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Targeted Therapeutic Strategy for Duchenne Muscular Dystrophy Leveraging RNA Editing

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Abstract:

Duchenne muscular dystrophy (DMD) is a devastation X-linked recessive genetic disorder involving progressive muscle tissue degeneration and premature death, primarily occurring in males. Current therapies such as corticosteroids and gene replacement are of limited value and suffer from the problems of enormous side effects and restrictions based on viral vectors. RNA base editing is a new-generation therapeutic strategy for genetic diseases like DMD with advantages of being reversible, less likely to lead to off-target risks than DNA editing, and direct disease-causing mRNA mutation correction. This study reviews the literature for RNA base editing as a therapy for DMD. Major RNA editing technologies, adenosine-to-inosine (A-to-I) editing by engineered ADAR enzymes , are introduced for their potential to edit out premature stop codons in the DMD mRNA to restore functional dystrophin protein expression. Key milestones include the miniaturization of editors suitable for adeno-associated virus (AAV) delivery, demonstration of efficacy in humanized DMD mouse models with dystrophin restoration and functional muscle improvement. The findings reveal the therapeutic utility of RNA base editing for DMD and propose new directions for its future clinical application.

Keywords: Duchenne muscular dystrophy; RNA base editing; adenosine-to-inosine editing; adeno-associated virus delivery.

1. Introduction

Duchenne muscular dystrophy (DMD) is a fatal, progressive, and disabling X-linked recessive neuromuscular disease, occurring in approximately 1 among every 3500-5000 live male births. Female carriers are common with an estimated prevalence of 1/50, but symptomatic cases are rare due to the X-linked recessive inheritance pattern; females possess two X chromosomes where a functional copy typically

compensates for the mutated allele, whereas males with a single mutated X chromosome develop the disease [1].

The condition manifests in early childhood (2-5 years) with delayed motor milestones, frequent falls, and failure to run or climb. It then progresses to loss of ambulation typically 6-12 years, followed by severe complications like scoliosis, joint contractures, and ultimately life-threatening cardiac and respiratory muscle failure in adolescence and early adulthood [2]. The underlying pathological process is a mutation of the gene DMD on the X chromosome (Xp21.2) resulting in absence or severe deficiency of dystrophin protein.

Dystrophin is crucial in maintaining muscle membrane structural integrity by spanning intracellular cytoskeleton to extracellular matrix. In the absence of the protein, muscle fibers are susceptible to cumulative damage with each contraction of the muscle, leading to a necrosis-fibrosis-fat and connective tissue replacement cycle that eventually manifests as cumulative muscle weakness and atrophy [2]. This pathological change affects not only the skeletal muscle but also cardiac and respiratory muscles, the root cause of the patient's development of cardiopulmonary failure.

The lack of dystrophin disrupts the stability of the dystrophin-associated protein complex (DAPC) and compromises the structural integrity of the muscle membrane. Therefore, muscle fibres are highly sensitive to mechanical stress damage during contraction, leading to a series of cyclical pathological events: membrane rupture, calcium influx, proteolytic activation, necrosis, chronic inflammation, and the replacement of functional muscle tissue with fibrotic and adipose tissue. It is this pathological cascade reaction that leads to the characteristic progressive muscle degeneration, and current therapeutic interventions for DMD remain palliative. Steroids such as prednisone and deflazacort can slow down functional decline, but they come with severe side effects, such as weight gain and osteoporosis. Emerging gene therapies face obstacles: immune responses to viral vectors and packaging limitations due to the large size of the DMD gene. Therefore, RNA editing, particularly adenosine-to-inosine (A-to-I) base editing, has become a transformative strategy. It directly corrects pathogenic mRNA transcripts without altering the genome. By utilising the cell's transcription-translation machinery, it repairs defective mRNA, showing promise for restoring dystrophin production and expanding treatment options for DMD [3].

This article critically examines the molecular mechanisms, preclinical advances, and translational potential of RNA editing for restoring dystrophin expression in DMD.

2. X - linked Recessive Muscular Disorder: Research Progress on DMD

2.1 Pathological Features and Pathogenesis of DMD

The genetic foundation of DMD lies in mutations within the gigantic DMD gene. Such mutations disrupt the normal synthesis of intact - length, functional dystrophin protein. Frequent forms of mutations include large deletions/duplications, which account for 70% of patients, and point mutations, making up 30% of patients [4]. Among point mutations, nonsense mutations insert a premature termination codon, like UAA, UAG or UGA, and frameshift mutations often create downstream PTCs; both are particularly pathogenic. These premature termination codons lead to premature termination of translation, thereby preventing the production of dystrophin.

