Susceptibility-associated genetic variation in *NEDD9* contributes to prostate cancer progression

Background: In the US, African American (AA) patients show higher prostate cancer (PCa) incidents and worse treatment outcomes in comparison to European Americans (EA). This PCa disparity is largely contributed by socioeconomic factors, but race-associated genetic variations may also play a role. From GWAS analyses, we identified a panel of PCa risk-associated single nucleotide polymorphisms (SNPs) with significantly different allele frequencies in AA versus EA. The top-ranked SNP, rs4713266, is located at an intronic region of the *NEDD9* gene and the risk allele frequency is significantly higher in AA. *NEDD9* encodes for a focal adhesion protein that is phosphorylated by FAK and Src and acts as a signaling hub to regulate multiple downstream pathways. Clinically, *NEDD9* amplification was found in castration-resistant PCa (~3%) and neuroendocrine PCa (~15%). The chromatin region containing this SNP is highly enriched for enhancer marks, indicating it may contain a putative enhancer (we named it NEDD9-Int1Enh) to drive *NEDD9* transcription.

Methods: To determine the role of rs4713266 in regulating *NEDD9* expression, we performed: (1) CRISPRa to determine if NEDD9-Int1Enh mediates *NEDD9* transcription; (2) reporter assays to determine if the nucleotide variation of this SNP can alter binding of specific transcription factors; (3) CRISPR editing to modify the nucleotide at the SNP to determine the effects on *NEDD9* expression. To determine NEDD9 function in PCa, we silenced *NEDD9* in VCaP cells and then assessed its effects on the global gene profile using RNA-seq and on PCa tumor growth and metastasis using *in vivo* and *in vitro* models, such as zebrafish embryo injection.

Results: We found that forced activation of NEDD9-Int1Enh can increase *NEDD9* expression and modifying the nucleotide of the SNP altered *NEDD9* expression by interacting with distinct transcription factors such as ERG (non-risk) and NANOG (risk). Moreover, RNA-seq results revealed that NEDD9 may promote several oncogenic pathways, including epithelial-mesenchymal transition (EMT), JAK/STAT3, and KRAS signaling pathways. Indeed, we show that *NEDD9* silencing decreased PCa tumor growth and metastasis *in vitro* and *in vivo*.

Conclusion: Our study indicates a critical role of rs4713266 on modulating *NEDD9* expression in PCa of different racial subgroups. We also demonstrate strong oncogenic activities of NEDD9 in promoting PCa progression and metastasis. Together, our study provides novel insights into genetic mechanisms driving PCa racial disparities.