The Role of Genome Editing in Targeted Therapy; Advances and Prospective Developments in Human Health

Ghunwa Javed[1,*], Qamar un Nisa[2], Ayah Talal Zaidalkilani[3], Ammar AL-Farga[4] and Shamreza Aziz[5]

[1]Department of Institute of Biochemistry and Biotechnology, University of Veterinary and Animal Sciences, Lahore, Pakistan
[2]Department of Pathology, University of Veterinary & Animal Sciences, Lahore, Pakistan
[3]Faculty of Pharmacy and Medical Sciences, Department of Nutrition, University of Petra, Amman, Jordan
[4]Department of Biological Sciences, College of Science, University of Jeddah, Jeddah, Saudi Arabia
[5]Department of Epidemiology and Public Health, University of Veterinary and Animal Sciences, Lahore, Pakistan
*Corresponding author: ghunwa.javed5@gmail.com

Abstract

The explosion of research that has come from the bacterial or synthetic nucleases has meant that nearly all eukaryotic organisms can have specific genomic regions targeted and modified provided that the accurate cell animal models of pathological events can be created. Moreover, in recent years only the genome editing has revealed great potential in many different areas starting from the basic research and finishing with the applied biotechnology together with the biomedical research. Newer commercially available nucleases like the zinc finger nuclease (ZFNs), transcription activator like effector nuclease (TALEN) and CRISPR – Cas associated nuclease has ensured a transition from idea to implementation. New modifications of three basic genome editing methods [TALENs, CRISPR/Cas9, ZFNs] and the use of derived products as actual tools for gene editing in a broad range of human pathologies and potential future treatments are examined, with special emphasis on the influence of the eukaryotic system and relevant animal models. Last, we briefly discuss clinical applications of the genome editing platforms for disease regimens and potential issues with the application of this technology.

Keywords: Genome Editing Tools, Mechanisms, Zinc finger nuclease, TALENs, CRISPR/Cas9, Disease modeling

Cite this Article as: Javed G, Nisa QU, Zaidalkilani AT, AL-Farga A and Aziz S, 2025. The role of genome editing in targeted therapy; advances and prospective developments in human health. In: Nisa ZU, Altaf S, Zahra A and Saeed K (eds), Nanobiotech in Holistic Health: Innovations for Integrated Well-being. Unique Scientific Publishers, Faisalabad, Pakistan, pp: 176-182. https://doi.org/10.47278/book.HH/2025.371

A Publication of Unique Scientific Publishers

Chapter No: 25-747

Received: 07-Jan-2025
Revised: 01-Apr-2025
Accepted: 07-May-2025

Introduction

New technologies in genome editing have improved how human genome study so that researchers are now in a position to determine how a particular product of a gene affects an organism's affliction. The advance of genetic engineering in the 1970s when it became possible to change DNA or RNA created a new field of genome editing (Rothstein, 1983). The awareness and application of B or S nucleases that have rapidly developed in the last decade have emerged into several aspects of basic and complex biomedical research and applied biotechnology (Cornu et al., 2017). Through the provision of genome editing infrastructure, it could be carried out both in vitro and in vivo since the infrastructure is delivered in situ. This process in effect means that it generates, reduces and 'edits' genes (Gaj et al., 2013; Ghosh et al., 2019). Pointed DNA modifications begin with nuclease induced double strand breaks (DSBs) and this initiated highly efficient cellular DNA recombination in mammals (Rouet et al., 1994; Kosicki et al., 2018). DNA generated by nucleases Double-strand breaks (DSBs) are repaired by nearly all cell types and species utilizing one of two main mechanisms: either nonhomologous end-joining (NHEJ) or homology-directed repair (HDR), which results in targeted integration or gene disruption (O'Driscoll & Jeggo, 2006).

Since fully matched DNA segments act as homologous templates, HR has mainly been employed to mediate gene targeting, such as gene knock-in or gene knock-out. Thus, its value is rather limited because HR does not function efficiently in non-model species and mammalian cells. An additional strategy to improve HDR-mediated genome editing's performance was to apply targeted nucleases because it was previously shown that DSB could increase the HDR frequency by orders of magnitude. The HDR though may use a template of foreign DNA that mimics the termination region sequence for damage repair once a specific DSB has been generated (Kaniecki et al., 2018).

This technique may be also used for targeted alterations initiated site specifically to fix mutations or to embed new sequences; in this case, the correctly constructed repair template is delivered directly to targeted cells (Kim & Kim, 2014; Verma & Greenberg, 2016). However, while NHEJ-mediated repair beget gene insertions or deletions (indels) of incalculable extent at the DSB site in NHEJ endogenous genes, which finally contribute to gene inactivation, it generally causes errors (Chang et al., 2017). Indications in coding sequence cause frameshift alterations that results in mRNA to degrade & nonsense mediated decay to form nonfunctional truncated protein (Lieber et al., 2010). This procedure is expected computationally to be easier than HR-based approaches as (a) no requirement for the repair matrix (b) type of cell seems to have minimal influence on the efficacy of change. Therefore, like RNAi, NHEJ may be used to apply gene-knock down in immortalized cell lines, but it might bring about 'knock out' mutations that are permanent and un reversing (Delacôte & Lopez, 2008).

