Stem and Progenitor Cells in Skeletal Muscle Development, Maintenance, and Therapy

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Satellite cells are dormant progenitors located at the periphery of skeletal myofibers that can be triggered to proliferate for both self-renewal and differentiation into myogenic cells. In addition to anatomic location, satellite cells are typified by markers such as M-cadherin, Pax7, Myf5, and neural cell adhesion molecule-1. The Pax3 and Pax7 transcription factors play essential roles in the early specification, migration, and myogenic differentiation of satellite cells. In addition to muscle-committed satellite cells, multi-lineage stem cells encountered in embryonic, as well as adult, tissues exhibit myogenic potential in experimental conditions. These multi-lineage stem cells include side-population cells, muscle-derived stem cells (MDSCs), and mesoangioblasts. Although the ontogenic derivation, identity, and localization of these non-conventional myogenic cells remain elusive, recent results suggest their ultimate origin in blood vessel walls. Indeed, purified pericytes and endothelium-related cells demonstrate high myogenic potential in culture and *in vivo*. Allogeneic myoblasts transplanted into Duchenne muscular dystrophy (DMD) patients have been, in early trials, largely inefficient owing to immune rejection, rapid death, and limited intramuscular migration—all obstacles that are now being alleviated, at least in part, by more efficient immunosuppression and escalated cell doses. As an alternative to myoblast transplantation, stem cells such as mesoangioblasts and CD133$^+$ progenitors administered through blood circulation have recently shown great potential to regenerate dystrophic muscle.

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INTRODUCTION

From the time of its initial description in 1961, the satellite cell, lying on the plasmalemmal surface of the muscle fiber but beneath the basement membrane surrounding the fiber,[1] was credited with the function of the cell responsible for the growth and maintenance of skeletal muscle, yet it was not proposed that it was, indeed, a stem cell. The gathering of formal evidence that satellite cells are a type of stem cell has been greatly hampered by the fact that the principal defining feature, their anatomical relationship to the muscle fiber, could be established unequivocally only by electron microscopy. However, over the past few years, a number of markers have been described that identify at least the majority of satellite cells. As a result, it has been possible to investigate the functions of this class of cells far more thoroughly. The consensus from a number of studies using these new markers has largely confirmed the initial proposition that the satellite cell is the principal, and possibly sole, source of muscle regeneration in the adult mouse.[2–4] At the same time, it has become clear that cells can occasionally enter the satellite cell position from other sources, in particular from the bone marrow, and participate to some extent in the regeneration of skeletal muscle fibers. When first discovered, this phenomenon caused much excitement because it held the promise of systemic delivery of myogenic cells to all the muscles of the body. Thus far, however, the efficiency of this mechanism has not approached that of the better-known mechanism of myogenesis based on the satellite cell. Furthermore, recent years have seen the partial identification and characterization of multi-lineage stem cells derived in culture from numerous adult tissues. The bone marrow–derived MSC (mesenchymal stem cell, or marrow stromal cell) can differentiate into mesodermal cells, including myoblasts,[5–8] and adult tissues host cells that can also contribute endodermal and ectodermal cell lineages.[9–12] A rare subset of multipotent adult progenitor

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cells, initially identified in adult bone marrow, was assumed to account for this multi-lineage potential.[13] Multipotent stem cells now appear to be broadly distributed, if not ubiquitous, within developed tissues; progenitor cells resembling mesenchymal stem cells have since been identified in the human umbilical cord,[13–15] and multipotent adult progenitor cell–like cells are present in mouse brain,[13] pancreas,[16] and skin dermis,[17] as well as in human skin[18] and white adipose tissue (**Table 1**).[19]

In this article, we first review current knowledge of the cellular and molecular biology of satellite cells. We then digest information pertaining to the myogenic potential of alternative adult multi-lineage progenitor cell populations, emphasizing those recently isolated from the skeletal muscle itself, such as side-population (SP) cells, $CD133^+$ progenitors, and muscle-derived stem cells (MDSCs). Owing to the growing evidence that at least some of these adult multipotent cells are ultimately derived from blood vessel walls, we have also described both the potential for and the interest in myogenesis of mesoangioblasts as well as prospectively purified pericytes, endothelial cells, and myo-endothelial cells. The sum of essential knowledge of satellite cells and less conventional myogenic cells has been, finally, put in the perspective of therapy, principally for Duchenne muscular dystrophy (DMD).

