BROAD-SPECTRUM THERAPEUTICS FOR VIRAL DISEASES: A MEDICAL COUNTERMEASURE PLATFORM FOR EMERGING THREATS

Use Of Crispr-based Antivirals As Broad-spectrum Therapeutics

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A vaccine for smallpox is no longer administered to the general public, and there is no proven, safe treatment specific to poxvirus infections, leaving a majority of people susceptible to infections by smallpox and other zoonotic Orthopoxviruses such as monkeypox. This is demonstrated by the recent monkeypox outbreak, which has resulted in 5,115 confirmed cases among 51 countries as of June 29, 2022. Furthermore, smallpox has historically been used as a bioweapon. It is classified as a Category A Select Agent by the CDC due to the national security risk posed by its ease of dissemination and transmission and its high mortality rates. To mitigate the risk posed by poxviruses, we have established a proof-of-concept for a CRISPR-based antiviral with the potential to cross-react among numerous other zoonotic Orthopoxvirus species.

Using vaccinia virus (VACV) as a model organism for other Orthopoxviruses, CRISPR–Cas9 technology was used to target three essential genes that are conserved across the genus, including A17L, E3L, and I2L. These genes are involved in different aspects of the virus lifecycle and host interaction. The gene A17L encodes a viral envelope protein that is essential for an early step in virion morphogenesis. E3L interferes with several cellular pathways, promotes cellular growth, and impairs antiviral activity and resistance to apoptosis. I2L is conserved in all orthopoxviruses and is a late protein that is essential for mature viral production, telomere binding, and entry into target cells. Three individual single guide RNAs (sgRNAs) were designed per gene to facilitate redundancy in rendering the genes inactive, thereby reducing the reproduction of the virus. The efficacy of the CRISPR targets was tested by transfecting human embryonic kidney (HEK293) cells with plasmids encoding both SaCas9 and an individual sgRNA. This resulted in a reduction of VACV titer by up to ~93% per target. Following the verification of CRISPR targets, safe and targeted delivery of the VACV CRISPR antivirals was tested using adeno-associated virus (AAV) as a packaging vector for both SaCas9 and sgRNA. Similarly, AAV delivery of the CRISPR antivirals resulted in a reduction of viral titer in HEK293 cells by up to ~93% for an individual target. Overall, we have identified highly specific CRISPR targets that significantly reduce VACV titer as well as an appropriate vector for delivering these CRISPR antiviral components to host cells in vitro.

Because the CRISPR targets were developed in essential, conserved poxvirus genes, this antiviral has the potential to affect emerging viruses within the poxvirus family. This approach is an important model for developing broad-spectrum viral therapeutics as it can be used as a paradigm to develop antivirals against other viral families as well.

SNL is managed and operated by NTESS under DOE NNSA contract DE-NA0003525.

Journal: Scientific Reports

Citation: Siegrist CM, Kinahan SM, Settecerri T, Greene AC, Santarpia JL. CRISPR/Cas9 as an antiviral against Orthopoxviruses using an AAV vector. Sci Rep. 2020 Nov 9;10(1):19307. doi: 10.1038/s41598-020-76449-9. PMID: 33168908; PMCID: PMC7653928.