# Interplay Among PI3K/AKT, PTEN/FOXO and AR Signaling in Prostate Cancer

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## Introduction

Phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K) belongs to a family of lipid kinases involved in phosphorylating the 3-position hydroxyl group of the inositol ring of phosphatidylinositol (PtdIns) [1]. Products of PI3K activity, i.e., the lipid second messengers phosphatidylinositol (3,4,5) trisphosphate [$PI(3,4,5)P_3$ or PIP3] and $PI(3,4)P_2$ (PIP2), promote membrane association and activation of serine/threonine kinases such as AKT (or termed protein kinase B (PKB)). There are three highly-homologous AKT isoforms: AKT1/PKBα, AKT2/PKBβ, and AKT3/PKBγ [2]. These isoforms encoded by three different genes possess both common and isoform-specific functions.

AKT is activated by phosphorylation of two serine (Ser)/threonine (Thr) residues, one (Thr$^{308}$ in AKT1) being phosphorylated by the phosphoinositide-dependent kinase 1 (PDK1) [3] and the other (Ser$^{473}$ in AKT1) being phosphorylated by the mammalian target of rapamycin complex 2 (mTORC2) [4]. Therefore, this pathway is also known as the PI3K/AKT/mTOR signaling pathway. Appropriately 40% of primary and 70% of metastatic prostate cancers harbor genomic alterations leading to the activation of the PI3K signaling pathway [5, 6].

The PI3K signaling cascade transduces extracellular signals to intracellular targets. The extracellular signals include peptide hormones and growth factors, such as insulin [7], epidermal growth factor (EGF) [8], sonic hedgehog (shh) [9] and insulin-like growth factor 1 (IGF-1) [9]. Mechanistically, upon the stimulus of the extracellular signals, the signaling transduction cascade is activated by PI3K phosphorylation. AKT acts as an important mediator via recruitment to the membrane by interaction with phosphoinositide docking sites, where it becomes fully activated through its phosphorylation by PDK1 and mTORC2. Activated AKT phosphorylates Ser and Thr residues of its targets, primarily within a minimal consensus recognition motif of R-X-R-X-X-S/T-f (X: any amino acid; f: a preference for large hydrophobic residues) [10]. This pathway leads to CREB activation [11], p27 inhibition [12, 13], FOXO phosphorylation and cytoplasmic localization [14, 15], and activation of downstream effectors of mTORC1 such as

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p70S6K and 4EBP1 [16]. There are myriad AKT targets with their phosphorylation sites listed in Fig. 1. Functionally, activation or inactivation of these downstream targets leads to nutrient metabolism, cell proliferation, survival, migration, and angiogenesis, which ensure prostate cancer cell survival and protection from apoptosis (Fig. 1).

## Activation of PI3K Due to PTEN Genetic Alterations

### PTEN Mutations Account for the Major Cause of PI3K Activation in Prostate Cancer

PI3K signaling in both primary and advanced prostate cancers is activated in a similar manner, mainly due to mutations in the tumor suppressor gene phosphatase and tensin homolog (*PTEN*). This gene encodes PTEN protein that acts as a $PIP_3$ phosphatase, therefore antagonizing the PI3K pathway [18]. Approximately 17% of primary prostate cancers in patients harbor *PTEN* mutations [19]. However, approximately 50% of metastatic castration-resistant prostate cancer (mCRPC) patients have somatic mutations in the PI3K pathway [20]. Among these mutations, *PTEN* mutations account for the highest frequency (approximately 40.7%), mainly biallelic inactivation of the phosphatase domain in the hotspots of this gene.

