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Advances in the Application of CRISPR-Cas9 in Stem Cell Therapy

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Abstract: Stem cell research has advanced rapidly, offering promising treatments for refractory diseases due to their unique capabilities for self-renewal and pluripotent differentiation. Stem cells play pivotal roles in treating genetic disorders, neurodegenerative diseases (NDDs), cardiovascular conditions, and cancer. In genetic diseases, combining stem cells with gene-editing tools like CRISPR-Cas9 enables precise correction of pathogenic genes, while healthy stem cells repair tissue by replacing diseased cells. For NDDs, iPSCs can differentiate into dopaminergic neurons to replace damaged brain cells and enhance neural regeneration. In cardiovascular diseases, they promote myocardial and vascular repair. In cancer, stem cells boost anti-tumor immunity and deliver drugs directly to tumor sites, improving treatment efficacy. Despite these breakthroughs, challenges persist. High-quality stem cell production is limited, and controlling differentiation to prevent tumorigenesis remains critical. Allogeneic transplants risk immune rejection, and using embryonic stem cells raises ethical concerns. Regulatory frameworks and clinical standards are needed to ensure safety and efficacy, alongside addressing ethical and patient rights issues. With continued innovation, stem cell therapy is going to revolutionize medicine, offering novel methods for complex diseases and improving global health.

Keywords: CRISPR-Cas9, cancer, stem cell, neurodegenerative disease.

1. Introduction

As a state-of-the-art modern treatment in life sciences study, stem cell therapy has emerged as world's most common specific therapy to the genetic diseases. Physicians can thus efficiently and safely treat the majority of patients with genetic diseases by targeting pathogenic mutations with somatic cell gene editing. In 2006, Shinya Yamanaka and his team achieved a breakthrough by importingOct4, Sox2, Klf4, and c-Myc into fibroblasts using viral vectors. Furthermore, this landmark discovery allowed for cells to take on such a pluripotent differentiation potential, which could then give rise to an iPSC. Back in 2007, Dr Kathrin Plath and her group were among the first to reprogramme skin cells into precursors of gametes, hepatocytes and skeletal cells using iPSCs. 18 19 iPSCs have unlimited proliferative potential and the capacity to differentiate into a range of homogeneous somatic cells, as well as tissues, making them highly applicable for cell therapy and tissue regeneration. On top of this, iPSCs bypass the ethical and immunogenic issues of embryonic stem cells [1].

CRISPR-Cas9 is regarded as a revolutionary gene-editing technology, which can assist scientists to modify genetic sequences more efficiently and precisely. CRISPR, a naturally occurring immune

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system found in bacteria and archaea, works in tandem with Cas9, a nuclease associated with the CRISPR system. By utilizing small RNA sequences complementary to target DNA, the system employs a guide RNA (sgRNA)—comprising crRNA and tracrRNA—to direct the Cas9 endonuclease to the desired DNA sequence. The Cas9 protein then induces double-strand breaks (DSBs) at specific target sites in the DNA. Subsequent DNA repair mechanisms can result in gene knockout or insertion. Compared to traditional homologous recombination methods, CRISPR-Cas9 offers significant advantages in speed and efficiency.

Applications of CRISPR-Cas9 include targeted gene editing, ranging from its initial role as a research tool to potential therapeutic interventions. CRISPR-Cas9 is gradually transitioning from a laboratory tool to clinical practice. For instance, Liu, et al. initiated clinical trials targeting gastric cancer and nasopharyngeal carcinoma, treating two gastric cancer patients with CRISPR-Cas9 [2]. On June 17, 2021, the UK reported the first clinical data demonstrating the safety and efficacy of in vivo CRISPR gene editing, showing that a single intravenous injection of CRISPR could precisely edit target cells within the body to treat genetic diseases.

Despite the high accuracy of CRISPR-Cas9 in targeting specific DNA sequences, off-target effects remain a concern, as unintended cuts in non-specific fragments could lead to cellular dysfunction or mutations, increasing safety risks. Consequently, CRISPR-Cas9 technology is evolving toward greater specificity. By integrating gene repair mechanisms—such as homologous recombination—following Cas9-induced DNA cleavage, the precision of DNA repair can be further enhanced, minimizing unintended effects and improving the overall safety profile.

