

Title: Integrated ctDNA and EV-RNA profiling reveals tumor evolution and phenotypic adaptation in metastatic Prostate Cancer

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Background

Therapy resistance and lineage plasticity pose major challenges in metastatic prostate cancer (mPC). While resistance to androgen deprivation therapy (ADT) and androgen receptor (AR)-directed therapies is often driven by genomic alterations, transcriptional reprogramming and lineage transitions also contribute to tumor adaptation. Liquid biopsy offers a minimally invasive strategy to longitudinally monitor tumor evolution through circulating tumor DNA (ctDNA) and extracellular vesicle RNA (EV-RNA), capturing complementary genomic and transcriptional alterations. Because therapy-induced transcriptional changes may precede clinical progression, longitudinal EV-RNA profiling may provide an early measure of treatment adaptation and emerging resistance.

Methods

We conducted an integrated longitudinal approach across different phenotypes: HSPC, CRPC, and NEPC; spanning multiple treatment lines. Plasma samples (n=262) underwent paired ctDNA and EV-RNA profiling, including low-pass whole-genome sequencing, deep targeted sequencing, fragmentomics, subclonality analysis, and EV-RNA whole-transcriptome sequencing. In parallel, transcriptomics from matched tumor biopsies from both patients and PDXs were analyzed. These molecular datasets were integrated with clinical annotations, treatment exposure, and outcome to characterize genomic evolution, transcriptional states, and therapy-induced adaptation.

Results

CtDNA profiling revealed stage-specific mutational patterns, recapitulating the genomic landscape and phenotypic diversity of mPC across HSPC, CRPC, and NEPC. Baseline ctDNA tumor fraction (TF) was associated with clinical outcome in both HSPC and CRPC. Beyond mutation profiling, ctDNA enabled comprehensive characterization through assessment of tumor mutational burden, homologous recombination deficiency, and subclonal dynamics. Tumor shedding was captured differently by ctDNA and EV-RNA, highlighting their complementary roles in monitoring tumor evolution. CtDNA-defined molecular subgroups showed distinct EV-RNA profiles: AR amplification correlated with androgen response, whereas PTEN loss was associated with PTEN deficiency, cell cycle and mTOR activation, and reduced inflammation.

EV-RNA enabled classification of disease phenotypes, distinguishing HSPC, CRPC, and NEPC through lineage-specific gene signatures. Importantly, longitudinal EV-RNA profiling in an HSPC PDX model identified early AR signaling activation preceding castration-resistant regrowth. Anticipating resistance based on this EV-RNA signal enabled treatment intervention before overt resistance and resulted in improved disease control, providing functional evidence that longitudinal transcriptional monitoring can identify an actionable window for therapeutic adaptation.

Conclusions

Integrating EV-RNA with ctDNA analysis provides complementary insights into tumor evolution and phenotypic diversity, enabling minimally invasive longitudinal monitoring, and capturing early adaptive changes during lineage transformation.

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