IN THE SPOTLIGHT

Prostate Power Play: Does *Pik3ca* Accelerate *Pten*-Deficient Cancer Progression?

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Summary: PI3K pathway alterations are frequently recurrent in metastatic prostate cancer and are associated with the development of currently incurable castration-resistant disease. Candidate inhibitors that target single PI3K pathway members lack efficacy as demonstrated in multiple clinical trials. In this issue, Pearson and colleagues examine the functional importance of co-occurring *PIK3CA* and *PTEN* aberrations using a novel mouse model and demonstrate a synergistic acceleration of tumorigenesis that may be responsible for *de novo* metastatic prostate cancer. *Cancer Discov; 8(6); 682–5. ©2018 AACR*

See related article by Pearson et al., p. 764 (6).

Second only to lung cancer, advanced prostate cancer is a major cause of cancer-related death in men. A dire need exists to develop a better understanding of how progression to advanced castration-resistant prostate cancer (CRPC) occurs. The dominant mechanism of resistance for targeted androgen receptor (AR)–based therapy is reactivation of AR signaling. However, hyperactivation of other key cancer-promoting signaling pathways has been suggested to accelerate the onset of hormone resistance to early-stage, or *de novo*, CRPC (1).

Large-scale collaborative efforts in the field have identified the PI3K–AKT signaling axis to be the most frequently altered pathway in advanced prostate cancer (2). PI3K is a lipid kinase made up of a regulatory and catalytic heterodimer that can be paired using different protein isoform combinations. Catalytic subunits are encoded by *PIK3CA* (p110α), *PIK3CB* (p110β), and *PIK3CD* (p110δ; Fig. 1A). PI3K catalyzes the conversion of phosphatidylinositol 4,5-bisphosphate (PIP_2) to phosphatidylinositol (3,4,5)-trisphosphate (PIP_3), and this results in the downstream activation of the protein kinase AKT. This subsequently activates mTORC1 and mTORC2, which are implicated in directing cell proliferation, migration, and cell survival. PTEN is an established tumor suppressor that is responsible for countering PI3K activity by converting PIP_3 back into PIP_2. Heterozygous or homozygous loss of *PTEN* occurs in roughly two fifths of patients with metastatic prostate cancer (2). Recently, the characterization of 1,013 tumor and matched germline prostate cancers detected PI3K pathway genomic alteration in 17% of primary tumors, and these were further enriched in 40% of cases with metastatic disease (1). Although genomic studies identify candidate drivers of CRPC progression, the functional characterization of these genetic changes is an enormous undertaking. Genetically engineered mouse (GEM) models that allow tissue-specific deletion of a gene of interest have become a mainstay in the field, especially the *PBiCre*$^{+}$;*Pten*$^{fl/fl}$ model that expresses androgen-stimulated Cre recombinase following puberty to homozygously delete *Pten* in prostate epithelium (3). The mouse model of *Pten* loss has been used to examine the functional importance of multiple key factors that drive prostate cancer, including *MYC, SOX2, TP53, KRAS, BRAF, SMAD4, TERT*, and recently *SPOP* (4, 5).

In this issue of *Cancer Discovery*, Pearson and colleagues utilize the *PBiCre*$^{+}$ system to functionally explore the biological impact of the *Pik3ca*^{H1047R} (*Pik3ca*^{HR})-activating mutation in the prostate and further explore if this aberration cooperates with *Pten* deletion to accelerate the progression of prostate cancer to CRPC (6). The motivation to develop this new prostate-specific *Pik3ca* model stems from initial observations in multiple prostate cancer genomic datasets. Examination of nine patient cohorts identified *PIK3CA* mutations and amplifications that have previously been suggested to increase p110α kinase activity. This gain of function is significantly correlated to attributes of poor patient outcome such as lymph node metastasis, Gleason grade, and recurrence-free survival. Importantly, around 45% of patients with *PIK3CA* mutation or amplification/gain also harbored mutation or loss of *PTEN*. What would drive apparently redundant genomic alterations? Pearson and colleagues addressed the following three questions: (i) What is the oncogenic role of *PI3KCA*^{H1047R} mutation in the prostate? (ii) Are there phenotypic differences between mutant *PI3KCA* and *PTEN*-null tumorigenesis? (iii) If non-redundant, do *PI3KCA* and *PTEN* aberration cooperate to produce CRPC?

