P39 Drivers and therapeutic vulnerabilities in AR indifferent anti-androgen resistant prostate cancer cells

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Introduction & Objectives: Inhibition of the androgen receptor (AR) by second-generation anti-androgens is a standard treatment for castration resistant prostate cancer (CRPC), but it inevitably leads to the development of resistance. Importantly, AR independent non-neuroendocrine resistance mechanisms are on the rise since the development of efficient AR signaling inhibitors and comprise approximately 20% of CRPC patients in the clinic nowadays. However, this clinical setting remains poorly understood to date due to a lack of accessible and well-characterized models.

Materials & Methods: Two anti-androgen resistant cell lines were generated by continuous treatment of LNCaP cells with increasing concentrations of enzalutamide (at the IC50, ResA) or a fixed concentration of RD-162 (10 μM, ResB). Proliferation and apoptosis assays were performed using live cell analysis (Incucyte). High-resolution clonogenic assays were analyzed with the CATCH-colonies software (www.catch-colonies.net). Transcriptome analysis was performed with the Qlucore software for ResA and ResB cells (RNASeq) and publicly available datasets. Altered E2F1/Rb expression was measured by subcellular fractionation and western blotting.

Results: ResA and ResB cells were fully resistant to enzalutamide in xenograft experiments. In addition, they were cross-resistant against apalutamide, darolutamide, abiraterone acetate, androgen deprivation, and docetaxel (partial) in vitro. Both cell lines are AR positive and androgen responsive but the activity of the AR is very low in presence of anti-androgens and not required for their anti-androgen resistant growth (AR indifferent). ResA and ResB cells have a high clonogenic self-renewal capacity, an altered morphology/EMT signature, and are neuroendocrine negative. We performed GSEA transcriptome analysis to identify the drivers of anti-androgen resistance in these cells and found several significantly enriched cell-cycle regulator associated pathways (E2F targets, G2M checkpoint, and Myc targets). On protein level we confirmed reduced Rb ($p=0.023$) and increased E2F1 ($p=0.029$) expression in ResA and ResB cells respectively. Importantly, metastatic CRPC samples with low AR activity also had significantly increased E2F activity compared to those with high AR activity (N=41 and 108 respectively, $p<0.001$). Interestingly, both anti-androgen resistant cell lines remained vulnerable against JQ1, olaparib, and obatoclax. In addition, ResB cells were strongly repressed by supra-physiological androgen therapy despite their AR indifferent phenotype.

Conclusions: The ResA and ResB cells are highly resistant against most clinically established therapies but remain sensitive to several therapeutic options that are currently evaluated in clinical trials. The model systems developed in this project may lead to a better understanding and the identification of therapeutic vulnerabilities of AR indifferent CRPC.