Targeting RET Kinase in Neuroendocrine Prostate Cancer

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Background: The advent of second generation androgen deprivation therapies have provided much needed life-extending treatments for metastatic castration resistant prostate cancer (mCRPC) patients. However, the implementation of these novel therapies has created a shift in the molecular characteristics of recurrent tumors, with a greater incidence of tumors that have lost androgen receptor (AR) signaling (collectively termed aggressive variant prostate cancers or AVPC). AVPC most commonly evolves from pre-existing prostate adenocarcinoma (AdCa), may be present in up to 35% of patients with mCRPC, and treatment provides modest 1-2 year survival rates due to rapid therapy resistance and disease progression. AVPC tumors may also gain neuroendocrine markers creating a distinct subclass of AVPC known as neuroendocrine prostate cancer (NEPC). However, the mechanisms related to the increase in the neuroendocrine (NE) cell component after hormonal therapy is unclear although it is postulated that AR splice variants or bypass kinase pathways may contribute to this rapidly progressing AVPC phenotype. Of specific interest to us, is the RET tyrosine kinase. RET was originally observed to be expressed in neuronal cell types and activating mutations of RET is observed in various NE tumors suggesting this kinase may be an important driver of the AVPC phenotype.

Methods: To understand how these AR negative AVPCs differ from their AR positive counterparts, we analyzed and compared the phosphoproteome of AdCa and AVPC cell lines to identify altered kinase activity between the two groups. We then utilized mRNA transcript data from multiple studies of patients with AVPC to determine if RET identified from our phosphoproteome screen was upregulated in NEPC patient tumors. We then used AD80 to pharmacologically inhibit RET kinase in NEPC tumor models including cell line models, ex-vivo cultured *PTEN/RB1* deleted mouse organoids, and NCI-H660 xenograft tumors to determine if RET kinase contributes to the survival and aggressive phenotype of NEPC.

Results: Our results revealed distinct phospho-serine/threonine and tyrosine phosphorylation patterns between AdCa and AVPC and predicted RET activity to be elevated in the AVPC cell lines, despite the absence of RET activating mutations. Large primary tumor transcript data sets revealed that NEPC patients had higher levels of neuroendocrine markers like SYP and CHGA, and also had increased levels of RET mRNA. We found that the RET pathway inhibitor, AD80, dramatically increased cell death in cultured mouse organoids and reduced xenograft tumor growth. Interestingly, in the organoid tumor model, treatment with both enzalutamide and AD80 further increased cell death beyond AD80 treatment alone.

Conclusions: These results implicate RET as an important kinase for NEPC tumor survival and progression and suggests that targeting RET kinase may be a treatment option in patients with AVPC containing NE features.

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