Investigating Regions of Interest in Chikungunya Virus Nonstructural Proteins

Ian Klevans, Jennifer Loome, Dr. Mark Heise
UNC Chapel Hill

THE UNIVERSITY of NORTH CAROLINA at CHAPEL HILL

Abstract

Chikungunya virus is a member of the Alphavirus genus that infects humans and may cause severe illness. The viral genome encodes viral proteins, one of which is nonstructural protein 2 (nsp2). One of the functions of nsp2 is its ability to shut off cellular transcription via the degradation of RNA Polymerase Binding Domain 1, or RPB1. Shutoff of cellular transcription is cytopathic, or cell-killing. This experiment explored which regions in nsp2 are necessary for this cytopathic effect. Here we mutated specific amino acid regions of interest within nsp2, created a pCMV plasmid vector system to express mutant nsp2, transfected these mutant nsp2 plasmids into cells, and observed cell death. In future experiments, we hope to co-express a fluorescent tagged RPB1 with nsp2, allowing for more specific determination of which mutants lost the ability to degrade RPB1 after transfection.

Preliminary Data

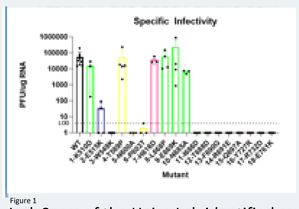


Figure 1

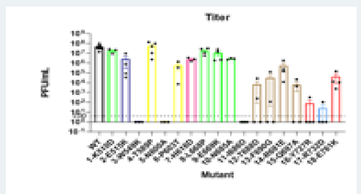


Figure 2

Jack Sears of the Heise Lab identified several nsp2 regions of interest that, when mutated, lowered specific infectivity and titer levels in mammalian cells. We hypothesize that these regions are essential for cell killing by nsp2.

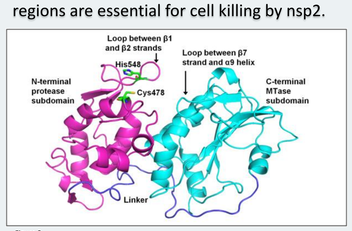


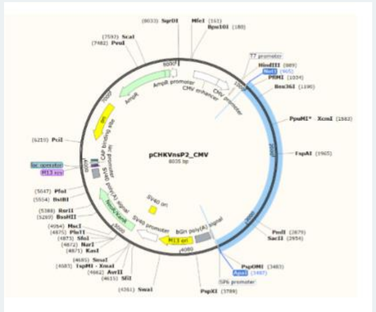
Figure 3

Figure 1. Specific Infectivity of Mutants of Interest: RNA encoding CHIKV with mutated or WT nsp2 was electroporated into BHK21 cells, and the number of cells that initiated productive infection per µg of RNA (specific infectivity) was measured. As demonstrated by the figure, mutants 12-16 had specific infectivity levels below the limit of detection.
Figure 2. Titer assay: Virus was produced by electroporating RNA encoding CHIKV with mutated or WT nsp2 into BHK21 cells. Supernatants were harvested, and viral titer was measured by plaque assay. Again, mutants 12-15 (shown in brown) generally had lower titer values than the rest of the mutants, indicating that the mutated amino acids are important for viral replication.
Figure 3. Shows the structure of CHIKV-nsp2 and its domains.

Objectives

The research carried out attempted to determine if the protein regions of interest would affect the virus' ability to kill mammalian cells.

Methods



pCMV Vector Synthesis

•A cytomegalovirus promoter was linked to our nsp2 and GFP proteins using around-the-world-PCR and Gibson Assembly to create the vector that would deliver said viral proteins. The pCHIKVnsp2_CMV plasmid contains antibiotic resistance genes that allow for selection with geneticin.

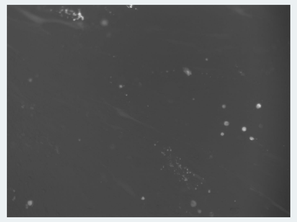
Cell Culture and Transfection

•BHK21 cells were transfected with 3 µg of plasmid per well with 9 µl lipofectamine. Selection was performed by adding geneticin 1 day post transfection at 3 µg/ml and changing the medium every 2 days.

