Review Article
PI-3 kinase p110β: a therapeutic target in advanced prostate cancers

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Abstract: Prostate cancers in the castration-resistant stage are life-threatening because they are not curable in clinic. The novel androgen receptor inhibitor Xandi (Enzalutamide) and the new CYP17 inhibitor Zytiga (Abiraterone) prolonged patient survival only a few months in advanced prostate cancers. Therefore, novel therapeutic agents for advanced prostate cancers are urgently needed. PI-3 kinases are major intracellular signaling molecules that regulate multiple signal pathways related to cellular metabolism, cytokinesis, growth and survival. Accumulating evidence in the literature indicates that some isoforms of this kinase family are oncogenic and abnormally expressed in various human cancers, including prostate cancers. Recent extensive studies from our group and others showed that PI-3 kinase p110β is aberrantly overexpressed in advanced prostate cancers and is critical for prostate cancer development and progression as demonstrated in cell-based and animal models. Importantly, novel p110β-specific inhibitors have been developed and are currently been testing in clinical trials. In this article, we will briefly summarize recent developments in this regard.

Keywords: Prostate cancer, p110β, cancer therapy, castration resistance

Introduction
Prostate cancer is the second most diagnosed cancer around the world with about 240K new cases annually in North America [1]. The natural history of prostate cancers varies individually, ranging from microscopic lesions, organ-confined diseases, to aggressive metastatic cancers that ultimately claim patient life [2]. The etiology of prostate cancer and subsequent disease relapse may have various molecular causes, but in each scenario, the androgen receptor (AR) expression is maintained and highly activated [3]. Since the seminal work of Huggins and Hodges in 1941, medical treatment for metastatic prostate cancer has relied heavily on androgen ablation to inactivate the AR [2]. However, most patients treated by androgen ablation ultimately relapse to more aggressive so-called ‘castration-resistant prostate cancers’ (CRPC) with a highly re-activated AR pathway. Although it is not fully clear how the AR is re-activated after androgen ablation (by either surgical or hormonal castration), accumulating evidence indicated that several intracellular signaling pathways including the phosphoinositide 3-OH kinase (PI-3 kinase, PI3K) family [4-10], mitogen-activated protein kinase family (MAP kinase, MAPK) [11-14] and Src kinase family [15-20] are involved. As a consequence of recent studies, targeting the AR activation pathways is becoming an intensive research topic in prostate cancer [21-27]. Despite recent developments of the novel anti-AR agent Enzalutamide [28-30] and the androgen synthesis enzyme CYP17 inhibitor Abiraterone [31-33], advanced CRPCs remain a fatal with no means of cure in sight [34]. In this mini-review, we will focus our discussion on the role of PI-3 kinase family, especially the class IA isof orm p110β, in prostate cancer development and progression, as well as the potential implication of targeting this PI-3 kinase as a novel therapy.

PI-3 kinase family

PI3Ks are a group of major cellular signaling molecules that regulate multiple pathways and
cellular functions [35-37]. In mammalian cells, there are three classes of PI3K isoforms (I, II and III), of which the class I PI3Ks are further divided into two subtypes, IA p110α/β/γ and IB p110γ. Class IA PI3Ks are associated with the p85 regulatory subfamily, while class IB is associated with p101/p84 regulatory subunits. Class I and III but not class II PI3Ks are dual functional kinases, namely lipid kinase and protein kinase. PI3K p110α and p110β are ubiquitously expressed and knocking out either gene in mice results in an embryonic lethal phenotype. In contrast, PI3K p110α and p110β are predominantly expressed in lymphocytes, and knockout animals display immune defects but the mice are viable. There is only one member of the class III PI3K family VPS34, and its major function is regulation of autophagy [38, 39]. Due to the inactivating mutation of PI3K signaling opponent PTEN gene (phosphatase and tensin homologue deleted on chromosome-10) or unknown mechanism after androgen withdrawal, elevated PI3K activity has been proposed as one of the major mechanisms for prostate cancer progression [5, 34].

**Property diversity of class I PI-3 kinase isoforms**

It is well documented that class I PI3Ks are activated upon hormone and growth factor stimulation through either receptor-tyrosine kinases (RTKs) or G-protein coupled receptors (GPCR). PI3K p110α and p110β share a high similarity in amino acid sequences, expression patterns and regulatory partners. In fact both of them produce the same phospholipid substrate of phosphatidylinositol 3,4,5-trisphosphate (PtdIns (3,4,5) P3), a well-established second messenger for AKT activation. However, these two class I kinases (p110α or p110β) exert distinct preferences in regulation and functionality [40]. The p110α kinase is mostly activated by receptor-tyrosine kinases and small GTPase Ras, while p110β kinase prefers GPCR and Rho GTPase RAC1/CDC42 [41]. GPCR-activated Gβγ subunit interacts with p110β on its C2-helical linker region (514KAAEIASSDSANVSSRGGKKFL-PV537) and disruption of this interaction by a decoy peptide derived from this region reduces PTEN-deficient cancer cell growth and invasion [42]. On the other hand, p110 catalytic activity is monitored by the regulatory p85 subunits, of which two inhibitory contacts between p110α C2/kinase domain and p85α nSH2/SH2 do-

