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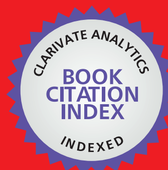

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Chapter 14

Novel Therapeutic Approaches for Skeletal Muscle Dystrophies

Emanuele Berardi and Maurilio Sampaolesi

Additional information is available at the end of the chapter

Abstract

Muscular dystrophies (MDs) are inherited diseases that affect skeletal and cardiac muscle tissues. Cases range from mild to very severe, resulting in respiratory or cardiac failures. No cures are available for MDs and corticosteroid treatments, mainly deflazacort and prednisolone, only help to control the inflammatory process and slightly delay the progression of the disease. This is due to the beneficial effect on pulmonary function and scoliosis. Walkers and wheelchairs are used to strengthen patients' independence and walking ability. When respiratory and/or cardiac muscles become weak, mechanical ventilation is mandatory. In addition, hypertension, cataracts, excessive weight gain and vertebral fracture are often serious side effects of deflazacort and prednisolone treatments.

This chapter deals with the advanced therapies used to treat muscle degenerations, ranging from pharmacological to gene and/or cell treatments. We review previous trials that use cell delivery protocols in mice and patients. Here, donor satellite cells and myogenic progenitors are isolated from the bone marrow. We then proceed to describe the recently identified stem/progenitor cells in relation to their ability to exist within a dystrophic muscle and to differentiate into skeletal muscle cells. In this perspective, different known features of various stem cells are compared including mesoangioblasts and mesoderm-derived stem cells, which are associated with the pericyte compartment.

This chapter also provides an outline of the latest techniques used for the isolation/generation and characterization of pluripotent and adult stem cells. We focus on their myogenic differentiation potential and the different strategies used for genetic manipulation including TALEN and CRISPR genome editing. We also explore the use of microRNAs as biological markers or as possible therapeutic targets to improve myogenic commitment of pluripotent and adult stem cells. Finally, based on the rapid advance in stem cell technology, we discuss a prediction of clinical translation for novel cell therapy protocols.

Keywords: Skeletal muscle regeneration, gene and cell therapy, TALEN and CRISPR genome editing, microRNAs

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1. Introduction

Muscular dystrophies (MDs) are genetic diseases caused by the continuous degeneration/ regeneration cycles of skeletal muscle tissue. Mutations in genes encoding for proteins, either at the plasma membrane or within internal membrane, are responsible for MDs. During contractions, the affected muscle fibres degenerate and the molecular mechanisms are not yet fully understood. Fibre loss is compensated by the regeneration of new fibres, mainly sustained by satellite cells. These are localized underneath the basal lamina of muscle fibres [1]. Damaged dystrophic muscles engage in a remodelling process to generate novel fibres and to produce abundant extracellular matrix (ECM). ECM is necessary for adequate tissue repair. During periods of degeneration/regeneration, myofibroblasts accumulate in dystrophic muscles and are responsible for large amounts of extracellular matrix proteins, generating fibrosis. Additionally, at the final stage, satellite cells become exhausted and are not able to generate new fibres. Cardiac muscle is less efficient in regeneration, compared to skeletal muscle and scar tissues in replacing damaged cardiomyocytes after injuries [2]. However, several research groups have demonstrated the presence of stem/progenitor cells that are able to differentiate into cardiac tissues [3-6], as well as skeletal muscle lineages [7-12]. This paper deals with novel therapeutic approaches for skeletal muscle dystrophies and explores pharmacological treatments. It also provides more recent gene and cell therapeutic protocols. Different sources of myogenic stem cells are discussed, highlighting their advantages and disadvantages, as well as underlining controversies in literature. Finally, we discuss autologous and heterologous cell therapy, considering the viral and non-viral technologies for *ex vivo* cell therapy in the treatment of muscular dystrophies.

2. Epidemiology, diagnosis and clinical management of muscular dystrophies

Muscular dystrophy was described for the first time in 1860s by the neurologist Guillaume-Benjamin-Amand Duchenne (de Boulogne). This followed a study of 13 boys who were affected by the most common type of muscular dystrophy, now carrying his name. Worldwide, Duchenne muscular dystrophy (DMD) affects 1/3,500 born males. Other isoforms of muscular dystrophies include Becker muscular dystrophy (less severe than DMD, with an incidence 3-6: 100,000 male births), limb-girdle muscular dystrophy (mainly affecting hip and shoulder muscles, occurring between 10 and 30 years of age, with an estimated range of incidence between 0.5-4:100,000), congenital muscular dystrophy (present at birth and not affecting the life span, incidence 1:21,500), facioscapulohumeral muscular dystrophy (inherited form of muscular dystrophy, initially affecting skeletal muscles of the face, scapula and upper arms, starting from teenage years. Incidence 4-12:100,000), myotonic dystrophy (an inherited form of muscular dystrophy, normally occurring in patients of any age. European incidence: 3-15:100,000) and finally, oculopharyngeal muscular dystrophy (a type of muscular dystrophy occurring in the middle age and, at the beginning, causes drooping of eyelids, dysphagia and

weakness of the extraocular muscles. This muscular dystrophy has been frequently observed in French Canadian patients, with a prevalence 1:1000). Distal muscular dystrophy is characterized by the onset observed in hands, feet lower arms or lower legs. Its incidence is unknown. Emery–Dreifuss muscular dystrophy affects muscles of the upper arms and lower legs. It causes multiple contractures, as well as heart problems (incidence: 1 for every 100,000).

Muscular dystrophies are typically diagnosed by physical exams, family medical history and tests. These may include muscle biopsies for the histological detection of dystrophin expression and electromyography tests to analyse the electrical activity of muscles at rest and during contraction. Furthermore, nerve conduction tests are conducted to detect possible injuries within the peripheral nervous system and genetic tests - mainly DNA analysis - reveal the presence of different mutated isoforms of dystrophin. Moreover, blood enzyme tests are carried out to detect the presence of creatine kinase, a known marker of fibre muscle damage.

Within the group of muscular diseases affecting the musculoskeletal system, muscular dystrophies represents a serious problem for human health, especially for its clinical management. Muscular dystrophies are characterized by a progressive weakness due to unrestrainable muscle degeneration. Since there are currently no real cures, occupational therapy represents the main tool adopted to ameliorate the patient's quality of life. This therapeutic line aims to assist dystrophic patients with MDs through the engagement of daily activities such as self-care, self-feeding and physical training. Specific instruments have been developed to help patients in their route along the disease including scooters/wheelchairs and some computer interface devices. Occupation therapy also aims to make changes in both the patient's occupational and home environments so as to improve the functionality of the inhabited places. Furthermore, physiological support for patients and relatives is also provided by occupational therapists. The totality of the therapeutic strategies, chosen at an individual level, represents a standard way for the clinical management of MDs in developed countries. Nevertheless, in low-income countries, socioeconomic reasons prevent the adoption of this course. Compared to the rest of the world, this causes a severe worsening of the patient's quality of life.

3. MDs and pharmacological treatments

Pharmacological treatments for MDs aim to stabilize the structural integrity of the muscle fibre membrane by counteracting chronic inflammation. Indeed, lack or genetic mutations of dystrophin causes a chronic influx of calcium into the myofibres. This is largely responsible for cell death and inflammatory response (Figure 1). Accumulation of fibrotic tissues in the replacement of damaged myofibres is another pathophysiological feature of MDs that is responsible for decreasing the contraction force and increasing fatigue. These events characterize dystrophinopathies because dystrophin plays a pivotal role in the anchoring of the dystrophin-associated protein complex, which, in normal conditions, can stabilize the structural integrity of membrane.