2. The origins of CRISPR/Cas9

The progress of CRISPR/Cas9 is shown in Figure 1. When regularly spaced palindromic repeats were discovered in DNA by Japanese scientists of E. coli K-12. This structure, later recognized as a series of regular palindromic repeats interspaced with unique sequences, serves as a defense mechanism for archaea and other microorganisms against viruses and foreign genetic elements. In 2002, this system was formally named CRISPR, alongside its associated proteins (Cas), particularly Cas9.

Such a groundbreaking milestone was achieved in 2012, when the Cas9 nuclease was first identified to possess both RNA-guided and DNA-cleaving functions. Subsequently, in 2013, researchers successfully applied this system to the genetic editing of eukaryotic cells, marking a transformative leap in the field of life sciences.

As of 2023, regulatory authorities in the United Kingdom have approved a CRISPR-based gene-editing therapy known as Casgevy. This approval underscores the immense potential of CRISPR/Cas9 and highlights its promising applications across diverse fields, including therapeutic development and beyond [3].

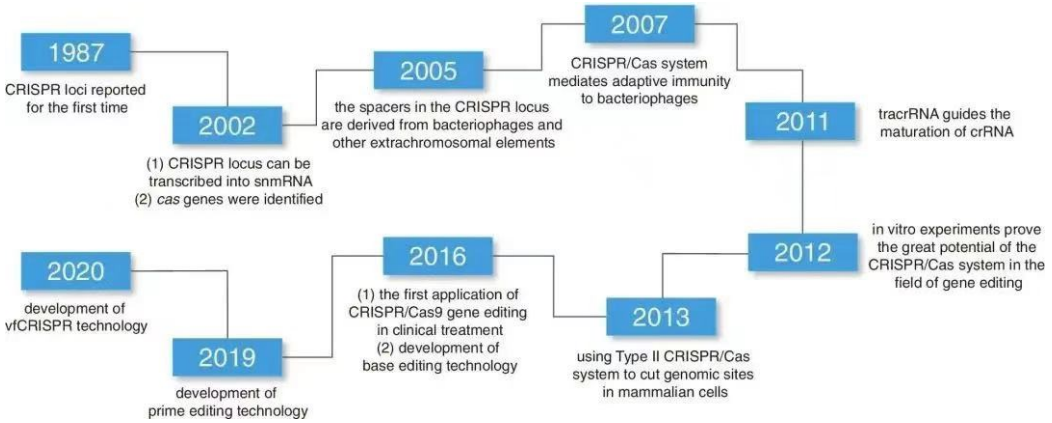

Figure 1: History of Development and the Evolution of Related Gene Editing Tools [4].

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Gene editing relies on two key components. The Cas9 enzyme contains two functional domains, HNH and RuvC. The HNH domain cleaves the DNA strand targeted by the sgRNA, while the RuvC domain cleaves the non-target strand. Guided by sgRNA, Cas9 induces a DSB at the target gene. The break is then repaired through the cell's endogenous DNA repair pathways—either non-homologous end joining (NHEJ) or homologous recombination (HDR)—resulting in gene knockout, insertion, or mutation.

Studies on the Cas9 molecular structure reveal that Cas9 first binds to sgRNA, and under the guidance of sgRNA, it further associates with the target double-stranded DNA. A conformational change in Cas9 unwinds the target DNA, allowing the sgRNA strand to invade and specifically bind to the DNA strand complementary to the sgRNA. Mechanistic studies also show that the protospacer adjacent motif (PAM) sequence at the 3' end of the target sequence is crucial for initial DNA binding. Without PAM, the target sequence, even if perfectly complementary to the sgRNA, will not be recognized by Cas9. Once sequence complementarity is achieved, Cas9 activates its nuclease activity. The HNH and RuvC domains each cleave one DNA strand, leading to the formation of a DNA DSB. By mutating either of these two domains, the Cas9 protein can be converted into a nickase (nCas9), which only cuts one strand. Mutating both domains results in a dead Cas9 (dCas9), which has no endonuclease activity. These two Cas9 variants, nCas9 and dCas9, are widely used for constructing various genetic manipulation tools [5].

