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Could gene therapy be the future for muscular dystrophy?

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Muscular dystrophy (MD) represents a group of hereditary disorders characterized by muscle weakness and wasting. The degree of disability depends upon the type of MD, and severe forms can lead to loss of ambulation, respiratory problems and death. Some genes linked to MD have been known to encode structural proteins in the muscle membrane-associated complex [1,2]. For example, the most common types of MD, Duchenne and Becker MD, are caused by mutations in the dystrophin gene that lead to either absence (Duchenne) or reduction (Becker) of the protein product [3,4]. Although the genetic causes for many types of MD have been identified, there is currently no cure for MD. Patients typically rely on physical therapy and oral corticosteroids for symptom management [5].

Recent research progress has offered great hope for cell and gene therapies. Since many forms of MD are monogenic disorders, gene replacement is an attractive approach. A wide array of strategies have been developed to deliver dystrophin or other affected genes to muscles, many of which have been highly promising in preclinical studies. In particular, the first report of viral vector-mediated gene delivery for MD recently demonstrated successful transgene expression in human muscle for up to 3 months [6].

Recombinant adeno-associated virus (AAV) has emerged as the most promising gene delivery vector owing to its high tropism for skeletal muscle and low immunogenicity. AAV is a replication-defective parvovirus that can be produced relatively efficiently. Although most adults have been infected with AAV, no pathogenicity has been associated with this virus, making it an ideal vector for gene transfer.

Despite the small packaging capacity of the AAV vector (<5 kb), dystrophin, the largest gene in the human genome (2600 kb) [4], has been modified into 'mini' and 'micro' forms and

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packaged [7,8]. These smaller versions of dystrophin retain their binding domains with actin in the cytoskeleton and dystroglycan at the cell membrane, but lack most of the central rod domain. The shortened dystrophin is thought to preserve critical regions for signaling and structural support. It is unclear whether these small forms of dystrophin could provide complete rescue in humans, but preclinical studies in the *mdx* mouse model of Duchenne MD have shown that mini- and micro-dystrophins can reverse the dystrophic phenotype [8,9].

One challenge for clinical translation is to deliver the vector to multiple muscle groups and potentially the affected cardiac muscle. Several serotypes of AAV (i.e., AAV6, AAV8 and AAV9) have shown considerable preclinical success in targeting muscle after vascular delivery in the *mdx* mouse and dystrophic canine [10–12]. Importantly, AAV9 appears to be particularly efficient in transducing cardiac tissue, which is of high interest considering the prevalence of cardiomyopathy in MD [13,14].

It seems likely that regional vascular delivery will be the first step toward systemic vector delivery. Isolating the circulation for as little as 10 min through balloon catheters or uniquely placed tourniquets on the extremity can target one to two critical muscle groups. This approach has recently been demonstrated to safely and efficiently transduce the limb muscle of the rhesus macaque, which has high anatomical similarity to humans [15]. Although AAV has not yet been administered systemically in humans, regional vascular delivery for muscle transduction is on the close horizon. Safety in regional vascular delivery will open the door for systemic approaches.

Another critical issue in gene therapy is evasion of the adaptive and innate immune systems. The primary concern is the role of the adaptive immune system in prohibiting delivery of the virus to the targeted cells. Since humans are a natural host for AAV, much of the population possess binding antibodies to AAV [16,17]. It is still unclear whether the majority of humans have a level of circulating neutralizing antibodies high enough to significantly affect viral gene delivery. However, by prescreening patients for neutralizing viral antibodies or using rare viral serotypes to deliver genes, this issue can potentially be circumvented.

A more unexpected observation has been the cytotoxic T-cell response to the AAV capsid peptides following vector administration. In a 2006 clinical trial for hemophilia, AAV expressing factor IX was delivered to the liver, but only resulted in transient gene expression [18]. The reduction in gene expression was attributed to T cells forming a cytotoxic response to AAV capsid peptides presented on the surface of transduced liver cells. This observation prompted discussions regarding the potential use of immunosuppressants in patients at least until after capsid peptides are fully cleared from transduced cells [16,19]. Interestingly, this damaging cytotoxic T-cell response has not been seen in all clinical trials where AAV has been administered. A recent clinical trial for limb-girdle MD type 2D successfully delivered the missing α -sarcoglycan protein to muscle and showed continued gene expression after 3 months [6]. Out of three subjects, only one displayed a minimal cytotoxic T-cell response to AAV capsid peptides, which did not preclude gene expression. Differences between these two trials include the AAV serotype, route of administration and the targeted tissue. Additional studies will be required to determine whether immunosuppression is needed to avoid a T-cell-mediated response.

