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Editorial

The Nucleus: The New Surgical Theater?

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Of the approximately 6000 known genetic disorders, about 10% affect the eye. Of these, up to 30% manifest as specific ocular disorders. Over the last 15 years major advances in gene therapy and other molecular approaches in the treatment of hereditary ocular diseases have led to successful treatments [1,2]. A number of clinical trials are currently underway for the treatment of diseases in both the posterior and anterior segments of the eye [3-6]. The most spectacular results, thus far, have been achieved in the treatment of one form of Leber Congenital Amaurosis (LCA) caused by mutations in the RPE65 gene using Adeno-Associated Virus (AAV)-mediated gene delivery, a form of gene replacement therapy [3,6]. In addition there are currently numerous other avenues of exploration in the development of more effective treatments for ocular disorders. These include optogenetics [7,8], cell/tissue replacement [9], embryonic stem cell-mediated development of new tissue [10], electronic prosthetics [11], expression interference [12,13], small molecule-mediated molecular transformation [14], and others.

The recent discovery and modification of targeting nuclease systems capable of making precise changes in the genome of any organism is the subject of this editorial [15,16]. I predict that this new technology, commonly called "genome editing" or "genome engineering", will not only provide a better way to approach the treatment and possible cure of many genetic diseases, but will also dramatically improve the development of many of the other types of treatment by facilitating more precise genome modification and reprogramming. Genome editing is also being developed for the possible treatment of non-genetic disorders through modulation of gene expression to enhance the body's natural defense mechanisms. Before the great potential of genome editing can be appreciated it is necessary to have a basic understanding of the current state of the technology and how it is different from the other technologies aimed at treating genetic disease. There are currently three genome editing technologies being studied in a number of model systems: 1) Zinc Finger Nuclease (ZFN [17,18]), 2) Tal Effector Nuclease (TALEN [19]), and 3) Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR [20]) coupled with a CRISPR-associated nuclease (Cas [21]). All three have been used extensively to modify the genomes of a very diverse number of non-human species. While it is not fully established yet, based on the fact that (according to PubMed) three times as many papers were published in 2013 using CRISPR/Cas systems compared to papers on TALEN and ZFN combined indicates that this technology may hold the most promise for the development of treatments for genetic disease. In the simplest sense, each of the technologies couples a DNA recognition region for genome target specificity with a DNA endonuclease that will cleave the DNA near the targeted site. Once a break is made at any site in the genome the organism's endogenous repair mechanisms are activated to rejoin the DNA using Homology Directed Repair (HDR) or Non-Homologous End Joining (NHEJ). Simultaneous inclusion of any donor DNA allows for site-specific integration into the genome, which could be used to substitute defective protein coding regions or to alter transcription regulation. Technologies for integrating DNA into the genome, both randomly and site-specifically, have been in use for decades in animal models and, as noted above, the use of AAV or lentivirus [21] to deliver active genes into the nucleus in patients is now in use in numerous clinical trials. What sets apart genome editing from existing technologies is the potential to alter the genome in a patient at the site of a mutation to permanently replace damaged DNA and restore genome integrity and function. Thus, it may be possible to not only *treat* a genetic disease, but to actually *cure* it. Two recent studies have demonstrated proof-of-principle in ocular [22] and hepatic tissues [23] of animal models of human diseases. In each study virus encoding a targeting nuclease was injected into the eye or tail vein and the nuclease was expressed and correctly cleaved the DNA at the targeted site. In addition to further enhancement and modification of the targeting systems, future studies need to be focused on improving delivery and minimizing the potential for off-target cleavage events that could trigger new disease. The use of genome editing to reverse the effects of hereditary retinal disorders is particularly attractive, since viral and nanoparticle based delivery methods are becoming well-established and significant cell coverage of the retina can be achieved. An additional advantage of genome editing is that it can be used to directly intervene in autosomal dominant disorders involving gain-of-function, such as autosomal dominant retinitis pigmentosa (adRP). To date, treatments in patients aimed at eliminating or reducing expression selectively of the dominant (disease-causing) allele have not been successful. Genome editing provides a means to replace the dominant allele with a normal copy to restore function in every cell that is targeted.

In summary, gene therapy since its inception more than 20 years ago has steadily improved and has recently led to success in the treatment of several human genetic disorders. The great potential for genome editing to not only treat, but cure, many diverse genetic diseases is just beginning to become clear; within the next 7-10 years, perhaps sooner, it is predicted that genome editing will realize this potential. Because of the accessibility, immune status and the advanced

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technology already developed for ocular gene therapy, the treatment of eye diseases will likely lead the way for this emerging technology. The ability to directly alter the genome *in vivo* with precision would be a major medical breakthrough that would establish the nucleus as the new surgical theater.

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