To date, PTEN deletion or mutations have been considered to be the major genetic alterations in PI3K/AKT signaling activation. Other genetic alterations, including amplifications and activating fusions in *PI3K3CA* and p.E17K activating mutations in *AKT1*, also contribute to the activation of PI3K signaling [20]. Mutations of another member of the PI3K catalytic subunit, *PI3K3CB*, were observed initially in a cohort of advanced prostate cancers [20]. In agreement with a previous study [21], mutations of *PI3K3CB* rather than *PI3K3CA* are most likely to occur in the context of *PTEN*-deficient cases, implying that some *PTEN* deficient cancers may depend on PIK3CB activation. The frequency of PI3K
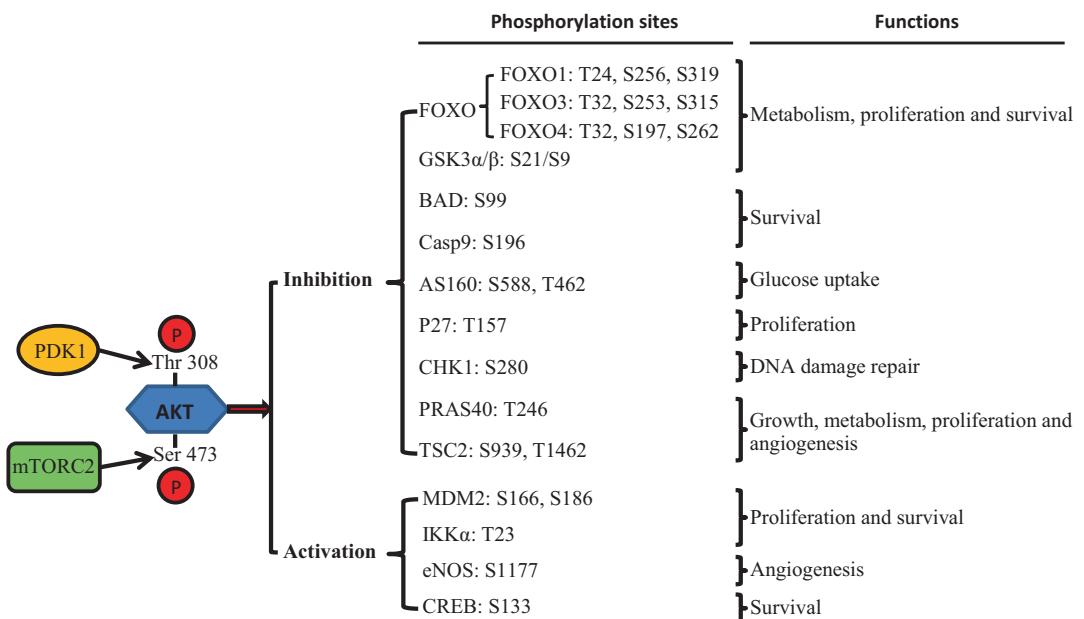


**Fig. 1** Selected AKT-phosphorylated proteins (modified from a previous report [17]). AKT is phosphorylated by PDK1 and mTORC2, resulting in the phosphorylation at Thr308 and Ser473 respectively. The phosphorylated AKT subsequently phosphorylates a group of proteins through a recognition motif R-X-R-X-X-S/T-f, which leads to the inhibition or activation of AKT targets.

**Table 1** The frequency of genetic alterations in PI3K signaling pathway genes in primary prostate cancer [22]

| Gene | Altered frequency | Missense | Truncating | Frameshift | Other |
|------|-------------------|----------|------------|------------|-------|
| PTEN | 167/1013 (16%) | 15 | 29 | 1 | 122 (Deep deletion) |
| PIK3CA | 69/1013 (7%) | 29 | 1 | 1 | 38 (Amplification) |
| PIK3CB | 41/1013 (4%) | 10 | 0 | 0 | 31 (Amplification) |
| AKT1 | 14/1013 (1%) | 6 | 0 | 0 | 8 (Amplification) |

**Table 2** The frequency of genetic alterations in PI3K signaling pathway genes in advanced prostate cancer [20]

| Gene | Altered frequency | Missense | Truncating | Frameshift | Other |
|------|-------------------|----------|------------|------------|-------|
| PTEN | 60/150 (40%) | 1 | 10 | 0 | 39 (Deep deletion) 8 (Fusion) 2 (Deep deletion and fusion) |
| PIK3CA | 8/150 (5%) | 5 | 0 | 1 | 1 (Amplification) 1 (Amplification and fusion) |
| PIK3CB | 10/150 (7%) | 3 | 1 | 0 | 5 (Amplification) 1 (Amplification and fusion) |
| AKT1 | 5/150 (3%) | 2 | 0 | 0 | 3 (Amplification) |

