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Selected issues of the Duchenne Muscular Dystrophy gene and cell therapy

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Abstract
Muscular dystrophies are a highly heterogeneous group of inherited disorders characterized by progressive skeletal muscle weakness and necrosis of the muscle tissue. Currently there is no specific treatment for any of the forms of muscular dystrophy. Most of the gene and cell therapy studies in this field have focused on Duchenne Muscular Dystrophy (DMD), one of the most frequent and fatal neuro-muscular diseases. This short review highlights the key points in these field, as well as main expectations and pitfalls concerning the gene therapy of DMD.

Keywords: Duchenne, DMD, gene therapy, HAC

Introduction

Muscular dystrophies are a highly heterogeneous group of inherited disorders characterized by progressive skeletal muscle weakness and necrosis of the muscle tissue. Currently, more than 20 different monogenous disorders, inherited in autosomal recessive, autosomal dominant, and X-linked ways, are referred to as muscular dystrophies. Their incidence varies from 1 in 3000 boys for Duchenne Muscular Dystrophy (DMD) to 1 in 200 000 for LGMD2B [9, Jain Foundation Data]. The onset of the inherited muscular dystrophies, as well as their natural history also can be very different. They can start in early childhood and rapidly develop, causing death in the early twenties as happens in DMD [1], or have an average onset in the late twenties, and mildly progress, not affecting the average lifespan, as for most LGMDs. This group of diseases is easily recognized, however differential diagnosis poses some issues for a physician, as in most cases it should be based on molecular and DNA sequence data. There is no specific treatment for any of the forms of muscular dystrophy. Physiotherapy and aerobic exercises may help to prevent contractures and maintain muscle tone, orthoses may be needed to improve quality of life in some cases, but no actual long-term therapy has been found [8]. Currently the two most promising approaches to treat muscular dystrophies in general, and DMD in particular, are gene and cell therapy or their combination. This short review highlights the key points in these fields, as well as main expectations and pitfalls in gene therapy of Duchenne myodystrophy.

Gene therapy for DMD

Muscular dystrophies, like all inherited monogenous diseases, are caused by mutations in various genes, and, thus, gene therapy is a very promising approach to their treatment. Most of the gene therapy studies in this field have been focused on Duchenne Muscular Dystrophy, one of the most widespread and fatal neuro-muscular diseases. Therefore, this particular condition is a very good model, which should help to elucidate the main issues, hopes, and pitfalls in the field.

DMD is caused by a vast spectrum of mutations, mainly the large-span deletions of the exons in the 2.3Mb dystrophin (*DMD*) gene [6,13]. The dystrophin mRNA spans nearly 1000kb and is primarily translated in the muscle tissue, where the protein of the same name plays a major role in muscle fiber contraction and maintenance. Mutations in the dystrophin gene impair normal protein synthesis, causing the consequent progressive myofiber necrosis resulting in progressive muscle weakness. Symptoms usually appear in male children before age 5 and may be visible in early infancy. Progressive proximal muscle weakness of the legs and pelvis associated with a loss of muscle mass is observed first. Eventually this weakness spreads to the arms, neck, and other areas.

Given this, the ideal vector for DMD gene therapy should be able to carry a 1000 kb of DNA and easily penetrate muscle fibers, and then effectively express functional dystrophin for an unlimited period of time. Moreover, it ought to be safe (non-mutagenic or immunogenic) and its expression should

be easily regulated. But for the carried DNA size, these requirements could be applied to the ideal vector to treat any muscular dystrophy. In this connection, let's try to evaluate the most promising vector types, taking into account the proposed criteria of safety and efficiency.

Currently all gene therapy delivery systems can be divided into two large groups: viral and non-viral. Viral vectors are the most commonly used: during the period from 1989 to 2010 they were employed in more than 60% of gene therapy clinical trials (Wiley Interscience Gene Therapy Trials Worldwide Database; Edelstein et al., 2007). Their major advantages are high effectiveness and specificity of transgene delivery.

Amongst the viral vectors, only lentiviruses and adeno-associated viruses (AAV) can be effectively used for muscular dystrophy's gene therapy, as only they are able to efficiently penetrate the myofibers and express the transgene there. The use of lentiviruses, despite their high transgene expression efficiency and relative safety, is connected with side effects that are too serious for them to be put into clinical practice. For instance, patients who underwent such gene therapy demonstrated a HIV-positive profile [3].

