Unraveling molecular heterogeneities in advanced prostate cancer at single cell resolution

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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. 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 an image-based single-cell transcriptomics toolbox that enables HIgh-Throughput Single-molecule Fluorescence In Situ Hybridization (HITSFISH), which combines automated super-resolution imaging of single RNA (nascent and mature, coding and non-coding) molecules across large areas (within and across cells) with computer vision. This toolbox is conceptualized to work in an array-based format, wherein the sample (eg - cultured cells) is distributed across a multi-well plate and each well is probed for a unique gene or unique a set of genes. Under the HITSFISH banner, we have developed a suite of unique assays that enable the hitherto possible in situ interrogation of transcripts, namely accurately quantifying gene expression, mapping gene localization, identifying RNA conformation and assessing RNA accessibility. HITSFISH discovered the rapid regulation of nascent, but not mature transcripts in response to several transcriptional and epigenetic modulators, identified that transcripts assemble into stress granules in response to several tyrosine kinase inhibitors and unraveled that "pre-resistant" plastic cell states may contribute to drug-resistance. We are using single-cell RNA sequencing and HITSFISH in a synergistic fashion to identify combinatorial gene expression patterns that spatiotemporally define distinct cell types in prostate cancer. In essence, this in situ toolkit enables single-cell, single-molecule systems biology of prostate cancer.

Acknowledgements/Funding:
We thank H. Johansson for initial help with FISH probe design. We thank J.D. Hoff and the SMART center, J. Ryu and I. Oh for assistance with HITSFISH instrumentation. A.M.C. is supported by the Prostate Cancer Foundation, and by the Howard Hughes Medical Institute. A.M.C. is an American Cancer Society Research Professor. S.P. was supported by AACR-Bayer Prostate Cancer Research Fellowship and is supported by the SPORE career enhancement award and a PCF young investigator award.