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Mesenchymal Stem Cells as Muscle Reservoir

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Abstract

Stem cell therapy is an attractive method to treat muscular dystrophy because only a small number of cells, together with a stimulatory signal for expansion, are required to obtain a therapeutic effect. Recently, it was published the isolation of human MSC-like cells from adult skeletal muscle. The aim of this review is to describe the recent progress in understanding MSC biology and their therapeutic applications. Given that these cells can be obtained with non- or minimally invasive-biopsy procedures, there is growing evidence that skeletal muscle may be an important clinical source of MSCs for use in therapeutic applications. Various in vitro and in vivo studies have evaluated the safety, feasibility and efficacy of transplanting MSCs for clinical trials. Currently, there are many registered clinical trial sites for evaluating MSC therapy throughout the world.

Keywords: Muscle; Mesenchymal stem cells; Muscular dystrophies; Cell therapy

Introduction

Skeletal muscle consists predominantly of syncytial fibers with peripheral, postmitotic myonuclei. Its repair potential in adulthood is due on the presence of a resident population of undifferentiated cells, named satellite cells. In mature skeletal muscle, most satellite cells are quiescent and are activated in response to injury; their activation mediates muscle regeneration. After division, satellite cell progeny, termed myoblasts, undergo terminal differentiation and become incorporated into muscle fibers [14]. Myogenesis is regulated by a family of transcription factors (myogenic regulatory factors [MRFs]), including MyoD, Myf5, myogenin, and MRF4 [103]. During muscle repair, satellite cells recapitulate the MRF expression program characteristic of embryonic development. Quiescent satellite cells do not express detectable levels of MRFs. After muscle injury, these cells proliferate and express Myf5 and MyoD [23,24]. Myogenin is expressed later and is associated with fusion and terminal differentiation [65,120].

Muscular dystrophies are a group of diseases characterized by the primary wasting of skeletal muscle. In many cases they are caused by mutations in the proteins that form the link between the cytoskeleton and the basal lamina [26], such as dystrophin in Duchenne Muscular Dystrophy (DMD) and Becker Muscular Dystrophy (BMD). Myoblasts represented the natural first choice in cellular therapeutics for diseases affecting skeletal muscle because of their intrinsic myogenic commitment [52]. However, myoblasts are recovered in low number from DMD muscle biopsies, are poorly expandable in vitro, and rapidly undergo senescence [25]. An alternative source of muscle progenitor cells is desirable. In the last years, stem cells received much attention for their potential use in cell-based therapies for various human diseases. According to these observations, various stem cells were used to treat muscular dystrophies, even if with limited advantage. Recently, different papers reported the isolation and characterization of mesenchymal stem cells (MSCs) from the synovial membrane (SM) of adult human donors [32]. Mesenchymal stem cells (MSCs) reside within the stromal compartment of bone marrow. They play a fundamental role in providing the stromal support system for haematopoietic stem cells in the marrow and constitute a very small fraction, 0.001–0.01% of the total population of nucleated cells in marrow [90]. Although they represent such a minor fraction, MSCs can be plated and enriched using standard cell culture techniques. In culture they show a fibroblastic morphology and adhere to the tissue culture substrate; primary cultures can be maintained for 12-16 days, during which time the non-adherent haematopoietic cell fraction is depleted. Isolation of MSCs from *in vivo* sources relies primarily on adherence to plastic and differentiation potential [90]. Although the purpose and function of MSCs *in vivo* have not yet been elucidated, the properties of MSCs are being exploited for therapeutic purposes. The aim of this review is to describe the recent progress in understanding MSC biology and their therapeutic applications. Moreover, we would want to highlight the work that has been done to characterize in particular the muscle-derived MSCs and their use in tissue engineering and regenerative medicine.

