Targeting Hepatitis B Virus with Zinc Finger Nucleases

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Hepatitis B virus (HBV) is a global pandemic with over 350 million chronically infected individuals. Annually, 600,000 people die from HBV-related disease in the forms of cirrhosis and liver cancer. Despite the existence of an effective vaccine for over thirty years, HBV prevalence is still astoundingly high, especially in Southeast Asia, China and sub-Saharan Africa. Current therapy can only improve symptoms for a limited time, but does not eradicate the virus from the infected individual. Researchers in the laboratory of Dr. Keith Jerome (Vaccine and Infectious Disease Division) have proposed a novel curative approach to HBV infection. Via the use of targeted endonucleases HBV DNA can be specifically recognized and mutated, in effect rendering the viral genome incapable of replication. The results of applying this methodology in an *in vitro* model of HBV infection were recently published in *PLoS ONE*.

In order to target the HBV genome in infected liver cells, several zinc finger nucleases (ZFNs) with specificity for HBV DNA sequences were designed. ZFNs consist of DNA recognition domains and cleavage domains, which dimerize upon recognition of their specific DNA target sequence, resulting in DNA cleavage. Following cleavage, DNA can undergo repair by a process called non-homologous end joining. However, this process can be error-prone, resulting in mutations such as small insertions or deletions, termed indels. If these indels appear in essential genes, the virus can be inactivated and replication halted.

Proof-of-principle tests using HBV-specific ZFNs in mammalian cells showed the ability of the enzymes to knock down the expression of a reporter gene designed to contain the HBV target sequences. Indels were found precisely in the middle of the target sequences, indicating that recognition and cleavage had occurred (see Figure).

Next, the potential for unwanted effects, such as cytotoxicity and off-target cleavage activity caused by the enzymes on host cells was examined. Cellular toxicity was detected for only one of the three enzymes. In an attempt to explain this toxicity, the researchers examined seven human DNA sequences with similarity to the HBV target sequences. Next generation sequencing was utilized to see if any of the sequences had been targeted and mutated when cells were treated with the ZFNs.

For the seven "off-target" sites, either minimal or no cleavage was detected. These data helped to establish whether the enzymes might be toxic or oncogenic to the host.

A big issue to be addressed with gene therapy strategies is the question of efficient delivery to the target cells. Hepatocyte cells in the liver are the site of HBV infection and are highly accessible via intravenous injection into the blood. In order to transduce hepatocytes with DNA expressing the ZFNs, the researchers used a viral vector called adeno-associated virus (AAV). Before being able to transition into animal models, the efficiency of delivery required testing in liver cells in culture. Highly efficient and long-lasting transgene expression was achieved following a single administration of the AAV vectors.

Lastly, ZFN-expressing AAV vectors were tested on an *in vitro* cell model for HBV infection. Following only a single dose of the AAV, HBV viral DNA was knocked down 200-fold and 26-fold in the supernatant and cells, respectively. The antiviral effect was also seen to last for at least 14 days.

"We were happy to witness a high level of viral inhibition by our enzymes that was specific and sustained," said Dr. Daniel Stone, a contributor to the research. "The lack of toxicity and off-target cleavage was also significant." Looking ahead to the next steps in their research, he expects this antiviral strategy to continue to show high potential in pre-clinical studies. "Having already designed the delivery vectors for the enzymes, testing of their efficacy in a humanized mouse model for HBV infection is our next logical step."

Weber ND, Stone D, Sedlak RH, De Silva Feelixge HS, Roychoudhury P, Schiffer JT, Aubert M, Jerome KR. 2014. AAV-Mediated Delivery of Zinc Finger Nucleases Targeting Hepatitis B Virus Inhibits Active Replication. *PLoS ONE.* DOI: 10.1371/journal.pone.0097579 [Epub ahead of print]

See also: Weber ND, Aubert M, Dang CH, Stone D, Jerome KR. 2014. DNA cleavage enzymes for treatment of persistent viral infections: recent advances and the pathway forward. *Virology.* 454-455:353-61.

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

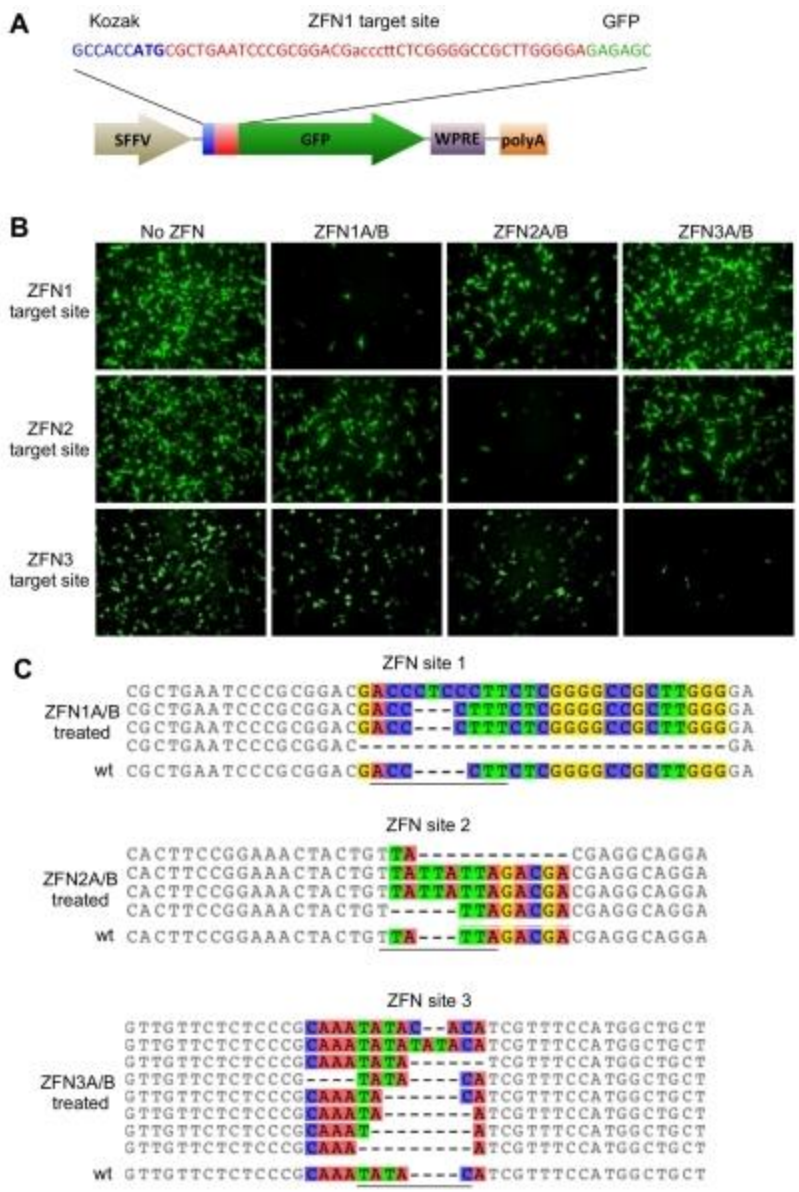

Image taken from the manuscript.

Tests on the ability to knock down the expression of a reporter gene by ZFNs. (a) Target sequences were cloned into plasmids expressing green fluorescent protein (GFP). (b) When cells expressing the GFP reporter were treated with ZFNs, specific GFP expression knockdown was observed. (c) DNA extracted from the treated cells revealed ZFN-induced insertions and deletions in the target sites. The centers of the target sites are underlined in the wild type (wt) sequences.