UNDERGRADUATE RESEARCH IN NATURAL AND CLINICAL SCIENCE AND TECHNOLOGY (URNCST) JOURNAL
Read more URNCST Journal articles and submit your own today at: https://www.urncst.com

ENCYCLOPEDIA ENTRY OPEN ACCESS

Zinc Finger Nucleases (ZFNs) and Gene Therapy Applications

Maya Cheit, BHSc Student [1]*, Rahaf Zeidan, BSc Student [2]

[1] Department of Health Science, University of Western Ontario, London, Ontario, Canada N6A 3K7
[2] Department of Science, University of Western Ontario, London, Ontario, Canada N6A 3K7

*Corresponding Author: mcheit@uwo.ca

Abstract

Introduction and Definition: Zinc finger nucleases (ZFNs) are a group of artificial restriction enzymes which are crucial for various processes in gene editing. They can target diseases based on gene therapy approaches and can be applied to a variety of eukaryotic cells through the precise introduction of double-strand breaks at specific genomic locations. Zinc Fingers were discovered in 1985 with ZFNs being discovered shortly after that in 1996.

Body: ZFNs are engineered by fusing DNA-binding domains with the nuclease domain of a FokI restriction enzyme. This results in custom-designed enzymes capable of binding to the target DNA sequence and inducing a double-strand break at the desired site. This binding is possible due to the high degree of specificity to recognize specific DNA sequences within the genome associated with disease-causing mutations. The DNA repair mechanisms, including both non-homologous end joining (NHEJ) and homology-directed repair (HDR), are triggered in response to double-strand breaks induced by ZFNs at specific genomic locations inside the cell. Cells possess innate mechanisms to mend such breaks. In the case of (NHEJ), the broken DNA ends are often rejoined, resulting in small insertions or deletions (indels) at the break site, potentially disrupting the function of the targeted gene. Alternatively, (HDR) can be employed, utilising an external DNA template to accurately introduce desired genetic modifications. ZFNs can be delivered to target cells using various methods and depends on factors such as the target tissue, specific diseases being targeted, and safety considerations. Viral vectors such as adeno-associated viruses or lentiviruses are used to deliver ZFNs into target cells. Lipid nanoparticles are synthetic encapsulates that allow repeat administration and transient delivery of ZFNs. Direct delivery involves physically introducing ZFNs which can allow a high degree of precision.

Keywords: zinc finger nucleases; ZFN; gene; delivery; cell

Introduction

ZFNs are engineered enzymes, which are a group of protein catalysts with unique structural features that play a vital role in gene editing. They are designed to target genetic disorders using gene therapy by making precise modifications to disease-causing genes through focusing and altering disease-causing genes by creating specific double-strand DNA breaks, which are subsequently repaired via non-homologous end joining or homologous recombination pathways. The discovery of Zinc Fingers in 1985 and ZFNs in 1996 marked significant advancements in gene therapies. Owing to the fact that they are a class of human proteins that are smaller than other gene therapy counterparts, which allow targeted modifications to specific gene sites across different species. Since then, ZFNs have developed into powerful tools for diverse applications, with continuous technological improvements enhancing their precision and safety in genetic disease treatment. As our molecular biology knowledge and technical capabilities progress, ZFNs remain central to gene editing innovations, promising better treatments and potential cures for genetic disorders previously deemed incurable.

Body

Applications in Gene Therapy

Zinc Finger Nucleases ZFNs are powerful tools that enable the introduction of precise DNA sequence changes into individual genes, which can then be experimentally manipulated to observe their effects. In comparison to other commonly used gene editing tools such as CRISPR/Cas-9, there is a higher degree of specificity that can be achieved using ZFN technology. Current challenges of common gene therapy tools such as CRISPR/Cas-9 include a lower precision of targeting specific genes as well as the risk of off-target effects, and the difficulty in effectively treating certain genetic disorders. ZFNs can address some of these unmet needs by offering a more customizable and specific approach to gene editing for future applications with high