The AAVs are the safest viral vectors due to their low immunogenicity, the absence of the insertional mutagenesis risk and other side effects. In addition, they are one of the most effective: stable transgene expression in primates could be detected even 18 months after injection [10]. The major issue in the use of this vector type for DMD gene therapy is the very small size of the therapeutic cassette — only 4 kb [15]. However, this has been solved by the use of a truncated version of the gene ("mini-dystrophin") instead of the complete one. This approach efficiently helped to ameliorate the disease, but not to cure, however it successfully passed through the Phase I clinical trial [5]. Unfortunately, is inapplicable for most of the muscular dystrophies, as not all the causative genes have "mini" versions.

Thus, none of the viral vectors, despite their efficiency and safety, fit the ideal criteria, for different reasons: the small size of the therapeutic cassette in the case of AAV, serious side effects in lentiviruses, and inefficiency of the other extensively studied viral vector types.

Non-viral vectors, such as plasmids and human artificial chromosomes (HACs), do have advantages over the viral ones, because of their safety and theoretically unlimited size of the therapeutic cassette. However, the efficiency of the plasmid-mediated transgene delivery is rather poor as well as the time of its expression [for instance, see 16], especially when it is delivered systemically.

HACs on the other hand are able to maintain expression of genomic-sized transgenes within target cells for an unlimited time. What's more, they do not need to integrate into the host genome to maintain the transgene expression, and the latter one's level is regulated by the intracellular mechanisms. In spite of the fact that their construction and delivery pose certain technical challenges, the recent advances in this field are very promising [4]. Moreover, the first successful results of the preclinical trial of the cell-based therapy exploiting HACs bearing DMD in mice have recently been published by Tedesco et al., 2011. The development of this strategy to treat other muscular dystrophies is a way to find the cure for this group of diseases.

So, non-viral vectors, and HACs in particular, represent the most promising direction in the DMD treatment research.

Cell therapy for muscular dystrophies

The main issue in developing an effective therapy for muscular dystrophies is that the muscle tissue is a non-dividing one, and its reparation is a very complicated and relatively rarely studied process. Even if one manages to find an effective and safe way to transfer genes to the muscle fiber and to obtain a stable transgene expression in the target cell, the results of such gene therapy aren't very promising. For instance, the presence of the transgenic dystrophin in the myofibrils will ameliorate the DMD and will prevent the disease progress, but will not help to restore them. Therefore such therapy only makes sense in the early stages of the disease.

The most effective way to solve this issue is to combine gene and cell therapy by introducing genetically modified stem cells, so that the restored myofibrils effectively express the transgene to prevent their damage and necrosis. However, not many types of stem cells fit the criteria necessary for the stem cell therapy of muscular dystrophies. Such cells should effectively fuse with the myofibers, they should be easily obtained from the patient's tissue and then be easily expanded in vitro, and finally they must be systemically deliverable. For instance, mesoangioblasts, currently one of the most promising cell types to treat DMD are easily cultivated, effectively fuse with the myofibers and can be delivered by intravenous infusion, but can only be harvested in the very early stages of perinatal development [11]. They could be obtained from an allogenic donor, but this poses the question of their availability, as well as ethical issues. Other populations of myogenic stem cells, such as CD133+ satellite cells, are very hard to obtain, and the use of embryonic stem cells and iPS cells raises ethical and safety issues respectively [7].

However there are two groups of the myogenic stem cells which partially suit all the criteria mentioned — these are CD133+ hematopoietic (HSC) and mesenchymal stem cells (MSC), which can be easily obtained from a patient, expanded in vitro, and have been shown to contribute to muscle regeneration [2,14]. Currently, the therapeutic potential of these two stem cells populations are being extensively studied.

Modification of these cell types with HACs bearing the necessary gene and their consequent transplantation to the patient via IV injection could be a very promising strategy to treat first DMD, and then muscular dystrophies in general. Thus, the use of HAC-modified autologous CD133+ HSC or MHC opens new perspectives for the treatment of the muscular dystrophies. However, much research must be done to bring this technology from paper into clinical practice.

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