

Genome Editing Technologies: Development, Mechanisms, and Biomedical Applications

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ABSTRACT

Genome editing allows researchers to make precise, targeted changes to the genome of an organism, hailed as one of the most revolutionary biotechnologies of the 21st century. This article provides a detailed overview of the developmental history of genome editing technologies, with a focus on comparing the working principles and technical advantages/disadvantages of ZFNs, TALENs, and CRISPR-Cas systems. It also elaborates on research advancements in areas such as cancer treatment, disease model construction, and genetic disorder therapy. By analyzing the logic and trends of technological iterations, this study aims to offer insights for in-depth research and clinical applications of genome editing technologies.

KEYWORDS

Genome editing technology; CRISPR technology; First-generation editing tools; ZFNs; TALENs

Genome editing technology is currently a hot topic, and this emerging platform has demonstrated significant potential in numerous applications. Given the widespread global recognition of genome editing technology and the continuous advancements in CRISPR-Cas9 technology, genome editing has once again reached a peak in development. This article provides an overview of the fundamental mechanisms of genome editing technology, compares the advantages and limitations of different genome editing techniques, explores improvement strategies for various genome editing technologies, and summarizes their applications in disease treatment. Through extensive literature collection and organization, this review aims to provide readers with a comprehensive data repository on genome editing, enhancing understanding of core tool iterations and advancements. The study systematically traces the evolution of genome editing technologies from homologous recombination, ZFNs, and TALENs to CRISPR-Cas and its derivative tools, evaluates the strengths and limitations of each generation, and summarizes major application progress in the biomedical field, offering comprehensive references for related research.

1 Exploration and Development of Genome Editing Technologies

1.1 Gene Targeting

Gene targeting via homologous recombination was the first technique in history capable of performing targeted and precise genomic modifications, laying the foundation for functional genomics research. However, its extremely low efficiency, long cycle time, and high cost have made genome editing a challenging issue, which has also spurred the development of novel technologies.

Homologous recombination (HR) is a genetic recombination mechanism that occurs between two DNA molecules with highly similar sequences, generating novel DNA sequence combinations through breaks, exchanges, and rejoining. This process includes chain end resection, single-stranded DNA invasion, Holliday Junction unwinding, and the introduction and pairing of homologous segments^[1]. Homologous recombination (HR) provides a precise mechanism for targeted genome modification in living cells. However, its designation as a standard tool does not imply that gene targeting is easily achieved or guaranteed success. When introduced into cells, the host's own recombination mechanisms may "replace" the vector sequence at the target genomic locus with an extremely low probability, thereby enabling directional gene modification.

The operational process of homologous recombination is complex and inefficient, with its success rate primarily dependent on the positivity or negativity of drugs in cell culture. This technology also exhibits drawbacks such as susceptibility to non-homologous end joining competition and potential for random integration/deoff-target effects. Collectively, these factors have driven the development of novel technologies including ZFN, TALEN, and CRISPR/Cas.

1.2 First-generation editing tools

The first-generation editing tools refer to the application of various nucleases, such as homing endonucleases, zinc finger nucleases, and transcription activator-like effector nucleases. These enzymes induce DNA double-strand breaks to

trigger error-prone non-homologous end joining or homologous directed repair at specific genomic sites, enabling extensive gene editing^[2]. While this technology has broken down technical barriers and accelerated scientific research progress, it also faces challenges including high off-target gene rates and cytotoxicity, which ultimately hastened the advent of the CRISPR-Cas era.

1.2.1 Zinc Finger Nucleases (ZFNs)

ZFN is a reagent specifically designed for DNA cleavage and has been widely applied in the field of gene-directed research. Achieving efficient targeted mutation and targeted gene replacement requires the induction of double-strand breaks by nucleases, and the repair of cellular DNA can affect the double-strand breaks induced by ZFNs^[3].

The primary limitations of ZFNs include complex design and low modularization. Additionally, ZFNs exhibit restricted target sequence selection and inability to target arbitrary pull points.

1.2.2 Transcription Activator-Like Effector Nucleases (TALENs)

TALEN can target nearly any sequence, and this unlimited targeting range makes TALEN an attractive platform for targeted gene editing^[4].

Compared to ZFNs, TALENs are easier to design and impose fewer site selection constraints. Compared to ZFNs, which require only 1 kb of DNA sequence, TALENs demand a much larger coding sequence—over 3 kb per TALEN—which presents a significant drawback. The larger size makes it more challenging to deliver plasmids expressing TALENs into cells. Additionally, the high repetitiveness of TALEN sequences due to their tandem array structure poses greater challenges in packaging them for viral vector delivery^[5].

1.3 The Era of CRISPR-Cas

The CRISPR-Cas era marks a groundbreaking chapter in genome editing. With its precision, efficiency, and cost-effectiveness, CRISPR-Cas has revolutionized life science research, ushering in an era of direct rewriting of life's genetic code. As CRISPR-Cas technology has been widely applied, scientists have conducted extensive optimizations, yielding more advanced and powerful tools.