main whereas three contacts between p110β C2/kinase domain and p85β nSh2/iSH2/cSH2 domain exist to restrict their basal activity [43-45]. These isoform-specific restrictions reflect functional diversity of class I PI-3 kinases in different cell/tissue types.

In terms of functionalities, p110α plays a pivotal role in insulin signaling and glucose metabolism, as well as G1 cycle entry, whereas p110β is important in DNA synthesis/replication and cell mitosis. While p110α is mainly localized in cytoplasm, p110β resides in the nucleus compartment [46]. Although either p110α or p110β is able to sustain cell proliferation and survival as a single viable kinase in immortalized mouse cells [47], there are many differences between these two Class I kinases in human cancer cells. First, their protein levels and corresponding kinase activities are variable in colon/urinary bladder tumor tissues and a significant difference between these two isoforms was found among various colon cancer cell lines [48]. Second, neutralizing antibodies against p110α induced apoptosis in multiple colon cancer cell lines but p110β antibodies only attenuated de novo DNA synthesis [48]. This is supported by a recent report [46] that p110β is mostly involved in DNA replication through both kinase-dependent and -independent mechanisms. Third, a nuclear localization sequence (NLS) has been identified in p110β C2 domain (K10KVNTKSTK118) but it is missing in p110α and this nuclear localized p110β is essential for cell survival [49] and chromosome segregation during mitosis [50]. In addition, p110β but not p110α was recently shown to be involved in osteoclastic resorption in bone [51, 52] and in male fertility as evidenced by testicular hypotrophy and impaired spermatogenesis in p110β inactivated mice [53]. Most strikingly, in spite of these diverse differences, a very recent report showed that p110α actually dimerizes with p110β upon serum stimulation in NIH3T3 cells and that their association depends on the p85 regulatory subunit [54]. Further analysis revealed that their kinase activity of this oligomerization in AKT phosphorylation is regulated by PTEN, which is also associated with the dimerized p110α/β complex. These data reflect another layer of PI3K regulation.

Autophagy is an evolutionary mechanism in response to nutritional stress and increased autophageic response is often associated with
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cancer progression and treatment resistance in many cancers [55]. Interestingly, in mice with conditional knockout of class I PI3Ks, p110α deletion resulted in a slight increase in autophagic response while p110β deletion resulted in a severe defect in autophagy response [56]. This p110β-mediated promotion of autophagy is associated with increased cellular levels of PtdIns(3)P that is required for the early event in autophagosome formation. Further analysis revealed that p110β kinase activity is not essential in promoting autophagy but its scaffolding property of the kinase protein is involved through its direct interaction with small GTPase Rab5 in responding to growth factor withdrawal [57]. These data provided a strong rationale for targeting p110β as a therapeutic biomarker in cancer management.

Oncogenic role of PI-3 kinase p110β in prostate cancer

It is conceivable that all class I PI-3 kinases are oncogenic when overexpressed or overactivated in mammalian cells [37, 58]. In human cancers, the most common gain-of-function mutations in PI3K family were from PIK3CA gene/p110α protein, whereas the los-of-function mutations were from PTEN gene/PTEN phosphatase protein [40, 59, 60]. In a small portion of human cancers, gene amplification was seen in PIK3CB gene/p110β protein although its wild-type form is also oncogenic [58, 61]. Only one tumor-associated p110β mutant E633K has been reported so far from a HER2-positive breast cancer patient [62]. This E633K mutant p110β had a higher basal activity that activates AKT-S6K1 pathway and promotes tumor cell proliferation/survival.

In prostate cancers, due to a higher prevalence of PTEN loss mutation, aberrant PI3K/AKT activation was proposed to play an important role in disease progression [34, 63]. In animal models, genetic approaches demonstrated that p110β but not p110α activation is required for PTEN-deletion or ERBB2-driven tumor development in the prostate and breast, respectively [9, 64]. Consistently, overexpression of a constitutive p110β or its downstream kinase AKT protein induces early lesions of prostatic tumor formation in mice [65, 66]. These animal studies were supported by data from cancer cell culture models, in which p110β activity was shown to be critical for cell proliferation and survival in PTEN-null prostate [10] and colon cancers [67]. These findings have been supported by additional animal model-based studies [68-71] and revealed that p110α but not p110β is critical in polyoma middle T antigen (MT) plus HER2-driven breast tumor development [69], PTEN-null derived thyroid tumor [68], or MT/Kras-driven ovarian tumor [70]. Those data indicate that PI3K isoform dependence in tumor development is not tissue- or organ-specific but due to the driving mode of carcinogens [71].

