Investigating DNAPK as a therapeutic target and a prognostic biomarker in castration-resistant prostate cancer.

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Background: Castration-resistant prostate cancer (CRPC) remains a leading cause of cancer related deaths in men worldwide despite paradigm shifts in the therapeutic advances. Thus, identification of biomarkers of aggressive disease early in the patient course to facilitate the desired treatment intensification; and identification of more effective therapeutic targets to reduce the mortality resulting from CRPC, are two critical needs in the management of this disease. The objective of this study is to address both of these needs.

Methods: We analyzed the expression of all known kinases in 545 prostatectomy samples, obtained from high-risk patients with long term (>10 years) clinical follow up, as kinases represent the most actionable class of therapeutic molecules. We ranked the kinases by their fold change in expression between prostate cancers (PCas) that subsequently metastasized vs those that did not. High-throughput as well as standard molecular and biochemical assays were used to carry out mechanistic, phenotype and *in vivo* studies.

Results: By systematically exploring the prognostic relevance of all known kinases in a large cohort of high-risk prostate cancer samples with a long term (<10 years) clinical follow up, we identified DNA dependent protein kinase (DNAPK) as the top kinase associated with metastatic progression in patients treated with prostatectomy. High expression of DNAPK is associated with poor clinical outcomes even when we stratified patiens into basal (less AR-driven) and luminal (more AR-driven) subtypes. Intriguingly, a combined GSEA analysis using expression data from patient samples and cell line models revealed that DNAPK regulates the Wnt signaling pathway. Further validation demonstrated that Wnt signaling is induced by castration (ADT) in cell line models and drives castration resistance, which is reversed after DNAPK inhibition. We show that DNAPK inhibits Wnt-induced cancer phenotypes in CRPC as well as enzalutamide-resistant cells. We show that DNAPK regulates Wnt signaling by its interaction with and transcriptional regulation of LEF-1. Pharmacologic inhibition of DNAPK by a laboratory-grade inhibitor NU7441 or clinical grade DNAPK inhibitor CC-115 significantly reduced the VCaP (AR-dependent), LNCaP-AR (CRPC), and PC3

(AR-independent) xenograft growth *in vivo*, indicating the therapeutic potential of DNAPK inhibitors in all the three spectrums of otherwise lethal aggressive prostate cancer.

Conclusions: Investigating DNAPK as both a biomarker for treatment intensification and a therapeutic target may allow for personalization of therapy for CRPC patients. DNAPK may promote aggressive prostate cancer via uprgulation of Wnt signaling. This provides rationale for investigating DNAPK as both a biomarker and a therapeutic target in aggressive prostate cancer.

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