CRISPR loci consist of spacer regions derived from bacteriophages and other extrachromosomal elements, separated by short repetitive sequences encoding small non-coding RNAs^[6]. CRISPR-Cas endows humans with the ability to directly read and write life's source code. Despite challenges, it is undoubtedly ushering biomedicine into a new era and driving the emergence and advancement of numerous technologies, such as CRISPR-Cas9. CRISPR-Cas9 is a programmable gene editing technology based on the natural immune systems of bacteria and archaea, which is easily scalable and highly cost-effective, making it applicable for broad-spectrum directed genome editing. The core of contemporary CRISPR-mediated genome engineering is Cas9, but it still faces challenges due to potential off-target effects and difficulties in delivering ribonucleoprotein particles. Cas12a, exhibiting significant differences from Cas9, provides an alternative molecular genome editing tool. To date, multiple studies have explored the application of Cas12a in genome editing across various cell types, with EedCas12a demonstrating higher gene silencing efficiency than SpdCas9 in the template strand of target DNA. Research also indicates that Cas12a's pre-crRNA processing activity makes it a standout candidate for multiplex gene regulation^[7].

Despite its revolutionary impact, first-generation CRISPR-Cas9 lacked the precision required for many advanced applications. Consequently, more sophisticated tools were needed. The emergence of technologies such as base editors and prime editors has enabled the development of a vast and diverse CRISPR toolset, capable of addressing various complex challenges in life sciences. Base editors do not cleave double strands but modify individual bases, requiring no DNA double-strand breaks, relying on homologous recombination (HR), and featuring simple operation. Prime editors, on the other hand, employ "templates" to directly rewrite DNA with precision down to arbitrary single-nucleotide mutations. They support all single-base mutations, exhibit minimal by-products, and demonstrate near-zero off-target effects. Together, these advancements will usher in an era of precision, in vivo, broad-spectrum, and AI-driven gene therapy.

2 Application of Genome Editing Technologies

The application fields of genome editing technology are extremely extensive. It has rapidly evolved from a fundamental research tool into an industrial revolution that disrupts multiple sectors, particularly technologies represented by CRISPR. This capability to rewrite life codes has exerted pivotal impacts across numerous domains.

2.1 Cancer Treatment

Abnormal cell division in cancer arises from genomic alterations and DNA damage due to multiple point mutations. Using a combination of Cre-LoxP technology and the CRISPR-Cas9 system, researchers can induce the loss of tumor suppressors like p53 and PTen^[8]. The tumor suppressor p53 is considered the most critical checkpoint in cell cycle regulation, as it triggers cell cycle arrest, cellular senescence, or apoptosis in response to DNA damage, thereby maintaining genomic stability. Therefore, it is reasonable to assume that the cell's response to DSB-dependent CRISPR/Cas9 editing involves activation of the p53 pathway. Genome editing techniques can achieve heritable inactivation of p53, thereby mitigating the toxicity induced by CRISPR/Cas9^[9].

Currently, the CRISPR/Cas9 system, as a groundbreaking technology, has been widely applied in the treatment of lung cancer. This technology operates through two approaches. The first method involves designing sgRNAs to introduce the CRISPR/Cas9 system into relevant patients, enabling DNA repair mechanisms to function and thereby inducing the breakdown of cancer cells. The second strategy for lung cancer treatment includes identifying immune cells such as lymphocytes. T cells are isolated from blood samples of lung cancer patients, and CRISPR/Cas9 technology is utilized to reduce genes encoding the PD-1 protein. These gene-edited cells are then expanded and reinfused into the same patient's bloodstream, allowing T cell receptors to recognize problematic cells and activate their functions^[10].

2.2 Treatment of Genetic Disorders

Similar to hematologic disorders, CRISPR gene editing holds potential applications in neuromuscular diseases, particularly in the field of Duchenne muscular dystrophy (DMD). Within the human genome, single-strand DNA breaks represent the most prevalent type of DNA damage. Cas proteins have been engineered to cleave only a single DNA strand, thereby overcoming the damage and imprecision associated with double-strand DNA breaks. DNA base editors introduce permanent changes, potentially requiring only one treatment after optimization. Some base editing tools, such as RNA-based base editors derived from CRISPR, can circumvent immunogenicity barriers and have a relatively small construct size, allowing them to be encapsulated into a single adeno-associated virus (AAV) vector. Consequently, researchers posit that ASO-dependent base editors may be particularly suitable for addressing diseases requiring targeted delivery to large organs or tissues^[11].

Compared with traditional gene therapy methods, gene editing technology has the potential to permanently modify pathogenic genes by precisely correcting, deleting, adding, or disrupting specific sequences.

2.3 Disease Model Construction and Drug Development

The CRISPR/Cas9 system enables effective correction of birth defects in animals. Researchers delivered Cas9 nuclease, specific sgRNA, and single-stranded DNA (ssDNA) oligonucleotides directly to the mouse liver, significantly reducing hepatic injury^[12]. Studies demonstrated that CRISPR-Cas12a exhibited minimal off-target effects. In preliminary experiments for cancer treatment, genome editing using the CRISPR-Cas13 system can edit RNA in rodents, with LwaCas13a demonstrating exceptional knockdown efficacy. The resulting knockout fragments serve as an effective tool for screening target gene expression regulatory elements. The CRISPR-Cas13a system successfully reduced mRNA levels of target genes and effectively slowed tumor growth rates^[13].