pathway gene mutations in primary and advanced prostate cancer is listed in Tables 1 and 2, respectively.
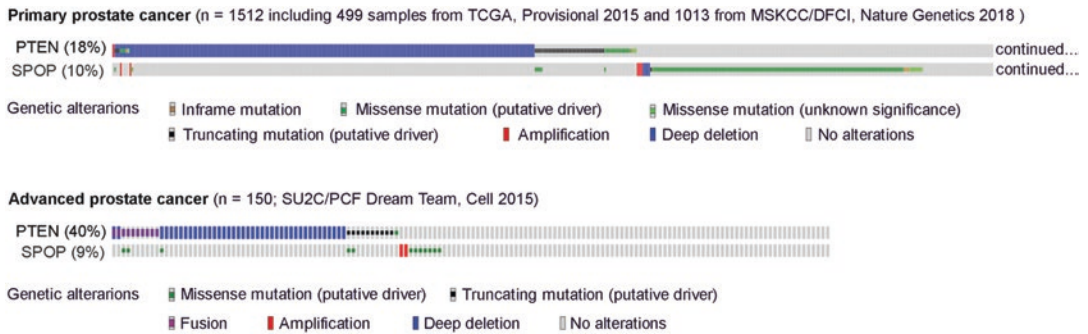
## Pten Deletion-Driven Prostate Cancer Mouse Models

Given that *PTEN* mutations comprise one of the most common genetic alterations in prostate cancer, it is important to assess its tumor suppressor function by generating *Pten* mutant mouse models. Conventional homozygous deletion of *Pten* causes embryonic lethality in mice [23]. Heterozygous loss of *Pten* in the mouse prostate results in a 100% penetrance of prostate intraepithelial neoplasia (PIN), a precursor of prostate cancer. However, on a Balb/c/129 genetic background, the latency of PIN is relatively long (approximately 10 months) and the PIN lesions rarely undergo metastasis [24, 25]. Thus, it is possible that mutations in other tumor suppressor genes or loss of another allele of *Pten* might be required for prostate tumorigenesis. Indeed, concomitant *Pten* heterozygous deletion and alterations in other genes, such as *p27* [26], *Nkx3.1* [27], *ERG* [28, 29], or *CREBBP* (*CBP*) [30] has given rise to prostate cancer in mice with various genetic backgrounds. Although these genetic alterations have accelerated formation of PIN

lesions and/or cancer, no metastatic prostate cancers have been observed in these models. Generation of a prostate-specific *Pten* homozygous deletion mouse model recapitulates the disease progression of human prostate cancer, mimicking the progression from PIN to invasive adenocarcinoma and, in very rare cases, metastasis [25]. This and other *Pten* deletion mouse models have been adapted to study the etiology of prostate cancer and the mechanisms of cancer progression [24, 25, 27, 30].

## Activation of AKT/mTOR Signaling Pathway in SPOP Mutated Prostate Cancer

Mutations in other genes that appear to be irrelevant to the PI3K pathway can indirectly promote activation of AKT/mTOR signaling. The most striking example is mutation of the tumor suppressor gene, speckle-type POZ (*SPOP*). *SPOP* is mutated in approximately 10–15% of prostate cancer patients [19, 20, 22, 31]. Intriguingly, there is a mutually exclusive relationship between PTEN mutation and SPOP mutation in patients with primary prostate cancer (Fig. 2), implying that these two genetic alterations share a common downstream pathway during prostate cancer pathogenesis. In advanced prostate cancer this

**Primary prostate cancer** (n = 1512 including 499 samples from TCGA, Provisional 2015 and 1013 from MSKCC/DFCI, Nature Genetics 2018 )



**Fig. 2** *PTEN* mutations are mutually exclusive with *SPOP* mutations in primary prostate cancer. The frequency of *PTEN* and *SPOP* mutations was analyzed from primary and advanced prostate cancers . The primary prostate cancer data was combined from two studies, including 1512 samples, whereas the advanced prostate cancer data from one study consisting of 150 samples. Mutual exclusivity was observed between PTEN and SPOP mutations in primary prostate cancer (***$P < 0.001$) but not in advanced prostate cancer ($P = 0.484$).

mutual exclusivity is not evident in the small number of CRPC cases (150) examined to date (Fig. 2). Therefore, further investigation of the relationship between these two genetic alterations in large cohorts of advanced prostate cancers is warranted.

## SPOP Mutations Induce AKT/mTORC1 Activation via Elevation of Bromodomain and Extra-Terminal (BET) Family Proteins

SPOP acts as an adaptor protein of the CULLIN3-based E3 ubiquitin ligase and promotes protein ubiquitylation and proteasome degradation. A number of prostate cancer relevant proteins such as bromodomain and extra-terminal motif (BET) proteins (BRD2, BRD3 and BRD4), SRC-3, TRIM24, ERG, and AR, are substrates of SPOP [32–40]. Almost all of these SPOP substrates are somehow involved in or associated with AKT signaling pathway and their relationships will be discussed throughout this chapter.

