

PSMA brings new flavors to PI3K signaling: A role for glutamate in prostate cancer

In this issue of JEM, Kaittanis et al. (<https://doi.org/10.1084/jem.20171052>) report a new signaling role for prostate-specific membrane antigen (PSMA), providing a mechanistic link between two major oncogenic pathways, as well as promising therapeutic implications for the diagnosis and treatment of prostate cancer.

Aberrant regulation of the androgen receptor (AR) pathway is one of the main drivers of tumorigenesis in prostate cancer and has been a promising therapeutic target in recent years (Ferraldeschi et al., 2015). Tumor growth is highly dependent on androgens, as activation of AR triggers the increased transcription of genes involved in cell growth and metabolism. Prostate cancer progresses through well-defined stages, leading up to metastatic carcinoma with a significant percentage of patients developing castration-resistant prostate cancer (CRPC). CRPC is an untreatable condition that develops despite low serum androgen levels, after androgen deprivation therapy (ADT). Milestones in the treatment of CRPC have been the discovery of inhibitors of AR signaling such as enzalutamide and abiraterone acetate (Yap et al., 2011), which are currently part of the standard course of treatment. However, despite clear clinical benefits, most patients ultimately develop resistance to these drugs and relapse. Although the mechanisms behind the development of CRPC are not yet fully understood, the consensus is that canonical sources of androgens are being replaced through genetic and nongenetic mechanisms, which continue to fuel tumor growth (Rodon et al., 2013). One of the nongenetic pathways involved in the development of CRPC is the phosphoinositide 3-kinase (PI3K) pathway, which is commonly deregulated in various human cancers. The PI3K–AKT–mTOR axis is abnormally activated in 70–100% of advanced prostate cancer patients (100% of CRPC patients; Taylor et al., 2010). This constitutive activation is attributed to loss of phosphatase and tensin homologue (PTEN), which has

been shown to play an important role in the development of AR-independent metastatic carcinoma (Wang et al., 2003). The tumor enhancing activity of PI3K in a PTEN-deficient background seems to be dependent on its p110 β catalytic isoform, rather than the p110 α isoform, more commonly mutated in human cancers (Jia et al., 2008). A solid body of evidence supports the establishment of a reciprocal feedback loop between the AR signaling pathway and the PI3K axis, which explains, at least in part, the development of CRPC and resistance to various therapeutic agents targeting these pathways. In this model, inhibition of PI3K in a PTEN-deficient background activates AR signaling, and vice-versa, inhibiting AR signaling activates PI3K-dependent AKT phosphorylation (Carver et al., 2011; Mulholland et al., 2011). This reciprocal negative feedback loop between AR and PI3K signaling remains a major challenge for future therapies targeting prostate cancer. In this current issue, Kaittanis et al. discover a new avenue to further our understanding of the mechanisms behind the AR-PI3K dynamics and the development of CRPC, consequently identifying a novel targetable oncogenic signaling cascade.

Prostate-specific membrane antigen (PSMA) has become a popular target for developing new diagnosis tools designed to improve stratification of patients for targeted personalized therapeutic regimens (Pillai et al., 2016). PSMA is moderately expressed in several tissues, including healthy prostate tissue; however, it is greatly up-regulated in prostate cancer (Israeli et al., 1994). PSMA has two types of catalytic activities: NAALDase and folate hydrolase,



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both resulting in the release of glutamate from the enzyme substrates. Its capacity to release glutamate from *N*-acetyl-L-aspartyl-glutamate (NAAG) is being explored for its therapeutic potential for brain ischemic injury and several neurodegenerative disorders. Kaittanis et al. (2018) investigate the folate hydrolase activity of PSMA in prostate cancer, its biological function (uncharted thus far), and, most importantly, its potential as a therapeutic target (see figure).

Kaittanis et al. (2018) observe a strong positive correlation between PSMA expression, disease aggressiveness, and phosphorylation of the AKT target in prostate tumor tissue from patients with localized disease. Based on this evidence, they hypothesized a significant role for PSMA in modulating signaling pathways implicated in the pathogenesis of prostate cancer, specifically the PI3K–AKT–mTOR pathway. This hypothesis is examined in detail in vitro through genetic and pharmacologic manipulation of expression and enzymatic activity of PSMA, using two different prostate cancer cell lines (LNCaP and PC3) that differ in their expression of PSMA. Taking advantage of these systems, a series of complementary experiments demonstrated PSMA-dependent activation of AKT

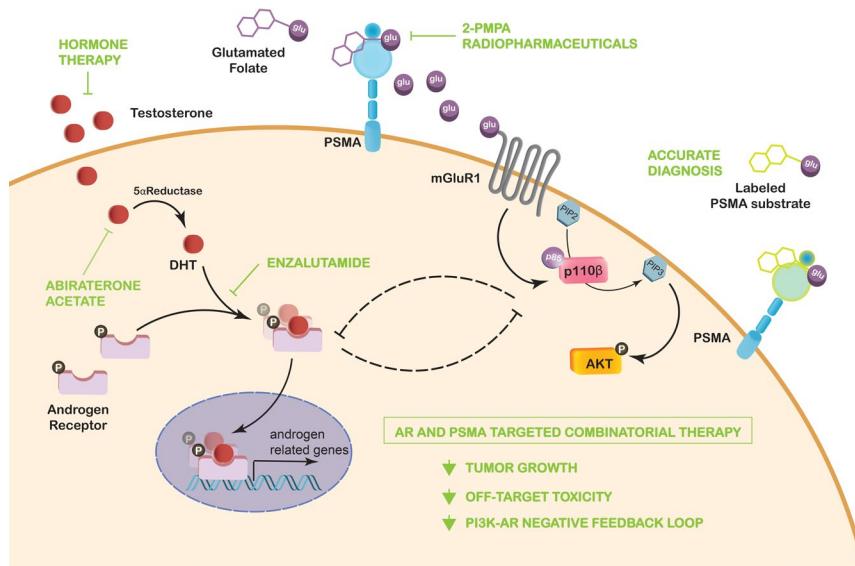
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and subsequent increased phosphorylation of downstream targets, 4EBP1 and S6, in the absence of any known intrinsic signaling properties (Kaittanis et al., 2018). Importantly, PSMA induces AKT signaling through its enzymatic activity and subsequent glutamate release. In fact, glutamate alone is shown to activate AKT signaling. Notably, the modulatory action of PSMA on this signaling pathway is dependent on the presence of an enzymatic substrate (ea. vitamin B9), which can be abolished by 2-PMPA, a known inhibitor of PSMA. Interestingly, Kaittanis et al. (2018) show that PSMA activates PI3K signaling through phosphorylation of p110 β , independent of PTEN status. Furthermore, PSMA expression in samples from 76 patients showed a strong correlation with AKT, but no correlation with PTEN expression was observed (Kaittanis et al., 2018).

