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**Figure 1.** Fzd2 and Wnt5a gene expression analysis using prostate cancer patient, Neuroendocrine Prostate Cancer (Mult-Institute, Nat Med 2016), Metastatic Prostate Adenocarcinoma (SU2C/PDFF Dream Team, PNAS 2019), Prostate Adenocarcinoma (Bread/Cornell, Nat Genet 2012). The Metastatic Prostate Cancer Project (Provisional, November 2019), Prostate Cancer (MDZ, Cancer Cell 2018). a) Fzd2 and Wnt5a were co-upregulated due to gene amplification, specifically for 27.27% (12 out of 44 cases) of prostate neuroendocrine carcinomas and 24.29% (17 out of 70 cases) of castration-resistant prostate cancer. b) Wnt5a is amplified in 23.89% (23/75) cases CRPC patients and 20.45% (9/44 cases) of NEPC (Prostate Neuroendocrine Carcinoma) patients. Fzd2 is amplified in 14.29% (10/70 cases) of NEPC database.

**Figure 2.** Downregulating Fzd2 decreases non-canonical Wnt and AR signaling. a) RNA of C4-28 parental and MDVR cells were collected and sent for RNA sequencing. Enrichment plot and heatmap depicting the non-canonical Wnt signaling and alterations in leading edge genes. MDVR cells were treated with Fzd2 siRNA for 3-5 days. GSEA enrichment plots (FDR) and heatmap (GSI) depicting the alteration of non-canonical Wnt and AR signaling pathway, AR-V7 signature genes (F) and cancer cell survival proliferation/proliferation related genes.

**Figure 3.** a) C4-28 MDVR cells were transiently transfected with 10nM and 20nM Fzd2 siRNAs and clonogenic assays and b) wound healing assay were performed. Images of the wound healing were taken at time 0 and 48 hours.

**Downregulating Fzd2 restores sensitivity to enzalutamide**

**Figure 4.** a) C4-2B parental and MDVR transiently transfected with control or Fzd2 siRNAs were subjected to Western blotting. b) C4-2B cells were transiently transfected with Fzd2 siRNA for 48 hours, total RNA was extracted and Fzd2 mRNA levels were analyzed by qRT-PCR. C) C4-2B MDVR cells were transiently transfected with Fzd2 siRNAs with or without 10μM enzalutamide. Cell proliferation was evaluated by CCK8 assay on the day 3, *p<0.05.

**Figure 5.** RNAi-siWnt5a inhibited CRPC PDx tumor growth.

**References**

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**Abstract**

Androgen receptor (AR) blockade using androgenics 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 stimulating 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 CRPC patients. Here, we determined the contribution of non-canonical Wnt5a/Fzd2 to enzalutamide treatment resistance, and explore the potential of targeting Wnt5a/Fzd2 to overcome androgen resistance in CRPC.

**Methods**

Wnt5a/Fzd2 expression was examined in enzalutamide resistant C4-28 CRPC cells. 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 RNAi engineered Wnt5a siRNA (RNAi-siWnt5a) was developed to target Wnt5a/Fzd2 signaling. The effect of RNAi-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 reconstituted C4-2B MDVR cells to enzalutamide treatment. Down regulation of Wnt5a using the RNAi engineered RNAi-siWnt5a inhibited the growth of enzalutamide resistant prostate cancer cells and restored 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.

**Introduction**

Wnt pathway has emerged from the studies showing a contributing role in tumorigenesis, progression and metastasis in various cancers. Non-canonical Wnt correlates with aggressiveness and malignancies in melanoma, breast cancer, lung cancer, gastric cancer and prostate cancer. Wnt5a is a representative ligand that activates Wnt-independent pathway in non-canonical Wnt signaling (Takahashi et al., 2011). The oncogenic role of Wnt5a was discovered in single circulating tumor cells from CRPC patients treated with enzalutamide, its upregulation correlating with high Gleason score andrelapse of prostate cancer. Wnt5a mediates non-canonical Wnt pathway by binding to its cognate receptor Frizzled-2 (Fzd2) which was reported to be closely associated with metastasis in late-stage and metastatic liver, breast, colon and lung cancer. In prostate cancer, Wnt5a/Fzd2 is the most common mode of non-canonical Wnt pathway detected in prostate cancer with EMT markers and higher Gleason score (Sandmark et al., 2017).