To produce the required DSBs at the different DNA target sites, developments in early genome editing have involved the creation of individualized zinc-finger nuclease (ZFN) and mega nucleases (Urnov et al., 2010). Since the desired proteins had to be generated as synthetic molecules with specifically encoded sequence recognition domains coupled with nonspecific nucleases to facilitate targeted DNA scission, these nuclease systems that we had developed entailed special skills to create targeted DNA cutting opportunities hitherto unimaginable with genetic manipulation tools (Cathomen & Joung, 2008; Silva et al., 2011).

The recently identified and highly effective gene editing technology called (CRISPR)-associated (Cas9) nuclease originates from bacterial immune response. Because this technique is capable to be well coded to alter the DNA sequence of integrated eukaryotic cells using an RNA guided DNA cutting module, this method outcompeted the ZFNs and TALENs to bring about preferred genetic changes. Since its first application in mammalian cells in 2013 for gene editing applications, the CRISPR/Cas9 system has quickly been adapted for gene expression regulation. This encompasses treatments as transcriptional or epigenetic changes, DNA repair or modification of any other aspect of the genome (Al-Attar et al., 2011; Cong et al., 2013).

Programmable nucleases have made the development of gene editing tools faster from discovery through clinical application and offered researchers precise control over most if not all genes across a broad spectrum of organisms and cell lines. Today's ongoing preclinical genomic editing is majorly related to developing T cell-based anticancer immunotherapy, hemophilia, muscular diseases, primary immune system defects, metabolic conditions, CVDs, viruses, and muscular diseases. Beyond preclinical research, some of these methods are presently undertaking phase I/II clinical trials. TALENs, CRISPR/Cas9, and ZFN are the three main genome editing technologies. Here, we look at their most recent developments and discuss how their corresponding reagents may be employed as tools for modifying genes for a variety of human illnesses and in exciting new treatments.

2. Structure and Mechanism of Genome Editing Tools

DNA interaction and ZFN structure are composed by fusing nonspecific cleavage domain to DNA target-recruiting domain, bound to zinc finger (Kim et al., 1996). Primarily discovered in 1985, the zinc-finger protein is a part of transcription factor IIIa in Xenopus oocytes; the factor is capable of binding to DNA at a definite locus (Diakun et al., 1986). A collection of Cys2His2 zinc fingers, which result from exceptionally stable interactions in their zinc-finger regions with corresponding DNA sequences, are employed to realize the essential specificity of the required zinc finger. About thirty amino acids and two anti-parallel β-sheets facing an α-helix make up the structural motif of the Cys2His2 zinc finger (Beerli & Barbas III, 2002).

The adaptive DNA recognition domain, Cys2-His2-ZF, is now believed to be the most common Genome-binding pattern in eukaryotic transcription factors, despite considerable uncertainty around the specificity of its DNA recognition mode. Each zinc-finger unit's selectivity may identify three base pairs in the DNA and establish base-specific connections by making direct contact with the main groove of the DNA through the finger's α-helical residues (Buck-Koehntop et al., 2012). In eukaryotic cells, the targeted DNA cleavage by ZFN generates DSBs on a specific site of the genome, which the body alters subsequent endogenous NHEJ / HDR repair pathways as required (Smith et al., 2000).

Three main factors impact ZFNs' sequence-based target identification and specificity:

(a) each finger of amino acid sequence

(b) How many fingers

(c) the interaction of the nuclease realm; Because ZFNs are modular, scientists can alter the catalytic and DNA-binding domains independently, even though each ZFN is made up of both. With this knowledge, scientists can create new modules that are both specific and attractive enough to manipulate a genome.