Isolation of MSCs

MSCs reside in diverse host tissues and organs, such as circulating blood, adult and foetal BM, spleen, amniotic fluid, cartilage, muscle tendons, placenta, adipose tissues, fetal tissues, periosteum, synovial fluid, thymus, trabecular bone, dermis, lung [1,11,69] and deciduous teeth [76]. Moreover, they differentiate not only into osteogenic, chondrogenic and adipogenic lineages but also others such as the mesodermal (myocyte, osteocyte, endothelium, adipocyte, cardiomyocyte), ectodermal (neuronal) and endodermal (hepatic, pancreatic, respiratory epithelium) lineages. In all cases the cells were shown to differentiate along several defined pathways.

Heterogeneity of MSCs

MSCs express CD44, CD73, CD90 and CD105 receptors while lacking hematopoietic stem cell markers such as CD14, CD31, CD33, CD34 and CD45. In addition, non-haematopoietic progenitor bone marrow stromal cells reacted with Stro-1 antibody while an antigen

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present on undifferentiated MSCs reacted with the SB-10 antibody [108]. Interestingly, it disappeared once the cells embarked upon the osteogenic pathway and began to express cell surface alkaline phosphatase [17]. Due to the lack of specific mesenchymal cell markers and the heterogeneity of the MSC populations, the Mesenchymal and Tissue Stem Cell Committee of the ISCT established three minimal criteria that MSCs isolated from human bone marrow and other mesenchymal tissues must have in vitro: i) adherence to plastic in standard culture conditions, ii) display of a specific surface antigen expression pattern (CD73+ CD90+ CD105+ CD34- CD45- CD11b- CD14- CD19- CD79a- HLA-DR-), and iii) multipotency, that is differentiation potential along the osteogenic, chondrogenic and adipogenic lineages should be demonstrated [67]. The heterogeneity of the MSC population is revealed by in vitro differentiation assays, where most of the population shows a differentiation potential towards one or two different cell types, whereas only very few cells harbor a tripotent differentiation capacity. The state of the art technology to isolate MSCs remains adherence to a plastic surface, followed by cytofluorometric analyses to determine the absence of HSC markers (CD34- CD117-), mature T-cell (CD45- CD3-), B-cell (CD19-) and monocyte (CD11b- CD14-) markers, as well as of co-stimulatory molecules (CD80- CD86-) in the proliferating population. MSCs express, however, HLA-ABC but not HLA-DR antigens.

Differentiation potential of MSCs

As mentioned above, MSCs can differentiate in a large variety of human tissues (Figure 1). They assume an osteoblastic morphology when cultivated in the presence of β-glycerol-phosphate, ascorbic acid-2-phosphate, dexamethasone and fetal bovine serum. As the MSCs are grown in a three-dimensional format with a serum-free nutrient medium and with a member of the TFG-β super-family, the cells rapidly lose their fibroblastic morphology and begin to initiate expression of a number of cartilage-specific extracellular matrix components, acquiring a chondrogenic phenotype [9]. Different molecules such as peroxisome proliferator-activated receptor- γ (PPAR- γ) and fatty acid synthetase can induce the adipogenic differentiation of MSCs, while IL-1 and TNF-α are suppressors. MSCs cultured in monolayer in the presence of isobutylmethylxanthine become adipocytes, forming large lipid-filled vacuoles. Moreover, when treated with 5-azacytidine and amphotericin B, MSCs can differentiate into myoblasts that fuse into rhythmically beating myotubes [9]. As published by Majumdar et al., MSCs express a large spectrum of cell adhesion molecules of potential importance in cell binding, homing interactions and migration of leukocytes, such as CCR1, CCR4, CCR7, CCR10 and CXCR5 [72]. They also exhibit high expression of integrins. Moreover, it was recently shown that MSCs express some of chemokine receptors and their chemokine ligands that are essential components involved in the migration of leukocytes into sites of inflammation.

