Zika Virus as a Potential Adjuvant in Medulloblastoma Treatment

Clairice M. Pearce, Andrew Crane, Maple L. Shiao, Christopher Sipe, Walter C. Low

Stem Cell Institute, Department of Neurosurgery, University of Minnesota, Minneapolis

UNIVERSITY OF MINNESOTA
Driven to Discover℠

Abstract

Zika Virus (ZIKV) has been thought to cause microcephaly in infants of infected mothers, because fetal neural stem cell death has been shown to be mediated by the virus. Since it is known that neural stem and brain tumor cells possess similar properties, we explored the feasibility of utilizing ZIKV as an oncolytic virus against human brain tumors. Specifically, we sought to characterize ZIKV infectivity in the medulloblastoma cell line (DAOY), as this cancer is the most prevalent amongst children and has been proven difficult to treat through chemotherapy, radiation, and surgical procedures. DAOY cells were incubated with ZIKV for 3 days, and RNA was isolated and used for qRT-PCR. Envelope, NS2, and NS5 proteins characteristic of the virus demonstrated a fold change of 10,000 or greater, indicating high viral infectivity. ZIKV presence within DAOY cells was also observed by immunocytochemistry. Viral kinetics were further assessed by means of a viral plaque assay. We observed an absence of plaques, indicating that ZIKV may not be released from infected cells. Thus, ZIKV could be better used as an adjuvant in stimulating the immune system for tumor targeting rather than as a solely oncolytic virus. We then tested the ability for ZIKV to act as an adjuvant in a murine model of glioma (GL261). Results demonstrated increased survival of mice injected with ZIKV in conjunction with a vaccine immunotherapy as compared to those only injected with ZIKV. These results provide support for the potential of ZIKV as a mechanism in medulloblastoma-targeted treatment.

Methods

- DAOY cells were grown on Fibronectin chamber slides and stained for AXL, TIM-1, DC-SIGN, and Tyro3 receptors
- DAOY cells were incubated with ZIKV for 3 days at an MOI of 0.5
- RNA was then isolated from infected and control cells and used for two-step RT-qPCR
- Primers tested for AXL, MERTK, and TYRO3 receptors as well as ZKV envelope, NS2, and NS5 gene expression
- cDNA obtained from RT-qPCR was analyzed by gel electrophoresis

ZKV Receptor Expression of DAOY

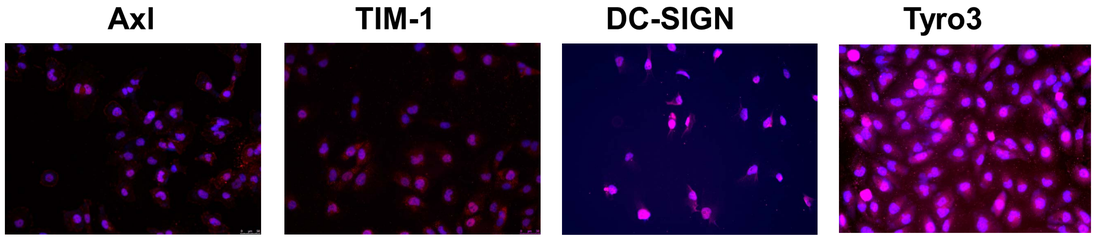

Figure 1. Presence of Receptors in DAOY cells by Immunohistochemistry
Suggested cell entry receptors were stained by immunohistochemistry and identified in DAOY.

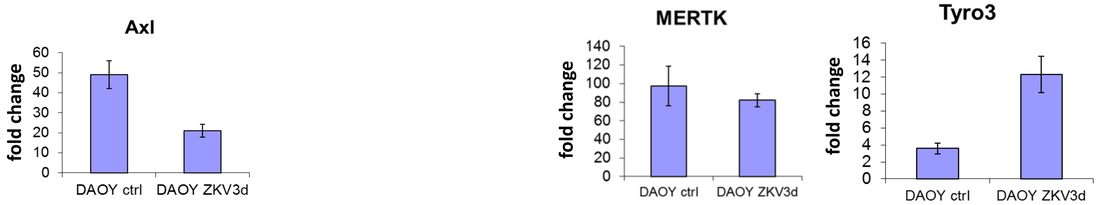

Figure 2. Presence of ZKV Entry Receptors in DAOY through RT-qPCR
Results indicate relative differences in fold change between control and 3 day ZKV-infected DAOY cells for Axl, MERTK, and Tyro3 receptors. For Axl and MERTK receptors, fold change decreased from uninfected to 3 days post infection. Conversely, the fold change in Tyro3 significantly increased from the time of infection.