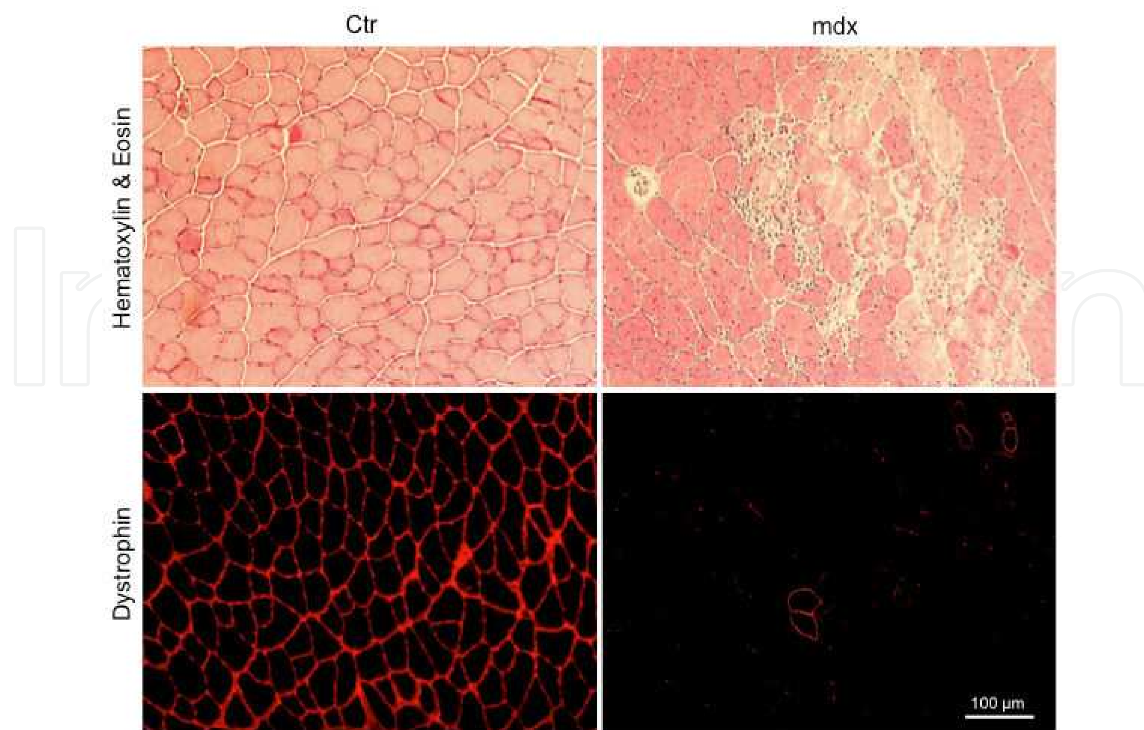

Figure 1. Histological features of muscular dystrophy. Upper panels show the histological architecture of the *tibialis anterior* muscle in a healthy control mouse (left panel) and dystrophic mouse model of human Duchenne muscular dystrophy (mdx, right panel). Haematoxylin and Eosin stain identifies the presence of a huge amount of infiltrating mononuclear cells within the *interstitum* of mdx muscle. Necrotic fibres, as well as centrally located nuclei fibres, are other hallmarks of dystrophic muscle (right panel) compared to healthy control (left panel). Lower panels show the immunofluorescence stain for dystrophin (red). Compared to the healthy control (lower left panel), muscle from mdx shows a strong reduction of dystrophin content. Only a small percentage of revertant fibres are positive for dystrophin expression (lower right panel).

Although there are no proficient cures for dystrophinopathies, several drugs have been used to delay their detrimental effects on muscle tissues, mainly through the attenuation of inflammation. In the treatment of MDs, several drugs are used for their ability to reduce circulating levels of TGF-β, known to play a crucial role in the fibrotic tissue deposition in dystrophic muscles. Among the drugs used to counteract the systemic burden of TGF-β are non-steroidal anti-inflammatory drugs (NSAIDs) such as nabumetone, ibuprofen and isosorbide dinitrate. These drugs have beneficial effects, especially in the treatment of Duchenne, Becker and Limb-Girdle muscular dystrophies [13]. Promising results were recently obtained from phase I studies in healthy volunteers. These studies revealed an optimal tolerability and safety profiles for a combined administration of isosorbide dinitrate, a nitric oxide donor and ibuprofen (NSAIDs) for the treatment of muscular dystrophies [14]. Other established pharmacological approaches aim to hamper the elevated muscle inflammation and necrosis events linked with mitochondrial dysfunction and altered metabolism. In particular, α-methylprednisolone (a synthetic glucocorticoid) stimulates the reduction of cytosolic calcium in dystrophic muscle and prevents apoptosis and/or necrosis events that normally occur during muscle degeneration in dystrophinopathies [15] (Figure 2). Another challenge in the pharmacological treatment of MDs is counteracting the high susceptibility to muscle damage

that characterizes dystrophinopathies. Indeed, among the corticosteroids drugs, prednisone and deflazacort show positive effects in reducing muscle damage and weakness, as well as in counteracting the loss of muscle contraction. Moreover, since the integrity of muscular membrane is a critical determinant of muscle degeneration during the illness progression, many efforts have been made to promote the sarcolemall stability of muscle fibres through the increase of native utrophin as a compensatory mechanism of dystrophin loss. In particular, nabumetone is a small molecule with anti-inflammatory properties, derived from its ability to inhibit cyclooxygenase. In addition, nabumetone can activate the transcription of utrophin. Aminoglycosides antibiotics, such as gentamicin and other small molecules under analysis (e.g., RTC13, RTC14 and ataluren, PTC124) aim to restore full-length dystrophin in patients with stop codon mutations. This promising therapeutic approach arises from the ability of such agents to stimulate the ribosomal read-through. This leads to the suppression of non-sense mutations in Duchenne/Becker muscular dystrophy [13].

