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The potential applications of CRISPR/Cas9 in treating Duchenne Muscular Dystrophy

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Abstract. The Duchenne Muscular Dystrophy (DMD) is a widespread genetic disorder caused by a mutation in the dystrophin gene. Current treatments, such as corticosteroids and genetic therapies like exon skipping, offer some symptom management but fall short of providing a cure. The CRISPR/Cas9 technology, which is related with Clustered Regularly Interspaced Short Palindromic Repeats-associated protein 9, could edit the genome with great precision. This technique holds potential in tackling the underlying issue of DMD by rectifying mutations in the dystrophin gene. Various strategies, including exon skipping, frame remodeling, and expression regulation, have demonstrated the potential to resumption dystrophin expression and improve muscle fitness in preclinical models. Even with these developments, there are still a number of important obstacles to overcome, such as enhancing the effectiveness of delivery, reducing off-target effects, and managing immune reactions. Future research will focus on refining these techniques and ensuring their safe and effective translation to clinical therapies, potentially revolutionizing the treatment of DMD and other genetic disorders.

Keywords: Duchenne muscular dystrophy, Genome therapy, CRISPR/Cas9, Gene editing.

1. Introduction

Duchenne muscular dystrophy (DMD) is a prevalent hereditary condition that impacts approximately 1 in every 3,500-5,000 male births [1]. DMD is a result of a mutation in the myotonic dystrophy protein gene found on chromosome X (Xp21.2). This gene is typically deleted in patients due to the mutation. Myotonic dystrophy proteins play a crucial role in preserving the structural integrity of muscle cells. Myotonic dystrophin has a role in stabilizing the plasma membrane during muscle contraction by being a part of the dystrophin-glycoprotein complex (DGC). The DGC establishes a connection between the extracellular matrix and the intracellular cytoskeleton [2]. The lack of this protein causes the activation of ion channels that respond to stretching, potentially causing higher levels of calcium ions inside cells. This, in turn, triggers the activation of protein hydrolases like calpain and increases the generation of reactive oxygen species. Consequently, this leads to harm to proteins and cell membranes, ultimately resulting in muscle damage and degeneration [3]. At present, the main treatments for DMD are Glucocorticoid therapy, exon skipping therapy, nonsense mutation suppression therapy and so on. These methods have certain clinical effects, such as glucocorticoid therapy can improve muscle strength, lung function and motor function, and delay the loss of walking ability; Exon skipping therapy and nonsense

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mutation suppression therapy can restore the expression of myotonic dystrophy proteins in part of the muscle. Nevertheless, there are certain constraints: glucocorticoid medication is accompanied by adverse effects, including weight gain and cataracts, which result in the termination of treatment in over 50% of patients. Additionally, gene replacement therapy needs to solve the problems of limited capacity of viral vectors and immune response. These treatments still need further research and optimization to improve efficacy and reduce side effects [4].

The CRISPR-Cas9 system consists of the single-guide RNA (sgRNA) and the Cas9 protein, enabling the accurate targeting and modification of specific gene sequences. The Cas9 protein possesses two nuclease domains, namely Histidine-Asparagine-Histidine and Resolvase of UV-induced recombination intermediates, which are responsible for cleaving the target DNA. Additionally, the sgRNA serves as a guide to direct the Cas9 protein to the specific target site. The CRISPR-Cas9 system has the ability to cause double-strand breaks (DSBs) in the specific DNA sequence being targeted. These breaks can be fixed using three different repair mechanisms: non-homologous end joining (NHEJ), homology-directed repair (HDR), or microhomology-mediated end joining (MMEJ) [5]. The CRISPR-Cas9 technology has produced remarkable advancements in the investigation and management of many diseases. CRISPR-Cas9 technology can be employed to specifically target the liver tumor suppressor genes P53 and PTEN in hepatocellular carcinoma. This targeting leads to the development of liver tumors that resemble those found in CRE-loxP knockout Pten and p53 transgenic animals. In addition, CRISPR/Cas9 can be employed to correct mutations in the Fah gene in a mouse model of hereditary tyrosinemia type I and to provide therapeutic treatment for the condition by restructuring the metabolic pathway. In colorectal cancer, CRISPR-Cas9 technology can be used to correct the Trp53 and APC(Antigen-presenting cells) tumor suppressor genes in a mouse model and to identify key genes leading to drug resistance; Renal cell cancer CRISPR-Cas9 technology can be employed to decrease the tumor suppressor gene VHL and detect additional genes associated with kidney cancer in renal cell carcinoma. Further, CRISPR-Cas9 has the capability to control the expression of long-stranded non-coding RNA genes that are linked to the development of tumors [6]. B-cell lymphoma can also be treated using gene editing using CRISPR-Cas9 technology. One way to stop tumor cell growth and metastasis is to knock down or control genes linked to B-cell lymphoma, such BCL67. Gene knockout libraries, such as GeCKO (genome-scale CRISPR knockout) libraries, can be created in the interim using CRISPR-Cas9 technology. These libraries provide fresh approaches to targeted therapy and can be used to assess possible therapeutic targets for B-cell lymphoma [7]. CRISPR-Cas9 technology can be utilized to treat DMD by binding to the target DNA. The body's endogenous repair mechanisms, primarily HDR and NHEJ, can effectively mend a DSB induced by the Cas9-sgRNA complex. Unlike HDR, which relies on a donor DNA template to create accurate genetic changes, NHEJ often leads to small insertions or deletions that might disrupt the reading frame of a gene. Due to its exceptional precision and adaptability, this method holds significant potential for application in the field of genome editing. The CRISPR-Cas9 system has novel prospects for utilizing genome editing technology in therapeutic applications and shows significant potential in the fields of disease treatment and personalized medicine [8]. This review aims to assess the potential of CRISPR/Cas9 as a therapeutic intervention for Duchenne muscular dystrophy.

