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Understanding the regulatory mechanisms governing host defense against flavivirus infections for biomarker identification

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Introduction

Rising global temperature is boosting vector (ticks, mosquitoes) populations, leading to more vector-borne flavivirus cases worldwide. Also, it is well documented that human bodily fluids, tissues, and cells can transmit flaviviruses. Flaviviruses such as Zika (ZIKV) and Dengue (DENV) virus can remain in infected tissues for many months even when the individuals are no longer viremic. Herein, we investigate the regulatory mechanisms governing host defense against flavivirus infections. We also aimed to identify host transcripts that could serve as biomarker panels for the detection of flaviviruses in host cells.

Materials and Methods

Vero (monkey) and SK-N-SH (human) cells exhibit cytopathy in response to ZIKV, but not DENV, infection. Cells were infected with ZIKV-MR766 and DENV3 strains at an MOI of 1. Persistently infected cultures were grown from acute infections and passaged weekly for 3+ months. Continued presence of flavivirus was determined with immunostaining via anti-flavivirus antibody (4G2). Control, acute, and persistent infection samples were prepared for RNA-seq via phenol/chloroform extraction, rRNA-depleted, cDNA libraries were generated, and samples were sequenced. RNA-seq was performed on the NovaSeq 6000, and differential gene expression was analyzed using DESeq2. Normalized RNA-seq data was visualized with Integrated Genomics Viewer (IGV).

Results

1. Transcriptomic profiles distinguish exposure to Zika and Dengue viruses in host cells during both acute and persistent infections.

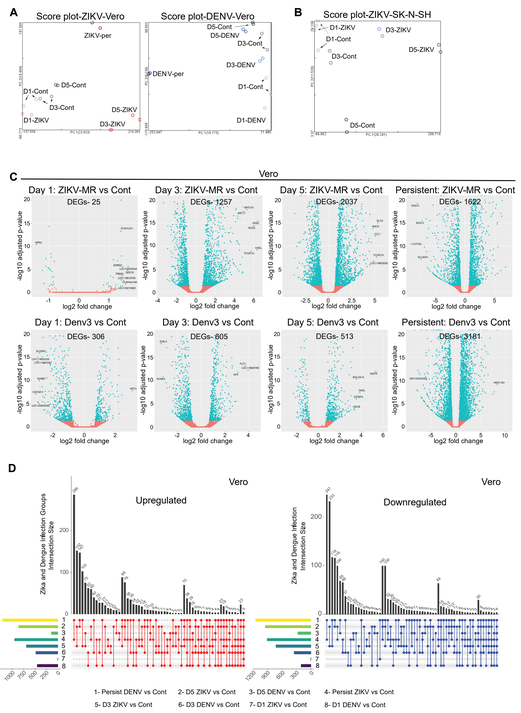


Figure 1. RNA-seq quality control indicates differences between Zika and Dengue infections during acute and persistent infections in Vero cells. (A, B) Principal component analysis between control and Zika- or Denv-infected Vero and SK-N-SH samples shows distinct differences between control and infected samples across 5 days post-infection as well as with persistently infected cells. (C) Volcano plots detail differentially expressed genes with log2 fold changes ± 0.58. (D) UpSet plots show shared differentially expressed transcripts between acute and persistent treatment conditions in Vero cells.

2. Several host regulatory circuits including mRNA processing factors are deregulated during flavivirus infections.

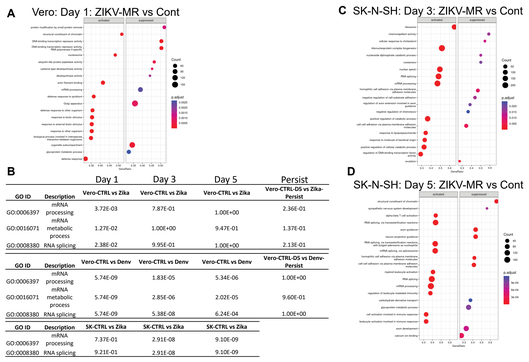


Figure 2. GSEA pathway analyses identified deregulation of mRNA processing factors in host cells upon flavivirus infection. A comparison of Day 1: Vero Zika acute infection vs Control (A), Day 3: SK-N-SH Zika acute infection vs control (C) and Day 3: SK-N-SH Zika acute infection vs control (D) GSEA data. GSEA adjusted p-values for mRNA processing (GO:0006397), mRNA metabolic process (GO:0016071) and RNA splicing (GO:0008380) pathways in control, Zika-, and Denv-infected Vero samples (B).

3. Nonsense-mediated decay, RNA degradation complex and nuclear pore complex genes are upregulated in a cell type-specific manner.

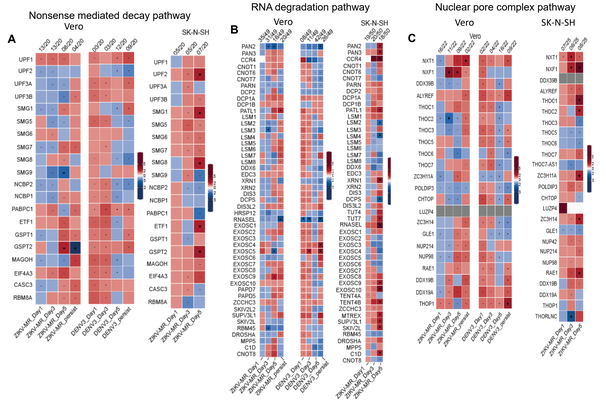


Figure 3. Heatmaps in Zika and Dengue virus infected host cells during acute and persistent infections. (A, B, C) Comparisons of Zika- and Denv-infected Vero and SK-N-SH sample transcripts for NMD, RNA degradation, and nuclear pore complex pathways.

