

Chapter 4

PTEN in Prostate Cancer

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Abstract PTEN is one of the most commonly deleted/mutated tumor suppressor genes in human prostate cancer. As a lipid phosphatase and negative regulator of the PI3K/AKT/mTOR pathway, PTEN controls a number of cellular processes, including survival, growth, proliferation, metabolism, migration, and cellular architecture. Over the past 15 years since its discovery, a number of mechanisms governing PTEN expression and function, including transcriptional and post-transcriptional regulation, post-translational modifications, and protein–protein interactions, have been shown to be altered in human prostate cancer. The functions of PTEN within the cell have been expanded to include phosphatase-independent roles and functions within the nucleus. The generation of genetically engineered mouse models (GEMs) with deletion of *Pten* has further revealed that varying degrees of *Pten* loss in combination with other genetic alterations are able to recapitulate all spectrums of human prostate cancer, from tumor initiation to metastasis. With new methods of genomic and transcriptional analysis of human prostate cancer specimens, PTEN loss can potentially be used as a diagnostic and prognostic biomarker for prostate cancer, as well as predict patient responses to emerging PI3K/AKT/mTOR inhibitors. Finally, deeper insight into communication between the PI3K/AKT/mTOR and Ras/MAPK signaling pathways has led to the creation of metastatic murine prostate cancer models that develop lethal metastases, while new understanding of a feedback loop between PTEN and androgen receptor (AR) controlled pathways has unveiled a new mechanism for the development of castration-resistant prostate cancer (CRPC). Our expanded knowledge of PTEN and its role in prostate cancer

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initiation and progression will inform the rational design of novel therapeutics that target PTEN-controlled pathways alone or in combination with other related pathways for the treatment of metastatic and castration-resistant prostate cancer.

Introduction

Although partial or complete loss of chromosome 10 in brain, bladder, and prostate cancers was identified as early as 1984 [1], it was not until 1997 that three independent groups, through mapping of mutations on chromosome 10 and cloning of a novel phosphatase, identified a tumor suppressor gene at the 10q23.31 locus named by different laboratories as the phosphatase and *tensin* homolog (PTEN), mutated in multiple advanced cancers 1 (MMAC1), and TGF- β -regulated and epithelial cell-enriched phosphatase 1 (TEP1) [2–4]. PTEN is a nonredundant phosphatase that antagonizes the phosphatidylinositol 3-kinase (PI3K)/AKT signaling pathway, one of the most important and well-studied cancer promoting pathways. As PTEN is the only known 3' phosphatase counteracting the PI3K/AKT pathway, it is not unexpected that loss of PTEN has a significant impact on prostate cancer progression. Indeed, loss of heterozygosity (LOH) of *PTEN* occurs frequently in many advanced stage sporadic tumors, including ~60 % of advanced prostate cancers [2]. Germline PTEN gene mutations account for the majority (80 %) of cases of Cowden Syndrome, an autosomal dominant multiple hamartoma syndrome that leads to an increased propensity for patients to develop breast [5], endometrial [5, 6], and thyroid cancers [7–9]. However, prostate cancer has not been associated with Cowden Syndrome and germline PTEN loss [10, 11], perhaps providing credence to the understanding that loss of PTEN is a late event in prostate carcinogenesis [12, 13].

In this chapter, we will review PTEN structure, function, and regulation. The consequences of loss of PTEN regulation and function in different stages of prostate cancer development, as well as the potential use of PTEN loss as a biomarker for prostate cancer prognosis and prediction of patient responses to PI3K/AKT/mTOR pathway inhibitors, will also be addressed. Due to space limitations, some important topics, such as the role of PTEN in prostate stem cell/cancer stem cell maintenance and crosstalk with the tumor microenvironment, cannot be covered. However, these topics have been covered extensively by several outstanding reviews [14–17].

PTEN Structure and Function

PTEN Structure

The *PTEN* gene comprises nine exons and encodes a protein of 403 amino acids [18]. The amino acid sequence of the PTEN tumor suppressor is considerably homologous to dual-specific protein phosphatases and tensin, a chicken cytoskeletal

protein [2]. The crystal structure of PTEN revealed an expanded active site pocket for binding to its substrates and a C2 domain, which mediates membrane attachment of cell signaling proteins. Three other functional domains have also been identified: a short phosphatidylinositol-4,5-bisphosphate (PIP2) binding domain on the N terminus, and PEST sequences and a PDZ interaction motif on the C-terminal tail that regulate protein stability and binding to PDZ domain-containing proteins, respectively [19]. The binding of PIP2 to PTEN produces a conformational change in the enzyme, leading to allosteric activation [20]. The positive charge of PTEN's substrate binding pocket is also important for accommodating larger acidic substrates such as phosphoinositides. The PTEN phosphatase domain is evolutionarily conserved, and is the recipient of 40 % of its cancer-associated mutations, which occur most commonly through either a C124S mutation that abolishes both lipid and protein phosphatase activity or a G129E mutation that abrogates only its lipid phosphatase activity [21–23]. Although the phosphatase domain is responsible for PTEN's physiological activity, other PTEN tumorigenic mutations occur on the C-terminal C2 domain and tail sequence, highlighting an important role of the C terminus in maintaining PTEN protein stability [24, 25]. The fact that tumor-associated mutations occur in all PTEN functional domains indicates that each of these regions is biologically relevant to PTEN function. In prostate cancer, PTEN loss most commonly results from a somatic mutation generated through copy number loss rather than point mutation [26], although recent exome sequencing has identified several recurrent mutations in the PTEN gene [27, 28].

PTEN and Regulation of the PI3K/AKT/mTOR Pathway

PI3K/AKT signaling plays a critical role in regulating growth responses, homeostasis, and longevity. At the cellular level, the PI3K/AKT pathway controls cell growth, migration, differentiation, and survival. Activation of the PI3K/AKT pathway is also frequently detected in human cancers [29]. PTEN is a unique lipid phosphatase that removes the phosphate from the D3 position of phosphatidylinositol-3,4,5-triphosphate (PIP3), a product of PI3K, thus directly antagonizing the action of PI3K [23, 30, 31]. PIP3 accumulation at the plasma membrane through PI3K activity results in recruitment and activation of important kinases involved in cell growth and survival, including phosphoinositide-dependent kinase-1 (PDK1) and AKT family members, via their pleckstrin homology (PH) domains [23, 30, 31]. In this manner, PTEN negatively regulates the PI3K/AKT pathway by inhibiting downstream AKT activation.

AKT isoforms (AKT1, AKT2, AKT3) are activated by phosphorylation at two different residues: Thr308 by PDK1 [32] and Ser473 by mammalian target of rapamycin complex 2 (mTORC2) [33]. Activated AKT drives cell survival, proliferation, growth, angiogenesis, and metabolism by phosphorylating downstream signaling proteins, which include inhibitory phosphorylation of GSK3 β , FOXO, BAD, p21, p27, and PGC1, and activating phosphorylation of mammalian target of

rapamycin complex 1 (mTORC1), IKK- β , MDM2, ENTPD5, SREBP1C, AS160, and SKP2 [33, 34]. AKT promotes cell cycle progression and proliferation by directly inhibiting p21 and p27 and alleviating GSK3 β -induced cyclin D1 degradation. Moreover, inhibition of GSK3 β has been shown to prevent the degradation of β -catenin, which can further stabilize cyclin D1 mRNA and promote G₁-phase/S-phase progression [34, 35]. Activation of AKT also helps evade apoptosis directly by phosphorylation of the pro-apoptotic protein BAD [36]. In this regard, it is not surprising that re-expression of WT PTEN in *PTEN* null prostate cancer cell lines leads to apoptosis [37].

AKT directly activates the mTOR pathway by phosphorylating TSC2, which dismantles the TSC1/TSC2 complex that normally inhibits Rheb. Rheb, now free from TSC1/TSC2 inhibition, can stimulate the phosphotransferase activity of mTORC1 [38]. AKT may also activate mTORC1 by phosphorylating and inhibiting PRAS40, a negative regulatory subunit of the mTORC1 complex [33, 39]. Active mTORC1 phosphorylates p70 ribosomal protein S6 kinase (S6K) and 4E-binding protein (4EBP1), which in turn initiates cap-dependent protein translation [40]. Therefore, as a consequence of PTEN inactivation, PI3K/AKT/mTOR pathway activation leads to enhanced translation of mRNAs involved in protein synthesis, cell growth, and proliferation.

Interestingly, mTORC1 signaling also triggers a negative feedback loop that inhibits the PI3K/AKT pathway. This occurs through the phosphorylation and degradation of insulin receptor substrate 1 (IRS1), a crucial effector of insulin signaling, by S6K [41, 42]. Conversely, inhibition of mTORC1 results in hyperactivation of the PI3K/AKT pathway, as well as increased signaling through the Ras/MAPK pathway. The growth factor receptor GRB10 is a novel mTORC1 substrate that mediates feedback inhibition of the PI3K/AKT and Ras/MAPK pathways by direct inhibition of IRS proteins [43, 44]. In contrast, PTEN loss can reverse mTORC1-mediated negative feedback inhibition of the PI3K/AKT/mTOR pathway by activating both the upstream and downstream arms of the PI3K/AKT/mTOR pathway. Therefore, effective inhibition of tumors with PTEN loss will require inhibition of both mTORC1 and other signaling molecules upstream in the pathway, including PI3K and AKT.

PTEN and Metabolism

Recent studies have suggested that metabolic reprogramming is a requirement for the rapid cell proliferation of cancer cells. As opposed to differentiated and nonproliferating cells, which primarily utilize mitochondrial oxidative phosphorylation to generate the ATP needed for cellular processes, rapidly proliferating cells, including stem cells and cancer cells, tend to convert most glucose to lactate, even in the presence of oxygen, through aerobic glycolysis and a phenomenon known as the Warburg effect [45]. In this way, cancer cells exhibit high rates of glycolysis with increased glucose and glutamine uptake and lactate production, as well as increased

biosynthesis of lipids, amino acids, and nucleic acids, macromolecules that are needed to compensate for anabolic growth [46].