3 Challenges and future prospects

From cell-based homologous recombination, to engineered nuclease-mediated targeted cleavage represented by ZFNs/TALENs, to revolutionary breakthroughs represented by CRISPR technology, genome editing has achieved progress from simple modification to precise editing, and it will inevitably produce greater potential in the treatment of future diseases. Despite this, there are still some challenges associated with genome editing technology, such as the off-target effects of genome editing and ethical issues. The off-target effect refers to the phenomenon that editing tools such as CRISPR-Cas9 mistakenly act on non-target sites during gene editing, leading to unintended genomic changes, which is one of the main risks faced by gene editing technology. The CRISPR-Cas9 system carries an off-target risk and may cleave non-target DNA. Based on this, CBE Editor, ABE Editor, and Prime Editor came into being. Adenine base editors (ABEs) for the conversion of adenosine (A) to guanosine (G) have a more compact structure, more precise editing, lower risk of off-target, and have validated their therapeutic potential in animal models of osteoarthritis and cell therapy. Prime editor is a gene editing technology that combines reverse transcriptase with master editing gRNA (pegRNA) and Cas-nicknase nuclease^[14]. This technology has extremely high versatility, editing purity and DNA target specificity, and has shown great application prospects in gene therapy, breeding improvement, basic research and other aspects. The emergence of these emerging technologies will lay a good foundation for the safer and more accurate use of genome editing technology in

the future. In addition, the rapid development of gene editing technology has raised many ethical questions, including the impact on human dignity, potential social inequality, and responsibility to future generations. It may be that in the future, human beings will not be able to completely eliminate the ethical and moral issues in gene editing technology, but human beings are also fearless, and we will hold confidence in genome editing technology, bravely explore the future, and continue to benefit mankind.

References

- [1] Wright, W. D., Shah, S. S., & Heyer, W. D. (2018). Homologous recombination and the repair of DNA double-strand breaks. *The Journal of biological chemistry*, 293(27), 10524–10535.
- [2] Gaj, T., Gersbach, C. A., & Barbas, C. F., 3rd (2013). ZFN, TALEN, and CRISPR/Cas-based methods for genome engineering. *Trends in biotechnology*, 31(7), 397–405.
- [3] Carroll D. (2011). Genome engineering with zinc-finger nucleases. *Genetics*, 188(4), 773–782.
- [4] Maeder, M. L., & Gersbach, C. A. (2016). Genome-editing Technologies for Gene and Cell Therapy. *Molecular therapy : the journal of the American Society of Gene Therapy*, 24(3), 430–446.
- [5] Khalil A. M. (2020). The genome editing revolution: review. *Journal, genetic engineering & biotechnology*, 18(1), 68.
- [6] Wang, S. W., Gao, C., Zheng, Y. M., Yi, L., Lu, J. C., Huang, X. Y., Cai, J. B., Zhang, P. F., Cui, Y. H., & Ke, A. W. (2022). Current applications and future perspective of CRISPR/Cas9 gene editing in cancer. *Molecular cancer*, 21(1), 57.
- [7] Paul, B., & Montoya, G. (2020). CRISPR-Cas12a: Functional overview and applications. *Biomedical journal*, 43(1), 8–17.
- [8] Afolabi, L. O., Afolabi, M. O., Sani, M. M., Okunowo, W. O., Yan, D., Chen, L., Zhang, Y., & Wan, X. (2021). Exploiting the CRISPR-Cas9 gene-editing system for human cancers and immunotherapy. *Clinical & translational immunology*, 10(6), e1286.
- [9] Conti, A., & Di Micco, R. (2018). p53 activation: a checkpoint for precision genome editing?. *Genome medicine*, 10(1), 66.
- [10] Allemailem, K. S., Alsahli, M. A., Almatroudi, A., Alrumaihi, F., Alkhaleefah, F. K., Rahmani, A. H., & Khan, A. A. (2022). Current updates of CRISPR/Cas9-mediated genome editing and targeting within tumor cells: an innovative strategy of cancer management. *Cancer communications (London, England)*, 42(12), 1257–1287.
- [11] Porto, E. M., Komor, A. C., Slaymaker, I. M., & Yeo, G. W. (2020). Base editing: advances and therapeutic opportunities. *Nature reviews. Drug discovery*, 19(12), 839–859.
- [12] Jacinto, F. V., Link, W., & Ferreira, B. I. (2020). CRISPR/Cas9-mediated genome editing: From basic research to translational medicine. *Journal of cellular and molecular medicine*, 24(7), 3766–3778.
- [13] Song, B., & Bae, S. (2024). Genome editing using CRISPR, CAST, and Fanzor systems. *Molecules and cells*, 47(7), 100086.
- [14] Naeem, M., Majeed, S., Hoque, M. Z., & Ahmad, I. (2020). Latest Developed Strategies to Minimize the Off-Target Effects in CRISPR-Cas-Mediated Genome Editing. *Cells*, 9(7), 1608.