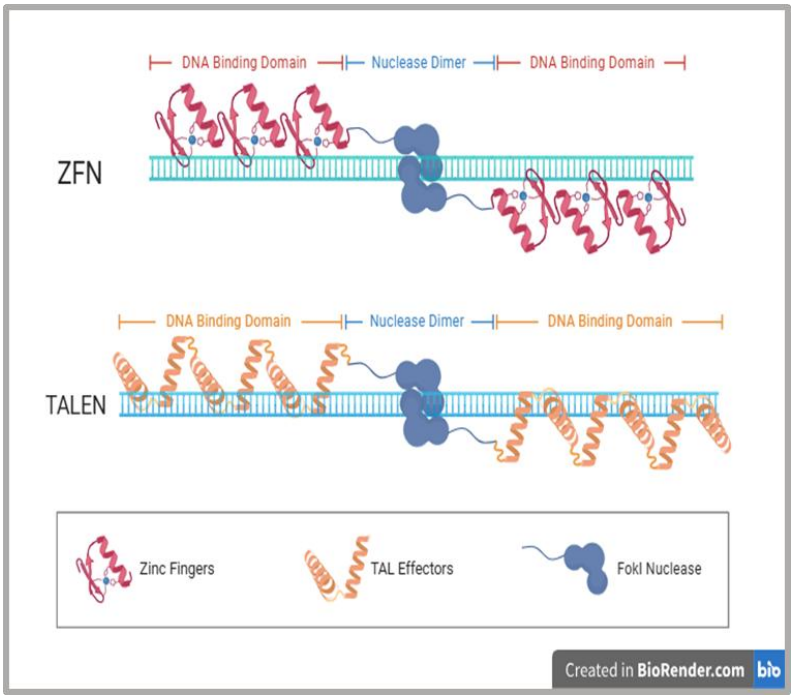

Fig. 1: Transcriptional Activator-Like Effector Nucleases and Zinc-Finger Nucleases attach to DNA using arrays designed to identify a target sequence

2.1. TALENs (a protein-based DNA targeting system)

TALENs are engineered nucleases, which performs in terms of functionality and specificity compared with ZFNs. Like ZFNs, TALENs use general DNA cleavage domain and domain specific for a certain DNA sequence in order to form DSBs. The repetitive sequence from transcription activator-like effector (TALE), a protein first discovered in phytopathogenic Xanthomonas bacteria that alters gene transcription in host plant cells on its own, forms this DNA-binding domain (Paschon et al., 2019). The C-terminal core domain of TALE, which is a member of the 33–35 aa sequence motif, interacts with DNA. The two hypervariable amino acids, or repeat variable di-residues (RVDs), at positions 12 and 13 make up the very similar but distinct amino acid sequence inside each repetition. Only C nucleotides are bound by ND, only A/G nucleotides by HN, only G nucleotides by NH, and all nucleotides by NP. RVDs mediate the specificity and severalty of DNA binding (Moscou & Bogdanove, 2009; Bogdanove & Voytas, 2011).

2.2 CRISPR/Cas9 (a versatile tool for genome editing)

First reported in *E. coli* in early 1987, CRISPRs have been identified in many more bacteria. The function of short repeat sequences stayed obscure for many years Until 2005, works showed that the sequences resembled phage DNA. Subsequent investigations showed that these sequences also protect bacteria and archaea from extraneous DNA by using RNA-guided DNA cleavage to provide an adaptive immune response. The CRISPR-Cas systems are often described according to the structural diversity of the Cas genes, as well as their arrangement (Liu et al., 2017).

It is now known that there are six classes for CRISPR-Cas, and at least 29 subtypes for each class, and the number is expanding rapidly. For instance, class 2 CRISPR-Cas systems contain only a single effector protein, while class 1 systems possess multiple-subunit effector com part. CRISPR-Cas systems were limited to class 2 systems that contained a single effector protein. Among them the most frequent subtype is type II CRISPR/Cas9 system that looks rather perspective as the gene editing because the method uses only one Cas protein (SpCas9) that targets certain DNA sequences. The means by which this system functions is constituted of a cas9 nuclease along with single-stranded guide RNA, ultimately abbreviated as the sgRNA (Hillary & Ceasar, 2023).

Three popular methods for CRISPR/Cas9 platform to modify genomes have been developed (Janik et al., 2020):

(1) The plasmid-based CRISPR / Cas9 method simultaneously combines Cas9 genes and sgRNA for extraction in inactive vitro, without multiple transfections and with increased Cas9 and sgRNA half-life.

(2) disadvantage of direct delivery of Cas9 mRNA & sgRNA into cells55 is the instable nature of mRNA used for Cas9 mRNA-based techniques leading to transient mRNA expression and temporary gene change.

(3) The Cas9 protein and sgRNA56 are directly introduced within the cell, which offers such advantages as immediate action, increased resistance to degradation and low immunogenicity.

Genetic information is permanently and irrevocably altered via DNA editing, which therefore raises inevitable ethical and security concerns. Additionally, gene therapy for nervous system illnesses is limited for some cell types since it is difficult to modify some cells, such neurons, utilizing CRISPR/Cas9-mediated editing. Thus, researchers have also proposed genome editing methods that just modify and change RNA (Asmamaw & Zawdie, 2021; Hillary & Ceasar, 2023). After the DNA encodes the essential information into a molecule known as RNA, then RNA basically controls the synthesis of protein. CRISPR technology can treat many diseases by repairing protein function in almost all cells and stop transformation by briefly changing RNA mutations (Asmamaw & Zawdie, 2021). The second approach is to of facing change in genetic variations is through the use of stem cell transplantation with CRISPR/Cas9 technology (Liu et al., 2020; Zhu et al., 2024).

3. Genome Editing for Gene Therapy and Disease Modeling

More realistically, the functions of genes and proper cellular behavior and function can be manipulated by modifying the targeted genes with chimeric tools as ZFNs, TALENs, and CRISPR/Cas9. Through animal model genetic changes, these genome editing techniques have made it easy to examine origin or causes of many diseases and come across molecular avenues that can be used to improve potent treatment approaches (Maeder & Gersbach, 2016).

