Targeted DNA Cleavage Can Inactivate Latent Virus

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Herpes Simplex Virus (HSV) infection is an incurable disease that afflicts as much as 50% of adults in the United States. This virus establishes a life-long infection in neuronal cells of the trigeminal ganglia and/or dorsal root ganglia. Periodic reactivation of the latent virus results in symptomatic cold sores on the mouth or genitals and viral shedding. Despite effectively reducing both the frequency and intensity of these episodes, antiviral medication does not eradicate the latent viral genomes from the infected cells. A novel curative approach involving rare-cleaving endonucleases to target viral DNA is being developed in the laboratory of Dr. Keith Jerome of the Vaccine and Infectious Disease Division. A recent study published in *Molecular Therapy–Nucleic Acids* shows promising results of this approach in an *in vitro* cell model for HSV latency.

Homing endonucleases (HE) are a type of rare-cleaving endonuclease that recognize and cleave large DNA sequences (usually around 20 base pairs). Upon recognition of their target sequence, HEs induce a double strand break in the DNA, which is typically repaired by the host cell via a DNA repair mechanism called nonhomologous end joining. This error-prone repair process can cause DNA mutations that can inactivate the gene that harbors the target sequence. By developing a homing endonuclease to specifically target a sequence contained in the HSV genome, the Jerome Lab hopes to successfully inactivate latent HSV and thus offer the possibility of a cure.

In their study, senior staff scientist Dr. Martine Aubert *et al*. first established an *in vitro* cell model for HSV latency. To create this model, primary human fibroblasts are infected with HSV and then treated with antiviral drugs to stop viral replication and establish latency. During latency, HSV persists in infected cells as a double-stranded circular DNA genome. The researchers can later induce HSV to come out of this latent state, a process called reactivation. With the HSV latency model in hand, they tested the effects of a HE that targets a sequence in the HSV U_L19 gene, which is essential for viral replication. Upon treatment of the cells with the HE, site-specific mutations were detected in the target site within HSV, indicating successful cleavage and mutagenic repair of the target sequence. Additionally, a drop in HSV production and viral DNA replication were observed in the HE-treated cells upon reactivation.

After confirming antiviral activity following treatment with HE, Aubert *et al*. examined additional methods to improve upon this therapeutic strategy. First, increased mutagenic activity was observed when HE was co-delivered with Trex2, a 3' exonuclease that increases the rate of mutagenic repair of double strand breaks. Second, delivery of the HE and Trex2 was accomplished via the use of an adeno-associated virus (AAV) vector. The efficient delivery of a transgene expressing a therapeutic enzyme is of fundamental importance when translating such an approach into animal testing and eventually the clinic. Third, accessibility of HSV DNA is affected by slight variations in the DNA that dictate its chromatin structure, or the extent to which the DNA forms tightly wound up coils. Through the use of histone deacetylase inhibitors, which function to relax tightly coiled DNA and allow better access to the sequence, the researchers increased the rate of HE-induced targeted mutations. This was accomplished by providing the HE with better access to the target sequence in the DNA.

These promising results highlight the initial advances being made in the field of targeted gene disruption. The Jerome Lab is also experimenting with utilizing this strategy to target other latent and persistent viral infections such as hepatitis B virus and human immunodeficiency virus (HIV). "While we've seen great excitement recently about the possibility of a cure for HIV, relatively little attention has been paid to cures for other latent viral infections like HSV," explains Dr. Jerome. "These findings show that attacking the latent virus where it hides in cells is possible." Indeed, the ability to specifically identify pathogenic viral DNA and inactivate it through DNA cleavage is a powerful notion. "We're excited to continue to develop this approach toward clinical application," says Dr. Jerome.

Aubert M, Boyle NM, Stone D, Stensland L, Huang ML, Magaret AS, Galetto R, Rawlings DJ, Scharenberg AM, and Jerome KR. 2014. In vitro Inactivation of Latent HSV by Targeted Mutagenesis Using an HSV-specific Homing Endonuclease. *Molecular therapy Nucleic acids*. 3, e146.

See also: Weber ND, Aubert M, Dang CH, Stone D, and Jerome KR. 2014. DNA cleavage enzymes for treatment of persistent viral infections: Recent advances and the pathway forward. *Virology*. pii: S0042-6822(13)00709-5.

Schiffer JT, Aubert M, Weber ND, Mintzer E, Stone D, and Jerome KR. 2012. Targeted DNA mutagenesis for the cure of chronic viral infections. *J Virol*. 86(17):8920-36

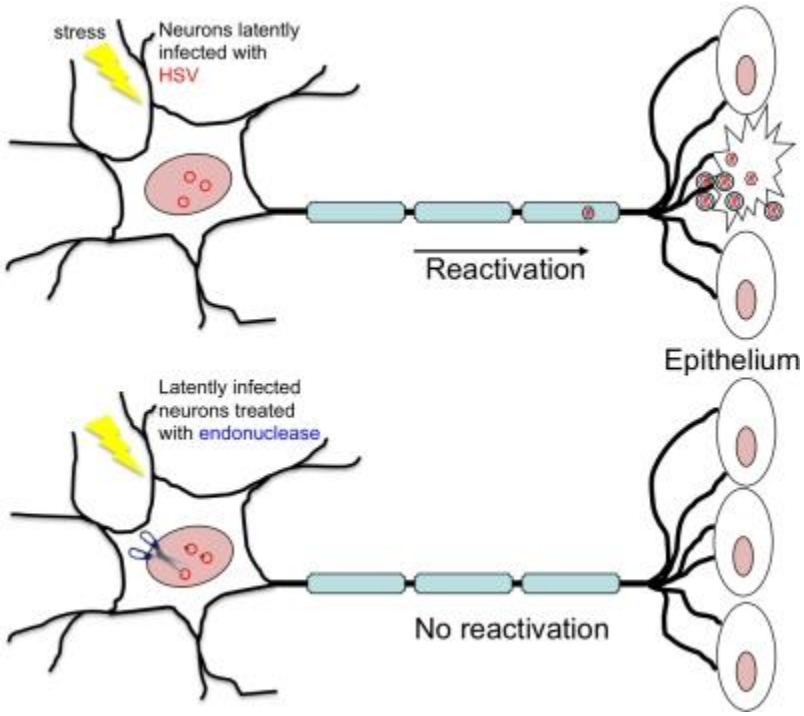

Image provided by Dr. Martine Aubert.

Latent HSV infection persists in neuronal cell bodies from where it can reactivate and cause lesions and viral shedding in the peripheral epithelium. By targeting the latent episomal HSV genome with an endonuclease, viral replication can be inhibited stopping reactivation and thus eliminating symptoms and transmission to new hosts, resulting in the possibility of a cure.