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Mononucleated Cells to Regenerate Skeletal Muscle Syncytial Tissues

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Abstract

Skeletal muscle is one of the most plastic tissues of vertebrates since it may able upon exercises to double in size due to a physiological hypertrophy. Despite the fact that it is mainly a syncytial tissue, it contains a relevant number of mononucleated cells that can be involved in its homeostasis and repair. Although the mononuclear cell types with the highest myogenic potential are the satellite cells located underneath the basal lamina of muscle fibres, other interstitial cells have been shown to contribute to muscle regeneration.

Adding complexity to this scenario is the fact that several authors revealed myogenic potential in pluripotent stem cells, which can be generated from patient somatic cells and eventually manipulated to correct the genetic defect.

Notwithstanding the copiousness of myogenic cell types, their use in ex vivo cell therapies for muscular degenerative diseases is still questionable. However, new discovers on their biological properties have advanced our comprehension in handling myogenic stem cells significantly.

In this review, we will focus on the myogenic potential of multi- and pluri-potent stem cells and their use in preclinical and clinical studies. New insights from direct reprogramming and epigenetic signalling to generate myogenic stem cells are also considered.

Keywords: Stem cells; Skeletal muscle; Regenerative medicine

Mimicking Human Muscle Pathology

Muscular dystrophies (MDs) are a group of muscle diseases that affect the musculoskeletal system and locomotion [1-5]. MDs are characterized by defects in muscle proteins, determining progressive death of muscle cells and tissue [2,3]. There are several forms of MDs, including the Duchenne muscular dystrophy (DMD), the Becker muscular dystrophy (BMD) and some forms of limb-girdle muscular dystrophy (LGMD).

DMD is the most severe syndrome and it is caused by mutations in the *dystrophin* gene located on the human X chromosome, with an inheritance pattern of 3 cases per 10000 live male births [5-7]. Satellite cells are localized underneath the basal lamina of terminally differentiated muscle fibres and in response to injury, they proliferate, fuse and give rise to regenerated muscle. Unfortunately, genetic mutations responsible for DMD are also present in satellite cells. Hence, the ability to restore normal muscle function remains obstructed. In DMD patients a small number of muscle fibres are able to produce functional *dystrophin*, mostly due to secondary mutations in myogenic precursor cells, which restore the reading frame [8,9]. However, these so-called revertant fibres are in a too small minority to alleviate the pathology of the *dystrophin*-deficiency. Exhaustion of the satellite cell pool due to degeneration and regeneration cycles is thought to critically contribute to the disease [10,11].

BMD is also caused by mutations in the *dystrophin* gene, but myofibrils retain a truncated and low-active form of the *dystrophin* protein, consequently the symptoms appear with a later, and much slower rate of progression [12,13]. Both BMD and DMD patients can show different degrees of cognitive damage, indicating that brain function is compromised. In the last stage of disease progression, the regenerative capacity of the muscles is lost, and muscle fibres are gradually replaced by adipose and fibrous connective tissue [1-13].

LGMD disease is a typically inherited disorder characterized by muscle weakness and cardiomyopathy, but in most case cognitive functions are not compromises. In LGMD, the function of several different proteins can be affected. LGMD can be autosomal dominant (LGMD1) or recessive (LGMD2). Among the proteins affected in the LGMD2 are γ, α, β, and δ sarcoglycans responsible for LGMD type 2C, 2D, 2E and 2F [14,15].

Several animal models have been developed during last 30 years to study muscular dystrophies, especially for DMD and LGMD. The *mdx* mouse model presents a X-linked mutation in the *dystrophin* gene, mimicking the genotype of DMD patients [16-19]. In the last 10 years, the *mdx* mice were extensively used for novel therapeutic approach studies. However, *mdx* mice show a decline in muscle regenerative capacity only at advanced age (>65 weeks), while necrotic processes persist with deposition of scar tissue. In addition, *mdx* mice show a very efficient muscle regeneration and normal lifespan, in contrast to human DMD, where skeletal muscle is progressively damaged and replaced by fibrotic tissue and patients die prematurely [19,20]. Besides, a double mouse mutant lacking both *dystrophin* and *utrophin*

(*mdx/utnr-*) has been generated, showing a closer phenotype to DMD patients, including cardiomyopathy [9,21].

