MAP3K11 promotes enzalutamide resistance in castration-resistant prostate cancer *in vitro*

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Background: Castration-resistant prostate cancer (CRPC) patient tumors can be treated with androgen receptor (AR) pathway antagonists such as enzalutamide that target AR signaling and transcriptional activity. CRPC patient tumors inevitably gain enzalutamide resistance. Our lab previously identified and validated *MAP3K11* as a putative driver of resistance in a short hairpin RNA screen. MAP3K11, also known as mixed lineage kinase 3 (MLK3), has been shown to be involved in multiple signaling pathways including the JNK, ERK, and p38 MAPK pathways. MAP3K11 has also been implicated in regulating AR activity via signaling cascades related to changes in phosphorylation of Serine 650 (AR-Ser650). The purpose of this study was to investigate the mechanism of MAP3K11 in CRPC to promote enzalutamide resistance.

Methods: To investigate the mechanism of MAP3K11 in enzalutamide resistance, we utilized enzalutamide sensitive (LNCaP, C4-2B) and enzalutamide resistant cells (22Rv1, CWR-R1-EnzR, C4-2B MDVR, PC-3, DU145). We used Western blotting to measure changes in MAP3K11, AR, and phospho-AR-Ser650. We furthermore investigated activation of JNK, p38, and ERK via Western blot. Finally, we performed cell survival assays using crystal violet to measure cell survival in response to MAP3K11 KD or inhibition with the inhibitor CEP-1347 in cells treated with DMSO or enzalutamide.

Results: Our studies show MAP3K11 KD or treatment with CEP-1347 decreased cell survival in CRPC and enzalutamide-resistant cell lines. Combination of enzalutamide with MAP3K11 inhibition further decreased cell survival. MAP3K11 KD and CEP-1347 treatment decreased phosphorylation of AR-Ser650 with no change in AR protein expression. To understand the cellular effects of phosphorylated AR-Ser650, we show phospho-AR-Ser650 localizes to the nucleus and is absent in the cytoplasm. We furthermore observed an increase in MAP3K11 expression and activation of JNK, p38, and ERK in enzalutamide-resistant compared to enzalutamide-sensitive cells.

Conclusions: This data suggests MAP3K11 plays a role in CRPC cell line survival and in enzalutamide-resistance mechanisms. We found MAP3K11 acts through the related JNK, p38, and ERK pathways in CRPC cells and effects AR localization via phosphorylation of AR-Ser650. Importantly, this data also shows MAP3K11 inhibition with CEP-1347 can sensitize cells to enzalutamide treatment. Future studies will further determine the mechanism of MAP3K11 in enzalutamide resistance, and evaluate MAP3K11 inhibition in pre-clinical models of CRPC.