Methods xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Methods

journal homepage: www.elsevier.com/locate/ymeth

Gene therapy in monogenic congenital myopathies

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ARTICLE INFO

Article history:
Received 22 April 2015
Received in revised form 10 September 2015
Accepted 7 October 2015
Available online xxxx

Keywords:
Gene therapy
Muscle disease
Animal models
Muscular dystrophy
Myopathy

ABSTRACT

Current treatment options for patients with monogenetic congenital myopathies (MCM) ameliorate the symptoms of the disorder without resolving the underlying cause. However, gene therapies are being developed where the mutated or deficient gene target is replaced. Preclinical findings in animal models appear promising, as illustrated by gene replacement for X-linked myotubular myopathy (XLMTM) in canine and murine models. Prospective applications and approaches to gene replacement therapy, using these disorders as examples, are discussed in this review.

1. Introduction to monogenic congenital myopathies (MCM)

Current treatment options for patients with monogenetic congenital myopathies (MCM) ameliorate the symptoms of the disorder without resolving the underlying cause. However, therapies are being developed where the mutated or deficient gene target is replaced. Thousands of clinical trials have been undertaken relating to gene therapy, with around 9% focused on monogenetic diseases such as Duchenne muscular dystrophy (DMD) and limb girdle muscular dystrophy (LGMD) [1]. Preclinical findings in animal models have been promising, as illustrated by studies of a potential treatment for X-linked myotubular myopathy (XLMTM) in canine and murine models [2]. We will therefore discuss the prospective applications and approaches of gene replacement therapy, using these disorders as examples.

Both limb girdle muscular dystrophy type 2C and Duchenne muscular dystrophy are part of a subclass of myopathies known as dystrophies, diseases where muscle degeneration is accompanied by replacement with fatty or connective tissue. DMD is caused by X chromosome linked genetic mutations leading to the absence of membrane-anchored dystrophin protein, the centerpiece of the large dystroglycan complex that plays a pivotal role in sarcolemma stability during muscle contraction [3] (Table 1). The symptoms are visible as early as 2–3 years of age, a progressive decrease in striated muscle function, starting from proximal muscle such as legs and pelvis and eventually involve the whole body. Most patients are wheelchair-dependent starting from early teen. The average life expectancy is around 25 years (<http://www.nlm.nih.gov/medlineplus/ency/article/000705.htm>), with respiratory failure and cardiac complications the highest causes of mortality.

Congenital centronuclear myopathies are inherited muscle diseases where the nucleus is located in the center of the muscle fiber instead of the periphery. X-linked myotubular myopathy (XLMTM) is the most common centronuclear myopathy, affecting an estimated 1 in 50,000 male births (Table 1) [4,5]. The disease is due to a mutation on the long-arm of the X chromosome, usually inherited by hemizygous boys from an asymptomatic carrier mother [6]. This mutation causes a deficiency of the protein myotubularin [7]. Myotubularin has been identified as a phosphoinositol phosphatase and may be critical to normal excitation–contraction coupling and remodeling of the sarcoplasmic reticulum in muscle [8]. When XLMTM patients are first born, they typically exhibit hypotonia and may be blue due to respiratory insufficiency [6]. The disease is often fatal in the first year of life and long-term survivors may require ventilatory support [9]. Affected boys are particularly susceptible to infection and respiratory dysfunction is the leading cause of death [10].

Although there are differences between the symptomatic presentations of these diseases, there are some shared difficulties to

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http://dx.doi.org/10.1016/j.ymeth.2015.10.004
1046-2023/

Table 1
A comparison of some of the monogenic congenital myopathies presently under study.

