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RNA Editing for Muscular Dystrophy Therapy

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Abstract Due to lack of effective therapies, muscular dystrophies became a focus for gene therapy. Multiple pre-clinical studies have shown successful restoration of dystrofin and dysferlin by RNA editing both in vivo and in vitro, but possibility of a clinical translation is still obscure. A number of new chemicals are being studied, and a search for new techniques is ongoing. This work is intended to give a brief overview of the current state of the RNA editing for treating muscular dystrophies.

Keywords RNA editing · Exon skipping · Trans-splicing · Duchenne muscular dystrophy · Dysferlinopathy

Abbreviations

AAV	Adeno-associated virus
ES	Exon-skipping
TS	Trans-splicing
DMD	Duchenne muscular dystrophy
BMD	Becker muscular dystrophy
LGMD2B	Limb girdle muscular dystrophy type 2B

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1 Introduction

RNA editing is a method that allows to remove or add genetic sequences mostly in the process of pre-mRNA splicing to obtain mRNA with no mutation and restored open reading frame, which can support, as the result, translation of a functional protein. Two main approaches for RNA editing are trans-splicing (TS) and exon skipping (ES). These methods are relatively well-studied in human cells and researchers are able to manipulate with small genetic constructs; therefore, small coding capacity, but safe and widely used AAV-vectors (for TS) or synthetic oligonucleotides (for ES), could be used. These approaches have become promising tools to correct mutations in genes responsible for a number of inherited diseases. Hemophilia A, spinal muscular atrophy, dystrophic epidermolysis bullosa, cystic fibrosis, Duchenne muscular dystrophy, dysferlinopathy (LGMD2b and Miyoshi myopathy), ataxia telangiectasia, neurofibromatosis (types 1 and 2), Pelizaeus–Merzbacher disease etc., have been successfully corrected in vitro or in vivo [1]. Muscular dystrophies are among the most prevalent inherited diseases causing premature death and disability, and RNA editing technology is being widely studied as a possible treatment.

2 Muscular Dystrophies

Muscular dystrophies are inherited myogenic disorders characterized by progressive muscle wasting and weakness of variable distribution and severity [2]. The chain "gene – protein – function – disease" underlies the pathogenesis of these diseases. Various mutations in gene encoding muscle proteins (missens mutations, nonsense mutations, codon repeats etc.,) lead to the gene structure changes which result in a protein structural and functional changes or absence of a protein. The

most affected proteins are dystrophin, dysferlin, integrins alfa-7 and alfa-9, caveolin 3, calpain 3, collagen type VI, lamins A/C, merosin, sarcoglycan, selenoprotein N1, emerin, and cholin kinase beta. Among the most prevalent—Duchenne muscular dystrophy (DMD) and dysferlinopathy (Miyoshi and LGMD2B)—are caused by mutations in *DMD* (dystrophin) and *DYSF* (dysferlin) genes, respectively [3].

DMD is a life-threatening disease, found in 1 out of 3500 boys. One out of 30,000 boys is being diagnosed with a milder form, Bekker myodystrophy, with a manifestation age of about 20 years old. DMD manifests at 2–5 years and rapidly leads to a proximal muscle weakness and a premature death due to a heart and lung muscle deficiency. Less prevalent and dramatic, though socially significant and leading to disability at an employable age, is dysferlinopathy with prevalence of 0.5–1 in 200,000 newborns. It includes a spectrum of muscle diseases characterized by two main phenotypes: Miyoshi myopathy with primarily distal weakness and limb-girdle muscular dystrophy type 2B (LGMD2B) with primarily proximal weakness. Other phenotypes are scapuloperoneal syndrome, distal myopathy with anterior tibial onset, elevated serum creatine kinase concentration only, and congenital muscular dystrophy [4].

