Unraveling molecular heterogeneities in advanced prostate cancer at single cell resolution

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Body:

Cellular heterogeneity adversely affects clinical stratification, treatment decisions and development of therapeutic resistance in cancer. Heterogeneity typically manifests as variability in gene expression and scales with the number of unique cell types and/or the extent of phenotypic plasticity. Moreover, recent reports have shown that rare, "pre-resistant" cells within a genetically homogeneous population of cancer cells can mediate drug resistance. Therefore, an incisive tool that effectively quantifies heterogeneity, robustly identifies rare cell populations and efficiently predicts cell state transitions, in addition to preserving the associated phenotypic manifestations will provide key insights into mechanisms of plasticity and development of drug resistance. To this end, we developed High-Throughput Single-cell analysis using single-molecule Fluorescence *In Situ* Hybridization (HITS-FISH) – a completely automated imaging-based tool that provides absolute quantification of gene expression (mature and immature transcripts that are coding or non-coding), while still preserving spatial and morphological information. Using a combination of HITS-FISH and high-throughput readout of gene expression signatures by single cell RNAseq (scRNAseq) we find that multiple, potentially plastic, cell states, some mediated by chromosomal instability, coexist within a seemingly homogeneous population of prostate cancer (PCa) cells. Within this heterogeneous cell mixture, we further found a rare population of untreated PCa cells that were enriched for HOX and HES transcription programs and displayed enzalutamide-resistant gene expression patterns, suggesting the presence of "pre-resistant" cells that preferentially adapt to the drug. We are currently performing lineage tracing and single-cell analysis to understand whether these are non-heritable, stochastic cell states or heritable cell types. We further propose to use scRNAseq and HITS-FISH in a synergistic fashion to identify combinatorial gene expression patterns that spatially define distinct cell types in PCa and translate this information into clinically relevant biomarker panels that robustly stratify PCa subtypes.

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