3.1. Cancer Research

High potential is offered by oncogenes and mutated tumor suppressor genes for application of gene modifying methods. With its early successful genome editing demonstrations in eukaryotic cells to the recent applications in developing the tumor targeted T lymphocytes and hematopoietic stem cells (HSCs), several targeted cleavage events have been reported in diverse important research. It has also invested in a promising area of cancer research and come up with original concepts for gene editing (Shankar et al., 2018).

While a three-finger protein has been designed to selectively inhibit the synthesis of human oncogene that has been transduced into mouse cell line, the ZFN-mediated targeting – a prototype platform for the programmed DNA scission and its applications – was unmasked in 1994 and used to modify genes in human and model organisms cells (Avesson & Barry, 2014). Through the provided DSB error repair, it is possible to access the telomeric area of the combined lineage leukemia (MLL) gene break points cluster region and examine chromosomal rearrangements linked to MLL leukemogenesis, a specially tailored ZFN was next used on a human lymphoblast line of cells from CML patients. Although different to the initial targeted regulation, successful regulation was also achieved at this locus employing ZFNs specifically designed to disrupt the β- and α-chain genes of the endogenous T cell receptor (TCR). Both CD3-TCR surface expression and cell viability of the ZFN-treated cells were significantly reduced to 0% whereas, the cell population showed an increase in the expression of interleukin. ZFN, a new therapy aimed at LTR promoter region of HTLV-1 (Tanaka et al., 2013; Aghamiri et al., 2020).

3.2. Cardiovascular Disease

CVD accounts for the highest mortality and increased risks are reported in many developed nations. Many forms of CVD are often linked to one gene alteration or to a set of several rare familial dominant mutations (Li et al., 2015). In fact, therapeutic cures are concerned more

with the prevention of the manifestation of sickness than with possible hereditary issues. This paper introduced and established the in vivo CVD model based on gene editing technology, as well as the survey of damaging genes and the molecular processes, enabling the evaluation of the therapeutic effect of gene therapy in controlling the expression of certain genes and enhancing gene functions. There are many research models of cardiovascular disease including the use of genome editing techniques (German et al., 2019).

Furthermore, CRISPR/Cas9 effectively knocked out MHCII in normal human endothelial cells by simultaneous knockout. To form vessels these cells do not need the stimulation of allogeneic CD4+ T lymphocytes. The effectiveness of this technique when it used in STEM- cell bioengineering of allografts: improving heart transplants has been helpful (Musunuru, 2017; Wu et al., 2024).

There has also been much interest in possible therapeutic strategies to reverse disease-causing mutations or to inactivate specific genes that, because of the advance of genome editing technology (Li et al., 2023). For instance, Long QT syndrome (LQTS) is a congenital disease of the heart electrical conduction system and this disease is dominant. It can be seen that LQTS can arise from hybrid mutations in several genes Some of these genes have obviously identifiable mutations in distinct sites with known biological function. For example, mutations in the alpha protein's hERG gene that codes for potassium voltage-gated channels and creates holes. A mutation in the hERG gene is likely to result in a life-threatening ventricular arrhythmia because its production and activation occurs mainly in smooth muscles and the myocardial tissues. Of course, the potential of such LQTS is that, one day, humans would fix mutations in hERG genes in cardiomyocytes using CRISPR technology (Zhou et al., 2021).

3.3. Metabolic Diseases

Metabolic illness is the term used to depict the pathological status of protein lipid and carbohydrate in the body hence metabolically disturbed. The term metabolic diseases may refer to a range of condition, which may be attributed to the individual's inherited background, as well as the external environment. Gene editing technologies might be used for genetics treatment, functional gene assay as well as for genetic model construction of metabolic diseases such as diabetes, obesity and hypertension. By interacting with delivery system receptor called LepR, the hormone leptin (Lep) was produced by the white fat tissue influencing the hypothalamus metabolic regulation center. Some uses of this natural herb include the following; it can control blood sugar levels and control or influence neuroendocrine functions. Besides, it has advantages of reducing caloric intake and stopping the formation of fat and decreasing appetite (Coppari & Bjørbæk, 2012).

On the basis of the present study, animal model of diabetes is valuable as an experimental tool for drug screening assays, preclinical investigation and pathophysiological observation (Hu et al., 2020). For Ins1 (insulin gene) recombinase Cre insertion was targeted at the stop codon and promoter (Naylor et al., 2016). Morphologically, PCR analysis indicated that the progeny F1 mice had cre-loxP recombination, and other organs although they stained negatively for the gene, all the islets with insulin positive cells were positive for this gene. However, these mice were similarly insensitive to glucose ingestion to that exhibited by wild type mice. Taking advantage of the experimental approaches then, one can use CRISPR/Cas9 system to knock down some of the genes that are implicated in diabetes in human iPSCs (Vethe et al., 2017).