The PI3K/AKT pathway plays a key role in the regulation of glucose metabolism given its position downstream of the insulin receptor. The PI3K/AKT pathway enhances insulin-mediated glucose uptake and membrane translocation of the glucose transporter GLUT1, which has been positively correlated with higher tumor grades and Gleason scores [47], by way of mTORC1 activation and cap-dependent translation [48], and GLUT4, by way of inhibition of AS160 [49]. As PI3K/AKT signaling leads to increased production of HIF1 α [50, 51], a transcription factor that regulates the transcription of the *Glut-1* gene [52], it is likely that both the PI3K/AKT pathway and HIF1 α activation contribute to higher levels of GLUT1 and enhanced glucose uptake [53]. Increased HIF1 α expression also upregulates expression of vascular endothelial growth factor (VEGF), a potent stimulator of angiogenesis that may further promote tumor metabolism by facilitating access to nutrients in the blood [54]. Conversely, stimulation of the PI3K/AKT pathway blocks gluconeogenesis by preventing both FOXO and PGC1 α activation [55, 56]. AKT may indirectly activate glycolysis as well by directly phosphorylating PKF2, whose product, Fru-1,6-P2, is a potent allosteric activator of the glycolysis rate-controlling enzyme PFK1 [32, 57]. A recent study using siRNA-mediated gene silencing in metastatic prostate cancer cell lines revealed that PFKFB4, an isoform of PFK2 that is required for glycolysis, is essential for survival of prostate tumor cells and that ablation of PFKFB4 inhibits tumor growth in a xenograft model [58].

In comparison to other epithelial cancers, primary prostate cancers are less glycolytic and, therefore, not sensitive to FDG-PET imaging until reaching the metastatic stage [59, 60]. On the other hand, prostate cancer is known to be lipogenic, and C-11-acetate and F18-choline have been used, although in limited scale, in prostate cancer imaging [59, 61]. Recent studies suggest that the PI3K/AKT pathway can regulate lipid metabolism as well to further promote anabolic growth through the Warburg effect. Upon PTEN loss and through inhibition of GSK3, the PI3K/AKT axis activates the transcription factor SREBP1C, which in turn transcribes genes involved in cholesterol and fatty acid biosynthesis [62, 63]. PTEN has also been shown to regulate the synthesis of long chain saturated fatty acids by inducing the downregulation of fatty acid synthase (FAS), a lipogenic enzyme overexpressed in many human cancers, including prostate cancer, in a lipid phosphatase-dependent manner [64]. Therefore, PTEN loss in prostate cancer cells may increase FAS protein expression, which is elevated in tumors with a poor prognosis [65]. Collectively, these data indicate that both upstream and downstream components of the PTEN regulated PI3K/AKT/mTOR pathway are involved in the metabolic reprogramming required to sustain the rapid growth and proliferation of tumor cells by (1) increasing glucose metabolism via aerobic glycolysis and (2) promoting macromolecule biosynthesis via lipogenesis.

The recent creation of a mouse model with global PTEN overexpression, the “Super-PTEN” model, has demonstrated that PTEN elevation at the organism level results in diminished glucose and glutamine uptake and increased mitochondrial

oxidative phosphorylation, resulting in a reversion to a more healthy metabolism [66]. PTEN elevation in this model coordinates this metabolic shift by negatively regulating both PI3K/AKT-dependent pathways, such as mTORC1 activation of PKM2, a controller of glycolytic flux [67], and PI3K/AKT-independent pathways, such as degradation of PFKFB3, a key regulator of glycolysis [68], through APC/Cdh1 activation [66]. Interestingly, these “Super-PTEN” mutants are resistant to oncogenic transformation, demonstrating that inhibition of the metabolic reprogramming to aerobic glycolysis through PTEN expression or inactivation of the PI3K/AKT pathway may be sufficient to obstruct tumor propagation [66]. These outcomes suggest that PTEN overexpression may indeed be an attractive option for cancer prevention and therapy.

PTEN in the Nucleus

It was initially assumed that PTEN is exclusively localized in the cytoplasm. However, following the discovery that PTEN contains dual nuclear localization signal-like sequences [69], it has been well recognized that PTEN can localize to the nucleus, and recent studies have illustrated the important functions of nuclear PTEN in regulating cell cycle progression and genomic integrity. Indeed, not only is there a marked reduction in nuclear PTEN in rapidly cycling cancer cell lines in comparison to resting or differentiated cells [70–73], but absence of nuclear PTEN has also been associated with reduced overall survival in prostate cancer patients [74].

Oxidative stress is one of the physiological stimuli that regulate the accumulation of nuclear PTEN [75]. Oxidative stress inhibits PTEN nuclear export, a process dependent on phosphorylation at Ser380. Nuclear PTEN, independent of its phosphatase activity, can regulate p53 stability and transcriptional activity [76, 77], leading to p53-mediated G₁ growth arrest, cell death, and reduction of reactive oxygen species (ROS) production [75]. Nuclear PTEN is also sufficient to reduce human prostate cancer xenograft growth in vivo in a p53-dependent manner [75], suggesting a unique role of nuclear PTEN to arrest and protect cells following oxidative damage and to regulate prostate cancer development.

Nuclear PIP3, unlike cytoplasmic PIP3, is insensitive to the lipid phosphatase activity of PTEN, implying nuclear functions for PTEN beyond its role as a negative regulator of the PI3K/AKT pathway [78]. This, however, is at odds with another finding that forced nuclear expression of PTEN can reduce nuclear levels of P-AKT, although it was not demonstrated whether this mechanism occurred through a PI3K-dependent or -independent pathway [79]. One proposed function of PTEN in the nucleus is to induce G₁ cell cycle arrest in part by reducing cyclin D1 levels through its protein phosphatase activity [80] or through controlling MAPK signaling [81]. Nuclear PTEN maintains chromosomal stability by physically associating with centromeres through docking onto CENP-C, a centromeric-binding protein [82]. Moreover, nuclear PTEN, through a phosphatase-independent mechanism, enhances DNA repair through increasing the activity of RAD51, a protein implicated in

double strand break (DSB) repair [82]. Not surprisingly, PTEN-null cells develop spontaneous DNA DSBs at a high rate [82]. Cytoplasmic PTEN can also contribute to DSB repair by inhibiting AKT-dependent sequestration of the cell cycle regulator CHK1 in the cytoplasm [83]. In this fashion, PTEN helps to maintain the G₂/S cell cycle checkpoint and likewise prevents genomic instability and DSBs. As PTEN loss leads to homologous recombination defects in human tumor cells through downregulation of RAD51 and CHK1 in the nucleus, tumor cells display increased sensitivity to inhibitors of PARP [84–86]. These findings provide evidence for the use of PARP inhibitors in patients with PTEN-deficient prostate cancer. However, a recent study of clinical prostate cancer specimens suggests that PTEN loss is not associated with reduced RAD51 mRNA or protein expression in primary prostate cancer, and that PTEN-deficient cells only exhibit mild sensitivity to PARP inhibition, casting doubt on whether PTEN is a useful biomarker for response to PARP inhibitors in prostate cancer [87].

Nuclear PTEN directly increases the antitumor and E3 ligase activity of APC/C through a phosphatase-independent mechanism by promoting the association of APC/C with its activator CDH1 [88]. The APC/C-CDH1 complex contains tumor suppressive activities that degrade oncogenic proteins such as PLK1 and Aurora kinases [89, 90]. In this regard, combining PLK and Aurora kinase inhibitors with PI3K/AKT inhibitors may provide increased efficacy in treating PTEN-deficient prostate cancer. Altogether, these findings suggest that the tumor suppressive functions of PTEN are in part due to its functions within the nucleus. New insights into the regulation of PTEN subcellular localization and the functions of PTEN in the nucleus may shed light on novel biomarkers and therapeutics for the treatment of prostate cancer.

PI3K/AKT/mTOR-Independent Functions of PTEN

Although most phenotypes associated with PTEN loss can be accounted for by the activation of the PI3K/AKT pathway, transgenic models with prostate-specific overexpression of p110 β or a constitutively active form of AKT develop only localized, precancerous PIN lesions, suggesting that PTEN possesses other tumor suppressor functions independent of the PI3K/AKT pathway [91–93]. Similarly, while conditional deletion of p110 β or *Rictor*, in addition to *Pten* conditional deletion, prevents the progression of tumor development from PIN to adenocarcinoma, they do not completely prevent prostate cancer initiation [94, 95].

One example of a PI3K/AKT/mTOR-independent mechanism of PTEN regulation is the interaction between PTEN and p53 [96]. PTEN inactivation is known to increase the expression [97] and activation of the p53 repressor MDM2 [98] by a PI3K/AKT-dependent pathway [99] and upregulate p53 through translational mechanisms mediated by mTORC1 [100, 101]. The PTEN C2 domain, which lacks phosphatase activity, can also regulate cell motility [102] and, interestingly, can interact directly with p53 in a phosphatase-independent manner to enhance p53-mediated

cell cycle arrest and apoptosis by promoting the stabilization, acetylation, and tetramerization of p53 [75, 76, 103]. Conversely, p53 can also regulate PTEN at the transcription level [104]. In the *Pten*-null mouse model, deletion of p53 accelerates *Pten*-null prostate cancer by reducing cellular senescence [105]. Concomitant mutations of PTEN and p53 have been detected within individual human tumors, supporting a selective advantage for combined inactivation of both tumor suppressors. However, whether the cooperation of PTEN and p53 loss in overriding cellular senescence promotes human prostate cancer progression needs to be further investigated.

PTEN can also regulate the expression of other tumor suppressors whose functions are commonly lost as an early event in prostate cancer initiation, such as Nkx3.1 [106]. Not only is Nkx3.1 expression downregulated in the PTEN-null murine prostate cancer model, but forced expression of Nkx3.1 in the PTEN-null prostate epithelium prevents prostate cancer initiation and progression [106]. Moreover, while a transcriptional profiling study has indicated that the JNK pathway is activated following PTEN loss in an AKT-independent manner [107], a recent report elucidated that JNK deficiency collaborates with PTEN loss in promoting CRPC [108].

Though the lipid phosphatase activity of PTEN is central to its role as a tumor suppressor, other, phosphatase-independent functions of PTEN are also important. PTEN is a dual-specificity protein phosphatase with activity toward acidic substrates. PTEN is capable of dephosphorylating phosphorylated serine, threonine, and tyrosine residues on peptide substrates in vitro [109], as well as protein substrates such as FAK [110], CREB [111], eIF2 [112], and SRC [113] in vivo, thereby directly inhibiting cell survival, proliferation, and migration. The activation of these PI3K/AKT-independent pathways after PTEN loss suggests that combining PI3K/AKT inhibitors with inhibitors of these other pathways may improve efficacy in treating patients with PTEN-deficient prostate cancer.

PTEN Regulation

Genetic Regulation

Germline PTEN mutations do not predispose men to prostate cancer [10, 11]. However, the 10q23 gene locus is a frequent target for somatic heterozygous deletion in primary, and, more frequently, in metastatic prostate tumors, where loss of heterozygosity (LOH) is found in 20–60 % of tumors [114]. However, the finding that the rate of PTEN LOH and mutations are far less frequent than the detected rate of PTEN loss at the protein level suggests that other, nongenomic alterations may occur that inactivate the second PTEN allele.

