Resolving Cellular and Metabolic Heterogeneity of Liver mCRPC with Single-Cell Spatial Transcriptomics

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Background: Metastatic castration-resistant prostate cancer (mCRPC) that spreads to the liver carries some of the worst prognoses among common metastatic sites, with markedly reduced overall survival. Much of the literature has focused on bone metastases, leaving a substantial gap in our understanding of how prostate cancer cells survive, grow, and resist therapies in the liver. In particular, despite potent inhibition of the androgen receptor (AR) pathway liver metastases often continue to progress. Given the liver's central role in controlling metabolic homeostasis, we hypothesize that it may select for or enrich prostate cancer cells that adopt distinct metabolic programs suited to its microenvironment. We are applying imaging-based single-cell spatial transcriptomics to probe the metabolic pathways that enable castration resistance within liver lesions.

Methods: We implemented a multiplexed single molecule Fluorescence In Situ Hybridization (smFISH) workflow on cryopreserved patient-derived xenograft (PDX) liver metastases. We designed a probe panel for 31 genes (including lineage markers, tumor suppressors, and metabolic stress/redox regulators). Primary probes were designed using PaintSHOP and filtered for off-targets via local BLAST. Uniformity of probe representation was tested using next-generation sequencing. We optimized cryopreservation and sample preparation, including fixation, sectioning, and clearing, to preserve RNA integrity in liver tissue. Probes were first tested on subcutaneous LuCaP136 tumors in intact mice to validate sensitivity, followed by application to liver metastasis PDX136, harvested ~50 days post-castration, for single-cell expression profiling.

Results: The 31-gene panel includes lineage/phenotypic markers (e.g. AR, KLK3/PSA, PSMA, EPCAM, KRT8, NKX3.1; neuroendocrine regulators like ASCL1, SOX2, INSM1, SYP, FOXA2), core tumor suppressors (PTEN, RB1, TP53), and metabolic stress/redox genes (e.g. NFE2L2/NRF2, G6PD, PGD, GPX2, GSR, GCLC, GCLM, SLC7A11). Sequencing confirmed probe pool fidelity. In liver PDX sections, optimized protocol yielded strong single-molecule signal with low background. Dual Eef2 probes showed consistent RNA-integrity, supporting reliable detection in cryopreserved liver tissue. Together, these results confirm end-to-end technical feasibility: probe design, QC, tissue processing, multiplexed imaging, and decoding in mCRPC liver metastases.

Conclusions: We have established a reliable multiplexed smFISH platform for single-cell, spatial gene expression mapping in cryopreserved, OCT-embedded, liver mCRPC PDX tissue. The method yields coordinated detection of lineage, tumor suppressor, and metabolic transcripts, enabling investigation of hepatic microenvironment-driven metabolic adaptations in castration resistance. These proof-of-concept results set the stage for wider profiling (including AR-low/negative models), integration with metabolomics, and functional testing of liver-adapted metabolic programs as therapeutic vulnerabilities in mCRPC.

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