THE SATELLITE CELL, A PROFESSIONAL PROGENITOR CELL IN SKELETAL MUSCLE

Satellite cell distribution in developed skeletal muscle

In adult skeletal muscle, satellite cells reside beneath the basal lamina of muscle, closely juxtaposed to muscle fibers, and make up 2–7% of the nuclei associated with a particular fiber. Satellite cells are normally mitotically quiescent, but are activated (*i.e.*, enter the cell cycle) in response to stress induced by weight bearing or by trauma such as injury.[20] The descendants of activated satellite cells, called myogenic precursor cells, or myoblasts, undergo multiple rounds of division before fusion and terminal differentiation. Satellite cells are distinct from their daughter myogenic precursor cells by biological, biochemical, and genetic criteria.[21] Activated satellite cells also generate progeny that restore the pool of quiescent satellite cells. Although it is clear that the satellite cell is the main source of myogenic cells in day-to-day maintenance of skeletal muscle, it also seems to be the case that satellite cells can move between adjacent muscle fibers and must, therefore, spend some time in the interstitial space during both growth and regeneration.[22,23] Currently, we have no estimate of the proportion of the total myogenic population that occupies this interstitial niche, and it is possible that investigation of the dynamics of this exchange between satellite cells and the interstitial myogenic cells will resolve some of the questions about interstitial myogenic stem cells. Their lack of immortality, as well as their key physiological role in normal post-natal growth of muscle fibers and regeneration after injuries, has made them a unique cell population for cell transplantation protocols that employ cells as gene delivery vehicles or as a source of cells for tissue build-up (see section Myoblast Transplantation As a Therapy for DMD).

Molecular markers of satellite cells

Although there are some marginal discrepancies among the individual markers as to the populations of cells identified as satellite cells, there is broad overall agreement that the majority of quiescent satellite cells in the mouse express low levels of Myf5, and most are positive for Pax7,[24] M-cadherin,[25,26] and CD34.[4,27] Among these, as detailed below, Pax7 seems to be crucial; although expression of this gene is not required for formation and development of pre-natal muscles,[24] it is required for persistence of the satellite cell population during post-natal life,[28,29] Satellite cells also express vascular cell adhesion molecule-1 (VCAM-1),[30] c-*met* (receptor for hepatocyte growth factor), neural cell adhesion molecule-1 (also known as CD56),[20] Foxk1,[31] and syndecans 3 and 4.[32] Several additional novel genes have been recently identified, including *IgSF4*, *neuritin*, *Hoxc10*, *TcR-β*, *Klra18*, *Itm2α*, *G0S2*, and *MEGF10*, that are expressed in satellite cells *in vivo* but are not expressed by primary myoblasts.[33] It has been previously reported that some of these markers are expressed at different levels between quiescent and activated satellite cells.[34]

In humans, the satellite cell markers do not fully correspond to those in the mouse. CD34 does not mark satellite cells in human muscle, and M-cadherin is not so consistent a marker of human as of mouse satellite cells. Among the more reliable markers of satellite cells in human muscle is neural cell adhesion molecule (CD56), which, however, also marks lymphocytes that may enter degenerating muscle in large numbers.[35]

Molecular control of satellite cell ontogeny and function

Early experiments using quail–chick chimeras suggested that satellite cells were derived from the somite.[36] Recent experiments support this work and indicate that the progenitors of satellite cells originate in embryonic somites as Pax3/Pax7-expressing cells.[37,38] However, as detailed below, studies by De Angelis *et al.* and others provided evidence that satellite cells may also be derived from cells associated with the embryonic and adult vasculature.[39] In the adult, results from several laboratories support the notion that satellite cells can be derived from so-called adult stem cells during regeneration.[40–42]

The maintenance of satellite cell numbers in aged muscle after repeated cycles of degeneration and regeneration has been interpreted to support the notion that satellite cells possess an intrinsic capacity for self-renewal.[20] Asymmetric distribution of Numb protein in daughters of satellite cells in cell culture has been implicated in the asymmetric generation of distinct daughter cells for self-renewal or differentiation.[43] However, whether satellite cells are true stem cells or, alternatively, are de-differentiated myoblasts[44] remains unresolved.

