Zika Infection Dysregulates Autophagy in Neural Stem Cells

Lani Tran 1,2, Lisa Henderson, Ph.D. 2, Avindra Nath, M.D. 2, Youssef Kousa, D.O., Ph.D. 1,2

1 Center for Neuroscience Research and Center for Genetic Medicine Research, Children’s National Research Institute, Washington, DC
2 National Institute of Neurological Disorders and Stroke, NIH, Bethesda, MD

Abstract

Zika virus emerged as a global threat due to its strong proclivity for the central nervous system, severely impacting brain development. However, our understanding of Zika pathogenesis is especially limited, and no vaccines or therapeutics have been approved. Preliminary data suggests a neurogenetic risk to prenatal viral infection when autophagy is disrupted. Autophagy is a host-defense, catabolic process that traps and delivers viral cargo, like Zika, to the lysosome for degradation. Interestingly, closely-related viruses can exploit autophagic machinery, promoting viral replication and propagation. We aim to characterize the relationship between Zika infection and autophagic molecular effectors, and investigate how prenatal genetic risks impact virally-induced brain injury.

Methods

Neural stem cells were infected with Zika at increasing multiplicities of infection (from 0.01 to 3). Cells were collected at timepoints spanning early, mid, and late infection and autophagy activation (12, 24, 36 hours). Autophagic flux and viral replication were quantified via RT-qPCR and Western Blotting. Transcriptional changes were also evaluated in four-week-old cerebral organoids 48 hours post-infection.

Zika Affects Autophagy in NSCs

A

B

C

RNA-Seq on Zika-Infected Organoids Highlights Autophagy

Figure 2. Zika infection affects multiple autophagy-related genes (A). Notably, despite an increase in relative gene expression, the protein level of p62 is reduced (B). Additionally, genes downstream of mTOR, such as 4E-BP1, are consistently reduced at 12, 24, and 36 hours post-infection, indicating sustained induction of autophagy (C).

A Cerebral Organoid System

Characterizing Endo-Cerebral Organoids

Figure 3. Four-week-old endo-cerebral organoids exhibit distinct 3D structure characterized by an inner cellular mass and cystic external covering (A). Immunostaining confirms presence of endothelial cells at periphery (CD31), neural cells centrally (β3-tubulin), and co-expression of mTOR/NeuN (C).

Discussion

Zika infection led to loss of phospho-4E-BP1, indicating early induction of autophagy. There was a corresponding increase in the expression of other autophagy-related genes, including p62, LC3B, WDRT45, and NBR1. RNA sequencing of virally-infected cerebral organoids also showed induction of autophagy pathway genes. Notably, p62—a protein that sequesters viral material for autophagic degradation and a marker of autophagic flux—showed drastically reduced protein levels despite increased gene expression, consistent with prior findings for Dengue (a closely-related flavivirus). As in Dengue, p62 may be acting as a viral restriction factor. We are now developing targeted pharmaceutical approaches to rescue p62 protein expression to ultimately boost neuronal survival and reduce injury.