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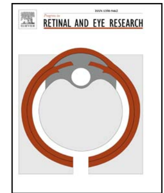

Personalised genome editing – The future for corneal dystrophies

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A B S T R A C T

The potential of personalised genome editing reaching the clinic has come to light due to advancements in the field of gene editing, namely the development of CRISPR/Cas9. The different mechanisms of repair used to resolve the double strand breaks (DSBs) mediated by Cas9 allow targeting of a wide range of disease causing mutations. Collectively, the corneal dystrophies offer an ideal platform for personalised genome editing; the majority of corneal dystrophies are monogenic, highly penetrant diseases with a known pattern of inheritance. This genetic background coupled with the accessibility, ease of visualisation and immune privilege status of the cornea make a gene editing strategy for the treatment of corneal dystrophies an attractive option. Off-target cleavage is a major concern for the therapeutic use of CRISPR/Cas9, thus current efforts in the gene editing field are focused on improving the genome-wide specificity of Cas9 to minimise the risk of off-target events. In addition, the delivery of CRISPR/Cas9 to different tissues is a key focus; various viral and non-viral platforms are being explored to develop a vehicle that is highly efficient, specific and non-toxic. The rapid pace and enthusiasm with which CRISPR/Cas9 has taken over biomedical research has ensured the personalised medicine revolution has been realised. CRISPR/Cas9 has recently been utilised in the first wave of clinical trials, and the potential for a genome editing therapy to treat corneal dystrophies looks promising. This review will discuss the current status of therapeutic gene editing in relation to the corneal dystrophies.

1. Introduction

Corneal dystrophies are a group of inherited, heterogeneous, bilateral disorders that affect the transparency or shape of the cornea (Klintworth, 2009). Historically, these dystrophies were sub-classified according to the corneal layer predominantly affected. Advances in genetic analysis and the completion of the human genome project gave researchers the capability to identify the causative genes (Shendure et al., 2017). These advances transformed our understanding of corneal dystrophies and revealed the extensive genetic heterogeneity that exists, leading to the necessity of a new classification system. In 2008, The International Committee for Classification of Corneal Dystrophies (IC3D) published a new classification system that aimed to preserve the traditional grouping while making way for the new era of genetic advancements; an updated version has since been published (Weiss et al., 2015).

The severity of the dystrophic phenotype can vary substantially, and therefore the treatment strategy required will need to be tailored to suit the individual patient accordingly (Klintworth, 2009). In some cases the corneal dystrophy can be asymptomatic and no treatment is required, while in other instances opacities which reduce visual acuity may result in complete loss of vision. Currently, corneal dystrophies are treated in a stage–related process (Seitz and Lisch, 2011). The decision of which treatment strategy will be most effective for the patient is made based on the current stage of the dystrophy. For milder cases, conservative therapies implemented include; gels/ointments, application of therapeutic contact lenses and/or conventional corneal abrasion (Seitz and Lisch, 2011). However, if these are not successful a surgical approach must be employed. The most effective surgical approach chosen will be based on the anatomical location of the opacities. Phototherapeutic keratectomy (PTK) can be considered for superficial dystrophies of the epithelium and, with less success, stromal dystrophies. However, it is not curative and in many cases the opacities may return (Chen and Xie, 2013; Dinh et al., 1999; Hafner et al., 2005). PTK is more often than not a temporary solution, it will likely require repeated treatments, with an ultimate goal of avoiding keratoplasty (NoRathi et al., 2016).

Unfortunately, in many instances sight deteriorates to the point where a keratoplasty is required. Although corneal transplantation is

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well-established, drawbacks include the perpetual shortage of corneal donors and graft rejection following transplant. Despite advancements in ocular surgery no significant improvements in graft survival rates have been observed in the last 30 years (Bidaut-Garnier et al., 2016). The 5 year survival rate of grafts from 2004 to 2014 is documented to be 76.5%; however, when in the presence of risk factors, this value falls to 57.1% (Bidaut-Garnier et al., 2016). Predisposing factors for high-risk keratoplasty include a preoperative vascularised cornea caused by inflammation related to infection or chemical injury; this leads to a disruption in the immune privilege status of the cornea, allowing entry of immunologically competent cells (Arentsen, 1983; Hill, 1994). Another critical issue is that patients harbouring a causative *TGFBI* mutation see a re-emergence or in some cases a novel occurrence of mutant protein in the corneal graft (Aldave et al., 2007; Han et al., 2016; Jun et al., 2004; Kim et al., 2008). There will always be an element of risk in undergoing a surgical procedure, and as such, keratoplasty, whether lamellar or penetrating, is reserved as the absolute last resort (Seitz and Lisch, 2011). Unfortunately, in practice this means that nothing curative may be done for the patient until they are effectively blind. Due to the complete penetrance observed with many of the corneal dystrophies an approach that tackles the underlying genetic cause permanently, with a minimally invasive technique, seems a very attractive option.

Gene therapy seeks to treat genetic diseases by the introduction of foreign therapeutic DNA into a patient's cells. Recent advancements in the field of gene therapy, such as the development of new tools coupled with improvements in delivery, safety and efficiency; have accelerated the possibility of gene therapy reaching the clinic as a treatment, the eye is central to this genetic revolution. This movement has been pioneered by developments in retinal gene therapy; currently there are several on-going clinical trials for retinal diseases including; Leber congenital amaurosis (LCA), choroideremia, Usher's syndrome and Stargardt disease. These developments will be discussed in section 7.2 ‘Ocular clinical trials.’