GL261 *in vivo* Studies

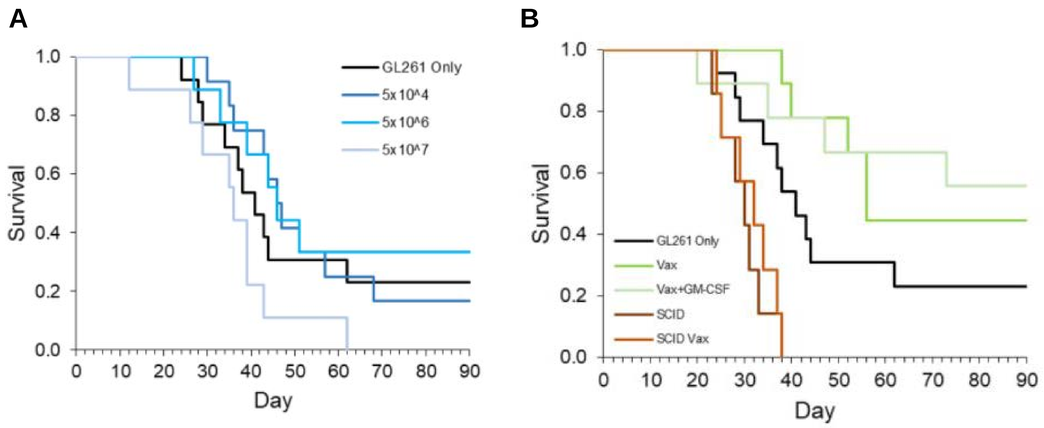

Figure 3. Survival Curves of *in vivo* murine models
A. ZIKV has a modest oncolytic effect against GL261 implanted tumors
B. Tumor vaccines with ZIKV adjuvants increase survival of GL261 tumor implanted mice

GL261 Viral Plaque Assay

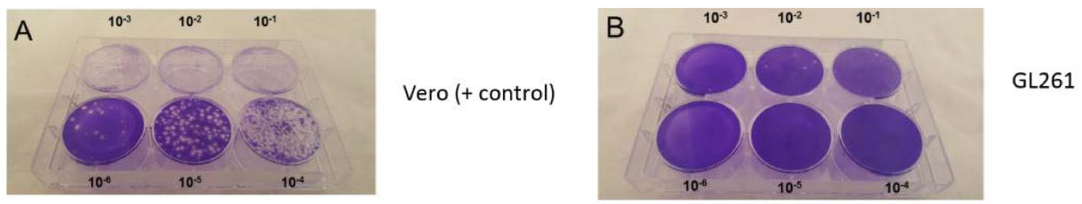

Figure 4. Viral Kinetics Assessed by Plaque Assay
A. Vero positive controls used to determine viral concentration in terms of infectious dose. Number of plaque
B. GL261 plaque assay demonstrates an absence of plaques, indicating ZIKV particles are not released from the host cell during replication

ZKV Infection of DAOY

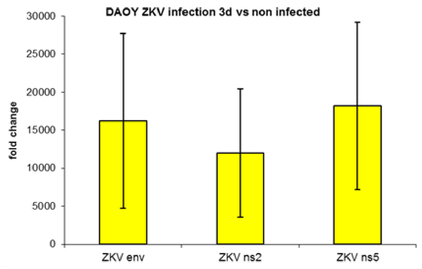

Figure 5. High ZKV Infectivity Characterized through RT-qPCR
Proteins characteristic of ZKV are present in DAOY cells, with a fold change of 10,000 and greater between uninfected and infected cells.

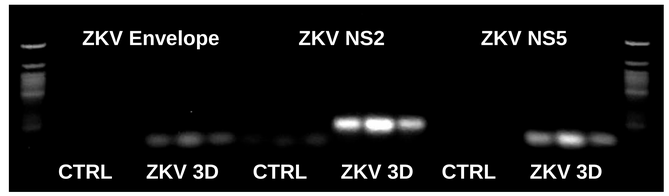

Figure 6. Validation of DAOY ZKV Infection
Control and 3 day ZKV-infected cDNA samples were run in triplicate on 2% agarose gel. Envelope, NS2, and NS5 genes characteristic of ZKV fluoresce, indicating infection.

Conclusions and Future Directions

Together, these results provide further evidence for eradication and treatment of the human medulloblastoma using ZKV:

- AXL, TIM-1, MERTK, and TYRO3 receptors characteristic of ZKV-targeted neural stem cells are present in DAOY cells
- DAOY demonstrates high infectivity after 3 days of exposure to ZKV
- Further analysis of ZKV kinetics in DAOY will be explored through viral plaque assay
- Apoptosis and cell death can be identified in DAOY cells by flow cytometry staining for Caspase 3
- Transcriptome analysis of RNA-Seq data will be used to explore mechanisms of ZKV infection in DAOY cells

Funding

This project was supported by the Randy Shaver Cancer Research Foundation and the University of Minnesota's Undergraduate Research Opportunities Program.

References

1. Fan, Xing, and Charles G Eberhart. 2008. “Medulloblastoma Stem Cells.” Journal of Clinical Oncology : Official Journal of the American Society of Clinical Oncology 26 (17). NIH Public Access: 2821–27. doi:10.1200/JCO.2007.15.2264.
2. Medulloblastoma. St. Jude Children’s Research Hospital. 2017.
3. Hamel, Rodolphe, Ophélie Dejarnac, Sineewanlaya Wichit, Peeraya Ekchariyawat, Aymeric Neyret, Natthanej Luplertlop, Manuel Perera-Lecoin, et al. 2015. “Biology of Zika Virus Infection in Human Skin Cells.” Journal of Virology 89 (17). American Society for Microbiology: 8880–96. doi:10.1128/JVI.00354-15.

UNIVERSITY OF MINNESOTA
Stem Cell Institute