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Hepatitis B virus serum RNA transcript isoform composition and proportion in chronic hepatitis B patients by nanopore long-read sequencing

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Introduction: Serum hepatitis B virus (HBV) RNA is a promising new biomarker to manage and predict clinical outcomes of chronic hepatitis B (CHB) infection. However, the HBV serum transcriptome within encapsidated particles, which is the biomarker analyte measured in serum, remains poorly characterized. This study aimed to evaluate serum HBV RNA transcript composition and proportionality by PCR-cDNA nanopore sequencing of samples from CHB patients having varied HBV genotype (gt, A to F) and HBeAg status.

Methods: Longitudinal specimens from 3 individuals during and following pregnancy (approximately 7 months between time points) were also investigated. HBV RNA extracted from 16 serum samples obtained from 13 patients (73.3% female, 84.6% Asian) was sequenced and serum HBV RNA isoform detection and quantification were performed using three bioinformatic workflows: FLAIR, RATTLE, and a GraphMap-based workflow within the Galaxy application. A spike-in RNA variant (SiRV) control mix was used to assess run quality and coverage. The proportionality of transcript isoforms was based on total HBV reads determined by each workflow.

Results: All chosen isoform detection workflows showed high agreement in transcript proportionality and composition for most samples. HBV pregenomic RNA (pgRNA) was the most frequently observed transcript isoform (93.8% of patient samples), while other detected transcripts included pgRNA spliced variants, 3' truncated variants and HBx mRNA, depending on the isoform detection method. Spliced variants of pgRNA were primarily observed in HBV gtB, C, E, or F-infected patients, with the Sp1 spliced variant detected most frequently. Twelve other pgRNA spliced variant transcripts were identified, including 3 previously unidentified transcripts, although spliced isoform identification was very dependent on the workflow used to analyze sequence data. Longitudinal sampling among pregnant and post-partum antiviral-treated individuals showed increasing proportions of 3' truncated pgRNA variants over time.

Conclusions: This study demonstrated long-read sequencing as a promising tool for the characterization of the serum HBV transcriptome. However, further studies are needed to better understand how serum HBV RNA isoform type and proportion are linked to CHB disease progression and antiviral treatment response.

Serum From A Patient With Suspected Chronic Hepatitis

Ensheng Dong



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