Correlation between MSCs and pericytes

Numerous experiments consistently suggested that MSCs are associated with the vasculature. MSC frequency could correlate with blood vessel density as the quantity of MSC per nucleated marrow cells decreased while vascular density also decreases with age [19] and adipose-tissue derived MSCs were much higher when tissue was highly vascularised [30]. Together with the fact that MSCs can express molecular markers such as NG2, Sca-1, α-SMA and PDGFβ-R [29], it was proposed the close correlation between MSCs and pericytes, multifunctional perivascular cells surrounding microvessels (arterioles, capillaries, and venules) [27,29,109]. Many studies revealed the potential plasticity of pericytes: they differentiated into a variety of mesenchymal populations ranging from osteoblasts, chondrocytes and adipocytes to fibroblasts [35,40]. In addition, pericytes located on the capillaries of the adult CNS responded to bFGF and differentiated into neural lineages expressing markers characteristic of pericytes, neurons and glial cells [37]. Under hypoxia stimulation, pericytes differentiated into smooth muscle cells [15,36]. More interestingly, pericytes isolated from muscle and non-muscle tissues from humans, are also myogenic in culture and in vivo [28], and transplantation of these culture-expanded pericytes was able to improve impaired cardiac function in vivo [27]. Progenitor cells of tissues such as bone, cartilage, adipocytes, myelosupportive stroma are easily isolated from

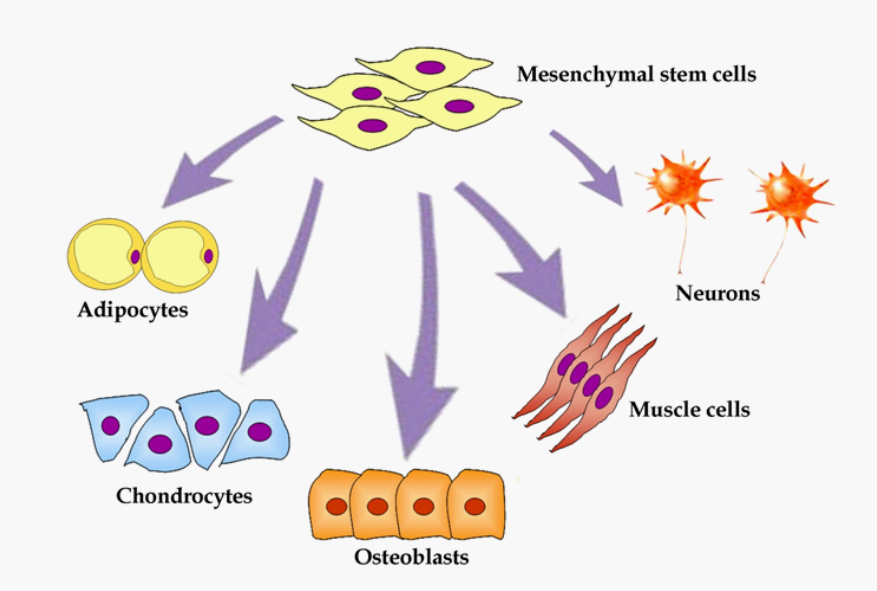

Figure 1: Differentiation potential of mesenchymal stem cells. MSCs can differentiate in a large variety of human tissues including osteogenic, chondrogenic, adipogenic and neuronal lineages. Recently, it was also demonstrated the presence of human MSC-like cells in adult skeletal muscle.

the bone marrow stroma as adherent, clonogenic, fibroblast-like cells (colony-forming unit-fibroblast, CFU-F). Recently, different groups demonstrated the multipotential nature of an individual CFU-F [2,55] so that arose the possibility of a marrow stromal "stem" cell feeding into multiple lineages, together composing the "stromal system" [86]. It is possible that the bone marrow (BM) includes skeletal progenitor stem and the BM stroma provides cues for homing, maintenance, proliferation, and maturation of haematopoietic progenitors. As an example of the multipotentiality of the stromal cells, Westen-Bainton (WB) cells represent the physical substrate of the haematopoietic microenvironment and provide an adventitial covering to marrow sinusoids. Moreover, they are able to express different markers of osteogenic commitment and can accumulate lipid and convert to adipocytes in vivo [12]. According to these experimental data, WB cells possess two interchangeable phenotypes, as they can differentiate in vivo into myelosupportive stromal cell and adipocytes, two "lineages" in the stromal system. Due to experimental limitations, the in situ counterpart of CFU-Fs remained unknown until the work of different labs suggested that WB cells could correspond to explanted clonogenic stromal progenitors or bone marrow stromal cells [53,54,61,62]. Moreover, the anatomical position of WB cells, their expression of the antigen CD146 and more in general their antigenic and trascriptomic profile, made them the exact counterpart of a family of cells that in other tissues are defined pericytes [53,54,61,62]. On the basis of the observations of Diaz-Flores regarding the role of pericytes in various tissues as tissue progenitors [34], the bone marrow stromal cells could represent a local subset of microvascular progenitors.

