the FEBS Journal
REVIEW ARTICLE

Perspectives of stem cell therapy in Duchenne muscular dystrophy

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Keywords
Duchenne muscular dystrophy; stem cells; therapy

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(Received 1 October 2012, revised 30 October 2012, accepted 31 October 2012)

doi:10.1111/febs.12083

Muscular dystrophies are heritable and heterogeneous neuromuscular disorders characterized by the primary wasting of skeletal muscle, usually caused by mutations in the proteins forming the link between the cytoskeleton and the basal lamina. As a result of mutations in the dystrophin gene, Duchenne muscular dystrophy patients suffer from progressive muscle atrophy and an exhaustion of muscular regenerative capacity. No efficient therapies are available. The evidence that adult stem cells were capable of participating in the regeneration of more than their resident organ led to the development of potential stem cell treatments for degenerative disorder. In the present review, we describe the different types of myogenic stem cells and their possible use for the progression of cell therapy in Duchenne muscular dystrophy.

Introduction

Skeletal muscle consists predominantly of syncytial fibres with peripheral, post-mitotic myonuclei. In postnatal life, the growth and repair of skeletal muscle fibres is mediated by a resident population of mononuclear myogenic precursors, the satellite cells (SCs), that are located between the sarcolemma and the basal lamina of the muscle fibre [1]. Following muscle injury, these cells divide and give rise to SC progeny, the myoblasts, that fuse to repair or replace the damaged fibres [2]. The progression of activated SCs toward myogenic differentiation is controlled by a family of transcription factors (myogenic regulatory factors; MRFs), including MyoD, Myf5, myogenin and MRF4 [3]. Even if it was considered that quiescent SCs did not express detectable levels of MRFs, Crist *et al.* [4] recently demonstrated that, in these cells, Myf5 mRNA is sequestered in mRNP granules, where the presence of miR-31 ensures silencing. Once SCs are

Abbreviations
DMD, Duchenne muscular dystrophy; GMRD, golden retriever muscular dystrophy; hSM-MSC, human synovial membrane-derived mesenchymal stem cell; iPSC, induced pluripotent stem cell; LGMD2D, limb-girdle muscular dystrophy 2D; MDSC, muscle-derived stem cell; MEC, mesenchymal stem cell; MRF, myogenic regulatory factors; SC, satellite cell; SDF, stromal-derived factor.

activated, mRNP granules dissociate and release Myf5 transcripts, leading to the rapid translation and accumulation of Myf5 protein, which promotes myogenesis [4]. After muscle injury, these cells proliferate and express Myf5 and MyoD [5,6], whereas myogenin is expressed later and is associated with fusion and terminal differentiation [7,8].

Numerous types of muscular dystrophy exist depending on their degree of severity and the muscle types affected [9]. Duchenne muscular dystrophy (DMD), the most common form of muscular dystrophy, is a lethal X-linked recessive disorder caused by a deficiency of dystrophin protein [10]. In the early phase of the disease, the self-renewal potential of DMD SCs is exhausted by a chronic regenerative process. This condition leads to muscular fibrosis in which most muscle tissue is lost and replaced by connective tissue and, consequently, progressive muscle weakness and atrophy arise [11]. Unfortunately, DMD patients are confined to a wheelchair before the age of 12 years and eventually die from heart and respiratory failure [9]. Patients with Becker muscular dystrophy (BMD) develop musculoskeletal symptoms at a much slower rate than those with DMD, strictly depending on mutations of the dystrophin gene and the amounts of dystrophin protein expressed in muscle. Many BMD patients remain ambulatory until the third or fourth decade or later, and develop dilated cardiomiopathy. The identification and characterization of the dystrophin gene led to the development of potential treatments for this disorder [12]. Growth-modulating agents [13], anti-inflammatory or second-messenger signal-modulating agents [14], and powerful molecular devices designed to skip mutations in the dystrophin gene [15,16] were attempted, although only corticosteroids were proven to be effective on DMD patients [17]. In recent years, stem cells received much attention for their potential use in cell-based therapies for various human diseases, such as leukaemia [18] and Parkinson's disease [19]. For several years after their discovery, the SCs were considered to be the only cells responsible for the growth and maintenance of skeletal muscle. However, the pioneristic work of Grounds *et al*. [20] demonstrated that the number of resident SCs in adult muscle is much smaller than the number of committed myogenic precursors populating the muscle tissue soon after injury. It was proposed that SCs could migrate from adjacent fibres or neighbouring muscles, and that nonmyogenic resident cells could be recruited to myogenesis [21]. Recently, several cellular markers were shown to identify and characterize muscular and nonmuscular multilineage stem cells that are able to actively participate in myogenesis. In the skeletal muscle itself, other than SCs, alternative adult multilineage progenitor cell populations retained myogenic potential, such as: muscle-derived stem cells (MDSCs) [22,23], mesoangioblasts [24] and muscle-derived $CD133^+$ progenitors [25], mesenchymal stem cells (MSCs) [26,27] and PW1 interstitial cells [28]. Because the stem cells noted above shared different peculiarities, we proposed that these committed progenitors represent the steps of differentiation of a common undifferentiated stem cell whose function is largely unknown. In this review, we describe several populations of resident and circulating myogenic stem cells and we examine how these cells could ameliorate the progression of the DMD clinical phenotype after stem cell treatment.

