Integrated ctDNA and EV-RNA analysis uncovers tumor evolution and lineage plasticity in metastatic Prostate Cancer

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Background

Tumor heterogeneity and lineage plasticity pose major challenges in the management of metastatic prostate cancer (mPC), particularly as the disease evolves under therapeutic pressure. While resistance is often driven by genomic mutations, transcriptomic adaptations also play a critical role. Liquid biopsy offers a minimally invasive strategy to longitudinally monitor both genomic (via circulating tumor DNA, ctDNA) and transcriptional (via extracellular vesicle RNA, EV-RNA) alterations in real time, providing insights into disease evolution and treatment response.

Methods

Longitudinal blood samples were collected from a prospective cohort of 145 mPC patients treated at Vall d'Hebron Hospital, spanning hormone-sensitive (HSPC), castration-resistant (CRPC), and neuroendocrine (NEPC) states, and across treatment lines (ARSI, taxanes, others). Plasma was processed to isolate ctDNA and EV-RNA. Mutational profiling of ctDNA (n=262) was performed by targeted deep sequencing. Whole-transcriptome profiling of EV-RNA (n=126) was conducted using a custom RExCuE-based library preparation enabling simultaneous gene expression analysis. EV-RNA from conditioned media of in vitro models with distinct AR and RB1 mutational backgrounds, was analyzed by RT-qPCR. Exome-capture RNA-seq was performed in matched tumor and blood samples from patient-derived xenografts (PDXs) representing HSPC, CRPC, and NEPC.

Results

ctDNA profiling revealed distinct, stage-specific mutational landscapes. In CRPC, AR alterations were predominant (51%), whereas NEPC was characterized by frequent MYC (70%), RB1 (60%), EZH2 (50%), and PI3K pathway mutations (30–70%). These genomic patterns were mirrored in matched EV-RNA, which showed concordant shifts in gene expression. Tumor fraction (TF) estimates derived from both variant allele frequency and methylation-based scores were strongly correlated ($r=0.8$, $p<0.0001$), although this relationship was less robust in NEPC. Notably, higher TF at treatment baseline was significantly associated with shorter progression-free survival (VAF HR=2.2, $p<0.0001$; methylation HR=2.5, $p<0.0001$).

Mutation calling across paired ctDNA-EV-RNA and RNA-EV-RNA demonstrates tumor- and patient-specific origins, with mutational status in ctDNA reflected in EV-RNA levels. AR and MYC amplifications lead to increased EV-RNA levels, while RB1 deletions aligned with transcript loss.

Finally, EV-RNA transcriptomes distinguished clinical states, with CRPC enriched for androgen response programs and NEPC defined by neuroendocrine signatures. Longitudinal profiling enabled real-time monitoring of lineage transitions, capturing early transcriptomic adaptations to therapy, including emergence of castration resistance and neuroendocrine features.

Conclusions

Combined analysis of ctDNA and EV-RNA provides complementary insights into tumor evolution, enabling minimally invasive, longitudinal genomic and transcriptomic monitoring, with EV-RNA uniquely capturing early adaptive changes in gene expression during lineage transformation.

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