SPOP mutations result in an increased expression of BET family proteins including BRD2, BRD3, and BRD4 [40]. Subsequently, the stabilized BRD4 activates the transcriptional expression of the Rho GTPase family member RAC1 and cholesterol synthesis genes [40]. RAC1 is a canonical small GTPase that acti-

vates the AKT-mTORC1 pathway by binding directly to mTOR [40, 41]. Cholesterol-rich lipid rafts are linked to AKT activation and prostate cancer cell survival [42, 43].

SRC-3, which is also known as amplified in breast 1(*AIB1*), is encoded by the nuclear receptor coactivator 3 (*NCOA3*) gene. SRC-3 is a transcriptional coactivator that contains several nuclear receptor interacting domains and possesses an intrinsic histone acetyltransferase activity, which facilitates the accessibility of transcriptional factors to chromatin. IGF-1, which is a target of SRC-3 [44], is a potent upstream regulator of the AKT signaling pathway [10]. Moreover, SRC-3 can also contribute to the activation of AKT in SPOP-mutant prostate cancer cells by functioning as a transcriptional coactivator to facilitate expression of *RAC1* and cholesterol synthesis genes [40].

TRIM 24, a known AR coactivator, binds to the *PIK3CA* promoter to regulate the transcription of *PIK3CA* gene, leading to the upregualtion of PI3K-AKT signaling [45]. Intriguingly, TRIM24 is regulated by SPOP via proteasome pathway [37]. Therefore, in SPOP mutant prostate cancer cells, TRIM24 is stabilized at the protein level. Taken together, SPOP mutations augment AKT signaling through multiple mechanisms, and further investigation is warranted to fully elucidate the signaling pathways through which SPOP mutations lead to activation of AKT signaling.

## *SPOP* Mutant Mouse Models

SPOP mutations represent a molecularly-distinct subtype of prostate cancer. Generation of mouse models that recapitulate the unique features of SPOP mutations is important for a full understanding of the etiology of these lesions and their role in prostate cancer. To mimic SPOP mutation-induced prostate cancer pathogenesis, the most-frequently occurring SPOP mutant, F133V, was knocked into the Rosa26 locus and specifically expressed in the mouse prostate through a lox-STOP-lox strategy [46]. Surprisingly, little or no histological or glandular architecture of the prostate was observed in this mouse model [46]. At the cellular level, proliferation was not significantly altered, and changes in AR expression were observed rarely, and rare cells exhibited cytological atypia with enlarged nuclei in a majority of all prostate lobes in mice at ≥12 months of age [46].

The above findings indicate that like many other known genetic alterations (*ERG*, *ETV1* and *TP53*) in human prostate cancer [28], SPOP mutations alone may not be sufficient to drive tumorigenesis, and other genetic alterations are required to promote or accelerate tumorigenesis and progression. As discussed above, *PTEN* heterozygous mutations alone results in minimal histologic changes in the prostate [25], even though *Pten* heterozygous mouse models have often been crossed with the other mutant mouse models to study the etiology of prostate cancer. Indeed, when the SPOP F133V mutation mouse was crossed with *Pten* heterozygous deletion mouse, high-grade PIN developed only in the compound mice [46]. However, it is worth noting that *SPOP* mutations and *PTEN* deletions are almost mutually exclusive in patients with primary prostate cancers (Fig. 2). Therefore, further development of clinically relevant *SPOP*-mutated prostate cancer mouse models is warranted to interrogate the molecular mechanisms underlying *SPOP* mutation-induced prostate tumorigenesis.

## FOXO1 Dysregulation in Prostate Cancer

The forkhead box-O protein 1 (FOXO1) belongs to the FOXO family that includes three other members (FOXO3, FOXO4 and FOXO6). FOXO1 is a transcription factor that acts as a tumor suppressor by transcriptionally regulating expression of genes involved in apoptotic cell death, cell cycle, DNA damage repair, glucose metabolism, and carcinogenesis [47, 48]. Multiple mechanisms regulate FOXO1 functions, including, but not limited to, genomic deletion, transcriptional downregulation, and phosphorylation. *FOXO1* gene deletion as well as transcriptional downregulation have been found in a substantial proportion of prostate cancers from patients [19, 20, 49–52]. FOXO1 is a direct phosphorylation target of AKT. AKT-mediated phosphorylation of FOXO1 induces its translocation from the nucleus to the cytoplasm, resulting in inhibition of the transactivation of its target genes. We have summarized three major mechanisms that lead to FOXO1 inactivation and the effects on its downstream pathways in Fig. 3.