Adult stem cells

SCs

SCs are small progenitor cells that lie between the basement membrane and sarcolemma of individual muscle fibres. They originate from somites [29,30], which are spheres of paraxial mesoderm that also generate skeletal muscle, although the exact progenitor that gives rise to SCs remains to be identified. SCs are normally present in healthy adult mammalian muscle as quiescent cells and, when activated by oxidative stress or specific stimuli, they can generate large numbers of new myofibres [31] (Fig. 1A). Starting from the work of Montarras *et al*. [32], several groups isolated pure populations of SCs using a combination of different markers. Cerletti *et al*. [33] isolated a population of skeletal muscle precursors from the SC pool; when transplanted into dystrophic mice, these cells restored dystrophin expression and improved contractile function. Moreover they entered into the SC niche so that they participated in subsequent rounds of injury repair [33]. Sacco *et al*. [34] demonstrated that freshly isolated SCs contributed extensively to muscle repair. Moreover, SCs were derived from the transplantation of one intact myofibre and, after transplantation into dystrophic mice, these cells were able to proliferate, contribute to muscle fibres and self-renew into muscle stem cells [34]. Tanaka *et al*. [35] identified a rare subpopulation of muscle side-population cells and transplanted them into regenerating muscle. Muscle side-population cells engrafted into the host SC niche adhered to isolated myofibres and gave rise both to SCs and myonuclear population [35]. The results obtained in the mouse model led to the investigation of SCs/myoblast injection in DMD patients in phase I clinical trials. Autologous transplantation of genetically corrected

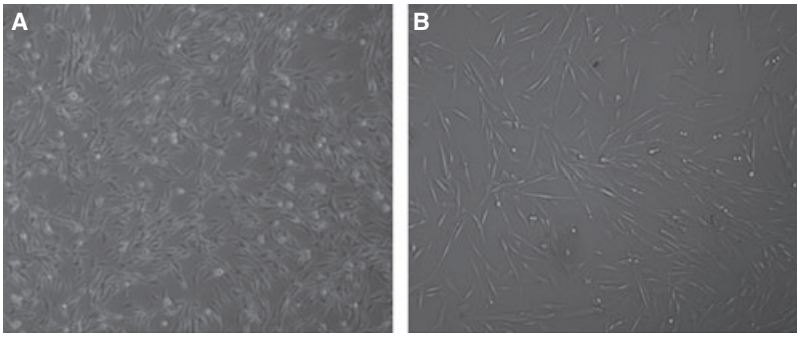

Fig. 1. (A) Human satellite cells isolated from normal muscle tissue in proliferation medium (×10 magnification; DMIR2; Leica Microsystems, Wetzlar, Germany). (B) Human MSC-like cells isolated from normal muscle tissue. Cells were visualized using IMAGEQUEST software (Thermo Fisher Scientific Inc., Waltham, MA, USA) (×10 magnification; DMIR2).