precision. These approaches have been applied to numerous organisms with eukaryotic cells, such as rats, rabbits and pigs [1]. However, human gene therapy applications are currently undergoing trials for future use to correct mutations within genetic diseases. The first clinical trial of ZFNs use in humans began in November 2017, aiming to assess the safety and tolerability of ZFN-based in vivo genome editing therapy. These diseases span from a wide range of diseases such as Human Immunodeficiency Virus (HIV), as ZFNs are being developed to disrupt the CCR5 gene, which is critical in making cells resistant to HIV infection [2]. Furthermore, cancer therapy studies are also being conducted in vitro, meaning in a lab setting rather than within a living organism, as ZFNs can knock out genes that decrease the abilities of cell adhesion, migration and invasion of hepatocellular carcinoma cells (Liver Cancer) [3]. Overall other diseases that are also being trialed for potential future use of ZFNs are Acquired Immunodeficiency Syndrome (AIDS), Cystic Fibrosis, Hemophilia and Sickle Cell Anemia [4].

Target Identification

Since ZFN specificity is not certain, it is important to select targets without similar genomic off-target sites, referring to locations in the genome where the ZFN might bind and induce unintended modifications. [5] Sequence specificity is the sequence of the Zinc Finger array that determines its binding specificity. Optimizing the arrangement of Zinc Fingers ensures high specificity for the target DNA sequence. ZFNs target specific DNA sequences through the precise binding of Zinc Finger arrays to their recognition sites within the genome [6]. Three to four Zinc Finger proteins will target 9 to 12 bases per ZFN and 19-24 bases for a ZFN pair. ZFN pairs are used for gene targeting at specific loci [7]. BLAST (Basic Local Alignment Search Tool) can be used to identify sequences within the genome that are suitable targets for genome editing using ZFNs. BLAST functions by using its bioinformatics tool used to search for sequences within its database. It works by searching for homologous sequences or confirming the uniqueness of potential target sites. BLAST also helps in selecting appropriate sequences for the ZFN design [8]. Once target sequences are identified, ZFNs are engineered to bind to and cleave these specific sequences. They enable precise modifications in the genome, facilitating applications such as gene knockout or correction based on the identified target sequence [9]. However, BLAST is not optimal in finding ZFN-off target sites and may miss some as they use seed-based as well as non-overlapping word indexes [10]. The seed-based method is efficient for identifying regions of high similarity but may miss potential off-target sites where mismatches occur within the seed regions. Seed regions referring to a short, initial segment of a nucleotide sequence that is used as a starting point for identifying regions of high similarity during sequence alignment or searching. Non-overlapping word indexes to quickly index and search through sequence databases is efficient for exact matches. However, this approach may overlook potential off-target sites where sequences are similar but not identical due to insertions, deletions, or mismatches that exceed the seed length [10].

ZFN Engineering

ZFNs are engineered by fusing zinc finger DNA-binding domains that target specific DNA sequences, with the nuclease domain of the FokI restriction enzyme, which cuts DNA at those sites [11]. This results in custom-designed enzymes capable of binding to the target DNA sequence and inducing a double-strand break (DSB), which is when the phosphorous sugar backbone of the DNA is broken at the desired site. This binding is possible due to the high degree of specificity to recognize specific DNA sequences within the genome associated with disease-causing mutations [12]. Achieving optimal activity and specificity in ZFNs involves carefully selecting and validating Zinc Finger modules that have both a high affinity for binding and targeting DNA and minimal off-target effects [13].

Selection of Zinc Finger Modules

Both Oligomerized Pool Engineering (OPEN) and Modular Assembly are used in the engineering of ZFNs. However, they differ in approaches and applications.

Oligomerized Pool Engineering (OPEN)

This is used to generate and select Zinc Finger arrays with high specificity and affinity for targeting and modifying DNA in eukaryotic genomes. The arrays are selected for by first selecting Zinc Finger modules through a vast module library. This library contains zinc finger arrays to select from, tailored to specifically target genomic sites of interest. This allows ease in identifying the most effective zinc finger for a given target. The individual Zinc Finger modules are than oligomerized, meaning linked together to form arrays. They are then screened, against the target DNA sequence, and final selection is based on the ability to bind specifically and strongly to the target DNA [14]. Modification of DNA through the array is achieved when fusing to a nuclease domain, typically the FokI endonuclease. This creates the ZFN, which cleaves DNA at the site bound by the Zinc Fingers. Targeted DNA cleavage occurs via the introduction of ZFNs to cells where the nuclease domain can cut at the specified location. It is a combinatorial selection-based research method to create Zinc Finger arrays to be used in ZFNs. Multiple customized ZFNs can be created in 8 weeks with this method as opposed to the slower “modular assembly”. The success rate is higher than modular assembly at around 80% [15].

