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Review Article

Hunting for a cure: The therapeutic potential of gene therapy in Duchenne muscular dystrophy

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ABSTRACT

Duchenne muscular dystrophy (DMD) is an incurable disease and the search for a cure is a challenging journey. However, with recent encouraging progress, we are seeing a light at the end of a long tunnel. This review focuses on several main strategies in gene therapy, including truncated dystrophin gene transfer via viral vectors, antisense mediated exon skipping to restore the reading frame, and read-through of translation stop codons. An exon skipping agent, eteplirsen, and a termination codon read drug, ataluren, are currently the most promising therapies. With better understanding of the molecular mechanism, gene therapy has improved with regard to the key areas of gene stability, safety, and route of delivery. Consequently, it has emerged as an exciting and hopeful means for novel treatment of this devastating disease.

1. Introduction

Duchenne muscular dystrophy (DMD) is a recessive X-linked disorder caused by mutations in the dystrophin gene. It is the most common and severe type of muscular dystrophy with an estimated incidence of 1 in 3500 live newborn boys [1]. The dystrophin protein is vital for structural stability of muscle tissue; therefore, its absence results in muscle degeneration. The prognosis for this multisystemic disease is bleak, as DMD patients become dependent and wheelchair bound by their teens. Cardiomyopathy and respiratory failure usually ensue as fatal complications in the early second and third decades of life, with a mean age at death of around 19 years [2]. Although it has been described since 1880, this fatal monogenic disorder is still incurable.

DMD patients typically begin to show symptoms of clumsiness and difficulty in walking at the age of 4–5 years. The diagnosis is suspected from the clinical picture with a serum creatinine kinase >10 times the normal limit. Muscle biopsy shows almost complete or total absence of the dystrophin protein [3]. The diagnosis of this rare disease is confirmed by genetic study [4].

2. Current available therapy

The current therapies for patients with DMD are based on an attempt to improve the phenotypes of the disease. Several methods have been tried, such as maintaining calcium homeostasis with calcium channel blockers, decreasing inflammation and increasing muscle strength using corticosteroids and beta-2 adrenergic agonist, and increasing muscle progenitor proliferation. However, only treatment with corticosteroids has been found to be effective to prolong ambulation and muscle strength [5]. Corticosteroids also have the proven advantages of cost-effectiveness and convenience of administration. The issues of the best choice of steroid and the dosing regimen remain controversial [2]. Evidence from randomized controlled trials has suggested that the most beneficial treatment is with prednisolone 0.75 mg/kg/day [5]. The disadvantage of this treatment is that it does not restore function that is already lost, and hence, early commencement of corticosteroid treatment is required [6]. Furthermore, the significant long-term adverse effects of corticosteroids are also a limiting factor, as life-long treatment is needed in this chronic progressive disease [5].

Because the current available therapy for DMD merely provides intermediate symptomatic benefit, extensive efforts have been

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made since the past decade to search for treatments addressing the underlying primary monogenic genotype defect.

3. Understanding the molecular mechanism

The dystrophin gene was discovered in 1986 by a positioning cloning technique. This gene has 79 exons and 2.6 million base pairs, with an enormous size of 2.4 Mb [7]. So far, it is the largest gene known in humans and consequently is at risk of sporadic mutations, with variable phenotypes ranging from the mild Becker muscular dystrophy (BMD) to the severe DMD [8]. These mutations occur from various mechanisms; about 65% are due to deletion, approximately 20% are from duplication, and the remaining 15% are nonsense and other small mutations [9]. Deletions can occur in one or more exons of the dystrophin gene. If the remainder of the gene can still be spliced together into RNA that avoids a frameshift "nonsense" codon (in-frame deletion mutation), a milder phenotype (BMD) is usually observed. Deletion mutations that result in new neighboring exons (junction) that do not share the same reading frame show a frameshift mutation, loss of dystrophin protein, and clinically severe DMD [10].

The dystrophin protein that is encoded by the dystrophin gene is important for the connection that links and secures the cytoskeleton of a muscle fiber to the sarcolemma with the surrounding extracellular matrix. Dystrophin prevents muscle damage from mechanical stress by acting like a spring, working with other muscle proteins in the event of stretching and contraction [11]. Therefore, without its protective function, muscle fibers are prone to damage, as the process of calcium influx, inflammation and necrosis will eventually cause destruction of the muscles [12].

