Genome-wide RNA interference Analysis of Viral Encephalitis Pathogenesis

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LABORATORY DIRECTED RESEARCH & DEVELOPMENT

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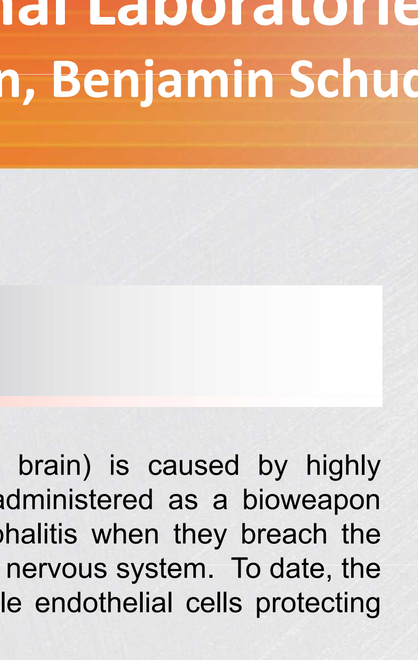
Problem

Viral encephalitis (acute inflammation of the brain) is caused by highly pathogenic viruses that could potentially be administered as a bioweapon threat. Such viruses can induce lethal encephalitis when they breach the blood-brain barrier to gain access to the central nervous system. To date, the host factors used by these viruses to dismantle endothelial cells protecting these blood vessels remain largely unknown.

Approach

RNA interference (RNAi) technology is a functional genomic approach that has recently emerged as a powerful tool to investigate host proteins involved in virus replication on a genome-wide level. By systematically silencing >20,000 individual host genes and analyzing their involvement in viral infection, a comprehensive portrait of virus-host interactions can be revealed. We will use genome-wide RNAi screening to investigate the host proteins involved in biodefense priority pathogens Rift Valley Fever virus (RVFV) and Nipah Virus (NiV) infections. The aim of this study is to elucidate the mechanisms by which these two viral pathogens induce lethal encephalitis pathogenesis.



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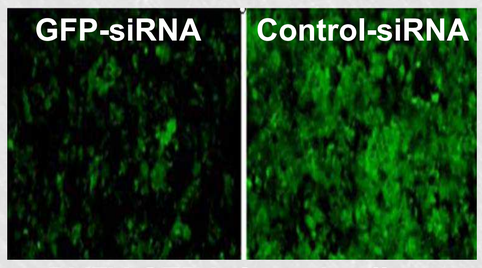
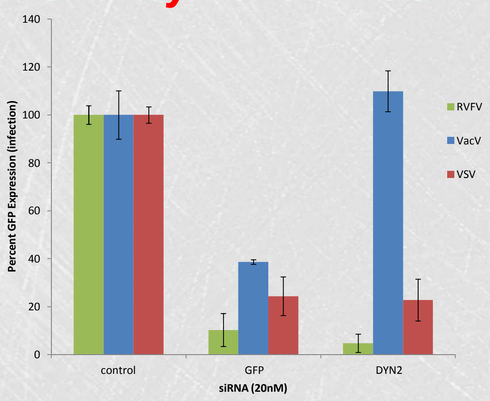
Results

Identifying siRNA controls for Genome-wide RNAi screening against Rift Valley Fever virus

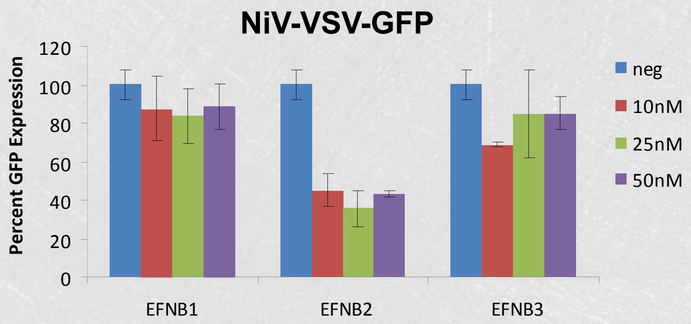


RVFV-GFP infected cells



We have identified siRNA controls targeted against a virus encoded gene (GFP) and a host protein (Dynamin) that block recombinant RVFV-GFP replication in human cell lines.

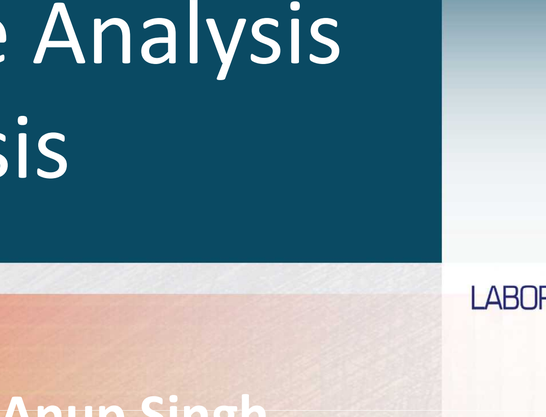
Developing Control siRNA for a Genome-wide RNAi screen against Nipah virus



siRNA targeted against the Nipah virus receptor ephrinB2 (EFNB2) blocks virus replication in human microvascular endothelial cells infected with pseudotyped Nipah virus (BSL-2) encoding GFP.