The absence of dystrophin disrupts the stability of the dystrophin - associated protein complex (DAPC) and compromises the structural integrity of the muscle membrane. As a result, muscle fibres are highly susceptible to mechanical stress damage during contraction, leading to a series of cyclical pathological events: membrane rupture, calcium influx, proteolytic activation, necrosis, chronic inflammation, and the replacement of functional muscle tissue with fibrotic and adipose tissue. It is this pathological cascade that causes the characteristic progressive muscle weakness and atrophy in Duchenne muscular dystrophy [1][2].

2.2 Current Therapies

Treatment of DMD aims at symptom relief, slowing disease progression, and improving quality of life but remains largely palliative.

For symptomatic or supportive care, it encompasses corticosteroids. Prednisone and deflazacort, the most effective therapies available currently, can notably delay muscle degeneration, preserving ambulation and respiratory function temporarily. Yet, long - term use brings serious side effects like weight gain, osteoporosis, growth retardation, cataracts, and behavioral changes [3]. Physical therapy, orthotics, respiratory support via non - invasive ventilation, and cardiac management with ACE inhibitors or beta - blockers are necessary supportive measures.

Regarding etiological therapies with a gene - targeted approach, DNA - level editing such as CRISPR/Cas9 seeks to permanently fix the underlying genomic mutation or trigger exon skipping via NHEJ. While it holds great promise for correcting the dystrophin reading frame, for instance in deletion cases amendable to exon skipping,

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challenges persist [1].

RNA - level interventions also play a role. In stop codon readthrough, small molecules like ataluren prompt ribosomes to bypass premature termination codons. Efficacy varies by mutation and is notably better for UGA premature termination codons, with clinical results remaining modest [2]. RNA base editing is an emerging strategy. It directly corrects faulty RNA sequences to avoid irreversible genomic DNA mutations. Engineered tools edit pathogenic mRNA transcripts, such as converting a premature termination codon to a sense codon. This allows ribosomes to produce full - length, functional dystrophin from the corrected mRNA.

The approach leverages the cell's own transcription and translation machinery to fix defective mRNA. It has advantages including transient effects, as corrected mRNA degrades naturally, and no double - strand DNA breaks. These features distinguish it from DNA editing and further expand the range of therapeutic strategies being explored for DMD.

3. The use of RNA editing strategy in DMD treatment

3.1 A to I RNA editing strategy

3.1.1 The ADAR protein family

The ADAR (adenosine deaminase acting on RNA) protein family is a class of proteins that are capable of catalyzing the deamination of adenosine, converting it into inosine. In the translation process, the transfer RNA will recognize inosine as guanosine (G). Consequently, ADAR proteins are capable of performing A - G editing [5].

ADAR proteins consist of a double - stranded RNA - binding domain at the N - terminus and a deaminase domain at the C - terminus. The RNA - binding domain will recognize the RNA sequences and structures while the deaminase domain is responsible for carrying out the deamination reaction [6].

3.1.2 The technical core of A to I RNA editing in the treatment

A to I RNA editing is catalyzed by adenosine deaminases which is combined with the RNA (ADARs). In this process, adenosine in double - stranded RNA regions will undergo deamination reaction and will be transformed to inosine. Since the transfer RNA will recognize inosine as guanine during translation, this editing can result in changes in the amino acid sequence of the resulting protein if it occurs in the coding region [7]. For example, in humans, A to I editing is a common physiological process in the human transcriptome, many of the editing processes occur in the Alu repetitive elements which are abundant in non - coding regions so it has little influence. However, some editing also occurs in coding regions and those events can lead to diseases such as neurological disorders. In the nervous system, specific A to I editing events will impact the function of ion channels and neurotransmitter receptors, thus affecting neuronal excitability and synaptic plasticity.