	Duchenne muscular dystrophy (DMD)	X-linked myotubular myopathy (XLMTM)	Facioscapulohumeral muscular dystrophy (FSHD)	Myotonic dystrophy (DM)	Limb-girdle muscular dystrophy (LGMD) 2C and 2D
Inheritance	Single gene mutation on the X chromosome	Single gene mutation at q28 on the X chromosome [17]	Autosomal dominant, contraction of D4Z4 repeat on chromosome 4q35 and toxic gain of function of the DUX4 gene [18]	Autosomal dominant. DM1 CTG triplet repeats expansion of DMPK gene locates on chromosome 19 [19]. DM2 CCTG tetranucleiotide repeat expansion of ZNF9 gene on chromosome 3 [20]	Autosomal recessive. Single gene mutation on chromosome 13 and 17 (2C and 2D, respectively)
Molecular biology	Deficiency of the protein dystrophin	Deficiency of the phosphatase myotubularin [17]	Toxic protein product of DUX4 [18]	Malfunctioned DMPK and ZNF9 proteins	Deficiency of gamma and alpha sarcoglycan (2C and 2D, respectively)
Clinical symptoms	Weakness of the skeletal muscles; respiratory insufficiency in teens; cardiac dysfunction; leading cause of death is cardiorespiratory failure	Weakness of the skeletal muscles [10], wheelchair dependence [10], respiratory insufficiency at birth [21]; no cardiac phenotype; leading cause of death is respiratory dysfunction [22]	Initial weakness of facial, scapula and humeral muscle, progressively involving other muscles; sparing respiratory muscle	Muscle wasting and myotonic; heart conduction block; cataract; infertility	Muscle wasting primarily involve proximal muscle such as hip and shoulder
Demographics	Presentation around 2–3 years of age; average life expectancy of 25 years	Affects 1 in 5000 live male births [4]; presentation typically at birth [23]; average life expectancy of 29 months [9]	Affects 12/100,000 [24]	Affects 1/8000 people worldwide. Type 1 most common in most countries [25]	Up to 68% of individuals with childhood onset and ~10% with adult onset [26]
Histology	Increased fiber size variability; cycling of fiber regeneration and degeneration	Centrally located nucleus[27]; variably-sized myofibers with an abnormally large number of small fibers; organelle abnormality and "necklace fibers" [28]	Non-specific fiber necrosis, increased variation in fiber size, internal nuclei, fiber type variability, connective tissue and fat proliferation. Mononuclear cell filtration [29]	Fiber atrophy, internal nuclei, pyknotic nuclear clumps, lipofuscin accumulation, increased fiber size variation [30]	Variation of fiber diameter, fiber degeneration and regeneration, split fibers, ring fibers

consider when design gene therapies. High vector titers may be required to reach an effective dose [11], increasing the chance of adverse effects in patients. In addition, the need to treat respiratory muscles as well as the heart in DMD and XLMTM may complicate delivery. Improvements in delivery methods [12] and in vector characterization to increase efficiency may address this problem [13]. Vector modification may also ensure more efficient delivery to the muscle and improve safety by reducing off-target delivery to organs like the liver [11,14]. Tissue-specific promoters is another strategy to secure tissue-specific transgene expression. Immune response is a major concern, particularly in genetically-null patients who may have antibodies against the gene product produced by the treatment [15] and immunosuppression before and during treatment may have to be considered. There are also challenges specific to each disease. For example, the large size of the dystrophin gene limits the choice of vector to be used in treatment. Overexpression of γ-sarcoglycan in LGMD patients may exacerbate the condition [16]. Significant wasting in XLMTM patients leaves very little muscle to treat and, due to the young age of the patients, selecting an appropriate and reproducible outcome measure may prove difficult. We will be discussing new developments that address these concerns, including modifications of the vector and the combination of gene therapy with other approaches.

2. Gene therapy

2.1. What is gene therapy

Gene therapy is defined as the introduction of nucleic acids, including DNA, RNA and their analogs into cells of living organism to treat diseases [31]. This occurs through modified expression of genes of interest to trigger alterations of certain biological functions. Gene therapy targets living cells, primarily because cell's intrinsic gene expression machinery is indispensible to mediate the production of therapeutic molecules, including protein, shRNA and microRNA.

Since classical gene therapy acts on native tissues, the abundance of target cells largely determines the effect of gene therapy. This is especially true in congenital myopathies. In the advanced stage of diseases, such as DMD and XLMTM, surviving myocytes are so limited that even if the function of individual myofibers were fully restored, there would be no appreciable functional improvement on tissue level. The advent of stem cell technology, especially the discovery of induced pluripotent stem cells (iPSCs) [32,33], has the potential to overcome this hurdle. Pluripotent stem cells may be able to replenish tissue loss through their indefinite self-replicating potential and capacity to be converted into nearly all cell types within the body. The advantages of combining stem cell therapy with gene therapy have been demonstrated in several animal studies, in which vectors were administered *ex vivo* and modified donor cells were later engrafted into native tissue [34–36].

Various gene therapy strategies target gene expression and regulatory network at different levels. For example, genetic sequence can be permanently inserted into genome for long-term expression, using retrovirus or lentivirus [31]. With the development of genome modification tools [37] such as clustered regularly interspersed short palindromic repeats (CRISPR) enzymes and Transcription activator-like effector nuclease (TALEN), the technical barrier of *in situ* editing eukaryotic genomic DNA has been substantially lowered. These techniques hold the potential to seam-

Please cite this article in press as: X. Guan et al., Methods (2015), http://dx.doi.org/10.1016/j.ymeth.2015.10.004