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Antiviral therapy of persistent viral infection using genome editing

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Chronic viral infections are often incurable because current antiviral strategies do not target chromosomally integrated or non-replicating episomal viral genomes. The rapid development of technologies for genome editing may possibly soon allow for therapeutic targeting of viral genomes and, hence, for development of curative strategies for persistent viral infection. However, detailed investigation of different antiviral genome editing approaches recently revealed various undesired effects. In particular, the problem of frequent and swift development of resistant viruses has to be thoroughly analysed before genome editing approaches become an established option for antiviral treatment.

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Introduction

Once established, persistent, and particularly latent virus infection considerably hampers virus clearance from infected organisms. Consequently, although current antiviral therapies, commonly administering small molecular weight inhibitors, routinely suppress progeny formation, they may ultimately fail in virus eradication, and thus in achieving a cure. However, the advent of advanced genome editing methods, such as homing endonucleases (HE; i.e. meganucleases), zinc finger nucleases (ZFN), transcription activator-like effector nucleases (TALEN), the CRISPR/Cas9 RNA-guided nuclease system, and engineered tyrosine recombinases (e.g. Cre variants), provide technologies for developing completely new therapeutic strategies primarily aiming at destroying virus genomes. In fact, these genome editing systems have already been successfully employed in various studies in cell cultures and small animal models to target several human pathogenic viruses, including human immunodeficiency virus (HIV) [1], hepatitis B and C virus (HBV and HCV) [2], herpesviruses (HSV, HCMV and EBV) [3–5], JC polyomavirus [6] and human papillomavirus (HPV) [7–10]; reviewed in [11–14]. Thus, genome editing represents the most direct anti-viral approach, and if proven to be safe in humans, may become a general antiviral strategy. Moreover, as a novel antiviral strategy, gene editing can also be employed to disrupt cellular genes that, for example, encode important virus receptor molecules [15,16], further expanding the possibilities for genome editing as an anti-viral approach. In this review, we contrast designer nuclease and designer recombinase genome editing technologies and provide an assessment of current prospects and challenges in the field, focussing on HIV and HBV genome editing as representative examples.

The toolbox for gene editing

Current gene editing technologies differ considerably in their mode of action, namely error-prone versus error-free DNA modification (for comparison see Table 1). Obviously, this may affect the likelihood of developing resistance and subsequent viral escape.

Error-prone repair is characteristic of HE, ZFN, TALEN and CRISPR/Cas9 (for more detailed description of their molecular action see [17–19]). In contrast, error-free repair is typified by site-specific recombinase systems, such as Cre [20].

HE, ZFN, TALEN and CRISPR/Cas9, commonly referred to as designer nucleases [13], differ mainly in how these programmable enzymes are recruited to their DNA target site(s). HE, ZFN and TALEN interact with their particular DNA target sequences via intrinsic DNA binding domains. In the case of HE, which recognize target sites of 14–40 bp (commonly ~18 bp), the DNA-binding and cleavage domains cannot be precisely separated [21]. Thus, *in vitro* engineering of such meganucleases to generate new binding specificities requires challenging, complex and tedious directed protein evolution technologies [22,23].

In contrast, ZFN and TALEN are characterized by a distinct modular structure, comprising artificial arrays of

Table 1

Comparison of the main features of genome editing systems

Editing system	Ease of DNA targeting	Precision of DNA repair	Dependence on host DNA repair	Multiplexing	Delivery	Typical target-site length (bp)	Typical protein size (kDa)
HE	–	–	+	–	+++	18	40
ZFN	+	–	+	–	++	18–36	45
TALEN	+	–	+	–	+	30–40	105
CRISPR/Cas	+++	–	+	+++	+	20	164
T-SSR	–	+++	–	–	+++	34	42

HE: homing endonucleases; ZFN: zinc finger nucleases; TALEN: transcription activator-like effector nucleases; CRISPR: clustered regularly interspaced short palindromic repeats; Cas: CRISPR-associated; T-SSR: tyrosine-type site-specific recombinase.