Modular Assembly

This is an approach to create multi-finger arrays that treat fingers like independent units. This refers to each Zinc Finger domain within the protein being considered as a modular, standalone unit that recognizes a specific DNA triplet (three base pairs). This allows for the designing and evaluation of the Zinc Finger module separately, rather than considering them in their final assembly. In doing so, it helps improve precision, customization as well as error reduction. This approach allows each Zinc Finger to be combined to form a multi-finger array, with each finger contributing independently to the binding of a longer DNA sequence. It is created using pre characterized Zinc Fingers, referring to zinc finger domains (small protein structures) that have already been studied and their DNA-binding properties determined to be used in recombinant DNA technology. The success rate of this method is lower than (OPEN) with a three-finger ZFN pairs having a 9.1% success rate whilst a four-finger pair at 26% [7] [16].

Fusion with FokI Nuclease Domain

The fusion of engineered Zinc Finger arrays with the FokI nuclease domain is a crucial step in the development of functional ZFNs. The FokI nuclease domain, derived from the FokI restriction enzyme, is particularly chosen for its ability to induce DSBs in DNA. Via its DBS, ZFNs can ultimately perform targeted mutagenesis, gene knock out, and targeted gene replacement. The FokI domain functions as a dimer, meaning two FokI domains must come together to cleave DNA. The orientation and spacing between individual Zinc Fingers within the array must be precisely controlled to ensure efficient dimerization of the FokI domains and accurate cleavage of the DNA. The engineered Zinc Finger arrays are fused to the FokI nuclease domain to engineer a protein that can bind then cleave the DNA of interest [17].

Structure of a ZFN

The structure of zinc nucleases is useful due to its specificity, versatility, customization and overall abilities within genome editing. They are chimeric nucleases made of a specific binding domain consisting of Zinc Finger protein motifs that bind to DNA fused to a nonspecific FokI endonuclease [18]. The Zinc Finger domains can individually recognize three to four nucleotide DNA sequences. The zinc nuclease is a modular construct of two functional domains linked via a peptide bond. They are made up of two modular domains, allowing the ability to link multiple Zinc Fingers and for the creation of custom DNA-binding proteins, tailored to bind virtually any DNA sequence. ZFNs can target DNA sequences using its custom-designed Zinc Finger proteins with a higher degree of specificity, reducing off target effects in comparison to other gene editing tools such as CRISPR/Cas-9. This modularity provides a high degree of flexibility and precision in targeting specific genomic loci [15]. Specificity and DNA recognition come from the individual Zinc Finger motifs, each recognizing a specific 3-base pair DNA sequence. By linking multiple Zinc Finger motifs in tandem, researchers can design Zinc Finger arrays that recognize longer and unique DNA sequences with high specificity [19]. In the engineered ZFN structure, the Zinc Finger DNA-binding domains provide specificity by recognizing and binding to the target DNA sequence. The FokI nuclease domains, on the other hand, are responsible for cleaving the DNA strand once dimerized. The FokI nuclease domain is a non-specific DNA cleavage domain derived from a bacterial restriction enzyme called Flavobacterium okeanokoites. It only becomes active when two FokI domains dimerize, which occurs when the Zinc Finger domains bind to adjacent DNA sequences. This ensures that the nuclease activity is tightly regulated and only occurs at the intended target site. The dimerization is necessary for two FokI domains to come together to cut the DNA, and provides an additional layer of specificity for the ZFN [12].

Mechanisms of Action & Delivery Methods

Zinc finger nucleases ZFNs have demonstrated immense promise in genetic engineering, not only owing to their accuracy in targeting specific DNA sequences, but also due to the different means for delivering them to target cells. The target tissue, the specific diseases being addressed, and safety considerations all influence the approach for delivery method chosen. ZFNs can be delivered to target cells using various techniques and depends on factors such as the target tissue, specific diseases being targeted, and safety considerations [15].