Epigenetic Regulation by DNA Methylation

Supporting its important physiological functions, PTEN is constitutively expressed in normal tissues, including infant and adult human prostates. However, PTEN expression can be downregulated on many levels in various physiological settings. Epigenetic inactivation of the PTEN promoter has been described in prostate cancer xenografts, where loss of PTEN protein is a result of promoter methylation [115]; however, this has yet to be shown in primary prostate cancer specimens. Additionally, the zinc-finger transcription factor SALL4 represses PTEN transcription in embryonic stem cells by recruiting an epigenetic repressor complex called the Mi-2/NuRD complex to the PTEN locus [116]. Despite these discoveries, epigenetic silencing of PTEN in prostate cancer has not been demonstrated in the in vivo and clinical setting.

Transcriptional Regulation

Suppression of *PTEN* transcription may have an important and understated role in prostate cancer initiation and progression. PTEN was originally cloned as a gene transcriptionally regulated by transforming growth factor β (TGF β) [4], which both suppresses and induces PTEN expression depending on the activation status of the Ras/MAPK pathway. When Ras/MAPK is activated, as is common in aggressive, late stage disease, TGF β suppresses PTEN expression through a Smad4-independent pathway [117]. Alternatively, when the Ras/MAPK pathway is blocked, TGF β induces PTEN expression through its canonical Smad-dependent pathway [118]. The Ras/MAPK pathway also suppresses PTEN levels through the transcription factor c-Jun [119]. Moreover, the MEK–JNK pathway suppresses PTEN transcription via activation of NF- κ B, which directly binds to and suppresses the PTEN promoter [120]. Expression of PTEN is also negatively regulated by the epithelial-to-mesenchymal transition (EMT) transcription factor SNAIL, which is itself activated by PI3K/AKT and RAS/MAPK pathways [121–123]. SNAIL competes for binding to the PTEN promoter with p53, which is a transcriptional activator of PTEN and leads to activation of PTEN transcription during p53-mediated apoptosis [96]. Activated NOTCH1 both positively and negatively regulates PTEN expression through MYC and CBF1, respectively [124, 125]. PTEN transcription can also be upregulated through several other transcription factors, including PPAR γ [126] and EGR1 [127], as well as downregulated by BMI1 [128], which regulates prostate stem cell self-renewal and malignant transformation [129]. All in all, transcriptional control of PTEN lies within a network of tumor suppressors and oncogenes controlling various signaling and development programs within normal and cancerous prostate cells.

Post-transcriptional Regulation

PTEN mRNA is also post-transcriptionally regulated by PTEN-targeting microRNAs (miRNAs), a class of endogenous 20–25 nucleotide noncoding RNAs that repress mRNA translation through imperfect base pairing between the seed sequence of the miRNA and the complementary seed match sequence in the 3' untranslated region of the target mRNA [130]. A number of miRNAs have been reported to promote tumorigenesis by downregulating PTEN expression. For example, miR-22 and the miR-106b-25 cluster, both PTEN targeting miRNA loci, are aberrantly overexpressed in human prostate cancer and are capable of initiating prostate tumorigenesis in vitro and in vivo [131]. The identification of these and other prospective PTEN-targeting miRNAs in serum of prostate cancer patients may be valuable as surrogate markers for PTEN status, and hence could correlate with both disease progression and the potential efficacy of PI3K/AKT inhibitor treatment.

In a newly emerging field of research, the PTEN pseudogene 1 (PTENP1) was found to influence PTEN expression through a coding-independent function, uncovering a new mechanism of gene regulation [132]. Since the PTENP1 mRNA transcript shares vast homology with PTEN mRNA, PTENP1 acts as a decoy for PTEN-targeting miRNAs and can thereby sequester and inhibit the negative regulatory effects of miRNAs on PTEN expression [14]. PTENP1 can, therefore, be considered a competing endogenous RNA or ceRNA. Recent research has uncovered a large network of ceRNA transcripts in prostate cancer that can control PTEN expression by blocking the action of PTEN-targeting miRNAs. These discoveries fortify the existence of a large and complex PTEN tumor suppressor network that can be regulated by coding and noncoding RNAs and can be used to explain the observance of partial or incomplete PTEN inactivation in human prostate cancer [133–135].

Post-translational Regulation

PTEN stability is regulated by various post-translational modifications. When inactivated, PTEN is phosphorylated at various serine and threonine residues on its C-terminal tail, which, in turn, increases PTEN stability [136–138]. This C-terminal phosphorylation results in a more stable yet “closed” state of PTEN, which reduces its plasma membrane localization [139] and its ability to form a complex with PDZ domain-containing proteins [138], thereby reducing its PIP3 lipid phosphatase activity [140–142]. As PTEN is activated, dephosphorylation of its C-terminal tail opens its phosphatase domain, increasing PTEN activity and enhancing its interactions with binding partners, but in turn making PTEN increasingly unstable [143]. Also located in the C-terminal tail, Ser370 can be phosphorylated by a downstream effector of SRC, CK2 [144], while Thr366 appears to be phosphorylated by GSK3 β [145]; however, the function of phosphorylation at these sites still remains unclear [146].

The targeting of PTEN to the plasma membrane can also be orchestrated through phosphorylation at Ser 229 and Thr321 on its C2 domain by the protein kinase ROCK [147–149]. Tyr336 of PTEN can also be phosphorylated by RAK, which can act as a tumor suppressor in its own right by regulating PTEN stability and function [150]. Future research may unveil other known and unknown kinases that are capable of phosphorylating PTEN and thereby regulate specific PTEN functions.

The open state of PTEN is also more prone to ubiquitin-mediated proteasomal degradation. Lys13 and Lys289 are conserved sites for PTEN ubiquitination, and monoubiquitination is necessary for the movement of PTEN from the cytoplasm to the nucleus [79]. NEDD4-1 is a recently identified E3 ligase of PTEN that induces both PTEN mono- and poly-ubiquitination [151]. However, NEDD4-1 knockout mice contain no differences in the expression level and subcellular localization of PTEN, hinting that other E3 ligases may be involved in the regulation and localization of PTEN [152]. Along these lines, two other E3 ligases, XIAP and WWP2, have been proposed to mediate PTEN ubiquitination [153, 154].

Similar to other phosphatases, the cysteine residues in the bottom of the PTEN catalytic pocket are very sensitive to oxidation [155]. The catalytic activity of PTEN is attenuated by reactive oxygen species (ROS) through the development of a disulphide bond between Cys71 and Cys124 that is induced during oxidative stress [156, 157]. Furthermore, PTEN can also be acetylated at Lys125 through Lys128 by PCAF and at Lys402 by CBP, inhibiting its catalytic activity while facilitating interactions with PDZ domain-containing proteins [158]. Finally, other forms of PTEN redox regulation have been suggested by research demonstrating the inactivation of PTEN through nitrosylation of cysteine residues in its phosphatase domain [159]. Together, these findings highlight the potential to manipulate mechanisms of PTEN post-translational modifications for use as therapeutics to enhance the tumor suppressive functions of PTEN.

Protein–Protein Interactions

A number of PTEN interacting proteins regulate the tumor suppressive abilities of PTEN by altering its conformation, stability, and subcellular localization. PTEN contains a 3 amino acid C-terminal region that binds to PDZ domain-containing proteins, and these PDZ domains are involved in multiprotein complex assembly [137, 160]. Indeed, the PDZ domain of PTEN mediates interactions with NHREF, which binds to and recruits PTEN to PDGFR to inhibit the activation of the PI3K–AKT pathway [161]. The PTEN PDZ-binding domain binds to several other proteins, including MAGI-2 and MAST205, which appear to enhance the stabilization of PTEN [160, 162, 163]. As PTEN can be found in high molecular mass complexes through size-exclusion chromatography, it was hypothesized that the PDZ-binding domain may be required for such complex formation [164]. However, mutagenesis studies demonstrated that neither PTEN's catalytic activity nor its PDZ binding domain are absolutely required for its complex formation. Instead, PTEN

phosphorylation status has a significant role in its complex assembly [165]. Using two-dimensional gel electrophoresis and mass spectrometry analysis, hnRNPC was identified as a novel PTEN-interacting protein [165]. Indeed, PTEN and hnRNPC are colocalized in the nucleus and may be involved in RNA regulation [165, 166].

Additional proteins are capable of binding to other domains on the PTEN protein. PICT-1 interacts with PTEN by binding to and promoting phosphorylation of the C-terminal tail, conferring PTEN stabilization [167]. Through a yeast two-hybrid screen, β -arrestin was identified as a PTEN-binding partner, binding to PTEN's C2 domain [168]. When PTEN is dephosphorylated at Thr383, this increases the binding affinity of β -arrestin to PTEN, which in turn allows PTEN to negatively regulate cell proliferation through its lipid phosphatase activity, as well as enhance cell migration by reversing the inhibitory effect of the C2 domain [168]. Furthermore, PTEN can directly interact with the regulatory subunit of PI3K, p85, which increases its lipid phosphatase activity and subsequent capability of downregulating the PI3K–AKT pathway [141, 169]. Therefore, p85 can regulate the PI3K/AKT pathway by both negatively regulating PI3K through direct binding to its catalytic subunit, p110, and by positively regulating PTEN activity. Under oxidative stress conditions, DJ-1, which was identified in *Drosophila melanogaster*, can also directly bind to PTEN, an action that is associated with increased P-AKT levels [170, 171]. Recent screens have identified novel PTEN regulators, including PREX2a [172] and SIPL1 [173], which bind to PTEN directly and inhibit its phosphatase activity against PIP3. MAN2C1 also binds to PTEN and inhibits its function in both prostate cancer cell lines and primary human prostate tumors [174]. Intriguingly, one study found that, of 60 % of primary human prostate tumors that were PTEN-positive, 80 % displayed overexpression of MAN2C1, uncovering a possible new mechanism of PTEN down-regulation without genomic loss of PTEN [174]. Despite the discovery of various PTEN protein-binding partners, further investigation is necessary to understand the physiological and clinical relevance of these interactions.

Subcellular Localization

The function of PTEN is also regulated by its subcellular localization. At the plasma membrane, PTEN can regulate directional chemotaxis. PTEN recruitment to the plasma membrane relies on electrostatic interactions with acidic lipids in the membrane, such as phosphatidylserine, PIP2, PIP3, and phosphatidic acid [175], as well as additional protein–protein interactions [143, 176]. PTEN interacts with several membrane-anchored proteins in its dephosphorylated form, including MAGI-2 [160], MAST205 [162], hDLG [138], MVP [177], and PDGFR and NHERF [161], which are thought to be potentially part of a larger PTEN complex, via its C-terminal PDZ domain. NEP has been shown to recruit PTEN to the plasma membrane, which in turn enhances its catalytic activity and subsequently hinders AKT activity [178]. Similarly, the motor protein myosin V regulates the migration of PTEN to the membrane by directly binding to PTEN [179].