The cornea offers the ideal candidate for targeted gene therapy due to its small surface area, accessibility and ease of visualisation. In addition, the cornea holds a unique immune privileged status which is critical for gene editing as it will minimise immune response to the gene-based therapeutics and delivery vehicles that are introduced (Charlesworth et al., 2018). Furthermore, as the cornea is avascular, any gene based therapeutics and delivery vehicle supplied to the cornea will not be able to reach other organs of the body minimising risk of off-target events in other tissues. Successful genome editing is reliant on i) strategic selection of a suitable gene therapy approach ii) efficient delivery to the targeted cell population iii) specific and efficient editing of the target gene in only the desired cell population. This review will discuss the current position of gene therapy in relation to the corneal dystrophies.

2. Genome engineering strategies

The concept of correcting disease status based on genetic information has fuelled decades of research. The most promising approaches for genetics based therapeutics that have emerged are; RNA interference (RNAi), gene augmentation and utilisation of genome engineering nucleases to achieve gene knockout or mutation correction. To-date the most utilised genome engineering strategy in ocular disease is that of gene augmentation, this will be discussed in sections 2.2 and 7.2.

2.1. RNAi

RNAi, first described in 1998 by Fire et al. (1998), utilises small interfering RNA (siRNA) molecules, 21 nucleotides in length, with complementarity to a specific gene's messenger RNA (mRNA) transcript. Upon target recognition the siRNA induces degradation of the complementary mRNA, preventing translation and protein expression (Fig. 2a). Currently, the database of clinical trials indicates that there are 40 ongoing clinical trials involving RNAi (RNAi clinical trials). At present 6 RNAi based therapeutic agents have progressed to phase 3 clinical trial stage (Sullenger and Nair, 2016). For example, vascular endothelial growth factor (VEGF) plays a well-established role in choroidal neo-vascularization (CNV), which leads to age-related macular degeneration (AMD). Bevasiranib, an siRNA targeted to the VEGF mRNA, reached phase 3 clinical trial, was administered by intravitreal injection every 12 weeks, almost 1/3 less frequently than current treatment options, such as Bevacizumab (Garba and Mousa, 2010). However, the trial was terminated as effects were not as potent as currently available therapies (Sullenger and Nair, 2016). It was shown that inhibition of Cas2, which is primarily activated by retinal ganglion cells, can prevent apoptosis in these retinal ganglion cells (Vigneswara et al., 2014). This interesting finding has now been translated to a potential therapy, QPI-1007, a siRNA targeted to Cas2, which is now in clinical trials to reduce retinal ganglion apoptisis in patients with Nonarteritic anterior ischemic optic neuropathy (NAION) (Sullenger and Nair, 2016).

2.2. Gene augmentation

Conventionally, gene therapy refers to the introduction of a functional copy of the gene to treat loss of function mutations, usually by viral transgene expression (discussed in detail in section 5.2 ‘Viral delivery to the eye’). The gene supplied by the virus allows the target cell to produce a functional protein in cases when the endogenous protein is defective (Fig. 2b). There are currently 2781 ongoing clinical trials for gene therapy listed on the clinical trial database, with 78 of these investigating gene therapy in eye diseases (Gene therapy clinical trials). Most ocular gene therapies tested to date target diseases of the retina, largely due to the fact that the majority of retinal diseases are caused by loss of function mutations (RetNet). For example, Leber's congenital amaurosis type II (LCA2), due to loss-of-function mutations in the *RPE65* gene, has been treated in three independent studies by delivery by single subretinal injection of the *RPE65* cDNA packaged in AAV2 (Bainbridge et al., 2008; Bennett et al., 2016; Hauswirth et al., 2008; Maguire et al., 2008). Improvement in vision that was stable for at least 3 years was observed in each study. The current stage of this therapeutic will be discussed in section 7.2 ‘Ocular clinical trials’.

2.3. Programmable nucleases

Programmable nucleases provide tools to manipulate the genome in a sequence specific manner, they consist of a nuclease that can be reprogrammed to cleave at a precise target sequence. They facilitate precise genome editing by inducing a double strand break (DSB) at a desired location. The cellular responses initiated to repair this damage are either non-homologous end-joining (NHEJ) or homology directed repair (HDR). Depending on which of the cellular responses that is employed, different modes of genome editing, such as gene knockout or gene correction, can be achieved. Which is discussed in detail in section 2.3.2 ‘Types of therapeutic genome modifications with CRISPR/Cas9’.

There are currently four classes of programmable nucleases that have been utilised: meganucleases (Belfort and Bonocora, 2014; Stoddard, 2011), zinc finger nucleases (ZFNs) (Urnov et al., 2010), transcription activator-like effector nucleases (TALENs) (Bogdanove and Voytas, 2011) and clustered regularly interspersed palindromic repeats (CRISPR) associated nuclease, Cas9 (Fig. 2 c–f). Although all of these programmable nucleases cause a DSB which mediates genome editing, the mode by which they achieve target recognition and their specific limitations differ, influencing which nuclease is most applicable for a given situation.

One of the major considerations is the ease with which a nuclease can be engineered for a specific target. For instance, meganucleases and ZFNs require extensive protein engineering, while CRISPR/Cas9 can be easily redirected with simple molecular cloning techniques. Due to this,

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