Cas9-induced double-strand breaks (DSBs) are typically repaired by the cell's DNA repair pathways: non-homologous end joining (NHEJ) and homology-directed repair (HDR). NHEJ is an error-prone repair mechanism that rapidly joins the broken ends of DNA, but it often results in insertions or deletions (indels) at the break site, leading to frameshift mutations and knockout of the target gene. In contrast, HDR allows precise repair by introducing a donor template. By providing a repair template, HDR can accurately modify endogenous genes near the Cas9 cleavage site.

Application of CRISPR/Cas9 in Establishing Animal Models

Currently, CRISPR/Cas9 is primarily used to establish cellular and animal models. In the field of cancer research, various tumor cell lines and animal models based on the CRISPR/Cas9 system have been successfully developed and applied for preclinical validation in areas such as cancer genes, drug targets, and drug resistance [6].

The establishment of cancer animal models is a crucial tool for studying the functions of cancer-related genes, and CRISPR/Cas9 has significantly simplified the process of creating these models, reducing costs and accelerating modeling timelines. By using CRISPR/Cas9, researchers can study the roles of specific genes in tumor initiation and progression. Currently, CRISPR/Cas9-based tumor models have been established for various cancers. For example, one study used CRISPR/Cas9 to silence the expression of the CDK11 gene in osteosarcoma cell lines KHOS and U-2OS, and found that silencing CDK11 significantly reduced osteosarcoma cell proliferation and invasion. This discovery not only revealed the critical role of CDK11 in osteosarcoma, but also provided a theoretical foundation for the development of new therapy strategies.

The development of chronic myelogenous leukemia (CML) is often associated with mutations in the tumor suppressor gene ASXL1, which impacts patient prognosis. In another study, researchers used CRISPR/Cas9 to target and repair the ASXL1 mutation in KBM5 cells, significantly reducing the proliferation rate of the cells and enhancing their differentiation. Mice with ASXL1-corrected tumors had a longer survival time compared to those with untreated tumors. This study not only confirmed the pivotal role of ASXL1 in CML but also offered new perspectives for future gene therapy.

Additionally, researchers from the University of Cambridge and other institutions conducted large-scale CRISPR/Cas9 screening of 324 tumor cell lines derived from 30 different malignancies, developing a database called Project Score. This database helps identify potential therapeutic targets

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for cancer. It contains extensive functional annotations of genes, as well as detailed experimental data and analysis tools, enabling researchers to more efficiently identify potential therapeutic targets.

3. Chimeric Antigen Receptor (CAR)-T Cell Therapy

Since the FDA approved two autologous CAR-T in 2017, numerous clinical trials have demonstrated the efficacy of CAR-T in treating various hematologic and non-hematologic malignancies. However, clinical applications are still limited by certain challenges, and CRISPR genome editing offers a potential solution to address these limitations (Figure 2). Recent studies have highlighted that CRISPR-based therapies can be used to target CAR-T directed against CD19 and CD70, providing a promising approach for the treatment of solid tumors, leukemia, and lymphoma [7].

