International Journal of Medical Science and Clinical Research Studies

ISSN(print): 2767-8326, ISSN(online): 2767-8342

Volume 02 Issue 10 October 2022

Page No: 1120-1122

DOI: https://doi.org/10.47191/ijmscrs/v2-i10-22, Impact Factor: 5.365

Gene Editing in Cancer Therapy

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ABSTRACT

The site-specific modification of an existing gene is known as gene editing. A section of DNA must be cut with an endonuclease (such as the CRISPR-Cas9 system) before the two severed ends are brought together, frequently with a new or improved sequence inserted between them. Somatic cell gene editing can be helpful in a variety of clinical contexts, and some preliminary preclinical and clinical trials have been carried out. Extremely high levels of precision are required for DNA recognition, excision, and repair; issues with publishing integrity must be resolved. Germline editing utilizing eggs, sperm, or embryos raises ethical concerns.

ARTICLE DETAILS

Published On:
25 October 2022

Available on:
https://ijmscr.org/

INTRODUCTION

As the name implies, gene editing involves directly altering the endogenous genomic sequence of a cell, and as such, is sequence-specific and allows for highly specific genomic sequence alterations. Methods are being developed to treat genetic disorders, including inherited conditions (e and acquired conditions such as cancer and infections. In theory, target cells might be any type of cell, such as pluripotent stem cells from an early-stage embryo, certain multipotent tissue stem cells, or adult somatic cells like T lymphocytes. All the offspring would be anticipated to contain the modified form of the gene after modifying the genome in a dividing cell. Preclinical research has been done on various cell types. [1]

GENE EDITING

A single-strand cut or double-strand break [DSB] caused by an endonuclease enzyme must be made in DNA and the severed ends must be reconnected or repaired in order to edit a genetic sequence. The DNA sequence that originated in the vicinity of the cut is replaced by a new, altered sequence throughout the repair process. A template and the enzyme endonuclease are supplied to the target cell together with the new sequence. The needed edit is present in the template. [1, 2]

The accuracy of DNA recognition, excision, and repair must be extremely high, with specific alterations in the frame and absence of off-target effects, for therapy to be effective and safe (changes in genes or sequences that are not theintended purpose). In cell and animal model systems, this strategy has been employed successfully. These capacities for editing endogenous double-stranded DNA are present in a variety of biological systems and nuclease types. [3]

CRISPR-Cas 9: Regularly interleaved clustered short palindromic repeats, or CRISPR, and CRISPR-associated protein endonuclease 9 (Cas9) are components of an old bacterial mechanism that has been preserved through evolution to react to bacteria-infecting viruses (bacteriophages). CRISPR arrays are areas of DNA in bacterial genomes that can be utilized to store the genetic material of mobile genetic elements and infectious viruses. The CRISPR sequence can be translated into RNA, which can direct effector proteins to the homologous area in the viral genome, where endonucleases can split them, when a previously discovered virus infects bacteria again. One of the many endonucleases that can find and break DNA with a particular sequence is Cas9 (e.g., Cpf1, Cas12). [4]

By altering the guide RNA (gRNA) sequence to match the DNA sequence of the other, Cas9 can nearly guide any DNA sequence in the lab. The gene can be made more specialized by using optimized gRNAs. These viral and non-viral vectors can be used to deliver Cas9 and gRNA into cells. [4]

Main editors: Main editing is a form of gene editing created in 2019 in which only one DNA strand is cut, with the new (edited) sequence being provided via a changed guide RNA. A Cas9

enzyme is subsequently used to cut the unaltered strand, and the new sequence is then used as a tilla plan to repair the damage. For editing minor DNA changes (point mutations or small deletions), such as those causing sickle cell disease or a common variety of cystic fibrosis, this approach appears to have very high fidelity. [5]

Zinc finger nucleases (ZFNs): The gene of interest can be targeted by a group of enzymes known as zinc finger nucleases (ZFNs), which have the ability to cleave particular DNA sequences. ZFNs were created as hybrid proteins by combining a FokI restriction endonuclease DNA cutting module with a zinc finger array-based DNA-binding module. The synthesis of dimers between FokI's catalytic domains, which is the basis for cleavage, necessitates the production of two hybrid FokI proteins containing zinc fingers. The direct DNA strand is bound by one monomer, whereas the reverse DNA strand is bound by the other monomer. [6]