The authors generated a *PBiCre*$^{+}$;*Pik3ca*$^{+/HR}$ (referred to as *Pik3ca*$^{+/HR}$) mouse mutant of prostate-specific, heterozygous p110α activation. Histologic characterization of individual prostate lobes over the duration of 400 days showed that *Pik3ca*$^{+/HR}$ mutation stimulated a progressive malignant

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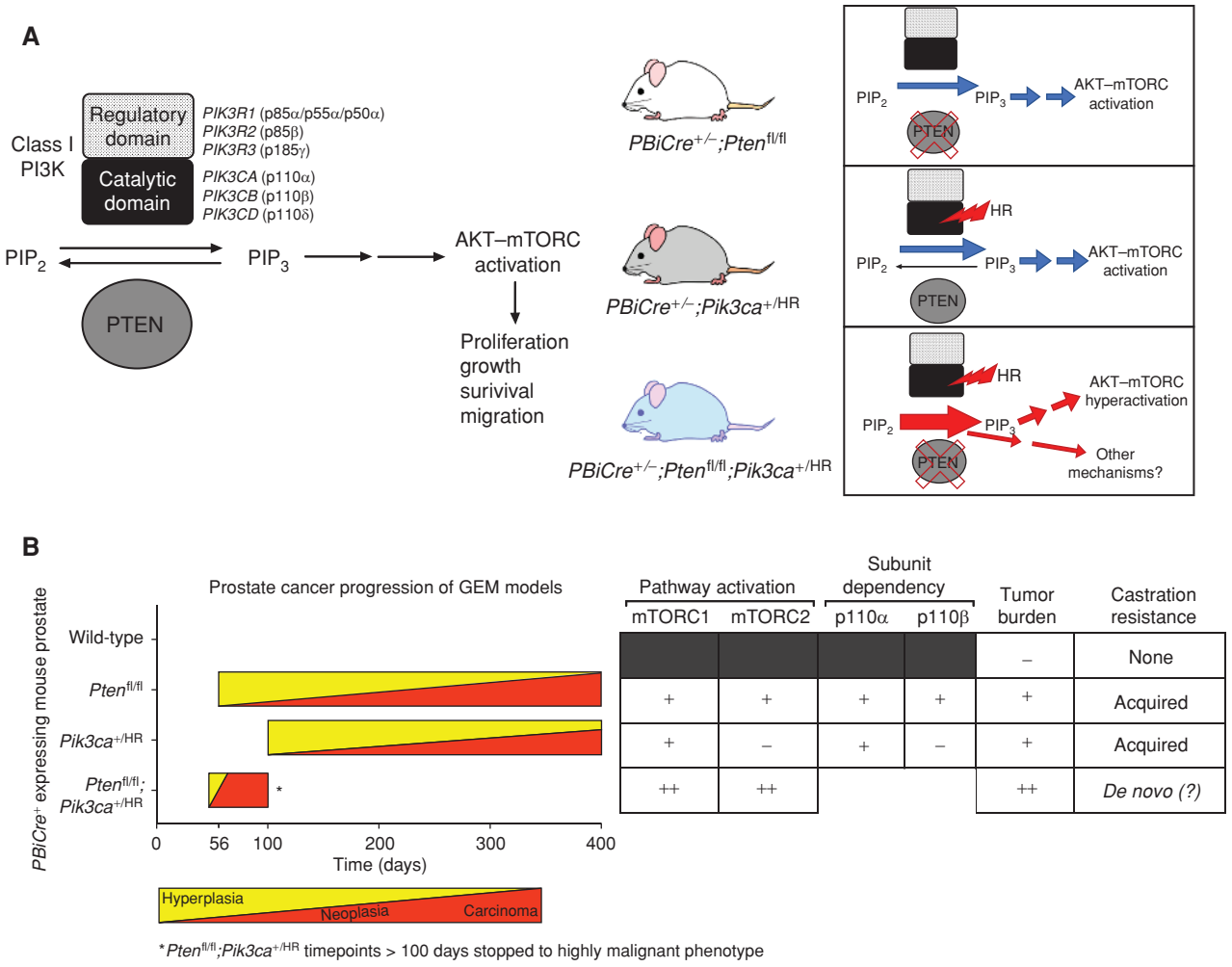

