Single-Cell Multi-Omics Reveals the Molecular Landscape and Heterogeneity of Metastatic Castration-Resistant Prostate Cancer

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Background: Metastatic castration-resistant prostate cancer (mCRPC) is invariably lethal and is the second- leading cause of cancer death in men. Distinct mCRPC lesions within an individual harbor extensive genomic, transcriptomic, and epigenomic heterogeneity over time and at distinct tumor sites. This complexity contributes to therapy resistance and challenges biomarker-driven selection of the next generation of antigen-directed treatments. A deeper understanding of the molecular heterogeneity of mCRPC is therefore critical for improving therapeutic strategies. Previous studies from our group and others have identified genomic and epigenomic subtypes of mCRPC using bulk sequencing approaches. These approaches lack the resolution needed to fully resolve intra-tumoral heterogeneity. Recent single-cell studies have begun to explore transcriptomic diversity in mCRPC, yet lack direct measurements of chromatin accessibility, an essential component for understanding upstream transcriptional regulation. Furthermore, many existing studies rely on cohorts with one sample per patient, limiting insights into intra-patient heterogeneity.

Approach: We performed single-cell multi-omics profiling using 10x Genomics technology on a prospectively banked cohort of 52 autopsy samples of mCRPC selected from 23 patients. This allowed us to simultaneously measure transcriptomes and chromatin accessibility at single-cell resolution, allowing for an integrated analysis of transcriptional and epigenomic landscapes. Sample selection was guided by immunohistochemistry to balanced sample selection between all subtypes of mCRPC. After quality control we analyzed 280,000 cells using gene module analysis.

Results: Approximately 90% of high-quality cells were identified as epithelial tumor cells using a combination of transcription and copy number analysis. Our integrative analysis of these cells identified co-expressed gene modules—termed expression meta-programs—that reflect diverse biological processes, including androgen signaling, neuroendocrine differentiation, responses to genomic alterations (e.g., *MYC* amplification), and environmental stressors (e.g., hypoxia and unfolded protein response). By integrating inferred copy number alterations, chromatin accessibility, and gene expression data, we

identified candidate genomic alterations and transcription factors that may regulate these distinct transcriptional programs. Analysis of intra-sample and inter-sample copy variation within individual patients identified subclonal somatic profiles linked to transcriptional variation.

Conclusion: Together, our findings underscore the molecular complexity of mCRPC, shaped by both genomic and epigenomic alterations. Further computational analyses and experimental validation are ongoing to delineate the regulatory networks uncovered in this study and to identify novel therapeutic opportunities for patients with mCRPC. Moreover, this dataset will represent a valuable resource for the prostate cancer research community.

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