Pax7 and the Pax family of developmental-control transcription factors. The paired-box family of transcription factors (Pax1–9) has important functions in the regulation of the development and differentiation of diverse cell lineages during embryogenesis.[45] *Pax7* and the closely related *Pax3* gene are paralogs with almost identical amino acid sequences and partially overlapping expression patterns during mouse embryogenesis.[46,47] Notably, *Pax3* plays an essential role in regulating the developmental program of MyoD-dependent migratory myoblasts during embryogenesis.[48,49] More recently, $Pax3^+/Pax7^+$ progenitors originating in the embryonic somite have been suggested to be the precursors of satellite cells in adult muscle.[37,38]

Table 1 Different types of stem cells investigated

	Developmental derivation	Anatomic localization	Lineage potential	Normal physiologic function	Molecular markers		Fate of human cells transplanted into immunodeficient mice
					M	H	
Myoblasts	Mesoderm	Cells proliferating after the activation of the satellite cells that are attached on the muscle fibers under the basal lamina	Myogenic Osteogenic	Regeneration of skeletal muscle fibers in injured and diseased muscle	$MyoD^+$ $Desmin^+$	$CD56^+$ $MyoD^+$ $Myf5^+$ $Desmin^+$	Fuse with mouse muscle fibers Form satellite cells
SP cells	60% somitic 40% unknown	Interstitial; perhaps associated with blood vessels	Myogenic	Unknown	$CD34^{+/-}$ $Sca\text{-}1^+$ $C\text{-}kit^-$ $CD45^-$ $CD31^{+/-}$	$CD34^{+/-}$ $CD133^{+/-}$ $C\text{-}kit^-$ $CD45^-$	Unknown
MDSC	Unknown	Myofiber periphery closely associated to blood vessels	Myogenic Osteogenic Hematopoietic Cardiogenic Chondrogenic	Unknown	$CD34^{+/-}$ $Sca\text{-}1^{+/-}$ $CD45^-$ $C\text{-}Kit^-$ $BCL\text{-}2^+$	Unknown	Unknown
Myo-endothelial cells	Unknown	Myofiber periphery close to blood vessels	Myogenic Cardiogenic Osteogenic Chondrogenic Other lineages not tested	Unknown	Unknown	$CD34^+$ $CD144^+$ $CD56^+$ $CD31^+$ $CD45^-$	Regeneration of muscle fibers when transplanted into CTX-treated skeletal muscle; improvement of cardiac function when injected into infarcted hearts
Pericytes	Mesectoderm in the head; mesoderm in the body; needs further characterization	Periphery of capillaries and microvessels in all tissues	Myogenic Osteogenic Adipogenic Chondrogenic Other lineages not tested	Blood flow regulation Control of angiogenesis	$\alpha\text{-}SMA^+$	$CD144^-$ $PDGFRB^+$ $CD146^+$ $NG2^+$ $CD34^-$ $CD45^-$	Myogenic when transplanted into CTX-treated skeletal muscle; improvement in cardiac function when injected into infarcted hearts
Mesoangioblasts	Mesoderm; walls of blood vessels	Associated with the microvessel wall	Myogenic (including smooth muscle) Cardiogenic (low) Osteogenic (low) Adipogenic	Unknown	$CD34^+$ $Sca\text{-}1^+$ $CD31^+$ $C\text{-}Kit^{+/-}$ $CD45^-$	$CD34^-$ $CD\ 133^-$ $CD31^-$ $NG2^+$ $CD45^-$	Regeneration of dystrophin + muscle fibers when injected into mdx/SCID mice
$CD133^+$ progenitor cells	Mesoderm	Myofiber periphery close to blood vessels	Myogenic Hematopoietic Vasculogenic (endothelium)	Angiogenesis in injured tissues Hematopoiesis	$CD34^{+/-}$ $Sca\text{-}1^+$	$CD34^{+/-}$ $Thy\text{-}1^{+/-}$ $CD133^+$ $CD146^+$	Regeneration of muscle fibers when transplanted into dystrophic skeletal muscle and promotion of angiogenesis

Abbreviations: MDSC, muscle-derived stem cell; Sca-1, stem cell antigen-1; SCID, severe combined immunodeficiency; SP, side population; M, mouse; H, human; CTX, cardiotoxin.

Pax7 and Pax3 proteins bind similar, if not identical, sequence-specific DNA elements, suggesting that they regulate similar sets of target genes.[50] Furthermore, increased expression and gain-of-function mutations in both *Pax3* and *Pax7* are associated with the development of alveolar rhabdomyosarcomas, indicating that the two molecules regulate similar activities in the myogenic program.[51] Although the Pax3 and Pax7 proteins are structurally similar, analysis of null mutations in mice indicates that they are required for the development of a number of distinct cell lineages[24,52–54] and appear to have only redundant roles in myogenesis.[24,28,37,38,55]