Viral vectors such as adeno-associated viruses or lentiviruses are modified viruses designed to deliver genetic material into cells and are used to deliver ZFNs into target cells. Adeno-associated viruses are attractive due to their ability to infect a wide range of cell types and their relatively low immunogenicity, which reduces the risk of triggering immune responses [20]. Lentiviruses have a larger cargo capacity compared to adeno-associated viruses, allowing for the delivery of larger ZFN constructs or additional regulatory elements. Lentiviruses can integrate their genetic material into the host cell genome, providing stable and long-term expression of ZFNs in target cells. Lipid nanoparticles are synthetic encapsulates that allow repeat administration and transient delivery of ZFNs [21]. These nanoparticles encapsulate ZFNs within lipid bilayers, facilitating their transport across cell membranes. Lipid nanoparticles are advantageous for their ability to protect ZFNs from degradation and to enable repeated administration, which is useful for treatments requiring multiple doses over time. Direct delivery involves physically introducing ZFNs which can allow a high degree of precision in delivering ZFNs directly to specific cells or tissues. This can include techniques such as electroporation, a process of using an electric field to introduce DNA into

the cells by creating temporary pores. Additionally, techniques such as microinjection can be used, which allows introduction of genetic material directly into the cytoplasm or nucleus of a cell [16].

New Research

Human immunodeficiency virus type 1 (HIV-1) remains a global health challenge, primarily targeting CD4+ T cells, which helps coordinate the immune response against infection and disease. HIV-1 enters these cells through interactions with surface receptors, notably the CD4 receptor and coreceptors CCR5 and CXCR4. This consists of attaching the virus envelope to the cell membrane and allowing for binding, leading to the formation of fusion-enabling complexes respectively. ZFN editing of the HIV coreceptors CCR5 and CXCR4 has revealed potential approaches to shielding CD4+ T cells against HIV-1 infection. Researchers effectively produced CD4+ T cells resistant to HIV-1 infection by genetically altering both CCR5 and CXCR4 at the same time using ZFNs. In laboratory trials, these genetically changed cells maintained normal proliferation rates, which is when a cancer cell copies its DNA and divides into two cells, but also showed strong resistance to viral infection. These findings indicate that altering HIV coreceptor function by genetic alteration might be a feasible technique for establishing long-term, medication-free therapy for controlling established HIV-1 infections [22].

Regulatory and Ethical Considerations of Gene Therapy

Regulatory Considerations: Approval and Oversight: Clinical Trials

Before ZFNs can be used in human therapies, they must undergo rigorous testing in clinical trials to demonstrate safety and efficacy. Regulatory bodies like the FDA (U.S. Food and Drug Administration) or EMA (European Medicines Agency) oversee these trials [18]. ZFN-based therapies are subject to gene therapy regulations, which can vary by country. For example, ZFNs must undergo various and through clinical trials to ensure they are safe and effective for future use. These regulations ensure that gene-editing techniques meet safety standards to protect patients and the environment [12]. Production of ZFNs must also be overseen for the entirety of its processing to maintain a consistency and purity.

Ethical Considerations of Gene Therapy

ZFNs can be used to edit both somatic, non-reproductive cells, and the germline which are sex cells such as sperm and egg. ZFNs have primarily been developed and used for somatic gene editing, which affect only the DNA of the individual treated. However, germline cell editing has many strict prohibitions and regulations as it raises ethical questions surrounding its multigenerational effects [17]. Additionally, ZFNs are some of the more expensive gene editing tools, which raises concerns over equity and accessibility to these treatments. Finally, the safety concerns of with ZFNs is the potential for off-target DNA cleavage, which can cause unintended genetic mutations. Regulatory frameworks often require comprehensive studies to assess and mitigate these risks [23]. While ZFN technology holds great promise for treating diseases and advancing scientific understanding, its use can undermine ethical principles related to safety, autonomy, and informed consent. A more cautious, ethically informed approach to ZFN applications is necessary to mitigate risks and ensure that its benefits do not come at the cost of ethical compromises.

Animal Use: Animal Welfare

Animals are often used in preclinical testing as to test the safety, efficacy and potential side effects of therapies before they are tested on humans. This is because there are model organisms that are genetically similar to humans, such as mice and rats. Although this raises concerns, the use of ZFNs on animals in research raises ethical questions about animal welfare and the potential for suffering [24]. Justification of use needs to ensure that ethical guidelines are followed, meaning the potential benefits outweigh the harm caused to the animals. Additionally, researchers must demonstrate the information obtained cannot be gained through other means.

