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Validation of Novel and Known Host Factors in CRISPR Genome-wide Screening for Zika Virus

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Abstract

Zika virus (ZIKV), a member of the *Flaviviridae,* is an emerging pathogen that was declared a Public Health Emergency by the World Health Organization. ZIKV is transmitted from mosquitos and symptoms are typically mild and do not require any necessary treatment. Some of the common symptoms are a mild fever, skin rashes, muscle and joint pain, and conjunctivitis. In addition to these mild symptoms, the CDC concluded that Zika virus infection of pregnant women can cause microcephaly and other severe brain defects in newborn babies. This initiated congress to divert about $589 million in unspent funds designated to fight Ebola in an effort to eradicate mosquitos and make an urgent push to develop vaccinations and anti-viral therapeutics against the Zika virus[1]. Since little is known about Zika, many researchers are looking for the host factors of ZIKV. Sandia National Laboratories is combating this research by utilizing CRISPR, a genome editing technology that was discovered in 2012 by teams led by Jennifer Doudna and Emmanuelle Charpentier[2]. CRISPR analysis utilizes Cas9, an enzyme that can target a section of DNA in the genome and cut the targeted DNA, leading to a specific knock out of a gene. This technique allows researchers to identify host factors that are important to Zika. We have performed genome-wide CRISPR screens for ZIKV and now our aim is to verify those hits. This will allow us to better understand the ZIKV life cycle and identify potential therapeutic targets to aid in countermeasures for ZIKV and potentially other closely related flaviviruses.

Introduction

Zika virus:

- First discovered in 1947, named after Zika forest in Uganda where it was found
- First human case in 1952
- February 1, 2016 the WHO declared Zika a Public Health Emergency of International Concern
- Common symptoms: fever, rash, and joint pain and CDC has confirmed that Zika infection during pregnancy can lead to microcephaly and various other brain defects in newborns[3]

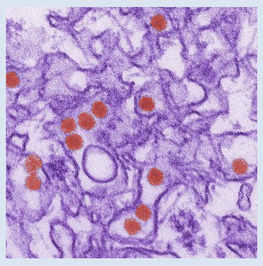

Figure 1. Zika virus under an electron microscope[5].

CRISPR/Cas9 technology:

- Short RNA sequence (guide RNA) is designed to target and silence a specific gene
- Cas9 (RNA-guided endonuclease) helps recognize the targeted site using a PAM (NGG) sequence
- Benefits: complete loss of function of gene targeted, easy to customize and optimize[4]

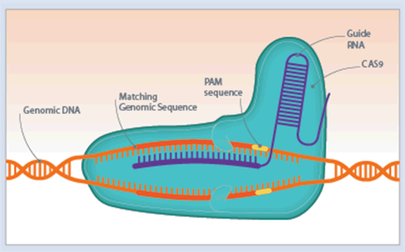

Figure 2. CRISPR/ Cas 9 mechanism Guide RNA attached to the complementary genomic DNA adjacent to the PAM sequence [6].

Screening overview:

- Genome-wide CRISPR screens to identify host factors for Zika and learn more about the virus's lifecycle

1. Use GeCKO plasmid library, packaged into lentivirus
2. Make Cas9-expressing cell line
3. Transduce Cas9 cells with GeCKO lentivirus
4. Infect cells with Zika virus
5. Amplify and deep sequence

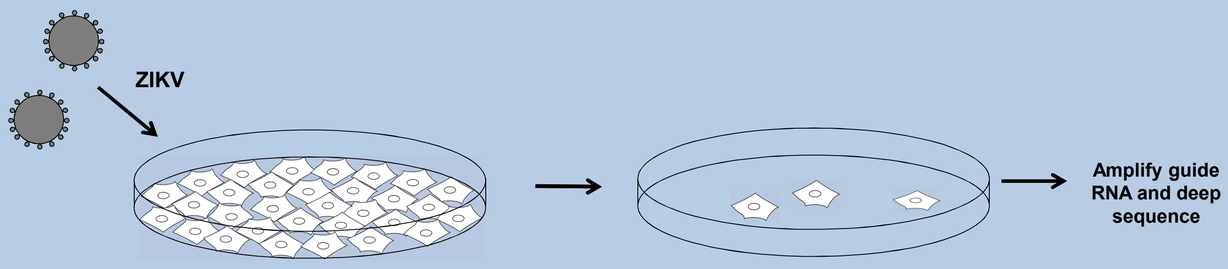

Figure 3. After infecting the pooled GeCKO cells (left dish) with ZIKV. We sequenced DNA from the surviving cells (right dish) to determine which genes are important host factors for Zika Virus infection.