To provide clinical relevance of PI3K involvement in prostate cancers, the expression profiles of class I PI3K family in human were examined. Gene expression of p110β and p85α but not p110α at the mRNA and protein levels were observed to be significantly higher in primary tumors compared to their benign counterparts [4]. These findings are supported by other reports from different groups [72, 73]. PI3K p110β activity and its downstream kinases AKT-SGK1 were found to be involved in AR-dependent gene expression, cell proliferation and survival in prostate cancer cells [74-76]. Most importantly, with a unique inducible AKT activation system [77], AKT activation was capable of driving the castration-resistant progression of prostate cancer in a mouse xenograft model [76]. These studies, together with others, provide a strong clinical rationale for targeting the PI-3 kinase p110β for prostate cancer management.

Isoform-specific p110β inhibitors in prostate cancer

Given the prominent importance of PI-3 kinases in multiple human cancers, targeting this pathway as a novel cancer therapy has drawn extensive attention in recent years as discussed in newly published review articles [34, 63, 78-83]. Most chemical inhibitors currently used in preclinical and clinical studies are targeting PI3K/AKT-mTOR pathways with either mono-, dual- or pan-specificities. The majority of inhibitors target p110α, AKT or mTOR and only two, GSK2636771 and SAR260301 (compound 28, discussed later), are p110β-specific inhibitors currently in early clinical trial [81]. It is obvious that non-isoform selective inhibitors for the PI3K pathway will yield higher efficiency but with severe side effects, while isoform-selective inhibitors definitely have higher toler-
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Figure 1. Chemical structures of the TGX221-derived (A) and structurally different (B) p110β-specific inhibitors. Groups circled in dashed lines are the major structural differences compared to TGX221.

able doses with fewer side effects [81]. In the era of personalized medicine, a potent inhibitor with precise target selectivity will be ideal for better patient management.

The first p110β-specific inhibitor TGX221 was reported in 2005 [84] and it is a derivative of the pan-PI3K inhibitor LY294002, which was synthesized in 1994 based on a natural flavonoid compound Quercetin that targets multiple protein kinases [85]. TGX221 has an IC\(_{50}\) of 5 nM against p110β, representing a high target selectivity and was initially used as anti-thrombotic agent in animals at 2.5 mg/kg intravenously without cardiovascular side-effect [86]. Due to the concern of its potential bleeding side-effect by inhibiting platelet function [84, 87] and its poor water solubility, a nanoparticle-based approach to specifically deliver TGX221 to cancer cells was designed [88, 89]. The cancer cell-specific targeting is based on a RNA aptamer-mediated binding against prostate-specific membrane antigen (PSMA), a cell surface protein highly expressed on prostate cancer cells [90]. TGX221 was modified (analog BL05, Figure 1A) so that the analog could be conjugated with polymers without affecting its inhibitory effect towards p110β/AKT activity. Early experiments in cell and animal models revealed excellent prostate cancer cell-specific uptake kinetics and sustained bioavailability in blood stream [90]. As tested in a nude mouse xenograft model, PSMA-targeted TGX221 nanoparticles dramatically reduced tumor growth of xenografts derived from multiple prostate cancer cells (Li et al, unpublished data highlighted at http://cdmrp.army.mil/pgrp/research_highlights/13li_highlight.shtml).
In addition, an alternative approach to target the TGX221 compound for HER2 overexpressing tumors was developed [89]. A HER2 binding peptide was conjugated to a TGX221 analog (TGX221-D1, Figure 1A) with a spacer peptide chain. The TGX-D1 analog was modified from TGX221 so as to be linked to the HER2-targeting peptide without affecting its inhibitory effect towards p110β kinase. To provide a prostate cancer-specific therapeutic effect, a short peptide derived from a substrate of prostate-specific antigen was inserted between HER2-binding peptide and TGX221 analog TGX-D1, so that the final peptide conjugates of TGX221 could be chopped by PSA inside prostate cancer cells after HER2-mediated uptake. The first phase study showed that the TGX221 peptide conjugate has a better prostate cancer cell-specific uptake rate and excellent water solubility compared to the parental compound TGX221 [89]. Further testing in animal models is required to determine its anti-tumor activity in xenograft models.