## FOXO1 and AR

FOXO1 can bind directly to the AR in a ligand-independent manner, thereby inhibiting the transcriptional activity of both full-length AR and constitutively active splice variants of AR [53–56]. However, this inhibitory effect is dependent largely on FOXO1 phosphorylation status. Specifically, upon the stimulus of IGF-1 or insulin, AKT signaling can induce FOXO1 phosphorylation and subsequent translocation from the nucleus to the cytoplasm, thereby impairing FOXO1 inhibition of ligand-induced AR activation in the nucleus. Similarly, in PTEN-mutated prostate cancer cells, AKT signaling is activated and FOXO1 is transported from the nucleus to the cytoplasm, thereby favoring transactivation of AR [48, 55]. However, AR regulation by the
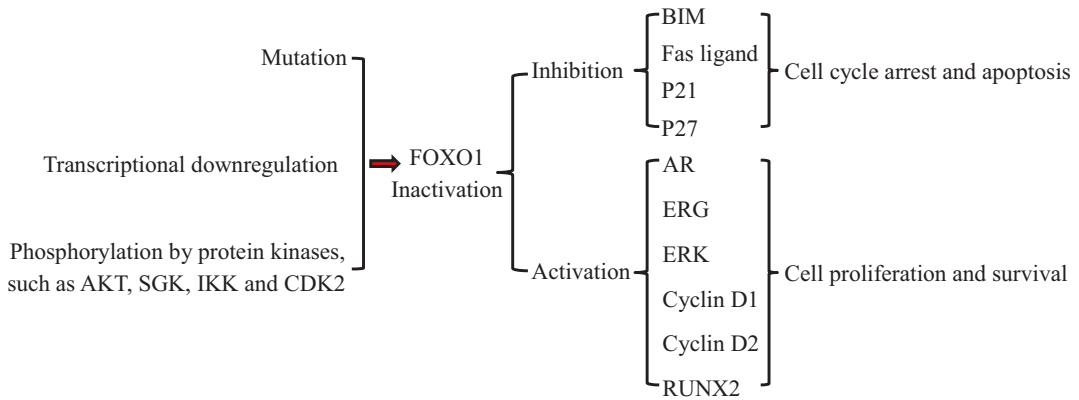
**Fig. 3** Diagram depicting three major mechanisms leading to FOXO1 inactivation. There are at least three mechanisms leading to the inactivation of FOXO1, which result in either the inhibition or the activation of its downstream targets and signaling.

PI3K/AKT pathway is very complex, including a negative feedback between AR and AKT signaling [5, 57]. For instance, AKT phosphorylates AR at Ser210, thereby inhibiting AR transactivation [57].

Aberrant activation of AR is associated with the progression of CRPC. The downregulation of FOXO1 in PTEN-negative prostate cancer contributes to the hyperactivation of AR [55]. Interestingly, FOXO1 interacts physically with HDAC3 and acts as a corepressor that inhibits androgen-independent activation of AR. Thus, co-transfection of FOXO1 and HDAC3 in prostate cancer cells results in a greater inhibition of AR activity than transfection with FOXO1 or HDAC3 alone [55]. Specifically, a putative transcription repression domain in the NH2-terminus of FOXO1 appears to be responsible for FOXO1 inhibition of the AR. FOXO1 can bind to the transcription activation unit 5 (TAU5) motifs in the AR NH2-terminal domain (NTD) that is required for recruitment of p160 coactivators including SRC-1, subsequently inhibiting the ligand-independent activation of AR splice variants [53]. Moreover, PI3K-AKT-FOXO1 signaling regulates AR variant 7 [56], further indicating that PI3K is a potential therapeutic target in CRPC patients.

## FOXO1 and ERG

The ETS-related gene (ERG) is a transcription factor belonging to the E-26 transformation-specific (ETS) family. It regulates a group of genes involved in vasculogenesis, angiogenesis, hematopoiesis, and bone development [58]. ERG is highly associated with prostate cancer development. Aberrant overexpression of ERG is found in approximately 50% of all human prostate cancer due to the fusion of the *ERG* gene body to androgen-regulated promoters and enhancers that normally regulate genes such as *TMPRSS2*, *SLC45A3*, and *NDRG1* [19, 20, 22, 59].

