Temporal evolution of cellular heterogeneity during the progression to advanced, AR-negative prostate cancer

Background:
The progression from castration-resistant prostate cancer (CRPC) to neuroendocrine (NE) prostate cancer (NEPC) is driven by a number of molecular events, such as the amplification and overexpression of *MYCN* (encoding the transcription factor N-Myc). In a cohort of advanced prostate cancer patients, we found an enrichment for the co-occurrence of N-Myc overexpression and the loss of the tumor suppressor *RB1*. Moreover, patients harboring both genetic events have significantly reduced overall survival. We have previously showed that N-Myc overexpression in a genetically engineered mouse model (GEMM) of prostate cancer can induce a transcriptional profile similar to what is observed in the context of RB1 loss-of-function. However, the molecular programs underlying these changes are not understood.

Methods:
We recently developed a novel GEMM with N-Myc overexpression in a Pten- and Rb1-null background that develops NE-like disease. We have extensively characterized tumor histology and metastatic spread over time. Moreover, we have used single-cell based approaches, such as scRNA-seq and scATAC-seq, in conjunction with genome-wide N-Myc ChIP-seq and DNA methylation studies, to fully characterize the development of NEPC. We have also compared the results from our GEMM to a cohort of clinical prostate cancer patients, including two scRNA-seq profiles from NEPC patient samples.

Results:
Tumors formed in the new GEMM significantly faster than animals with wild-type *Rb1*, beginning as early as 8 weeks of age. The tumors contained foci of AR-negative, poorly differentiated disease. Distant metastases were found in the lymph nodes, lungs and liver and consisted entirely of AR-negative tumor cells that were positive for NE markers. Additionally, surgical castration followed by longitudinal MRI revealed that these tumors were resistant to castration compared to control groups. Bulk RNA-seq, N-Myc ChIP-seq, and RRBS performed on regions of conventional adenocarcinoma compared to poorly differentiated tumor regions revealed an enrichment for a number of neural- and stem cell-related genes and clinically relevant changes to the methylome. To understand the heterogeneity in the developing tumors, we performed scRNA-seq and scATAC-seq on mouse prostates collected at 6 and 8 weeks of age. While a large number of cells at these early time points were N-Myc^{+} and AR^{-}, a distinct subpopulation of cells was present that were N-Myc^{+} and AR^{+} but showed low expression of AR target genes, suggesting a transition away from an AR-dependent state. Combined analysis of scRNA-seq and scATAC-seq data revealed the existence of a population of cells expressing a novel transcription factor which may be implicated in the transition to an NE-like state.

Conclusions:
We have identified that N-Myc overexpression in conjunction with Rb1 loss-of-function is enriched in NEPC patients and confers reduced overall survival. Using a novel GEMM, we have shown that N-Myc overexpression and Rb1 loss-of-function can synergize to dramatically accelerate tumorigenesis, promote castration resistance, and drive the acquisition of an NE-like state. Future studies will assess the temporal regulation of transcriptional programs following castration and reveal the mechanistic underpinnings of the development of NEPC.