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Genetic Therapies for Duchenne Muscular Dystrophy and Beyond

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Progressive weakness of skeletal muscle is the hallmark of muscular dystrophies. It is often accompanied by cardiomyopathy and respiratory insufficiency. It has generally been perceived as incurable diseases, while the advent of genetic therapy is changing the paradigm. Most research and achievements have been for the treatment of Duchenne muscular dystrophy, while it is promising to hope for therapies for other myopathies. Drugs for nonsense read-through and exon skipping are already approved for clinical use in Europe and the United States, respectively. Gene therapy using adeno-associated virus is in early phase of clinical trial. In this review, most promising genetic therapies will be briefly described.

Key words: Duchenne muscular dystrophy, Gene therapy, Exon skipping, Antisense oligo, Read-through

REVIEW ARTICLE

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INTRODUCTION

The initial description of muscular dystrophy dates back to the mid-19th century. It causes progressive skeletal muscle weakness, accompanied by heart failure or respiratory insufficiency depending on the types. In case of Duchenne muscular dystrophy (MIM#310200), the most frequent childhood form, the first symptom of hip girdle weakness develops before 5 years of age. The weakness aggravates slowly but relentlessly, to make the child limited to a wheelchair in late childhood or early teen. It is usual to have fatal complications from respiratory insufficiency or cardiomyopathy in their late teens or early twenties without active intervention. Use of corticosteroid and ventilator support in concert with the other supportive management have greatly improved the length and quality of life with Duchenne muscular dystrophy, but it is still perceived as an incurable disease.

However, the effort in medical research has been as unyielding as the nature of the disease. Dystrophin (DMD, MIM*300377), the causative gene of Duchenne muscular dystrophy, was cloned in 1986. It was the first pathogenic gene identified by positional cloning, which opened a new era of human molecular genetics. One after another, pathogenic genes have been characterized to differentiate each myopathy by the gene involved. Animal models were established, and the research in molecular mechanism followed. Now, two of the innovative therapies have been approved for clinical use, and many of them are under development with promising results.

READ-THROUGH OF NONSENSE MUTATION

The aminoglycosides antibiotics bind to the aminoacyl site of ribosomal RNA

and lead to misreading of the genetic code and inhibition of translation (Fig. 1). This error-prone protein synthesis by aminoglycoside brings broad-spectrum bactericidal activity. It also induces error in termination of protein elongation on stop codon, which can be exploited to promote read-through of nonsense mutation. Approximately 15% of Duchenne muscular dystrophy patients harbor nonsense point mutation.

Gentamicin has been tested if it can achieve desired read-through effect in Duchenne muscular dystrophy patients with nonsense mutation. It could successfully reduce creatine kinase level and restore dystrophin expression at sarcolemma of Duchenne muscular dystrophy patients with nonsense mutation, unlike those with frameshift mutation [1]. Nevertheless, the toxicity of aminoglycoside and need for intravenous injection discourage long-term use of gentamicin in these patients.

Ataluren is a low-molecular-weight compound screened from orally available non-toxic chemical library. It makes ribosome less sensitive to premature stop codon to facilitate read-through of nonsense mutation, though the exact mechanism of action is still unknown [2]. European Medical Agency conditionally approved ataluren for clinical use in Duchenne muscular dystrophy with nonsense mutation in 2014. A phase 3 study revealed significant benefit in Duchenne patients with moderate disability over 48 weeks with excellent safety profile [3].

Theoretically, ataluren should be effective in any disease by nonsense mutation. It is actively being tested experimentally and clinically in several diseases including dysferlin-deficient muscular dystrophy, aniridia [4], metabolic diseases, and ion

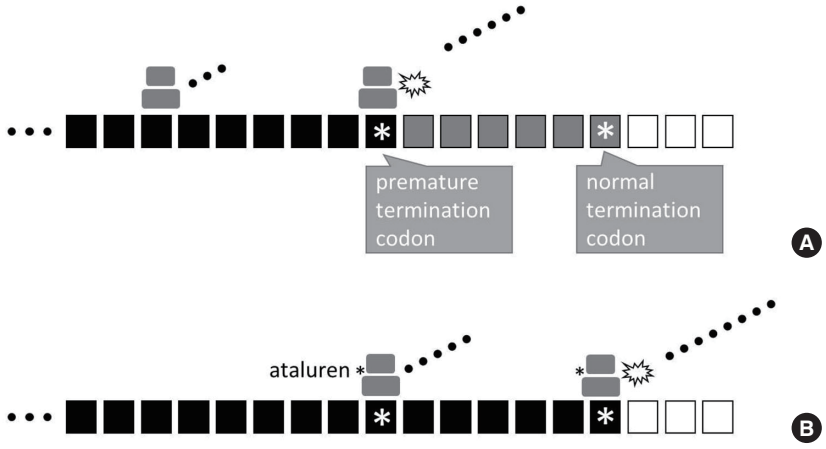

Fig. 1. Read-through of nonsense mutation. (A) Nonsense mutation is a premature termination codon that truncates the translation process of protein synthesis. Shorter than expected size of protein is produced. (B) The affects the translation machinery to ignore premature stop codon. Full-length protein is produced and released at the normal termination codon.