Top Hits

Gene Name	Function	Guide RNA
AXL	Receptor tyrosine kinase	CAGAGCCCGTGGACCTACTC
EMC3	Subunit of ER membrane complex that is required for nuclear fusion	TCCGAAGCCCAAATACATTG
ATP6V0C	ATPase transporting subunit. Acidification of intracellular organelles	GCGCTCACCGCTGAAGACCA
MMGT1	Mediates magnesium transport	CAGGCACTTACGCTGCGCAG
WDR7	Binding, involved in cell cycle progression	GTGACATCCTGTTACGATCG
EMC6	Subunit of ER membrane complex that is required for nuclear fusion	ACGGCCGCCTCGCTGATGAA
PAQR5	Receptor activity, progesterone binding	CGAACAGGATGCCTTGCTCA
PTK7	WNT signaling pathway, cell polarity	CACGGAGCGGCGTTTCGCCC
DKKL1	Interacts with WNT pathway	CAGCCACCTCCAGATCGACA
DYTN	Downregulation of WNT pathway	TTCTCACGACAATGTACAAC

Table 1: Top hits from a genome-wide CRISPR screen with Zika Virus. Guide RNAs used to create the knockout are listed on the right. *Underlined Gene names are novel hits.

Validating Hits

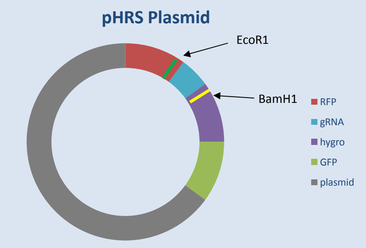

Figure 4: pHRS Plasmid Composition. The pHRS plasmid is transfected into cells to act as a reporter for the pCas9 guide plasmid. The guide RNA is inserted using restriction enzymes EcoR1 and Bam H1.

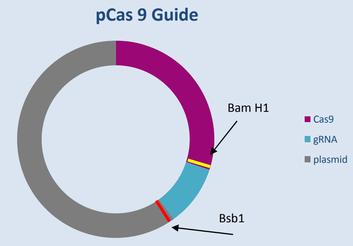

Figure 5: pCas 9 Guide Plasmid Composition. The pCas9 Guide plasmid is transfected into cells to knock out the specific genes using the Cas9 and a guide RNA encoded in the plasmid. The guide RNA is inserted using restriction enzymes Bsb1 and Bam H1.

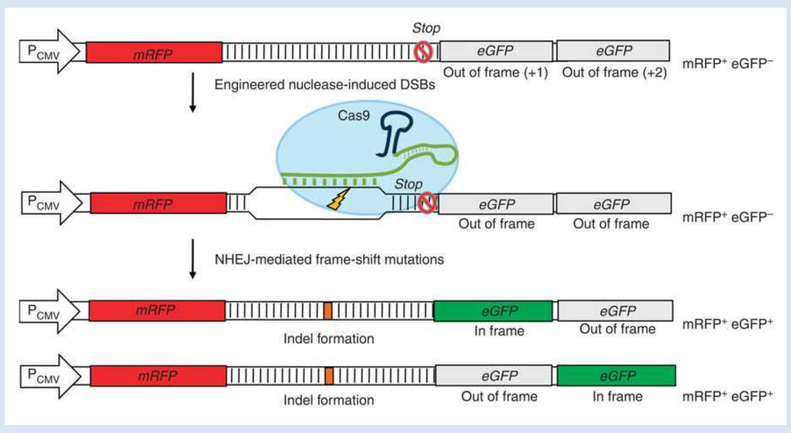

Figure 6: Cas9/Reporter Line interaction. Cas9 utilizes the guide RNA to find the complementary DNA sequence adjacent to a PAM sequence and knocks out the gene due to formation of an indel. This causes the GFP to move into frame and express a green fluorescence signaling that the CRISPR knockout was successful[7].

Future Experiments

- Continue confirming novel and known top hits for ZIKV screen with single clone knock-outs using cell reporter lines.
- Use found host factors to develop or screen for drugs to create anti-viral therapeutics.

Acknowledgements

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References

1. Kelley, Erin. Top Health Officials Fear Congress will leave without appropriating Zika Funds. USA Today. 2016.
2. Park, Alice. The Gene Machine: What CRISPR experiments mean for humanity. TIME magazine. 2016.
3. CDC website. About Zika.
4.Riordan, Sean. Application of CRISPR/Cas 9 for biomedical discoveries. *Cell and Bioscience. 2015.*
5. Wappes, Jim. Local Zika cases in 33 nations as GBS numbers climb. *University of Minnesota CIDRAP*. 2016.
6. Genome Engineering using CRISPR (Cas 9). *AG Scientific Blog*. 2014.
7. Ramakrishna, et al. "Surrogate reporter-based enrichment of cells containing RNA-guided Cas9 nuclease-induced mutations." Nature communications 5 (2014).

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