PTEN is predominantly localized to the nucleus in differentiated and resting cells in comparison to rapidly cycling cancer cells [72]. The nuclear localization of PTEN is also dependent upon the cell cycle stage, with nuclear PTEN levels highest at the G₁ phase and lowest at the S phase [71]. While some studies have shown that PTEN nuclear localization is dependent upon noncanonical nuclear localization sequences on PTEN and major vault protein-mediated nuclear transport [69], others have shown that nuclear localization of PTEN occurs through passive diffusion through the nuclear membrane [180]. It has been further suggested that PTEN contains a type of cytoplasmic localization signal (CLS) in its N-terminal region that, when mutated, induces the nuclear import of PTEN [181]. Other so-called nuclear exclusion motifs and NLS sequences have been identified that control PTEN localization through a RAN-dependent mechanism [182]; however, how they regulate the shuttling of PTEN between the nucleus and the cytoplasm is not understood. More conclusively, PTEN monoubiquitination by the E3 ligase NEDD4-1 induces the nuclear localization of PTEN [151], while the deubiquitinase HAUSP controls PTEN deubiquitination and nuclear exclusion [183]. Oxidative stress induces the accumulation of PTEN in the nucleus, where it associates with p53 to trigger cell cycle arrest and reduce ROS [75]. Nevertheless, mechanisms involved in the nuclear export of PTEN are still waiting to be uncovered. The use of models utilizing PTEN proteins with mutations that disrupt PTEN localization but maintain PTEN phosphatase activity may provide new understandings into the role of nuclear PTEN.

PTEN Loss as a Biomarker for Human Prostate Cancer

Despite recent and past findings firmly establishing loss of *PTEN* as one of the most common somatic genetic alterations in prostate cancer, prostate cancer specimens are not routinely screened for PTEN loss in the clinical setting. Fluorescence in situ hybridization (FISH) has been used to identify genomic *PTEN* loss, which is found in 9–23 % of high-grade prostatic intraepithelial neoplasia (PIN) lesions [184, 185] and 10–70 % of prostate cancers [12, 13, 186–190], and is correlated with an overall poor prognosis [26, 74, 190–193]. Loss of PTEN expression in the cytoplasm as well as in the nucleus, as determined by FISH and immunohistochemistry (IHC) analysis, is independently associated with decreased disease-specific survival [74, 194]. Part of the reason for these variations may be due to the subjective nature and tediousness of counting the number of fluorescent signals and positive antibody stains relative to control signals and stains to quantify PTEN expression. FISH analysis also lacks the sensitivity to identify minor mutations/perturbations in the PTEN gene locus, as well as other epigenetic and post-transcriptional changes that may influence PTEN expression [13, 195]. Moreover, current research, through the use of “break apart” FISH technology, has revealed that gross chromosomal rearrangements of the PTEN locus occur in prostate cancer, which could very well explain the absence of PTEN expression in tumors designated as harboring genomic loss of only one PTEN allele using conventional single probe FISH [196].

Despite discrepancies in reported rates of genomic *PTEN* loss, a general finding of these studies is that loss of one *PTEN* allele is significantly more frequent than loss of both *PTEN* alleles, although homozygous deletions are associated with advanced disease and metastasis [197, 198]. Haploinsufficiency for *PTEN*, as well as inactivation of the second *PTEN* allele through nongenomic alterations, may explain why heterozygous *PTEN* deletions outnumber homozygous *PTEN* deletions in human prostate cancer and also result in poor outcomes [184, 190, 199]. Indeed, nearly 70 % of primary human prostate tumors do not contain inactivation of both copies of *PTEN* [105]. In terms of disease progression, the frequency of *PTEN* loss is higher in surgical cohorts enriched for high Gleason grades and aggressive disease stages [192]. *PTEN* loss is more common in hormone refractory and metastatic prostate cancer than in hormone-dependent primary tumors, with homozygous *PTEN* loss in 10 and 50 % of hormone-dependent and metastatic/hormone refractory cases, respectively [74, 184, 185, 189–191, 193, 200, 201]. Therefore, *PTEN* could serve as a prognostic marker for hormone refractory and metastatic disease. *PTEN* genomic loss is also associated with the *TMPRSS2-ERG* fusion [26, 185], and recent reports have concluded that these events cooperate to stratify patients with a poorer prognosis in the clinic [192]. A close association between *PTEN* loss and therapeutic resistance, as demonstrated by a decreased time to biochemical recurrence after radical prostatectomy, adjuvant docetaxel treatment, and radiotherapy, has also been observed [74, 190, 191, 202, 203].

The possibility that FISH and other genomic analyses may fail to detect some cases of *PTEN* inactivation calls for alternative methods to detect *PTEN* loss. Considering the role of post-transcriptional and post-translational modifications in *PTEN* protein expression and subcellular localization as discussed above, quantification of *PTEN* protein levels using immunohistochemistry (IHC) may be a better indicator of *PTEN* expression. In a recent study using a rabbit monoclonal antibody against *PTEN* for IHC analysis, *PTEN* protein loss was detected in 75–86 % of samples with genomic *PTEN* loss and was even discovered at times in the absence of genomic *PTEN* loss [194]. Interestingly, 45 and 37 % of tumors with *PTEN* protein loss did not show genomic deletions measurable by FISH or SNP microarray analysis, respectively, further suggesting that alternative mechanisms of *PTEN* inactivation exist beyond the genomic level [194]. Moreover, IHC analysis has correlated *PTEN* protein loss with high Gleason scores, as well as decreased time to metastasis in a cohort of patients having undergone surgical resection [194]. Other studies using large prostate cancer cohorts combining genomic analysis, through comparative genomic hybridization (CGH) and whole-exome sequencing, and transcriptome analysis have uncovered frequent alterations of the PI3K/AKT pathway in prostate cancer [26–28], which correlated with 42 and 100 % of human primary and metastatic prostate cancer, respectively, as well as high-risk disease [26]. Using network component analysis, 20 transcription factors have been identified whose activities, as deduced from their target gene expression, are immediately altered upon the re-expression of *PTEN* in a *PTEN*-inducible system [204]. Notably, the activity of these transcription factors can be used to predict *PTEN* functional status in human prostate, breast, and brain tumor samples with increased reliability when compared to basic expression-based analysis [204]. With improved mechanisms for detecting

PTEN functional status, PTEN loss could be used not only as a prognostic biomarker for men with prostate cancer but also as a potential predictive marker to identify patients who could benefit from emerging PI3K/AKT pathway therapies.

PTEN in Prostate Cancer Initiation, Progression, CPRC, and Metastasis

Mouse Models of Prostate Cancer

Prostate cancer research has been limited, in part, by the lack of animal models that develop spontaneous prostate tumors in a manner that mimics human prostate cancer. Mouse xenograft models reconstituted from primary human metastatic prostate cancer cells and cell lines have been developed and used extensively in research as preclinical models. However, these xenograft models cannot be used for studying the underlying mechanisms involved in prostate cancer initiation and progression since they are derived from late stage disease. Moreover, many of the key features of the disease, especially the resident tumor microenvironment and the stromal and immune cells that occupy it, are lacking in this immune incompetent system and, therefore, engrafted tumors cannot recapitulate the whole spectrum of human prostate cancer [205]. Genetically engineered mouse (GEM) models of prostate cancer have advanced significantly over the past decade [206–210], and the strong implication of PTEN loss in prostate cancer progression in humans has prompted the expansion of GEM models based on PTEN inactivation. Greater knowledge of the role of PTEN loss as an individual and cooperative agent in prostate cancer development, including initiation, progression and invasion, castration-resistant prostate cancer (CRPC), and metastasis, has been uncovered using mouse models that recapitulate the human disease through genetic loss of the murine homolog of the *Pten* gene (Fig. 4.1).

PTEN Dosage in Mouse Models of Prostate Cancer

PTEN dosage appears to be an important determinant in the development of many epithelial cancers, as demonstrated in various mouse models of *Pten* loss [211]. In the prostate, a hypomorphic PTEN allele, which leads to ~20 % reduction of PTEN levels, shows no sign of neoplastic lesions in the prostate epithelium, while conditional or conventional deletion of one *Pten* allele causes a 50 % reduction of PTEN levels and leads to precancerous PIN lesions but not cancer, indicating that inactivation of one allele of *Pten* is sufficient to initiate tumorigenesis but not tumor progression [76, 212–216]. Interestingly, by combining a hypomorphic allele with a knockout allele, and thereby reducing PTEN levels by 70–80 %, these mice progress to invasive adenocarcinoma of the prostate [214], indicating that a more

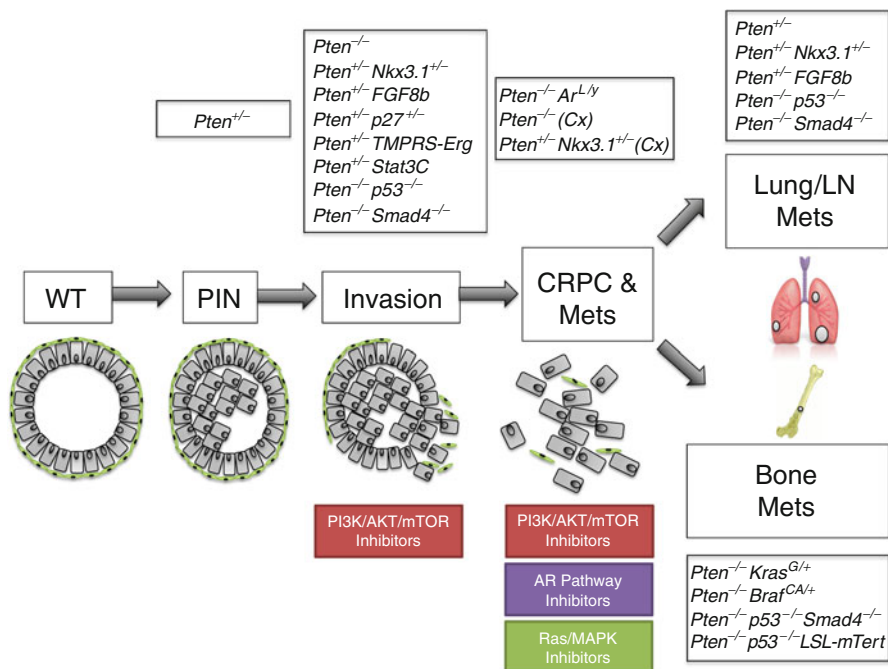


Fig. 4.1 *Pten* knockout mouse models of prostate cancer. *Pten* heterozygous (*Pten*^{+/-}) or homozygous (*Pten*^{-/-}) loss, alone or in combination with other pathway alterations, is able to recapitulate all stages of human prostate cancer, including initiation (PIN), cancer progression/invasion, CRPC, and metastasis. Studies in these murine models provide credence for the use of PI3K/AKT/mTOR, AR, and Ras/MAPK inhibitors for the treatment of metastatic CRPC. In the figure, gray squares represent luminal cells, while green ovals represent basal cells. Cx castration, PIN prostatic intraepithelial neoplasia, LN lymph nodes, Mets metastases, WT wild type, CRPC castration-resistant prostate cancer

profound downregulation of PTEN is needed for cancer progression to occur in the prostate [217]. These findings counter the canonical “two-hit hypothesis” of cancer and suggest that slight variations in PTEN expression, induced through genetic alterations as well as nongenetic changes in PTEN expression, are able to recapitulate varying stages of prostate tumor initiation and progression [218]. Despite the evidence for PTEN haplosufficiency in the mouse, evidence for this in humans still remains to be determined.

