Elucidating Zika Virus and Host Mitochondria-Interacting Protein Interaction Networks Using Amino Acid-Based Algorithms

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Abstract

Zika virus (ZIKV) is a neuroinvasive pathogen capable of infecting both neurons and astrocytes. Following infection, p53 has been shown to promote apoptosis in the infected central nervous system. Most cell types can activate either the p53 transcription-dependent or the p53 mitochondrial pathway to induce apoptosis. Given the lack of expression of p53 transcriptional targets upon ZIKV infection in previous experiments, this project focuses on exploring potential molecular substrates of the mitochondria-dependent p53 apoptotic pathway using predictors of subcellular localization. We performed these analyzes with three ZIKV isolates: Uganda MR766, Brazil Fortaleza, and mouse-adapted Dakar. For all isolates, the predictions identified the Zika capsid protein to directly localize to the mitochondria in host cells, and its potential conserved mechanisms of mitochondrial localization in ZIKV infection were identified from a study that compared flavivirus-host interactions of ZIKV and Dengue Virus (DENV) in human and mosquitoes[1].

Introduction

ZIKV infects humans and animals through bites of *Aedes* mosquitoes and can spread via contact with blood and bodily fluids. **By using amino-acid-based predictors, TargetP[2] and MitoFates[3], potential mitochondrial targeting regions of the protein sequences of each ZIKV protein can be determined.** TargetP predicts proteins location by scoring N-terminal sequence motifs directing proteins to the mitochondria, and MitoFates predicts the cleavage sites of mitochondrial presequence. To identify mitochondria-interacting proteins that interact with ZIKV proteins and look for overlaps in their interactions, potential mechanisms of mitochondrial localization in ZIKV infection were determined. This work elucidating flavivirus-host protein interaction networks is important for understanding conserved mechanisms of ZIKV neuropathogenesis.

References

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MTS Probability Prediction Scores

Table 1: TargetP Results for ZIKV MR766

Zika (MR766)	Other (no targeting peptide)	Signal Peptide (sp)	Mitochondrial transfer peptide (mTP)
protein E	0.9996	0.0003	0.0001
protein C	0.9344	0.0107	0.055
protein prM	0.9973	0.0026	0.0001
NS1	0.9998	0.0001	0.0001
NS2A	0.9998	0.0002	0
NS2B	0.0138	0.9861	0.0001
NS3	0.9994	0.0002	0.0004
NS4A	0.9997	0	0.0003
NS4B	0.9996	0.0004	0
NS5	0.9995	0.0003	0.0002

Table 2: MitoFates Results for ZIKV MR766

Zika (MR766)	Probability of Presequence	Cleavage site	Prediction
protein E	0	58MPP, 59lcp55*	No mitochondrial presequence
protein C	0.658	56 MPP	Possessing mitochondrial presequence
protein prM	0.005	93MPP	No mitochondrial presequence
NS1	0	42MPP	No mitochondrial presequence
NS2A	0	28MMP	No mitochondrial presequence
NS2B	0	74MPP	No mitochondrial presequence
NS3	0.008	30MPP	No mitochondrial presequence
NS4A	0.003	40MPP	No mitochondrial presequence
NS4B	0.01	9MPP	No mitochondrial presequence
NS5	0.04	43MPP	No mitochondrial presequence

For all three isolates of ZIKV, TargetP and MitoFates predicted the Zika capsid protein to be targeted to the mitochondria by detecting its mitochondrial targeting sequence (MTS). The other ZIKV proteins were not considered to contain MTS by the two prediction programs.

Protein-Protein Interactions

Table 3: Comparison of Protein-Protein Interactions of Cellular Proteins with ZIKV & DENV Proteins

Virus	Protein	Host	Number of Cellular Proteins	Proteins for Mitochondrial Localization	Proteins for Organelle Targeting and Organization
Zika (MR766)	protein C	Human	63	12	0
Zika (MR766)	NS4A	Human	157	0	58
DENV	protein C	Aedes aegypti	77	12	0
DENV	protein C	Human	4	2	0
DENV	prM	Human	53	7	0

The enrichment analysis, by the study that compares flavivirus-host interactions of ZIKV and DENV, revealed that the ZIKV capsid protein was enriched for interaction partners involved in mitochondrial localization.

Conclusion/ Future Direction

Our previous studies, focusing on elucidating roles for p53 signaling in neuroinflammation and host defense in vivo using mouse models of ZIKV encephalitis, found a lack of expression of p53 transcriptional targets upon ZIKV infection. In this study, amino acid-based algorithms were used to explore potential molecular substrates of the mitochondrial-dependent p53 apoptotic pathway. In future studies, we plan to design siRNAs to repress cellular protein expression or introduce mutations into the Zika capsid protein determine its role in mitochondria-dependent p53 pathway to induce apoptosis. Overall, comparing protein-protein interactions across ZIKV and DENV helped reveal potential conserved mechanisms of mitochondrial localization in ZIKV pathogenesis.

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