DNA-binding motifs linked to the non-specific catalytic domain of the restriction enzyme *Fok*I [17–19]. Importantly, the *Fok*I nuclease domain must dimerize to cut DNA, therefore two ZFN or TALEN molecules are required to target a single site [24]. The respective DNA binding domains of the proteins are customized using *in vitro* platforms to assemble three to six zinc finger modules (one module/3 bp of target DNA) or 15–20 TALE modules (one module/1 bp of target DNA) [25]. Thus, target sites typically vary from 18–36 bp for ZFN and 30–40 bp for TALEN. Notably, targeting of longer sites generally improves the specificity of these programmable nucleases (i.e. minimizes off-target effects).

Unlike HE, ZFN and TALEN, the CRISPR-associated Cas9 nuclease is recruited to a DNA site via its association with a short guide RNA (gRNA) that hybridizes to a target DNA site of about 20 bp [17,19,25]. Thus, the DNA binding specificity solely depends on RNA-DNA base pairing. This is a huge advantage for easily targeting the Cas9 nuclease to sites of interest, since it only requires constructing a specific gRNA. Furthermore, this feature allows multiplexing and the use of Cas9 nickase dimer mutants to improve specificity, and hence prevent off-target effects [26••,27].

However, upon target site recognition, all of these nucleases, HE, ZFN, TALEN and CRISPR/Cas9 alike, introduce free DNA double-strand breaks (DSBs) at the target locus as the first step in gene correction/inactivation. Since DSBs represent one of the most dangerous lesions for a cell [28], these breaks activate the intrinsic cellular error-prone non-homologous end-joining (NHEJ) repair mechanism, typically inducing an abundance of random insertions and deletions (indels) at the target locus, which usually inactivate the target gene [17,19,24]. Since indel formation cannot be controlled, NHEJ has a considerable negative impact on genomic fidelity, and therefore, particularly when targeting highly active replicating systems such as viruses, facilitates the loss of target sites and development of resistance.

Tyrosine-type site-specific recombinase (T-SSR) systems, such as Cre recombinase, enable highly predictable and accurate genome editing, since they act in an error-free manner independently of endogenous DNA repair pathways (e.g. NHEJ) [20]. Thus, T-SSRs mediate precise DNA cleavage and ligation without the gain or loss of nucleotides. Drawbacks include the fact that recombination requires the presence of two identical target site sequences of 34 bp. When in the same orientation, their recombination results in accurate removal of the intervening sequence. Such an arrangement exists naturally in the case of integrated retroviruses (i.e. proviruses), which are flanked by identical long terminal repeat (LTR) sequences. Furthermore, similar to HE, engineering T-SSRs to have new binding specificities necessitates rather complex directed molecular evolution technologies [29–31].

Targeting episomal HBV cccDNA

Hepatitis B virus (HBV) infection is a global health problem, putting ~350 million infected people at risk of developing cirrhosis of the liver or hepatocellular carcinoma [32]. The HBV genome persists in infected hepatocytes as an episomal cccDNA (covalently closed circular DNA) [12]. Since cccDNA is not addressed by current therapy principles, a cure of HBV infection would require developing novel therapeutic approaches to eradicate the viral cccDNA. Obviously, genome editing technologies could be perfectly suited to provide therapeutic options targeting HBV cccDNA [12].

A series of studies have investigated targeting various cccDNA-derived sequences using ZFN [33,34], TALEN [35,36] and the CRISPR/Cas9 wildtype [37–45] or 'nickase' systems [46], a mutated version of Cas9 that generates a single-strand DNA break (nick) at the specific target site [26••]. These studies demonstrated HBV inactivation in cultured cell lines and HBV mouse models in the short term (up to a maximum of 10 days of follow-up) and provided proof of principle for HBV treatment by genome editing technologies. To further develop these approaches towards clinical application, long-term analysis of potential viral escape is imperative. This is particularly important, since HBV genomes are characterized by considerable sequence variability. For example, at least