For LMGD2 diseases, γ, α, β, and δ sarcoglycan knock-out (KO) mice were generated [22,23]. These mice show chronic skeletal muscle degeneration and, in the case of γ, β, and δ *sarcoglycan*-KO mice also heart is affected, developing a dilated cardiomyopathy [23]. LGMD mice are considered a better animal model than *mdx* mice for preclinical studies because of their lack of revertant fibres.

There are also canine models of DMD that exhibits similar clinical symptoms to human DMD [24]. Canine X-linked muscular dystrophy models include the golden retriever muscular dystrophy [25] dog and German shorthaired pointer. The canine models more accurately phenocopy the human disease than other animal models [26].

Facio-scapulo-humeral muscular dystrophy (FSHD) is a severe form of muscular dystrophy and it is the third most common human myopathy. The disease is characterized by a progressive degeneration of a highly selective set of muscle groups [27]. As its name suggests, muscles weakness regards facial mimic and shoulder girdle muscles. The FSHD derives for the interplay of complex genetic and epigenetic events [28]. In 95% cases, the pathology is caused by the overexpression of the gene called *FRG1 (FSHD region gene 1)*. Overexpression of *FRG1* in mice causes a myopathy with some similarities to FSHD. For this reason, FSHD is the ideal candidate for the using of RNAi-mediated gene silencing therapeutic approaches [29].

Multi- and Pluri-potent Stem Cells for Myogenic Commitment

Stem cells are mononucleated cells able to self-renew and differentiate into diverse specialized cell types. We can divide them in two categories: pluripotent stem cells such as iPS (see below) and embryonic stem cells (ES), isolated from the inner cell mass of blastocysts, and multipotent stem cells, present in various adult tissues. While they are in principle to act as repair tissue system in adulthood, in a developing embryo they allow the generation of specialized tissues maintaining the normal turnover of regenerative organs, especially for blood, skin and intestine. Embryonic stem cells (ESCs) are considered the most promising source for cell therapy, but complex biological and ethical problems correlated with ESCs have hampered their use in favour of adult stem cell strategies [30]. In mammals, there are several stem cell types with varying myogenic potential that could be eventually used for therapeutic approaches, as discussed in the following paragraphs.

Stem Cells from Bone Marrow, Adipose Tissue and Amniotic Fluid

Among the cell types used in preclinical studies for the regeneration of muscle tissue, mesenchymal stem cells (MSCs) can be isolated from different tissues, such as adipose tissue, dental pulp, placenta, umbilical cord and fallopian tube [31-34]. However, an important question is whether MSCs from different sources are comparable in their differentiation potential *in vivo* or whether the stem cell niche of their origin influences their plasticity. It has been previously suggested that hMSCs from bone marrow and human circulating AC133+ stem cells, when co-cultured with mouse skeletal myoblasts, form myotubes by fusion and commit functionally in the myogenic environment [35-37]. However, obtaining cells from bone marrow is not a very easy procedure and yields a low number of hMSCs.

A great impulse in the studies of MSCs in dystrophies was given by the multipotent adipose-derived stem cells (hASCs). hASCs are similar to MSCs, they maintain mesenchymal lineage and they are more easily available. hASCs can differentiate into skeletal muscle cells *in vitro* either in co-culture with skeletal myoblasts, or when cultured in medium supplemented with horse serum and/or under reduced serum conditions. In particular, spontaneous fusion of ASCs and subsequent myotube-like formation was observed in early culture passages at high cell density [38,39].

Recently, it was described a population of mesenchymal progenitors, distinct from satellite cells, in the skeletal muscle. These progenitors have many similarities with hASCs, they do not generate myofibres spontaneously, but they enhance the rate of differentiation of primary myogenic progenitors, and they have adipogenic differentiation potential both *in vitro* and *in vivo* [40]. The interaction between muscle cells and these mesenchymal progenitors has a considerable impact on muscle homeostasis since adipogenesis is strongly inhibited by the presence of satellite cell-derived myofibres.

