

Loss of Notch signaling facilitates neuroendocrine differentiation in advanced prostate cancer

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Background: One emerging mechanism of acquired resistance to androgen receptor (AR) targeted therapies in prostate cancer is evolution towards an AR-low or AR-negative state. This may be associated with clinical and molecular features of small cell /neuroendocrine carcinoma including loss of the *RB1* and *TP53* tumor suppressor genes and expression of neuroendocrine/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 benign prostate (n=132) localized prostate cancer (PCa, n=254), castration resistant prostate adenocarcinoma (CRPC-Adeno, n=90), and NEPC (n=76) patient cohorts, patient-derived models, and mouse models of NEPC (Ku et al, Science 2017); and performed immunohistochemistry (IHC) for the validation. 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 found in the skins of grapes known to activate Notch.

Results: Gene expression profiles of human samples, patient-derived models, and mouse NEPC showed significant downregulation of NOTCH1, NOTCH2, and HES1 compared with benign, PCa as well as CRPC-Adeno. IHC confirmed low AR, Krt8, Notch1/2, and Hes1 in NEPC together with upregulation of neuroendocrine markers Syp and Ascl1. Ectopic expression of NOTCH1/2 intracellular domain (NICD1/2) induced lethality 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. To further translate these findings, we treated (*Pten/Rb1/Trp53*)^{-/-} transgenic mice with resveratrol. Our data demonstrated that resveratrol was also capable of switching an AR-low NEPC phenotype towards an AR-positive luminal phenotype by restoring Notch signaling and inhibiting neuroendocrine differentiation.

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 mechanism underlying acquired resistance to ADT and open the door to new therapeutic opportunities for treating an aggressive, non-AR driven variant of prostate cancer.

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