SCs to DMD patients is the theoretical ideal approach for minimizing the host immune rejection of donor cells [36]. However, the growth of freshly isolated SCs *in vitro* significantly reduced their *in vivo* myogenic potential so that it was very difficult to obtain a sufficient quantity of such cells. These trials demonstrated that myoblast transplantation was an inefficient technique, as a result of the very low expression of dystrophin in DMD muscle fibres (approximately 1%), and there was no functional or clinical improvement in the children [37–39].

MDSCs

MDSCs were identified within skeletal muscle: they possessed the ability to self-renew and differentiated into mesodermal cell types. From muscle tissue, Sarig *et al.* [22] isolated a subpopulation of slow adherent myogenic cells that formed a monolayer of $MyoD^+$ cells. They differentiated into mononucleated contractile fibres, expressing muscular proteins such as myosin heavy chain and myogenin. Moreover, when exposed to certain signalling molecules, these cells trans-differentiated into osteogenic and adipogenic cells [22]. Similarly, Tamaki *et al.* [23] isolated $CD34^-$ $CD45^-$ cells from skeletal muscle: following transplantation, they exhibited the capacity to proliferate into myogenic, vasculogenic and neural cell lineages. Interestingly, Qu-Petersen *et al.* [40] isolated three different subpopulations of cells from skeletal muscle: although two of them exhibited SCs-like characteristics, the third population comprised cells that retained their phenotype for several passages and showed the ability to differentiate into muscle, endothelial and neural lineages.

The crucial question arose as to whether these cells contained a subpopulation of committed muscle stem cells that display a better ability to regenerate skeletal muscle compared to muscle SCs. We demonstrated that $Sca\text{-}1^+CD34^+$ stem cells purified from the muscle tissues of newborn mice were multipotent *in vitro* and differentiated into both myogenic and multimyeloid lineages. Following intra-arterial transplantation into dystrophic mice, they adhere to the endothelium of microvessels of host muscles and participate in muscle regeneration [41]. Alessandri *et al.* [42] identified a subpopulation of muscle-derived cells different from SCs. The cells expressed desmin, vimentin and CD133, whereas they were negative for endothelial and haematopoietic markers such as CD45, Von Willebrand factor and Ve-cadherin. Moreover, they were able to differentiate *in vitro* into skeletal muscle fibres, expressing actin and desmin, astrocytes and neurones [42]. Among the muscle-derived stem cells, a poorly adherent subfraction was isolated, which generated a heterogeneous population composed of spindle-shaped flat cells and a low percentage of round cells that performed atypical division pattern. These cells were called MuStem cells [43]. They expressed the SC-markers Pax7, CD56 and β1-integrin, as well as the myogenic regulatory factors Myf5 and MyoD, and it was suggested that MuStem cells originated from the SC niche and corresponded mainly to early myogenic progenitors. Their capacity of differentiation was not limited to myogenic lineage, and they gave rise to osteocytes, adipocytes and interstitial cells [43]. When transplanted into muscles of golden retriever muscular dystrophy (GRMD) dogs, MuStem cells were abundantly detected in recipient muscles, recovered the expression of dystrophin and generated SCs. Importantly, following intra-arterial injection of MuStem cells, numerous $dystrophin^+$ fibres clustered over the entire section of several muscles. Accordingly, Rouger *et al.* [43] suggested that the transplantation of MuStem cells could exert an important role in the regeneration of dystrophic muscles even

if, before extending these results to prospective human trials, a more detailed functional characterization of the phenotype of treated GRMD dogs should be required.

Although other studies are needed to better understand the physiological location of MDSCs, human MDSCs represent an alternative source for isolating pluripotent stem cells with respect to the development of cell-based therapies because they are easy to proliferate *in vitro* and to migrate through the vasculature [44,45].