It has also been demonstrated that the transplantation of haematopoietic cell alone does not report any improvement in restoring dystrophin or other muscle genes in dystrophic mice [41]. In addition, the use of immunosuppressants in heterologous transplantation of hematopoietic stem cells (HSCs) may cause toxicity to many organs, such as kidney, liver, muscle and bone. However, an interesting study demonstrated an increased in the dystrophin gene in DMD dogs up to 7% of wild-type levels and it was maintained for at least 24 weeks, without any pharmacological immunosuppression [42].

Another promising source of stem cells for myogenic restoration is the amniotic fluid. In fact, human amniotic fluid stem (hAFS) cells is a novel class of stem cells that shares characteristics of both embryonic and adult stem cells and they have been regarded as promising candidate for cell therapy [43]. Many groups have confirmed that AFS cells have the potential to differentiate into hematopoietic, neurogenic, osteogenic, chondrogenic, adipogenic, renal, hepatic, and muscular lineages [44,45]. Although, regarding their biological properties and marker expression pattern, AFS cells appear to be more similar to embryonic stem (ES) cells than, for example, to trophoblast cells, the precise origin of AFS cells remains elusive. Many therapeutic approaches using AFS cells are currently under investigations and, recently, results demonstrated how AFS cells represent a powerful tool for regenerative medicine [46]. As regards muscle regeneration, De Coppi et al. [44] demonstrated that vein transplantation of mouse amniotic fluid stem cells enhances the muscle strength and improves the survival rate of dystrophic mice [47]. AFS cells integrate in the stem cell muscle compartment and they show a great capacity to regenerate muscle injuries as well as satellite cell pool.

Muscle Myogenic-committed Stem Cells

To restore muscles affected by Duchenne and Becker dystrophies several types of muscle-derived cell transplantation strategies have been tested in animals and in a few DMD patients [48]. Muscle precursor cells, known as myoblasts and derived from quiescent satellite cells, were one of the first cell types explored in pioneer transplantation studies, but intramuscular injections of myoblasts did not show great results, because of the rapid death of injected cells and the failure of the migration of injected myoblasts from the infusion site [49,50].

Nevertheless, satellite cells are still considered the major candidate

for muscle regeneration in muscular dystrophies. It was demonstrated that a single satellite cell could reform new myofibres with the recovery of the physiological function of contraction. Initially quiescent satellite cells expressing *Pax7* and *Pax3* migrate to the site of damage, and start to up-regulate *MyoD* and *Myf5*, and become proliferative myoblasts.

Finally, *Mrf4* and *Myogenin* positive myoblasts form multinucleated myofibres or fuse to damaged myofibres [51-54].

Recently, interesting studies revealed the presence of muscle-derived stem cells (MDSCs), as possible predecessor of satellite cells [55]. These cells, isolated from late pre-plating mononucleated muscle cells are not limited to the myogenic lineage; they can differentiate into other lineages (bone, cartilage, tendons) *in vivo* and *in vitro*. These cells present high-level proliferation, high self-renewal, immune-privileged behaviour, and a superior capacity to regenerate skeletal muscle. In addition, MDSCs can be easily infected with viral vectors for gene therapy strategies, which is a very important attribute in the development of tissue engineering applications towards muscular dystrophies [55,56]. There are also muscle side population (SP) cells, which are being investigated as potential myogenic progenitors. They are associated with the muscle vascular system and are spontaneously committed towards the haematopoietic lineage [57]. In co-cultures with myoblasts, SP cells are able to form myotubes and, if injected intramuscularly into crushed tibialis of a *scid* immunodeficient mouse, can restore up to 1% of regenerating fibres [58].