Phenotypes Associated with Homozygous Deletion of *Pten* in the Prostate Epithelium

A number of studies have been performed through the use of conditional mutants with prostate-specific deletion of one or both *Pten* alleles [211, 213–215, 219–221]. Conditional homozygous *Pten* deletion (*Pten*^{-/-}) driven by the *PB-Cre4* promoter

results in invasive adenocarcinoma in 100 % of mice at 9–12 weeks [215]. Importantly, the *Pten*^{-/-} prostate cancer model mimics the course of human prostate cancer formation, progressing from hyperplasia to PIN to invasive adenocarcinoma with defined kinetics [215]. Interestingly, homozygous deletion of other tumor suppressors in the murine prostate, including *p53* [222], *retinoblastoma* (*Rb*) [223], and *Nkx3.1* [224], leads to PIN lesions but never an adenocarcinoma phenotype, solidifying the importance of PTEN function in the prostate gland. Moreover, although *Pten*-null tumors are initially responsive to androgen ablation, eventually mice will develop CRPC, as is commonly seen in human prostate cancer [215]. *Pten* homozygous deletion driven by other promoters in the mouse, including *PSA*^{Cre}, *MMTV*^{Cre}, and *Nkx3.1*^{CreERT2}, also results in the development of invasive adenocarcinoma, albeit over a longer latency [225–228].

Compound Pten Knockout Transgenic Mouse Models of Prostate Cancer

Pten Loss Combined with Alterations in Other Tumor Suppressors

Several studies carried out with compound transgenic mice have shown that mono-allelic or biallelic deletion of tumor suppressor genes such as *Nkx3.1* [229, 230], *p27*^{KIP1} [231], and *p53* [105] can cooperate with *Pten* loss in promoting prostate cancer (Fig. 4.1). While loss of a single allele of *Nkx3.1* [224, 232] and *p27*^{KIP1} [233], both of which occurrences have been implicated in advanced stage prostate cancer and poor disease-free survival in humans [234, 235], is sufficient to promote prostate cancer initiation and PIN lesions, concomitant loss of *Pten* is needed to promote prostate tumorigenesis and cancer progression [211, 230, 231, 236]. Moreover, while the TRAMP mouse model alone, which contains inactivation of the *p53* and *Rb* tumor suppressor genes through expression of the large/small SV40 tumor T antigens under the *probasin* promoter, is capable of inducing the development of aggressive prostate tumors [209], loss of heterozygosity of *Pten* in TRAMP mice demonstrated an increased rate of tumor development, with a subsequent decrease in overall survival from 245 days to 159 days [237]. In the same way, conditional ablation of one or two alleles of *p53* leads to the development of PIN lesions, while *Pten*^{-/-};*p53*^{-/-} double mutants exhibit invasive prostate cancer as early as 2 weeks after puberty that is invariably lethal by 7 months of age [105, 238]. Also, deletion of *Smad4*, a tumor suppressor known to regulate the TGF- β signaling pathway, cooperates with *Pten* deletion in the prostate to enhance tumor cell proliferation and drive invasion to produce fully penetrant prostate cancer and metastases to the lymph nodes and lungs [239]. Finally, combining *Pten* and *p53* loss with loss of *Smad4* or reactivation of murine telomerase (*mTert*) produces prostate cancer metastases in the bone [240], indicating that additional pathway alterations are necessary to drive prostate tumor cells to form metastases in the microenvironment of the bone, an important feature of human prostate cancer.

Pten Loss Combined with Alterations in Oncogenes and Oncogenic Signaling Pathways

Activation of oncogenes and oncogenic signaling pathways cooperates with PTEN loss to promote invasive prostate cancer. In prostate cancer, the *ERG* gene is frequently translocated to the *TMPRSS2* promoter region, with the resulting *TMPRSS2-ERG* fusion protein expressed in 50 % of human prostate cancer specimens [241–243]. Whereas mice expressing *TMPRSS2-ERGA* under the control of the *ARR2Pb* promoter only develop PIN lesions [242], this translocation collaborates with *Pten* haplosufficiency to cause invasive adenocarcinoma of the prostate [244]. Similarly, cooperation between *FGF8b* overexpression and *Pten* haplosufficiency in a murine model leads to adenocarcinoma of the prostate, as well as lymph node metastases, in comparison to *FGF8b* overexpression alone, which leads to only hyperplastic and PIN lesions [245]. The 8q24 chromosomal region comprising the *MYC* oncogene is somatically amplified in a cohort of advanced human prostate tumors [246]. While mice engineered to express high levels of human *c-Myc* in the prostate (*PB-Cre4 Myc^{hi}*) develop invasive adenocarcinomas with 100 % penetrance [247], focal expression of *c-Myc* specifically in luminal epithelial cells of the prostate of mice (*PB-Cre4 Z-Myc*) results in only a mild pathology [248]. However, when combined with deletion of *Pten*, *PB-Cre4 Z-Myc* mice develop high-grade PIN and prostate cancer [248]. Although further investigation is needed to fully understand the synergistic effect of *c-Myc* activation and *Pten* loss in prostate cancer, evidence from this study and others suggests that loss of *Pten* may have differential effects depending on the cell types and regions/lobes/zones of the prostate where genetic deletion occurs. With the advent of cell type specific promoters in the prostate, future murine models will be able to tease out the effects of PTEN loss in specific cells in the prostate. For now, these models confirm that concomitant loss of *Pten* and genetic activation of oncogenes such as *ERG*, *FGF8b*, and *Myc* accelerate initiation and progression in human prostate cancer (Fig. 4.1).

Pten Loss Combined with Alterations in Inflammatory Pathway Regulators

Various lines of evidence suggest that chronic inflammation is linked to prostate tumorigenesis [249–251]. Indeed, expression of specific cytokines can be used as a prognostic indicator of biochemical recurrence in human prostate cancer [252]. One of the most prevalent inflammatory mediators clearly implicated in prostate cancer is IL-6, a cytokine that has not only been associated with tumor growth, proliferation, and angiogenesis in many cancers [253], but whose high levels in the circulating plasma of prostate cancer patients have also been correlated with advanced stages of the disease, therapeutic resistance, and an overall poor prognosis [254]. Although the foremost effect of IL-6 is activation of the JAK/STAT3 pathway [255], the PI3K/AKT pathway can also directly activate and phosphorylate STAT3 at Ser727 [256], which induces metastatic behavior of prostate cancer cells both in vitro and in vivo through stimulation of angiogenesis and suppression of

antitumor immune responses [257]. While transgenic mice that constitutively express *Stat3* under the control of the *ARR2Pb* promoter develop only PIN lesions, when crossed with *Pten*^{+/-} mice, the subsequent compound mutants develop invasive prostate tumors [258].

Many inflammatory cytokines and chemokines promote tumor progression by converging on and stimulating the IKK2/NF- κ B signaling axis [259]. The main function of IKK2 is the phosphorylation of I κ B molecules, which act as inhibitors of NF- κ B, thus rendering them subject to degradation and allowing NF- κ B to remain activated. Constitutive activation of the transcription factor NF- κ B in prostate cancer has been correlated with disease progression [260], and inhibition of NF- κ B activity in prostate cancer cells can suppress angiogenesis and subsequent tumor invasion and metastasis by downregulating expression of downstream NF- κ B targets such as VEGF and MMP9 [261]. Interestingly, while a mouse model containing a constitutively active version of IKK2 alone is insufficient in promoting prostate tumorigenesis, in combination with heterozygous loss of *Pten*, IKK2 activation leads to an increase in tumor size, accompanied by increased inflammation [262]. These studies demonstrate that inflammatory cytokines secreted from the stromal microenvironment of the prostate cooperate with PTEN loss to drive epithelial prostate tumor cells toward invasive disease.

PTEN and Tumor Cell Migration and Invasion

As demonstrated in various models of conditional *Pten* deletion in the prostate, homozygous *Pten* loss leads to progression from PIN lesions to invasive adenocarcinoma, a process that requires disruption of the basement membrane and junctional integrity in epithelial acinar structures to allow the invasion of tumor cells through the surrounding basement membrane and into the stromal microenvironment (Fig. 4.1). PTEN and PIP3 play conserved roles in the determination of cell polarity in diverse cell types. From data first obtained in *Dictyostelium discoideum* [263–266], a unicellular amoeba, and later from neutrophils undergoing chemotaxis [267, 268], enrichment of PIP3 at the leading edge of migrating cells and localization of PTEN in the lateral and trailing edges of the cell has been observed. The PI3K pathway also promotes membrane ruffling, cell motility, and cellular spreading through downstream effectors such as RHO, RAC1, and CDC42 [269]. Consequently, forced expression of PTEN in tumor cell lines inhibits tumor cell invasiveness in vitro and in xenografts in vivo through both phosphatase-dependent [110, 270] and phosphatase-independent [102] mechanisms. In normal glandular development, PTEN concentrates to the apical plasma membrane during epithelial morphogenesis, where it catalyzes the conversion PIP3 into PIP2, which recruits ANX2, CDC42, and aPKC to the membrane to establish cellular polarity [271]. In this regard, loss of PTEN expression may block the development of the apical surface and lumen of epithelial structures. Therefore, activation of the PI3K/AKT pathway upon PTEN loss may lead to the loss of epithelial features, and thereby increase the likelihood

of cells developing the properties of increased motility and invasive capacity through an epithelial-to-mesenchymal transition (EMT) [128]. In all, these findings raise the possibility that the considerable increase in the *PTEN* mutation/deletion rate in metastatic tumors might result from a selective metastatic advantage acquired through the loss of PTEN regulation of motility and invasion.

Pten Loss in Metastatic Prostate Cancer Mouse Models

It is clear from these models that *Pten* LOH is required for cancer progression and invasive adenocarcinoma development. Although biallelic *Pten* deletion, alone or in combination with homozygous deletion of *p53* [105, 238], *Nkx3.1* [230], or *Smad4* [239] or activation of *FGF8b* [245], does lead to the occurrence of small micrometastases in the lymph nodes and lungs, it fails to produce significant metastatic burden, particularly in the bone [215]. Therefore, other genetic alterations and signaling pathway abnormalities must collaborate with activation of the PI3K/AKT pathway to promote metastatic prostate cancer to the bone.