In the treatment of DMD, the principle of this A to I RNA editing strategy is to recruit ADARs to targeted regions of the DMD mRNA through engineered system. Designing antisense oligonucleotides (ASOs) that can hybridize with the DMD mRNA and guide the ADAR enzymes to the desired sites. After combination, the ADARs will convert adenosine to inosine, which is recognized as guanine during translation. This can be possible to correct point mutations or splicing errors in the DMD mRNA and lead to the production of a more functional dystrophin protein. For example, Our study presents a technique utilizing mini-dCas13X-mediated RNA adenine base editing (mxABE) to address monogenic diseases caused by nonsense mutations, through A-to-G editing in a genetically humanized mouse model of Duchenne muscular dystrophy (DMD). The research first identified a nonsense point mutation (c.4174C>T, p.Gln1392*) in the DMD gene of a patient and confirmed that this mutation leads to the development of the disease in humanized mice.In this model, when mxABE was encapsulated in a single adeno-associated virus (AAV), its in vivo A-to-G editing efficiency could reach up to 84%, a value that is at least 20 times higher than the reported results of previous other RNA editing methods. Moreover, mxABE can enable read-through of premature termination codons (PTCs) in multiple muscle tissues, thereby significantly increasing the expression level of dystrophin to over 50% of the wild-type level.More importantly, after systemic delivery of mxABE via AAV, the expression levels of dystrophin in the diaphragm, tibialis anterior, and heart muscle were restored to an average of 37%, 6%, and 54% of the wild-type levels, respectively, and muscle function was also improved accordingly. These data fully demonstrate that the mxABE-based therapeutic strategy is expected to become a new and effective method for treating DMD and other monogenic diseases [8].

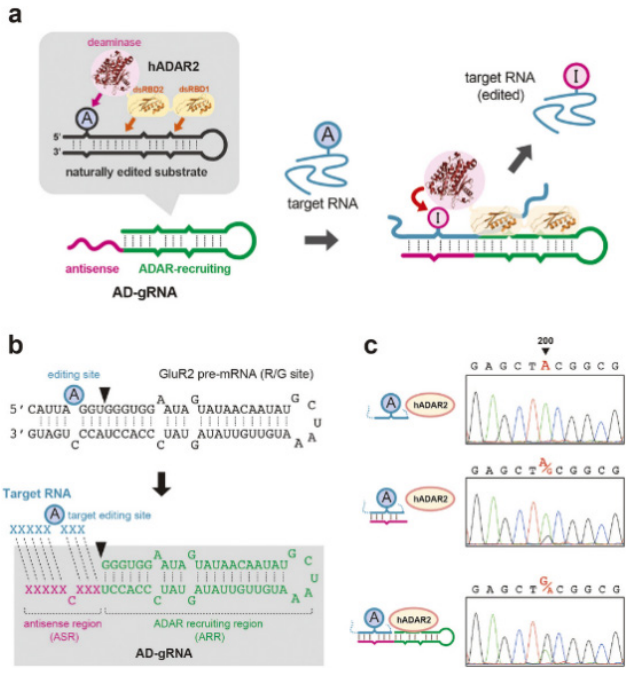

Fig. 1 The process of editing. (a) Technical principle of RNA editing (b) Design and practice (c) Effect verification [9]

Fig. 1(a) has shown the process of A to I RNA editing. The deaminase represents the deaminase domain of hADAR2, which catalyzes the deamination reaction. The hADAR2 is Human Adenosine Deaminase Acting on RNA 2, an enzyme that mediates A - to - I RNA editing. It has double - stranded RNA - binding domains and a deaminase domain. The naturally edited substrate will combine with the hADAR2 and it's a will operate deamination reaction.

Fig. 1(b), it has shown a more precious figure of the RNA part. The editing site is the position on the RNA where A - to - I editing occurs. The GluR2 pre - mRNA (R/G site) is pre - messenger RNA of the GluR2 gene, with a specific region (R/G site) that undergoes A - to - I editing. The sequence shows the wild - type RNA before editing, with the adenosine at the editing site. The target RNA is the RNA molecule to be edited and the target editing site is where we want A - to - I conversion occurs.

As can be seen in Fig.1(c), the results measured after the reaction. The sequence electropherograms can display DNA sequencing results, the peaks represent different nucleotides: The top one (without effective editing) has a clear A peak at the target site. The middle and bottom ones (with AD - gRNA - mediated editing) show reduced A peaks and emergence of peaks corresponding to inosine (functionally like G, so G - related signals appear), indicating A - to - I conversion at the target site. These data validate the success of AD - gRNA - directed A - to - I editing by hADAR2.

