Molecular Therapy
Nucleic Acids
Commentary

Minicircle DNA vectors: A breakthrough in non-viral delivery of CRISPR base editors?

Masamitsu Kanada[1,2,3] and Assaf A. Gilad[4,5]

https://doi.org/10.1016/j.omtn.2024.102275

The recent approval of the world's first CRISPR-Cas9 gene-editing therapy for sickle cell disease marks a new era in human medicine.[1] This groundbreaking technology has revolutionized disease treatment by enabling the direct correction or modification of genes at the cellular level, leveraging its high specificity, versatility, and efficiency. While CRISPR-Cas9 represents a significant advancement, it builds upon a foundation of earlier gene therapy technologies. These include the use of viral vectors to deliver functional genes, successfully applied in disorders such as spinal muscular atrophy and hemophilia, and the development of targeted gene-editing tools like zinc finger nucleases and transcription activator-like effector nucleases (TALENs). The broader field of gene therapy has demonstrated promising results in treating various genetic disorders, certain types of cancer, and even some infectious diseases. The approval of CRISPR-based therapy for sickle cell disease represents a significant milestone in these ongoing efforts, and it opens new possibilities for treating a wide range of genetic conditions, potentially offering hope to patients with previously untreatable diseases.

Researchers have developed diverse strategies for delivering CRISPR-Cas9 components to somatic and stem cells, both *ex vivo* and *in vivo*. These methods include the use of viral vectors, such as adeno-associated viruses and lentiviruses, and synthetic nanomaterials like lipid or polymer nanoparticles for gene delivery. While viral vectors have demonstrated superior CRISPR-Cas9 delivery efficiency and more effective gene-editing outcomes, they face two significant challenges. First, the manufacturing of viral vectors is costly, requiring specialized facilities to ensure safety and efficacy. Second, viral vectors pose inherent safety risks, including immunogenicity and insertional mutagenesis. Overcoming these challenges is crucial for fully realizing viral vector-mediated gene-editing therapies. Non-viral vector technologies offer promising alternatives, potentially providing safer and more cost-effective gene delivery options. In addition to the lipid or polymer nanoparticles, researchers have developed various non-viral systems for therapeutic DNA delivery. These include the injection of recombinant plasmid DNA combined with physical methods such as gene gun, electroporation, hydrodynamic delivery, sonoporation, and magnetofection techniques. Each approach aims to enhance the delivery and integration of genetic material into target cells. However, despite these advancements, conventional plasmid DNA vectors often face limitations, primarily low efficiency in gene delivery and expression.

Base editors, a refinement of CRISPR technology, offer precise genomic modifications without causing double-strand breaks.[2] These tools combine a catalytically impaired Cas9 with a deaminase enzyme, enabling targeted nucleotide substitutions. By offering a potentially safer alternative to traditional CRISPR-Cas9 systems, base editors have shown promise in correcting point mutations associated with various genetic disorders. In a paper published in this issue, Evans et al. (2024)[3] introduce non-viral minicircle DNA vectors as a delivery vehicle for CRISPR base editors. These bacterial backbone-free plasmids are produced from parental plasmids utilizing site-specific recombination techniques in engineered bacteria. Minicircles offer several advantages over conventional plasmid vectors.[4] Their smaller size enables higher gene delivery efficiency, while the absence of bacterial components reduces immunogenicity. Moreover, minicircles mitigate the risk of unintended antibiotic resistance gene transfer to host bacteria, a concern associated with general plasmid vectors.

The authors' work builds upon the "Gene On" (GO) optical reporter system, introduced in 2020, which evaluates base editor activity at the single-cell level.[5] This system detects successful edits by converting a premature TAG stop codon to TGG (tryptophan) or ACG to ATG (start codon), thereby activating gene expression. By combining minicircle technology with the GO reporter system, Evans et al.[3] present a promising approach to enhance the efficiency and safety of CRISPR base editor delivery. Key improvements to the GO system include the introduction of Akaluc, a highly sensitive luciferase with red-shifted emission, enabling deeper tissue penetration in bioluminescence imaging.[6] This AkaBLI GO reporter system will be crucial for sensitively evaluating base editor delivery in animal models, providing valuable *in vivo* data on editing efficiency and specificity in future research. Such preclinical assessments are essential steps toward potential clinical translation.