2. Current views of Duchenne Muscular Dystrophy

2.1. Pathophysiology of DMD

Covering roughly 2.4 megabases of DNA and composed of 79 exons, the dystrophin gene is one of the largest in the human genome. This gene encodes a 427 kDa protein that features four major functional domains: an N-terminal actin-binding domain, a central rod domain with spectrin-like repeats, a cysteine-rich domain, and a C-terminal domain [9]. Alterations in the DMD gene, such as deletions, duplications, point mutations, and minor insertions or deletions, alterating the reading frame leads to a truncated, non-functional dystrophin protein [10]. Current therapeutic approaches for DMD, involving medications like corticosteroids and genetic interventions such as exon skipping, offer improvements in specific symptoms but have yet to yield a groundbreaking solution. While these treatments assist in

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managing symptoms and slowing disease progression, an ideal solution is still not achieved. Researchers continue to face challenges and are actively seeking more effective strategies. Presently, common treatments aim to balance symptom management with addressing the genetic roots of the disease. Corticosteroids, like prednisone and deflazacort, are frequently utilized to manage DMD. These medications help slow muscle weakness progression and extend ambulation by decreasing inflammation and stabilizing muscle cell membranes [10]. However, prolonged corticosteroid use is linked with significant adverse effects, including weight gain, osteoporosis, growth delay, and a higher risk of infections and diabetes [11].

2.2. Current gene therapy for DMD

Gene therapy for DMD seeks to restore dystrophin expression through several methods, such as exon skipping, gene replacement, and genome editing. Exon skipping uses antisense oligonucleotides (ASOs) to omit specific exons during the splicing of pre-mRNA, by restoring the reading frame, this approach permits the generation of a truncated, yet functional dystrophin protein. FDA-approved ASOs like eteplirsen (exon 51), golodirsen (exon 53), and viltolarsen (exon 53) have shown modest increases in dystrophin levels but are limited to patients with certain exon mutations [10]. Gene replacement therapy entails the insertion of a functional dystrophin gene copy via viral vectors such as adeno-associated viruses (AAV). Due to the dystrophin gene's large size, mini- or micro-dystrophin constructs are used. Early clinical trials have shown promise, but challenges remain in immune responses and efficient delivery to muscle tissues [12]. The CRISPR/Cas9 genome editing technique has becomes a potential curative approach for DMD by directly correcting genetic mutations. Preclinical studies have shown the feasibility of using CRISPR/Cas9 to remove mutated exons or correct point mutations, restoring dystrophin expression in animal models. Nonetheless, issues like delivery efficiency, off-target effects, and immune responses need to be resolved prior to clinical use [13].

3. Application of CRISPR/Cas9 in DMD

CRISPR/Cas9 is a groundbreaking genome-editing technology tool that allows for the accurate alteration of DNA sequences within living organisms. This tool shows significant potential for treating DMD by correcting mutations in the dystrophin gene. Various strategies have been investigated to accomplish this, including exon skipping, frame remodeling, and expression regulation.

3.1. CRISPR/Cas9 used for exon skipping

Exon skipping uses CRISPR/Cas9 to initiate NHEJ repair, allowing the deletion or skipping of exons with disease-causing mutations, thus This restores the reading frame and results in the production of partially functional dystrophin proteins. In a notable study, Long and colleagues employed CRISPR/Cas9 to repair the dystrophin gene in mdx mice, which have a point mutation in exon 23. The researchers injected AAV vectors with Cas9 and sgRNA targeting exon 23 into the mice, leading to successful exon skipping and dystrophin expression restoration in muscle tissues. Histological analysis revealed reduced muscle fiber necrosis and inflammation, while functional tests showed enhanced muscle strength and decreased serum creatine kinase levels [15].

3.2. CRISPR/Cas9 used for frame remodeling

For frame shift mutations, CRISPR/Cas9 can adjust the reading frame via NHEJ repair, enabling dystrophin protein expression. Nelson et al. showcased this method by targeting exon 51 in DMD patient-derived iPSCs. By utilizing CRISPR/Cas9, the researchers generated small insertions and deletions (INDELs) at the exon 51 splice site, which corrected the frame shift and enabled the production of functional dystrophin. The modified iPSCs were differentiated into muscle cells, which exhibited restored dystrophin expression and normal cellular function [16].

