

Targeting Wnt5a/FZD2 signaling overcomes resistance to enzalutamide in advanced CRPC

Shu Ning, Cameron M Armstrong, Chengfei Liu, Wei Lou, Alan P Lombard, Leandro S D'Abronzio, Jinge Zhao, Neelu Batra, Aiming Yu, Allen C Gao*
Department of Urology · University of California Davis

Background Androgen receptor (AR) blockade using antiandrogens is a mainstay for the treatment of castration resistant prostate cancer (CRPC). Unfortunately, drug resistance occurs frequently due to mechanisms that are not completely understood. Wnt5a, a representative ligand of non-canonical Wnt signaling, is expressed in circulating tumor cells from CRPC patients treated with enzalutamide. FZD2, the cognate frizzled receptor for Wnt5a, is the most commonly co-upregulated non-canonical Wnt signaling molecules in prostate cancer. Here we determine the contribution of non-canonical Wnt5a/FZD2 to enzalutamide treatment resistance, and explore the potential of targeting Wnt5a/FZD2 to overcome antiandrogen resistance in castration resistant prostate cancer.

Methods Wnt5a/FZD2 expression was examined in enzalutamide resistant C4-2B cells (C4-2B MDVR). Wnt5a and FZD2 expression were modulated using specific siRNAs. Cell growth, colony formation, and migration were studied *in vitro*. RNA sequencing analysis was performed on C4-2B MDVR cells with FZD2 knocked down; gene expression of non-canonical Wnt signaling, AR activity and AR-V7 related genes were analyzed. A novel RNA bioengineered Wnt5a siRNA (tRNA-siWnt5A) was developed to target Wnt5a/FZD2 signaling. The effect of tRNA-siWnt5a on tumor growth and sensitivity to enzalutamide treatment was evaluated *in vitro* and *in vivo*.

Results Wnt5a and FZD2 are highly co-upregulated in castration resistant prostate cancer patients. Wnt5a and FZD2 are overexpressed in enzalutamide resistant C4-2B MDVR cells compared to parental C4-2B cells. Knocking down FZD2 abrogates the increase of full-length AR and AR variant expression and diminishes the enrichment of genes involved in the non-canonical Wnt signaling pathway. Blocking FZD2 using specific siRNAs suppresses prostate cancer cell growth, colony formation, and migration. FZD2 knockdown with siRNA resensitized C4-2B MDVR cells to enzalutamide treatment. Down regulation of Wnt5a using the bioengineered tRNA-siWnt5A inhibited the growth of enzalutamide resistant prostate cancer cells and resensitized cells to enzalutamide treatment *in vitro*, and resistant CRPC PDX tumor growth *in vivo*.

Conclusions Our studies suggest that Wnt5a/FZD2 confers enzalutamide resistance and prostate cancer survival and proliferation. Targeting the non-canonical Wnt5a/FZD2 pathway could provide benefit for CRPC patients with tumors expressing high level of Wnt5a and FZD2, not only overcoming resistance but potentiating anti-tumor effects of enzalutamide in CRPC patients.