Mesoangioblasts

Mesoangioblasts are multipotent progenitors of mesodermal tissues, physically associated with the embryonic dorsal aorta in avian and mammalian species [46]. Brunelli *et al.* [47] demonstrated that clonal mesoangioblast lines expressed α-smooth muscle actin and also that they were induced to differentiate into mature smooth muscle expressing smooth muscle myosin upon treatment with transforming growth factor-β. Moreover, Dellavalle *et al.* [48] isolated pericyte-derived cells from human muscle that, when transplanted into dystrophic mice, generated various fibres expressing human dystrophin. These myogenic progenitors were found to be associated with microvascular walls and so it was suggested that these cells represented a correlate of embryonic 'mesoangioblasts' present after birth [48]. Mesoangioblasts were transduced with a lentiviral vector expressing human microdystrophin and injected into animal models of DMD. Cossu and Sampaolesi [49] and Sampaolesi *et al.* [50] notably obtained the recovery of dystrophin and also improvements in muscle function and mobility, especially in GRMDs. From a clinical point of view, they attempted to ameliorate the migration ability of mesoangioblasts by exposing them to stromal-derived factor (SDF)-1 tumour necrosis factor-α and by enhancing the expression of cytokines such as α4-integrins and L-selectin [51]. In this way, and after injection into dystrophic mice, these mesoangioblasts allowed the reconstitution of more than 80% of α-sarcoglycan-expressing fibres, with a five-fold increase in efficiency compared to control cells [51]. More recently, Tedesco *et al.* [52] treated mdx-derived mesoangioblasts with a human artificial chromosome vector containing the entire (2.4 Mb) human dystrophin genetic locus. Once transplanted into dystrophic mice, genetically corrected cells engrafted robustly and differentiated into dystrophin-positive muscle fibres and muscle-SCs, giving rise to a morphological/functional amelioration of the dystrophic phenotype that lasted for up to 8 months after transplantation [52].

Based on the evidence suggesting that mesoangioblasts ameliorated the pathological phenotype of different animal models of muscular dystrophy [53], Tedesco *et al.* [54] studied the effects of these cells in related diseases, such as limb-girdle muscular dystrophy 2D (LGMD2D). Because LGMD2D patients have a reduced numbers of pericytes, such that it could be impossible to obtain sufficient mesoangioblasts for autologous cell therapy, fibroblasts and myoblasts were reprogrammed from LGMD2D patients. Accordingly, human-induced pluripotent stem cells (iPSCs) were generated, leading to the development of a protocol for the derivation of mesoangioblast-like cells from these iPSCs. Once obtained, the iPSC-derived mesoangioblasts were genetically corrected *in vitro* and transplanted into α-sarcoglycan-null immunodeficient mice, generating α-sarcoglycan$^+$ muscle fibres [54]. Because it is well known that mesoangioblasts could be useful for treating DMD, as well as also other forms of muscular dystrophies, these cells are currently undergoing a phase I/II clinical trial (EudraCT no. 2011-000176-33).

Blood- and muscle-derived CD133$^+$ cells

CD133 is a member of a novel family of cell surface glycoproteins [55–57] and was recognized to be the homologue of mouse prominin-1. Miraglia *et al.* [56] and Yin *et al.* [57] identified, for the first time, the expression of CD133 antigen in haematopoietic system-derived CD34$^+$ stem cells. Subsequently, CD133 expression was demonstrated in several different tissues, including retinoblastoma [56], myogenic cells [58], endothelial progenitors and foetal brain neural stem cells [59]. In particular, we isolated and characterized a novel stem cell population (i.e. human circulating CD133$^+$ cells) that restored dystrophin expression and eventually regenerated the SC pool in dystrophic scid/mdx mouse [58].

More recently, we compared the behaviour of two distinct CD133$^+$ cell populations isolated from blood and skeletal muscle tissues, and we characterized their ability to express an exon-skipped version of human dystrophin after transduction with a lentivirus carrying a construct designed to skip exon 51 [60] (Fig. 2). Transplanted into scid/mdx mice, these cells differentiated into muscular and endothelium lineages [25]. In particular, they allowed the expression of a functional human dystrophin and restructured the dystrophin-associated protein complex, as shown by plasmalemmal re-expression of α- and β-sarcoglycans proteins. Muscle-derived CD133$^+$ cells showed a better muscle regeneration compared to the results obtained with

blood-derived stem cells [25]. Interestingly, we assessed the possible SC-like differentiation of transplanted cells because they were located beneath the basal lamina and distributed along freshly isolated fibres, expressing M-cadherin. In parallel, we planned a Phase I clinical trial injecting autologous muscle-derived CD133 stem cells into DMD muscles. Transplanted cells remained as mononucleated cells around vessels and muscle fibres in the dystrophic muscle environment, promoting an increase in the number of capillaries per muscle fibres [58,61]. More importantly, we showed, for the first time, that intramuscular transplantation of muscle-derived $CD133^+$ stem cells in DMD muscle patients is a safe and feasible procedure [58].