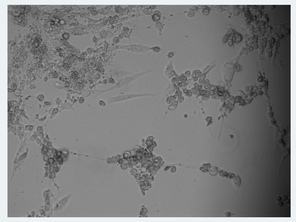
Mutagenesis

•Mutations in nsp2 were induced using around-the-world PCR using primers (designed by Jack Sears).

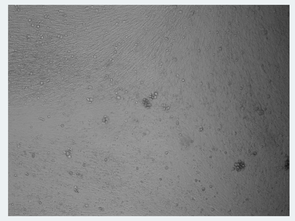
Results



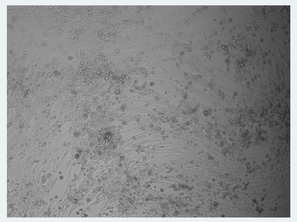
2.1 Transfection Optimization



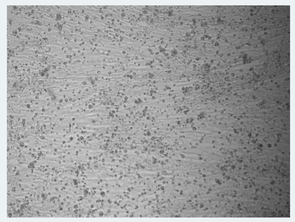
2.2 Antibiotic Optimization



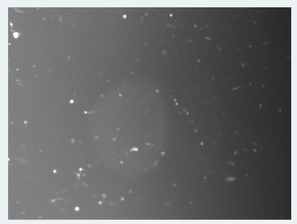
2.3 Untransfected, healthy BHK-21 Cells



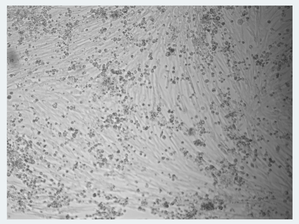
2.4 Lipofectamine only



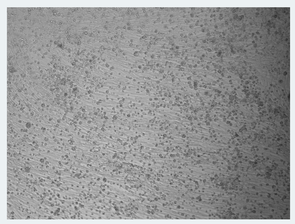
2.5 pCMV GFP plasmid, bright field microscope



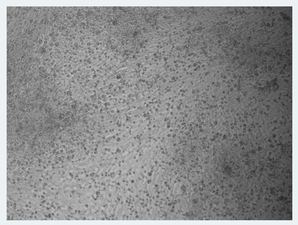
2.6 pCMV GFP plasmid, GFP-specific laser



2.7 nsp2 wild type middle

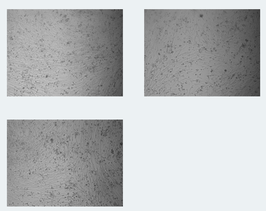


2.8 nsp2 mutant 12 middle

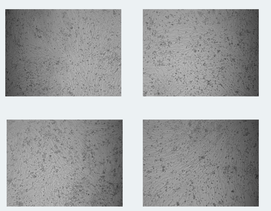


2.9 nsp2 mutant 13 middle

Results from this experiment are preliminary and only serve to demonstrate the ability to observe cell death from the fully optimized assay. A pCMV GFP plasmid is shown in figures 2.3 and 2.6 to show the expression of GFP in BHK-21 cells. Figure 2.2 shows the ability of geneticin antibiotic to kill cells. Figure 2.7 shows the ability of the nsp2 wild-type protein to kill cells. The cell death assay of mutants 12 and 13 are shown in figures 2.8-2.14. Note: the results of this experiment are not conclusive and future testing will need to be done to conclusively determine the killing ability of nsp2 with mutations 12 and 13. Additionally, images were taken 24 hours post antibiotic treatment instead of 48 hours.



nsp2 mutant 12 (additional images) 2.10-2.12



nsp2 mutant 13 (additional images) 2.13-2.14

Future Directions

Identifying potential regions of interest in chikungunya virus' nsp2 may help identify targets for antiviral drugs that can fight the virus' ability to kill mammalian cells. A western blot will be used to confirm appropriate expression of viral protein in the transfected cells. The degradation of RBP1 can also be analyzed to determine the cell killing mechanism of nsp2.