Progress in Retinal and Eye Research 53 (2016) 1–20

Contents lists available at ScienceDirect

Progress in Retinal and Eye Research

journal homepage: www.elsevier.com/locate/prer

# Genome engineering in ophthalmology: Application of CRISPR/Cas to the treatment of eye disease

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ARTICLE INFO

*Article history:*
Received 26 August 2015
Received in revised form
30 April 2016
Accepted 4 May 2016
Available online 13 May 2016

*Keywords:*
Gene editing
Ethics
Cas9
Cpf1
Inherited retinal disease
Genetic ophthalmology

ABSTRACT

The Clustered Regularly Interspaced Short Palindromic Repeat (CRISPR) and CRISPR-associated protein (Cas) system has enabled an accurate and efficient means to edit the human genome. Rapid advances in this technology could results in imminent clinical application, and with favourable anatomical and immunological profiles, ophthalmic disease will be at the forefront of such work. There have been a number of breakthroughs improving the specificity and efficacy of CRISPR/Cas-mediated genome editing. Similarly, better methods to identify off-target cleavage sites have also been developed. With the impending clinical utility of CRISPR/Cas technology, complex ethical issues related to the regulation and management of the precise applications of human gene editing must be considered. This review discusses the current progress and recent breakthroughs in CRISPR/Cas-based gene engineering, and outlines some of the technical issues that must be addressed before gene correction, be it *in vivo* or *in vitro*, is integrated into ophthalmic care. We outline a clinical pipeline for CRISPR-based treatments of inherited eye diseases and provide an overview of the important ethical implications of gene editing and how these may influence the future of this technology.



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http://dx.doi.org/10.1016/j.preteyeres.2016.05.001
1350-9462/

## 1. Introduction

The Clustered Regularly Interspaced Short Palindromic Repeat (CRISPR) and CRISPR-associated protein (Cas) system is used by bacteria to counter viral intrusion and has recently been adapted to allow efficient editing of the mammalian nuclear genome. Its targeted manipulation has opened exciting avenues for the study and treatment of human disease. Indeed, CRISPR/Cas-based technology is being championed as a relatively straightforward technology for *in vitro* correction of genetic mutations, with *in vivo* genomic editing not lagging far behind (Hsu et al., 2014; Pennisi, 2013). Genome engineering is particularly attractive for treating inherited conditions caused by genes with very specific spatial and stoichiometric expressions, such as those found in many of the inherited, non-syndromic eye diseases (Tucker et al., 2014). By direct editing of genomic DNA, through the induction of DNA nicks or breaks and subsequent repair, the corrected gene remains under its normal endogenous expression control elements. CRISPR/Cas technology potentially avoids ectopic expression and abnormal gene transcription, which can result in adverse events, such as changes in immune modulation or tumour development. Additionally, the limits of viral vector capacity, which impede the widespread utility of current gene-replacement therapies for many diseases, could be circumvented by a CRISPR-based approach (Ran et al., 2015; Zuris et al., 2015).

Rapid advancements in CRISPR technology have led to extensive improvements to the specificity and efficiency in targeted genome editing. Given that the eye presents favourable anatomical and immunological characteristics due to the blood-retinal barrier and ocular immune privilege (Zhou and Caspi, 2010), it is envisaged that ocular diseases will be at the forefront of the clinical translation of CRISPR/Cas-based therapies. However, as with all emerging biotechnologies, the utility of CRISPR/Cas will not be based on its therapeutic potential alone. There are important ethical and regulatory factors that will have a major influence over what applications are deemed acceptable. Nonetheless, as CRISPR/Cas begins transitioning from the laboratory bench to the clinic, there is a pressing need for a clearly defined, safe and standardised clinical pathway for the therapeutic implementation application of this technology.

There is no doubt that improved understanding of disease mechanisms and novel therapeutic targets will be identified through the application of genome-wide screens using CRISPR-based knock-out or synergistic activation mediators, as well as Cas9 transgenic mice (Chen et al., 2015; Konermann et al., 2015; Parnas et al., 2015; Shalem et al., 2014); however, it is foreseen that the direct clinical application of CRISPR/Cas is likely to come from individualised gene editing regimens. This review outlines the current progress of CRISPR-based genomic engineering, specifically exploring its applications and future prospects in the treatment of ophthalmic disease. We describe key advances in the field that have addressed technical issues of CRISPR/Cas, expanding its utility for future ophthalmic therapies. Whilst there is a diverse range of potential applications of CRISPR/Cas genome engineering, we focus on the correction of disease-causing variants in the clinical setting. Finally, we provide an overview of the ethical considerations pertinent to this new technology.

### 1.1. Genome engineering: more than meets the eye

Current DNA editing methods can be broadly divided into two groups based on their targeting mechanism: protein- and nucleotide-directed (Esvelt and Wang, 2013). Zinc Finger Nucleases (ZFNs) and Transcription Activator-Like Effector Nucleases (TALENs) are the best studied protein-directed DNA editing techniques, whilst CRISPR/Cas technology is a nucleotide-directed DNA editing technique (Xu et al., 2014). Nucleotide-directed techniques are more easily applied than protein-directed techniques.

Underpinning genome-editing technology, regardless of the precise method used to target and cleave DNA, is the fact that various mechanisms are used by cells for homeostatic DNA repair. As displayed in Fig. 1, there are two principal, endogenous pathways used for repair after a double stranded DNA break (DSB). Homology Directed Repair (HDR) occurs when the break is repaired using a homologous sequence, or in the case of directed gene editing, a custom template containing the desired sequence (Christian et al., 2010). Alternatively, Non-Homologous End Joining (NHEJ) occurs in the absence of a homologous sequence, when the two broken ends are joined together inaccurately. This method frequently results in insertion and deletions (indels), leading to frameshift mutations that can be used to effectively create gene knockouts (Christian et al., 2010). CRISPR/Cas can capitalise on HDR to edit genomes by providing a repair template containing the desired sequence to be inserted into the genome (Cong et al., 2013; Mali et al., 2013b).

#### 1.1.1. Protein-directed gene editing

ZFNs comprise programmable, sequence-specific DNA-binding modules connected to a FokI endonuclease domain (Bibikova et al., 2001; Kim et al., 1996). The ZFN DNA-binding domains contain individual zinc finger repeats which recognise the target sequence at which the DNA nuclease domain cuts the DNA. The modular structure of the DNA binding domains in ZFNs can be customised to target desired sequences in the genome. The most commonly used