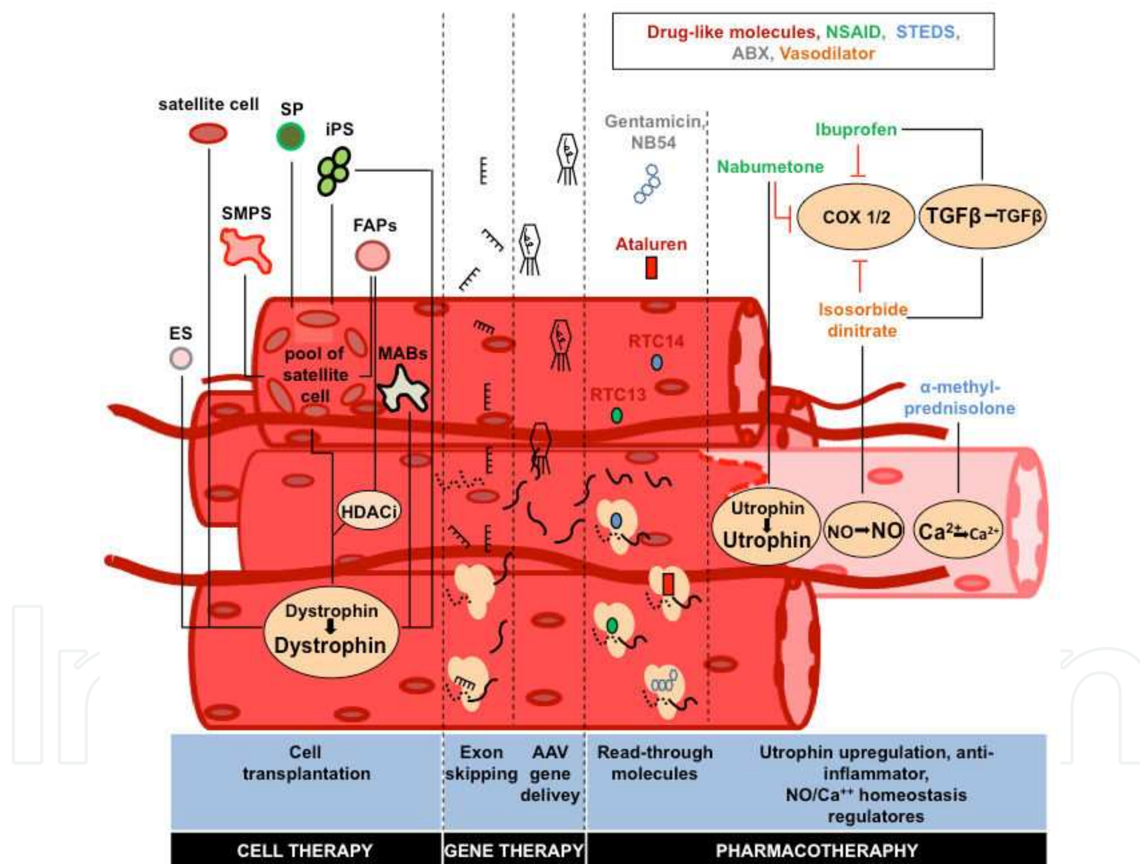

Figure 2. Schematic representation of the strategies adopted in the study/treatment of muscular dystrophy. Pharmaco-therapeutics approaches include drugs that can increase the expression of utrophyn, anti-inflammatory and No/Ca^{2++} homeostasis regulator drugs and small molecules that can promote the read-through of dystrophin. NSAIDs= non-steroidal anti-inflammatory drugs; STEDs= steroid drugs; ABX= antibiotics; COX 1/2= cyclooxygenase type 1 and 2. Gene therapy is based on both exon skipping and Adeno- associated virus (AAV) gene delivery strategies aiming to the increase of dystrophin content. Cell therapy investigates the myogenic potential observed in adult stem cells (such as satellite cells, FAPs, SP, MABs and iPS) and in ES cells, through the increase of both dystrophin content and the pool of resident satellite cells.

4. Inducing muscle hypertrophy as therapeutic strategy for MDs

In adult muscles, satellite cells are quiescence and blocked in the G0 phase of the cell cycle. After activation, these satellite cells move outside of the basal lamina and express Pax7 and MyoD. These cells are now known as myoblasts. They extensively divide and fuse to differentiate and form multinucleated myofibres. During this late differentiation process, myoblasts down-regulate Pax7 and express myogenin. MyoD and Myf5 factors are involved in the early stages of transitioning from undifferentiated myogenic precursors to myoblasts [16]. On the contrary, myogenin and MRF4 regulate the transition from myoblast to mature fibres.

In adulthood, a skeletal muscle can enlarge or reduce its own mass through a complex interplay in which several molecules are involved. During skeletal muscle hypertrophy, the myofibrils increase in number and size to increase muscular strength (Figure 3). While sarcoplasmic hypertrophy is a characteristic of body-builders' muscles, myofibrillar hypertrophy is typically found in weight lifters. In contrast, muscle atrophy, also known as muscle wasting, is the result of muscle protein loss with a reduction in fibres. At a molecular level, signals control both muscle growth and atrophy. These are finely interconnected and the biochemical pathways can be altered by increasing or decreasing specific growth factors [17]. Insulin Growth Factor-1 (IGF-1) is the most reliable muscle growth-promoting factor. IGF-1 is largely produced in the liver. However, skeletal muscle also contributes to the production of two distinct IGF-1 isoforms. Different isoforms of IGF-1 exist due to different RNA spliced variants. Human skeletal muscle has been found to express at least two isoforms [18]. These are IGF-1Ea, which is the liver type or systemic form and IGF-1Ec, also called Mecano Growth Factor (MGF). MGF is an autocrine/paracrine form that is particularly interesting as it is expressed in response to mechanical stimuli and cellular damage.

Increased muscle loading leads to augmented expression of the IGF-1 encoding gene, both in humans and animal models. Several authors have indicated that IGF1Ea induces proliferation and differentiation of satellite cells and muscle hypertrophy [19, 20]. Transgenic mice overexpressing IGF-1Ea display, indeed, skeletal muscle hypertrophy associated with increased muscle strength [21].

IGF-1Ea and other isoforms act via a tyrosin-kinase receptor IGFR-1, enabling AKT1 to be activated by the generation of phosphatidylinositol-3,4,5-triphosphates (PIP3). PIP3 Kinase (PI3Kinase) and the phosphatases PTEN and SHIP2 regulate the formation of PIP3, which recruits AKT1 on the plasma membrane. They can activate the mammalian target of rapamycin (mTOR) or FK506-binding protein 12-rapamycin-associated protein 1 AKT1 (PKBa). Furthermore, mTOR generates two complexes: the Rapamycin-sensitive Ternary complex mTORC1 and Rapamycin-insensitive mTORC2. These complexes control pathways that determine the mass/size (mTORC1) and the shape (mTORC2) of the cells. The general activation of mTOR results in the phosphorylation of several downstream targets in a signalling cascade. In this view, AKT1 is responsible for modulating the muscle growth and protein up-regulation signals in skeletal muscle tissue.

Met-Activating Genetically-Improved Chimeric Factor 1 (Magic-F1) is a recombinant protein that also triggers AKT pathway [22]. Magic-F1 is constructed as a bivalent ligand from HGF,

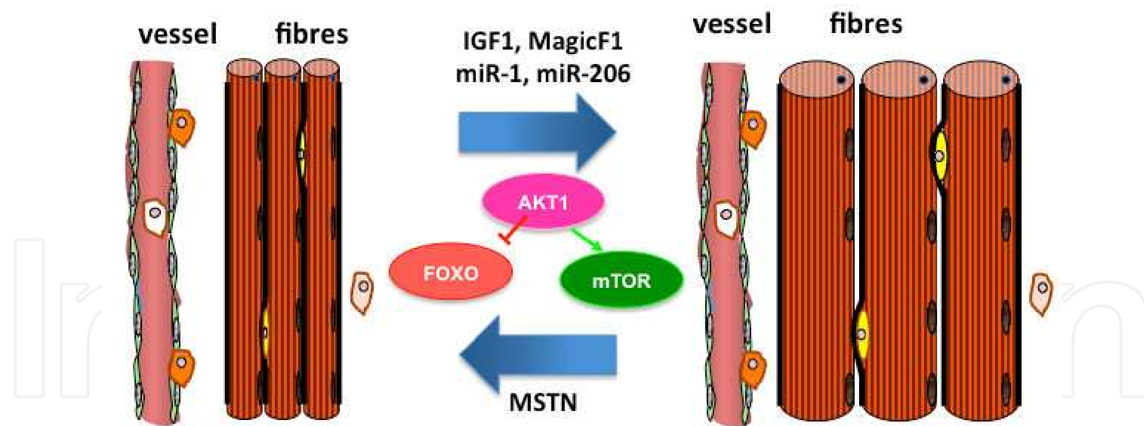

Figure 3. Muscle remodelling is a complex process and is among many key factors that modulate both satellite cells activation and protein synthesis. As a final result, fibre size and nuclear content can be increased (hypertrophy, right panel). In the cartoon, molecules can induce muscle growth and the key players in the hypertrophy-signalling pathway are indicated.

containing the signal peptide, the N-domain and the first two kringle-domains K1 and K2 of HGF. However, the kringles repeat in tandem and are joined by a linker. Magic-F1 binds c-Met and the HGF receptor activates Akt but not the Erk signalling pathway. Therefore, this recombinant molecule, which enhances the myogenic differentiation process, is a safe molecule. It does not have the potential risk of stimulating uncontrolled proliferation observed in several growth factors including IGF1. Previous studies in transgenic mice expressing Magi-F1 under muscle specific promoter showed that the recombinant protein cooperates with Pax3 signal pathway in early embryogenesis. This generates a more active skeletal muscle progenitors in early embryogenesis [23]. This results in a constitutive muscular hypertrophy in the adulthood of transgenic mice, since Magic-F1can down-regulate myostatin, a potent muscle mass regulator. Furthermore, it can directly activate MyoD, Myf-5 and several anti-apoptotic pathways.