Splotch (*Sp*) mice, lacking a functional *Pax3* gene, do not survive to term and fail to form limb muscles owing to impaired migration of Pax3-expressing cells originating from the somite.[53,56] Compound mutant *Sp/Myf5*$^{-/-}$ mice do not express MyoD in their somites, suggesting that Myf5 and Pax3 function upstream of MyoD in myogenic determination.[49] Forced expression of Pax3 induces MyoD expression and subsequent myogenesis in non-muscle tissues in avian embryos.[48] However, ectopic expression of Pax3 in C2C12 myoblasts efficiently inhibits myogenic differentiation.[57] Co-expression of MyoD and Pax3 is not observed in the mouse myotome.[58] Therefore, Pax3 was suggested to function as an indirect upstream factor that induced migration or other cellular changes to facilitate subsequent induction of *MyoD* transcription.[59] Contrary to this notion, a *Pax3–FKHR* fusion gene was observed to activate many muscle regulatory genes, including *Myf5*, following expression in NIH-3T3 cells.[60] These data, together with the co-expression of Pax3 and Pax7 in somite-derived pro-satellite cells,[37,38] suggest that Pax3 mediates the migratory phase of the lineage, whereas Pax7 is required to achieve myogenic potential.

Pax7 is required for the myogenic specification of satellite cells. Using representational difference analysis, the paired-box transcription factor Pax7 has been cloned as a gene specifically expressed in the satellite cell myogenic lineage.[24,33] Pax7 is specifically expressed in satellite cells in adult muscle and their daughter myogenic precursor cells *in vivo* and primary myoblasts *in vitro*. Cell culture and electron microscopic analysis indicated ablation of satellite cells in *Pax7*$^{-/-}$ skeletal muscle. Fluorescence-activated cell sorting/Hoechst analysis demonstrated that the proportion of muscle-derived SP cells, a putative adult stem cell population further described below, was unaffected. These results demonstrate that satellite cells and muscle-derived SP (side population) cells represent distinct cell populations and reveal an essential role for Pax7 in specifying the satellite cell myogenic lineage functioning upstream of the MyoD family of bHLH factors (**Figure 1**).[24,42,55]

An extensive analysis of *Pax7*$^{-/-}$ mice has confirmed the progressive ablation of the satellite cell lineage in multiple muscle groups.[24,28] Small numbers of Pax7-deficient cells do survive in the satellite cell position, but these cells arrest and die upon entering mitosis. *Pax7*$^{-/-}$ muscles are reduced in size, the fibers contain approximately 50% of the normal number of nuclei, and fiber diameters are significantly reduced. Together, these data confirm an essential role for Pax7 in regulating the myogenic potential of satellite cells.[61]

In previous studies, the potential of atypical non-satellite cell progenitors to participate in muscle regeneration has been investigated. CD45^{+}/Sca1^{+} cells purified from regenerating wild-type muscle express Pax7 and give rise to skeletal myoblasts. In contrast, CD45^{+}/Sca1^{+} cells from regenerating *Pax7*$^{-/-}$ muscle do not undergo myogenic progression unless exposed to Wnt proteins.[42] Retroviral expression of Pax7 in CD45^{+}/Sca1^{+} cells from uninjured muscle induced the formation of myogenic progenitors expressing Myf5 and MyoD, which differentiated into myogenin and myosin heavy chain–expressing myocytes.[55] Together, these results demonstrate that Pax7 is required for the myogenic specification of muscle-derived adult stem cells during regenerative myogenesis.[42,55] It is important to note that, although atypical myogenic cell progenitors have the proven potential to participate to some degree in muscle regeneration under conditions of severe trauma, these experiments, when taken together, strongly support the contention that, under physiological conditions, the growth and regeneration of skeletal muscle is mediated largely, if not exclusively, by muscle satellite cells.[62]

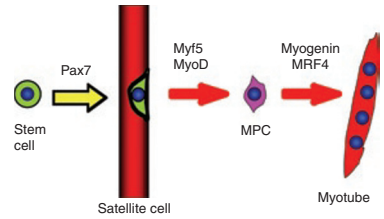

Figure 1 Pax7 has an essential role in regulating the myogenic potential of muscle satellite cells. In the absence of Pax7, satellite cells die during activation following first mitosis. MPC, myogenic precursor cell.

In addition, numerous growth factors such as fibroblast growth factor 6, bone morphogenetic protein, and NO have been suggested to play roles in stimulating satellite cell activation.[21] Nevertheless, the precise molecular mechanisms regulating satellite cell function remain poorly understood.