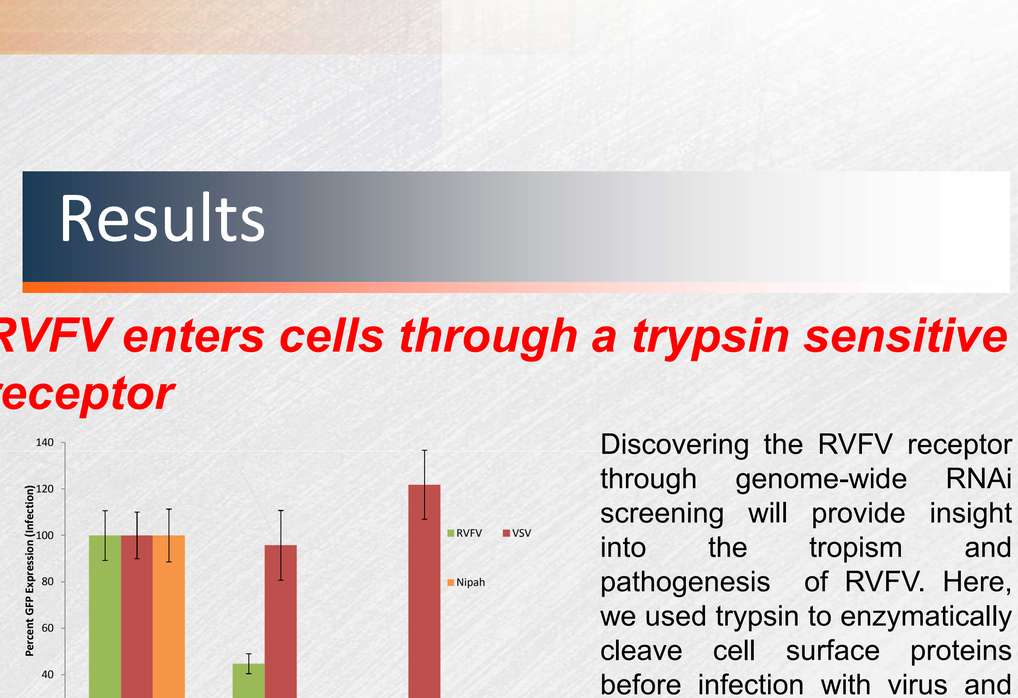
Results

RVFV enters cells through a trypsin sensitive receptor



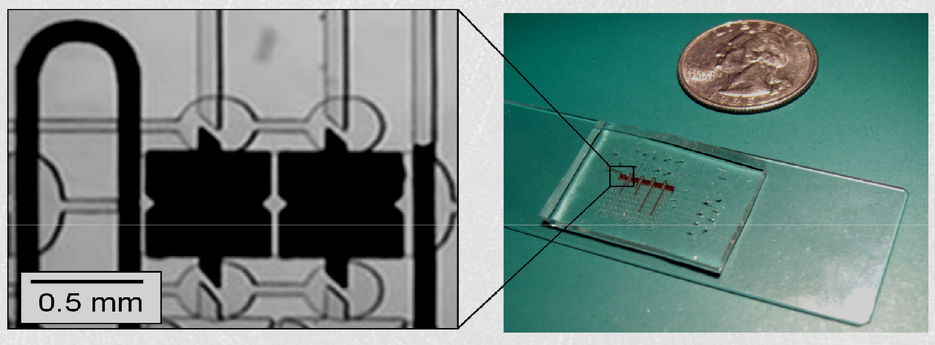
Discovering the RVFV receptor through genome-wide RNAi screening will provide insight into the tropism and pathogenesis of RVFV. Here, we used trypsin to enzymatically cleave cell surface proteins before infection with virus and found that RVFV requires a trypsin sensitive receptor for entry and infection.

Testing small molecule inhibitors of RVFV entry



Host proteins identified through genome-wide RNAi will be further characterized by small molecule inhibitors to dissect the pathways involved in infection and to aid in the development of therapeutics. Upon evaluating small molecule inhibitors of dynamin-dependent and dynamin-independent endocytosis, RVFV was found to enter cells through dynamin-dependent, caveolin-mediated endocytosis.

Developing microfluidic platforms for high throughput screening in BSL-3/4 containment



Microfluidic-based devices provide an optimal method to introduce RNAi libraries into primary cells within a portable and cost-effective platform. To this end, we are using microfluidic platforms that combine cell and siRNA array for the development of a high-level biocontainment compatible RNAi screening chip.

Significance

- Genome-wide RNA interference offers the opportunity to identify host proteins involved viral encephalitis pathogenesis
- Performing multiple genome-wide RNAi screens against viruses that induce a similar pathogenesis can identify common host protein targets for the development of *broad spectrum therapeutics*
- Developing microfluidic technology for high throughput screening in BSL3/4 containment will greatly improve the speed at which host proteins involved in highly pathogenic viral infections are identified

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