In addition to the generation of AAV capsid immunogenesis, there may also be an immune response mounted to the AAV-expressed therapeutic protein. In the case of Duchenne dystrophy, the dystrophin peptides expressed by gene transfer could be seen as a foreign antigen. Scientists had anticipated that the revertant fibers found in many patients would be protective. These revertant fibers result from spontaneous second-site mutations that skip exons and restore the open reading frame, producing dystrophin. It has been the hope that dystrophin

expression on revertant fibers would generate tolerance (i.e., recognition as ‘self’ and not ‘foreign’). Instead, recent findings in a Duchenne MD gene therapy clinical trial have revealed that novel dystrophin epitopes on revertants are immunogenic rather than protective, threatening newly expressed microdystrophin. An advantage of translational therapy is that these immunogenic epitopes on revertant fibers can be assessed prior to gene transfer. Their presence favors alternate approaches, including gene delivery of utrophin, an endogenous homolog of dystrophin [20].

While AAV has been the most promising gene therapy vector for MD, other considerations influence its use depending on the target tissue and specific goals to be achieved. AAV vectors infect both dividing and nondividing cells and express the inserted transgene DNA as an episome [21]. However, the episomal transgene will eventually be diluted out in dividing cells, making AAV less desirable for rapidly dividing cell populations. Overall, it is advantageous that AAV does not integrate into the host DNA since integrating retroviral vectors can present a significant risk for cancerous transformation related to insertional mutagenesis [22,23]. Fortunately for MD gene therapy, integration does not appear to be a required goal because muscle fibers are terminally differentiated, nondividing cells. Concerns have been raised that in cases of Duchenne MD where there are prolific cycles of muscle fiber degeneration and regeneration, the AAV episome could be in jeopardy. Nevertheless, it is worth emphasizing that robust and stable AAV gene expression has been a reproducible finding in *mdx* mouse studies for well over a year [24], even in the face of pronounced muscle regeneration and degeneration. Should this issue become a concern for human gene therapy, it might be necessary to reconsider viral vectors that integrate into the genome, such as lentivirus. A lentiviral vector carrying microdystrophin has been demonstrated to infect muscle stem cells and muscle fibers after intramuscular injection in the *mdx* mouse [25]. The transduced muscle stem cells were able to differentiate into new muscle fibers expressing microdystrophin. Although lentivirus is less efficient than AAV in transducing muscle, a scenario could be envisioned where AAV is utilized to transduce existing muscle fibers while muscle stem cells are infected by lentivirus either *in vivo* or *ex vivo*.

The genetic cause of some forms of MD, such as facioscapulohumeral MD, is still unclear. One gene replacement strategy for such a muscle wasting disease is to develop approaches to increase muscle mass and strength. Disruption of the myostatin signaling pathway has been shown to significantly enlarge muscle mass [26]. Myostatin is a member of the TGF- β superfamily and is a negative regulator of muscle growth [27]. A wide variety of both pharmacological and gene therapy approaches have been developed to inhibit myostatin [28–30]. We recently demonstrated that AAV delivery of a myostatin antagonist protein, follistatin, increases muscle mass and strength in the *mdx* mouse and in the cynomolgus macaque [24, 31]. Significantly, AAV expressing follistatin proved to be safe and effective in the nonhuman primate, setting the stage for clinical translation for treating muscle diseases.

Adeno-associated virus-mediated gene therapy is presently at the forefront of promising treatments for MD. Certainly, the development of gene therapy for these diseases has not been without challenges. Translational scientists have used creativity and persistence to develop novel approaches, such as truncated dystrophin isoforms and optimized viral vectors for gene delivery. This is further exemplified in the efforts to achieve success in the human gene transfer trial for limb-girdle MD type 2D [6]. Collectively, these achievements provide the impetus to move forward, optimizing regional and systemic delivery approaches creating the potential to change the quality of life for patients with these devastating muscle disorders.

Biographies

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