Medical Hypotheses 110 (2018) 97–100

Contents lists available at ScienceDirect

Medical Hypotheses

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

Effective regeneration of dystrophic muscle using autologous iPSC-derived progenitors with CRISPR-Cas9 mediated precise correction

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ARTICLE INFO

Keywords:
CRISPR-Cas9
Precise correction
DMD
Myogenic progenitor cells
Myogenesis

ABSTRACT

Duchenne muscular dystrophy (DMD) is a lethal muscle wasting disease caused by a lack of dystrophin, which eventually leads to apoptosis of muscle cells and impaired muscle contractility. Clustered Regularly Interspaced Short Palindromic Repeats/CRISPR associated protein 9 (CRISPR/Cas9) gene editing of induced pluripotent stem cells (IPSC) offers the potential to correct the DMD gene defect and create healthy IPSC for autologous cell transplantation without causing immune activation. However, IPSC carry a risk of tumor formation, which can potentially be mitigated by differentiation of IPSC into myogenic progenitor cells (MPC). We hypothesize that precise genetic editing in IPSC using CRISPR-Cas9 technology, coupled with MPC differentiation and autologous transplantation, can lead to safe and effective muscle repair. With future research, our hypothesis may provide an optimal autologous stem cell-based approach to treat the dystrophic pathology and improve the quality of life for patients with DMD.

Introduction

Duchenne Muscular Dystrophy (DMD) is a genetic disorder which is characterized by the absence of dystrophin, a cytoskeletal structural protein. This disease predominately affects males, as the mutations in the gene which cause this condition are located on the X chromosome [1–3]. Most patients are diagnosed between the ages of 2 and 5, ambulation is commonly lost in the teen years, and death is usually caused by cardiac or respiratory failure in the third decade [4]. Lacking the dystrophin protein is problematic for a number of reasons, the most obvious being dystrophin's role in muscle stabilization. Dystrophin is responsible for protecting the muscle sarcolemma from contraction-induced damages. Without it, the dystroglycan complex, which resides at the cellular surface, is not able to join with the cytoskeleton of myocytes. This inability to link the dystroglycan complex and the cytoskeleton leads to muscular destabilization, and eventually weakness and myopathy [1,5].

In the early stages of DMD, the damage caused by the absence of dystrophin is counteracted to a certain degree by stem cells in muscle which facilitate repair of the damaged tissue. However, as the disease progresses and the patient ages, this stem cell-mediated repair is insufficient, leading to recruitment of macrophages into the muscle, inflammation, and fibro-fatty replacement of muscle fibers [6–8]. The associated muscle wasting poses major therapeutic challenges, as delivering therapeutic agents into fibrotic, inflamed tissues is difficult and considered a large obstacle in developing curative treatments for DMD [8].

The DMD gene is one of the largest in the human genome, consisting of 79 exons, and one of the more commonly mutated genes [9]. DMD can be caused by in-frame, out-of-frame, and frameshift mutations, with frameshift being the most common. Each of these mutations results in the deletion of exons in the DMD gene, which either generates a premature stop codon or disrupts the reading frame [10–12]. Approximately 60% of these mutations occur between exons 45–55 of the DMD gene, making this section of the gene an ideal target for gene therapy [12,13]. In-frame deletions between exons 45–55 typically trigger a milder form of dystrophy known as Becker Muscular Dystrophy, which allows patients to live much longer, less affected lives [12]. In-frame deletions between exons 45–46 can result in severe DMD; however these mutations are uncommon [14]. Intragenic mutations affecting more than one exon are typical in at least 60% of DMD patients [15].

Hypothesis

Using autologous iPSC-derived myogenic progenitor cells, in which the dystrophin gene is precisely corrected by CRISPR/Cas9 technology, will efficiently and safely regenerate muscles in patients with Duchenne muscular dystrophy (Fig. 1).

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https://doi.org/10.1016/j.mehy.2017.11.009
Received 22 October 2017; Accepted 22 November 2017