The therapeutic potential of Magic-F1 was evaluated on α-sarcoglycan null mice (Scga-/-), an established model of limb-girdle muscular dystrophy type 2D. The Scga-/-/Magic-F1 transgenic mice showed a stronger muscle phenotype than their Scga-/- counterparts. Furthermore, the physiological benefit of muscular hypertrophy partially recovered the dystrophic phenotype [22].

Mutations occurring in the myostatin gene, also named growth and differentiation factor 8 (GDF8), are responsible for a hypertrophic phenotype. There is a great increase in muscle mass in a breed of beef cattle known as Belgium Blue [24]. Mdx mice that do not express myostatin are stronger and more muscular than their mdx counterparts [25]. Fibrosis and fatty remodelling are less evident in the diaphragm of those transgenic mice, suggesting improved muscle regeneration. In 2004, the first mutation of the myostatin gene in humans was reported and correlated with an enlargement of the skeletal muscle apparatus [26].

In summary, inducing muscular hypertrophy is relevant in clinical applications as a potential treatment of muscle diseases, including muscular dystrophy and cachexia, which cause

wasting in muscle mass and force. Insulin-like Growth Factor-1 (IGF-1), MAGIC-F1 and myostatin regulate the key steps during muscle regeneration (Figure 3). In animal models for Duchenne muscular dystrophy [22, 25, 27], these molecules have demonstrated a therapeutic value, without redressing the primary cause of the lesion and, in principle, could be adopted in patients suffering from muscular dystrophies. The delivery strategies of these molecules and potential side effects require more investigation. So far, their translational potential has been hindered in clinical trials.

5. Gene therapy and gene editing for MDs

In previous decades, significant advancements in direct gene replacement approaches in genetic muscle diseases have been achieved. Two strategies are currently being tested in the dystrophic animal model and have already entered - or are ready to enter - clinical experimentation. These are exon skipping and the expression of dystrophin variants of reduced size (Figure 2).

For the exon-skipping experiments, adeno associated viral vectors (AAV) were engineered to produce small nuclear U7 RNA targeting exons [28, 29]. These excluded the mutation of dystrophin from an in-frame transcript that is translated in a 'quasi' normal dystrophin protein. High-pressure intravenous delivery was adopted to guarantee an efficient systemic delivery. Phase I and II clinical protocol in patients was designed by scaling up AAV production for total body delivery and transient immune suppression to enable reinfusions.

As an alternative strategy, dystrophin variants of reduced size were considered, since the large size of the transcripts (14 kb) is an impediment to generate viral vectors. Indeed, a mild phenotype was observed in Becker muscular dystrophy patients, characterized by a huge deletion of dystrophin gene. This resulted in the expression of truncated, yet partially functional, dystrophin. This observation led to the idea of using truncated dystrophins for therapeutic use in mdx mice via rAAV vector-mediated gene transfer (for a detailed review see [30]). Recombinant adeno-associated viral (rAAV) vector-mediated gene transfer represents a promising approach for genetic diseases of muscles. Despite the limited DNA packaging capacity (~4.8 kb), its transduction efficiency is very impressive. Recently, Chamberlain group showed that functional dystrophin transgenes could be reconstituted *in vivo* by homologous recombination (HR), following intravascular co-delivery of two independent rAAV6 vectors [31]. Systemic delivery of dystrophin variants of reduced size has also been effective in pigs (Pichavant et al., 2010). Unfortunately, a phase 1/2 clinical trial using intramuscular injections of AAV2 into DMD patients did not result in the restoration of dystrophin expression. This is likely due to T cell immunity to dystrophin proteins. An alternative strategy is the delivery of utrophin, a dystrophin related protein normally present at neuromuscular junctions, which should not elicit an immune response. Utrophin has 3,433 amino acids, with a predicted molecular mass of 395 kDa. It is slightly smaller than dystrophin (427 kDa) and ubiquitously expressed. In addition, while dystrophin is expressed throughout the sarcolemma of skeletal muscle fibres, utrophin is restricted at the neuromuscular and myotendinous junction.

A significant inverse correlation between utrophin expression and disease severity in DMD has been observed [32]. Davies group developed a range of strategies to up-regulate utrophin for therapeutic approaches in muscular dystrophies [33]. A number of drugs were tested in a stable H2K mdx myoblast cell line. Here, a luciferase reporter gene was under control of the mouse utrophin promoter to identify an effect on utrophin expression [34]. This long screening study allowed the identification of SMT C1100, which also showed therapeutic potential in the mdx mouse [35]. Since the drug also demonstrated a synergistic effect when administered with prednisolone [35], the gold standard in treatment of DMD patients in the clinic, SMT C1100 was tested in phase I trials by BioMarin Pharmaceuticals (as BMN-195; Novato, CA, USA). Unfortunately, the plasma levels of the drug were not high enough for the trials to continue. Although there were no safety issues, new formulations are necessary.

In conclusion, the systemic delivery of AAV, plasmids and molecules to counteract muscle muscular dystrophy still face significant technical hurdles and alternative strategies are necessary.

6. Multipotent and pluripotent stem cells for the treatment of MDs

Satellite cells are quiescent mononucleated myogenic cells, located between the sarcolemma and basement membrane of terminally-differentiated muscle fibres [36]. For a long time, adult muscle was considered as a static tissue. Furthermore, due to its histological nature, it was considered formed by spatially-oriented post-mitotic multinucleated muscle fibres. Since 1961, the discovery of satellite cells by Katz and Mauro, together with vast studies about their biological role, revealed the existence of plasticity potential in adult skeletal muscle tissue. Their name derived from the distinctive location, wedged between the basal lamina and sarcolemma of myofibres, in a separate place from the fibre. With an estimated number between 1×10^{10} to 2×10^{10} satellite cells per person, these cells represent the main source of muscle progenitors within the adult skeletal muscle tissue [36]. Indeed, they are unipotent stem cells that, in the case of acute muscle damage and during muscle regeneration/degeneration, are able to re-enter the cell cycle and contribute to muscle repair by offering new myogenic progenitors capable to fuse and form new fibres. Pax7 (a paired box transcription factor) was the first identified marker required for myogenic specification of satellite cells. However, in the last decade, several surface markers were identified within the pool of satellite cells. These include CD34, M-cadherin, syndecan-3/4, c-met and the chemokine receptor CXCR4, as well as $\alpha7\beta1$-integrin (a transmembrane domain protein), Pax3, barx2, myocyte nuclear factor (MNF) (the latter three are known transcription factors) and caveolin-1 (a scaffolding protein within caveolar membrane) (Table 1). Since these markers are all expressed by satellite cells and not by post-mitotic myonuclei of fibres, their identification within the muscle microenvironment outside the fibres has been relatively easy. These markers were investigated both *in vitro* and *in vivo* for the marked regenerative ability potential of satellite cells [13]. Pre-clinical studies of DMD in the mdx mouse model, which recapitulates the pathophysiological features of human DMD such as the absence of dystrophin (Figure 1), contributed to the increase in knowledge of the therapeutic potential of satellite cells. It was

demonstrated that the satellite cells derived from a single fibre of a healthy donor and transplanted into a muscle of mdx mouse can actively contribute to the repopulation of the satellite cell pool of dystrophic muscle, as well as to the regeneration of new dystrophin expressed fibres. Several combinations of markers have been used to identify donor satellite cells and their myogenic contribution in dystrophic mice, including Pax7/CD34 [37] and CD34/integrin-α [38]. In 2004, a distinct subpopulation of satellite cells, named skeletal myogenic precursors (SMPS), positive for Cxcr4 and β1 integrin and negative for CD45 and Sca1 (a known maker of hematopoietic progenitor cells), were discovered, highlighting the heterogenic nature of satellite cells [39] (Table 1). SMPS showed a robust regenerating ability, as well as a strong capability of repopulating the satellite cell poll when injected in the muscles of mdx mice. Additionally, their potential role in counteracting muscle wasting was confirmed by a strong improvement of muscular contractile properties such as contractile force, observed in treated dystrophic muscles compared to the healthy controls [7].