4. Protein profiles distinguish exposure to Zika and Dengue viruses in host cells during acute and persistent infections.

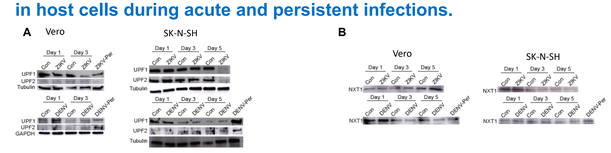


Figure 4. The NMD pathway master regulators UPF1 and UPF2 proteins are degraded by Zika, but not Dengue.
(A) Western blot results of NMD pathway proteins UPF1, UPF2, and housekeeping Tubulin and GAPDH in control and infected samples at various time points. (B) Western blot results of nuclear pore complex protein NXT1 involved in nuclear export in control and infected samples at various time points.

5. Degradation of NMD factors drives intron accumulation in host cells upon flavivirus infection.

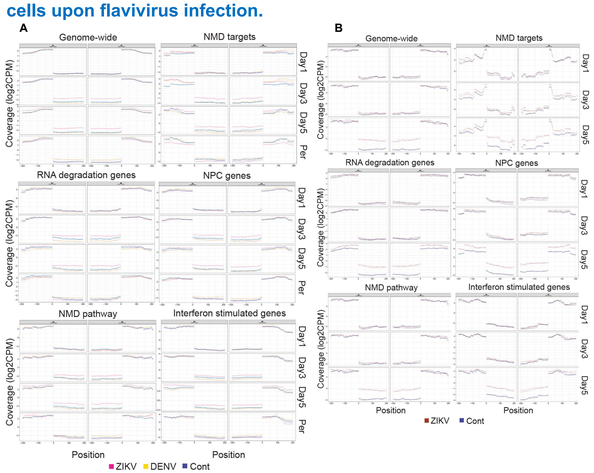


Figure 5. Accumulation of intronic reads in ZIKV-infected samples. Comparison of intron/exon and exon/intron boundaries genome-wide as well as for NMD targets, RNA degradation genes, NPC genes, NMD pathway, and ISGs within 200bp for control (blue), Zika- (red), and Denv-infected (yellow) in Vero (A) and SK-N-SH (B) samples.

6. Disruption of nuclear export impairs Zika virulence

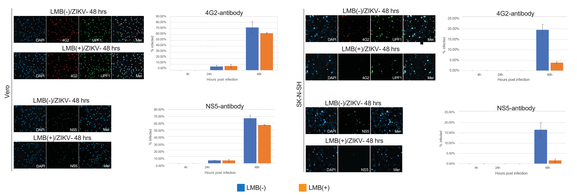


Figure 6. 20nM leptomycin B (LMB) treatment disrupts Zika infection. We counted cells exhibiting positive infected signal in LMB-treated and –untreated Zika-infected Vero and SK-N-SH cells. Samples were fixed, immunoassayed, and counted at 4-, 24-, and 48-hours post-infection using two antibodies against Zika (Zika NS5, 4G2). Two samples per condition were counted and averaged to determine infectivity. LMB-treated Zika-infected cells exhibited a lower rate of infectivity at 48 hours post-infection particularly in SK-N-SH cells.

7. Identification of NMD targets as potential biomarkers for flavivirus infection in host cells

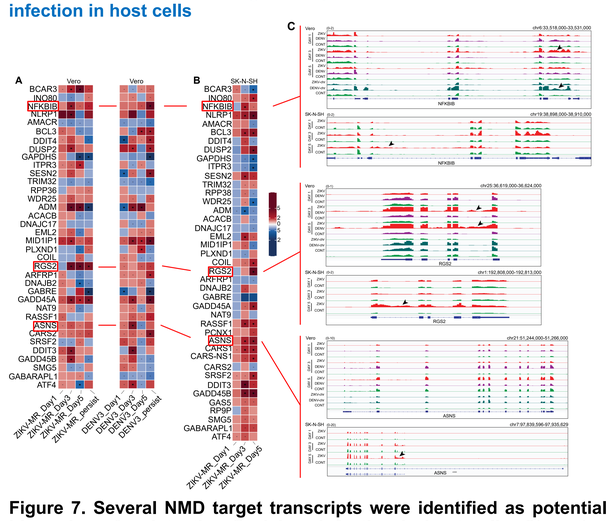
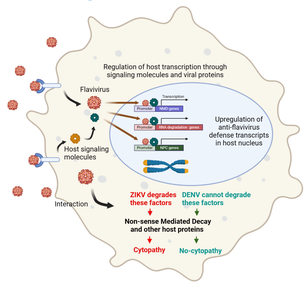


Figure 7. Several NMD target transcripts were identified as potential biomarkers for detecting flavivirus infection in host cells. Given that NMD is dysregulated in flavivirus-infected cells, we analyzed NMD target transcripts and created heat maps from Vero (A) and SK-N-SH (B) RNA-seq data. An IGV snapshot shows normalized RNA-seq signals for three candidate biomarkers (C).

8. Proposed model of molecular basis of flavivirus virulence concerning host defenses.



The diagram illustrates the interaction between flaviviruses and host cells. Upon viral invasion, host signaling molecules activate, upregulating viral defense transcripts in cell type specific manner. In Zika, unlike Dengue, viral proteins infiltrate the host cells and degrade UPF1 and UPF2, weakening host defenses and increasing Zika's virulence. This targeted degradation of essential host proteins leads to cytopathy.

Future directions

1. Validation of the identified candidate biomarker transcripts will be conducted in additional human cell lines infected with various flaviviruses, such as WNV.
2. Further validation will also extend to donor cells and tissues to ensure their reliability as biomarkers.

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