Zika Infection Dysregulates Autophagy in Neural Stem Cells

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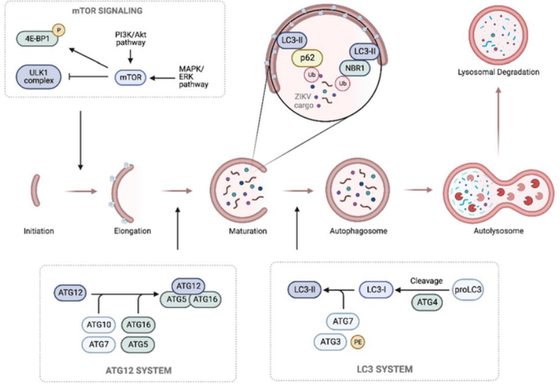
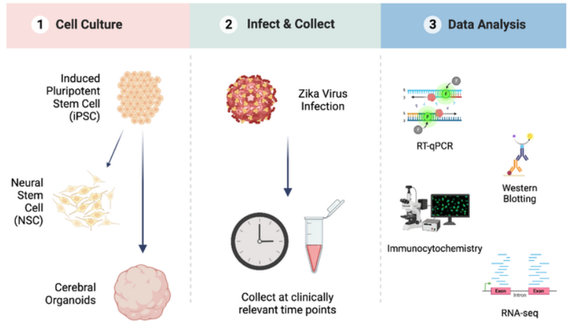
Abstract

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.



From iPSCs to Neural Stem Cells

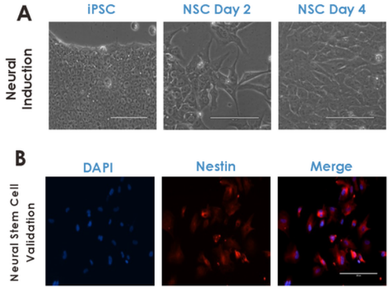


Figure 1. *Developing a successful monolayer neural stem cell culture from iPSCs. Phase microscopy of cell morphology during induction of iPSCs into NSCs (A). Immunocytochemical analysis of NSCs confirm stem cell markers (B).*

Zika Affects Autophagy in NSCs

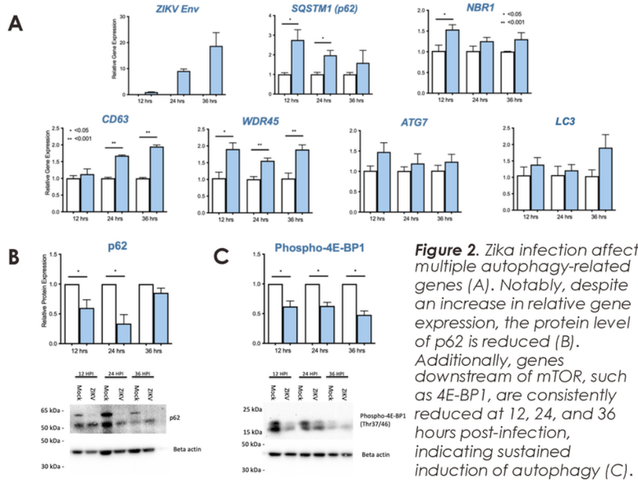


Figure 2. *Zika infection affects multiple autophagy-related genes (A). Notably, despite an increase in relative gene expression, the protein level of p62 is reduced (B). Additionally, genes downstream of mTOR, such as 4E-BP1, are consistently reduced at 12, 24, and 36 hours post-infection, indicating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

A Cerebral Organoid System

Characterizing Endo-Cerebral Organoids

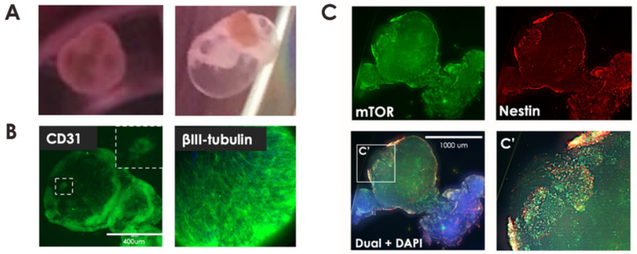


Figure 3. *Four-week-old endo-cerebral organoids exhibit distinct 3D structure characterized by an inner cellular mass and cystic external covering (A). Immunostaining confirms presence of endothelial cells at periphery (CD31), neuronal cells centrally (βIII-tubulin), and co-expression of mTOR/Nestin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

RNA-Seq on Zika-Infected Organoids Highlights Autophagy

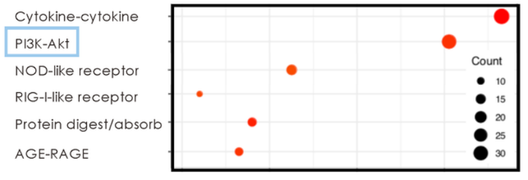


Figure 4. *Analysis of bulk RNA sequencing data reveals differential gene expression of the mTOR pathway.*

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, WDR45, 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.