Loss of Notch signaling facilitates neuroendocrine differentiation in advanced prostate cancer

Sheng-Yu Ku[1], Loredana Puca[2], Spencer Rosario[3], Sylvan Baca[1], Yanqing Wang[3], Aram Vosoughi[2], Matthew Freedman[1], David W. Goodrich[3], Himisha Beltran[1]
[1]Department of Medical Oncology, Dana Farber Cancer Institute, Boston, MA, USA; [2]Department of Medicine, Weill Cornell Medicine, New York, NY, USA; [3]Department of Pharmacology & Therapeutics, Roswell Park Comprehensive Cancer Center, Buffalo, NY, USA

Background: One emerging mechanism of acquired resistance to androgen receptor (AR) targeted therapies in prostate cancer is evolution from an AR-driven luminal prostate cancer to an AR-low or AR-negative neuroendocrine prostate cancer (NEPC) phenotype. This lineage plasticity is associated with loss of *RB1* and *TP53* tumor suppressor genes and expression of neuroendocrine and neuronal lineage markers including Achaete-scute homolog 1 (ASCL1) and Delta-like 3 (DLL3). As ASCL1 and DLL3 function to suppress Notch signaling, we sought to further investigate the role of Notch signaling in NEPC.

Methods: We evaluated the expression of Notch signaling genes by RNA-seq in a cohort of 735 samples (423 patients) including benign prostate (n= 173) localized prostate cancer (PCa, n=324), castration resistant prostate adenocarcinoma (CRPC-Adeno, n=127), and NEPC (n=111), patient-derived organoid models (n=6), and mouse models of NEPC (Ku et al, Science 2017), and generated a scoring method to calculate Notch activity. We performed immunohistochemistry (IHC) to confirm protein expression in select cases. We conducted ChIP-seq analysis of $H3K4me_3$ and $H3K27me_3$ on Notch related genes using LuCaP PDXs. We introduced the active form of either NOTCH1 or NOTCH2 in human NEPC (Puca et al, Nature Comm 2018) and (*Pten*|*Rb1*|*Trp53*)$^{-/-}$ prostate cancer organoids. We treated (*Pten*|*Rb1*|*Trp53*)$^{-/-}$ transgenic mice with resveratrol, a dietary supplement that activates Notch.

Results: Gene expression profiles of human samples, patient-derived organoid models, and mouse NEPC showed significant downregulation of NOTCH1, NOTCH2, and HES1 compared with benign, PCa as well as CRPC-Adeno. Notch signaling score was also significantly lower in NEPC and negatively correlated with higher NEPC score. IHC confirmed low Ar, Krt8, Notch1/2, and Hes1 protein expression in NEPC together with expression of neuroendocrine markers Syp and Ascl1. ChIP-seq analysis of LuCaP PDXs demonstrated high H3K27me3 and low H3K4me3 on Notch related genes in NEPC models but not adenocarcinoma PDXs, suggesting a possible mechanism of Notch signaling downregulation. Ectopic expression of NOTCH1/2 intracellular domain (NICD1/2) reduced RNA and protein expression of neuroendocrine markers, such as SYP as well as CHGA, and impaired cell growth in human NEPC organoids. In addition, NICD1/2-expressing (*Pten*|*Rb1*|*Trp53*)$^{-/-}$ organoids reversed a neuroendocrine phenotype towards a luminal phenotype in vivo. Importantly, AR expression was also rescued. Resveratrol treatment of (*Pten*|*Rb1*|*Trp53*)$^{-/-}$ transgenic mice demonstrated switching from an AR-low NEPC phenotype towards an AR-positive luminal phenotype along with restoration of Notch signaling.

Conclusions: These data demonstrate that downregulation of Notch signaling is associated with the NEPC phenotype in prostate cancer. Molecular or pharmacological Notch reactivation modulates NEPC and AR signaling and may potentially restore sensitivity to AR-targeted therapies. These findings underscore a potential reversible mechanism underlying acquired resistance to androgen deprivation therapy and open the door to new biomarker and therapeutic strategies.

Funding Acknowledgements: PCF YIA (SK, LP), R01CA207757 (DWG, Multi-PI), R21CA179907 (DWG), R01CA70292 (DWG), DOD PC160264 (HB)