Summary

Widespread and long-term use of first and second line androgen deprivation therapy is changing the molecular and phenotypic landscapes of metastatic castration-resistant prostate cancer (mCRPC).

Observations made through our longstanding rapid autopsy program (1998-2018; over 150 rapid autopsies performed) supports a shift in mCRPC towards androgen receptor (AR)-null phenotypes.

Our objective was to assess the intratumoral heterogeneity comprising intrinsic tumor cell characteristics and alterations in the tumor microenvironment in metastatic sites. We used classical immunohistochemical methods and bulk RNA-Seq to assess the phenotype of mCRPC metastases and then assessed the same metastases using GeoMx™ Digital Spatial Profiling (DSP), a novel, highly multiplexed assay that digitally characterizes protein and RNA expression from spatially discrete regions of interest (ROIs) within tissue sections.

Introduction

Metastatic Castration-Resistant Prostate Cancer: a Continuum of Disease

AR decreasing

A (A) Representative immunohistochemical stains for AR, PSA, synaptophysin and chromogranin used to classify metastases from rapid autopsy patients as adenocarcinoma (Aden), AR-low, amphicrine, neuroendocrine (NEPC), or double-negative (DNPC). (B) RNAseq of prostate epithelial and neuroendocrine-associated genes in patient specimens. Mean-centered ratios of genes are color coded according to scale. Results are expressed as mean-centered log, FPKM (C) Multidimensional scaling plots of patient CRPC metastases using expression profiles of genes governed by AR and REST activity or associated with adenocarcinoma, NEPC or squamous phenotypes.

Hypothesis – a Continuum of Disease Progression

Schematic of cell phenotypes following AR pathway-directed therapy:

GeoMx™ DSP Reagents and Workflow

Process slides for protein or RNA

Conclusions

1. Tumor ROIs cluster strongly by expected mCRPC subtype and phenotype.
2. Tumor ROIs from phenotypically diverse AR+ vs. NE- subtypes show spatially distinct RNA expression profiles that are not resolved in bulk analysis.
3. Molecular profiling of tumor microenvironment shows abundance and expression differences of key immune-related pathways in mCRPC subtypes.
4. RNA analysis shows expected differential expression between spatially distinct tumor and microenvironment compartments.
5. Our study establishes a methodology for future high-pex spatial profiling of the mCRPC rapid autopsy cohort, including comprehensive inter- and intra-patient molecular profiling.

Results

Experimental Design

Five Serial Sections per Patient

Four ROI Types

Tumor ROIs Cluster Strongly by Case Type and Phenotype

Sensitivity & Reproducibility

Correlation of 1,412 genes MOR

Tumor ROIs 7-12 in 15-06-M1 cluster differentially with tumor ROIs from the AR+/NE- and AR-/NE+ cases. This differential clustering correlates with 15-06-M1 AR+/NE+ mixed phenotype. Counts were normalized to the geometric mean of 12 housekeeper genes included in the panel. Clustering was performed in R using the Pearson distance measure & complete linkage clustering.

Characterizing the AR-NE Axis and Immune Signaling Genes in Liver Metastases

Stromal Immune Signaling Correlates with Tissue of Origin

Differential expression analysis of tumor (left) or stromal (right) ROIs between AR+ liver metastasis (17-036-H2) and AR-/NE+ liver metastasis (17-017-H3). The AR-/NE axis is differentially expressed between the two cases, as expected, while immune signaling genes are higher in the AR+ metastasis.