Although the proliferation rate of these cells and the strategy for delivering myogenic cells to the affected muscles need to be ameliorated, these results could represent a first step in future clinical trials for DMD based on the autologous transplantation of engineered $CD133^+$ stem cells.

A major limitation in these studies is the heterogeneity of stem cell populations because all cells express two or three antigens and this intrinsic characteristic determines the efficacy (or not) in clinical protocols [62]. For this reason, another important point supporting muscular tissue functionality is the ability to recruit resident and circulating progenitors for myogenesis: in this sense, $CD133^+$ cells were characterized for their regenerative potential *in vivo*, as well as their ability to repopulate the satellite cell niche.

Negroni *et al.* [63] identified a highly myogenic $CD133^+CD34^+$ subpopulation. They demonstrated that, in the interstitial space of muscle injected with these cells, more human mononuclear cells remained compared to human myoblasts. The percentage of undifferentiated interstitial cells confirms that all $CD133^+$ stem cells are not at the same stage of myogenic commitment, and that some of cells never advance to terminal differentiation [63].

$CD133^+$ cells and mesoangioblasts displayed similar behaviours under experimental conditions: mesoangioblasts [24] and $CD133^+$ cells [64] share the ability to migrate through the vasculature, meaning that these cells are good candidates for cell therapy. Such evidence suggests that there may be a probable connection between the origin and development of these two kind of progenitors cells.

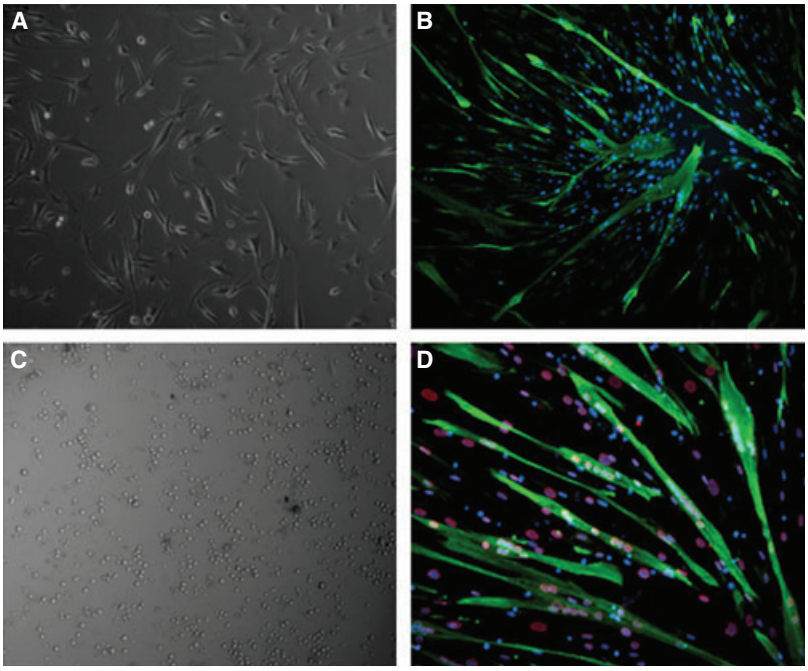

Fig. 2. (A) Human $CD133^+$ stem cells isolated from normal muscle tissue in proliferation medium. Cells were visualized using IMAGEQUEST software (Thermo Fisher Scientific Inc.) (×10 magnification; DMIR2). (B) Muscle-derived $CD133^+$ cells were plated in differentiation medium and differentiated into myotubes expressing myosin heavy chains (green). (C) Human $CD133^+$ stem cells isolated from normal blood in proliferation medium (×10 magnification; DMIR2). (D) Blood-derived $CD133^+$ stem cells were plated in the presence of murine myotubes. Human cells expressing lamin A/C (red) fused with murine myotubes expressing myosin heavy chains (green), giving rise to multinucleated heterozygous murine/human myotubes (green).

