Culturing Viruses from Wild Deer in Utah

Brennan Winkler, Department of Biology, Utah State University; Justin Moscon, Department of Chemistry and Biochemistry, Utah State University; Eric Delwart, Department of Laboratory Medicine, University of California-San Francisco; Annette Roug, Utah Division of Wildlife Resources; Bart Tarbet, Department of Animal, Dairy and Veterinary Sciences, Utah State University

Abstract

In the Spring of 2016, two deer showing clinical signs of neurological illness were reported to the Utah Division of Wildlife Resources. The animals were euthanized and post-mortem tissues collected for identification of any disease causing agents. Brain and spleen samples were sent to Dr. Eric Delwart, University of California-San Francisco for metagenomics evaluation. Mobukvirus (described once) and novel picornavirus and bovine parvovirus were tentatively identified. However, cell culture of the viruses is necessary for further characterization and genomic sequencing. If these novel viruses can be cultured, it may be possible to develop serologic tests for additional field investigations in wildlife.

We (USU) received tissue samples in June and have started viral evaluation in a number of cell lines. The cell lines being evaluated include: MDBK cells (bovine kidney), MDCK cells (canine kidney), BHK-21 cells (hamster kidney), Vero cells (African green monkey kidney), ST cells (swine testis), SK-RST cells (swine kidney), RD Cells (human rhabdosarcoma), Hela cells (human adenocarcinoma), and the mosquito C636 cell line.

In this experiment MDBK, SK-RST, and ST cells were used. Lipofection was performed to produce more virus using viral RNA extracted from the brain tissue. Supernatant from the lipofection was used to infect fresh confluent cells. Supernatant from the first infection were used to infect more cells using dilutions. The results are inconclusive as CPE has not been clearly characterized within the samples.

Methods

Tissue Lysis and RNA Extraction
- Whole frozen deer brain tissue is excised and placed in MEM media and brought to room temperature
- Tissue is Sonicated to disrupt brain cell membranes in preparation for RNA extraction
- Viral RNA extraction is performed on prepared tissue using Qiagen’s QIAamp Viral RNA Mini Kit
- RNA concentration and purity is determined using a NanoDrop Spectrophotometer

RNA Lipofection of different Cell Lines
- Cells are cultured and seeded in a 12 well plate and incubated for 24 hrs to achieve confluency
- Cells are infected using supernatant from the Lipofection plate and incubated at 37°C and checked for CPE
- Supernatant from infected cells with observed CPE on the first plate are used to infect a second plate using a 1:3, 1:10, and 1:30 dilution for each cell type.

Subsequent Infections

Visualizaion was used to determine the successfulness of the experiments.

Results

1st Infection
- MDBK Sample
- MDBK Control
- SK-RST Sample
- SK-RST Control

2nd Infection
- SK-RST Control

Conclusions/Discussion

The results of this experiment are inconclusive concerning whether or not any virus derived from the deer brain have been cultured using these methods. Further testing is necessary to determine whether cells are dying of toxicity or viral infection. Cytopathic affect (CPE) occurs when a host cell is infected with virus and the cell dies or is lysed open (broken open) as a result of infection. This is able to be visualized under a microscope to determine whether cells are potentially infected with viral material. This visualization was used to determine the successfulness of the experiments.

Lipofection was first used to introduce extracted viral RNA from the deer brain into cultured cells in order to produce more virus; visible CPE should have been apparent. Cell density in the 12-well plate was too low to determine whether the lipofection successfully worked and CPE could not be ascertained. Cell death may have consequentially resulted from low cell density.

The first infection has some visible cell death. This result could be due to toxicity of residual lipofection reagent and dead cells. The second infection also shows inconsistent results as the 1:10 dilution appears to have more cell death than the 1.3 dilution. Although virus could be producing CPE, the results are indefinite.

References


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