In addition, in recent years, a new type of vessel-associated muscle-derived stem cells, termed mesoangioblasts (MABs), has been investigated as a potential therapy for muscular dystrophy. Mesoangioblasts isolated from murine embryonic dorsal aorta differentiate rapidly into tissues of mesodermal origin, including smooth, skeletal and cardiac muscle, and also into osteogenic, chondrogenic and adipogenic lineages [59,60]. MABs display high proliferation rate in vitro, without any tumour transformation potential. Incorporated into skeletal muscle fibres, they contribute to the satellite cell compartment and promote expression of muscle-specific proteins. In the environment of severe muscle damage in the *mdx/utrn$^{-/-}$* mouse, mesoangioblasts continue to proliferate and promote regeneration, migrate to adjacent damaged muscles and differentiate into multiple tissue types [59-61].

iPS Cells

In recent years, induced-pluripotent stem (iPS) cells have been used instem cell-based therapies for muscular and cardiac diseases. It has been demonstrated that mouse and human fibroblasts can be reprogrammed into an ESC-like state by introducing combinations of several transcription factors, including *Oct-3/4*, *Sox2*, *c-Myc*, and *Klf4* [62,63]. The cells have been shown to exhibit ESC-like characteristics in terms of self-renewal, expression of ESC markers, and differentiation towards three germ layers *in vitro* and *in vivo* [64]. iPSCs are able to differentiate spontaneously into cardiomyocytes, providing a donor cell source to treat a mouse model of acute myocardial infarction and also having the interesting feature of reducing tumour formation after transplantation. iPSCs may bypass the ethical problems correlated with ESCs and their potentiality could represent a powerful tool for muscular regenerative therapy [65]. Nevertheless, the *in vivo* safety of iPSCs remains controversial [66]. In fact, Wang et.al, for example, demonstrated that tail-tip fibroblasts prepared from 14-month-old *mdx* mice were highly proliferative and efficiently reprogrammed,

and stable in culture, but fibroblasts isolated from muscle of *mdx* mice at different ages showed lower proliferative activity and lower reprogramming efficiency, compared with those from younger *mdx* mice [67]. Therefore, iPSCs generated from patients with Duchenne and Becker dystrophies represent a good source in terms of plasticity and life span, but further studies will be necessary to elucidate cellular and molecular mechanisms of iPSCs that could be the basis for the cell therapy of human disorders.

Stem Cell Therapy with Multi- and Pluripotent Stem Cells

Cell-based therapies have been largely considered a promising therapeutic tool for the treatment of muscular dystrophies and for the regeneration of a mature muscle tissue [5]. Satellite cells, the stem cell of muscle tissue, represent a good cell model for the treatment of dystrophies. In fact, *mdx* irradiated mice transplanted intramuscularly with Pax7$^+$ CD34$^+$ GFP$^+$satellite cells, demonstrated a good restoration of *dystrophin* expression in many skeletal fibres and a real contribution to the resident satellite compartment [68]. Nevertheless, satellite cells have a low migration capability and they show impaired engraftment potential when expanded *in vitro*. Besides, *wt* myoblasts transplantation in dystrophic muscles restored *dystrophin* expression, but it has been observed a poor cell survival and a limited distribution of the transplanted cells [68,69].

MDSCs and SP cells in co-culture with myoblasts, they can form myotubes *in vitro* and, if injected intramuscularly into crushed tibialis of a *scid/bg* immunodeficient mouse, can give rise to up to 1% of regenerating fibres [48]. Cell therapy with MDSCs and muscle side population (SP) cells demonstrated the re-establishment of *dystrophin* expression in the *mdx* mouse more efficiently than myoblasts during an extended period [55-57].

Vessel-associated muscle-derived stem cells (MABs) represent another example of stem cells used in preclinical animal models of muscular dystrophy. In fact, as well as the application of MABs therapy on *mdx/utrn$^{-/-}$* mice [68-70], Sampaolesi et al. [60] demonstrated the effectiveness of MABs in a canine model of muscular dystrophy. The intra-arterial delivery of wild-type canine mesoangioblasts in DMD Golden Retriever dogs produces an extensive recovery of *dystrophin* expression, normal muscle morphology and function. In addition, similar results have been achieved by human MABs transplantation into *scid-mdx* immunodeficient dystrophic mice [59]. It is very evident that MABs cell therapy shows long-term benefits in dystrophic animals, in terms of general motility restoration and whole muscle regeneration. Nevertheless, the problem of the use of steroids and immunosuppressant drugs remains critically for the use of those cells in heterologous cell therapies.