Although *Ras* mutations [272–274] and *Ras* fusion events [275] in prostate cancer are uncommon, strong evidence suggests that Ras/MAPK activation plays a substantial role in human prostate cancer progression, particularly in metastasis and CRPC development. Indeed, the Ras/Raf/MAPK pathway has been recently shown to be altered in 43 and 90 % of primary and metastatic lesions, respectively [26]. P-MAPK levels, as assessed in tumor microarrays (TMAs) from human prostate cancer samples, are significantly elevated in neo-adjuvant treated, recurrent, and CRPC patients as compared to benign prostatic hyperplasia (BPH) specimens, corresponding with a significant reduction in PTEN expression [122]. These findings have prompted the development of two recent murine models of prostate cancer that combine homozygous *Pten* loss with activation of the Ras/Raf/MAPK pathway: the *PbCre;Pten^{L/L};Kras^{G12D/+}* model [122], and the *Nkx3.1^{CE2/+};Pten^{fl/fl};Braf^{CA/+}* model [276]. In both models, activation of the MAPK pathway through either *Braf* or *Kras* conditional overexpression resulted in overt macrometastases to the lymph nodes, lungs, liver, and, importantly, the bone marrow, in around 30 % [276] and 100 % [122] of cases, respectively. In the *PbCre;Pten^{L/L};Kras^{G12D/+}* model, treatment with a MEK inhibitor alone was able to fully ablate metastatic spread to the lungs and other distant organs, implicating the RAS/RAF/MAPK pathway as a driver of metastasis in *Pten*-deficient prostate cancer [122]. Interestingly, an EMT phenotype is also observed at the primary tumor site in the *PbCre;Pten^{L/L};Kras^{G12D/+}* model [122]. As EMT has been postulated to play a critical role in the process of metastasis [277], this new model provides a unique opportunity to study the impact of EMT in prostate cancer metastasis in vivo in the context of *Pten* loss and Ras/MAPK activation. With these novel metastatic models of prostate cancer, a better understanding of the contribution of PTEN to the metastatic cascade, including localized invasion, intravasation into the blood stream, survival as circulating tumor cells (CTCs), extravasation out of the blood stream, and metastatic seeding to distant

organ sites, can be further uncovered. Overall, past and present murine models of prostate cancer induced by *Pten* loss have demonstrated that loss of PTEN, to varying degrees and in combination with other genetic alterations, can recapitulate the entire spectrum of prostate cancer, from initiation (heterozygous *Pten* loss), through progression (homozygous *Pten* loss), and, finally, to metastasis (homozygous *Pten* loss and Ras/MAPK activation) (Fig. 4.1).

PTEN and CRPC

Androgens are indispensable for prostatic glandular development and homeostasis and contribute to prostate cancer development through activation of the androgen receptor (AR). Androgen deprivation therapy remains the most common treatment for advanced prostate cancer. However, therapeutic effects are short lived, and patients usually succumb to CRPC within 18–24 months, leaving the disease essentially untreatable [278]. New generation androgen deprivation therapies (ADTs), such as abiraterone [279] and MDV3100 [280], that more effectively ablate androgen production and AR signaling, are rapidly being developed and approved for patients with metastatic CRPC. Similar to human prostate cancers, while castration initially results in massive apoptosis of the prostate epithelium in the *Pten*-null murine model of prostate cancer, the ki67 proliferation index remains constant, indicating that a select population of cells remains resistant to androgen withdrawal [215].

AR is expressed in CRPC and may function through autocrine signaling or cross-talk with other prosurvival and proliferation pathways [281, 282], including the PI3K/AKT pathway, which has been shown to induce AR expression in the absence of PTEN [283–285]. Multiple studies have found an association between the loss of PTEN and the development of CRPC [201, 215, 286, 287]. Moreover, loss of PTEN and AR expression has been correlated clinically with increased mortality in CRPC patients [193]. aCGH analysis on metastatic prostate cancer samples has also demonstrated frequent amplification of AR (73 %), coinciding with aberrant deletion of PTEN (87 %) [288].

While some studies have proposed that PTEN deletion activates AR through PI3K-mediated stabilization of AR protein levels or AKT-mediated phosphorylation and activation of the AR [231, 289, 290], other reports have revealed that PI3K/AKT pathway stimulation promotes degradation of AR and inhibits AR transcriptional activity [291]. Supporting the later claim, levels of AR are heterogeneous, and, in many cases, absent in late stage, metastatic disease [292–295]. These observations raise the possibility that loss of AR expression and activity may serve as a means of evading androgen withdrawal through simultaneous activation of other signaling pathways. Indeed, two independent laboratories have recently demonstrated that PTEN loss inhibits androgen-responsive gene expression by regulating AR activity [296, 297], indicating that castration-resistant growth is an intrinsic property of *Pten* null prostate cancer cells regardless of cancer stage [296]. These studies further suggest a reciprocal feedback loop that exists between AR and PTEN

in prostate cancer, in which conditional deletion of *Ar* in the prostate epithelium promotes the proliferation of *Pten*-deficient cancer cells in *PbCre;Pten^{+/L};Ar^{+/L}* mice through the downregulation of the androgen-responsive gene *Fkbp5* and preventing PHLPP-mediated AKT inhibition [296]. Moreover, inhibition of the PI3K/AKT pathway was shown to upregulate the receptor tyrosine kinase HER3 [297]. As suppression of HER2/HER3 heterodimers has been linked to inhibition of AR transcriptional activity through an AKT-independent mechanism [298], it is plausible that PI3K/AKT inhibition upregulates AR transcriptional activity by increasing HER3 expression.

In all, it is probable that AR suppresses the PI3K/AKT pathway in order to promote differentiation of the prostate epithelium and keep prostate cancer cells sensitive to androgens. When AR activity is downregulated upon ADT treatment, the PI3K/AKT pathway takes over to promote cell proliferation and cell survival in the absence of androgen or AR activity, further driving tumor progression toward metastatic CRPC [296, 297]. These findings may explain why clinical trials that inhibit the activation of the PI3K/AKT signaling axis, as well as its downstream effector mTOR, failed to have a substantial effect on tumor progression in men [299, 300], as inhibition of the PI3K/AKT/mTOR pathway causes an upregulation of AR transcriptional activity that promotes cell survival [297]. Since, in the background of PTEN-deficient prostate cancer, AKT regulates proliferation, while AR regulates survival, inhibition of both signaling pathways is necessary for effective tumor reduction. Indeed, combined therapy targeting both PI3K and AR pathways reduces tumor growth in *Pten*-null mice [296, 297], suggesting the possible efficacy of combined PI3K/AKT and AR inhibitor treatment in the clinic (Fig. 4.1).

PI3K/AKT/mTOR Pathway Inhibition as a Treatment for Prostate Cancer

Current Prostate Cancer Treatment

Treatment resistance is a major issue in the management of prostate cancer, as it is estimated that 30,000 men in the USA will die in 2012 alone from metastatic and CRPC, for which there is currently no cure. Although androgen-deprivation therapy (ADT) remains the standard treatment of metastatic prostate cancer, progression to castration-resistant disease occurs in the majority of patients [301–303]. Among available therapeutic approaches for the treatment of CRPC, conventional chemotherapy with docetaxel and other agents has limited efficacy and has yet to produce long-term benefits [304, 305]. Although agents that specifically inhibit the AR, androgen synthesis, and/or AR-regulated pathways, such as MDV3100 and Abiraterone, have recently entered the clinic and have shown promising results [279, 280], their therapeutic effects are short lived, and patients eventually develop CRPC [278]. Another novel therapy, sipuleucel-T, which is the first ever FDA-approved therapeutic cancer vaccine for the treatment of metastatic prostate

cancer, also only modestly improves the survival of late-stage patients by a few months [306, 307].

The current trend in medicine has been to exercise a personalized treatment approach that is based on molecular and genetic profiling of individual patients to determine the best therapeutic strategy. A considerable number of novel therapeutics are presently undergoing clinical trials, including small molecules that target common genetic or pathway alterations found in human cancers. These inhibitors have been FDA approved for treatment of various solid tumors including, renal, GIST, breast, pancreatic, colorectal, and NSCLC cancer [308–314], and thus hold promise for the treatment of prostate cancer. As it is clear that PI3K/AKT/mTOR pathway activation plays a prominent role in prostate cancer initiation and progression, CRPC, and metastatic disease, the loss of PTEN expression in individuals with prostate cancer could be used as a biomarker to stratify populations of patients that may benefit from treatment with PI3K/AKT/mTOR pathway inhibitors.

PI3K Inhibitors

Class I PI3Ks are heterodimers composed of one catalytic subunit of p110 α , p110 β , or p110 δ , collectively known as p110, and a regulatory subunit, p85. PI3K isoform selectivity may be essential to boost therapeutic efficacy and minimize off-target toxicity. Recent research suggests a dominant role for the PI3K isoform p110 β in PTEN-deficient tumors. In the *Pten*-null prostate cancer model, loss of p110 β , but not p110 α , decreased PI3K signaling and prevented prostate carcinogenesis [94]. In a similar fashion, inducible depletion of p110 β , but not p110 α , using shRNA in PTEN-deficient human cancer cell lines quenches PI3K-mediated signaling and inhibits growth both in vitro and in vivo [315].

The most studied PI3K inhibitors to date are the first-generation PI3K inhibitors LY294002 and wortmannin. LY294002 treatment results in cell-cycle arrest and sensitizes the LNCaP prostate cancer cell line to radiation therapy, decreases the invasive properties of LNCaP, PC-3, and DU145 prostate cancer cell lines, and inhibits angiogenesis in PC-3 prostate cancer cells by way of decreased levels of HIF1- α and VEGF. Similarly, wortmannin induces apoptosis and radiosensitizes DU145 cells [316, 317]. However, both wortmannin and LY294004 show limited selectivity for individual PI3K isoforms, nonspecifically target multiple other signaling molecules [318–321], and demonstrate significant toxicity in animals [317, 322], limiting their effectiveness in vivo.