Dual-Use Concerns: Bioterrorism

The powerful capabilities of ZFNs could be misused for harmful purposes, such as bioterrorism. This is because ZFNs can be used to modify human genes in ways that can cause harm and introduce genetic vulnerabilities that can be exploited. Furthermore, gene editing can also be used to modify organisms in the environment, which can lead to ecological disruptions. Ethical considerations include the development of safeguards and international agreements to prevent such misuse [12].

List of Abbreviations

AIDS: acquired immunodeficiency syndrome
BLAST: basic local alignment search tool
173DNA: deoxyribonucleic acid
HDR: homology-directed repair
HIV: human immunodeficiency virus
NHEJ: non-homologous end joining
OPEN: oligomerized pool engineering
ZFN: zinc finger nuclease

Conflicts of Interest

The author(s) Maya Cheit and Rahaf Zeidan declare that they have no conflict of interests.

Authors' Contributions

MC: Performed comprehensive literature review, selected and analyzed relevant data, wrote initial drafts, made critical revisions to the manuscript, and gave approval of the final version.
ZA: Performed comprehensive literature review, selected and analyzed relevant data, wrote initial drafts, made critical revisions to the manuscript, and gave approval of the final version.

Acknowledgements

I would like to thank Neha Dhanvanthry for assisting me and advising me throughout the process of writing this manuscript. Additionally, ChatGPT-3.5 was used to assist in the writing process.

Funding

The development of this encyclopedia entry was not funded.

References

[1] Didigu CA, Wilen CB, Wang J, Duong J, Secreto AJ, Danet-Desnoyers GA, et al. Simultaneous zinc-finger nuclease editing of the HIV coreceptors ccr5 and cxcr4 protects CD4+ T cells from HIV-1 infection. Blood (2014 January 2;123(1), 61–69. https://doi.org/10.1182/blood-2013-08-521229
[2] Gaj T, Gersbach CA, Barbas CF. ZFN, TALEN, and CRISPR/Cas-based methods for genome engineering. Trends in Biotechnology. 2013 July;31(7):397-405. https://doi.org/10.1016/j.tibtech.2013.04.004
[3] Reyon D, Kirkpatrick JR, Sander JD, Zhang F, Voytas DF, Joung JK, et al. ZFN Genome: A comprehensive resource for locating zinc finger nuclease target sites in model organisms. BMC Genomics. 2011 January 28; 12:83. https://doi.org/10.1186/1471-2164-12-83
[4] Lee J, Ha S, Kim MJ, Park JH, Kim K. Modeling hepatocellular carcinoma in vitro using cell culture systems. Journal of Gastrointestinal Cancer. 2021 December 20;52 :1294–1308. https://doi.org/10.1007/s12029-021-00772-1
[5] Cradick TJ, Ambrosini G, Iseli C, Bucher P. ZFN-Site searches genomes for zinc finger nuclease target sites and off-target sites. BMC Bioinformatics. 2011; 12:152. https://doi.org/10.1186/1471-2105-12-152
[6] Joung JK, Sander JD. TALENs: A widely applicable technology for targeted genome editing. Nature Reviews Molecular Cell Biology. 2012 November 21;14(1):49-55. https://doi.org/10.1038/nrm3486
[7] Urnov FD, Rebar EJ, Holmes MC, Zhang HS, Gregory PD. Genome editing with engineered zinc finger nucleases. Nature Reviews Genetics. 2010 September; 11(9):636-46. https://doi.org/10.1038/nrg2842
[8] Cathomen T, Joung JK. Zinc-finger nucleases: The next generation emerges. Molecular Therapy. 2008; 16(7):1200-7. https://doi.org/10.1038/mt.2008.114
[9] Yamamoto T, editor. Targeted Genome Editing Using Site-Specific Nucleases: ZFNs, TALENs, and the CRISPR/Cas9 System. 1st ed. Springer. 2015 January 5;1(1) 20. https://doi.org/10.1007/978-4-431-55227-7
[10] De Graeff N, Jongsma KR, Johnston J, Hartley S, Bredenoord AL. The ethics of genome editing in non-human animals: A systematic review of reasons reported in the academic literature. Philosophical Transactions of the Royal Society B. 2019;374(1772): 1471-2970. https://doi.org/10.1098/rstb.2018.0106
[11] Klug A. The discovery of zinc fingers and their development for practical applications in gene regulation and genome manipulation. Quarterly Reviews of Biophysics. 2010 May 18;43(1):1-21. https://doi.org/10.1017/S0033583510000089
[12] Carroll D. Genome Engineering with Zinc-Finger Nucleases. Genetics. 2011 August 1 ;188(4):773-782. https://doi.org/10.1534/genetics.111.131433
[13] Maeder ML, Thibodeau-Beganny S, Osiak A, Wright DA, Anthony RM, Eichtinger M, et al. Rapid "open-source" engineering of customized zinc-finger nucleases for highly efficient gene modification. Molecular Cell. 2008 July 25;31(2):294-301. https://doi.org/10.1016/j.molcel.2008.06.016
[14] Sander JD, Reyon D, Maeder ML, Foley JE, Thibodeau-Beganny S, Li X, et al. Predicting success of oligomerized pool engineering (OPEN) for zinc finger target site sequences. BMC Bioinformatics. 2010; 11:543. https://doi.org/10.1186/1471-2105-11-543
[15] Maeder M, Thibodeau-Beganny S, Sander J, Sander J, Voytas D, Joung JK. Oligomerized pool engineering (OPEN): an 'open-source' protocol for making customized zinc-finger arrays. Nature Protocol. 2009; 4:1471-1501. https://doi.org/10.1038/nprot.2009.98
[16] Conway A, Mendel M, Kim K, McGovern K, Boyko A, Zhang L, et al. Non-viral Delivery of Zinc Finger Nuclease mRNA Enables Highly Efficient In Vivo Genome Editing of Multiple Therapeutic Gene Targets. Molecular Therapy. 2019;27(4):866 77. https://doi.org/10.1016/j.ymthe.2019.03.003
[17] Jo YI, Kim H, Ramakrishna S. Recent developments and clinical studies utilizing engineered zinc finger nuclease technology. Cellular and Molecular Life Sciences. 2015 Jun 19;72(20):3819–3830. https://doi.org/10.1007/s00018-015-1956-5
[18] Joseph AM, Karas M, Ramadan Y, Joubran E, Jacobs RJ. Ethical Perspectives of Therapeutic Human Genome Editing From Multiple and Diverse Viewpoints: A Scoping Review Ther. Cureus. 2022 Nov 27;14(11). https://doi:10.7759/cureus.31927