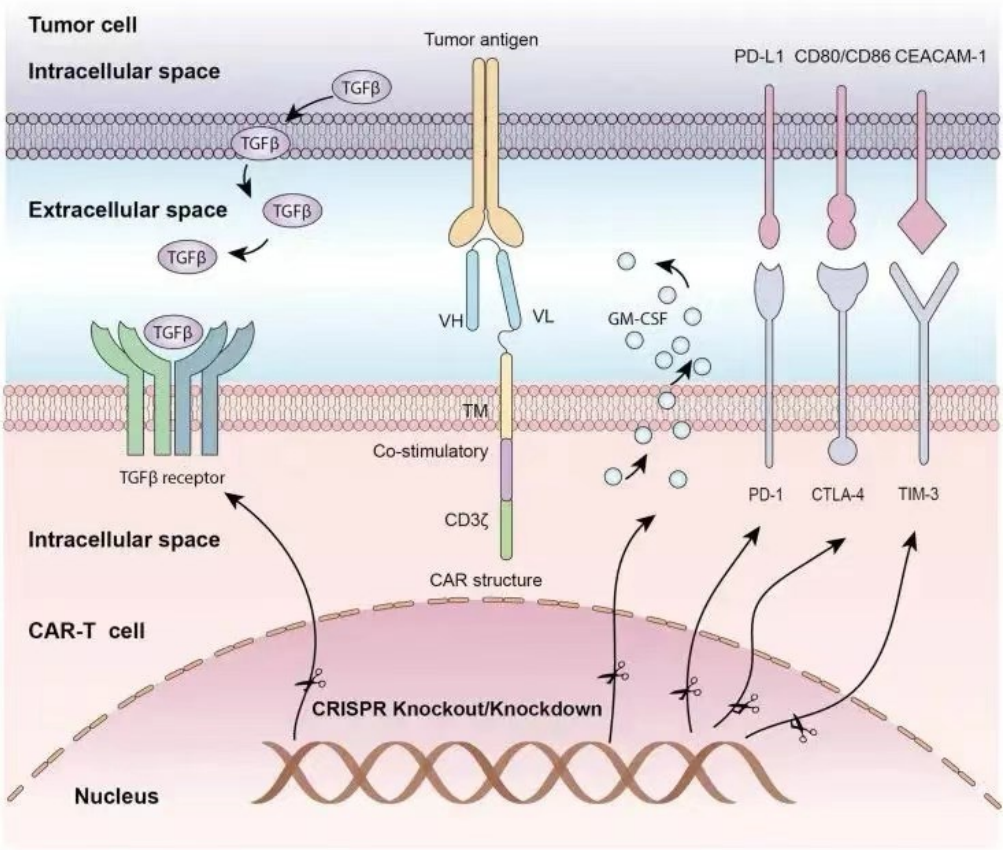

Figure 2: Application of the CRISPR/Cas9 System in CAR-T Cell Editing [8].

4. Transgenic T Cell Receptor (TCR)-T Cell Therapy

CRISPR/Cas9-mediated knockout of endogenous TCR-β in T cells, combined with the transduction of cancer-reactive receptors, significantly increases the expression of transgenic T cell receptors (tgTCR), enhancing the engineered T cells' ability to target and kill cancer cells. Compared to standard TCR-transduced T cells, TCR-transduced and CRISPR-edited T cells exhibit a thousand-fold greater sensitivity to the antigen [9].

The first clinical trial report of 2020 revealed the use of multiple CRISPR/Cas9 edits to target T cell genes in order to enhance anti-tumor immune responses. Additionally, synthetic cancer-specific TCR transgenes (which recognize tumor cells) were introduced. Engineered T cells persist in patients for up to 9 months, providing preliminary evidence that combining TCR transfer with genome editing may lead to more effective and safer cancer immunotherapies [10].

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5. Conclusion

Significant progress has been made in stem cell therapy within current life sciences research, particularly in the treatment of major diseases such as genetic disorders and cancer. Through somatic gene editing technologies, scientists are now able to precisely repair or replace pathogenic genes, offering new therapeutic hope for patients. Moreover, breakthroughs in iPSC technology have made it possible to reprogram adult cells into pluripotent stem cells, providing abundant cellular sources for regenerative medicine and opening new avenues for disease model establishment and drug screening.

Despite the many achievements in stem cell therapy, several challenges remain. First, the safety and efficacy of gene editing still require further validation, especially regarding long-term effects and potential side effects. Additionally, the mechanisms governing stem cell differentiation are not yet fully understood, and efficiently and stably directing stem cells to differentiate into specific types of somatic cells remains a major focus of research. Ethical and legal issues also pose significant barriers to the widespread application of stem cell therapy.

To overcome these challenges, it is essential to optimize gene editing tools such as CRISPR/Cas9, reduce off-target effects, improve editing efficiency, and ensure the safety and reliability of treatments. Further research into the key signaling pathways and molecular mechanisms involved in stem cell differentiation is critical to developing more effective differentiation induction strategies, enabling precise and personalized cell therapies. Establishing a robust ethical review system and legal framework is crucial to ensure the regulatory and ethical integrity of stem cell research and applications, protecting patient rights and promoting the healthy development of the technology. We can accelerate the translation of basic research findings into clinical applications and conducting more human clinical trials are essential to validate the security and efficacy of stem cell therapies and deliver tangible benefits to patients.

In the future, iPSC is expected to make breakthroughs in several key areas: achieving precision medicine through genetic sequencing and individualized stem cell culture; combining gene editing, immunotherapy, and other technologies to enhance therapeutic efficacy; utilizing biomanufacturing technologies to enable large-scale production and standardized preparation of stem cells; and establishing comprehensive ethical review and regulatory mechanisms to safeguard patient rights.

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