MSCs

'Bona fide' MSCs are isolated from adult and foetal bone marrow, and comprise clonogenic and adherent cells. Moreover MSC-like cells were found to reside in several host tissues and organs [65–67] and differentiated not only into osteogenic, chondrogenic and adipogenic lineages, but also into other mesodermal (myocyte, endothelium, cardiomyocyte), ectodermal (neuronal) and endodermal (hepatic, pancreatic, respiratory epithelium) lineages (Fig. 1B). Recently, human MSC-like cells were isolated from healthy muscle tissue biopsies [68] and surgical waste tissues [69]. Because they were obtained with non- or minimally invasive-biopsy procedures, skeletal muscle could be an important clinical source of MSC-like cells for therapeutic applications [70]. MSCs migration to injured tissue is strictly controlled by several chemokine signals. SDF-1 is an ubiquitous MSC chemo-attractant associated with several diverse tissue injuries [71–73]. SDF-1 is able to stimulate the homing of stem cells to the areas of hypoxia: in myocardial infarction or stroke, SDF-1 was associated with the mobilization of stem cells to the periphery and homing to the site of injury [74,75]. Goncalves *et al.* [76] genetically modified human mesenchymal stem cells with a full-length dystrophin coding sequence so that these cells were able to participate in myogenesis through cellular fusion. Based on this important evidence, Gang *et al.* [77] isolated MSC-like cells from human umbilical cord blood and showed that they differentiated into skeletal muscle, rather than osteoblasts and adipocytes [78,79]. Cultured in myogenic medium, more than half of umbilical cord blood-MSC-like cells were positive for myosin and expressed myogenic markers such as MyoD and myogenin [77]. As the development of inflammation and, consequently, the chronic wound healing response is one of the most important problems in dystrophic patients, the use of mesenchymal stem cells as inhibitors of inflammation is conceptually appealing.

Riordan *et al.* [80] demonstrated that, in the bone marrow, one of the main functions of MSCs is the protection of haematopoietic precursor from inflammatory damage. Nemeth *et al.* [81] showed that these cells were capable of inhibiting one of the most potent inflammatory processes, septic shock, through modulation of macrophage activity. Furthermore, it was reported that the injection of syngeneic (or in some cases allogeneic) MSCs inhibited chronic inflammatory processes in animal models of autoimmune arthritis and diabetes [82,83], multiple sclerosis [84,85] and lupus [86]. Different studies have explained the multifactorial roles played by MSCs in controlling inflammation. Song *et al.* [87] described the role of synovial fluid in regulating the migration of MSCs and their control of the inflammation process in chronic inflammatory diseases such as rheumatoid arthritis. Rafei *et al.* [88] studied the behaviour of MSCs in reversing symptomatic neuroinflammation in experimental autoimmune encephalomyelitis and found that their role was exerted through the paracrine conversion of CCL2. Subsequently, MSCs were used in DMD patients because, in addition to their anti-inflammatory activity, they possessed the ability to fuse with recipient muscle fibres and genetically complement dystrophic muscle, allowing the production of trophic factors stimulating the activity of endogenous cells [89]. As described above, DMD pathology is characterized by an irreversible muscle degeneration caused by a progressive decrease in the number of SCs. Accordingly, the most important aim of transplanted stem cells could be replenishment of the SC compartment and restoration of the regeneration potential necessary for muscle tissue homeostasis and repair [40,90,91]. De Bari *et al.* [92] demonstrated that adult human synovial membrane-derived mesenchymal stem cells (hSM-MSCs) had myogenic potential *in vitro*. More recently De Bari *et al.* [93] transplanted hSM-MSCs into nude mouse muscles and found that these cells contributed to myofibres and functional SC formation. It was also shown that their differentiation was regulated by specific cues because hSM-MSCs were found in several tissues of the recipient animal but differentiated only within skeletal muscle. Injected into mdx mice, they ameliorated the dystrophic phenotype of these mice and restored the expression of dystrophin [93].

