Cellular Defense Against Vesicular Stomatitis Virus

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Objectives

The project involves identifying the role of RNA interference (RNAi) as a mechanism of antiviral response against Vesicular Stomatitis Virus (VSV). The gene, *Dicer-1*, is an integral component of the RNAi response. Our objective is to create cell lines that lack the *Dicer-1* gene, using a novel method called CRISPR/Cas9 genome editing, and compare the infectivity rate of VSV in cells that maintain the *Dicer-1* gene to cells that lack the gene. We will also examine if the expression of two other genes - *miR-23* and *miR-93* - are increased due to VSV infection, as they have been recently reported to mediate antiviral effects to other viruses through an RNAi dependent mechanism. Finally, we will be building on data collected over the summer that demonstrated the antiviral properties of curcumin - a compound found in the spice, turmeric - in robustly controlling VSV infection. We will analyze if the antiviral effect of curcumin is mediated by RNAi genes, specifically *Dicer-1*, *miR-23* and *miR-93*.

Description

This project involves understanding the molecular interaction of VSV with mammalian cells, and how cells may be able to resist infection by VSV. One cellular mechanism of antiviral defense that has been recently shown in animal cells is RNA interference (RNAi) (Huppi et al. 2005). The effectiveness of RNAi as an antiviral mechanism varies depending on the type of virus and on the cell type being infected. We are studying if RNAi can protect mammalian cell lines from VSV, for which there are no current reports in the literature. *Dicer-1* is a gene that codes for a protein that cleaves long RNA molecules into very small RNAs (only 20-22 nucleotides long), to be used to initiate a RNA-induced silencing complex (RISC). The RISC complex thereby targets the other copies of the original long RNA for degradation, using the small RNAs as "guides" to help locate the long RNA copies, which is the basis for RNAi (Tijsterman and Plasterk 2004). Thus, by deleting the *Dicer-1* gene, no "guide" RNAs can be generated for the RISC complex, and so no RNAi can be induced.

This project can be divided into 3 related but independent goals.

GOAL 1. Create *Dicer1* "knock-out (KO)" Vero cells using CRISPR/Cas9 technology, and test if *Dicer-1* deficient cells are most susceptible to infection by VSV.

Since 2013, a genome editing method called CRISPR/Cas9 has revolutionized the field of genetics. It is currently the fastest, cheapest, and most reliable method of genome editing that has ever been available for commercial use (Zhang et al. 2014). I propose to use this technology to generate Vero cells (a cell line derived from monkey kidney cells, commonly used for viral assays) that have the *Dicer-1* gene deleted. Using a genetically modified form of VSV that expresses a green fluorescent protein (VSV-GFP) when it infects cells, I will infect *Dicer-1* deficient Vero cells with VSV-GFP and compare infectivity rates to control Vero cells, using different concentrations of virus and different end timepoints. Infection rates will be measured

using (a) fluorescent microscopy and quantitative PCR (qPCR) to detect early infection times - 1h, 6h, and 24h; and (b) plaque assays to quantify viral infections for longer infection times - 48h and 72h. Based on previous research of RNAi in viral infection, I hypothesize that *Dicer-1* knock-out Vero cells will show higher rates of infection than control cells, since they will not have the ability to use RNAi as a defense system.

GOAL 2. Measure the expression of RNAi related genes in response to VSV infection.
If RNAi is a response induced by VSV infection, I hypothesize that genes related to RNAi should be "turned on" during infection. *Dicer-1* is a key gene for RNAi, and thus I predict increased levels of DICER-1 will be found after VSV infection. The gene Argonaute-1 is also a key gene in the RISC complex (Ikeda et al. 2006), and so I predict levels of ARGONAUTE-1 will also be increased after infection. Finally, two other genes - *miR-23* and *miR-93* - had increased expression after infection of VSV, but in non-mammalian cells (Otsuka et al. 2007). These two genes are part of a class of RNA molecules called "microRNAs," which use the RISC complex to target larger RNA molecules, and so are part of the RNAi system. I predict these two microRNAs will be increased in response to VSV infection in Vero (mammalian) cells. The expression levels of these genes will be measured by qPCR. Vero cells will be infected with VSV-GFP virus, and RNA from the cells will be collected 1h, 6h, and 24h after infection.

GOAL 3. Test if curcumin antiviral effects on VSV are mediated through RNAi.
Curcumin is from a class of compounds called curcuminoids, that are found in the extract of turmeric, a rhizome that is commonly used in Asian curries as a spice. It has been used in traditional Indian medicine (Ayurvedic medicine) for hundreds of years to treat cuts, wounds, and gastrointestinal problems (Hatcher et al. 2008). Modern research with curcumin has found very powerful effects of curcumin in controlling inflammation and as an antioxidant (Hatcher et al. 2008), and clinical trials have shown beneficial effects in helping to treat cancer (specifically colon cancer) (Kawamori et al. 1999). Recent studies have shown the ability to control viral infections, although there are no published reports of curcumin with VSV in mammalian cells. Over the summer, I worked with Dr. Ambegaokar on the antiviral properties of curcumin, and I found a very strong antiviral effect of curcumin with VSV in Vero cells. There are many ways in which curcumin may prevent infection, including an ability to induce RNAi. To test this, I will treat Vero cells with the same concentration of curcumin that showed antiviral properties, and will measure the expression of the RNAi genes listed in Goal 2: *Dicer-1*, *Argonaute-1*, *miR-23*, and *miR-93*. RNA from curcumin-treated and control Vero cells with be collected at 1h, 6h, and 24h time points, and expression of these genes will be measured by qPCR.

