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Genome-wide CRISPR screens to identify host factors important for Zika Virus infection

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ABSTRACT

Zika virus is an emerging pathogen, and has been identified as a Public Health Emergency of International Concern by the World Health Organization. Zika virus is a member of the *Flaviviridae*, whose close relatives include Dengue virus and Yellow Fever virus, and is transmitted by mosquitos. Infections with Zika virus are asymptomatic in a majority of cases, but can lead to fever, rash, muscle, and joint pain. However, as confirmed recently by the CDC, Zika virus infections in pregnant women can lead to the development of microcephaly in fetuses. This has prompted an urgent push to develop vaccines and anti-viral therapeutics against Zika virus. Because there is so little known about the life cycle of this emerging pathogen, studies to investigate which host factors are important for Zika virus are warranted. To this end, we are performing genome-wide CRISPR screens to identify host targets for Zika virus infection. The advantage of CRISPR screens is that they provide complete loss-of-function, whereas traditional genome-wide screens using RNA interference may only partially suppress the genes of interest. We have previously performed genome-wide CRISPR screens on another important viral pathogen, Rift Valley fever virus, which revealed that the virus was dependent on several genes key to the heparan sulfate biosynthesis pathway. This has been shown in the literature to be a possible important entry factor for the virus. By performing CRISPR screens and validating hits, we aim to not only learn more about the life cycle of Zika virus, but identify potential therapeutic targets to aid in the development of countermeasures for Zika virus and other closely related flaviviruses.

INTRODUCTION

- Zika virus (ZIKV) was originally isolated from a Rhesus monkey in 1947 at the African Zika forest in Uganda
- The current ZIKV outbreak in Brazil began in March of 2015
- Centers for Disease Control has confirmed that ZIKV is the cause of fetal abnormalities including microcephaly, congenital blindness, and stillbirth
- A recent study looking at possible viral entry factors for ZIKV implicated AXL, a protein expressed on the surface of neural stem cells
- To date, little is known about the life cycle of ZIKV and what host factors are important for the viral infection

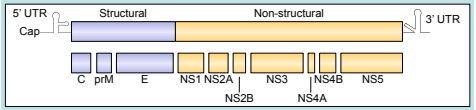

Figure 1: Zika virus genome. Zika virus is a positive-sense single-stranded RNA virus of the *Flaviviridae* family. The genome encoded a single 10kb polyprotein that is post-translationally cleaved into three structural and 7 nonstructural proteins.

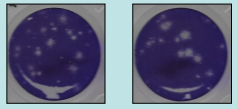

Figure 2: Zika virus plaques. Strain PRVABC59 from Puerto Rico (left) and strain Honduras (right), titered on Vero cells and stained 5 days post infection.