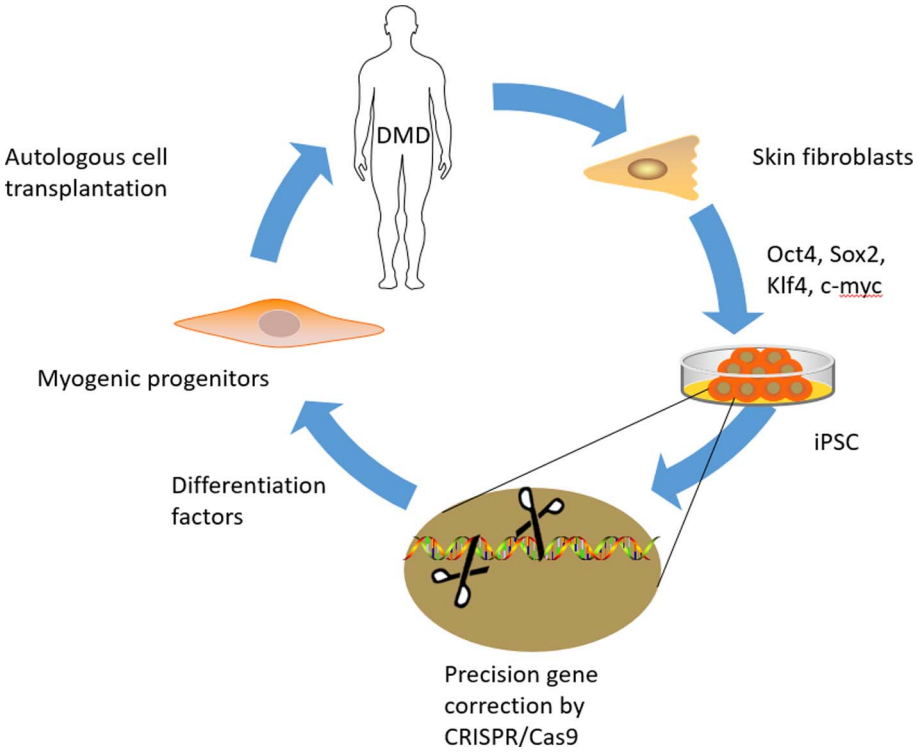

Fig. 1. Schematic of repairing muscular dystrophy by combination of autologous iPSC-derived myogenic progenitor cell transplantation with CRISPR/Cas9-mediated precise gene correction.

Basis for hypothesis

As mentioned above, DMD is a genetic disorder involving the mutation of one or more exons in the DMD gene. The use of IPSCs edited by CRISPR/Cas9 technology has been found to be an appropriate and effective method for gene editing [1]. Long et al., reported use of CRISPR/Cas9 technology *in vivo* led to the repair of damaged muscular tissue and elimination of potential future consequences [1]. The use of autologous IPSCs is an attractive option considering the potentially unlimited availability of these cells [9]. Li HL et al., reported some success correcting the dystrophin gene in DMD patient derived iPSC by CRISPR-Cas9, and can detect full-length dystrophin protein after differentiating iPSC to skeletal muscle cells [16]. Young et al. have conducted imprecise use of CRISPR/Cas9 in the treatment of DMD, in which one single pair of guide RNAs can restore dystrophin expression in 60% of DMD patients [17,12].

Current therapies to treat DMD

The current approach to DMD involves a combination of glucocorticoids and physical/occupational therapy to maintain strength and flexibility for as long as possible. While these treatments are quite effective at improving symptoms, a large number of undesirable side effects are associated with the chronic use of glucocorticoids [18,19]. Many alternative remedies have been proposed, both to manage and potentially cure the disease. These alternatives include, but are not limited to gene replacement therapies; manipulation of inflammatory cascades; compensatory protein expression; collagen producing cells; activation/inhibition of key growth factors; and manipulation of mRNAs [8,19–21]. The most prevalent method currently under investigation is gene editing therapy.

The majority of treatments aimed at managing symptoms and improving quality of life target the chronic inflammation which is a major causative factor for DMD complications. The IL-1β pathway is thought to be the initiator of muscular degradation in DMD. As such, a variety of therapies have been developed to target this pathway [22]. The two most commonly used steroids to reduce inflammation in DMD patients are deflazacort and prednisone; both medications are used chronically and carry a substantial number of side effects. The most common effects are weight gain, stunted growth, and increased risk of respiratory infection, with the latter being the most serious. One study found deflazacort to be safer than prednisone [23].

Treatment with taurine has recently been shown to be effective in a mouse model of DMD; the therapy prevented both necrosis and inflammation in juvenile mice [7]. Inhibition of PKCθ inhibits both inflammation and muscle atrophy through modulation of immune cell responses. Pharmacological intervention with C20, a PKCθ inhibitor, has proven efficacious at reducing contraction-based injuries and increasing muscular function [19]. Another common target for anti-inflammatory medications is the transcription factor NF-kβ. Flavocoxid, which targets NF-kβ, is currently undergoing testing in phase 1 clinical trials [24].

Follistatin, a therapy developed for management of symptoms rather than to provide curative treatment, activates the Akt/mTOR/S6K signaling pathway, leading to an increase in strength and muscular mass [25]. Follistatin has proven effective in increasing distance in the 6-min walk test of patients suffering from Becker muscular dystrophy; it is currently under clinical trial for use with DMD patients as well [26,27]. Resveratrol, a natural compound which induces antioxidant effects, decreases oxidative damage and reduces fibrosis and was found to have cardioprotective functions in mouse models [28]. Another drug that targets fibrosis and oxidative stress is idebenone, which has shown efficacy in DMD [29]. Therapies targeting fibrosis are by no means limited to the previously mentioned drugs; crenolanib, simvastatin, flavocoid, and others not listed here have also been tested experimentally. Most of these agents have antioxidant properties, and nearly all target the TGF-β pathway [8,29–31].

Drugs such as eteplirsen and ataluren are undergoing various phases of clinical trials; they have been designed to skip exon 51 and disrupt premature nonsense mutations, respectively [9]. Eteplirsen was found to increase dystrophin expression in muscle fibers by 23% compared to the control groups, and it is currently undergoing FDA review [32]. One major downfall of eteplirsen, however, is the cost, which is estimated at $300,000–$400,000 per year [33]. The administration of tricyclo-DNA