Transcriptome analysis of de novo metastatic castration-sensitive prostate cancer using long-read sequencing technologies

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Background: The treatment landscape of metastatic castration-sensitive prostate cancer (mCSPC) has recently become complicated, and classifications based on clinical information, such as tumor volume, are insufficient for treatment selection. Rational biomarkers based on biological evidence may enable optimal treatment for each patient and improve treatment outcomes. We aimed to evaluate the transcriptome features of mCSPC using long-read sequencing (LRS) technologies.

Methods: Diagnostic prostate biopsy tissues were prospectively collected from treatment-naïve de novo mCSPC patients between 2020 and 2023. Fresh tumor tissue specimens were divided into two pieces for pathological diagnosis and molecular profiling. The pieces for molecular profiling were stored in RNA-stabilized reagents immediately after biopsy (within 30 s) to avoid nucleic acid degradation. Benign prostatic tissue samples were collected from patients with benign prostatic hyperplasia as controls. RNA was extracted from tissue samples, and the following criteria were required for LRS: RNA Integrity Number (RIN) ≥7.0, A260/280 >1.5, A260/230 >1.0, and total RNA amount >500 ng. Sequencing was performed using the MinION platform (Oxford Nanopore Technologies) with the flow cell FLO-MIN106. Transcript isoform detection and quantification were performed using the SPLICE pipeline, which was developed to analyze LRS data.

Results: RNA was extracted from 104 tumor tissues, and 77% (80/104) of the samples met the quality control criteria for LRS. After filtering low-quality reads, an average of 13 million reads was obtained, with an average read length of 1,286 per tissue sample. Unsupervised clustering of transcripts demonstrated clear segregation between mCSPC tumor and benign prostatic hyperplasia tissues. In differential expression genes (DEGs) and differential expression transcripts (DETs) analyses, 2173 DEGs and 3361 DETs were identified after adjusting for multiple testing (FDR < 0.01). Among the DETs, we identified alternative splicing (AS) in the GABARAP gene, which is associated with autophagy. One of the splicing variants in *GABARAP*, ENST00000577035, was expressed at significantly higher levels in mCSPC than in benign prostate tissues ($p = 8.4\times10^{-12}$). High expression levels of ENST00000577035 were also associated with worse castration-resistant prostate cancer-free survival time in mCSPC patients (median: not reached vs. 16 months, $p = 0.0043$).

Conclusions: LRS has the advantage of reading longer reads compared to SRS. This new technology may advance the understanding of AS, and functional analyses will help optimize the therapeutic selection of mCSPC.

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