Cell type	Localizaton	Surface markers expression (+ or -)	Transcription factors	Cytoplasmatic markers
satellite cells	between the sarcolemma and basal lamina of muscle fiber	$CD34^+$, M-cadherin$^+$, syndecan-3/4$^+$, c-met$^+$, $Cxcr4^+$, $\alpha7\beta1$-integrin$^+$	Pax7, Pax3, Barx2, myocyte nuclear factor (MNF)	caveolin-1
skeletal myogenic precursors (SMPS)		$Cxcr4^+$, $\beta1$-integrin$^+$, $CD45^-$, Sca-1$^-$		
muscle side population (SP)	Interstitial	Sca1+/Cd45+/Abcg2+ Syndecan4+	Pax7	
fibro-adipogenic progenitors (FAPs)	Interstitial	$CD45^-$/$CD31^-$ $CD34^+$, Sca-1$^+$ $PDGFR\alpha^+$		
mesoangioblasts (MABs)	Interstitial	SMA^+, $PDGFR^+$, $PDGFR\beta^+$, $Ng2^+$		alkaline phosphatase

Table 1. Cell markers identifying adult stem cells currently used in cell therapy.

Although satellite cells are the main source of myogenic renewal in adult skeletal muscle tissue, in recent years, other adult stem cells have been discovered. A subpopulation of muscle precursors associated with skeletal muscle tissue, named muscle side population (SP) cells, is a rare source of multipotent stem cells that contribute to muscle regeneration, upon transplantation. SP cells are characterized by a complete permeability to the Hoechst 33342 dyes, derived from their high expression level of Abcg2 transporter (Table 1). The myogenic potential ability of SP cells has been tested by *in vitro* co-culture with myoblast cells. In these conditions, SP cells were able to fuse with myoblast to form mature myotubes. At the same time, *in vivo* experiments confirmed their involvement in myogenic differentiation (see below). Interestingly, as observed in satellite cells, SP cells also show certain heterogeneity inside their population. Analysis of the expression pattern of specific markers revealed that 80% of SPs are positive for the vascular endothelial marker CD31, while 2-10% of total muscle SPs are blood-

derived and positive for the immune marker CD45. In the case of muscle damage and during the followed early phase of regeneration, a fraction of SP cells have been identified as highly positive for CD45, Abcg2 and CD31 (the latter two suggested a possible intervention in modulation of both vascularization and immune system) [40]. Furthermore, a third fraction of SP cells, representing 5% of total population, were recently identified. These cells are characterized by the absence of both CD45 and CD31 expression, while they may express Pax7, Sca1 and Syndecan4 [40-42] (Table 1). Interestingly, although in physiological conditions this subpopulation of muscle resident SP cells represents the smallest fraction within the rest of population, if engrafted in a regenerating muscle (pre-treated with cardiotoxin to induce acute tissue damage), they showed the highest myogenic differentiation potential [13].

In 2010, a new population of muscle interstitial stem cells were identified. These are characterized by their ability to undertake both fibrogenic and adipogenic differentiation [43, 44]. These mesenchymal fibro-adipogenic precursors (FAPs) show an intriguingly functional crosstalk with satellite cells. The nature of this relationship is mutually exclusive within the homeostatic processes of skeletal muscle and, indeed, the presence of FAPs during muscle regeneration enhances the myogenic potential of satellite cells. At the same time, the presence of satellite cells derived from new myofibres inhibits the adipogenic differentiation of FAPs [45]. FAPs were also investigated in the pre-clinical models of MDs. In particular, the treatment of FAPs with histone deacetylase inhibitors (HDACi) increased their *in vitro* myogenic differentiation (Figure 2), while, if transplanted in advanced dystrophic muscle (old mdx mouse), FAP cells can enhance the regenerative potential of resident satellite cells [46].

Another important source of muscle progenitors associated with vasculature, named mesoangioblasts (MABs), were investigated for their therapeutic potential in the treatment of muscular dystrophies (Figure 2). Mesoangioblasts were originally isolated from embryonic aorta of a quail and mouse and, later on, in the adult skeletal muscle of a mouse, dog and human. MABs are multipotent stem cells positive for CD34, SMA, Pdgfrα, Pdgfrβ, Ng2, AP and, accordingly with such expression pattern, can undertake several differentiation fates (Table 1). These include myogenic, osteogenic, chondrogenic and adipogenic. Studies of *Scga*-null mice (a limb-girdle muscular dystrophy mouse model) and GRMD (golden retriever muscular dystrophy) dogs showed that intra-vein injection of MABs can restore both the histological structure and function of large areas of dystrophic muscles [11, 12]. These promising results provided important knowledge regarding the therapeutic use of MABs for the treatment of muscular dystrophies. Recently, these efforts were finalized in a phase I/II clinical trial of donor mesoangioblasts transplantation from HLA-identical donors in five DMD patients, nearing completion (EudraCT Number: 2011- 000176-33). This clinical trial will provide useful information regarding the safety for the systemic delivery of stem cells in dystrophic patients, as well as the assessment of the ability of MABs to increase the dystrophin expression (Figure 2).

The scaling up of the research on pluripotent stem cells of the last decades has offered the interesting prospective to adopt this new precious source of stem cell in the treatment of MDs.

Embryonic stem (ES) cells are pluripotent stem cells, originally isolated from the inner cell mass of the blastocyst in a mouse in 1981 and from human in 1998. Their efficient pluripotency arises from the ability of ES cells to differentiate in all three germ layers - meso-

derm, ectoderm and endoderm. It was demonstrated in the early 1990s that, if cultured *in vitro*, murine ES cells can develop aggregates of cells (embryoid bodies) and differentiate in skeletal muscle cells expressing myogenic markers in the same muscle-specific determination genes order observed during embrional development: myf5, myogenin, myoD and myf6 [47]. Later on, both *in vitro* and *in vivo* studies confirmed their myogenic differentiation potential [13]. Nonetheless, the possibility of a therapeutic adoption of ES cells met the criticisms of both civil and scientific communities (see below). In 2006, Yamanaka published a revolutionary, paradigm-shift study. For the first time, a fate conversion of somatic cells (fibroblasts) into pluripotency was demonstrated [48]. As a result of this study, Yamanaka won the Nobel Prize in 2012 and began a new era for pluripotent stem cells-based therapeutic approach of chronic illness. So far, the myogenic potential of the induced pluripotent stem (iPS) cells, either from mouse or human origin, have been provided to counteract muscle degeneration in MDs [13] (Figure 2). In particular, *in vitro* and *in vivo* analyses showed that myogenic precursors generated from iPS cells could produce chimeric myotubes if co-cultured with C2C12 myogenic cell line. Furthermore, if transplanted in dystrophic muscles, their contractile properties could also be improved [49].