3.2 U-to-Ψ RNA editing strategy

3.2.1 U-to-Ψ RNA editing strategy

U to Ψ RNA editing, on the other hand, involves the isomerization of uridine to pseudouridine. This process is carried out by a group of enzymes called pseudouridine synthases. Pseudouridine has distinct structural and chemical properties compared to uridine, which can influence the folding, stability, and function of RNA molecules. For instance, in ribosomal RNA (rRNA), U to Ψ editing can enhance the accuracy and efficiency of translation. The presence of pseudo uridine in rRNA can optimize the interaction between ribosomes and messenger RNA (mRNA), as well as transfer RNA (tRNA), during the translation process. In transfer RNA, U to Ψ editing often occurs in the anticodon loop, which is crucial for the correct decoding of mRNA codons. This editing can fine - tune the base - pairing properties of tRNA, ensuring accurate translation of the genetic code [10].

3.2.2 The technical core of U-to-Ψ RNA editing strategy in the treatment

This technology utilizes engineered guide snoRNA to recruit endogenous pseudouridine synthase complexes in cells, enabling efficient and accurate conversion of uridine (U) to pseudouridine (Ψ) at specific sites of RNA. By precisely introducing pseudouridine modifications at nonsense mutation sites in mRNA, premature termination codons are converted into ΨAA, ΨAG, or ΨGA, thereby achieving readthrough of premature termination codons and full - length expression of functional proteins. Pseudouridine modification does not alter base complementarity and will not affect the coding information of codons; the little off - target effects generated by RESTART will not impact RNA stability or protein translation [10].

4. Methods to improve editing efficiency

4.1 Improvement of Editing Efficiency

RNA editing has been extensively used in disease treatment, fundamental research, and many other domains, with its application breadth progressively broadening. Nonetheless, it remains predominantly in the developmental phase.

Numerous investigations have been conducted to investigate approaches to the optimization of RNA Editing Systems. According to research on Leveraging Endogenous ADAR for Programmable Editing of RNA (LEAPER), the average editing efficiency of the developed circular RNA (circ-arRNA) is over three times higher than that of the

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linear version, suggesting that RNA editing optimization greatly benefits from increased editing efficiency [11][12]. Three main approaches are applied to improve editing efficiency, namely optimizing protein components in the editing system, modifying guide RNA structure, and modifying guide RNA termini [13][14][15].

4.2 Specific Delivery Method

Specific delivery refers to the precise transport of therapeutic molecules such as drugs, gene-editing tools, nucleic acids, and proteins to target tissues, cells, or subcellular structures, while minimizing effects on non-target sites. Its core objectives are to enhance therapeutic efficacy and reduce off-target effects, and it is particularly crucial in fields such as gene editing, gene therapy, and cancer therapy.

Previous research shows that through the engineering of lipid nanoparticles, the selective organ targeting (SORT) strategy is able to selectively edit a range of therapeutic-related cell types and precisely deliver editing tools to various mouse organs, indicating that specific delivery is a crucial component in maximizing the use of RNA editing in precision therapy [16].

Base editors or Cas9 ribonucleoproteins are packaged efficiently and delivered by engineered virus-like particles (eVLPs) [16]. Using ribonucleoproteins instead of nucleic acids to deliver gene editing medications in vivo is safer. eVLPs reduce the possibility of off-target editing or DNA integration while achieving effective base editing in a range of cell types [17]. A single injection of eVLPs lowered serum Pcsk9 levels, partially restored visual function, and effectively edited the brain, liver, and retina in a mouse model of inherited blindness [17]. These findings highlight the benefits of specific delivery in enhancing editing efficiency, lowering risk, and curing illness [17].

4.2.1 Adeno-associated Virus

Adeno-associated virus (AAV) is a small, non-enveloped parvovirus with a single-stranded DNA genome, characterized by its dependence on helper viruses (such as adenoviruses or herpesviruses for productive replication. Due to its unique biological properties, gene therapy with AAV as a vector has emerged as an innovative treatment approach with the potential for significant disease change in numerous monogenic illnesses, or possibly even cures.

