trial in patients with CRPC, and although generally well tolerated, it showed no evidence of significant activity. Simultaneous targeting of AKT-1 and AKT-2 was shown to be superior to the inhibition of a single isoform for induction of caspase-3 activity in tumor cells, suggesting that pan-AKT inhibitors such as the ATP-competitive inhibitor GSK690693 (GlaxoSmithKline) are therefore likely to be more promising, although toxicity may be a potential issue (62). Several other small-molecule AKT inhibitors are now in early clinical trials, including a potent highly selective pan-AKT allosteric inhibitor MK2206 (Merck, Inc) that is currently in Phase I trials, both as a single agent and also in combination with other agents including docetaxel (63).

**mTOR inhibitors.** Proof of principle that the PI3K pathway can be successfully targeted for clinical use in cancer has been demonstrated by the development of rapamycin analogs (temsirolimus, everolimus) that inhibit the mTORC1 kinase. However, preliminary results to date have been disappointing when these analogs have been administered as single agents in CRPC. Superior single-agent activity may potentially be seen with dual mTORC1/mTORC2 ATP-competitive kinase inhibitors, which are currently in clinical development. These broader spectrum inhibitors may, however, be more toxic. mTORC1/mTORC2 inhibitors should, nonetheless, abrogate the reported negative-feedback loops associated with rapamycin analog administration, resulting in activation of upstream targets such as IGF1R and p-AKT (64).

**Combination Studies: Vertical and Horizontal Blockade**

At least two potential combination targeting strategies are envisioned: “vertical” or “horizontal” blockade. The concept of “vertical blockade,” or using multiple inhibitors targeting a specific pathway, may be of particular importance to alleviate the issue of negative-feedback loops. Dual PI3K and mTOR inhibitors such as PI-103, XL765, and BEZ-235 are therefore attractive (54). Preliminary data on XL765 have recently been presented and encouragingly show no significant toxicity concerns and evidence of pharmacodynamic modulation (65). “Horizontal blockade,” or the combined use of inhibitors of multiple signaling pathways, may also be important. Of particular importance for PI3K pathway inhibitors is the activation status of the RAS/RAF/MEK pathway. Recent data have suggested that deregulation of the RAS/RAF/MEK pathway is a key regulator in cancer cell resistance to PI3K inhibitors and suggest combined targeting of PI3K and MEK as an effective anticancer strategy (66). PI3K/AKT and B-RAF-ERK have been shown to act combinatorially in a mouse model of androgen-independent prostate cancer, suggesting particular importance for combination blockade in CRPC; however, the clinical relevance of these findings needs to be evaluated (67). There is also evidence to suggest cross-talk between AR and PI3K signaling. Rational combinations of PI3K/AKT...
Pharmacodynamic Biomarkers: Selecting Appropriate Dose and Schedule

The use of PK-PD profiling is important to optimize drug dosing and scheduling and demonstrates proof of principle as well as the downstream impact of target modulation on cancer cell proliferation and survival (69). Evaluation of the downstream phosphorylation status of key pathway proteins is widely done, usually by western blotting, immunohistochemistry or ELISA-based assays, and is likely to be relied on for some time (55, 57). Pharmacodynamic studies of mTOR inhibitors have highlighted some potential challenges of such studies (70) relating to intraintrapotent heterogeneity and the labile nature of the phosphorylation signal, presumably due to phosphatase activity. Evaluating molecular imaging strategies is therefore attractive, albeit more costly.

FDG-PET scanning in this setting is based on the rationale that signaling through the insulin receptor activates PI3K and AKT (71, 72). This stimulates glucose uptake and glycolysis through activation of mTOR and HIF-1α. The same glycolytic enzymes that are regulated by PI3K/mTOR/HIF-1α signaling are responsible for uptake and retention of the labeled PET tracer FDG (73). Studies investigating the use of FDG-PET with these drugs are ongoing. [18F]-fluorothymidine (FLT)-PET may also be a biomarker for PI3K blockade, with studies suggesting that this may have some utility in prostate cancer (74). Quantitative [18F]-FLT uptake correlates with the proliferation marker Ki-67 expression and may be superior to [18F] FDG-PET for detecting tumor proliferation (75).

Magnetic resonance spectroscopy (MRS) can detect concentrations of endogenous metabolites in a minimally invasive manner. The nonselective, prototypic PI3K inhibitors LY294002 and wortmannin induced a significant reduction in endogenous metabolites, associated with decreased AKT phosphorylation. MRS may therefore also provide a minimally invasive readout of PI3K inhibition. PI3K pathway blockade is anti-angiogenic, with p110α reported as being key to endothelial cell migration (77). Clinical imaging studies to evaluate the impact of these agents on tumor vasculature by dynamic contrast enhanced (DCE)-MRI, diffusion-weighted MRI (DWI), and DCE-CT may also have utility in proof-of-concept studies.

Conclusions

PI3K/AKT signaling appears to be critical to prostate cancer cell survival and proliferation. Our increasing understanding of the biology of this disease has led to the hope that novel inhibitors of the pathway will result in therapeutic benefit. However, for this to be achieved, it is now critical that well designed clinical trials that can question and answer key hypotheses are conducted to ensure that these drugs are placed both in their correct clinical context (e.g., neoadjuvant, adjuvant, metastatic) and in appropriate combinations (e.g., radiotherapy, hormonal therapy, and chemotherapy). The evaluation of predictive and pharmacodynamic biomarkers is likely to be crucial to the successful accelerated development of these agents.

Disclosure of Potential Conflicts of Interest

The authors have received commercial research grants from Genentech and Merck. The authors have served as consultants for Novartis, Arno, Merck, Exelixis, and Genentech. The Institute of Cancer Research has an interest in developing PI3K/AKT inhibitors.

References


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