7. Muscle progenitor cell transplantation: Causes of failure and new perspectives

So far, in pre-clinical models, the exploration of cell-based therapy for muscle degeneration in MDs showed promising results. However, both technical and ethical issues are still the main determinants that hinder this therapeutic approach in clinics. The low abundance of satellite cells in human muscle, their heterogeneity and the reduced myogenic potential when expanded *in vitro* are the reasons why this source of stem cell is not ideal for the treatment of muscular dystrophies. Previously, satellite cells showed a general limited cell migration from the transplantation site and were not able to cross the endothelial barrier when injected systemically [12]. To overcome this important issue, MABs were proposed as an alternative source of cells. This is due to their ability to cross the vessel walls after intra-arterial delivery in dystrophic mice [11, 12]. Nevertheless, a large number of cell deaths can still occur if the immune response occurs within a few hours of the treatment. Thus, immune suppression is needed in the heterologous transplantation, as planned in the ongoing phase I/II clinical trial where Duchenne patients were transplanted intra-arterially with HLA-identical allogeneic MABs (EudraCTno. 2011-000176-33). This clinical trial faced three problems: the age of enrolled patients (frequently in advanced stage of diseases), the low dose of cells transplanted (from 1/5 to 1/10 of that administered to the GRMD dogs) and the intra femoral arteries delivery (limiting the treatment only for the muscle located downstream of femoral artery). For the treatment of muscular dystrophies, pluripotent stem cells have been recently explored. In such pre-clinical studies, the major problem encountered was controlling their myogenic differentiation and avoiding tumour formation. In addition, the therapeutic use of ES cells has been strongly contested within the scientific community and from public opinion. This is because

of ethical concerns (due to the embryonic origin of ES cells) and it does not seem that the employment of iPS cells will help this.

In conclusion, many scientific issues can be solved by further investigations in the cell biology of myogenic stem cells. With this in mind, further studies on mechanisms regulating skeletal muscle regeneration in basic and applied research are needed in order to solve several practical problems, as described above.

8. New frontiers for the treatment of MDs: Exosomes, MicroRNAs and gene editing

MicroRNAs (miRs) are non-coding RNA transcripts, ~22 nucleotides long that promote mRNA degradation by annealing to complementary sequences in the 3'untranslated regions (UTR) of specific target mRNA. Furthermore, miRs can target several transcripts and system individual mRNAs can be targeted by multiple miRs.

The biogenesis of miRNAs starts with the generation of pri-miRs by RNA polymerase II. These pri-miRs are transformed in pre-miRs by the microprocessor complex. They then transport them in the cytosol, where Dicer cleaves pre-miRs in ~22nt-long double-stranded molecules [50]. The guide strand responsible for the recognition of target mRNAs is loaded on the RNA-induced silencing complex (RISC), which contains multiple proteins including a ribonuclease enzyme.

Several biological processes, including muscle growth and differentiation, are mediated by a collection of specific miRs. These miRs can be released from the cells in the surrounding areas or in the circulation and circulating miRs (circ-miRs) appear resistant to harsh conditions [51]. Circ-miRs are protected by carriers, making them stable and valuable biological markers. Among the different carriers identified, exosomes are small vesicles (50-100nm diameter) that act as important regulators of long-range miR shuttling [52]. After the unknown processes of maturation, exosomes are released from the plasma membrane and are identified by specific markers, as Hsp-60/70 in the lumen and CD9/63/81 and tissue-specific membrane proteins on the surface [53]. Despite the lack of detail concerning receptors and intracellular processing generate debate and controversies, it is largely accepted that pre- or mature miRs are delivered to other cells, eliciting their regulation in target non-miR-originating cells.

The importance of miRNAs in the muscle development was established in a study involving conditional transgenic mice lacking Dicer in myogenic progenitors. This study resulted in aberrant muscle differentiation, accompanied by hyperplasia [54].

Furthermore, miR-206 is the most abundant miRNA in adult vertebrate skeletal muscle and promotes muscle skeletal muscle development and differentiation [55].

Interestingly, a mutation in myostatin gene that causes a dramatic muscle increase in textil sheep creates a target site for miR-206 and miRNA1. In these sheep, myostatin down-regulation determines a phenocopy of the double muscling Belgiun Blue cattle previously described (see

Myostatin section). Our knowledge of miRNA biology is still in its infancy and future investigation needs to be carried out in order to clarify the molecular mechanism and the precise involvement of these miRNAs in muscle development and regeneration.

Recently, several groups tested gene editing to correct point mutation using TALEN and CRISPR genome editing. TALENs are endonucleases that possess two domains: a TAL effector DNA binding domain and a DNA cleavage domain. Left and right TALENs can induce a double strand break (DSB) in the DNA that allows homologous recombination of the target DNA.

Also, CRISPR (clustered regularly interspaced short palindromic repeats) is a RNA-guided gene-editing system that can introduce a double strand break at any desired location by delivering the Cas9 protein and appropriate guide RNAs. Olson group used CRISPR/Cas9-mediated genome editing to correct the dystrophin gene mutation in the germ line of mdx mice [56]. This procedure produced genetically mosaic animals, containing 2-100% correction of the dystrophin gene. In principle, this technology could facilitate the genome editing of post-natal somatic cells, avoiding the use of viral vectors. Li et al. recently performed three correction methods (exon skipping, frame-shifting and exon knockin) in DMD-patient-derived iPSCs to restore the dystrophin protein. In their study, exon knockin was the most effective approach and identified clones with a minimal mutation load. We further investigated the genomic integrity by karyotyping, copy number variation array and exome sequencing to identify. TALEN and CRISPR-Cas9 corrected iPSCs were able to differentiate into skeletal muscle cells and express full-length dystrophin protein [57]. This is innovative technology that will be further investigated. However, no field trials have been planned.

9. Concluding remarks

To date, many efforts have been made to increase the understanding of genetic and molecular mechanisms of different types of muscular dystrophy. Historically, the flux of knowledge achieved from patients to laboratories of research, or from bed to bench, allowed the design, test, production and administration of new molecules that only perform a partial restoration of dystrophin expression. Nonetheless, the use of common drugs, such as non-steroidal anti-inflammatory drugs, corticosteroids and aminoglycosides antibiotics administered alone or combined with new drugs, showed poor beneficial effects regarding the restoration of dystrophin or the up-regulation of utrophin as compensatory mechanisms.

Thus, despite the limited results achieved by the pharmacological approaches tested so far, pharmacotherapy is still considered to be a useful tool in delaying the process of muscular degeneration and palliate the symptoms of the late stage of diseases.

An alternative approach for the treatment of dystrophies derives from the specific strategies adopted in gene therapy. Adeno-associated virus and lentiviral vector technologies have been studied in pre-clinical models to mediate the delivery of micro-dystrophin or mini-utrophin. Furthermore, an exon skipping strategy was proposed to restore the endogenous expression of dystrophin. Unfortunately, this also produced poor results in terms of dystrophin restoration and safety.

However, in recent decades, the stem cell therapy for muscular dystrophies has represented a new field of interest. In particular, it has sparked an increase in the understanding of biology in both multipotent (*i.e.* SP, MABs and FAPs) and pluripotent stem cells (*i.e.* ES and iPS cells), leading to the discovery and identification of new muscle progenitor cells. The exploitation of their myogenic potential has been investigated in several animal models including the mouse and dog. Extraordinary results were obtained in terms of dystrophin expression, decrease in inflammatory burden and increase in muscular function. More studies are likely to be conducted on myogenic derivatives from pluripotent stem cells and, possibly, in combination with miRNA and gene editing (CRISPR and TALEN) technologies for the treatment of muscle diseases. However, stem cell-based protocols still rely on adult stem cells. Many practical issues negatively interfere with their potential use in clinics. Low cell motility after transplantation, as well as the high immune rejection observed in pre-clinical models, are the main problems for obtaining a systemic treatment for cell-based therapy of dystrophies. A combined approach between pharmacotherapy and cell-based therapy can increase the beneficial effects of cell transplantation and give hope for the treatment of muscle degenerations. This could be achieved by increasing the myogenic differentiation potential of both multipotent and pluripotent stem cells by exosome, as well as miRNAs and gene editing technologies associated with pharmacological anti-inflammatory effects.

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References

[1] Mauro A. 'Satellite Cell of Skeletal Muscle Fibers'. *J Biophys Biochem Cytol*. 1961 Feb; 9: 493-5.

[2] Anversa P, Leri A, Kajstura J. 'Cardiac Regeneration'. *J Am Coll Cardiol*. 2006 May 2; 47(9): 1769-76.

