Genome-wide RNA interference Analysis of Viral Encephalitis Pathogenesis

Sandia National Laboratories

Oscar Negrete, Brooke Harmon, Benjamin Schudel, Anup Singh

LDRD LABORATORY DIRECTED RESEARCH & DEVELOPMENT

Problem

Highly pathogenic viruses such as Rift Valley Fever virus (RVFV) and Nipah virus (NiV) can induce lethal encephalitis (acute inflammation of the brain) when they breach the blood-brain barrier to gain access to the central nervous system. It has been postulated that this happens because these viruses dismantle endothelial cells protecting these blood vessels. However, the host factors involved in the pathogenesis induced by RVFV and NiV remain largely unknown. The discovery of such host factors will greatly increase the development of therapeutic strategies to reduce RVFV and NiV replication.

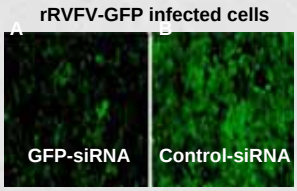
Approach

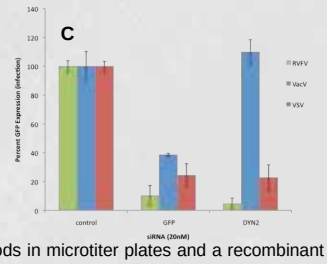
The aim of this study is to develop an innovative microfluidic RNAi screening platform to discover the host proteins involved in lethal encephalitis induced by Rift Valley Fever virus (RVFV) and Nipah virus (NiV) infections. We have chosen to use RNA interference (RNAi) technology, a functional genomic approach that has recently emerged as a powerful tool to investigate host proteins involved in virus replication on a genome-wide level (*Nature* 2010 **463**, 813-817). By systematically silencing >20,000 individual host genes and analyzing their involvement in viral infection, a comprehensive portrait of virus-host interactions should be revealed.

Example of genome-wide RNAi screening for virus-host interactions. Intensity of red color indicates the significance of enrichment for proteins required by a virus within different protein complexes grouped by function. (*Nature* **463**, 813-817).

Results

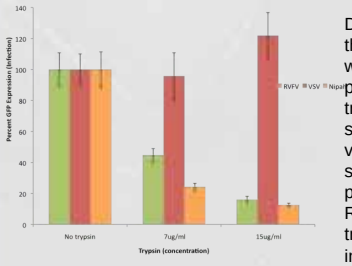
Identifying siRNA controls for Genome-wide RNAi screening against Rift Valley Fever virus





Using traditional siRNA delivery methods in microtiter plates and a recombinant RVFV that expresses green fluorescence protein (rRVFV-GFP) as a reporter of infection, we have shown siRNA targeted against the virus encoded GFP gene (GFP) blocks rRVFV-GFP replication (A) in human cell lines as compared to (B) control. The bar graph (C) clearly demonstrates the specificity of the inhibition of RVFV infection with host protein Dynamin, in addition to the GFP-siRNA control.

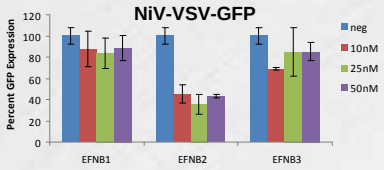
Identification of specific receptors of virus entry through genome-wide RNAi screen



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. Compared to viruses with cell surface protein -dependent (NiV) and protein-independent (VSV) entry, RVFV was found to require a trypsin sensitive receptor entry and infection.

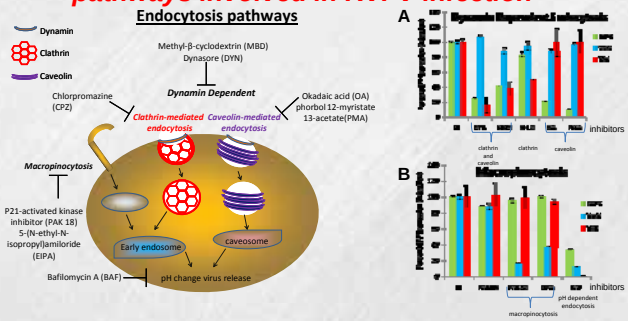
Results

Identifying siRNA controls for Genome-wide RNAi screening against Nipah virus



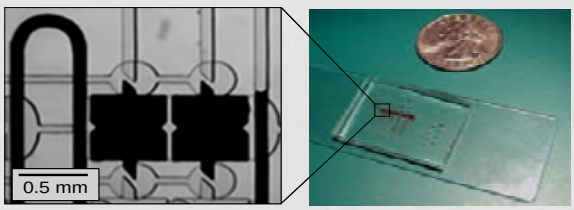
siRNA targeted against the Nipah virus receptor ephrinB2 (EFNB2), but not the related ephrinB1 (EFNB1) and ephrinB3 (EFNB3) genes, inhibited virus replication in human microvascular endothelial cells infected with pseudotyped Nipah virus (BSL-2) encoding GFP.

Characterization of dynamin-dependent endocytosis pathways involved in RVFV infection



We used dynamin-dependent small molecule inhibitors to further to dissect the pathways involved in RVFV infection. Endocytosis pathways are shown in the cartoon on the left. Using small molecule inhibitors of dynamin-dependent (A) and dynamin-independent endocytosis (B), RVFV was found to enter cells through dynamin-dependent, caveolin-mediated endocytosis.

A prototype microfluidic platform for high throughput screening in BSL-3/4 containment



We are currently developing microfluidic platforms, as the prototype demonstrates, that combine cell and siRNA array for high-level biocontainment compatible RNAi screening. Microfluidic-based devices provide an optimal method to introduce RNAi libraries into primary cells within a portable and cost-effective platform.

Significance

- Genome-wide RNA interference offers the opportunity to identify host proteins involved in 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 novel microfluidic technology for siRNA screening that performs in BSL3/4 containment for the study of highly pathogenic viruses

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