Potentiality of adipose-tissue derived MSCs

Following the emerging field of regenerative that required new sources of stem cells, MSCs with similar characteristics to bone marrow-derived MSCs were isolated from different tissues in addition to bone marrow stroma [78]. Adipose tissue was found to be an abundant and easily accessible source of adult stem cells, termed adipose-derived stem cells (ASCs), able to differentiate into adipogenic, osteogenic, chondrogenic and myogenic cells [127,128]. Together with the fact that ASCs could be manufactured in accordance with current Good Manufacturing Practice guidelines, these cells seemed to be ideal for clinical applications. ASCs can be obtained from adipose tissue using collagenase digestion: when freshly isolated, fASCs were reported to be heterogeneous, as they included not only a significant number of CD44+ and CD105+ multipotent stem cells, but also hematopoietic cells expressing CD11b, CD34 and CD45; endothelial cells positive for CD31 and smooth muscle cells (SMCs) expressing smooth muscle actin [6]. However, cultured ASCs were homogeneous, being all positive for the stem cell markers CD44 and CD105, but negative for CD11b, CD45, CD34 and CD31 [6]. These findings demonstrated that care must be taken in the manipulation and culture of ASCs and that fASCs could be safer and more practical than cultured ASCs for clinical use. ASCs were investigated for their potential of *in vivo* tissue regeneration: in a rat ischemic model, transplantation of ASCs improved the vascular supply [75,77] and ameliorate myocardial regeneration [18,118]. Fortunately, no authors reported cases of arrhythmia or tumorigenesis in any studies regarding the myocardial regeneration of ASCs. Human ASCs were described to express four types of ion channels including delayed rectifier-like K+ current , Ca2+ activated K+ current, transient outward K+ current, and TTX-sensitive transient inward sodium current and in any case their transplantation increased electrical stability of the heart [7]. Moreover, administration of ASCs onto skin ulcers accelerated the healing process of the skin wound [79]. Recently, several groups demonstrated the ability of hASCs to differentiate into muscle *in vitro* [70,100] while i*n vivo* studies showed that implantation of ASCs in *mdx* mice restored dystrophin expression in murine cells [99]. The contribution of ASCs to muscle regeneration could be related to *de novo* generation of muscle-specific cells from themselves or modification in gene expression after their direct fusion with host cells. Vieira and collaborators co-cultured hASCs with human DMD myoblasts and myotubes observed that the cells fused into myoblasts as well as myotubes and expressed dystrophin [119]. Interestingly, they also found some similarities between ASCs and mesoangioblasts, regarding their fibroblast-like morphology, their efficiency of proliferation and their protein expression. However, while mesoangioblasts are not easily obtainable, human liposuctioned fat is available in large quantities.

MSCs and skeletal muscle

Recently, it was published the isolation of human MSC-like cells from adult skeletal muscle. The muscle tissue used to harvest the cells was obtained from healthy muscle tissue biopsies [125], surgical waste tissue from orthopaedic reconstructions [81], or surgically debrided muscle tissue following orthopaedic trauma [81]. Given that these cells can be obtained with non- or minimally invasive-biopsy procedures, there is growing evidence that skeletal muscle may be an important clinical source of MSCs for use in therapeutic applications [60].

Similar specific differentiation of non-induced MSC into injured tissue has been demonstrated in post-myocardial infarct models [63,93], in stroke [21], kidney damage [94], pulmonary fibrosis [101], and bone fractures [16]. Several chemokine signals appear to be associated with MSC migration to injured tissue. Stromal derived factor (SDF)-1 seems to be a ubiquitous MSC chemoattractant associated with a plethora of diverse tissue injuries ranging from noise induced auditory spiral ligament damage in the cochlea of the ear [113], to burn injury [5,44,64], to bone fractures . Most commonly studied is the critical role of SDF-1 stimulation of stem cell homing to areas of hypoxia. In many injury situations such as myocardial infarction or stroke, SDF-1 has been demonstrated to be associated with mobilization of stem cells into the periphery and homing to the site of injury [89,107].