Myoblast transplantation as a therapy for DMD

Myoblast transplantation is a possible treatment for several muscular dystrophies. The initial demonstration that myoblast transplantation could restore the expression of dystrophin in nude/mdx mouse muscle fibers came as early as 1989.[63] This promising result triggered a rapid series of clinical trials of myoblast transplantation to DMD patients.[64–71] However, these clinical trials produced either negative or very limited positive results. Tremblay's group reported the presence of dystrophin-positive muscle fibers in a few patients, but was not able, at the time, to rule out the possibility that these were revertant muscle fibers, as the mutation in the patients had not been identified.[64,67] Karpati's group used cyclophosphamide as an immunosuppressive agent;[68] however, experiments on mice in subsequent years demonstrated that this anti-tumor drug killed the transplanted myoblasts, as well as any other rapidly proliferating cells.[72] Gussoni *et al.* reported the detection, by reverse-transcriptase polymerase chain reaction, of the normal messenger RNA, but this did not translate into an increase in the percentage of dystrophin-positive fibers following myoblast transfer therapy.[69] Mendell's group reported the presence of 10% dystrophin-positive fibers in one patient.[71] These fibers were definitively of donor origin as they were identified with a dystrophin monoclonal antibody detecting an epitope encoded by an exon deleted in the patient's genome.

In the years that followed these initial trials, several research teams identified three problems that were responsible for the limited results observed: (i) at least 75% of the transplanted myoblasts die in the first 3 days after transplantation;[73–75] (ii) myoblasts do not migrate more than 200 μm away from the intramuscular injection trajectory;[76] and (iii) if immunosuppression is not adequate, the myoblasts are rapidly rejected in less than 2 weeks.[77] Moreover, cyclosporine, which was used for immunosuppression in several clinical trials, induces apoptosis of the myoblasts at the time of their differentiation.[78,79] There are now some solutions to overcome these problems. The rapid death of a large percentage of myoblasts can be compensated for by the transplantation of a high number of cells. Indeed the transplantation of 30 million cells per mm^3 produced very good results in monkeys.[76] The low migration distance of myoblasts requires a high number of adjacent intramuscular injections to obtain good transplantation results in monkeys. Immunosuppression with FK506 (Tacrolimus or Prograf®; ASTELLAS Pharma, Deerfield, IL, USA) permitted Kinoshita *et al.*[80,81] to obtain very good transplantation results not only in mice but also in monkeys. Other technologies, such as the modulation of MyoD expression[82] and the use of matrix metalloproteinase,[83] have also been used to improve myoblast migration within the injected muscle. Similarly, it has been observed that increased muscle regeneration and, potentially, myoblast migration often occur in the environment of the irradiated muscle, suggesting that factors released with the irradiated muscle can eventually be used to improve the success of myoblast transfer therapy.[84]

These solutions are not ideal, but they nevertheless permitted the restoration of dystrophin expression in 26–30% of muscle fibers in a recent clinical trial.[85–87] These are the best results obtained in DMD patients by any therapeutic approach so far. These results in DMD patients required a high number of injections (*i.e.*, 100 injections per cm^2 of muscle surface). Although this procedure seems scary at first, it was very well tolerated by monkeys and the 11 patients who have so far received such transplantations under local anesthesia. One patient has received a total of 4,000 intramuscular injections without any complication.[87] This patient indicated that the procedure was not any worse than going to the dentist, and that a simple over-the-counter medication was sufficient to control the pain. There was no infection or other complication associated with the procedure. Given the severity of this disease, and the fact that there is no alternative therapy, this treatment seems acceptable to most patients.

Successful myoblast transplantation in mice, monkeys, and humans currently requires sustained immunosuppression therapy with *FK506*. This agent may induce adverse effects in patients (nephrotoxicity, diabetes, increased risk of cancer) if used on an ongoing, long-term basis. Two possible alternative solutions exist to avoid these problems. The first solution is to induce specific immunological tolerance toward donor myoblasts, as has already been done in mice using two different protocols to induce mixed chimerism and central tolerance.[88,89] The second of these protocols used no more than two drugs (cyclophosphamide and treosulfan), which have already been approved for clinical use and do not require immunosuppressive therapy. A second alternative to sustained immunosuppression therapy is the transplantation of genetically modified autologous myoblasts. Successful transplantation of myoblasts genetically altered with a lentiviral vector containing the micro-dystrophin gene has been recently reported in mice and in monkeys.[90] Introduction of the full dystrophin gene in myoblasts with an adeno/adeno-associated virus vector is, according to other reports, also possible.[91,92] In addition, it is feasible to skip one exon following genetic modification of myoblasts with a lentivirus coding for an appropriate short hairpin RNA. The transplantation of autologous, genetically corrected myoblasts is potentially limited by the gradual senescence of the satellite cells in patients as they age. A possible solution to this problem would be to derive myoblasts from other autologous pluripotent stem cells such as those found in adipose tissue[93] or from the other stem cells discussed in this review.