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3.3. CRISPR/Cas9 used for expression regulation

CRISPR/Cas9 can also modulate the expression of compensatory proteins such as utrophin to mitigate the consequences of dystrophin deletion. Research indicates that increasing utrophin levels in muscle cells can partially restore muscle function in DMD models. For example, researchers utilized CRISPR activation (CRISPRa) to increase utrophin expression in muscle cells from DMD patients, which led to improved muscle cell stability and function [16].

3.4. Models for CRISPR/Cas9 in DMD

3.4.1. Mouse models

In preclinical studies, CRISPR/Cas9 has been utilized to correct DMD mutations in mouse models. For example, in the mdx mouse model carrying a point mutation in exon 23, researchers used CRISPR/Cas9-mediated exon skipping to restore dystrophin expression. This CRISPR/Cas9 system was delivered using AAV vectors, proven to be safe and effective. Mdx mice treated with CRISPR/Cas9 showed significant improvements in muscle function and histological markers, such as increased dystrophin-positive fibers and reduced muscle degeneration [16].

3.4.2. Induced pluripotent stem cells (iPSCs) modle

CRISPR/Cas9 has been employed to edit iPSCs derived from DMD patients' fibroblasts to correct dystrophin gene mutations. For instance, researchers applied CRISPR/Cas9 to correct a mutation in exon 44 of the dystrophin gene in iPSCs derived from patients. Corrected iPSCs were then differentiated into muscle cells that expressed functional dystrophin. These edited cells showed improved muscle cell function and reduced signs of DMD pathology in vitro, providing a valuable platform for disease modeling and therapeutic development [14].

3.4.3. Direct muscle cells modle

Direct gene editing of muscle cells has also been investigated. In vitro studies have demonstrated that CRISPR/Cas9 can effectively target and correct dystrophin mutations in human muscle cells. For example, researchers used CRISPR/Cas9 to fix a mutation in the dystrophin gene in human muscle cells, which restored dystrophin expression and improved cellular function. This approach has shown potential for therapeutic applications, as corrected muscle cells exhibited enhanced stability and reduced susceptibility to damage [17].

3.5. Delivery system of CRISPR/Cas9 in DMD

A dual AAV delivery approach, with the Cas9 nuclease in a single-stranded AAV (ssAAV) and the CRISPR guide RNA (sgRNA) in a self-complementary AAV (scAAV), has been employed to enhance CRISPR/Cas9 genome editing efficiency. In a study by Olsen et al., this dual AAV system was applied to a DMD mouse model. The findings showed that this method required at least 20-fold lower viral doses for effective genome editing compared to traditional ssAAV vectors. Mice treated with this dual AAV system exhibited significant dystrophin expression restoration and enhanced muscle contractile function, demonstrating the potential of this approach for clinical application [18].

4. Challenges and Future Directions

Despite the significant progress made in CRISPR/Cas9-based therapies for Duchenne Muscular Dystrophy (DMD), several challenges remain. These include delivery efficiency, off-target effects, immune responses, and long-term safety and efficacy. Delivery methods often struggle with efficiency, as current vectors like adeno-associated viruses (AAVs) have limited capacity and can trigger immune reactions, while off-target effects pose risks of unintended genomic alterations, potentially leading to instability and mutations. Additionally, immune responses to CRISPR components can reduce therapeutic effectiveness and pose safety concerns, and long-term safety issues, such as the stability of gene edits and the risk of oncogenesis, need thorough investigation.

Future research will focus on optimizing delivery methods, such as developing non-viral vectors and tissue-specific delivery systems, and improving the specificity and efficiency of gene editing by using high-fidelity Cas9 variants and advanced guide RNA designs. Thorough preclinical and clinical studies are crucial to ensure the safe and effective application of CRISPR/Cas9 therapies in patients, addressing both short-term and long-term safety issues while improving therapeutic results.

While current treatments for DMD provide symptomatic relief and slow disease progression, CRISPR/Cas9 offers a potential curative approach by directly addressing the underlying genetic cause. Continued advancements in genome editing technologies and delivery systems will be crucial in overcoming the remaining hurdles and making this therapy a reality for DMD patients [14].

5. Conclusion

The application of CRISPR/Cas9 technology in treating Duchenne Muscular Dystrophy (DMD) has shown remarkable promise by precisely targeting and correcting mutations in the dystrophin gene. This approach offers potential curative solutions that address the root cause of DMD, moving beyond symptomatic treatments. Despite the progress, challenges remain, such as improving delivery efficiency, minimizing off-target effects, and addressing immune responses. Future research will focus on overcoming these hurdles and refining genome editing techniques and delivery systems to translate promising preclinical results into safe and effective clinical therapies, potentially revolutionizing gene therapy and personalized medicine for various genetic disorders

Authors Contribution

All the authors contributed equally and their names were listed in alphabetical order.

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