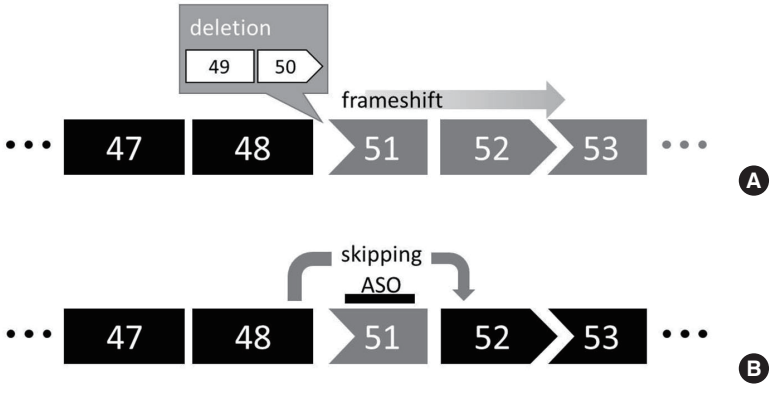

Fig. 2. Skipping of dystrophin exon 51. (A) In patients with deletion of dystrophin exon 49 & 50, shift in reading frame occurs from exon 51 and downstream. It results in the loss of functionally critical region of dystrophin. (B) Antisense oligonucleotide binds to splicing signal sequence of exon 51. Splicing machinery fails to recognize exon 51 and splices exon 48 to exon 52, skipping exon 51. This restores reading frame from exon 52 and downstream, while information of exon 51 is omitted. ASO, antisense oligonucleotide.

channel diseases. But contrary to the expectation, its effect on cystic fibrosis was not superior to the inhaled tobramycin [5].

Exon skipping

Duchenne muscular dystrophy is peculiar in that majority of cases are due to large deletions involving one or more exons. Some exons are in-frame containing multiple of 3 nucleotides; deletion of those exons causes loss of information corresponding to the deletion, more likely to cause less severe phenotype, Becker muscular dystrophy (MIM#300376). Other exons are naturally composed of nucleotides not in line with codon. Deletions of those exons disrupt reading frame in the codons of following exons, leading to classic phenotype of Duchenne muscular dystrophy.

It is possible to restore reading frame by covering another out-of-frame exon and resulting in the skipping of the exon (Fig. 2). Anti-sense oligonucleotide, actually a synthetic nucleotide analogue, is used to promote this exon skipping. The anti-sense oligonucleotide is designed to bind to a specific region of pre-mRNA critical to the splicing. Deletions of DMD gene in Duchenne patients are concentrated between exon 45 to 55. It is estimated that exon 51 skipping can be applied to approximately 13% of Duchenne patients. Another 8% of patients will be amenable to skipping of exon 53 and exon 45 respectively [6].

Eteplirsen for exon 51 skipping is approved in 2016 by the US Food and Drug Administration. Clinical trials for exon 53 skipping and for exon 45 skipping are ongoing (Table 1). As exon skipping depends on the specific exon structure, the design of antisense oligonucleotide should be customized according to the deletions. Potentially, exon skipping strategy can be applied to more than 70% of Duchenne muscular dystrophy cases with large deletion, while clinical trials for minor mutations may face difficulty in recruiting subjects as well as funding.

Antisense oligonucleotides with next-generation chemistry are under development to achieve higher expression of dystrophin, ideally also in heart. Myopathies other than Duchenne muscular dystrophy may benefit from exon skipping [7, 8], though it is not actively under clinical trial.

Gene replacement therapy

Replacement of defective gene is the most intuitive way to cure genetic disease. Gene replacement therapy holds an advantage over other strategies in that it can be effective regardless of mutation type. The history of gene therapy development proves it is not as easy as its concept. Sustained high-level expression of exogenous gene is often not without immunologic rejection.

Adeno-associated virus (AAV) is a vector closest to this goal. It was first isolated as a contaminant of adenovirus preparation. It is not pathogenic in nature and dependent on other viruses for replication. It infects non-dividing cells in high energy tissues including muscle, heart, liver, and nervous system, and mostly stay unintegrated to the genome as an episome. Recombinant AAV is devoid of any wild type viral gene except for the short strip of inverted terminal repeats. AAV is also actively under investigation for the treatment of diseases other than muscular dystrophy [9-11].

Small packaging capacity is the only downside of AAV. Coding sequence for dystrophin spans up to 11 kb, while the recombinant AAV can deliver less than 4.9 kb in a single capsid (Fig. 3). Micro-dystrophin with minimal functional component of dystrophin just fits in AAV capsid. Experiments in mice [12] and dogs [13] ensured functional improvement with long-term expression [14]. Early phases of clinical trials is ongoing to show safety and exploratory clinical efficacy. Success in gene therapy for Duchenne muscular dystrophy will accelerate clinical trials for other muscle gene therapy [15,16].