Pathway activation		Subunit dependency		Tumor burden	Castration resistance
mTORC1	mTORC2	p110α	p110β		
				–	None
+	+	+	+	+	Acquired
+	–	+	–	+	Acquired
++	++			++	*De novo (?)*

Figure 1. Graphical abstract summary of Pearson et al. (6). **A,** Class 1 PI3K is composed of a regulatory and catalytic subdomain coded for by any of the *PIK3CA*, *PIK3CB*, or *PIK3CD* genes. PI3K activity converts PIP_2 to PIP_3 and is reversed by PTEN. AKT and mTORC signaling are substrates of PI3K pathway activation promoting downstream modulation of cell proliferation, growth, survival, and migration. GEM models can alter this pathway specifically in prostate tissue using *PBiCre* expression and a *Pten*$^{fl/fl}$ allele and/or *Pik3ca*$^{+/HR}$ activating mutant of the p110α catalytic subunit. *Pik3ca*^{HR} and *Pten* deletion both function to activate PI3K–AKT–mTORC signaling, and together appear to synergize. **B,** Histologic characterization of the GEM models demonstrates differences in onset of hyperplasia and progression to invasive prostate carcinoma. A summary of the molecular findings of this is shown.

phenotype that developed from mild hyperplasia (day 56) to locally invasive prostate carcinoma (days 300–400). This is the first *in vivo* evidence to confirm that single-allele *Pik3ca*^{HR} mutation is sufficient to induce prostate cancer in mice (6).

Next, Pearson and colleagues considered the *Pik3ca*$^{+/HR}$-mutant phenotype relative to the established *PBiCre*$^{+/-}$;*Pten*$^{fl/fl}$ (referred to as *Pten*$^{fl/fl}$) model as both alterations activate the PI3K pathway. They found the *Pten*$^{fl/fl}$ animals had earlier onset of hyperplasia and a more rapid progression to invasive carcinoma, as well as greater tumor burden and significantly more proliferating cells compared with *Pik3ca*$^{+/HR}$ hyperplastic tissues, and higher expression of the cytokeratin-8 marker of basal cell lineage. These data show that the *Pik3ca*$^{+/HR}$ single mutant is an activating mutation that promotes murine cancer progression and—although comparable to the *Pten*-null model—there are distinct phenotypic differences that suggest that these key PI3K pathway components do not phenocopy. Understanding these differences may reveal unique functions of *Pik3ca* and *Pten* that may offer novel therapeutic opportunities.

Stemming from the initial animal model development, the authors questioned if *Pik3ca* mutation and *Pten* loss models parallel in downstream molecular signaling. Using immunohistochemistry (IHC), both models showed activation of the AKT–mTORC1 signaling pathway relative to wild-type prostate tissue. Expression of *Pten* was maintained in the *Pik3ca*$^{+/HR}$ model but did not impair *Pik3ca* downstream signaling through the mTORC1 phospho-targets. Although both models activated mTORC1 substrates, mTORC2 target activation was elevated almost exclusively in the *Pten*$^{fl/fl}$ tumors. Further, both mutants displayed active p110α signaling (high pERK) relative to wild-type, but only *Pten*$^{fl/fl}$ prostate tumors had active markers of p110β signaling (active RAC-GTP), therefore

identifying another nonredundant phenotype whereby *Pten*-deleted tumors may preferentially drive malignancy via p110β activation (6).

Credentialing the importance of PI3K catalytic subunit dependency (p110α or p110β) has therapeutic implications given the active development of drug inhibitors in this class, many of which are being tested in clinical trials. Pan-PI3K inhibitors, such as BKM210 and BYL719, are being tested for treatment of breast, colon, ovarian, and more recently metastatic prostate cancers (clinical trial NCT012196999). Pearson and colleagues directly explore p110α and p110β dependency of the $Pten^{fl/fl}$ and $Pik3ca^{+/HR}$ models using BKM120, A44 (p110α-specific inhibitor), and TGX-221 (p110β-specific inhibitor) treatment on mice that were stage-matched for prostate carcinoma. $Pik3ca^{+/HR}$ tumor burden regressed with A66 and BKM120, suggesting p110α dependency for this driver mutation, whereas $Pten^{fl/fl}$ is thought to be p110α/p110β codependent, as tumor regression was observed only with pan-inhibition of both isoforms. These data are well aligned with previous work that shows that PI3K isoform–specific monotherapies are ineffective for the treatment of *PTEN*-null prostate cancer (7).