Other adult-derived stem cells have been isolated and characterized for the use in animal transplantation experiments, such as bone marrow-derived stem cells (BMSCs), adipose-derived stem cells (ASCs), hematopoietic stem cells (HSCs) and amniotic-fluid stem cells (AFSCs). After bone marrow transplantation in dystrophic mice, bone marrow-derived stem cells (BMSCs) are able to migrate and contribute to the formation of new *Myf5$^+$* fibres [71]. In addition, it has been demonstrated that injections of hASCs into tibialis anterior of immunocompetent and immunosuppressed *mdx* mice allowed the restoration of fused myofibres and, after 6 month from transplantation, chimaericmyofibres expressing human *dystrophin* appeared [38,39,72].

In fact, mesenchymal stem cells in general have a positive effect when interacting with the host muscle, but there are also evidences in the literature describing the immunosuppressive properties of MSCs [40,73]. Inflammatory infiltration is observed in the dystrophic muscles but little is known about the mechanisms involved in mesenchymal immunomodulation. Finally, the use of hematopoietic stem cells (HSCs) may cause toxicity to many organs, and their use is very restricted.

The interest of applying iPSCs technology to generate autologous cells for therapeutic purposes is a key challenge in future therapy. The iPS cells can be obtained through direct reprogramming of different human somatic cells to a pluripotent state with viral and/or non-viral methods [67]. There are several protocols to differentiate murine IP cells to myoblasts, such as a transfection with plasmid expressing myogenic factors. In particular, MABs reprogrammed to a pluripotent state (MAB-iPSCs) and subsequently transfected with *Pax3* and *Pax7* are capable to form myotubes *in vitro* more efficiently than fibroblast-derived iPS cells (f-IPSCs) [74]. Quattrocelli et al. [74] concluded that MAB-iPSCs contain an intrinsic myogenic cell memory compared to iPSCs obtained from fibroblasts (f-iPSCs). Another important study focused on the effectiveness of human ES/iPS cells reprogrammed from fibroblasts to repair dystrophic muscle. In fact, the intramuscular transplantation of TA muscles with ES- or iPSCs transfected with *Pax7* showed not only a restoring in the expression of *dystrophin* gene in *mdx* mice, but also showed an improving in the strength generation of engrafted muscles [75]. The future goal for the use of iPSCs in cell therapy is to discover non-genetic methods of delivering factors to generate myogenic progenitors. These methods could include the use of safer transient vectors, recombinant proteins, or the use of synthetic modified mRNAs.

As regards, human artificial chromosome (HAC) vector has many advantages in gene therapy, including the ability to carry large gene inserts including the regulatory elements. It has been demonstrated that a HAC vector containing the entire *dystrophin* gene (DYS-HAC) is able to correct mutation and deletion of *dystrophin* in iPSCsderived from *mdx* mice [76]. In addition, an improvement of the dystrophic phenotype in the *mdx* mouse model was achieved by a combination of HAC-mediated gene replacement MABs transplantation protocols [77]. Corrected mesoangioblasts showed an increase in the re-establishment of *dystrophin*-positive muscle fibres and in the pool of muscle satellite cells in *mdx* mice. Therefore, HAC-mediated gene transfer shows positive effects in preclinical model of DMD and could offer potentials for future clinical translation in clinical therapy (Figure 1).

New Insights from Direct Reprogramming and Epigenetic Signalling (miRNAs and RNAi)