Transplantation potential of MSCs

The expression of cell adhesion molecules, integrins and chemokine receptors support the idea that MSCs can home to tissues, particularly when injured or inflamed, involving migration across endothelial cell layers where they can enhance wound healing, support tissue regeneration and restore the BM microenvironment. The mechanism by which MSCs home to tissues and migrate across endothelium is not yet fully understood, but it is likely that injured tissue expresses specific receptors or ligands to facilitate trafficking, adhesion, and infiltration. For these reasons, MSCs are used as therapeutic delivery agents to repair damaged tissues [20]. However there are still many questions to be addressed concerning the behaviour of MSCs in transplantation experiments. First of all, host responses to allogeneic MSC therapy has to be defined. The exact signaling events driving MSCs to repair the damaged areas are as yet unknown, even if the expression of chemokine receptors and adhesion molecules exert a fundamental role. Moreover, the response of MSCs to local differentiation signals in vivo has not been clarified. Finally, as MSCs are expanded in large-scale culture for human applications it could be important to identify defined growth media to ensure more reproducible culture techniques and enhanced safety [20].

Various in vitro and in vivo studies have evaluated the safety, feasibility and efficacy of transplanting MSCs for clinical trials [33,69,91]. Cellular transplantation into animal models have demonstrated that

MSC can engraft into organs like liver, bone, lung and kidney after infusion. Several groups used these cells to repair infracted myocardium [59,84,85], some of them injected isolated murine MSCs directly into healthy adult myocardium and noted neoangiogenesis near the injection site within 1 week after transplantation [47]. Transplantation of MSCs together with erythropoietin treatment in rat models of acute myocardial infarction favoured capillary density, reduction of infarct size and fibrotic areas, compared with groups that received only MSC [124]. MSCs were shown to enhance the survival of existing myocytes in mice through paracrine mechanisms [82]. Interestingly, Hofstetter and colleagues injected rat MSCs into the spinal cords of rats rendered paraplegic 1 week after injury. They showed that MSCs formed bundles that bridged the epicenter of the injury, guiding regeneration through the spinal cord lesion, thus promoting recovery [56]. These data open the hypothesis that the beneficial effect of MSCs in sites of injury could not necessarily involve their differentiation into the regenerating tissue type but rather the local production of growth or other factors or physical attributes such as forming guiding strands in the injured spinal cord.

Clinical applicability of MSCs in muscular dystrophies

Thus the ability of MSC to differentiate into various injured tissue, including muscle, as well as ability to complement dystrophin deficiency [49], makes them an attractive therapeutic candidate for DMD. The use of mesenchymal stem cells as inhibitors of inflammation is conceptually appealing. In the bone marrow it has been speculated that one of their main functions is the protection of hematopoietic precursor from inflammatory damage [98]. This potent activity of MSC is best exemplified in an experiment where these cells were capable of inhibiting one of the most potent inflammatory processes, septic shock. The investigators demonstrated that administration of bone marrow-derived MSC was capable of increasing survival in the lethal cecal-puncture ligation murine model through modulation of macrophage activity [80]. Moreover, inhibition of chronic inflammatory processes such as models of autoimmune arthritis and diabetes [42,71], multiple sclerosis [22,96], and lupus [126], was well documented by syngeneic, or in some cases allogeneic MSCs. Mechanistically, MSCs play multifactorial roles in controlling inflammation. They have the ability to selectively home towards damaged tissue via expression of receptors for SDF-1, lysophosphatidic acid [110], and CCL2 [97]. Given the regenerative and anti-inflammatory effects of MSC, several studies have used this population in animal models of DMD [58].