[3] Beltrami AP, Barlucchi L, Torella D, Baker M, Limana F, Chimenti S, et al. 'Adult Cardiac Stem Cells are Multipotent and Support Myocardial Regeneration'. *Cell.* 2003 Sep 19; 114(6): 763-76.

[4] Galvez BG, Covarello D, Tolorenzi R, Brunelli S, Dellavalle A, Crippa S, et al. 'Human Cardiac Mesoangioblasts Isolated from Hypertrophic Cardiomyopathies are Greatly Reduced in Proliferation and Differentiation Potency'. *Cardiovasc Res*. 2009 Sep 1; 83(4): 707-16.

[5] Galvez BG, Sampaolesi M, Barbuti A, Crespi A, Covarello D, Brunelli S, et al. 'Cardiac Mesoangioblasts are Committed, Self-renewable Progenitors, Associated with Small Vessels of Juvenile Mouse Ventricle'. *Cell Death Differ*. 2008 Sep; 15(9): 1417-28.

[6] Nadal-Ginard B, Anversa P, Kajstura J, Leri A. 'Cardiac Stem Cells and Myocardial Regeneration'. *Novartis Found Symp.* 2005; 265: 142-54; discussion 55-7, 204-11.

[7] Cerletti M, Jurga S, Witczak CA, Hirshman MF, Shadrach JL, Goodyear LJ, et al. 'Highly Efficient, Functional Engraftment of Skeletal Muscle Stem Cells in Dystrophic Muscles'. *Cell.* 2008 Jul 11; 134(1): 37-47.

[8] Costamagna D, Quattrocelli M, Duelen R, Sahakyan V, Perini I, Palazzolo G, et al. 'Fate Choice of Post-natal Mesoderm Progenitors: Skeletal Versus Cardiac Muscle Plasticity'. *Cell Mol Life Sci.* 2014 Feb; 71(4): 615-27.

[9] Crisan M, Yap S, Casteilla L, Chen CW, Corselli M, Park TS, et al. 'A Perivascular Origin for Mesenchymal Stem Cells in Multiple Human Organs'. *Cell Stem Cell*. 2008 Sep 11; 3(3): 301-13.

[10] Quattrocelli M, Costamagna D, Giacomazzi G, Camps J, Sampaolesi M. Notch 'Signaling Regulates Myogenic Regenerative Capacity of Murine and Human Mesoangioblasts'. *Cell Death Dis.* 2014; 5: e1448.

[11] Sampaolesi M, Blot S, D'Antona G, Granger N, Tonlorenzi R, Innocenzi A, et al. 'Mesoangioblast Stem Cells Ameliorate Muscle Function in Dystrophic Dogs'. *Nature.* 2006 Nov 30; 444(7119): 574-9.

[12] Sampaolesi M, Torrente Y, Innocenzi A, Tonlorenzi R, D'Antona G, Pellegrino MA, et al. 'Cell Therapy of Alpha-sarcoglycan Null Dystrophic Mice Through Intra-arterial Delivery of Mesoangioblasts'. *Science*. 2003 Jul 25; 301(5632): 487-92.

[13] Berardi E, Annibali D, Cassano M, Crippa S, Sampaolesi M. 'Molecular and Cell-based Therapies for Muscle Degenerations: A Road Under Construction'. *Front Physiol.* 2014; 5: 119.

[14] Cossu MV, Cattaneo D, Fucile E, Pellegrino P, Baldelli S, Cozzi V, et al. 'Combined Isosorbide Dinitrate and Ibuprofen as a Novel Therapy for Muscular Dystrophies: Evidence from Phase I Studies in Healthy Volunteers'. *Drug Des Dev Ther.* 2014; 8: 411-9.

[15] Ruegg UT, Nicolas-Metral V, Challet C, Bernard-Helary K, Dorchies OM, Wagner S, et al. 'Pharmacological Control of Cellular Calcium Handling in Dystrophic Skeletal Muscle'. *Neuromuscul Disord.* 2002 Oct; 12 Suppl 1: S155-61.

[16] Rudnicki MA, Schnegelsberg PN, Stead RH, Braun T, Arnold HH, Jaenisch R. 'MyoD or Myf-5 is Required for the Formation of Skeletal Muscle'. *Cell.* 1993 Dec 31; 75(7): 1351-9.

[17] Sartorelli V, Fulco M. 'Molecular and Cellular Determinants of Skeletal Muscle Atrophy and Hypertrophy'. *Sci STKE.* [Review]. 2004 Aug 3; 2004(244): re11.

[18] Hameed M, Lange KH, Andersen JL, Schjerling P, Kjaer M, Harridge SD, et al. 'The Effect of Recombinant Human Growth Hormone and Resistance Training on IGF-I mRNA Expression in the Muscles of Elderly Men'. *J Physiol.* 2004 Feb 15; 555(Pt 1): 231-40.

[19] Adi S, Bin-Abbas B, Wu NY, Rosenthal SM. 'Early Stimulation and Late Inhibition of Extracellular Signal-regulated Kinase 1/2 Phosphorylation by IGF-I: A Potential Mechanism Mediating the Wwitch in IGF-I Action on Skeletal Muscle Cell Differentiation'. *Endocrinology*. 2002 Feb; 143(2): 511-6.

[20] Allen RE, Boxhorn LK. 'Regulation of Skeletal Muscle Satellite Cell Proliferation and Differentiation by Transforming Growth Factor-beta, Insulin-like Growth Factor I, and Fibroblast Growth Factor'. *J Cell Physiol.*1989 Feb; 138(2): 311-5.

[21] Musaro A, McCullagh K, Paul A, Houghton L, Dobrowolny G, Molinaro M, et al. 'Localized Igf-1 Transgene Expression Sustains Hypertrophy and Eegeneration in Senescent Skeletal Muscle'. *Nat Genet.* 2001 Feb; 27(2): 195-200.

[22] Cassano M, Biressi S, Finan A, Benedetti L, Omes C, Boratto R, et al. 'Magic-factor 1, A Partial Agonist of Met, Induces Muscle Hypertrophy by Protecting Myogenic Progenitors from Apoptosis'. *PLoS One.* 2008; 3(9): e3223.

[23] Ronzoni F, Bongio M, Conte S, Vercesi L, Cassano M, Tribioli C, et al. 'Localization of Magic-F1 Transgene, Involved in Muscular Hypertrophy, During Early Myogenesis'. *Journal of biomedicine & biotechnology*. 2011; 2011: 492075.

[24] McPherron AC, Lee SJ. 'Double Muscling in Cattle Due to Mutations in the Myostatin Gene'. *Proc Natl Acad Sci U S A.* 1997 Nov 11; 94(23): 12457-61.

[25] Wagner KR, McPherron AC, Winik N, Lee SJ. 'Loss of Myostatin Attenuates Severity of Muscular Dystrophy in Mdx Mice'. *Ann Neurol.* 2002 Dec; 52(6): 832-6.

[26] Schuelke M, Wagner KR, Stolz LE, Hubner C, Riebel T, Komen W, et al. 'Myostatin Mutation Associated with Gross Muscle Hypertrophy in a Child'. *N Engl J Med.* 2004 Jun 24; 350(26): 2682-8.

[27] Rizzuto E, Catizone A, Musaro A, Del Prete Z. 'Dystrophic Tendon Functionality is Recovered by Muscle-specific Expression of Insulin-like Growth Factor in Mdx Mice'. *J Biomech.* 2013 Feb 1; 46(3): 604-7.

[28] Douglas JT. 'Adenoviral Vectors for Gene Therapy'. *Mol Biotechnol*. 2007 May; 36(1): 71-80.

[29] Wang Z, Kuhr CS, Allen JM, Blankinship M, Gregorevic P, Chamberlain JS, et al. 'Sustained AAV-mediated Dystrophin Expression in a Canine Model of Duchenne Muscular Dystrophy With a Brief Course of Immunosuppression'. *Mol Ther.* 2007 Jun; 15(6): 1160-6.

