Poster 38: Therapeutic Opportunities in the Master Transcription Factor Circuitries of Clear Cell Carcinomas

Nolan J. Kinne, MD– Cedars Sinai Medical Center

Topic: Ovarian

Objectives
Transcription factors are emerging as targets for novel therapeutics for the treatment of malignancies. We sought to evaluate functions of PAX8 and HNF1B in both clear cell ovarian cancer (CCOC) and clear cell renal cell carcinoma (ccRCC) to ask whether these factors control tissue-specific or shared molecular functions and to assess their candidacy for targeted therapy.

Methods
We used two prototypic human cell lines for each cell type. We determined differential expression of PAX8 and HNF1B by quantification of protein in western blots. HNF1B and PAX8 expression was depleted using siRNA and the Incucyte® Live-Cell Analysis System to monitor continuous growth of cells grown as monolayers and as three-dimensional spheroids. RNA-sequencing and CUT&RUN was performed to determine the transcriptional targets and binding sites of these factors.

Results
PAX8 was found to be more highly expressed in CCOC than ccRCC however there was no difference in expression of HNF1B. Knockdown of PAX8 caused decrease in expression of HNF1B in both cell lines but no change in expression of two other factors ETS2 and EPAS1. Knockdown of HNF1B did not affect expression of the other transcription factors tested. The knockdown of HNF1B and PAX8 caused a significant decrease in growth rate of both cell lines in monolayer culture and had heterogeneous effects on spheroid growth. Depletion of these factors resulted in varying degrees of global transcriptional repression or activation, with evidence of a hierarchy dominated by HNF1B and PAX8, and negative crosstalk between ETS2 and EPAS1. Key downstream pathways include immune signaling, PDGF signaling and autophagy.

Conclusions
PAX8 is more highly expressed in CCOC than ccRCC. PAX8, HNF1B, ETS2 and EPAS1 exhibit a strong degree of connectedness, suggesting they form a core regulatory circuit in both CCOC and ccRCC. Critically PAX8 and HNF1B are required for normal cellular proliferation in both CCOC and ccRCC in both monolayer and spheroid culture. Ongoing integrated multi-omic analyses have identified the autophagy pathway as a novel therapeutic opportunity in PAX8 and HNF1B-driven clear cell tumors.