DMD pathology is also characterized by a progressive decrease of the number of satellite cells and their decline is related with the age. The depletion of the satellite cell pool, with consequent irreversible muscle degeneration, is believed to be responsible for terminal muscle failure in DMD [25]. The ability of transplanted stem cells to replenish the satellite cell compartment may ensure long term efficacy by restoring the regeneration potential necessary for muscle tissue homeostasis and repair. Different groups demonstrated that injected muscle cells can persist as quiescent satellite cells [3,95,123,87]. Recently, Blau and co-workers showed that BM cells were able to do the same [68]. Moreover, De Bari et al published that a small population of the implanted MSCs isolated from human synovial membrane persisted as functional satellite cells in muscle tissues for over 6 months [32]. Human mononuclear cells recovered from first recipient mice displayed in vitro phenotypic and functional properties of primary myoblasts and retained their myogenic capacity into second recipient mice [32]. In addition, transplanted hSM-MSC restored the dystrophin expression at the sarcolemma of muscle fibers and rescued, at least in part, the capacity of mdx mouse muscle cells to produce MGF, a critical factor controlling local muscle maintenance and repair [121] not detectable in dystrophic mdx muscles [48,122]. Starting from previous work demonstrating their capacity to differentiate into osteoblasts and adipocytes [39,50,102], Gang and collaborators showed that MSCs isolated from human umbilical cord blood (UCB-MSCs) were capable to develop into skeletal muscle [45]. They demonstrated that these cells - during myogenic induction by myogenic medium - can express two fundamental muscle-specific transcription factors, such as MyoD and myogenin. Moreover they observed weak myosin expression in 3-week cultures after myogenic induction and significantly enhanced expression at 6 weeks, when more that half of the cells were myosin-positive [45].

Therapeutic application

Stem cell therapy is an attractive method to treat muscular dystrophy because only a small number of cells, together with a stimulatory signal for expansion, are required to obtain a therapeutic effect [92]. The clinical relevance candidate stem cell population must be easily extracted, must remain capable of efficient myogenic conversion, and when transplanted must integrate into the muscles allowing the functional correction of the dystrophic phenotype [92]. Haematopoietic stem cells were widely used in transplantation experiments, especially in the treatment of leukemia and other cancers [112]. Orlic et al. showed that locally delivered bone marrow cells generated de novo myocardium [85] while Stamm et al. demonstrated a dramatic improvement in global heart function following the delivery of bone marrow cells into the infarct zone in patients suffering for myocardial infarction [111].

Considering the possible common origin between MSCs and HSCs and the similar properties in term of expression of certain markers and capacity of differentiation, some striking examples of the therapeutic use of marrow-derived MSCs were reported, including cardiovascular repair, treatment of lung fibrosis, spinal cord injury and bone and cartilage repair. Saito and co-workers demonstrated that MSCs are tolerated in a xenogeneic environment while retaining their ability to be recruited to the injured myocardium and undergo differentiation to a cardiac phenotype [105]. They also demonstrated the in vivo differentiation of MSCs to a skeletal muscle phenotype [105].

Moreover, although clinical interest in cultured mesenchymal stem cells initially focused on the potential of their stem cell-like properties for tissue regeneration and repair, the discovery of their paracrine properties markedly increased the range of therapeutic applications for which they were studied [83]. Many clinical trials plan the administration of MSCs systemically and assume that MSCs engraft and provide long-term support by either directly replenishing damaged tissue or interacting with neighboring cells to promote endogenous repair. In fact systemic infusion of mesenchymal stromal cells has proved beneficial in different preclinical models of acute lung injury, myocardial infarction, diabetes and multiple sclerosis [10,115]. Although the mechanisms underlying the therapeutic effects of mesenchymal stromal cells in these disease models are not well characterized, probably it's depend also from the release of a combination of multiple molecules with anti-inflammatory, antiproliferative, anti-apoptotic and angiogenic properties. One of the hypothesis is that factors secreted by mesenchymal cells determine a environment that promote repair by local tissue-resident progenitor populations; this behaviour would explain the presence of favourable effects even without prolonged mesenchymal cell engraftment at sites of injury [73,10,115].