3.4. Neurodegenerative Diseases

The foregoing neurodegenerative diseases progressively strip afflicted individual of the ability to reason, move, and exist autonomously. These illnesses result from Neuronal degeneration and death and Neurons are the cells in the body that keep our bodies and minds functioning. Every ailment has unique causes of its own: Huntington's is due to a toxic protein from a mutated HTT gene; Parkinson's leads to the death of dopamine producing cells caused by aggregation of the α-synuclein protein; Alzheimer's has amyloid-β plaques and toxic tau tangles in the brain; and ALS comes from mutation in genes such as SOD1 or C9orf72 which leads to the degeneration of motor neuron (Fan et al., 2018; Raikwar et al., 2019).

Base editing reduces the likelihood of off-target issues by editing single letter changes in DNA while not wholly deleting a strand of DNA. In addition, there are other forms of RNA therapy, this include RNA interference (RNAi), and antisense oligonucleotides (ASOs) which help to lessen the production of toxic proteins (Nojadeh et al., 2023).

3.5. Viral Diseases

Organic diseases such as the colds, HIV/AIDS, hepatitis B among many others have remained a formidable force in the competitive and ever shrinking world. While traditional therapies often only cure those manifestations of the disease, genome editing technologies, and especially systems based on the CRISPR-Cas, open new horizons for targeted and effective treatment (Kim & Lee, 2022).

To prevent the virus from replicating, the genetic makeup has been modified and through use of CRISPR-Cas9 the DNA of the HIV virus was extracted from AIDS affected cells. The second approach relies on the protection of the immune cells from HIV by changing certain gene signaling known as CCR5. Hepatitis B may yet have a treatment since genome editing may pinpoint and reduce the virus's DNA within liver cells. By making the use of CRISPR-Cas9, the researchers have succeeded in knocking out the HSV DNA that has been known to cause recurring outbreaks in nerve cells. CRISPR-Cas13 attacks the RNA of RNA chain viruses, which include SARS-CoV-2, to reduce their ability to replicate (De Silva Feelixge et al., 2018; Kim & Lee, 2022).

However, issues such as effective delivery and hence minimizing off-target effects have remained as existing problems even as these other developments continue apace. For the treatment of viral diseases, genome editing may simply remain a theory but as it is advanced genome editing may be one of the most important tools to unlock permanent and powerful cures.

3.6. Hereditary Eye Diseases

Due to progress in the gene-sequencing technology, conditions such as congenital corneal dystrophy, Leber congenital amaurosis (LCA), congenital glaucoma, congenital cataract, retinitis pigmentosa (RP), Usher syndrome, retinoblastoma (RB), and many other hereditary eye ailments can now be diagnosed genetically more comfortably (Hung et al., 2016).

Animal models of RP have previously been produced using CRISPR/Cas9. Named after the receptor enhancer protein that aids in the development of the endoplasmic reticulum, REEP6 is a newest member of the human REEP/Yop1 protein family. The potential for using AAV and iPSCs to treat associated eye illnesses is increased when CRISPR/Cas9 technology is combined with other methods. It has been demonstrated that CRISPR/Cas9 may fix pathogenic mutations in the retinal pigmentosa GTPase regulator (RPGF) that cause X-linked RP in patient-specific iPSCs. This implies that a tethered iPSC transplantation strategy for different retinal degenerations may involve combining CRISPR with self-directed iPSCs (Benati et al., 2020). According to patient-specific imaging and physiological characterization of three RP individuals carrying frameshift variations in RPGR gene, photoreceptors from RP patients exhibit aberrant shape, location, and electrical activity in iPSC-derived retinal organoids. As a result, CRISPR/Cas9 is the fixed mutation of RPGR that can reverse ciliopathy and stop photoreceptor loss as a mutant repair method (Gallego et al., 2020).

3.7. Other Hereditary Diseases

The most prevalent kind of muscular dystrophy linked to a malfunction in the DMD gene is Duchenne muscular dystrophy. It has been discovered that not all aspects of human disease can be modeled in mice with X-linked muscular dystrophy (mdx). Their diminutive stature, low levels of long-term muscular deterioration and weakening thus hinders research and study of illness. As a result, larger species such as rat rabbit, or pigs are more useful in preclinical researches (Olson, 2021).

More of monkey dystrophin gene was disrupted through CRISPR/Cas9 where it introduced mutations that are characteristic of DMD. Further, the relative targeted rate detection indicates that mosaic mutations resulting from CRISPR/Cas9 may be present in 87 % of the potential dystrophin genotypes in monkey muscle (Ousterout et al., 2015). Notably, three research groups recently reported finding they had erased variations in dystrophin gene that encodes for DMD gene and affects the production of proteins. The researchers employed CRISPR/Cas9 Gene editing to delete altered DMD region from the mdx animal model to generate shorter isoform of dystrophin protein in muscle fibers and to optimize the muscular activity to a certain degree. This gave a possible means of helping to rectify disease causing mutations in peoples muscular tissue (Mani, 2021; Kan & Doudna, 2022).