Dystrophin protein is located on the cytoplasmic surface of the sarcolemma, and is integrated in a protein connection known as the dystrophin glycoprotein complex (DGC) [13,14]. This protein complex consists of other membrane-associated proteins such as sarcoplasmic proteins, transmembrane proteins, and extracellular proteins, which bind to one of the protein domains of dystrophin. It provides mechanical links to the extracellular matrix that are vital for maintaining stability of the muscle membrane [15].

Dystrophin has four major domains with different functions [16]. The first domain is the N-terminal, which binds to the cytoskeleton via F-actin (filamentous actin). Many patients who lack this domain exhibit a moderate to severe BMD phenotype, although the remaining protein domains are intact [10]. The second domain is 24 spectrin-like repeats, and is a central rod domain. Most of the deletion mutations occur in this domain, but fortunately, this appears to be the least critical for dystrophin function. Deletion and duplication of this region result in mild Becker's dystrophy phenotypes, although the mutations are extensive. The third domain is the most important domain for dystrophin function. This cysteine-rich domain, which binds together with beta-dystroglycan, is a significant component of the DGC. The phenotypes of severe DMD are the consequences of lacking in this domain [10]. The fourth domain has only has a minor role in membrane integrity [7]. This C-terminal domain binds to alpha-dystrobrevin and DGC [17].

This knowledge of the dystrophin gene and protein, with the associated mutations, has provided essential understanding of the genotype-phenotype relation. In the same dystrophin gene, different mutations can result in different phenotypes. This concept is very important to strategize the therapeutic approach for DMD.

4. Gene therapy and viral vector technology

4.1. Gene therapy

Several promising strategies have been described in gene therapy for DMD. The main approach is to either replace or repair the mutated dystrophin gene or transcript. The three main approaches described here are gene transfer or replacement, antisense-mediated exon skipping and read-through stop codon [18]. Table 1 summarizes the different approaches in gene-based therapy.

4.2. Viral vectors

The success of gene transfer therapy depends on the efficiency of the gene transfer vector. The usual vector for gene transfer therapy in neurological disorders, including DMD, is a virus. Virus has been chosen instead of a synthetic vector or *ex vivo* gene transfer because of its capability to evolve and infect specific cell populations. Different types of viruses have been used as gene transfer vectors for DMD, such as herpes simplex virus, lentivirus, adenovirus and adeno-associated virus (AAV). Adenovirus was the early preferred delivery vehicle to muscle [19], but because of the limited duration of gene expression in adenovirus [20], it was later replaced by AAV. AAV is a type of parvovirus that is not associated with human disease. This small virus has a better safety profile than adenovirus since it is less immunogenic [7]. However, a single stranded genome of AAV demands a lytic helper virus for its production via replication [21]. With the advances in recombinant technology, this shortcoming has been overcome by combining these different viruses into a new recombinant virus, known as a recombinant AAV (rAAV). At present, this rAAV is the most common vector used and has been proven effective in a Phase I study [22].

The rAAV has 12 known serotypes and they have been used via different routes and targets. The most utilized serotypes for direct gene delivery to skeletal muscle, mainly for localized treatment, are rAAV-1 and rAAV-2. The gene also can be distributed systemically using the serotypes rAAV-6, rAAV-8, and rAAV-9 [23]. Long-term stable gene expression has been reported in mice, dogs, and rhesus monkeys after intramuscular rAAV injection [24]. At the same time, intravenous injection has also been proven stable for at least a 2-year duration for rAAV6 subtypes in *mdx* mice [25]. As the human

Table 1
Summary of the different approaches in gene therapy.

	Main approaches in gene therapy		
Approach	Antisense-mediated exon skipping strategy	Read-through stop-codon strategy	Gene transfer strategy
Aim	To restore the reading frame at the pre-mRNA level by modification of dystrophin mRNA splicing via AO	To ignore the premature stop-codons, allowing the production of functional protein	To replace the mutated dystrophin gene
Discussed drugs/techniques	Exon 51 skipping AO compounds (1) PMO (i.e. eteplirsen) (2) 2′-O-MeAO (i.e. drisapersen)	(1) Gentamicin (2) Ataluren (PTC124)	(1) Truncated gene (mini-genes) transfer via viral vectors (2) Trans-splicing gene strategy

AO = antisense oligonucleotide; PMO = phosphorodiamidate morpholino oligomer.