Evans et al.[3] also uncovered significant challenges in the GO system. First, they identified an unexpected difficulty in accurately controlling reporter gene expression through premature stop codon conversion. Their data caution against using viral internal

[1]Institute for Quantitative Health Science and Engineering (IQ), Michigan State University, East Lansing, MI 48824, USA; [2]Department of Pharmacology & Toxicology, Michigan State University, East Lansing, MI 48824, USA; [3]College of Human Medicine, Michigan State University, East Lansing, MI 48824, USA; [4]Department of Chemical Engineering & Materials Science, Michigan State University, East Lansing, MI 48824, USA; [5]Department of Radiology, Michigan State University, East Lansing, MI 48824, USA

Correspondence: Masamitsu Kanada, Michigan State University, 775 Woodlot Dr., East Lansing, MI 48824, USA.

E-mail: kanadama@msu.edu

Correspondence: Assaf A. Gilad, Michigan State University, 775 Woodlot Dr., East Lansing, MI 48824, USA.

E-mail: gilad@msu.edu

© 2024 The Author(s).
Published by Elsevier Inc. on behalf of The American Society of Gene and Cell Therapy.
This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

ribosome entry site (IRES) elements and 2A peptides in expression vectors to control reporter gene expression. This finding may be related to the natural properties of read-through permissive sequences, which require further investigation. Second, they observed cell-type-dependent base conversion efficiency. Minicircle-based delivery of adenine base editors achieved a 60.6% average A-to-G conversion in HeLa cells but less than 3% in other cancer cell lines. Similarly, cytosine base editors showed an 11.6% average C-to-T conversion in HeLa cells but less than 2% in other cell lines. These findings highlight the value of the sensitivity and quantitative bioluminescence GO reporter system as a powerful tool for accurately monitoring base editor activity *in vitro*.

Future research on *in vivo* base conversion efficiency in animal models will be critical for assessing gene delivery efficiency, understanding tissue-specific responses, and evaluating potential off-target effects. These studies will provide crucial insights for developing effective therapeutic base editors. Minicircle technology may prove to be a crucial option in generating cost-effective and safe non-viral delivery systems for CRISPR gene editing and beyond. As the field progresses, integrating these research avenues will be essential for translating gene-editing techniques from laboratory discoveries to clinical applications.

DECLARATION OF INTERESTS

The authors declare no competing interests.

REFERENCES

1. Frangoul, H., Altshuler, D., Cappellini, M.D., Chen, Y.S., Domm, J., Eustace, B.K., Foell, J., de la Fuente, J., Grupp, S., Handgretinger, R., et al. (2021). CRISPR-Cas9 Gene Editing for Sickle Cell Disease and beta-Thalassemia. N. Engl. J. Med. *384*, 252–260.
2. Gaudelli, N.M., Komor, A.C., Rees, H.A., Packer, M.S., Badran, A.H., Bryson, D.I., and Liu, D.R. (2017). Programmable base editing of A*T to G*C in genomic DNA without DNA cleavage. Nature *551*, 464–471.
3. Evans, M.M., Liu, S., Krautner, J.S., Seguin, C.G., Leung, R., and Ronald, J.A. (2024). Evaluation of DNA Minicircles for Delivery of Adenine and Cytosine Base Editors using Activatable Gene On "GO" Reporter Imaging Systems. Mol. Ther. Nucleic Acids *35*, 102248.
4. Kay, M.A., He, C.Y., and Chen, Z.Y. (2010). A robust system for production of minicircle DNA vectors. Nat. Biotechnol. *28*, 1287–1289.
5. Katti, A., Foronda, M., Zimmerman, J., Diaz, B., Zafra, M.P., Goswami, S., and Dow, L.E. (2020). GO: a functional reporter system to identify and enrich base editing activity. Nucleic Acids Res. *48*, 2841–2852.
6. Iwano, S., Sugiyama, M., Hama, H., Watakabe, A., Hasegawa, N., Kuchimaru, T., Tanaka, K.Z., Takahashi, M., Ishida, Y., Hata, J., et al. (2018). Single-cell bioluminescence imaging of deep tissue in freely moving animals. Science *359*, 935–939.