Defining the spectrum of resistance to androgen ablation therapy in prostate cancer

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Background. Despite many significant advances in the treatment of prostate cancer (PCA), this disease is still a leading cause of cancer death among men. PCA cells require androgen receptor (AR) signaling for their growth and survival. Indeed, androgen deprivation therapy (ADT) has remained the main therapeutic option for patients with advanced PCA for about 70 years. However, the major cause of death in men with metastatic prostate cancer involves progression to castration-resistant prostate cancer (CRPC), which ultimately leads to development of metastasis in 50% to 70% of patients. The current treatments for PRPC have demonstrated only limited efficacy, making CRPC a formidable medical challenge.

Methods. Characterizing mechanisms of resistance to ADT could enable the development of more effective therapeutic strategies. To address this need, we took a systematic and unbiased approach to identify mechanisms of resistance. Using the androgen-sensitive LNCaP cells, we performed a systematic genome-wide suppressor small-hairpin RNA (shRNA) screen. Cells were infected with a 98,000-shRNA lentiviral library, targeting approximately 16,000 human genes, and cultured in absence of androgens for several weeks. Cells showing the ability to proliferate upon androgen deprivation were harvested at different time points and shRNA sequences were identified and quantitated through next-gen sequencing. Hit genes were those with at least 2 shRNAs enriched >4 fold, and whose silencing exerted the most robust effects on cell proliferation.

Results. In vitro validation experiments identified several genes whose silencing allows LNCaP cells to proliferate in the absence of androgen. Among these, the top hit of the RNAi screen, the membrane-associated type I inositol-1,4,5-trisphosphate (InsP3) 5-phosphatase (INPP5A), was also found to be significantly deleted in CRPC patients. In accordance, in vivo validation experiments showed that knockdown of INPP5A promoted LNCaP xenografts growth in castrated mice. Interestingly, while INPP5A silencing results in re-activation of the androgen receptor signaling in these cells, we found that the AR inhibitor enzalutamide only partially prevented the growth of LNCaP cells that harbor INPP5A knockdown under androgen deprived conditions.

Conclusions. INPP5A loss may confer resistance to ADT through a combined AR-dependent and AR-independent mechanism. Characterizing the mechanisms through which INPP5A loss promotes ADT resistance may uncover new biological insights and therapeutic avenues.

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