These findings, together with the accumulating evidences of the hypo-immunogenic nature of MSCs, prompted clinical studies on the therapeutic potential of mesenchymal stem cells. Allogeneic bone marrow transplantation in children with Osteogenesis Imperfecta resulted in engraftment of donor-derived MSCs and an increase in new bone formation [57]. Infusion of allogeneic MSCs into patients with Hurler's syndrome or metachromatic leukodystrophy showed no evidence of alloreactive T cells and no incidence of graft-versus-host disease (GVHD) [66]. Engraftment of allogeneic MSCs has also been demonstrated in a patient with severe idiopathic aplastic anaemia with improvement of marrow stromal function [43]. On the basis of their immunoregulatory properties, mesenchymal stem cells are also tested for the treatment of graft-versus-host disease, Crohn's disease and some haematological malignancies [4,83,106,114]. One of the major problems that are to be solved concern the standardization of protocols for the isolation of mesenchymal stem cells and their expansion *in vitro* [83].

Clinical trials

Currently, there are 79 registered clinical trial sites for evaluating MSC therapy throughout the world (**http://clinicaltrials.gov/**). The United States has the highest concentration of registered trial than half of the total number indicating strong international interest in MSCs as a potential therapy. The majority of trials are sponsored by academic medical centers exploring novel applications of MSCs to treat conditions as diverse as critical-limb ischemia (NCT00883870), spinal cord injury (NCT00816803), and liver cirrhosis (NCT00420134). Now two other clinical studies, not yet open for participant recruitment, plan the intramuscular injection of mesenchymal stem cell for treatment of children with idiopathic dilated cardiomyopathy and autologous cultured mesenchymal bone marrow stromal cells secreting neurotrophic factors (MSC-NTF), in Amyotrophic Lateral Sclerosis [40] patients. The main aim of the first study is to determine whether intramuscular injection of umbilical cord mesenchymal stem cells can improve the ventricular function of children with idiopathic dilated cardiomyopathy (IDCM). Secondary end-points will be the exploration of the possible mechanism of the improvement of ventricular function in children with IDCM and also the evaluation of the safety of intramuscular injection of umbilical cord mesenchymal stem cell. The second study will evaluate the safety, tolerability and therapeutic effects (preliminary efficacy) of injection of autologous cultured mesenchymal bone marrow stromal cells secreting neurotrophic factors (MSC-NTF), as a possible treatment for patients with ALS at the early and progressive disease stages.

Conclusion

The most important requisite of the cell-based therapeutic approach is to replace damaged cells with stem cells to improve patient's pathology. Mesenchymal stem cells treatments have the potential to ameliorate the symptoms of various neurodegenerative diseases. In recent years, skeletal muscle emerged as a promising tissue source for mesenchymal stem and progenitor cells that can be used in a variety of therapeutic applications. Skeletal muscle is one of the most plentiful tissues in the body, accounting for approximately one third of body weight in a healthy individual [46]. The incredible capacity of muscle to repair itself after injury suggests that it serves as a reservoir for cells that participate in tissue regeneration processes [117]. Several research groups characterized different muscle-derived stem cell populations for their ability to differentiate into multiple cell types, including osteoblasts, adipocytes, chondrocytes, myoblasts and endothelial cells. In addition, there is evidence that these cells exhibit the same trophic properties (i.e., pro-growth, antiinflammation and anti-apoptotic) that are attributed to the pro-regeneration effects of bone marrow-derived MSCs. These cells could be obtained via minimally invasive muscle biopsy from healthy muscle but also from muscle tissue following traumatic injury: MSCs could provide surgeons with clinically versatile populations of stem cells. The wide presence of perivascular cells throughout the body and the peculiarity of the MSCs – that, at the same time, share generic properties but they are tissue-specific – produces a paradox regarding the developmental origin of mesenchymal stem cells. As demonstrated by various authors, tissues of mesodermal origin constitute the motile scaffold of the vertebrate body also including the vascular system. However, mesodermal lineages and tissues neither are rooted into a single embryonic precursor cell – mesenchymal stem cell - nor originate from a single embryonic structure. In fact, skeletogenic, myogenic, and angiogenic/haematopoietic lineages diverge early in development [13].

