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Using Small Interfering RNAs, the Venezuelan Equine Encephalitis Virus is Inhibited

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Abstract

Due to a lack of efficient therapeutic intervention options, acutely contagious new world alphaviruses like the Venezuelan Equine Encephalitis Virus (VEEV) pose significant risks to the human population. Several in vitro and in vivo models of acute viral infections, including those involving alphaviruses like the Chikungunya virus and filoviruses like the Ebola virus, have shown that small interfering RNAs (vsiRNAs) that can specifically target the viral genome provide survival advantages. In this study, novel vsiRNAs were created and tested for antiviral efficacy in mammalian cells during VEEV infection. These vsiRNAs targeted conserved areas in the nonstructural and structural genes of the VEEV genome. The results show that vsiRNAs could successfully lower the infectious virus titer at earlier stages after infection. The inhibition was overcome at subsequent time points in the context of the virulent Trinidad Donkey strain and the attenuated TC-83 strain. The RISC complex's catalytic component, Argonaute 2 protein (Ago2), was depleted, negating the inhibitory effect of the vsiRNAs and highlighting the role of the siRNA route 3 were depleted, suggesting that the RNAi pathway plays a role in the development of a successful infection.

Keywords: Argonaute 2 • Venezuelan equine encephalitis virus • Host proteome • Mass spectrometry • Viral proteome • RNA interference • RISC complex

Introduction

The National Institutes of Health (NIH) and the Centers for Disease Control and Prevention categorise new world encephalitic alphaviruses, such as the Venezuelan Equine Encephalitis Virus (VEEV), as category B select agents (CDC). The 11.4 Kb genome of the Togaviridae family member VEEV identifies it as a positive-strand RNA virus. The genome contains the genes for five structural proteins and four nonstructural proteins (Capsid, 6K, E1, E2 and E3). The transmission of VEEV-related illness by infected mosquitoes causes it to spontaneously occur in people in many regions of the world. Infections have been noted in the Americas for numerous decades, mostly linked to natural transmission. In 1972, Columbia reported one of the biggest naturally occurring VEEV epidemics. 960 occurrences of neurological symptoms, 23,283 human infections, and 156 fatalities. Due to infection of the central nervous system, VEEV is known to produce encephalitic illness and is highly contagious in an aerosol form [1].

There are no FDA-approved vaccines, therapeutics or prophylactics available to protect from or treat encephalitic disease due to VEEV exposure. TC-83, a live attenuated strain of VEEV, and C-84, a formalin inactivated preparation based on TC-83, are used to vaccinate at risk personnel. However, while TC-83 induces robust seroconversion, immunoreactivity concerns persist, with several vaccinees reporting experiencing disease-like symptoms. C-84 is a poor immunogen without robust seroconversion and is used as a booster if required. available to protect from or treat encephalitic disease due to VEEV exposure [2]. TC-83, At-risk employees are immunised using two different vaccines: C-84, a formalin-inactivated preparation based on TC-83 and a live attenuated strain of VEEV. Although TC-83 generates a strong seroconversion, there are still concerns about immunoreactivity because many vaccine recipients report suffering disease-like symptoms. Poor immunogen C-84 is used as a booster if necessary because it has weak seroconversion [3].

Immunoprecipitation

Two milligrammes of total protein were incubated with either 2 g of mouse IgG3 isotype control (Abcam, 18394, Cambridge, UK), anti-VEEV nsP2 antibody (KeraFast, EU015), or anti-MATRN3 antibody for an overnight incubation at 4°C with rotation (Novus, NB100-1761). Protein-antibody IP complexes were loaded to magnetic Dynabeads coated with protein G (FisherSci, 10-003-D) after being washed in citrate phosphate buffer pH 5.0 (50 mM Tris-HCL pH 7.5, 120 mM NaCl, 5 mM EDTA, 0.5% NP-40, 50 mM NaF, 0.2 mM Na3VO4, Protease cocktail tablet) (Sigma-A Rotation at room temperature proceeded for 40 min followed by 1× wash with TNE100 + 0.1% NP-40, 1 wash with TNE50 + 0.1% NP-40 and 2 wash with PBS. TNE buffers consisted of 100 mM Tris-HCl pH 7.5 and 0.2 mM EDTA, using either 50 mM or 100 mM NaCl for TNE50. Laemmli buffer supplemented with 100 mM DTT was added for Western blot imaging, and beads were boiled for 10 min. The final PBS wash was removed from samples used for mass spectrometry, and Dynabeads were then kept at 80 °C until being used for analysis [4].