The mutation of Dystrophin gene in DMD patients is associated with a dysfunction of several proteins correlated with the *dystrophin* gene, such as *α, β, γ, and δ sarcoglycans, sarcospan*, and other proteins, such as *α-dystrobrevin, α1, β1, β2, γ1, γ2, calveolin*, growth factor receptor–bound protein-2(Grb2), and neuronal *Nitric Oxide Synthase* (*nNOS*) [78]. It has been found that mRNA levels are altered in the human DMD pathology, and in this background an important role in regulating several biological responses in muscle cells, ranging from proliferation, differentiation and modification of mRNA was given by the microRNAs (miRNAs) [79,80]. miRNAs are a class of post-transcriptional regulators and they are short ~22 nucleotide RNA
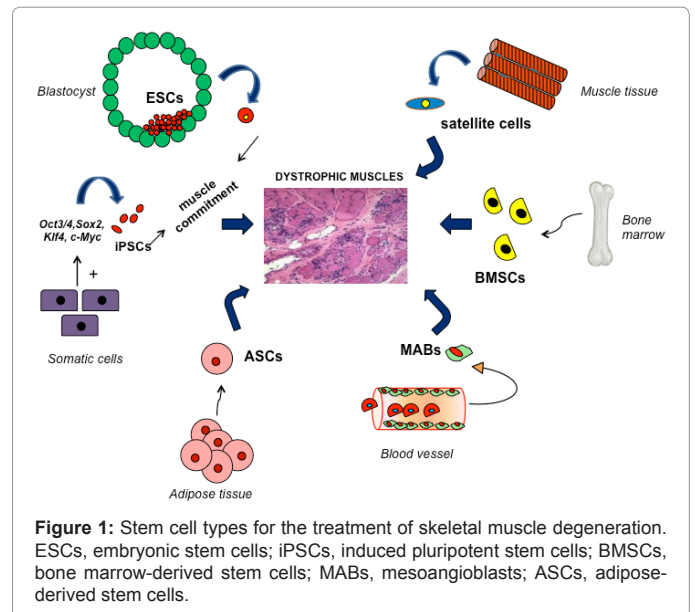
Figure 1: Stem cell types for the treatment of skeletal muscle degeneration. ESCs, embryonic stem cells; iPSCs, induced pluripotent stem cells; BMSCs, bone marrow-derived stem cells; MABs, mesoangioblasts; ASCs, adipose-derived stem cells.

sequences that bind to complementary sequences in the 3' UTR of multiple target mRNAs, usually resulting in their silencing. It has been shown that myosin genes [81] encode miRNAs controlling muscle performance. Hence, all the identified muscle miRNAs indirectly promote myogenesis, rather than acting directly on key regulatory factors for muscle differentiation. Crippa et al. [82] demonstrated that among the miRNAs known that regulate skeletal myogenesis, only miR669a and miR669q directly inhibit the MyoD 3′ untranslated region (UTR) and, consequently, skeletal myogenesis [82,83]. miR669a and miR669q are the first identified miRNAs that act upstream of MyoD, thus indirectly regulating all MyoD targets. The novelty of this study is that the dysregulation of both these miRNAs is necessary to activate skeletal myogenesis in *β -sarcoglycan* know-out cardiac progenitors. On the contrary, in *wt* cardiac progenitors miR669 family is capable of repressing skeletal myogenesis by inhibiting *MyoD* expression [82]. So far, the understanding of the regulatory network of the miRNAs in dystrophic diseases and their role in the activation of satellite cells in a damage muscle represents an important starting point to be focused on future research and therapies.

In addition, another important approach to display new therapy for muscular dystrophies is the RNAi-based technique [84]. RNAi is a process within living cells that moderates the activity of their gene targets; it is an RNA-dependent gene silencing process, that cells use to defend themselves against parasitic genes, viruses and transposons, but also in directing development as well as gene expression in general [84]. Bortolanza et al. [29] explored the therapeutic potentials of RNAi gene silencing. In fact, the FSHD is caused by the over-expression of the *FRG1* gene, and, therefore, its inhibition would be expected to lead to a therapeutic benefit in FSHD. The group demonstrated that injected *FSHD-/-* mice with a viral vector containing AAV6-sh1 *FRG1* doses displayed a reduction in *FRG1* expression in all the analyzed muscles and a positive general physiological recovery, without any evidence of toxicity in any of the treated mice. This work opens the doors to future investigations on RNAi-based techniques and their clinical applications and treatment for particular muscular dystrophies in which aberrant up-regulation of specific genes is involved. This new

therapy allowed significant improvement of disease in *FSHD⁻ᐟ⁻* mice and could potentially be translated to human patients to develop new therapies.

