Integrative multi-omic analysis of extracellular vesicle transcriptomics profiling combined with cfDNA methylation reveals improved stratification of low-risk and high-risk prostate cancer patients in urine-based liquid biopsy.

Short Title: EV multi-omics based liquid biopsy for prostate cancer risk stratification.

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Background: Prostate cancer (PCa) is the second most commonly diagnosed malignancy and the sixth leading cause of cancer-related mortality among men worldwide. In this study, we employ a multi-omic strategy that integrates analytes derived from both urinary cell-free DNA (cfDNA) and extracellular vesicle (EV) RNA to enhance the risk stratification of prostate cancer patients. This approach is evaluated against the performance of multi-parametric MRI and established risk calculators within the same patient cohort.

Methods: This interim analysis includes results from urine samples collected from 95 individuals being evaluated for prostate biopsy. The cohort consisted of 51 low-risk (Grade Group [GG] 0-1), 10 intermediate-risk (GG2), and 44 high-risk prostate cancer (PCa) patients (GG 3-5). EV-RNA and cfDNA were concurrently isolated from each urine specimen to leverage complementary molecular information. We developed an EV-RNA sequencing platform targeting mRNAs and lncRNAs, aiming for 50 million reads per sample. Similarly, cfDNA methylome profiling was conducted at an equivalent sequencing depth. We analyzed the expression of EV-RNA, splice variant Differential Transcript Usage (DTU), and cfDNA methylation patterns using Bio-Techne's multi-omic platform. Machine learning-based feature selection algorithms were employed to identify biomarker signatures from each analyte. Receiver operating characteristic (ROC) curves were generated using leave-one-out cross-validation with naïve Bayes classifier models to calculate the area under the curve (AUC). Finally, the individual signatures were integrated to develop a multi-omic classifier.

Results: Differential gene expression (DEx) analysis identified genes with distinct expression patterns between low- and high-risk patients, revealing both established and novel associations with prostate cancer (PCa). Splice variant analysis uncovered 60 genes previously linked to PCa that exhibited Differential Transcript Usage (DTU). Additionally, over 18,000 differentially methylated bases were detected between high- and low-risk PCa patients. Feature selection analysis highlighted the top ten features across EV-RNA expression, splice variants, and cfDNA methylation, leading to the development of an integrative multi-analyte signature. This multi-omic signature achieved an area under the curve (AUC) of 0.92, outperforming the individual analytes, as well as the AUCs achieved by PSA (0.52) and MRI-PIRADS (0.65).

Conclusions: Although biomarker signatures from individual analytes in this study effectively stratified PCa risk, the multi-omic signature further enhanced discriminatory power, highlighting the complementary nature of these signals. This multi-omic biomarker discovery approach, which integrates cfDNA and EV cargo, shows substantial promise as a risk assessment tool for high-grade prostate cancer. By providing a more comprehensive assessment, this strategy has the potential to inform and improve decision-making for PCa.

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