Patients suffering from primary immunodeficiencies, direct consequences – immunologic defect, or absence of any component of the immune system, can undergo allogeneic HSC transplantation. This might be a dangerous surgery if individuals seeking leukocyte antigen-matched donors are incompatible for tissue matching. Gene therapy is another tempting way of dealing with a patient's own HSCs. Auxiliary HSCs can be applied in situ for regeneration of the deficient cells and for creating animal or cell models that will manifest the adverse impact of mutations inherent in immunocompromised individuals. One of the worst known immunodeficiencies – SCID – results from a gene mutation that opens for IL2RG. This leads to T cell line development arrest and augmented primary or secondary damage to the main B cells (Ameratunga et al., 2024).

4. Conclusions and Future Perspectives

The CRISPR/Cas9 system has shown a remarkable ability to directly interfere with target gene loci or to generate versatile platforms, as well as the astonishing capacity, if the evidence gathered so far is considered, to address diseases influencing human existence through employing genome editing systems for therapeutic intervention. With the present understanding of the genetic and epigenetic characteristics of cancer cell lines, future studies can use pooled CRISPR screening to uncover different synthetic lethal links in the genome and discover new targets for therapeutic intervention. Moreover, noncoding sections of the cancer genome can be reduced by the use of the CRISPR/Cas9 method in cancer research; this advances functional analysis of uncharacterized genotypes. In particular, cell visualization; epigenetic modification; gene expression regulation; medicinal drug development; operational genome screening; gene diagnostics benefited from gene editing techniques. More complexed new genome editing machineries and much more targeted nano structured transports have enhanced transport capacity and reduced toxicity, bringing the genome editing technology at the clinical interfaces though in its collective use; the off-target effects are yet to be minimized.

References

Aghamiri, S., Talaei, S., Roshanzamiri, S., Zandsalimi, F., Fazeli, E., Aliyu, M., & Ghanbarian, H. (2020). Delivery of genome editing tools: A promising strategy for HPV-related cervical malignancy therapy. *Expert Opinion on Drug Delivery, 17*(6), 753-766.

Al-Attar, S., Westra, E. R., Van Der Oost, J., & Brouns, S. J. (2011). Clustered regularly interspaced short palindromic repeats (CRISPRs): the hallmark of an ingenious antiviral defense mechanism in prokaryotes. *Biological Chemistry, 392*(4), 277-289.

Ameratunga, R., Leung, E., Woon, S.-T., Lea, E., Allan, C., Chan, L., & Lehnert, K. (2024). Challenges for gene editing in common variable immunodeficiency disorders: Current and future prospects. *Clinical Immunology, 258*, 109854.

Asmamaw, M., & Zawdie, B. (2021). Mechanism and applications of CRISPR/Cas-9-mediated genome editing. *Biologics: Targets Therapy, 15*, 353-361.

Avesson, L., & Barry, G. (2014). The emerging role of RNA and DNA editing in cancer. *Biochimica et Biophysica Acta -Reviews on Cancer, 1845*(2), 308-316.

Beerli, R. R., & Barbas III, C. F. (2002). Engineering polydactyl zinc-finger transcription factors. *Nature Biotechnology, 20*(2), 135-141.

Benati, D., Patrizi, C., & Recchia, A. (2020). Gene editing prospects for treating inherited retinal diseases. *Journal of Medical Genetics, 57*(7), 437-444.

Bogdanove, A. J., & Voytas, D. F. (2011). TAL effectors: customizable proteins for DNA targeting. *Science, 333*(6051), 1843-1846.

Buck-Koehntop, B. A., Stanfield, R. L., Ekiert, D. C., Martinez-Yamout, M. A., Dyson, H. J., Wilson, I. A., & Wright, P. E. (2012). Molecular basis for recognition of methylated and specific DNA sequences by the zinc finger protein Kaiso. *Proceedings of the National Academy of Sciences, 109*(38), 15229-15234.