TGX221 analog KN-309 was synthesized as a racemic mixture in 2004 (described in a patent document of Jackson, et al. W02004016607). Its active enantiomer AZD6482 (Figure 1A) was tested for its anti-thrombotic effect in animals and human volunteers [91]. AZD6482 has an IC_{50} of 10 nM towards p110β and was verified as the first anti-platelet agent in humans without increased bleeding time (clinical trial NCT00688714 and NCT00853450). Interestingly, a different group also identified AZD6482 (named KIN-193) as a potent p110β-specific inhibitor from a collection of 19 PI3K inhibitors [92]. From a panel of 422 human cancer cell lines, AZD6482 (KIN-193)-induced suppression of cell proliferation was associated with PTEN-null status. Intraperitoneal delivery of AZD6482 (KIN-193) at a dose of 20 mg/kg (twice a day) suppressed PTEN-null cancer cell (prostate cancer PC-3 and breast cancer HCC-70)-derived but not PTEN-positive breast HCC1954 cancer cell-derived xenograft growth in nude mice. These data strongly suggest that targeting PTEN-deficient cancer cells with TGX221 analogs has a bright future as a therapeutic agent in cancer therapy.

Very recently, scientists from Sanofi reported a new series of p110β inhibitory compounds with major structural differences from TGX221 [93]. Compound 28 (SAR260301, Figure 1B) exerted preferred drug-like properties, including high water-solubility (0.928 mM), oral bioavailability and low IC_{50} specific to p110β (23 nM) more than 20-fold less compared to other PI-3 kinases. In animal experiments, oral administration of Compound 28 at a dose of 150 mg/kg bidaily suppressed AKT phosphorylation and reduced xenograft tumor growth derived from human prostate cancer PC-3 (PTEN-null) and melanoma UACC-62 (PTEN-null/BRAF^{V600E} mutant) cells. These data provided the biological foundation for compound 28/SAR260301 entering early clinical trials (NCT01673737) as a therapeutic drug for advanced cancers. Meanwhile, research scientists from AstraZeneca also reported a new series of p110β-specific inhibitory compounds that exert better in vivo anti-thrombotic effect devoid of bleeding and insulin resistance [94]. Of which Compound R-16 (Figure 1A) exerted a reliable oral pharmacokinetic profile and a safe margin towards p100α to avoid interfering insulin pathway. However, its use as anti-tumor agent is currently unknown.

GSK2636771 is a p110β-specific inhibitor developed by GlaxoSmithKline and is now in early phase of clinical trials for PTEN-null cancers (NCT01458067). Like SAR260301, GSK2636771 is largely different from TGX221 (Figure 1B) and has a superb IC_{50} towards p110β (1.3 nM) [95]. GSK2636771 inhibited cell proliferation and AKT phosphorylation in PTEN-null prostate cancer (PC-3) and breast cancer (BT549 and HCC70) cell lines but not in PTEN-positive breast cancer HCC1954 cells. Also, it had no inhibitory effect on endometrioid endometrial cancer cell lines, neither PTEN-null nor -positive cells [96]. There is no published data in the literature for its use in animal experiments with human prostate cancer cells.

Based on GSK2636771 and another closely related series of TGX221 analogs [97], a third series of thiazolopyrimidinone compounds were reported as p110β-specific inhibitors [98]. Compound 18 (Figure 1B) in this series showed the most preferable properties as an oral anticancer agent, including high specificity against p110β (IC_{50} 0.6 nM), orally bioactive for 6 h suppression of AKT phosphorylation at a dose of 100 mg/kg, and a complete blockade of tumor growth derived from prostate cancer PC-3 cells at both doses of 100 or 300 mg/kg per day in nude mice. These data strongly suggest that
this well-designed small chemical compound with high specificity towards p110β represents a highly effective treatment for advanced prostate cancers.

Conclusion and prospective points

Prostate cancer at the advanced stage is a lethal disease without means to cure in clinic. Extensive studies demonstrated that aberrant activation of the PI3K pathway plays a critical role in disease progression. Current preclinical data suggest that small chemicals specifically targeting PI3K/p110β kinase are capable of suppressing tumor growth. A series of p110β-specific inhibitory compounds were reported in the literature and two of them, SAR260301 and GSK2636771, are in early phase of clinical trials. In this personalized medicine era, prostate cancer-specific targeting of a specific kinase represents the cutting edge trend in drug discovery.

Although current data from animal models did not reveal severe side effects of p110β-specific inhibitors, theoretically, systemic inhibition of p110β kinase might cause dysfunctions in off-target organs or tissues, such as insulin metabolism [64], cardiovascular [83] or brain/neuronal system [99]. Therefore, a tissue-targeted delivery system coupled with a pro-drug strategy as described previously [88, 89] will provide protection from potential systemic side effects. In addition to ATP-competitive inhibitors, alternative strategies to disrupt p110β interaction with its upstream activators like G-protein sub-units or p85 proteins [4, 8, 100] will open novel avenues for even better drugs to be developed in the near future.

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