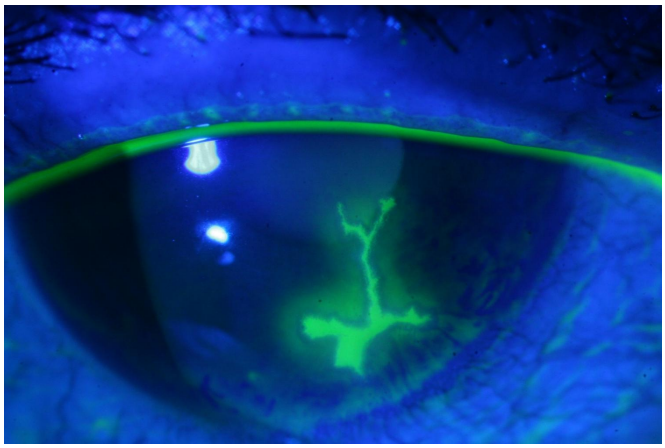
Towards a cure for herpesviruses: Targeting infection with CRISPR/Cas9

30 June 2016



Human eye with herpes keratitis, a disease caused by reactivation of latent HSV-1 that can lead to impaired vision and blindness. Credit: Hilda Schenk, University Medical Center Utrecht.

Most adults carry multiple herpesviruses. Following the initial acute infection, these viruses establish life-long infections in their hosts and cause cold sores, keratitis, genital herpes, shingles, infectious mononucleosis, and other diseases. Some herpesviruses can cause cancer in man. During the latent phase of infection, the viruses remain dormant for long periods of time, but retain the capacity to cause occasional reactivations, that may lead to disease. A study published on June 30th in *PLOS Pathogens* suggests that attacking herpesvirus DNA with CRISPR/Cas9 genome editing technology can suppress virus replication and, in some cases, lead to elimination of the virus.

The CRISPR/Cas9 system targets specific DNA sequences and induces clean cuts across both strands of the DNA. In mammalian cells, such cuts are flagged and quickly repaired by an emergency repair system called NHEJ (for non-homologous end-joining). NHEJ is efficient but not very accurate and often results in insertion or deletion of a few DNA bases at the repair site. Because DNA is read in codons of three bases at a time, such small changes in critical positions often destroy the function of the respective gene and its protein product.

Robert Jan Lebbink, from the University Medical Center in Utrecht, The Netherlands, and colleagues reasoned that CRISPR/Cas9 could target and mutate latent herpesvirus DNA in infected human cells and so potentially prevent herpesvirus-associated diseases. To test this, the researchers devised specific guide (g)RNAs—sequences that are complementary to vital parts of the viral genome and function as 'molecular addresses'. These gRNAs, combined with the 'molecular scissors' part of the CRISPR/Cas9 system, should induce specific cuts and subsequent mutations in the herpesvirus DNA, and so cripple the viruses.

In their systematic approach, the researchers looked at three different members of the herpesvirus group: herpes simplex virus type 1 (HSV-1) causing cold sores and herpes keratitis; human cytomegalovirus (HCMV), the most common viral cause of birth defects (when the virus is transmitted from mother to fetus); and Epstein-Barr virus (EBV) causing infectious mononucleosis and multiple types of cancer.

Working with lymphoma cells latently infected with EBV, the researchers showed that introduction of gRNAs that target specific EBV DNA sequences can introduce mutations at the targeted sites. Such mutations can eliminate essential functions of the virus as well as de-stabilize the viral DNA molecules. Consistent with this, the researchers report that by using two different gRNAs targeting an essential EBV gene, they can induce loss of over 95% of EBV genomes from the host cells.

During latent infection, HCMV genomes exist as circular DNA molecules in the nucleus of host cells.

Upon virus reactivation, HCMV replication proceeds slowly. With appropriate gRNAs, the researchers found that CRISPR/Cas9 editing can efficiently impair HCMV replication. However, they also observed emergence of escape variants that bypass CRISPR/Cas9 editing, suggesting that simultaneous editing at multiple critical sites in the HCMV genome is necessary to avoid the development of resistant genomes.

Compared to HCMV, HSV-1 multiplies much faster. When the researchers tested various gRNAs targeting different essential HSV-1 genes in conjunction with CRISPR/Cas9, they found that many of them were able to reduce virus replication. When they combined two of those gRNAs, thereby simultaneously targeting two essential genes, they were able to completely suppress HSV-1 replication. On the other hand, they were unable to induce editing during the latent phase, i.e. when the viral DNA was not actively multiplying.

"We observed highly efficient and specific clearance of EBV from latently infected tumor cells and impairment of HSV-1 and HCMV replication in human cells", the researchers summarize. They go on to say, "although CRISPR/Cas9 was inefficient at directing genome engineering of quiescent HSV-1, virus replication upon reactivation of quiescent HSV-1 was efficiently abrogated using anti-HSV-1 gRNAs". Their results, they hope, "may allow the design of effective therapeutic strategies to target human herpesviruses during both latent and productive infections."

More information: van Diemen FR, Kruse EM, Hooykaas MJG, Bruggeling CE, Schürch AC, van Ham PM, et al. (2016) CRISPR/Cas9-Mediated Genome Editing of Herpesviruses Limits Productive and Latent Infections. *PLoS Pathog* 12(6): e1005701. DOI: 10.1371/journal.ppat.1005701