Research to increase the migration distance of myoblasts injected into muscle is still ongoing.[94,95] Although this limited distance is a problem, intramuscular delivery of cells does prevent complications associated with systemic delivery (*i.e.*, the risk of embolism in the lungs, heart, brain, kidneys, and liver). The risks associated with repeated, localized intramuscular injections may, thus, be lower than those associated with systemic delivery of stem cells.

MULTIPOTENT STEM CELLS IN SKELETAL MUSCLE DEVELOPMENT AND REPAIR

Muscle SP cells as a source of myogenic stem cells

SP cells were first isolated from mouse skeletal muscle[96,97] by adapting a method optimized for the purification of bone marrow SP cells.[98] Muscle SP cells are heterogeneous and differ from bone marrow SP cells with respect to expression of surface antigens.[96,99] The heterogeneity of SP cells increases when low Hoechst dye concentrations are used for the isolation procedure, as cells that would normally be within the main population fall within the SP gate.[100] The main population is thought to be enriched in lineage-committed cells.[98] When isolated using high Hoechst dye concentrations, the majority of mouse muscle SP cells (>90%) are positive for stem cell antigen-1 (Sca-1) and are negative for the hematopoietic SP markers CD45, CD43, and c-kit.[100] Genes expressed by murine bone marrow and skeletal muscle SP cells have been studied, highlighting transcriptional similarities and differences between these two tissue-specific "stem" cell populations.[101]

Early experiments demonstrated that muscle SP cells have the ability, upon intravenous injection, to engraft in the skeletal muscle of mice with muscular dystrophy,[96] whether or not host mice had been lethally irradiated.[102] When host mice were lethally irradiated, muscle SP cells appeared to provide short-term hematopoietic reconstitution of the bone marrow.[96] Subsequent studies have indicated that the hematopoietic potential of muscle SP cells resides in a small fraction that expresses the hematopoietic cell marker CD45, whereas the "myogenic" fraction is CD45-negative.[103,104]

With regard to their position in the myogenic cell lineage, muscle SP cells appear to be different from satellite cells. First, muscle SP cells are present in *Pax7*$^{-/-}$ mice, which exhibit a severe deficiency in satellite cells.[24] Second, muscle SP cells cultured *in vitro* adopt a myogenic fate and express markers that are present on quiescent and activated satellite cells upon culture with myogenic cells.[40] Finally, after injection into diseased or injured muscle *in vivo*, muscle SP cells give rise to Myf5-positive cells or to Pax7-positive, desmin-positive myogenic cells—all markers

that are also expressed by activated or quiescent satellite cells.[102,105] The developmental origin of muscle SP cells appears to be, at least partly, located within the somites, as demonstrated via retroviral somite labeling in chick–quail chimeras and analyses of Pax3–green fluorescent protein transgenic mice.[106] Further, it appears that muscle SP cells do not receive a contribution, at least post-natally, from cells within the bone marrow, as trafficking from the bone marrow to muscle SP cells has not been detected after bone marrow transplantation followed by cardiotoxin treatment of the host muscle.[107]

Muscle SP cells are still under investigation to better understand their potential for targeting dystrophic muscle after injection into the circulation. Recent studies have demonstrated that cultured muscle SP cells (as opposed to freshly isolated SP cells) can more efficiently engraft in dystrophic muscle after delivery via the femoral artery.[108] Although the levels of engraftment reported thus far are not therapeutically significant, promising studies are ongoing to further test the efficacy of serial intra-arterial injections and to identify the molecule(s) that may be responsible for the efficient extravasation of SP cells from the circulation to dystrophic skeletal muscle (Bachrach *et al.*, manuscript in preparation).

Finally, muscle SP cells have also been isolated from human muscle and are being studied to determine their possible role in this tissue.[109] In fetal skeletal muscle, recent evidence suggests that muscle SP cells may act as paracrine cells, secreting factors that might promote the proliferation of other myogenic cells located nearby.[110] It has recently been found that human fetal muscle SP cells express high levels of bone morphogenetic protein-4, which stimulates the proliferation of $Myf5^+/BMPR1A^+$ myogenic cells.[110] This proliferation can be inhibited by gremlin, a known bone morphogenetic protein-4 antagonist that is highly expressed by muscle main population cells.[110]

In summary, muscle SP cells are a rare cell type within skeletal muscle and share markers with mesoangioblasts[111] and MDSCs.[112] Future studies on muscle SP cells will address the relationships between these cells and other progenitors found in muscle. By consolidating the ties between different "stem" cells within muscle, it will be possible to merge knowledge on how to better propagate and manipulate these cells, with the goal of using them efficiently for therapeutic applications.