One potential consequence and side effect of PI3K pathway inhibition is the development of insulin resistance in patients. While both p110 α and p110 β play specific roles in insulin signaling, research suggests that glucose homeostasis is predominantly mediated by p110 α [94, 323]. Indeed, p110 α inhibitors, but not p110 β or p110 δ inhibitors, alter insulin-dependent glucose regulation in mice [323]. Thus, in the setting of PTEN-deficient tumors, p110 β -specific inhibitors, in contrast to pan-PI3K inhibitors, may offer enhanced efficacy with a reduced likelihood of insulin resistance. Together, these studies suggest that effective treatment of

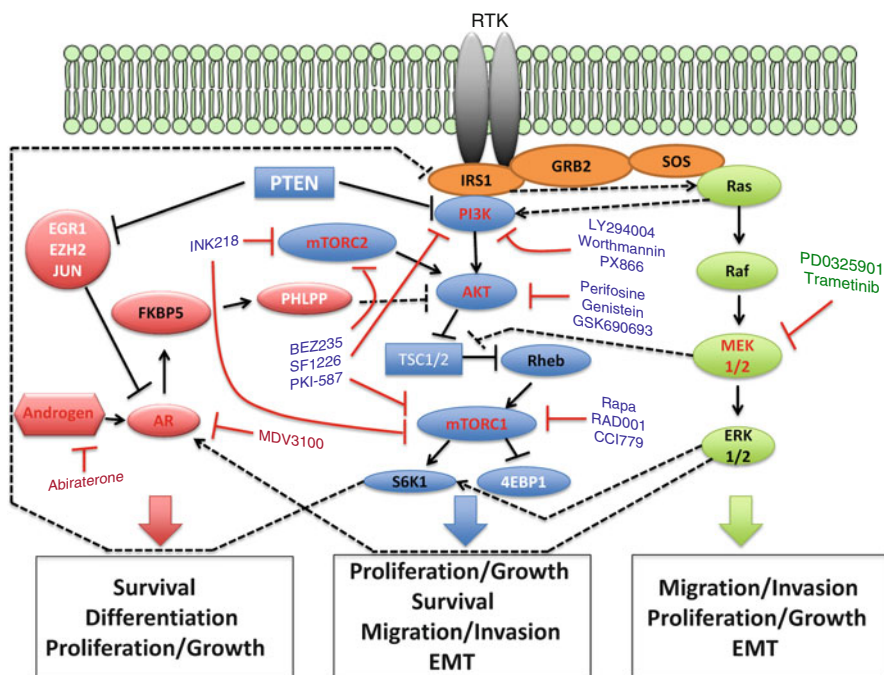


Fig. 4.2 PI3K/AKT/mTOR, Ras/MAPK, and AR signaling pathways converge to promote prostate cancer development. Although all three pathways promote cell proliferation/growth, AR signaling maintains prostate cells in a differentiated state, while PI3K/AKT/mTOR and Ras/MAPK signaling promotes EMT and cell migration/invasion. Red, blue, and green ovals represent AR, PI3K/AKT/mTOR, and Ras/MAPK signaling molecules, respectively. Orange ovals denote adaptor molecules. Pathway activators are in black letters, and pathway suppressors are in white letters. Solid black lines depict signaling within a pathway, and dotted black lines depict crosstalk between pathways or feedback loops. Red lines denote the drug targets. Signaling molecules in these pathways that are the targets of drug inhibitors are in red letters

PTEN-deficient prostate tumors may necessitate the use of therapeutic agents that successfully target p110 β . However, even in cancers that may be specifically reliant on either p110 α or p110 β , there remains the possibility that other, noninhibited p110 isoforms may make up for the decreased activity of the targeted isoform. Moreover, not all tumors that are driven by PTEN loss are dependent on p110 β , and the presence of other genetic modifications and pathway alterations is likely to change the PI3K-isoform reliance of these tumors. Interestingly, PTEN loss appears to be a predictive marker for sensitivity to PX-866, an oral derivative of wortmannin, despite the fact that PX-866 displays a high efficacy against p110 α and p110 δ but not p110 β [324]. Therefore, although PI3K signaling is an obvious target for therapy, especially in PTEN-deficient prostate cancer, given the redundancy and complex feedback regulation existing in the PI3K/AKT/mTOR pathway, as well as a need for a more in-depth understanding of the pathway, the clinical efficacy of using PI3K inhibitors as single agents is modest (Fig. 4.2).

AKT Inhibitors

The significance of the individual AKT isoforms in prostate cancer has yet to be fully uncovered, despite findings that AKT-1 isoform expression may be a prognostic marker for biochemical recurrence in patients with prostate cancer [325]. There are several classes of AKT inhibitors currently in development, including isoform-selective AKT catalytic-domain inhibitors and inhibitors of its PH domain, and many have been tested in prostate cancer. Perifosine, an alkylphospholipid that targets the PH domain of AKT and prevents it from binding to PIP3, decreases AKT phosphorylation, inhibits growth, and induces cell-cycle arrest of PC-3 cells [326]. Although there are no published preclinical studies investigating perifosine activity against prostate cancer, perifosine has gone to clinical trials for patients with CRPC. Though generally well tolerated, perifosine showed no evidence of significant inhibitory activity [300, 327]. Genistein, a non-specific AKT inhibitor, causes significant growth inhibition and apoptosis of cancer cells [328, 329]. While genistein has demonstrated significant potential in vivo, decreasing the incidence of lung metastasis in an orthotopic model using PC-3 cells [330] and inhibiting tumor growth when combined with docetaxel in an experimental model of bone metastasis [331], another report claimed that genistein increased the size of metastatic lymph nodes in a PC-3 orthotopic model [326]. Concomitant targeting of AKT-1 and AKT-2 with ATP-competitive inhibitors, such as GSK690693, has been shown to be more effective than inhibition of single isoforms for the induction of apoptosis in tumor cells, suggesting that these Pan-AKT inhibitors are likely to have more promise in the clinic, although increased toxicity may be a potential issue [332]. However, AKT inhibitors will not block the non-AKT effectors downstream of PI3K signaling. Paradoxically, AKT inhibitors could increase upstream receptor tyrosine kinase activities by alleviating downstream negative feedback loops [333]. Therefore, the importance of AKT-independent effectors of PI3K signaling and downstream negative feedback loops in the pathway might considerably affect the clinical effectiveness of AKT inhibitors (Fig. 4.2).

mTOR Inhibitors

mTOR inhibitors have been the most effective among the inhibitors of the PI3K/AKT/mTOR pathway in treating solid tumors and have received the most consideration in the treatment of prostate cancer. Rapamycin, the prototypical mTOR inhibitor, associates with its intracellular receptor, FKBP12, which then binds directly to mTORC1 and suppresses mTOR-mediated phosphorylation of its downstream effectors, S6K and 4EBP1. Rapamycin induces cell-cycle arrest in PC-3 and DU145 prostate cancer cell lines in vitro [334–337] and reduces tumor volume and blocks growth and proliferation in tumors with activated AKT or loss of PTEN in vivo [338, 339]. Although limited, there have been reports on in vitro and preclinical

studies demonstrating the efficacy of the rapamycin analogs (rapalogs) CCI-779 and RAD001 in the treatment of prostate cancer. CCI-779 inhibits growth of PC-3 and DU145 cells in vitro, and, in vivo, and reduces tumor volumes in PC-3 and DU145 xenografts [340]. Likewise, RAD001 treatment decreases proliferation of prostate cancer cells in vitro [341, 342] and reverses PIN lesions in AKT-1 transgenic mice [343].

Despite these preclinical findings, mTORC1 inhibitors, including rapamycin and rapalogs, have demonstrated little success as single agent treatments in the clinic [299, 344, 345]. Although rapamycin and rapalogs are effective at inhibiting mTORC1 kinase activity, inhibition of mTORC1 eventually leads to AKT activation and increased P-AKT levels due to the loss of the S6K to IRS-1 feedback loop and reactivation of PI3K signaling [41, 345, 346]. Moreover, while mTORC1 is sensitive to rapamycin treatment, mTORC2 is generally considered to be resistant to rapamycin. In this regard, mTORC2 phosphorylation and activation of AKT may further limit the efficacy of mTORC1 inhibitors like rapamycin [38]. Therefore, the use of rapamycin and rapalogues as single treatments could potentially cause the hyperactivation of the PI3K/AKT pathway (Fig. 4.2).

To achieve a significant clinical effect, mTORC1 inhibition with rapamycin and rapalogs may require the addition of upstream inhibitors, such as insulin-like growth factor signaling or PI3K signaling inhibitors [347–350], or, alternatively, more effective inhibition of both TORC1 and TORC2 activity. mTOR catalytic site inhibitors, which are currently in clinical development, target the kinase domain of mTOR and have the advantage of blocking the activity of both mTORC1 and mTORC2. The additional inhibition of mTORC2 provides the benefit of blocking AKT activation through S473 phosphorylation, and therefore, these catalytic site inhibitors would be expected to inhibit the mTOR pathway more effectively than rapamycin. Current research has described torkinibs and torin1, two selective ATP-competitive inhibitors of mTOR that impede cellular growth and proliferation more effectively than rapamycin [351, 352]. Interestingly, however, the enhanced activity of these mTOR kinase inhibitors seems to be due to more complete inhibition of mTORC1 activity rather than mTORC2 inhibition, as measured by decreased levels of 4EBP1 phosphorylation and cap-dependent translation compared to rapamycin treatment [351, 352]. In support of the efficacy of these mTOR catalytic site inhibitors, a recent preclinical study using the mTOR catalytic site inhibitor INK218 in the *Pten*-null murine prostate cancer model demonstrated that INK218 is able to inhibit AKT and 4E-BP1 phosphorylation in addition to S6K1 phosphorylation, lead to a 50 % decrease in PIN lesions, reduce overall tumor volume, and promote tumor cell apoptosis, as opposed to RAD001 treatment, which results in inhibition of S6K1 but not AKT and 4E-BP1 phosphorylation, only partial regression of PIN lesions, and no significant effect on tumor cell apoptosis [353]. Remarkably, treatment with INK218 blocks progression of invasive prostate cancer locally in the prostate and even inhibits the total number and size of distant metastases [353]. Although new generation mTOR catalytic site inhibitors have the capacity to reduce prostate tumor invasion and metastasis by more effectively disabling mTORC1 signaling and inhibiting mTORC2 activation, treatment with these inhibitors alone

does not inhibit PI3K activity, and, therefore, would need to be combined with other PI3K antagonists to fully ablate distant metastasis and lead to complete tumor regression (Fig. 4.2).

