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Review

DNA cleavage enzymes for treatment of persistent viral infections: Recent advances and the pathway forward

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ABSTRACT

Treatment for most persistent viral infections consists of palliative drug options rather than curative approaches. This is often because long-lasting viral DNA in infected cells is not affected by current antivirals, providing a source for viral persistence and reactivation. Targeting latent viral DNA itself could therefore provide a basis for novel curative strategies. DNA cleavage enzymes can be used to induce targeted mutagenesis of specific genes, including those of exogenous viruses. Although initial *in vitro* and even *in vivo* studies have been carried out using DNA cleavage enzymes targeting various viruses, many questions still remain concerning the feasibility of these strategies as they transition into preclinical research. Here, we review the most recent findings on DNA cleavage enzymes for human viral infections, consider the most relevant animal models for several human viral infections, and address issues regarding safety and enzyme delivery. Results from well-designed *in vivo* studies will ideally provide answers to the most urgent remaining questions, and allow continued progress toward clinical application.

Contents

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General overview

Recent advances in our ability to cleave and modify DNA at a desired locus have energized the field of genome editing. Restriction enzymes have served for years as the workhorses for recombinant DNA manipulation in a vast array of molecular biology applications. However, the short DNA recognition sequences of restriction enzymes limit their use in whole genome editing, due to the high frequency in which their recognition sites occur by chance within a given genome. In the 1980s the first member of the homing endonuclease (HE) family of DNA endonucleases I-SceI was isolated (Colleaux et al., 1986). HEs have much larger DNA recognition sequences (12–40 bp) than restriction enzymes (Chevalier and Stoddard, 2001; Belfort and Roberts, 1997), and thus the likelihood of cleavage occurring at unwanted sites of the genome is much lower, allowing HEs to be used as DNA-targeting enzymes in live cells without lethal toxicity. Thus, the identification of HEs introduced the potential for using DNA cleavage enzymes to manipulate the genome in living cells. In recent years, the ability to engineer the DNA specificity of HEs has vastly increased their utility in genome editing applications (Gao et al., 2010; Grizot et al., 2010; Baxter et al., 2012).

In parallel with the development of HEs, a number of other artificial site-specific DNA cleavage enzymes have been developed that can also be designed to target desired DNA loci, including the zinc finger nucleases (ZFNs), transcription activator like effector nucleases (TALENs), and the CRISPR/Cas9 system (Schiffer et al., 2012; Stone et al., 2013; Gaj et al., 2013). The development of multiple classes of targeted site-specific endonucleases that cleave large DNA sequences with high specificity has expanded the scope of genome manipulation technology to the point that it is now possible to cleave DNA at almost any sequence, and this is aiding new efforts towards previously impossible therapeutic strategies.

Much of the research being carried out in the field of genome editing is based on gene correction, in which an endonuclease is used to introduce a site-specific DNA double strand break (DSB) that is repaired *via* homologous recombination (HR) using a HR donor template that corrects the faulty genomic locus. Targeted HR may also be used to introduce a missing gene or foreign sequence into a targeted locus. However, site-specific endonucleases can also be utilized for gene disruption applications. In mammalian cells, the predominant mechanism for DNA DSB repair is non-homologous end-joining, which is error-prone. Through imprecise repair that results in frame-shift mutations or the deletion of essential DNA sequences, genes can be targeted for disruption. This mechanism offers a possible strategy for targeting exogenous DNA sequences present in cells that have been infected by viruses. By specifically targeting viral DNA for disruption, host cells might effectively be “cured” of viral infection.