Timeline
The project will start during the summer of 2016 and continue through the end of the fall 2016 semester.

May-June 2016: Create *Dicer-1* "knock-out (KO)" Vero cells using CRISPR/Cas9 technology.

July 2016: Test if *Dicer-1* deficient cells are more susceptible to infection by VSV.

August 2016: Measure the expression of RNAi related genes in response to VSV infection.

September 2016: Treat Vero cells with curcumin and collect RNA from treated & non-treated cells.

October 2016: Measure the expression of RNAi related genes in response to curcumin.

November 2016: Analyze data and prepare for presentations.

Evaluation and Sharing

If I get the funding for this project, I will be the first to use the CRISPR/Cas9 genome editing technology in OWU. I hope to work out any difficulties with this new technology, so that I can introduce it to the OWU scientific and nonscientific communities. Since it is a relatively inexpensive, it has the potential to become a more standard technology that can be used and taught at OWU. My project can act as a foundation for future students who have similar research interests. The *Dicer-1* knock-out cell lines I will generate can also be used in the future for a wide variety of research projects.

Research of this caliber has potential to be published in a peer reviewed scientific journal. Other scientists will be able to evaluate my work during the peer review process, and can perform follow-up research on what I publish. The project can also be presented at meetings sponsored by research societies like the Ohio Branch American Society for Microbiology (OBASM). This conference brings together microbiologists from all over Ohio, *where we engage in an idea-sharing process about new kinds of breakthrough research in microbiology.* The poster will also be public for OWU students as it will be posted in the hallways of the science center. It would be a pleasure for me to have my research shared with fellow OWU students, science majors and non-science majors. It would be my contribution, however small, to OWU's learning environment.

Personal Statement

Growing up in Ethiopia, I had a deep, internalized understanding of diseases. In many instances, I saw infectious diseases affect people I know. I saw first-hand the symptoms that pathogens can cause. My experiences with diseases have inspired me to understand them and learn about how to counteract them, and that is the basis of my research. Attending OWU, I have been able to learn about microbes down to their detailed mechanisms. I started to think of them as human tools for further knowledge rather than just pathogens. OWU has given me a deeper understanding of microbes, and with this project, I want to take that understanding a step further to make an original contribution to the field of microbiology.

Working with cells from spring semester of my freshman year has made me very knowledgeable about their life cycles. The more I understood *about them, the more I realize* there is *still so* much to learn about them. When I learned that I could apply for a grant as an OWU student, I

thought of filling in the gaps of knowledge that exists in science. Scientific research is my passion, and to be able to have some meaningful impact in my field is very significant to me. I have taken many upper level science courses with labs. I learned about RISC and CRISPR/Cas9 in my Genetics course (BIOL 271) during my sophomore year. I also learned about research methods from other science classes such as Cell and Molecular Biology (ZOOL 351). I used the theory I learned from these classes to come up with this project idea. I am also doing a microbiology independent study this semester that involves handling, growing and reviving bacteria. Dr. Ambegaokar mentored me in cell culture techniques during my summer research. I practiced DNA extraction and PCR in my advanced biology classes. During the course of the research, if I have any questions regarding theory or experimental procedures, Dr. Ambegaokar is more than happy to *guide* me. My OWU education has given me a strong conceptual foundation which I want to take to the next level with substantial research in my field of interest. This is a perfect example of Theory to Practice.

References

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Budget

Product	Provider (Cat #)	Quantity	Price
GeneArt gRNA Synthesis Kit	Thermo Fisher	1 Kit	$500.00
GeneArt CRISPR Nuclease mRNA	Thermo Fisher	1 Kit	$309.00
Mirvana	Ambion	1 Kit	$314.00
Quick-RNA MiniPrep Kit (200)	Genesee (#11-328)	1 Kit	$662.00
Taqman Advanced miRNA cDNA Synthesis kit	Thermo Fisher (# A28007)	1 Kit	$425.00
Taqman Fast Advanced Master Mix	Thermo Fisher (#4444557)	1 Kit	$465.00
Sybr green Master Mix (EvaGreen, 200 X 20uL rxns)	Bio-Rad (#1725200)	1 Kit	$180.00
Curcumin	Sigma Aldrich	500 mg	$130.00
DMEM powdered media	Sigma Aldrich	1 Carton	$116.50
6-well cell culture plates	VWR	50 plates	$135.20
Fetal Bovine Serum	VWR (#1500-500)	(500mL)	$544.20
L-glutamine	Sigma Aldrich	1 L	$134.00
Penicillin /Streptomycin	Sigma Aldrich	100 ml	$23.00
miR-23 FAM-MGB primer/probe (250 rxns)	ThermoFisher (#A25576: 478602_miR)	1 Kit	$250.00
miR-93 FAM-MGB primer/probe	ThermoFisher (#A25576:	1 Kit	$250.00

	#478209_miR:)		
miR FAM-MGB controls	ThermoFisher (#A25576: #478056_miR:)	1 Kit	$250.00
qPCR primers for Dicer1, Argonaute1, and GFP	Integrated DNA Technologies	12 Primers	$10.00

Total **$ 4,697.90**