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Review

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Advancing Approaches of Gene Correction in Therapeutic Applications: Exploring Front-Line Technologies Beyond CRISPR-Cas9

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Abstract

Gene correction technologies have revolutionized the field of genetic medicine, offering promising avenues for the treatment of various genetic diseases. This manuscript delves into the advancements in gene correction approaches beyond CRISPR-Cas9, shedding light on the challenges and opportunities in this rapidly evolving field. The review encompasses a comprehensive analysis of nuclease and nickase genome editing methods, highlighting the complexities encountered in translating these techniques to clinical settings. Furthermore, the exploration of base editors for correcting point mutations in monogenic diseases underscores the potential of precise genetic engineering tools in addressing a wide range of disorders. The integration of transcription activator-like effector domain (TALE) base editors and the development of piggyPrime for high-efficiency prime editing showcase innovative strategies for multiplex gene engineering and genomic integration, respectively. Additionally, the study on off-target editing outcomes in non-human primate embryos emphasizes the critical need for thorough sequencing-based techniques to evaluate editing outcomes and ensure the safety of gene editing approaches. Overall, this communication underscores the importance of advancing gene correction technologies to bring effective therapeutic interventions closer to patients in need, while also addressing the challenges associated with unintended editing effects and safety considerations.

Keywords: Gene editing; CRISPR-Cas9; Therapeutic applications

Gene Correction in Therapeutic Applications

By directly correcting disease-causing genetic variants, gene editing has enormous potential as a permanent cure for genetic diseases. Initial clinical trials, however, tended to concentrate on simpler targets using editing techniques that rely on double-strand DNA breaks to disrupt genes, leading to insertions and deletions (indels) through the nonhomologous end joining (NHEJ) pathway. In comparison to homology-directed repair (HDR), NHEJ is the most effective conventional gene editing technique because it is a DNA repair pathway that is consistently active throughout the cell cycle and acts as the default pathway for repairing DNA breaks. On the other hand, HDR is only active during the S and G2 phases of the cell cycle and calls for the delivery of an external repair template. Due to exogenous DNA's potential toxicity toward the majority of therapeutically important cell types and the natural competition between NHEJ and HDR, these characteristics present difficulties for the clinical application of HDR. However, HDR has the benefit of allowing for precise genome editing, which is true gene editing with control over the desired result. However, due to the possibility of unintended edits and chromosomal abnormalities such as translocations and chromothripsis, both NHEJ and HDR approaches involve DNA breaks, which are thought to be potential sources of genotoxicity [1, 2].

To lessen the likelihood of harmful events caused by DNA breaks, next-generation gene editing tools such as base and prime editing have been developed. These tools rely on DNA single-strand nicking, which lowers the possibility of chromosomal aberrations and off-target edits. They are still constrained in the types of edits they can make, however. To enable more extensive edits, novel editors based on CRISPR-associated transposases or CRISPR-directed integrases have been created. These editors are not yet ready for use in clinical settings as they are still in the developmental stage. However, it is anticipated that the application of CRISPR-based tools and other site-specific engineered nucleases for the treatment of human diseases will increase given the rapidly evolving toolbox of gene editing techniques. There are still a number of issues to resolve and problems to be solved in the pursuit of precise gene correction. This research topic on therapeutic gene correction strategies based on engineered site-specific nucleases or CRISPR systems seeks to address some of these issues and offer new scientific insights. The subject is covered by a number of contributions, including important scientific developments in precise genetic engineering and professional opinions on current developments in the area. By making these efforts, we hope to hasten the application of therapeutic gene correction techniques to patients who are in need [3].

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An overview of the most recent developments in nuclease and nickase genome editing methods for the treatment of genetic diseases is provided in an article by Lu et al. The article gives a thorough overview of the advancements made to these methods and identifies the major difficulties encountered when implementing these methods in clinical settings [4].

Reshetnikov et al reviewed the state of base editors (BEs) for the correction of point mutations in monogenic diseases in a separate study. BEs for adenine and cytosine allow for the conversion of C•G to T•A and A•T to G•C, respectively. The article provides a comprehensive description of these editing tools and offers in-depth insights into studies that have used BEs *in vivo* and *in vitro/ex vivo* for a variety of monogenic disorders, including retinal, neuromuscular, blood, and metabolic disorders [5].

The genome editing repertoire has been exciting since the recent addition of transcription activator-like effector domain (TALE) BEs. Boyne et al combined a nuclease and a BE, two different molecular tools, to demonstrate the viability of effective multiplex gene engineering in their original paper. They also defined the ideal activity window for TALE BEs. Utilizing such a multiplex strategy has a number of important benefits, including improved control over editing results by preventing translocations, which frequently occur when using multiple nucleases at once. Moreover, this method allows for gene knock-ins at the nuclease target site, which can be used to achieve more than just knock-outs [6].