[19] Wang L, Li F, Dang L, Liang C, Wang C, He B, et al. In vivo delivery systems for therapeutic genome editing. International Journal of Molecular Science. 2016 February 15;17(5):626. https://doi.org/10.3390/ijms17050626
[20] Naso MF, Tomkowicz B, Perry WL, Strohl WR. Adeno-Associated Virus (AAV) as a Vector for Gene Therapy. BioDrugs. 2017 July 1;31(4):317-34. https://doi.org/10.1007/s40259-017-0234-5
[21] Gaj T, Liu J, Anderson KE, Sirk SJ, Barbas CF. Protein Delivery Using Cys2-His2 Zinc-Finger Domains. ACS Chemical Biology. 2014 June 17;9(8):1662-7. https://doi.org/10.1021/cb500282g
[22] Fraser HB, Schadt EE. The quantitative genetics of phenotypic robustness. PLoS One. 2010;11(3):253-64. https://doi.org/10.1371/journal.pone.0008635
[23] Rothschild, J. Ethical considerations of gene editing and genetic selection. Journal of General and Family Medicine. 2020 May 29; 21(3), 37- 47. https://doi.org/10.1002/jgf2.321
[24] Goldberg AD, Allis CD, Bernstein E. Epigenetics: A Landscape Takes Shape. Cell. 2020;144(5):646-74. https://doi.org/10.1016/j.cell.2007.02.006

Article Information
Managing Editor: Jeremy Y. Ng
Peer Reviewers: Neha Dhanvanthry, Afnan Munshi
Article Dates: Received Jul 07 24; Accepted Nov 09 24; Published Mar 06 25

Citation
Please cite this article as follows:
Cheit M, Zeidan R. Zinc finger nucleases (ZFNs) and gene therapy applications. URNCST Journal. 2025 Mar 06: 9(3). https://urncst.com/index.php/urncst/article/view/680
DOI Link: https://doi.org/10.26685/urncst.680