Various works suggested two possible models: a precursor pericyte could give rise to several tissue-specific pericytes, which will form distinct MSC populations. Alternatively, pericytes from different origins could provide common MSCs with tissue specific functions due to differences in their niche environments [12,13,41,104]. Based on these observations, it was proposed that pericytes serve as a reservoir of multipotent cells that can be recruited from the vasculature as needed to repair the tissue in response to injury. One limitation to the use of muscle-derived stem and progenitor cells in therapeutic application is the lack of standards in the cell types that are currently being investigated. There are at least three distinct populations of cells harvested from human skeletal muscle (i.e., pericytes, myoendothelial cells and traumatized-muscle-derived MPCs) and one murine cell population (MDSC) that have the characteristics of an MSC population. Additionally, there are several other stem cell populations - i.e., mesoangioblasts [74], side population cells [116], and CD133+ cells [88] - that do not meet the requirements for MSCs, and were therefore not included in this review. Finally, there are muscle-specific stem cells (i.e., satellite cells and myoblasts) that are capable of only myogenic differentiation. Each of these cell types is characterized primarily on the basis of their *in vitro* characteristics after they have been harvested from the body. However, it is evident that the phenotype of these cells may shift *in vivo* as they migrate through different tissues and are exposed to different extracellular and environmental signals. The origin of these proliferating cells is not currently known, although it is possible that the population of pericytes serves as a cellular reservoir for MPCs as well as the myoendothelial and satellite cell types. In case of injury, skeletal muscle contains several stem cell types, which can respond to the severity of injury and repair minor muscle damage or remain as a more plastic stem cell type to promote regeneration following major tissue damage. The distinctions made between the MDSCs, myoendothelial cells, pericytes and traumatized-muscled-derived MPCs are based on their in vitro characteristics and species of origin.

Another important question arose regarding the origin of satellite cells. These cells were presumed to be somitic; however, it is surprising that despite a low number of resident, quiescent satellite cells in adult healthy muscle, hundreds of activated - MyoD-positive - satellite cells are seen hours after an injury to the tissue [51]. Moreover, the appearance of muscle cells in a variety of tissues and the work of Emery and collaborators demonstrating that a circulating skeletal myogenic progenitor resided in the bone marrow of adult mice and participated in muscle regeneration [38] provided an indication for potential myogenic precursors in sites other than muscle. The observation that the large majority of clones with the typical morphology of

mouse adult satellite cells were derived from dorsal aorta and not from somites and the demonstration that aorta-derived myogenic progenitors participate in muscle regeneration and fuse with resident satellite cells [31] suggested that a subset of postnatal satellite cells could be rooted in a vascular lineage. It could be important to assess whether these myogenic vascular cells arise from a primordial pericyte or from endothelial, as suggested by the expression of endothelial markers. In more general terms, myogenic precursors found a common denominator in a developmental relationship with the vasculature. Multipotent mesodermal progenitors able to give rise to myogenic and skeletogenic lineages could be formed in development, as perithelial elements physically associated with the outer wall of a forming vessels, directly from an endothelial precursor. In summary, several multipotent adult progenitor cells have been identified in the non-haematopoietic compartment of bone marrow, expressing early endothelial/pericyte markers. Although the molecular characterization of these stem cells is still in progress, their multipotency suggests a possible common origin in the developing vasculature. The vessel wall itself could be the source of mesodermal progenitors: it could be the easiest way to bring progenitors inside any developing tissue and could also explain the presence of rare circulating progenitor cells expressing one of the possible phenotypes [74].

In this review, we have described several applications using stem cells derived from human skeletal muscle to regenerate a variety of tissue types, many of which are nearing clinical translation. Taken together, these findings strongly illustrate the great potential of this approach for the development of therapeutic applications in the near-term, and underscore the critical importance of research efforts directed at elucidating and harnessing the regenerative properties of skeletal-muscle-derived stem and progenitor cells. Among them, although more efforts are needed to better elucidate their origin and to ameliorate their capacity of differentiation, MSCs are one of the most suitable subpopulation for clinical use.

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