Cathomen, T., & Joung, J. K. (2008). Zinc-finger nucleases: the next generation emerges. *Molecular Therapy, 16*(7), 1200-1207.
Chang, H. H., Pannunzio, N. R., Adachi, N., & Lieber, M. R. (2017). Non-homologous DNA end joining and alternative pathways to double-strand break repair. *Nature reviews Molecular Cell Biology, 18*(8), 495-506.
Cong, L., Ran, F. A., Cox, D., Lin, S., Barretto, R., Habib, N., & Marraffini, L. A. (2013). Multiplex genome engineering using CRISPR/Cas systems. *Science, 339*(6121), 819-823.
Coppari, R., & Bjørbæk, C. (2012). Leptin revisited: its mechanism of action and potential for treating diabetes. *Nature Reviews Drug Discovery, 11*(9), 692-708.
Cornu, T. I., Mussolino, C., & Cathomen, T. (2017). Refining strategies to translate genome editing to the clinic. *Nature Medicine, 23*(4), 415-423.
De Silva Feelixge, H. S., Stone, D., Roychoudhury, P., Aubert, M., & Jerome, K. R. (2018). CRISPR/Cas9 and Genome Editing for Viral Disease—Is Resistance Futile? *ACS Infectious Diseases, 4*(6), 871-880.
Delacôte, F., & Lopez, B. S. (2008). Importance of the cell cycle phase for the choice of the appropriate DSB repair pathway, for genome stability mintenance: the trans-S double-strand break repair model. *Cell Cycle, 7*(1), 33-38.
Diakun, G., Fairall, L., & Klug, A. (1986). EXAFS study of the zinc-binding sites in the protein transcription factor IIIA. *Nature, 324*(6098), 698-699.
Fan, H.-C., Chi, C.-S., Lee, Y.-J., Tsai, J.-D., Lin, S.-Z., & Harn, H.-J. (2018). The role of gene editing in neurodegenerative diseases. *Cell Transplantation, 27*(3), 364-378.
Gaj, T., Gersbach, C. A., & Barbas, C. F. (2013). ZFN, TALEN, and CRISPR/Cas-based methods for genome engineering. *Trends in Biotechnology, 31*(7), 397-405.
Gallego, C., Gonçalves, M. A., & Wijnholds, J. (2020). Novel therapeutic approaches for the treatment of retinal degenerative diseases: focus on CRISPR/Cas-based gene editing. *Frontiers in Neuroscience, 14*, 838.
German, D. M., Mitalipov, S., Mishra, A., & Kaul, S. (2019). Therapeutic genome editing in cardiovascular diseases. *JACC: Basic to Translational Science, 4*(1), 122-131.
Ghosh, D., Venkataramani, P., Nandi, S., & Bhattacharjee, S. (2019). CRISPR–Cas9 a boon or bane: the bumpy road ahead to cancer therapeutics. *Cancer Cell International, 19*, 1-10.
Hillary, V. E., & Ceasar, S. A. (2023). A review on the mechanism and applications of CRISPR/Cas9/Cas12/Cas13/Cas14 proteins utilized for genome engineering. *Molecular Biotechnology, 65*(3), 311-325.
Hu, M., Cherkaoui, I., Misra, S., & Rutter, G. A. (2020). Functional genomics in pancreatic β cells: recent advances in gene deletion and genome editing technologies for diabetes research. *Frontiers in Endocrinology, 11*, 576632.
Hung, S. S., McCaughey, T., Swann, O., Pébay, A., & Hewitt, A. W. (2016). Genome engineering in ophthalmology: Application of CRISPR/Cas to the treatment of eye disease. *Progress in Retinal Eye Research, 53*, 1-20.
Janik, E., Niemcewicz, M., Ceremuga, M., Krzowski, L., Saluk-Bijak, J., & Bijak, M. (2020). Various aspects of a gene editing system—crispr–cas9. *International Journal of Molecular Sciences, 21*(24), 9604.
Kan, M. J., & Doudna, J. A. (2022). Treatment of genetic diseases with CRISPR genome editing. *Jama, 328*(10), 980-981.
Kaniecki, K., De Tullio, L., & Greene, E. C. (2018). A change of view: homologous recombination at single-molecule resolution. *Nature Reviews Genetics, 19*(4), 191-207.
Kim, H., & Kim, J.-S. (2014). A guide to genome engineering with programmable nucleases. *Nature Reviews Genetics, 15*(5), 321-334.
Kim, T. H., & Lee, S.-W. (2022). Therapeutic application of genome editing technologies in viral diseases. *International Journal of Molecular Sciences, 23*(10), 5399.
Kim, Y.-G., Cha, J., & Chandrasegaran, S. (1996). Hybrid restriction enzymes: zinc finger fusions to Fok I cleavage domain. *Proceedings of the National Academy of Sciences, 93*(3), 1156-1160.
Kosicki, M., Tomberg, K., & Bradley, A. (2018). Repair of double-strand breaks induced by CRISPR–Cas9 leads to large deletions and complex rearrangements. *Nature Biotechnology, 36*(8), 765-771.
Li, A. H., Morrison, A. C., Kovar, C., Cupples, L. A., Brody, J. A., Polfus, L. M., & Veeraraghavan, N. (2015). Analysis of loss-of-function variants and 20 risk factor phenotypes in 8,554 individuals identifies loci influencing chronic disease. *Nature Genetics, 47*(6), 640-642.
Li, Z.-H., Wang, J., Xu, J.-P., Wang, J., & Yang, X. (2023). Recent advances in CRISPR-based genome editing technology and its applications in cardiovascular research. *Military Medical Research, 10*(1), 12.
Lieber, M. R., Gu, J., Lu, H., Shimazaki, N., & Tsai, A. G. (2010). Nonhomologous DNA end joining (NHEJ) and chromosomal translocations in humans. *Genome Stability Human Diseases, 50*, 279-296.
Liu, C., Zhang, L., Liu, H., & Cheng, K. (2017). Delivery strategies of the CRISPR-Cas9 gene-editing system for therapeutic applications. *Journal of Controlled Release, 266*, 17-26.
Liu, G., Zhang, Y., & Zhang, T. (2020). Computational approaches for effective CRISPR guide RNA design and evaluation. *Computational Structural Biotechnology Journal, 18*, 35-44.
Maeder, M. L., & Gersbach, C. A. (2016). Genome-editing technologies for gene and cell therapy. *Molecular Therapy, 24*(3), 430-446.
Mani, I. (2021). CRISPR-Cas9 for treating hereditary diseases. *Progress in Molecular Biology Translational Science, 181*, 165-183.
Moscou, M. J., & Bogdanove, A. J. (2009). A simple cipher governs DNA recognition by TAL effectors. *Science, 326*(5959), 1501-1501.
Musunuru, K. (2017). Genome editing: the recent history and perspective in cardiovascular diseases. *Journal of the American College of Cardiology, 70*(22), 2808-2821.
Naylor, J., Suckow, A. T., Seth, A., Baker, D. J., Sermadiras, I., Ravn, P., & Coghlan, M. P. (2016). Use of CRISPR/Cas9-engineered INS-1 pancreatic β cells to define the pharmacology of dual GIPR/GLP-1R agonists. *Biochemical Journal, 473*(18), 2881-2891.