Gene editing

Clustered regularly interspaced short palindromic repeats

Table 1. Dystrophin deletions amenable to skipping of exon 51, 53, and 45

Exon 51 skip-amenable	Exon 53 skip-amenable	Exon 45 skip-amenable
3-50, 4-50, 5-50, 6-50, 9-50, 10-50, 11-50, 13-50, 14-50, 15-50, 16-50, 17-50, 19-50, 21-50, 23-50, 24-50, 25-50, 26-50, 27-50, 28-50, 29-50, 30-50, 31-50, 32-50, 33-50, 34-50, 35-50, 36-50, 37-50, 38-50, 39-50, 40-50, 41-50, 42-50, 43-50, 45-50, 47-50, 48-50, 49-50, 50-52, 52-58, 52-61, 52-63, 52-64, 52-66, 52-76, 52-77	3-52, 4-52, 5-52, 6-52, 9-52, 10-52, 11-52, 13-52, 14-52, 15-52, 16-52, 17-52, 19-5,2 21-52, 23-52, 24-52, 25-52, 26-52, 27-52, 28-52, 29-52, 30-52, 31-52, 32-52, 33-52, 34-52, 35-52, 36-52, 37-52, 38-52, 39-52, 40-52, 41-52, 42-52, 43-52, 45-52, 47-52, 48-52, 49-52, 50-52, 52, 54-58, 54-61, 54-63, 54-64, 54-66, 54-76, 54-77	7-44, 12-44, 18-44, 44-46, 46-47, 46-48, 46-49, 46-51, 46-53, 46-55, 46-57, 46-59, 46-60, 46-67, 46-69, 46-75, 46-78

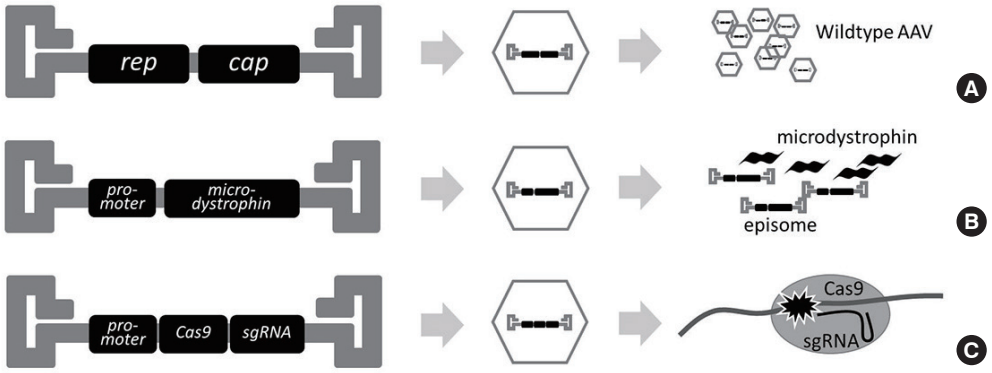

Fig. 3. AAV genome and application. (A) Wildtype AAV genome contains rep and cap protein. With the help of other virus like adenovirus, it can replicate to complete life cycle. (B) Recombinant AAV genome containing micro-dystrophin is devoid of wildtype AAV gene except for inverted terminal repeats at the ends. It can be produced without any use of wiltype virus. Once transduced into muscle cells, it sustains long-term expression of microdystrophin as episome. (C) Cas9 system can be packaged into recombinant AAV. It can be efficiently delivered into target cells with guide RNA to make a break at the target sequence (AAV, adeno-associated virus; sgRNA, guide RNA).

(CRISPR) and CRISPR-associated proteins (Cas) technology enabled revolutionized gene editing method. It makes breaks in the specific sequence of the genome guided by RNA sequence. The CRISPR-Cas system was trimmed to fit into the AAV vector, that it can be efficiently delivered to muscle. Experiments in mice with dystrophin mutation could successfully restore dystrophin expression by cutting the mutant exon out [17].

Gene editing is expected to be the most versatile tool for autosomal dominant muscle diseases [18]. However, prolonged enzymatic activity and potential off-target effects should further be investigated before it can be used in clinical trials. Base editing is a technology in an even earlier stage of development than gene editing. It can correct a missense mutation unlike gene editing cutting a few nucleotides out of the target sequence [19].

CONCLUSIONS

The enzyme replacement therapy of Pompe disease [20], approved in 2006, was the first specific therapy for muscle disease. It is now established as the standard treatment, and modifications to enhance the efficacy is under active investigation. Genetic therapies are expanding the list of treatable muscle diseases, starting from Duchenne muscular dystrophy. These therapies are harnessing cutting-edge sciences leading the medical research in inherited disorders. Other approaches specifically targeting biochemical defects are upcoming, for example, nucleoside supplementation for myopathic mitochondrial DNA depletion syndrome [21] and sialic acid supplementation for GNE myopathy [22].

Hope for new therapies for muscular dystrophy is more promising than ever. This warrants special emphasis for the patients and the doctors to stick to the standard care. It includes corticosteroid in Duchenne muscular dystrophy and the multidisciplinary support [23]. Early diagnosis and meticulous genotype-phenotype correlation is an essential step to be extended to national/international registry and patient support group, which will feed forward to promote the development of new therapies in this field.

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