[30] Goyenvalle A, Davies KE. 'Engineering Exon-skipping Vectors Expressing U7 snRNA Constructs for Duchenne Muscular Dystrophy Gene Therapy'. *Methods Mol Biol*. 2011; 709: 179-96.

[31] Odom GL, Gregorevic P, Allen JM, Chamberlain JS. 'Gene Therapy of Mdx Mice With Large Truncated Dystrophins Generated by Recombination Using rAAV6'. *Molecular Therapy*. 2011 Jan; 19(1): 36-45.

[32] Kleopa KA, Drousiotou A, Mavrikiou E, Ormiston A, Kyriakides T. 'Naturally Occurring Utrophin Correlates with Disease Severity in Duchenne Muscular Dystrophy'. *Hum Mol Genet.* 2006 May 15; 15(10): 1623-8.

[33] Fairclough RJ, Bareja A, Davies KE. 'Progress in Therapy for Duchenne Muscular Dystrophy'. *Exp Physiol.* 2011 Nov; 96(11): 1101-13.

[34] Morgan JE, Beauchamp JR, Pagel CN, Peckham M, Ataliotis P, Jat PS, et al. 'Myogenic Cell Lines Derived from Transgenic Mice Carrying a Thermolabile T Antigen: A Model System for the Derivation of Tissue-specific and Mutation-specific Cell Lines'. *Dev Biol.* 1994 Apr; 162(2): 486-98.

[35] Tinsley JM, Fairclough RJ, Storer R, Wilkes FJ, Potter AC, Squire SE, et al. 'Daily Treatment with SMTC1100, A Novel Small Molecule Utrophin Upregulator, Dramatically Reduces the Dystrophic Symptoms in the Mdx Mouse'. *PLoS One.* 2011; 6(5): e19189.

[36] Morgan JE, Partridge TA. 'Muscle Satellite Cells'. *Int J Biochem Cell Biol.* 2003 Aug; 35(8): 1151-6.

[37] Montarras D, Morgan J, Collins C, Relaix F, Zaffran S, Cumano A, et al. 'Direct Isolation of Satellite Cells for Skeletal Muscle Regeneration'. *Science*. 2005 Sep 23; 309(5743): 2064-7.

[38] Sacco A, Doyonnas R, Kraft P, Vitorovic S, Blau HM. 'Self-renewal and Expansion of Single Transplanted Muscle Stem Cells'. *Nature.* 2008 Nov 27; 456(7221): 502-6.

[39] Sherwood RI, Christensen JL, Conboy IM, Conboy MJ, Rando TA, Weissman IL, et al. 'Isolation of Adult Mouse Myogenic Progenitors: Functional Heterogeneity of Cells Within and Engrafting Skeletal Muscle'. *Cell.* 2004 Nov 12; 119(4): 543-54.

[40] Penton CM, Thomas-Ahner JM, Johnson EK, McAllister C, Montanaro F. 'Muscle Side Population Cells from Dystrophic or Injured Muscle Adopt a Fibro-adipogenic Fate'. *PLoS One.* 2013; 8(1): e54553.

[41] Perez AL, Bachrach E, Illigens BMW, Jun SJ, Bagden E, Steffen L, et al. 'Cxcr4 Enhances Engraftment of Muscle Progenitor Cells'. *Muscle Nerve.* 2009 Oct; 40(4): 562-72.

[42] Tanaka KK, Hall JK, Troy AA, Cornelison DDW, et al. 'Syndecan-4-Expressing Muscle Progenitor Cells in the SP Engraft as Satellite Cells During Muscle Regeneration'. *Cell Stem Cell.* 2009 Mar 6; 4(3): 217-25.

[43] Joe AWB, Yi L, Natarajan A, Le Grand F, So L, Wang J, et al. 'Muscle Injury Activates Resident Fibro/Adipogenic Progenitors that Facilitate Myogenesis'. *Nat Cell Biol.* 2010 Feb; 12(2): 153-U44.

[44] Uezumi A, Fukada S, Yamamoto N, Takeda S, Tsuchida K. 'Mesenchymal Progenitors Distinct from Satellite Cells Contribute to Ectopic Fat Cell Formation in Skeletal Muscle'. *Nat Cell Biol*. 2010 Feb; 12(2):143-52.

[45] Shadrach JL, Wagers AJ. 'Stem Cells for Skeletal Muscle Repair'. *Philos Trans R Soc Lond B Biol Sci*. 2011 Aug 12; 366(1575): 2297-306.

[46] Mozzetta C, Consalvi S, Saccone V, Tierney M, Diamantini A, Mitchell KJ, et al. 'Fibroadipogenic Progenitors Mediate the Ability of HDAC Inhibitors to Promote Regeneration in Dystrophic Muscles of Young, but Not Old Mdx Mice'. *EMBO Mol Med*. 2013 Apr; 5(4): 626-39.

[47] Rohwedel J, Maltsev V, Bober E, Arnold HH, Hescheler J, Wobus AM. 'Muscle-Cell Differentiation of Embryonic Stem-Cells Reflects Myogenesis in-Vivo - Developmentally-Regulated Expression of Myogenic Determination Genes and Functional Expression of Ionic Currents'. *Dev Biol.* 1994 Jul; 164(1): 87-101.

[48] Takahashi K, Yamanaka S. 'Induction of Pluripotent Stem Cells from Mouse Embryonic and Adult Fibroblast Cultures by Defined Factors'. *Cell.* [Research Support, Non-U.S. Gov't]. 2006 Aug 25; 126(4): 663-76.

[49] Tedesco FS, Dellavalle A, Diaz-Manera J, Messina G, Cossu G. 'Repairing Skeletal Muscle: Regenerative Potential of Skeletal Muscle Stem Cells'. *J Clin Invest.* 2010 Jan; 120(1): 11-9.

[50] Crippa S, Cassano M, Sampaolesi M. 'Role of MiRNAs in Muscle Stem Cell Biology: Proliferation, Differentiation and Death'. *Curr Pharm Des*. 2012; 18(13): 1718-29.

[51] Cheng G. 'Circulating miRNAs: Roles in Cancer Diagnosis, Prognosis and Therapy'. *Adv Drug Deliv Rev.* 2015 Jan; 81C: 75-93.

[52] Atay S, Godwin AK. Tumor-derived 'Exosomes: A Message Delivery System for Tumor Progression'. *Commun Integr Biol*. 2014 Jan 1; 7(1): e28231.

[53] Yellon DM, Davidson SM. 'Exosomes: Nanoparticles Involved in Cardioprotection?' *Circ Res.* 2014 Jan 17;114(2):325-32.

[54] O'Rourke JR, Georges SA, Seay HR, Tapscott SJ, McManus MT, et al. 'Essential Role for Dicer During Skeletal Muscle Development'. *Dev Biol.* 2007 Nov 15; 311(2): 359-68.

[55] McCarthy JJ, Esser KA, Andrade FH. 'MicroRNA-206 is Overexpressed in the Diaphragm but not the Hindlimb Muscle of Mdx Mouse'. *Am J Physiol Cell Physiol.* 2007 Jul; 293(1): C451-7.

[56] Long C, McAnally JR, Shelton JM, Mireault AA, Bassel-Duby R, Olson EN. 'Prevention of Muscular Dystrophy in Mice by CRISPR/Cas9-mediated Editing of Germline DNA'. *Science.* 2014 Sep 5; 345(6201): 1184-8.

[57] Li HL, Fujimoto N, Sasakawa N, Shirai S, Ohkame T, Sakuma T, et al. 'Precise Correction of the Dystrophin Gene in Duchenne Muscular Dystrophy Patient Induced Pluripotent Stem Cells by TALEN and CRISPR-Cas9'. Stem Cell Reports. 2015 Jan 13; 4(1): 143-54.

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