On the basis of these discoveries, Wolff et al present piggyPrime, a novel high-efficiency prime editing tool. The genomic integration of all prime editing genetic components into cells is made possible by this tool's use of the piggyBac transposon system, which enables the gradual accumulation of prime edits over time. Surprisingly, using this technique, up to 100% of the desired alleles can be targeted in some cell lines, allowing the creation of transgenic cell lines that are an accurate model for disease-causing genetic variants [7].

Usher et al also explored the process of creating disease model cell lines with pathogenic variants by using traditional CRISPR/Cas HDR-based gene editing to introduce these variants into the cellular genome. The authors compare various HDR-related parameters using data from 95 transfections, including changes to the donor template, concentration, HDR enhancers, and the application of cold shock. They also noted a weak correlation between guide RNA activity predicted by online algorithms and actual activity in cells. The authors offer a workflow for planning and carrying out gene editing experiments that produce and characterize clonal lines for use in disease modeling in response to these findings [8].

Furthermore, the articles by Houghton et al concentrate on methods for gene editing to prevent monogenic inborn errors of immunity (IEI). A thorough analysis of DOCK8 immunodeficiency syndrome, an instance of autosomal recessive hyper IgE syndrome brought on by defects in the DOCK8 gene, is provided by Ravendran et al. Ravendran et al discuss various genome-editing strategies that may be used to treat this crippling immunodeficiency syndrome [9, 10].

However, X-linked lymphoproliferative disease (XLP), an IEI caused by mutations or deletions in the SH2D1A gene, is the focus of Houghton et al. The research by Houghton et al compares the application of TALENs, CRISPR/Cas9, and CRISPR/Cas12a in conjunction with AAV6-mediated delivery of a repair template. The components, which focus on exon 1 close to the start codon, make it easier to integrate a nearly full SH2D1A cDNA sequence, allowing for its physiological expression and regulation by the endogenous promoter. The study shows integration frequencies in T cells ranging from 30% to 50%, demonstrating the recovery of immune function and SH2D1A gene expression in patient T cells to levels comparable to those seen in healthy individuals [10].

Despite the fact that genome editing has considerable potential, it is important to address any security issues this technology might raise. The potential side effects of CRISPR nuclease activity in human clinical trials are examined by Wienert and Cromer. In their review, they give a summary of the most recent sequencing-based techniques that can identify both minor and major unintended effects of genome editing, even at low frequencies. The risk of unintended editing in unintended cell types and the possibility of germline transmission are highlighted by the authors, who also emphasize the safety and ethical issues surrounding the *in vivo* delivery of CRISPR tools. They also describe sophisticated mitigation techniques that can guarantee that CRISPR's safety keeps up with its effectiveness [11].

Schmidt et al and Atkins et al explored crucial facets of unintended on- and off-target editing outcomes in a similar manner. Using whole-genome sequencing (WGS) analysis, Schmidt et al assessed the on- and off-target editing outcomes in Mauritian cynomolgus macaque embryos with CRISPR-Cas9 targeting of the CCR5 gene. The WGS analysis of CRISPR-Cas9-targeted nonhuman primate embryonic cells presented in this study is the first to show large deletions at the target site and *de novo* mutations at predicted off-target sites. These results unequivocally show the need for thorough sequencing-based techniques to assess editing outcomes in primate embryos, underscoring the dangers of editing human embryos. In a review, Atkins et al reviewed the advantages and disadvantages of various methods for identifying off-target cleavage events. They also discuss how these methods can be used clinically to evaluate the safety of cutting-edge CRISPR/Cas9 HIV curing approaches that are currently being tested in clinical trials [12, 13].

Compliance With Ethical Standards

In the realm of genetic medicine, the ethical considerations surrounding gene correction for therapeutic purposes are paramount. Similarly, the technical challenges and ethical concerns regarding gene editing in human germlines and embryos have sparked debates on the implications of altering heritable traits and the potential risks of unintended consequences. In the United States, the National Institutes of Health (NIH) guidelines emphasize the importance of informed consent, privacy protection, and the responsible conduct of research in genetic interventions. In the same context, the European Union's General Data Protection Regulation (GDPR) mandates stringent data protection measures to safeguard individuals' genetic

information and ensure transparency in genetic research. In the United Kingdom, the Human Fertilisation and Embryology Authority (HFEA) sets forth regulations governing the use of gene editing technologies in human embryos, emphasizing the need for rigorous oversight, transparency, and adherence to ethical standards [14-17]. These guidelines underscore the necessity of upholding ethical principles, respecting individual autonomy, and promoting the responsible application of gene correction technologies in therapeutic settings to ensure patient safety and uphold societal values. Such frameworks underscore the ethical imperative of upholding principles of beneficence, autonomy, and justice in the application of gene correction techniques, ensuring that advancements in genetic medicine are pursued ethically and morally.

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Conflict of Interest

The author has no conflict of interest to declare.

Data Availability

The author declares that data supporting the findings of this study are available within the article.

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