Nojadeh, J. N., Eryilmaz, N. S. B., & Ergüder, B. İ. (2023). CRISPR/Cas9 genome editing for neurodegenerative diseases. *EXCLI Journal, 22*, 567.

O'Driscoll, M., & Jeggo, P. A. (2006). The role of double-strand break repair—insights from human genetics. *Nature Reviews Genetics, 7*(1), 45-54.

Olson, E. N. (2021). Toward the correction of muscular dystrophy by gene editing. *Proceedings of the National Academy of Sciences, 118*(22), e2004840117.

Ousterout, D. G., Kabadi, A. M., Thakore, P. I., Majoros, W. H., Reddy, T. E., & Gersbach, C. A. (2015). Multiplex CRISPR/Cas9-based genome editing for correction of dystrophin mutations that cause Duchenne muscular dystrophy. *Nature Communications, 6*(1), 6244.

Paschon, D. E., Lussier, S., Wangzor, T., Xia, D. F., Li, P. W., Hinkley, S. J., & Truong, L. N. (2019). Diversifying the structure of zinc finger nucleases for high-precision genome editing. *Nature Communications, 10*(1), 1133.

Raikwar, S. P., Kikkeri, N. S., Sakuru, R., Saeed, D., Zahoor, H., Premkumar, K., & Ahmed, M. E. (2019). Next generation precision medicine: CRISPR-mediated genome editing for the treatment of neurodegenerative disorders. *Journal of Neuroimmune Pharmacology, 14*, 608-641.

Rothstein, R. J. (1983). One-step gene disruption in yeast. In *Methods in Enzymology* (Vol. 101, pp. 202-211). Elsevier.

Rouet, P., Smih, F., & Jasin, M. (1994). Expression of a site-specific endonuclease stimulates homologous recombination in mammalian cells. *Proceedings of the National Academy of Sciences, 91*(13), 6064-6068.

Shankar, S., Sreekumar, A., Prasad, D., Das, A. V., & Pillai, M. R. (2018). Genome editing of oncogenes with ZFNs and TALENs: caveats in nuclease design. *Cancer Cell International, 18*, 1-11.

Silva, G., Poirot, L., Galetto, R., Smith, J., Montoya, G., Duchateau, P., & Pâques, F. (2011). Meganucleases and other tools for targeted genome engineering: perspectives and challenges for gene therapy. *Current Gene Therapy, 11*(1), 11-27.

Smith, J., Bibikova, M., Whitby, F. G., Reddy, A., Chandrasegaran, S., & Carroll, D. (2000). Requirements for double-strand cleavage by chimeric restriction enzymes with zinc finger DNA-recognition domains. *Nucleic Acids Research, 28*(17), 3361-3369.

Tanaka, A., Takeda, S., Kariya, R., Matsuda, K., Urano, E., Okada, S., & Komano, J. (2013). A novel therapeutic molecule against HTLV-1 infection targeting provirus. *Leukemia, 27*(8), 1621-1627.

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.

Verma, P., & Greenberg, R. A. (2016). Noncanonical views of homology-directed DNA repair. *Genes Development, 30*(10), 1138-1154.

Vethe, H., Bjørlykke, Y., Ghila, L. M., Paulo, J. A., Scholz, H., Gygi, S. P., & Ræder, H. (2017). Probing the missing mature β-cell proteomic landscape in differentiating patient iPSC-derived cells. *Scientific Reports, 7*(1), 4780.

Wu, X., Yang, J., Zhang, J., & Song, Y. (2024). Gene editing therapy for cardiovascular diseases. *MedComm-Future Medicine, 5*(7), e639.

Zhou, J., Ren, Z., Xu, J., Zhang, J., & Chen, Y. E. (2021). Gene editing therapy ready for cardiovascular diseases: Opportunities, challenges, and perspectives. *Medical Review, 1*(1), 6-9.

Zhu, M., Xu, R., Yuan, J., Wang, J., Ren, X., Cong, T., & Wang, H. (2024). Tracking-seq reveals the heterogeneity of off-target effects in CRISPR–Cas9-mediated genome editing. *Nature Biotechnology*, 1-12.