Endonuclease can only cut DNA when two ZFNs bind to both strands of the target DNA in the proper orientation. Each "finger" recognizes around three base pairs of DNA. To allow for the synthesis of dimers, catalytic activity, and the production of double-stranded breaks, the two FokI monomers must be near to one another (DSBs).[6]

Although the two hybrid proteins and the intermediate linker sequence provide ZFN a high degree of binding specificity, extensive engineering is needed to generate zinc fingers that can bind to any desired DNA sequence, making the procedure expensive, time-consuming, and difficult to replicate. [6]

TALEN: A plant pathogen (Xanthomonas) makes proteins called transcription activator-like proteins (TALs), which are able to change the expression of genes in plants. By joining a DNA-binding block of the TAL effector, which is made up of 33–35 amino acid repeats, to a DNA cleavage domain, TALs can be altered to have endonuclease activity. The name of these complexes is TALEN (transcription-activator-like effector nucleases). The sequence-independent FokI enzyme performs as a site-specific nuclease similarly to ZFNs, and TALENs can effectively cause DNA breaks that need to be repaired by joining non-homologous ends (NHEJ) and homology-directed repair (HDR) in human pluripotent stem cells. somatic cells, too. [7]

Two hypervariable amino acid residues at positions 12 and 13, known as repeating variable residues, allow TALENs to detect particular DNA sequences (VDR). Although TALENs can be created for almost any sequence, their application is constrained by the requirement to create new proteins for each target site, much like ZFNs. TALENs are introduced into cells using the same delivery vectors as CRISPR-Cas9 and ZFN. However, the size of TALEN (around 3 kb for a single TALEN) and the repetitive nature of TALEN arrays make it challenging to distribute in vivo, leading to packaging issues in some viral delivery systems. [7]

The cell creates the changes as the endogenous DNA sequence is repaired once it has been damaged. The editing processes that result in DSBs activate one of the two primary cellular DNA repair mechanisms (homology-directed repair or non-homologous end-joining). There is only a single-thread break in the main edition, thus no DSB repair is necessary. [7]

Homology-Directed Repair: In order to repair DSB, HDR employs a new sequence template, resulting in the introduction of a new sequence that can fix damaging variations. At the DNA level, this method enables precise editing. The cellular DNA repair machinery uses the template during DSB repair and can be supplied with the donor DNA (for example, single-stranded oligonucleotide DNA with the desired sequence) and endonuclease. Due to its greater precision, this approach is preferred in most situations. The most effective ways to ensure the accuracy and specificity of editions are still being studied. [8]

Non-homologous end bonding: The two cut ends of DNA are repaired by NHEJ so that they can be reattached, although this process frequently introduces tiny insertions or deletions (indels) at the repair site. Indels may affect target genes by changing the reading framework, which may result in the breakdown of RNA or the generation of an unusable protein. A disease-causing gene that is overexpressed or operates via a dominant-negative mechanism can be altered utilizing NHEJ. Alternately, NHEJ can be employed to fix a mutated gene's reading frame. As an illustration, NHEJ was utilized to induce frame deletion of exons 20 to 23 in a mouse model of Duchenne muscular dystrophy (mdx mice), preventing transcription of a nonsense variant in exon 23, which would have resulted in premature codon arrest and altered dystrophin production. [9]

Gene editing clinical research: The two cut ends of DNA are repaired by NHEJ so that they can be reattached, although this process frequently introduces tiny insertions or deletions (indels) at the repair site. Indels may affect target genes by changing the reading framework, which may result in the breakdown of RNA or the generation of an unusable protein. A disease-causing gene that is overexpressed or operates via a dominant-negative mechanism can be altered utilizing NHEJ. [10, 11]

Potential adverse effects of gene editing: Gene editing raises serious safety concerns regarding the possibility of off-target effects, such as the cleavage or genetic modification of DNA regions other than the intended target site; disruption of oncogenes, tumor suppressor genes, and/or DNA repair genes may cause significant cellular toxicity and/or the emergence of cancer. The specificity and fidelity of genome editing methods are being improved while negative effects are being lessened. The amount of gene therapy construction used, the pattern of the gene-editing system's expression, and the number, kind, and stage of the edited cells' development all have an impact on how specific the gene editing process is. [12]