GENOME-WIDE CRISPR SCREENING METHOD

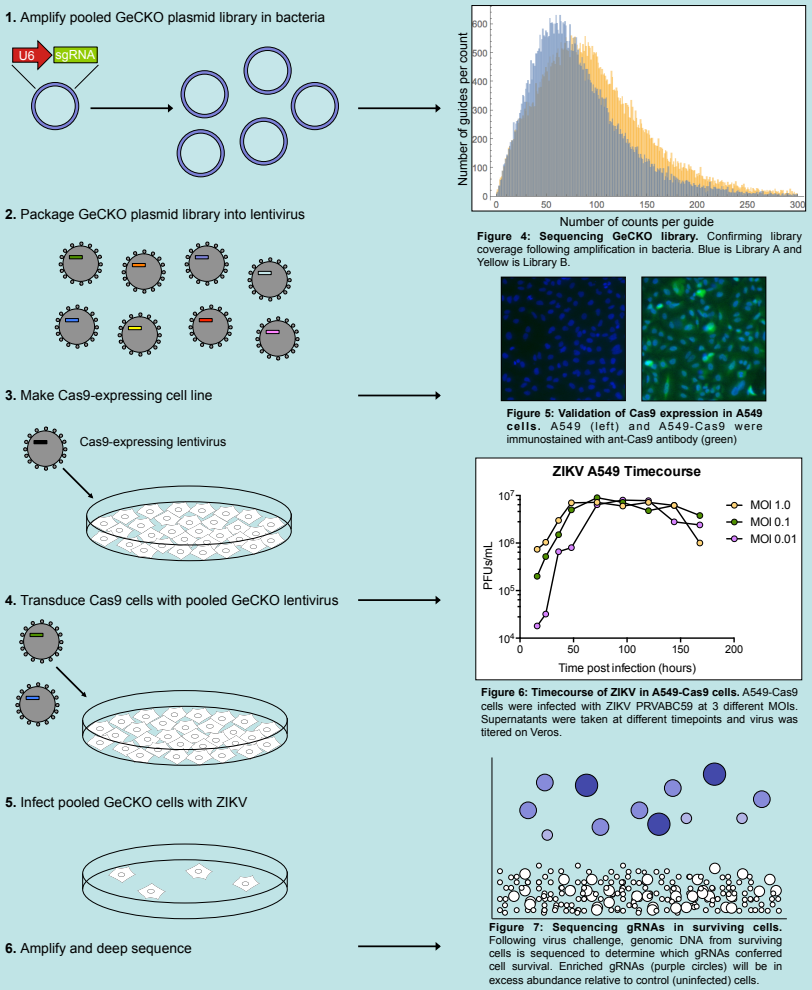

Figure 4: Sequencing GeCKO library. Confirming library coverage following amplification in bacteria. Blue is Library A and Yellow is Library B.

Figure 5: Validation of Cas9 expression in A549 cells. A549 (left) and A549-Cas9 were immunostained with ant-Cas9 antibody (green)

Figure 6: Timecourse of ZIKV in A549-Cas9 cells. A549-Cas9 cells were infected with ZIKV PRVABC59 at 3 different MOIs. Supernatants were taken at different timepoints and virus was titered on Veros.

Figure 7: Sequencing gRNAs in surviving cells. Following virus challenge, genomic DNA from surviving cells is sequenced to determine which gRNAs conferred cell survival. Enriched gRNAs (purple circles) will be in excess abundance relative to control (uninfected) cells.

Figure 3: Genome-wide knockout screen using the two vector GeCKO (Genome-scale CRISPR Knock-Out) Library (V 2.0). We are using the two library CRISPR system from the Zhang lab (genome-engineering.org). Following amplification of the library and generation of Cas9-expressing cells, pooled GeCKO lentivirus is made and used to transduce Cas9 cells. After allowing gene editing to take place, pooled GeCKO cells are infected with virus until cytopathic effect has occurred and surviving cells have formed pools. The cells are harvested and genomic DNA amplified and sequenced for gRNAs.

CRISPR SCREENING FOR RVFV

Genome-wide CRISPR screen for RVFV-MP12 reveals several genes involved in the heparan sulfate biosynthesis pathway

- 293T pooled GeCKO cells were infected with RVFV-MP12 at an MOI of 0.3
- Surviving colonies of cells were harvested and the genomic DNA amplified
- Replicate experiments revealed several genes involved in the heparan sulfate biosynthesis pathway

Experiment 1	Experiment 2
SLC35B2	SLC35B2
B3GAT3	PEX13
EXT2	ACAT2
B3GALT6	TPD52L3
COG2	

Table 1: Top hits from a genome-wide CRISPR screen with RVFV-MP12 in 293T cells. Genes involved in the heparan sulfate biosynthesis pathway are underlined.

FUTURE EXPERIMENTS

- Continue primary screens for ZIKV using the GeCKO V2.0 library
- Confirm top hits for both ZIKV and RFVF-MP12 screens with single clone knock-outs
- Screen for new viral host factors in heparan sulfate-deficient cell lines

REFERENCES

1. Nowakowski, T.J. et al. Expression Analysis Highlights AXL as a Candidate Zika Virus Entry Receptor in Neural Stem Cells. Cell Stem Cell. 2016. 18: 1-6.

2. Shalem, O, et al. Genome-Scale CRISPR-Cas9 Knockout Screening in Human Cells. Science. 2013. 343: 84 – 87.

3. A Haploid Genetic Screen Identifies Heparan Sulfate Proteoglyans Supporting Rift Valley Fever Virus Infection. J Virology. 2016. 90: 1414 – 1423.

4. Boer, et al. Heparan Sulfate Facilitates Rift Valley Fever Virus Entry into the Cell. J Virology. 2012. 86: 13767 – 13771.

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Top Hits # of reads Gene_guide 2158921 >PPIL3_1 2087217 >SLC35B2_3 217015 >CCL14_2 136467 >B3GAT3_1 67423 >EXT2_2 18338 >CDC42BPA_2 16553 >COG2_1 11693 >SLC35B2_2 3364 >EXT2_3 3155 >B3GALT6_2 1057 >FGF11_2

Top Hits # of reads Gene_guide 2783196 >SLC35B2_1 2172151 >SLC35B2_2 456246 >PEX13_3 346627 >TPD52L3_2 108728 >ACAT2_3