In this review we will discuss recent advances in the field of genome engineering toward using sequence-specific DNA cleavage enzymes to suppress or eliminate viral infections. Recent *in vitro* data suggest that essential viral genes from viruses including hepatitis B virus (HBV), human immunodeficiency virus (HIV), human papilloma virus (HPV), human simplex virus (HSV), and human T cell lymphotropic virus (HTLV) can be disrupted, which can lead to disruption or elimination of viral replication. Furthermore, initial *in vivo* studies suggest that antiviral therapies using DNA cleavage enzymes can also disrupt certain viruses in animal models of infection. It is clear from these studies that further research will need to be performed in increasingly realistic and relevant animal models of viral disease. The implementation of genome-directed antiviral therapies faces many hurdles, including the efficiency of enzyme delivery, rates of off-target enzyme cleavage activity, and the development of therapy-associated toxicity. As the field continues to move forward, both the current limitations of existing animal models and the potential hurdles to DNA cleavage enzyme therapy will need to be appropriately addressed.

Current progress in targeting viral infections

In vitro results

Many laboratories have now tested engineered DNA cleavage enzymes using informative *in vitro* models. The first enzyme designed to target integrated HIV provirus was a Cre recombinase-based enzyme specific for the HIV long terminal repeat (LTR), referred to as Tre-recombinase (Buchholz and Hauber, 2011; Sarkar et al., 2007). The LTR is present on both ends of the HIV genome, and by successfully targeting both LTR sites, excision of the viral DNA can be achieved (Sarkar et al., 2007). Tre-recombinase has demonstrated significant activity toward LTR sequences in both episomal and stable integrated reporter constructs, including proviral excision from chromosomal integration sites in transfected cells (Mariyanna et al., 2012). The enzyme has been packaged into a lentiviral self-inactivation vector, allowing delivery to HIV-infected cells and antiviral activity in the absence of cytopathic effects (Hauber et al., 2013). Unfortunately, Tre-recombinase was created to recognize the LTR from a rare HIV strain of subtype A chosen for its sequence similarity to the wild-type loxP sequence, which is not present in most HIV strains and complicates broader application for other HIV strains.

The first efforts targeting integrated provirus with a non-recombinase DNA cleavage enzyme was a proof-of-principle study that achieved HE-induced gene disruption of an integrated lentiviral reporter provirus in which the wild-type recognition site for the HE Y2 I-*Ani*I was inserted into a GFP open reading frame (Aubert et al., 2011). Treatment with the enzyme resulted in a loss of GFP fluorescence due to misrepair of the cleaved target site. Although these results are promising, they were obtained by targeting a wild-type HE recognition sequence rather than a viral sequence. More recently, engineered ZFNs targeting HIV proviral DNA have been utilized to target and cleave the HIV LTR sequences in latently infected cells as well as in HIV-infected human primary cells, *in vitro* (Qu et al., 2013). These ZFNs, like Tre-recombinase, were capable of excising the integrated provirus. In a similar study, therapeutic ZFNs directed toward the LTR of HTLV-1 resulted in disruption of LTR promoter activity and removal of the proviral genome from infected cells (Tanaka et al., 2013). These ZFNs also caused a drop in cell proliferation and resulted in DNA double strand break-induced apoptosis. Comparable outcomes have been achieved by targeting the HIV LTR with the CRISPR/Cas9 system in infected cell lines (Ebina et al., 2013). Despite these advances, targeting the LTR as a therapeutic strategy may have drawbacks, since simultaneous cleavage of both LTR binding sites will be needed for provirus excision. Additionally, the LTR does not include coding sequences but instead functions as a promoter region, so that the introduction of mutations within the LTR may not be optimally detrimental to viral fitness.

DNA cleavage enzymes can also target viruses with persistent episomal forms. In targeting HPV, Mino et al. fused a staphylococcal nuclease to an engineered zinc finger protein to cleave episomal genomes, which inhibited HPV-18 DNA replication in a cell culture model (Mino et al., 2013). Likewise, ZFNs and TALENs have been used to induce mutations in HBV genomic sequences, which exist in hepatocytes in an episomal covalently closed circular (cccDNA) form. First, ZFNs were used to target a plasmid containing HBV sequences resulting in a decrease in viral replicative intermediates in transfected cells (Cradick et al., 2010). Later, TALENs targeted to conserved regions in different HBV genotypes showed disruption of the target site and knock down of viral