Ethical concerns with germline genome editing [13]: Contrary to gene editing in somatic cells, the idea of permanently altering the germline through gene editing in human gametes (eggs or sperm) or embryos poses significant ethical issues. Several consensus papers published between 2017 and 2018 and an op-ed from 2015 both addressed these issues, which include the following:

- The inability to express assent before to birth.
- Lack of distinction between applications for research and those for clinical use.
- Resource allocation and fair access
- Exploitation for alterations that are not therapeutic (such as "enhancing" a trait rather than curing or preventing an illness)
- Potentially unanticipated negative effects
- Possible consequences for future generations, such as the need for monitoring and a lack of consent
- Possibility of discrimination because of health risks or better performanceSome of these concerns also apply to gene therapy and adult stem cell gene editing.

CONCLUSIONS

There is broad agreement in the medical and scientific communities that genome editing of somatic cells has the potential to treat inherited illnesses and possibly some types of cancer. As such, its pursuit is justifiable, provided that these problems are well addressed. Despite these concerns germline genome editing may one day be used to "fix" a pathogenic mutation or to counteract its negative consequences (for example, by changing a water-to-water component in a pathway), curing or at least improving the disease. This contrasts with the use of germline genome editing, which is not thought to be medically ethical, to improve or change features unrelated to the condition.

REFERENCES

I. Mao, Y., Botella, J. R., Liu, Y., & Zhu, J. K. (2019). Gene editing in plants: progress and challenges. National Science Review, 6(3), 421-437.

II. Mosbach, V., Poggi, L., & Richard, G. F. (2019). Trinucleotide repeat instability during double-strand break repair: from mechanisms to gene therapy. Current genetics, 65(1), 17-28.

III. Salsman, J., & Dellaire, G. (2017). Precision genome editing in the CRISPR era. Biochemistry and cell biology, 95(2), 187-201.

IV. Singh, V., Braddick, D., & Dhar, P. K. (2017). Exploring the potential of genome editing CRISPR-Cas9 technology. Gene, 599, 1-18.

V. Anzalone, A. V., Koblan, L. W., & Liu, D. R. (2020). Genome editing with CRISPR–Cas nucleases, base editors, transposases and prime editors. Nature biotechnology, 38(7), 824-844.

VI. Urnov, F. D., Rebar, E. J., Holmes, M. C., Zhang, H. S., & Gregory, P. D. (2010). Genome editing with engineered zinc finger nucleases. Nature Reviews Genetics, 11(9), 636-646.

VII. Sun, N., & Zhao, H. (2013). Transcription activator-like effector nucleases (TALENs): a highly efficient and versatile tool for genome editing. Biotechnology and bioengineering, 110(7), 1811-1821.

VIII. Moreno-Mateos, M. A., Fernandez, J. P., Rouet, R., Vejnar, C. E., Lane, M. A., Mis, E., ... & Giraldez, A. J. (2017). CRISPR-Cpf1 mediates efficient homology-directed repair and temperature-controlled genome editing. Nature communications, 8(1), 1-9.

IX. Prado, G. S., Pinheiro, T. T., de FARIA, J. C., & VIANELLO, R. (2021). Genome editing via non-homologous end-joining (NHEJ) and ribonucleoproteins (RNP).

X. Holt, N., Wang, J., Kim, K., Friedman, G., Wang, X., Taupin, V., ... & Cannon, P. M. (2010). Human hematopoietic stem/progenitor cells modified by zinc-finger nucleases targeted to CCR5 control HIV-1 in vivo. Nature biotechnology, 28(8), 839-847.

XI. Li, Y., Glass, Z., Huang, M., Chen, Z. Y., & Xu, Q. (2020). Ex vivo cell-based CRISPR/Cas9 genome editing for therapeutic applications. Biomaterials, 234, 119711.

XII. Lee, B. C., Lozano, R. J., & Dunbar, C. E. (2021). Understanding and overcoming adverse consequences of genome editing on hematopoietic stem and progenitor cells. Molecular Therapy, 29(11), 3205-3218.

XIII. Rossant, J. (2018). Gene editing in human development: ethical concerns and practical applications. Development, 145(16), dev150888.