Dual PI3K–mTOR Inhibitors

The use of multiple inhibitors to target the PI3K/AKT/mTOR pathway may be of particular importance to alleviate the issue of negative-feedback loops in the pathway. As the catalytic domains of the p110 subunits and mTOR are similar in structure, there are a number of small molecule inhibitors currently being tested that can block both PI3K and mTOR. Compared to other PI3K pathway inhibitors, dual PI3K–mTOR inhibitors, which include NVP-BEZ235, BGT-226, XL765, SF1126, PKI-402, and PKI587, have the possible advantage of inhibiting all PI3K isoforms, as well as both mTORC1 and mTORC2. Therefore, these inhibitors should effectively turn off the PI3K/AKT/mTOR pathway completely and overcome feedback inhibition normally observed with mTORC1 inhibitors such as rapamycin and other rapalogs [347]. BEZ235 is capable of simultaneously inhibiting multiple class I PI3K isoforms and mTOR kinase activity by binding to their respective catalytic sites [349]. BEZ235, unlike PI3K inhibitors alone, is able to lower levels of both P-S6 and P-AKT, demonstrating that dual inhibition of both mTOR and PI3K is capable of preventing an increase in P-AKT levels [349, 350]. BEZ235 exhibits greater antiproliferative effects compared with rapamycin treatment in cancer cell lines in vitro and slows tumor growth and vasculature development in PTEN-deficient cell line engrafted mice, where it is well tolerated with no significant changes in body weight [349, 350, 354]. In preclinical studies, SF1126, a conjugate of LY294002, reduces cell growth, proliferation, and angiogenesis, and exhibits lower toxicity than LY294002 [355]. Furthermore, PKI-587, another dual PI3K/mTOR inhibitor, induces tumor regression in several cancer cell line xenograft models, and has a favorable drug safety profile in toxicology studies [356]. Importantly, in contrast to PI3K inhibitors that cause cytostatic effects through tumor cell G_0 – G_1 arrest [357–359], PKI-587 inhibition of PI3K and mTOR can fully ablate AKT activation and cause the induction of apoptosis, the preferred outcome against tumor cells [356]. Despite these preclinical findings, a major issue with dual PI3K/mTOR inhibitors is their efficacies in vivo in the clinical settings.

Combination Therapy with PI3K/AKT/mTOR and Ras/MAPK Inhibitors

One explanation behind the limited success of PI3K/AKT/mTOR pathway inhibition in the clinic is that blockade of PI3K signaling may shift the tumor survival signaling to a Ras/MAPK-dependent pathway [360]. Analyzes of human prostate cancer

microarrays have demonstrated that the PI3K/AKT and Ras/MAPK pathways are often coordinately dysregulated during prostate cancer progression in humans [122, 361]. Although BEZ235 is effective against PI3K-driven tumors as a single agent, the inhibitor responds poorly to tumors harboring Kras mutations [350]. Indeed, BEZ235 is only effective against Kras-driven tumors when combined with a MEK inhibitor [362]. Humans with advanced prostate cancer treated with RAD001 experience increased activation of MAPK signaling, probably due to the loss of the S6K-IRS1 feedback loop that leads to Ras activation [360] (Fig. 4.2). In addition, neoadjuvant hormone therapy can lead to increased P-MAPK activation and N-cadherin expression, both of which have been implicated in the induction of the EMT program and metastatic prostate cancer [122, 363]. Ras activation can also play a direct role in moving prostate cancer cell lines toward decreased androgen dependence [364]. Indeed, PI3K/AKT and Braf/ERK pathway activation acts combinatorially in a mouse model of CRPC [365]. These studies suggest the importance for combined PI3K/AKT and Ras/MAPK pathway blockade in the treatment of CRPC and metastatic prostate cancer.

A number of studies conducted with *Pten* knockout mice have shown that combined pharmacological targeting of mTOR and MEK may lead to reduced primary prostate tumor progression [122, 361]. Combination therapy using the mTORC1 inhibitor rapamycin and the MEK inhibitor PD0325901 inhibits not only growth in prostate cancer cell lines [361] but also reduces tumor burden in castrated, androgen-insensitive prostate tumors in the *Nkx3.1^{-/-};Pten^{+/-}* murine model [365]. Dual mTOR and MEK inhibition also completely ablates the dissemination of distant metastases in the *PbCre;Pten^{L/L};Kras^{G12D/+}* murine prostate cancer model, which exhibits 100 % penetrable macrometastasis [122], as well as reduces tumor and metastatic burden in *Nkx3.1^{Cre-ER};Pten^{fl/fl};Braf^{CA/+}* mice [276]. Thus, in late stage, metastatic prostate cancer, dual PI3K/AKT and Ras/MAPK inhibition may be necessary to reduce metastasis, as well as slow primary tumor growth (Fig. 4.1).

Combination Therapy with PI3K/AKT/mTOR and AR Pathway Inhibitors

Recent studies using the *Pten*-null murine model of prostate cancer have demonstrated a reciprocal feedback loop that exists between AR and PI3K pathways in the prostate cancer, whereby inhibition of the PI3K pathway in *Pten*-deficient prostate cancer results in reactivation of AR signaling by modulating AR corepressor activities or through feedback signaling to the receptor tyrosine kinase HER2/HER3 [296, 297]. Therefore, the efficacy of PI3K inhibitors for the treatment of *PTEN*-deficient prostate cancer may be improved through combined AR pathway inhibition. Another recent study utilizing surgical castration in *Pten*-null mice to model CRPC demonstrated that dual targeting of both AKT and mTOR with inhibitors MK-2206 and MK-8869, respectively, is highly effective at inhibiting CRPC in vivo [366]. Moreover, the AR agonist MDV3100, which has shown promise in the clinic,

has improved efficacy in combination with BEZ235 [350], a dual inhibitor of PI3K and mTORC1/2, in castration-resistant GEM mice [297]. Other laboratories have also documented beneficial effects of combined AR and mTORC1 inhibition with rapamycin in *Pten*^{-/-} models [296, 367]. Thus, more effective inhibition of the AR signaling axis with new generation inhibitors such as abiraterone and MDV3100 in combination with mTOR or PI3K/mTOR dual inhibitors may prove to be more beneficial in treating CRPC patients displaying alterations in PI3K/AKT pathway signaling (Fig. 4.2).

In all, although dual PI3K/mTOR inhibitors now offer the advantage of complete PI3K/AKT/mTOR pathway inhibition, with signaling feedback loops present in the PI3K/AKT/mTOR pathway that negatively control both Ras/MAPK and AR signaling, it is likely that PI3K inhibition alone will not be able to achieve full regression of tumors in patients with prostate tumors driven by PTEN loss. A better understanding of pathway dynamics gained from recent preclinical studies prompts the rationale for combining inhibition of the PI3K/AKT/mTOR pathway with inhibition of either the Ras/MAPK or AR signaling pathways for the treatment of metastatic CRPC. However, better surrogate biomarkers that predict patient responses to PI3K inhibitors, as well as more high-throughput systems to molecularly profile and detect PTEN loss or PI3K/AKT/mTOR activation in patients, will be needed to accurately assess the efficacy of PI3K/AKT/mTOR inhibition as a treatment in individual patients.

Conclusions and Perspectives

In the 15 years since the discovery of PTEN as a frequently mutated gene in cancer, great progress in understanding the function and regulation of PTEN has been made. While PTEN was first identified as a lipid phosphatase with tumor suppressive activity against the PI3K/AKT pathway, recent studies have revealed that PTEN has additional protein phosphatase and lipid phosphatase-independent activities, as well as functions in the nucleus. Further understanding of the mechanisms behind PTEN post-transcriptional regulation, post-translational modifications, and protein–protein interactions offers novel therapeutic opportunities, as well as explanations of why, clinically, loss of PTEN expression can occur without genetic deletion or mutations at the *PTEN* locus. With improved methods for detecting PTEN status, such as CGH, whole-exome sequencing, and transcriptome analysis, PTEN loss can more readily and more accurately be used as a prognostic biomarker for men with prostate cancer, as well as a potential predictive marker to identify patients who could benefit from emerging PTEN/PI3K/Akt pathway therapies. Moreover, studies with large human prostate cancer cohorts have revealed that alterations in the PI3K/AKT/mTOR pathway are more common in advanced, metastatic prostate cancer and CRPC compared to primary, androgen-dependent tumors and are associated with a poorer overall prognosis and increased chance of biochemical recurrence. Recent works using mouse models with varying degrees of *Pten* loss have helped to

reveal the role of PTEN dosage in prostate tumorigenesis and the collaborative effects of PTEN loss and other genetic and pathway alterations in prostate cancer initiation, progression, and metastasis. A better understanding of the interactions between the PI3K/AKT pathway and Ras/MAPK, p53, and TGF- β -Smad pathways has facilitated the development of metastatic models of prostate cancer with bone metastasis potential, an important feature of human prostate cancer. As bone tropism of prostate cancer metastasis is not well understood, these mouse models should provide better insights into the cell types and molecular pathways involved in metastasis to the bone. Better systems, via lineage-tracing and cell type-specific deletion, are needed to address which cell types are responsible for different stages of the disease, including prostate cancer progression, castration resistance, and metastasis. As previous studies have suggested that prostate luminal, transit amplifying (TA), and basal cells can serve as a cell-of-origin and as cancer stem cell (CSC) populations in prostate cancer [368], it will be important for future models to employ more restrictive prostate-specific promoters allowing the potential for tumor initiation from basal, TA, neuroendocrine, and luminal cell types. Two recent reports have also elucidated a reciprocal feedback loop between the PI3K/AKT and AR signaling pathways that directly regulates CRPC, offering an explanation for how loss of androgen dependence may further strengthen PI3K/AKT signaling in PTEN-deficient prostate cancer, as well as a rationale for the combined use of AR signaling and PI3K/AKT/mTOR inhibitors in the treatment of CRPC.

Although beyond the scope of this review, emerging research in other solid tumors has demonstrated that the tumor microenvironment itself may play a defined role in tumor propagation and progression, and it will be interesting to see if aberrations in the PI3K/AKT/mTOR signaling in stromal-specific subtypes themselves in the prostate may contribute to tumorigenesis. Moreover, PTEN alterations in the tumor epithelium, which have been demonstrated to induce the release of paracrine signals, including chemokines and cytokines that may attract immune cell types to the prostate and contribute to the development of a tumor-permissive rather than a tumor-suppressive microenvironment, suggest that immunotherapy may be a possible treatment for prostate cancer patients. Again, the specific stromal cells and immune cells that contribute to the prostate tumor microenvironment will need to be further pursued with the use of lineage-specific promoters and tracking systems in immune competent models that preserve the tumor's native microenvironment.

Finally, while past clinical trials using rapamycin and rapalogues to treat human prostate cancer have shown little efficacy, due in part to an inability to inhibit PI3K and AKT signaling, dual PI3K/mTOR inhibitors have the capacity to completely inhibit all strands of the PI3K/AKT/mTOR pathway, and thus deserve further study in preclinical models of prostate cancer. However, with signaling feedback loops present in the PI3K/AKT/mTOR pathway that negatively control both Ras/MAPK and AR signaling, it is unlikely that PI3K inhibition alone will be able to achieve full regression of PTEN-deficient prostate tumors. Further understanding of pathway dynamics gained from recent preclinical studies prompts the rationale for combining inhibition of the PI3K/AKT/mTOR pathway with inhibition of either the Ras/MAPK or AR signaling pathways for the treatment of metastatic CRPC. In the

end, though much progress has been made in understanding the role PTEN and its regulation of the PI3K/AKT/mTOR pathway in prostate cancer, in the future, more basic and preclinical mechanistic studies that further elucidate the complexity of the PI3K/AKT/mTOR signaling pathway and can be translated from bench to bedside will help to design better treatment options for patients with metastatic CRPC, for which there is still no cure.

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