Conclusions and Perspectives

Skeletal muscle tissue offers several advantages to exploit and explore stem cell therapy approaches. It is a multinucleated tissue that efficiently regenerates after injury by a very defined biological program characterized by progenitor cell proliferation, cell fusion and differentiation. Nevertheless, this fantastic regenerative process cannot successfully counteract skeletal muscle chronic degeneration. Several animal models were generated or discovered in animal colonies mimicking the human diseases. In the early 60' Mauro described for the first time satellite cells, which have been hypothesized as remaining embryonic myoblasts from the developing somites [85]. Several following studies have demonstrated that progenitor cells from the dermomyotome give rise to satellite cells, although it still questionable if those cells are the unique precursors of adult satellite cells. Skeletal muscle regeneration is possible for the ability of satellite cells to react to appropriate stimuli, in which stem cell niche, growth factors and many regulatory molecules, including miRNAs play a major role. After an injury, HGF and IGF activate and stimulate satellite cell proliferation, while specific miRNAs and TGF-β1 negatively influenced this process. In addition, besides satellite cells, a wide range of resident stem cells, seem to be primary involved in the process.

In muscular dystrophies the regenerative machinery is altered and exhausted by degeneration and regeneration cycles. For possible cell-based therapeutic approaches satellite cells are unsuitable since they are not able to cross endothelial barrier, and more than 95% of injected satellite cells die the first days after injection. For these limitations it appears clear that other stem cells, including the MDSCs, MABs, MSCs, ASCs, AFSCs and iPS cells, may turn out to be more suitable for the treatment of skeletal muscle degenerations. Ongoing research will possibly reveal the stem cell niche components of skeletal muscles, with the ultimate goal to obtain biological substrates and molecules to better maintain stem cells in culture. The possibility to generate myogenic stem cells from patient somatic cells is also an attractive strategy to bypass immunological issues. In addition, further elucidation on epigenetic memory could help in the choice of cell sources to be reprogrammed. However, it is largely unknown whether those newly generated stem cells are able to home to the satellite cell niche, prolonging the regeneration capacity of newly formed myofibres. Finally, several recent studies reveal the possibility to use miRNA technology to guarantee a better myogenic commitment of stem cells. Several clinical trials have been already started, involving myoblast or perycite injection from HLA-matched donors. Unfortunately, the current stringent regulatory constraints will prolong significantly the clinical translation of novel approaches, including miRNA and iPS cell technologies for muscular dystrophies. In addition, any stem cell injected into a patient has to be produced under stringent and very expensive GMP (good manufacture practice) conditions. In the nearly future, in order to accelerate translational studies it is critical to promote and support multidisciplinary research networks, involving industrial and academic laboratories (Table 1).

Stem cell	Origin	Characteristics	Preclinical/clinical studies
ES cells	Inner cell mass of the blastocysts	pluripotent stem cells biological and ethical problems teratoma formation graft versus-host disease	on mdx mice [30]
iPS cells	Reprogrammed mouse or human somatic cells	pluripotent stem cells no ethical problems autologous transplantation possible teratoma formation	on mdx mice,alphaSG-null and alphaSG-null/Scid/bg [67,73,74]
Amniotic fluid stem cells	Amniotic fluid	multipotent stem cells no ethical problems easy to isolate and expand in culture	dystrophic mice [44-47]
Muscle -SP Cells	Muscle tissue	multipotent cells high proliferation	Scid/bg immunodeficient mice [48,57,58]
Satellite cells	Muscle fibres, under the basal lamina	high self-renewal high capacity to generate skeletal muscle limited engraftment and migration	Nude mice, pigs, trial on humans [51-54].
Mesoangioblast cells (MABs)	Muscle blood vessels, pericyte	high proliferation *in vitro* no tumor transformation able to pass the endothelial layer	alphaSG-null, mdx/scid mice, GRMD dogs [59-61]; phase I/II clinical trial ongoing.
Bone marrow stromal cells (BMSCs)	Bone marrow and also from different tissues (adipose, tendon, dental pulp, placenta)	multipotent stem cells easy to isolate and expand in culture immunomodulatory properties anti-inflammatory properties low engraftment	mdx mice [56-58] tissue engineering applications (biomaterials, bioreactors, bone implants)
Adipose-derived stem cells (ASCs)	Adipose tissue	very easy to isolate and multipotent cells high proliferation in vitro immunosuppressive properties	mdx mice, small-scale studies on tissue engineering, as BMSCs (bone implants, biomaterials) [38,39,71]

Table 1: Origin and characteristics of stem cells involved in muscle regeneration.

Acknowledgment

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