ERG genetic rearrangements and loss of PTEN often co-occur in human prostate cancers [19, 28], indicating an association between PI3K signaling and ERG. Indeed, the combination of transgenic expression of prostate cancer-associated *TMPRSS2-ERG* and heterozygous deletion of *Pten* induces high grade PIN and cancer in the mouse prostate [28, 29], although how the loss of PTEN works in concert with ERG overexpression to promote prostate tumorigenesis was unexplored in these studies. Also, FOXO1 binds directly to the DNA binding domain of ERG and inhibits ERG transcriptional activity in prostate cancer cells [60]. However, FOXO1

inhibition of ERG is abolished by AKT due to AKT mediated phosphorylation and exclusion of FOXO1 from the nucleus [60]. Importantly, homozygous deletion of *FOXO1* cooperates with overexpression of *TMPRSS2-ERG* to induce formation of HGPIN and cancerous phenotypes in the mouse prostate [60]. Therefore, functional loss or genetic deletion of FOXO1 results in an aberrant activation of ERG fusions and abnormal expression of ERG target genes, thereby contributing to prostate tumorigenesis.

## FOXO1 and RUNX2

The Runt-related transcription factor 2 (RUNX2), also known as core-binding factor subunit alpha-1 (CBFA1), regulates many cellular proliferation genes, such as *c-Myc*, *C/EBP* [61], *TP53* [62], and the CDK inhibitor *p21$^{cip1}$* [63], at the transcription level. The DNA-binding affinity of RUNX2 is most likely dependent on its phosphorylation state [64], which is correlated with cellular proliferation. Thus, RUNX2 phosphorylation is related to RUNX2-mediated cellular proliferation and cell cycle control. In support of this concept, RUNX2 is phosphorylated at Ser451 by CDK1, which facilitates cell cycle progression through the regulation of G2 and M phases [65]. Phosphorylation of RUNX2 at Ser301 and Ser319 by MAPK-dependent activation also promotes RUNX2 transcriptional activity [64, 66].

Intriguingly, RUNX2 protein level fluctuates throughout the cell cycle, and this is most likely due to its regulation by both gene transcription and protein degradation. RUNX2 can also interact with several protein kinases that facilitate cell-cycle dependent dynamics. Thus far, most studies relating to RUNX2 have been focused on osteoblast proliferation and differentiation; however, the role of RUNX2 in prostate tumorigenesis is poorly understood.
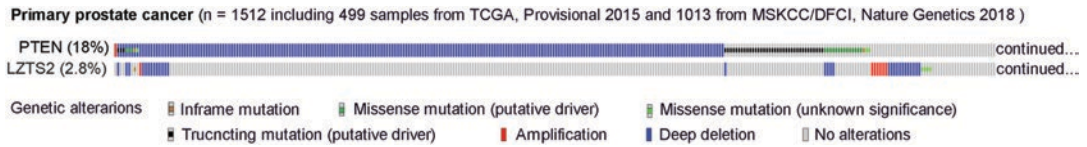
RUNX2 forms a protein complex with AR in prostate cancer cells [67, 68]. AR can inhibit RUNX2 binding to DNA through protein-protein interaction [67]. AKT phosphorylates AR at Ser-210 and subsequently inhibits AR transactivation [57]. This supports the finding that PI3K signaling can stimulate the transcriptional activity of RUNX2. In contrast, FOXO1 acts as a repressor of RUNX2. Thus, loss of PTEN or FOXO1 leads to the upregulation of RUNX2 transcriptional activity and increased migration and invasion of prostate cancer cells [69]. FOXO1 inhibition of RUNX2 also occurs in osteoblasts [70]. Thus, FOXO1 is an important negative regulator of RUNX2. This concept is further supported by a recent study, which identified a signaling axis of AKT-FOXO1-RUNX2-OCN-GPRC6A-CREB, activation of which results in upregulation of cytochrome P450 (CYP) enzymes (CYP11A1, CYP17A1) and increased synthesis of testosterone in PTEN-null prostate cancer cells [71]. Abnormal RUNX2 activation plays a pivotal role in PTEN loss-induced intratumoral androgen synthesis and tumor microenvironment remodeling [71]. Deletion of *Runx2* in *Pten* homozygous knockout prostate decreased *Cyp11a1* and *Cyp17a1* gene expression, testosterone levels, and tumor growth in castrated mice [71]. Therefore, under AKT activation conditions, the cytoplasm exportation of FOXO1 results in the loss of its function and inhibition of the transcriptional activity of RUNX2. Moreover, aberrant activation of RUNX2 promotes intratumoral androgen biosynthesis through the RUNX2-OCN-GPRC6A-CREB signaling cascade.