As a therapeutic strategy, AAV-based gene therapy offers a number of noteworthy benefits. It has proven tremendous potential for substantial disease alteration and even cures in various monogenic illnesses, signifying a positive shift in therapy approaches [18]. The safety profile is usually favorable, with no direct deaths related to transgenes or capsids in reviewed trials, and most completed studies satisfying their safety endpoints [18]. Efficacy is shown across multiple indications, with increased success probabilities in clinical development compared to historical norms for medications in relevant therapeutic areas, particularly in haematology, where the IND-to-NDA success rate exceeds 56% [18].

Furthermore, shorter study durations have been made possible by improvements in trial designs and regulatory familiarity, which have allowed for more effective advancement [18]. The application of AAV vectors in a variety of target organs, including the liver, muscles, eye, and central nervous system, demonstrates their adaptability [18]. Intravenous and intrathecal delivery of AAV vectors have demonstrated comparatively good safety profiles.

4.2.2 Lipid Nanoparticle

Lipid Nanoparticles (LNPs) are a class of colloidal delivery systems composed of ionizable lipids, phospholipids, cholesterol, and polyethylene glycol (PEG)-lipids, designed to encapsulate and protect nucleic acids such as mRNA, siRNA and DNA for intracellular delivery.

To effectively distribute the CRISPR-Cas9 ribonucleoprotein to cells, a modified LNP was created. While intravenous injection restored dystrophin expression in Duchenne muscular dystrophy mice and decreased serum PCSK9 levels in C57BL/6 mice, it also allowed for tissue-specific multiplex editing of six genes in the lungs of mice and the development of organ-specific cancer models in the liver and lungs [19].

According to another research, a team created a universal platform for CRISPR gene editing in vitro and in vivo [20]. The technology is built on a newly created LNP formulation and a designed thermostable iGeoCas9 gene-editing enzyme, which allows for effective encapsulation, transport, and tissue-specific gene editing of the gene editor in RNP form [20]. Following a single intravenous injection of iGeoCas9 RNP-LNP, the degree of genome editing in the liver and lungs of reporter mice increased from 16% to 37% when tissue-selective LNP formulations were used [20].

4.3 Applications in Animal Models

Utilizing a genetically modified mouse model of DMD, researchers show how to use mini-dCas13X–mediated RNA adenine base editing (mxABE) to treat monogenic disorders linked to nonsense mutations through A-to-G editing [21]. While in another research, scientists applied the ceRBE system to restore the gene expression, treating DMD [22]. The differences are summarized in Table 1.

Table 1. Differences between two systems [21][22]

Systems	Key Component	Size (Amino acids)	Editing Efficiency
mxABE System	Mini-dCas13X	830	84%
ceRBE System	EcCas6e	199	68%

5. Conclusion

In previous researches, RNA editing has been proven to be an efficient method to treat DMD. Compared to DNA editing, RNA editing demonstrates prominent benefits, including lower risks, lower probability of off-target effects, advantages in specific delivery, and so on. As diverse editing strategies and delivery tools are involved, editing efficiency can be considerably improved. These studies provide a crucial function in discovering practical therapy strategies for DMD. Initially, they break traditional therapeutic limitations, offering precise strategies through specific delivery. In addition, by encompassing more mutation types, they expand applicability to diverse genetic variations and drive the clinical translation of RNA editing, thereby benefiting other genetic disorders. Moreover, the improvement of patients' life quality and relief of social burden both result from addressing the root cause and reducing long-term care needs.

Despite the significance of RNA editing, it still needs to be refined and enhanced for broader clinical use. Recent researches almost focused on DMD of non-human primates instead of applying to humans. Furthermore, DMD affects muscles throughout the body, including respiratory and cardiac muscles, yet current technologies struggle to achieve efficient systemic editing. It is necessary to overcome physiological barriers such as the blood-muscle and blood-brain barriers, and develop efficient systemically deliverable carriers like novel lipid nanoparticles, to ensure that critical muscle groups, including the diaphragm and myocardium, reach therapeutic editing levels. Expanding coverage of complex mutations by combining editing tools such as the mxABE system and the ceRBE system is expected to be promising, ultimately improving the feasibility of systemic treatment.

In summary, advancements in RNA editing technologies harbor transformative potential for Duchenne muscular dystrophy. With ongoing refinements in targeting precision, editing efficiency, and systemic delivery systems, these innovations may ultimately evolve into a curative strategy that alleviates the profound burden of this devastating disease.

Authors Contribution

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

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