Mesoangioblasts as a potential source of stem cells to regenerate skeletal muscle

Among different types of mesodermal stem cells recently identified and characterized to different extents are mesoangioblasts. Mesoangioblasts were first identified in the wall of the mouse embryonic dorsal aorta as cells expressing early endothelial (as well as several pericyte) markers[39] that were able to proliferate extensively *in vitro* and, in due course, differentiate into different types of solid mesoderm.[113] Mesoangioblast-like cells were later isolated from vessels of post-natal tissues in the mouse, rat, dog, and human. Post-natal cells generally express pericyte rather than endothelial cell markers but are otherwise similar to their embryonic counterparts in terms of proliferation and differentiation potency. It is hypothesized that dog and human cells proliferate to a limited extent and undergo senescence at variance with rodent cells, which eventually become aneuploid and immortal. When wild-type or dystrophic, genetically corrected mesoangioblasts were delivered intra-arterially to dystrophic muscle of α-sarcoglycan-null mice (a model for limb girdle muscular dystrophy), they induced a dramatic functional amelioration of the dystrophic phenotype.[114] This was due to the widespread distribution of donor cells through the capillary network and to an intrinsic defect of proliferation in the resident satellite cells, a situation that created a selective advantage for the injected donor cells.

To proceed to clinical experimentation it was considered to be crucial that delivery and muscle homing of mesoangioblasts be optimized to characterize human cells in depth and that the protocol be tested in a large-animal model. Recently, it was reported that enhancing delivery of mesoangioblasts leads to the complete reconstitution of downstream skeletal muscles in α-sarcoglycan-null dystrophic mice. Mesoangioblasts, exposed *in vitro* to either stromal cell derived factor-1 or tumor necrosis factor-α showed enhanced transmigration *in vitro* and migration into dystrophic muscle *in vivo*. Transient expression of α-4 integrins or L-selectin also produced a several-fold increase in migration both *in vitro* and *in vivo*. Thus, combining stromal derived factor-1 with expression of α-4 integrin resulted in reconstitution of more than 80% of α-sarcoglycan-expressing fibers, with a five-fold increase in efficiency over control cells.[115]

Similar cells could be isolated from blood vessels of human post-natal skeletal muscle that expressed markers for pericytes, such as alkaline phosphatase, and that differentiated into myotubes *in vitro* with high efficiency. When transplanted into severe combined immunodeficiency (SCID) mdx mice, human mesoangioblasts generate numerous fibers expressing human dystrophin. Cells similarly isolated from DMD patients and engineered to express human mini-dystrophin also gave rise to many dystrophin-positive fibers *in vivo*.[116] Finally, arterial delivery of wild-type canine mesoangioblasts in dystrophic dogs led to extensive recovery of dystrophin expression and amelioration of muscle morphology and function.[117] Together, these data contribute to a detailed characterization of mesoangioblasts and lay the foundation for future clinical experimentation.

Repairing skeletal muscle with $CD133^+$ progenitor cells

As mentioned above, attempts to repair muscle damage in DMD patients by transplanting myogenic progenitors directly into muscles faced problems of cell survival and limited intramuscular migration. The delivery of myogenic stem cells to the sites of muscle lesions via the systemic circulation is a potential alternative approach to treating this disease, but intravenously injected cells may be trapped in other organs (*e.g.*, liver, spleen, lung) so that only a small portion enter muscle capillary circulation and migrate into dystrophic muscle. Recent works support the idea that stem cells may reach the site of muscle regeneration and contribute to muscle repair as well as replenish the satellite cell pool after arterial injection, suggesting that this technique is particularly suited for treating muscle dystrophy.[114,115,118,119] The success of this protocol was largely due to the widespread distribution of donor stem cells through the muscle capillary network, a distinct strategic advantage over previous approaches. The molecular pathways involved in muscle stem cell homing should be carefully considered in

any attempt to treat DMD patients by arterial delivery. Dystrophic muscle damage results in the release of various substances, including intracellular proteins, cytokines, and chemokines, ultimately resulting in an inflammatory response.[21,120–123] This condition creates a microenvironment that may regulate the expression of chemo-attractant receptors (*e.g.*, L-selectin and very late antigen-4) and stimulate stem cell homing.[96,115,119,124] Sca-1$^+$/CD34$^-$/L-selectin$^+$ MDSCs did "home" to the muscle tissues; in fact, these cells were prevalently found to adhere firmly and cross the endothelium of mdx dystrophic muscles after intravenous injections. Muscle homing was probably related to the high expression of L-selectin ligands on the vessels of inflamed muscles, as is the case in dystrophic muscles.[125,126] Of particular significance, endothelium from dystrophic muscle expresses the mucosal addressin cell adhesion molecule-1, which is a ligand for the L-selectin expressed selectively at venular sites on chronically inflamed endothelium in muscle dystrophy. Interestingly enough, only blood vessels from young mdx mice express mucosal addressin cell adhesion molecule-1, whereas blood vessels from older mice were observed to have down-regulated mucosal addressin at their surface.