## Cross Talk Between PI3K Signaling and Other Pathways in Prostate Cancer

In prostate cancer, it is well accepted that a number of signaling pathways cross talk with each other either in an orchestrated fashion or in a negative feedback manner. An understanding of the cross talk among these pathways is critical for an effective treatment of prostate cancer.

**Primary prostate cancer** (n = 1512 including 499 samples from TCGA, Provisional 2015 and 1013 from MSKCC/DFCI, Nature Genetics 2018 )

PTEN (18%)
LZTS2 (2.8%)

Genetic alterarions  ▮ Inframe mutation      ▮ Missense mutation (putative driver)      ▮ Missense mutation (unknown significance)
                     ▮ Truncting mutation (putative driver)      ▮ Amplification      ▮ Deep deletion      ▯ No alterations

**Fig. 4** *PTEN* mutations are concurrent with *LZTS2* mutations. The primary prostate cancer data was combined from two studies, including 1512 samples. The co-occurrence of *PTEN* and *LZTS2* mutations was analyzed and displayed significant co-occurrence (***$P$ < 0.001).

## AKT Signaling and AR

AKT and AR signaling are two major drivers of prostate cancer. AKT can phosphorylate AR at Ser-210 and subsequently inhibit AR transactivation [57]. On the other hand, AR inhibition activates AKT signaling by reducing expression of the AKT phosphatase PHLPP1 [5, 72]. Because inhibiting either of these two pathways often activates the other, the development of a dual inhibitor might be advantageous for therapy. Notably, HDAC3 can regulate both AKT signaling [39, 73] and AR transcriptional activity [74]. Moreover, a HDAC3-specific inhibitor (RGFP966) inhibits both AKT and AR pathways in prostate cancer in vitro and in vivo, including prostate cancer organoid and mouse xenograft models [39].

## AKT Signaling and WNT/β-Catenin Signaling

WNT/β-catenin signaling is mediated by the extracellular signals of WNT proteins via cell membrane receptors, which converge on the transcription factor β-catenin. Genetic and epigenetic alterations have been identified in components of WNT signaling pathway in both primary and advanced prostate cancer [19, 20, 22], further suggesting that WNT signaling contributes prostate tumorigenesis. Notably, inhibition of WNT signaling in mice prevents prostate cancer progression [75].

There are at least three proposed mechanisms by which WNT signaling is activated in prostate cancer. Firstly, tumor stromal cells can secret WNT proteins to maintain the tumor microenvironment and support self-renewal or expansion of prostate cancer stem-like or progenitor cells and drug resistance via WNT/β-catenin signaling [75]. Secondly, AKT signaling can phosphorylate β-catenin and promote its transcriptional activity, which drives tumor cell invasion [76]. In the context of prostate cancer cells, the WNT co-receptor LRP6 increases aerobic glycolysis in a β-catenin-independent manner by directly activating AKT-mTORC1 signaling [77]. Thirdly, the leucine zipper tumor suppressor-2 (LZTS2), a β-catenin-binding protein, is a negative regulator of WNT signaling [78]. The *LZTS2* gene is approximately 15 Mb from the *PTEN* gene locus and is frequently deleted in a variety of human malignancies, including prostate cancer. Interestingly, *PTEN* deletions and *LZTS2* deletions frequently co-exist in primary prostate cancer (Fig. 4), implying a novel mechanism for the dysregulation of WNT/β-catenin signaling during prostate tumorigenesis [79].

## AKT Signaling and MAPK/ERK Signaling

The mitogen-activated protein kinase (MAPK) (or called extracellular signal-regulated kinase (ERK)) pathway is also known as the Ras-Raf-MEK-ERK pathway. It transduces the extracellular signals from the cell surface to the DNA in the nucleus. In prostate cancer, AKT and MAPK signaling pathways are frequently activated in androgen-independent cancer types. The activation of either AKT or ERK signaling in an androgen-responsive prostate cancer cell line promotes hormone-independent