Conclusions

Subsequent to the discovery of the dystrophin gene, it was assumed that characterization of the molecular defects causing DMD and the association with dystrophin would result in the development of a therapy for this neuromuscular disorder. Unfortunately, we have yet to find an effective therapy for the dystrophic process. Although numerous approaches have been investigated, many suffer from a variety of drawbacks. Stem cell therapy is an attractive method for treating muscular dystrophy because only a small number of cells, together with a stimulatory signal for expansion, is required to obtain a therapeutic effect. From a clinical point of view, a candidate stem cell must possess a high rate of proliferation and remain capable of efficient myogenic conversion [36]. As described above, a stem cell population with myogenic potential at various

stages of development has been found and characterized from multiple regions of the body.

Unfortunately, one of the most important problems to be overcome is survival and subsequent migration from the site of injection to the compromised muscles of the body. Determination of the mechanisms involved in the muscle homing of stem cells will aid the development of a potential therapy for muscular dystrophies based on the systemic delivery of stem cells. Accordingly, several research groups have described the role of adhesion molecules in mediating both rolling and arrest *in vivo* in blood vessels [94,95], whereas others have revealed certain mechanisms and identified specific growth factors that allow the survival and proliferation of transplanted stem cells [96]. Mesoangioblasts [24], muscle side-population [97] and blood derived $CD133^+$ [64] have the ability to migrate through the vasculature, whereas most other suitable stem cells do not. Recently, Doherty *et al.* [98] demonstrated that pericytes, which are endothelial-derived cells that line the capillaries, have osteogenic potential. Moreover, they showed that pericytes represent a population of primitive precursors cells or multipotent mesenchymal progenitors because they are capable of differentiating into several cellular lineages [98]. If pericytes took part in myogenic regeneration, this could explain the widespread distribution of atypical stem cell populations with myogenic potential [36]. According to the results obtained in animal models [99,100], SCs were used in cell-based therapy of muscular dystrophies, although no promising results were obtained. Nevertheless, Skuk *et al.* [101] reported a study conducted in a 26-year-old DMD patient. The patient was immunosuppressed with tacrolimus and received normal muscle-precursor cells in biceps brachii: following implantation of stem cells, 27.5% of the myofibre profiles expressed donor-derived dystrophin 1 month post-transplantation and 34.5% expressed donor-derived dystrophin 18 months post-transplantation. The method used in this trial was termed a 'high-density injection' protocol but, unfortunately, it is applicable only to skeletal muscles accessible from the body surface [101]. In a double-blind phase I clinical trial, we transplanted autologous $CD133^+$ cells extracted from muscle biopsies by intramuscular injection into eight boys with DMD and took samples after 7 months. The experimental plan aimed only to test the safety of implanted cells and no adverse effects were reported [102]. The most important goal of therapeutical approaches is the reconstruction of functional tissues in skeletal muscles that have been severely replaced by fat and fibrosis, offering the possibility of restoring strength in advanced and severely affected patients. A clear possibility is that the effective management and therapy of DMD could only be achieved through a combination approaches, such as cellular therapy associated with gene therapy or pharmacological treatment. We applied this concept in the DMD field: we used an exon-skipping technique to allow the expression of human dystrophin within the DMD $CD133^+$ cells to permit the use of the patient's own stem cells, thus minimizing the risk of immunological graft rejection [103]. Van Deutekom *et al.* [104] described the safety and local dystrophin-recovery achieved following a single intramuscular injection of the antisense nucleotide PRO051 that was specifically designed to skip exon 51. The limited studies performed so far suggest that intramuscular injection of myoblasts [105–107] and, above all, muscle-derived $CD133^+$, appeared to be safe [102]. However, in our opinion, the intra-arterial injection of a patient's own transduced stem cells is the best way to treat degenerative muscular diseases such as DMD. Indeed, the systemic distribution of the cells is the only way to deliver them to the whole body musculature and, consequently, treat severely affected patients who have a reduced body mass. Whatever strategy is used, additional efforts will be necessary aining to increase the proliferation and capacity of stem cell migration and the amelioration of safety procedures with respect to gene modifications.

Acknowledgements

This work was supported by the Association Monégasque contre les Myopathies (AMM), Associazione La Nostra Famiglia Fondo DMD Gli Amici di Emanuele, the Associazione Amici del Centro Dino Ferrari, EU's Framework programme 7 Optistem 223098 and Provincia di Trento Fondo 12-03-5277500-01. Conflict of interests no longer exist.

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