Kaittanis et al. (2018) further explore the molecular mechanisms behind PI3K modulation after PSMA activation, uncovering that PSMA colocalizes with and activates the metabotropic glu-

tamate receptor group 1 (mGluR1). In the absence of mGluR1, neither PSMA activation nor glutamate supplementation was sufficient to induce p110 β phosphorylation and activate AKT. Although previously suggested that the p110 β isoform is activated by a GPCR (Cizmecioglu et al., 2016), it had not been identified until now. Furthermore, Kaittanis et al. (2018) demonstrate that inhibiting PSMA, mGluR1, or p110 β equally suppresses AKT signaling. Their data establish a direct relationship between the AR pathway and PSMA enzymatic activity. Moreover, the authors show that 2-PMPA reduces tumor growth of PSMA-expressing xenografts in mice. However, a more marked effect was observed when using the AR inhibitor enzalutamide in combination with inhibition of PSMA. This is in line with previous reports (Carver et al., 2011) that a combinatorial approach with PI3K and AR inhibitors could potentially be more efficient than monotherapies because of the reciprocal regulation between these two major oncogenic pathways.



PSMA: A versatile tool for prostate cancer therapy. PSMA is expressed with high specificity at the membrane of prostate cancer cells. Through its unique position and enzymatic function, it constitutes a notable target for radiolabeling. Because of its strict correlation with AKT expression, it could prove to be the ideal tool for diagnosis and patient stratification. Moreover, targeting PSMA inhibits PI3K signaling in prostate cancer cells; thus, combinatorial approaches with androgen pathway inhibitors and PSMA inhibitors could lead to a powerful therapeutic tool, overcoming the off-target toxic effects associated with other therapies, such as PI3K inhibitors. Combining these two applications may pave the way toward innovative PSMA-targeted theranostic approaches.

The work presented by Kaittanis et al. (2018) enhances our understanding of the mechanism behind the activation of the PI3K pathway in prostate cancer, by uncovering PSMA as a modulator of this major driver of tumor growth transition to CRPC. Likewise, they provide evidence supporting a novel role for glutamate as a signaling molecule, linking mGluR1 to activation of the p110 β isoform of PI3K (see figure). Metabolizing glutamate through the activation of PSMA offers cancer cells an advantage by activating the PI3K pathway and establishing a negative regulatory loop between the PI3K and AR pathways. The interplay between PSMA and the androgen axis begs to be explored further. It was previously shown that androgens inhibit PSMA expression (Meller et al., 2015), whereas inhibition of PSMA is shown here to activate the AR pathway, corroborating the previously described AR-PI3K interaction. Additionally, AR has linked PI3K activation to PTEN inhibition; however, Kaittanis et al. (2018) demonstrate that PSMA's function in prostate cancer is independent of PTEN. It is possible that two different mechanisms maintain the interplay between AR and PI3K and therefore enhance resistance to therapy and promote the transition to CRPC. Further understanding of the mechanisms that drive the exacerbated activation of PSMA is necessary to better evaluate its role in the development of the PI3K-AR regulatory loop and its therapeutic potential. To date, there are more than 50 drugs targeting the PI3K-AKT-mTOR pathway being exploited in different cancer contexts, including prostate cancer. Although there are clear benefits associated with inhibiting PI3K in the treatment of prostate cancer, the effects of PI3K inhibitors as monotherapy have been disappointing. However, combinatorial therapies with AR inhibitors seem to have better efficacy. In this context, because PSMA is highly expressed in prostate cancer, it may prove to be a more specific target, eliminating some of the undesired off-target effects of PI3K inhibitors.

A promising way of capitalizing on the advantages offered by a target such as PSMA is through the development of more efficient theranostic tools. Because of its low expression in normal cells, compared with cancer cells, and mostly because of its localization at the cell surface, PSMA is ideal for immune radiolabeling, or radiolabeling through pharmaceutical inhibitors, overcoming some of the disadvantages of previous similar targets (Evans et al., 2016). Because of its strong correlation with AKT expression and metastasis shown here by Kaittanis et al. (2018), PSMA offers improved diagnostic accuracy over other documented biomarkers. To date, efforts to develop such tools have brought several candidate molecules into clinical trials, which are showing promising results. Elucidating the biology and mechanisms

of action of PSMA will catalyze these efforts and lead the way to improved patient care. The findings of Kaittanis et al. (2018) are a significant step forward as they link two major pathways involved in oncogenesis, resistance to therapy, and transition to the untreatable stage of prostate cancer.

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