There is no effective treatment for these diseases yet, and the only method that is able to implement etiotropic treatment is an intervention into the gene expression process which includes recombinant cDNA transfection, genomic DNA editing technologies, and mRNA editing. Most of these approaches are limited due to several reasons: small coding capacity of AAV vector (which is considered one of the safest viral vectors for clinical application)—under 4.7 kb—cannot accommodate large size of gene encoding muscle proteins. *DMD* is the largest gene found in nature, measuring 2.4 million base pairs and consisting of 79 exons. *DYSF* gene has 55 exons and 6.2 thousand base pair nucleotides in an open reading frame [5]. Thereby, RNA editing has a good potential due to the relatively small size of therapeutic molecules and has an ability to use only one vector (no need of co-transfection), and it is affecting only those cells where a protein's mRNA is being synthesized.

3 Exon Skipping

ES is a process that allows to bypass a mutated part of mRNA by antisense oligonucleotides (AONs) to restore the reading frame of a gene. As the result of ES, truncated mRNA and thereafter truncated protein are being produced, unless AONs are targeted to splicing silencers, pseudo or nonsense exons [6, 7]. Thus, ES is only effective when protein structural changes do not affect its functionality.

AONs are complex molecules targeted to particular complimentary regions of pre-mRNA. To date, a number of AONs have been developed with increased specificity and stability: 2'OMePS (2'O-methyl-phosphorothioate oligonucleotide), PMO (phosphorodiamidate morpholino oligomer), LNA (locked nucleic acid), PNA (peptide nucleic acid), tcDNA (tricyclo DNA), PPMO (peptide-conjugated PMO), Pip-PMO (PMO internalization peptide-conjugated PMO), and vPMO (vivo-morpholino). PMO and 2'OMePS studies have reached clinical trial stage, but none has been approved yet.

AONs are promising therapeutic agents for DMD as they can restore the open reading frame of mutant *DMD* gene. The structure of dystrophin allows deletion of internal parts of the protein without a significant loss of functionality and protein synthesis; therefore, exon skipping could produce partially functional dystrophin protein isoforms in skeletal muscle and convert the DMD phenotype into a milder BMD phenotype. Studies of exon skipping have shown that it is theoretically applicable in up to 83% of all DMD mutations [8]; moreover, multiexon skipping from exons 45 through 55 could rescue phenotype in up to 63% of patients with Duchenne muscular dystrophy [9].

To date, eight constructs have been studied in phases 1–3 clinical trials. They are Kyndrisa (Drisapersen) (2'OMePS targeting exon 51, phase 3, NCT01803412) by BioMarin Pharmaceutical, USA; PRO044, PRO045, PRO053 (2'OMePS targeting exon 44 (phase 2, NCT02329769), 45 (phase 2b NCT01826474), 53 (Phase 1/2 NCT01957059) respectively) by BioMarin Pharmaceutical; Eteplirsen (PMO targeting exon 51, phase 3 NCT02255552) by Sarepta Therapeutics, USA; SRP-4045, SRP-4053 (PMOs targeting exon 45 (phase 1/2, NCT02530905) and exon 53 (phase 1/2 NCT02310906) respectively) by Sarepta Therapeutics; NS-065/NCNP-01 (PMO targeting exon 53, phase 1, NCT02081625) National Center of Neurology and Psychiatry, Japan [10]. All studies have been suspended due to low efficiency of abovementioned AONs when applied in humans. Registration of applications by Kyndrisa and Eteplirsen has been rejected by the Food and Drug Administration (USA) due to the same reasons. In 2016, BioMarin has announced discontinuation of their current research on experimental drugs for DMD, but will continue to explore the development of the next generation oligonucleotides [11].Later this year FDA granted accelerated approval to Exondys 51 (eteplirsen). Under the accelerated approval provisions, the FDA is requiring Sarepta Therapeutics to conduct a clinical trial to confirm the drug's clinical benefit. The required study is designed to assess whether Exondys 51 improves motor function of DMD patients with a confirmed mutation of the dystrophin gene amenable to exon 51 skipping. If the trial fails to verify clinical benefit, the FDA may initiate proceedings to withdraw approval of the drug [12].

