**Multi-omic profiling of circulating tumor DNA in patients with CRPC**

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**Background:** Circulating tumor DNA (ctDNA) from liquid biopsies provides a non-invasive route to profile tumor genomes and to study genomic changes during therapy. Current research and clinical efforts have focused on the detection of mutations in select genes from ctDNA, demonstrating potential for clinical utility. However, approaches to recapitulate genomic alterations in ctDNA, including those in non-coding regions, remain rudimentary because of extreme variability in the amount of ctDNA. Recent advances in ctDNA analysis suggest the potential to predict transcriptional patterns using ctDNA fragment patterns and nucleosome profiling without the need for additional specialized assays. Our goal is to develop a "multi-omic" approach to detect and track the evolving landscape of alterations in ctDNA during therapy, including non-coding alterations in the enhancer regulatory elements of the androgen receptor, genomic signature of tandem duplications, and AR binding activity in CRPC.

**Methods:** We analyzed ctDNA data from 13 deep whole genome sequencing (WGS) samples and >280 low-coverage (0.1x-1x) WGS samples collected from patients with CRPC. We applied computational approaches to analyze multiple "omics" features from ctDNA to predict tissue-of-origin, genome-wide alteration profiles, AR locus alterations, and AR binding activity. We also analyzed data from >300 clinical targeted gene panel sequencing samples of tumor tissue DNA and ctDNA to identify genome-wide and AR locus alterations using off-target read analysis.

**Results:** From the analysis of low-coverage WGS of patient ctDNA, we were able to predict both the genome-wide tandem duplicator phenotype status and the AR locus alterations, including at the AR gene body and the distal enhancer. We also show that these genome-wide and non-coding alteration events can be predicted in the off-target analysis of clinical panel sequencing data of ctDNA.

To determine transcriptional activity from ctDNA, we explored several approaches to analyze AR transcription factor binding activity. We observed that the binding activity at ~500 AR binding sites for various ctDNA fragment sizes is associated with the tumor fraction during progressive disease. Across a cohort of CRPC patients, the ctDNA fraction is positively correlated with AR binding activity but is negatively correlated with the activity of blood-cell-specific transcription factors.

**Conclusions:** The ability to detect genomic and transcriptomic features from ctDNA can enable "multi-omic" clinical applications for non-invasive therapeutic surveillance in CRPC. Integrative monitoring of AR alterations and transcription factor binding activity from ctDNA can provide insights into tumor plasticity, subtyping, and the activity of targeted therapies.

**Conflict of Interest:** GH - patent application (US20190078232A1)

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