Western blot

293T cells that had been transfected or infected were made into whole cell lysates. In a nutshell, the cell culture medium was withdrawn, and cells were lysed in 1 mM phenylmethylsulphonyl fluoride and 1 clear lysis buffer (CLB, Cell Signaling Technology, 9803, Danvers, MA, USA) (PMSF, Cell Signaling Technology, 8553S). Supernatants were collected in a separate microcentrifuge tube after cell lysates were centrifuged at 10,000 rpm for 10 min at 4°C. The standard curve of 1 mg/mL bovine serum albumin (BSA, Fisher Scientific, BP1600, Hampton, NH, USA) was used to measure the protein concentrations (VWR, E530-1L). Protein samples were divided equally into two parts Laemmli buffer (1610737, Bio-Rad, Hercules, CA, USA) and then boiled for ten minutes [5,6]. Samples of proteins were electrophoresed on a 4–20% gel. From infected or transfected Tris-Glycine Gels (ThermoFisher, XP04122), whole cell lysates were produced and transferred to polyvinyl difluoride (PVDF) membranes using a wet transfer method at 4°C for 2 hours

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at 250 mA. PVDF membranes were blocked with 3% nonfat dried milk in 0.1% Tween-20-buffered saline at room temperature for 30 min [7].

Primary antibodies such as anti-Ago2, anti-DICER1, anti-MATRN3, anti-MOV10, anti-TRBP2, and anti-MATRN3 were diluted in 3% BSA in TBS-T at a 1:1000 dilution before being incubated on separate membranes overnight at 4°C. PVDF membranes were washed three times for 10 minutes before being incubated for 1 hour at room temperature with the corresponding secondary HRP-conjugated antibody (Fisher Sci, PI32460) diluted in 3% nonfat dry milk in TBS-T at a 1:10,000 dilution [8]. Membranes were then swiftly cleaned three times with TBST for five minutes and three times with TBS for five minutes. Then, using a Bio-Rad Molecular Imager ChemiDoc XRS system, membranes were photographed using SuperSignal West Femto Maximum Sensitivity Substrate Kit (ThermoFisher, 34095). Actin expression was measured as a loading control for all samples. After blocking (3% BSA in TBS-T for 30 min.), incubating with HRP-conjugated anti-actin antibody (Abcam, ab49900), diluted in 3% BSA, TBS-T for 30 min and washing with TBS-T three times, membranes were reprobed with a mild stripping buffer composed of 0.1 M glycine, 0.2 M NaCl, and 0.1% Tween-20 at pH 2.5. The ChemiDoc XRS system was used to photograph membranes (Biorad, Hercules, CA, USA). Signals were standardised to actin and band densities were determined using NIH ImageJ programme [9-10].

Discussion

After blocking (3% BSA in TBS-T for 30 min), incubating with HRP-conjugated anti-actin antibody (Abcam, ab49900), diluted in 3% BSA, TBS-T for 30 min and washing with TBS-T three times, membranes were reprobed with a mild stripping buffer composed of 0.1 M glycine, 0.2 M NaCl, and 0.1% Tween-20 at pH 2.5. The ChemiDoc XRS system was used to photograph membranes (Biorad, Hercules, CA, USA). Signals were standardised to actin and band densities were determined using NIH Image J programme. Both in vitro and in vivo investigations demonstrated antiviral activity to be a more effective method of stopping CHIKV replication. In order to overcome increased inhibition, ongoing research in our lab is concentrating on the encapsulation of several vs iRNAs.

Conclusion

Inhibiting infectious viral titers in cell culture and animal models using vsiRNA as an antiviral method is possible, according to the current study. While this proof-of-concept study backs up the strategy, further work on the strategy is necessary to deliver effective countermeasure solutions. To do this, vsiRNAs that can target different regions of the viral genome must be combined logically in order to increase the extent of inhibition and reduce the risk of suppressive mutations in the viral genome. By boosting bioavailability in difficult-to-reach target tissues like the brain and giving enhanced stability in vivo, optimization of delivery techniques like encapsulation can advance the countermeasure development path.

Acknowledgement

None.

Conflict of Interest

None.

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