After discovering that *PIK3CA* mutation and *PTEN* loss can co-occur in patients, the investigators developed a $PBiCre^{+}$;$Pik3ca^{+/HR}$;$Pten^{fl/fl}$ (referred to as $Pik3ca^{+/HR}$;$Pten^{fl/fl}$) double-mutant mouse model. Combination of prostate-specific *Pik3ca*-activating mutation and *Pten* loss showed 100% incidence of invasive carcinoma with significantly greater tumor burden relative to age-matched single mutants (Fig. 1B). Double-mutant tumors had elevated IHC staining for PCNA-positive proliferating cells and unaltered markers of apoptosis. Additionally, $Pik3ca^{+/HR}$;$Pten^{fl/fl}$ tumors had a hyperactivation of mTORC1 and mTORC2 signaling targets compared with stage-matched single mutants (6). This model convincingly suggests that *Pten* deletion together with *Pik3ca* mutation can synergistically accelerate cancer progression, and do this by cooperatively increasing proliferative mechanisms without rewiring survival pathways.

Clinically, loss of *PTEN* is associated with resistance to androgen deprivation therapy (7, 8). The authors therefore sought to determine if $Pik3ca^{HR}$ mutation can confer castration resistance in mice. Surgical castration of $Pik3ca^{+/HR}$ and $Pten^{fl/f}$ mice reduced prostate tumor volume compared with noncastrate controls but did not entirely eliminate tumors due to the acquired development of CRPC. In contrast, castration of $Pik3ca^{+/HR}$;$Pten^{fl/fl}$ double mutants did not significantly alter tumor burden relative to noncastrate controls. Double-mutant tumors showed no change in already-elevated markers of cell proliferation and displayed attributes of CRPC (i.e., androgen receptor nuclear localization) earlier than the single-mutant models of acquired resistance. There was also hyperactivation of mTORC1 and AKT, which was maintained following castration of the double mutant. Taken together, these observations reflect the properties of *de novo* CRPC that is nonresponsive to androgen ablation from the early or beginning stages.

Finally, Pearson and colleagues delve into the potential mechanisms responsible for promoting the synergy between *Pik3ca*-mutant and *Pten*-deleted CRPC using a reverse-phase protein array (RPPA). Distinct signaling events were identified between $Pik3ca^{+/HR}$ and $Pten^{fl/fl}$ tumors that involve the PI3K cascade, MAPK, and tyrosine kinase–mediated signaling. Although the single-mutant samples showed RPPA profile differences between castrated and uncastrated tumors, the double-mutant mice had little variation between castrated and controls. Providing further insight, the authors nominate NDRG inactivation as a potential mechanism of *de novo* CRPC, as increased phospho-NDRG1—a substrate of the mTORC2 pathway—is detected in post-castrated $Pik3ca^{+/HR}$;$Pten^{fl/fl}$ mice. These data highlight the existence of distinct signaling functions of *Pik3ca* mutation and *Pten* loss that contribute to the progression of prostate cancer and suggest novel cooperative mechanisms that drive castrate-resistant disease.

The phenotypic nonredundancy of $Pten^{fl/fl}$ and $Pik3ca^{+/HR}$ models highlights the likelihood that alternative molecular functions of these factors are influencing cancer development. This is exemplified by recent work revealing novel substrates of PTEN in addition to PIP_3 (9). As well, the field has yet to determine the biological impact of the lesser-known type 2 phosphatidylinositol-5-phosphate 4-kinase network, and the extent to which it may influence efficacy of PI3K–AKT–mTOR targeted therapies in prostate cancer (10).

In summary, this work by Pearson and colleagues reports that the $PIK3CA^{H1047R}$ mutation is sufficient to produce locally invasive prostate cancer *in vivo* that is accelerated in combination with *PTEN* loss. Although many genomic events accumulate with progression to CRPC, *PIK3CA* alteration offers an actionable target that ideally can be used to inform the individualization of patient treatment.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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