Despite failure of most of the clinical trials of AONs, some molecules demonstrated promising results in ongoing pre-

clinical research. According to Goyenvalle et al., a systemic delivery of tcDNA-AONs promotes a high degree of rescue of dystrophin expression in skeletal muscles, the heart, and, to a lesser extent, the brain [13]; LNA-modified AONs exhibit better mismatch discrimination and high resistance to nucleases [14]. The work of Peccate et al. demonstrated that the PPMO pre-treatment allowed efficient maintenance of AAV genomes in mdx muscles and enhanced the AAV-U7 therapy effect by ten-fold increase of the protein level after 6 months. PPMO pre-treatment was also beneficial to AAV-mediated gene therapy with transfer of micro-dystrophin cDNA into muscles [15].

Thus, ES has become a common approach in DMD treatment; however, its application in *DYSF* gene correction is limited. Unlike dystrophin, truncated and functional dysferlin has been demonstrated only in one study. Exon 32 skipping has allowed to obtain functional dysferlin in myoblasts generated from patient MyoD transduced fibroblasts [16]. New exon skipping targets are being assessed by in silico modeling [17]; however, no results have been published yet.

4 Spliceosome-Mediated RNA Trans-Splicing

RNA trans-splicing is a process in which exons originating from two distinct pre-mRNA molecules are joined, and single chimeric mRNA molecule is produced. There are currently three approaches for TS: endonuclease, ribozyme-mediated, and spliceosome-mediated trans-splicing. Spliceosome-mediated trans-splicing is the most well studied, and along with other types, it could be induced artificially.

Spliceosome-mediated RNA trans-splicing (SMaRT) allows to reprogram RNA splicing process to substitute a mutation containing exon to a normal one via pre-trans-splicing molecules (PTMs) delivered by viral vectors (usually AAV). PTMs are complex molecules consisting of three domains: binding domain, targeted to an exon of interest; splicing domain, initiating splicing process on the PTM; and a coding domain, a sequence, coding new exon. Three types of SMaRT have been described: 1) 3′ TS, the most well-studied type, occurs when 3′ splice site of PTM and a 5′ target site are linked together; 2) 5′ TS, which is opposite to 3′ TS and is less studied; and 3) internal type, when PTM has both 3′ and 5′ binding domains in its structure [1].

Pre-clinical studies have shown SMaRT to successfully correct mutations causing such diseases as hemophilia A, spinal muscular atrophy, dystrophic epidermolysis bullosa, and cystic fibrosis in vivo. [1].

DMD pre-mRNA editing has been performed by Lorain et al. in vitro by using co-transfection of PTMs along with a reporter minigene containing part of the dystrophin gene harboring the stop-codon mutation found in the mdx mouse model of DMD. Accurate and efficient trans-splicing of up to 30% of the transcripts encoded by the co-transfected minigene has been achieved.

Correction of *DYSF* gene has been described in two works. Monjaret et al. had used RNA trans-splicing molecules designed to target the 3′ end of human dysferlin pre-mRNA transcripts (in HER911 cells). Trans-splicing of pre-mRNA generated from minigene constructs and from the endogenous genes was achieved. However, in addition to corrected mRNAs, they detected undesirable PTMs spliced directly from open reading frames present on the molecule or associated with internal alternative cis-splicing [18]. Philippi et al. had successfully trans-spliced DYSF gene pre-mRNA in vitro in human LGMD2B myoblasts as well as in vivo in skeletal muscle of wild-type and Dysf(−/−) mice [19]. This was the first demonstration of Dysf protein rescue by SmaRT in vivo.

Over the recent years, there has been great progress in muscular dystrophy therapy development. Despite failure of clinical trials so far, new promising molecules for ES are being intensively studied both by academia and industry. To date, no clinical trials involving TS have been announced; therefore, its efficiency in patients is not clear and its safety needs to be assessed. Though new DNA editing technologies (CRISPR/Cas systems and NgAgo-gDNA) are considered as new approaches for gene therapy in human, RNA editing should be studied more thoroughly. RNA editing is a versatile tool for mutation corrections, which can be used independently or in combination with other gene therapeutic techniques to boost its efficiency.

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Compliance with Ethical Standards

Conflict of Interest The authors were supported by the Human Stem Cells Institute PJSC, Moscow, Russia.

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