**Nanopore Direct RNA Sequencing Reveals Virus-Induced Changes in m6A Methylation Patterns in Human Cells**

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Nanopore direct RNA sequencing provides a powerful method for characterizing the changing transcriptional landscape of viruses and their host. The findings from this study highlight the role of m6A RNA methylation in virus-host interactions. The identification of differentially methylated RNA sites during viral infection provides new insights into the mechanisms of viral pathogenesis and host defense against viral infections. Our hypothesis is that m6A methylations alter RNA folding and binding partners in virus-host interactions. Our results show that m6A patterns vary with the type of human cell and the virus. This study focused on influenza-infected human bronchial epithelial cells (HBEC) and sindbis-infected A549 epithelial cells.

Adaptive viral pathogens successfully repurpose the host transcriptional machinery. RNA modifications are fundamental in regulating several cellular processes and play a critical role in cancer, cell-fate determination, development, and diseases. Despite their importance, technical limitations have often hampered transcriptome-wide mapping of RNA modifications. Nanopore direct RNA sequencing is a third-generation sequencing technology that allows the direct sequencing of native RNA molecules, providing a direct way to detect modifications at single-molecule resolution. The Oxford Nanopore Technologies MinION platform was used to directly sequence polyadenylated host and viral RNAs from infected human cells. m6A is one of the most common in human virus infections. Guppy and m6Anet software use machine learning to identify m6A nucleotides in RNA nanopore data. The average length of RNA reads was above 6kb, with high quality data evidenced by an average identity and read accuracy above 0.95 and 95%, respectively.

The number of m6A sites ranged from 206,128 to 632,965 across all four datasets. In the HBEC cells, the number of m6A sites in influenza-infected cells was 4.2% higher than that in uninfected cells, while in the A549 cell line, the number of m6A sites in Sindbis virus-infected cells increased by 7.9% compared to uninfected cells, suggesting that viral infection promotes m6A methylation of RNA in their host cells. Moreover, the number of m6A varies in different types of RNA, including protein coding, introns, antisense, and lincRNA.

The study found that m6A conserved motifs vary across different cell types. In the HBEC cell line, the motif "GGACU" was dominant in all types of detected RNA, and the number of this motif in infected cells decreased in protein-coding RNA, processed transcript, and LincRNA but significantly increased in the intron. In contrast, the A549 cell lines showed that the "GGACU" motif was dominant in protein-coding RNA, introns, lincRNA, and antisense RNA, but the number of this motif in infected cells decreased. In processed transcripts in uninfected A549 cells, the dominant m6A motif was "GGACA", with the number of conserved m6A motif "GGACU" and "GGACA" in infected cells increased. A change in the host preference for m6A writers is one possible explanation for the change in the dominant motif. Our collaborative research will continue to study the correlations of m6A modification with alternative splicing, polyA tail length, RNA folding, gene expression network, and RNA function in virus response.