**TROP2 regulates prostate cancer growth and metastasis through distinct molecular mechanisms**

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**Background:** Prostate cancer is the most common non-cutaneous cancer and second leading cause of cancer related deaths in men in the United States. The first line of treatment for men with advanced prostate cancer is androgen deprivation therapy. Although initial responses are observed, prostate cancer commonly relapses in its lethal metastatic form referred to as castration resistant prostate cancer (CRPC) with 1-2 years mean survival time. Neuroendocrine prostate cancer (NEPC) is highly aggressive, AR independent subtype and usually emerges post castration resistance. We recently identified that the cell surface receptor TROP2, is a new driver of NEPC. Moreover, we demonstrated that TROP2 regulates prostate cancer growth and metastasis. In this study, we set out to delineate the molecular mechanisms through which TROP2 regulates prostate cancer growth and metastasis.

**Methods:** Proximity-dependent Biotin Identification (BioID) was performed by expressing TROP2-Biotin ligase fusion protein in prostate cancer cell lines to identify TROP2 interactome with biotinylated labels utilizing pull-down methods following with mass spectrometry. Lentiviral infection was used to generate prostate cancer cell lines with over-expression of TROP2 and knock-down of NOTCH1, SLC4A7, PLEC, and OCLN shRNA to modulate gene expression levels. *In vitro* functional assays were performed including colony formation, and Matrigel drop 3D cell invasion assays.

**Results:** The TROP2 membrane interactome was identified utilizing Proximity-dependent Biotin Identification (BioID) in living cells and uncovered that TROP2-mediated prostate cancer growth and metastasis are orchestrated by distinct downstream pathways including Notch signaling (NOTCH1), control of intracellular pH (SLC4A7), exosome secretion (PLEC), and tight junctions (OCLN). Interaction of TROP2 binding partners including NOTCH1, SLC4A7, PLEC and OCLN with TROP2 were further validated by fluorescence resonance energy transfer (FRET) using confocal microscopy. Moreover, knocking down the TROP2 interacting partners in TROP2 over-expressing prostate cancer cells suppressed TROP2-driven growth and invasion ability.

**Conclusions:** In a previous study, we identified cell surface receptor, TROP2, as a novel driver of metastatic NEPC. Herein, our new findings reveal that TROP2 interacts with NOTCH1, SLC4A7, PLEC, and OCLN, which may highlight novel biological functions of TROP2 in prostate tumorigenesis and provide new understanding of the potential mechanism of neuroendocrine differentiation and metastasis to develop new therapeutic strategy for metastatic CRPC with neuroendocrine features.