**Table 3** PI3K/AKT inhibitors tested in prostate cancer [84]

| Therapeutic regimen | Indication | Clinical trial status | Patient status | Reference or ClinicalTrials.gov identifier |
|---|---|---|---|---|
| BKM120 | PI3K inhibitor | Phase II | mCRPC | NCT01385293 |
| GSK2636771 | PI3K inhibitor | Phase I, GSK2636771 ± Enzalutamide | mCRPC | NCT02215096 |
| AZD8186 | PI3K inhibitor | Phase I, AZD8186 ± Abiraterone or AZD2014 | mCRPC | NCT01884285 |
| LY3023414 | PI3K + mTOR inhibitor | Phase II, Enzalutamide ± LY3023414 | mCRPC | NCT02407054 |
| AZD5363 | AKT inhibitor | Phase I/phase II clinical trials | mCRPC | [85] |
| MK2206 | AKT inhibitor | Phase II, Bicalutamide ± MK2206 | mCRPC | NCT01251861 |

but AR-dependent growth in culture [80]. It is hypothesized that epithelial-stromal competition leads to androgen independence during prostate tumorigenesis, in which activation of AKT and ERK promotes AR activity in the prostate epithelium while counteracting the antagonistic effects in the stroma [80].

Inhibition of the PI3K/AKT signaling activates the MAPK pathway [81, 82], whereas activation of AKT by phosphorylation leads to FOXO1 exclusion from the nucleus and abolishment of its tumor suppressor functions in the nucleus. Intriguingly, AKT-phosphorylated FOXO1 can inhibit the MAPK pathway by binding to the scaffold protein IQGAP1 in the cytoplasm, thus impeding IQGAP1-dependent activation of ERK1/2 [83]. Thus, FOXO1 possesses tumor suppressor functions in both the nucleus and the cytoplasm.

## Targeting PI3K/AKT Signaling for Prostate Cancer Treatment

Due to the critical role of PI3K/AKT in maintaining prostate cancer progression and cell survival, targeting the PI3K/AKT pathway represents a promising strategy for prostate cancer treatment. However, the inhibitors that are currently under testing often encounter issues such as low efficacy and acquired drug resistance. Thus, development of new single-targeting or dual inhibitors is urgently needed.

## PI3K/AKT Inhibitors Tested in Prostate Cancer

A number of PI3K/AKT signaling pathway inhibitors have been tested, or are currently under investigation in clinical trials (Table 3). Mechanistically, most of them act on PI3K and AKT, with few on mTORC1 or mTORC2. Hereby, we have summarized a few of PI3K/AKT inhibitors which are currently under clinical trials to treat mCRPC patients either by administration alone or in combination with AR inhibitors as seen in Table 3. Specifically, BKM120 (NCT01385293), a PI3K inhibitor, is administrated alone and is currently under phase II clinical trial. GSK2636771 (NCT02215096) and AZD8186 (NCT01884285), two PI3K inhibitors, are treated in the combination with Enzalutamide or Abiraterone respectively and are under phase I clinical trials. LY3023414 (NCT02407054) is a dual inhibitor, which targets both PI3K and mTOR in an ATP-competitive manner [86]. AZD5365 [85] and MK2206 (NCT01251861) are two AKT inhibitors. Currently, AZD5363 is administrated alone and is under Phase I/phase II clinical trials. Interestingly, a preclinical study has found that AZD5363 significantly delayed Enzalutamide-resistant prostate cancer when it is combined with Enzalutamide [87]. MK2206 is under phase II clinical trial, and is administrated with or without Bicalutamide. Taken together, PI3K/AKT inhibitors appear to present a limited clinical outcome as single agents.

## A Limitation of Monotherapy with the PI3K Inhibitors

In prostate cancer, PI3K and AR signaling pathways are the most frequently activated pathways in which a reciprocal feedback exists between these two pathways. The monotherapy with AR or AKT inhibitors often leads to activation of the other pathway to sustain the cell survival or drive acquired resistance in prostate cancer [5, 72]. In addition, both PI3K/AKT and AR signaling are extremely important for prostate pathogenesis, the combination treatment of PI3K/AKT inhibitors and AR signaling inhibitors is required for a better therapeutic perspective [72, 88]. Indeed, as described above (section "PI3K/AKT Inhibitors Tested in Prostate Cancer"), several clinical trials have been conducted by using the combination of PI3K inhibitors (such as GSK2636771 and LY3023414) and AR-inhibitory agents (such as Enzalutamide and Abiraterone).

## Conclusions

The PI3K/AKT signaling pathway has been identified as a key driver of prostate tumorigenesis and drug resistance. Studies on the underlying mechanisms by which this pathway promotes prostate tumorigenesis have focused primarily on the phosphorylation of downstream target proteins. Cross talk between AKT signaling and the other parallel pathways has been under-investigated. Thus, further exploration in this area could shed new light on our understanding of the molecular basis for prostate tumorigenesis and progression, and identify novel therapeutic targets for prostate cancer.

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