Global transcriptome and alternative splicing analysis of Zika virus infections in pediatric patients

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Zika virus (ZIKV) is a re-emerging mosquito-borne flavivirus that is closely related to Dengue virus (DENV) and West Nile virus. The striking feature of the 2015 ZIKV outbreak in the Americas was the correlation between intrauterine ZIKV infection and developmental and neurological defects in newborns. To date the ZIKV-directed mechanisms contributing in the unusual neuropathologies are poorly understood. The Pager lab recently completed a global analysis of changes in gene expression and alternative splicing of neuroblastoma cells infected with the contemporary ZIKV strain isolated from Puerto Rico in 2016, the African ZIKV strain isolated from Uganda in 1947, and the closely related DENV. They observed notable changes in immune-related genes in ZIKV-infected cells but not in DENV infection. Moreover, differential gene expression and alternative splicing events in cells infected with the Puerto Rican ZIKV isolate were significantly higher compared to the Ugandan ZIKV strain and DENV. Recently Michlmayr *et al*. (2020), employed a systems-wide immunology approach of RNA-seq, CyTOF and cytokine/chemokine arrays to examine responses by the innate immune system during acute ZIKV infection. While Michlmayr *et al*., reported that prior DENV infection did not change the innate immune response to ZIKV, the authors did not investigate alternative splicing events as they relate to the regulation of RNA modification. We posit that changes in alternative splicing could have profound effects on mRNA stability and translation. Thus, the goal of this bioinformatic analyses is to reanalyze the differential gene expression studies undertaken by Michlmayr and colleague, as well as to investigate changes in alternative splicing. To this end, we gathered RNA-seq data available from Michlmayr *et al* (2020) Cell Reports 31, 107569 and GEO:GSE12982. First, we used DESeq2 for differential gene expression analysis followed by gene enrichment analysis of the statistically significant differential gene expression using Panther. We found that the vast majority of differentially expressed genes were highly upregulated in the early acute phase as compared to the convalescent phase of ZIKV infection. Next, splicing differences were investigated using replicate multivariate analysis of transcript splicing (rMATS), and splicing events filtered using a custom Python script. These analyses revealed changes in alternative splicing patterns in multiple genes, including DUS2 and RBM15, which code for proteins that regulate uridine modifications on tRNA and downstream alternative splicing through interactions with the splicosome, respectively. Interestingly, studies previously conducted in the Pager lab have consistantly revealed uridine modifications on tRNA molecules following ZIKV infection, indicating that regulation of genes such as DUS2 may be important in host-virus interaction. Future studies will focus on the functional role of alternatively-spliced DUS2 protein products and their potential impact on the host immune response to ZIKV infection.