Freshly isolated human CD133$^+$ cells express CD44, lymphocyte function-associated antigen-1, P-selectin glycoprotein ligand-1, very late antigen-4, and L-selectin, a pattern of adhesion molecules defining cells potentially able to migrate through the blood vessel wall. When injected into the circulation of dystrophic *scid/mdx* mice, the CD133$^+$ cells contributed to muscle repair and the replenishment of the satellite cell pool after arterial injection.[119] Human dystrophin expression was significantly increased when muscle exercise was performed 24 hours before the arterial injection of human CD133$^+$ cells. In these experiments, muscle exercise produced acute inflammation in the dystrophic muscle, massively increasing the expression of VCAM-1 on murine endothelium. However, low expression of VCAM-1 in vessels of unexercised dystrophic muscle was not sufficient to mediate efficient recruitment of CD133$^+$ cells in this experimental model (**Figure 2**). Intravital microscopy analysis confirmed that CD133$^+$ stem cells were able to roll and firmly adhere in the dystrophic vessels of *scid/mdx* mice as a result of the interaction between VCAM-1 and very late antigen-4 (**Figure 2**). Development of methods to manipulate the expression of VCAM-1 and its ligands might improve muscle stem cell homing and open new therapeutic perspectives for muscular dystrophy patients.

Isolation of stem cells from skeletal muscle via the pre-plate technique

A modified pre-plate technique was used to isolate various populations of muscle-derived cells from mice, including a population of early myogenic progenitor cells. In fact, through this pre-plate technique, which separates myogenic cells based on their adherence to collagen-coated flasks, a population of early myogenic progenitor cells was isolated from the late pre-plate fraction of cells and a population of more committed myogenic cells was isolated from an earlier fraction of cells as observed by others. On the basis of their marker profiles (stem cell and myogenic cell marker profiles) as well as their proliferation/fusion behavior *in vitro*, earlier fraction of cells are likely a population of satellite cells, whereas the

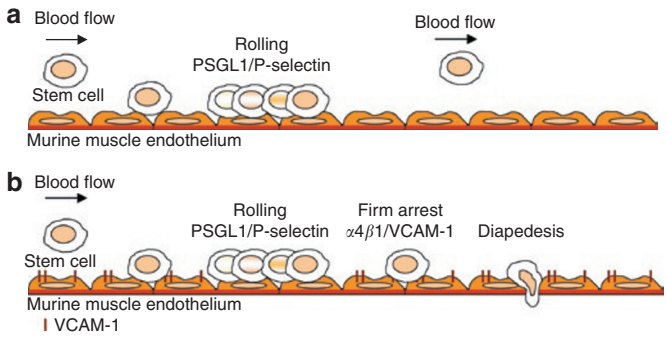

Figure 2 Recruitment of stem cells in inflamed murine muscle endothelium is mediated by adhesion molecules. The expression of high levels of VLA-4 on human CD133$^+$ stem cells and up-regulation of the endothelial vascular cell adhesion molecule 1 (VCAM-1) in dystrophic inflamed vessels are critical for the capture and migration of the injected stem cells. (**a**) In murine muscle that was not inflamed, the interaction of P-selectin glycoprotein ligand-1 (PSGL-1) (expressed on the CD133$^+$ stem cells) and the corresponding endothelial receptor P-selectin contributes to rolling but is not relevant for the capture and migration of the CD133$^+$ stem cells. (**b**) However, dystrophic inflamed endothelium enriched in VCAM-1 adhesion molecules mediates frequent rolling events and the migration of the CD133$^+$ stem cells.

long-term proliferating cells derived from the late pre-plate fraction of cells appear to be MDSCs.[127, 128, 129, 130]

The transplantation of these early myogenic cells (MDSCs) into the skeletal muscle of mice yields a better outcome than the transplantation of late myogenic progenitor cells, such as satellite cells. Experiments have been also conducted to determine the mechanism by which mouse MDSCs display an improved regeneration capacity in skeletal muscle (when compared with satellite cells). The ability of MDSCs to proliferate *in vivo* for an extended period of time—combined with their strong capacity for self-renewal, their multi-lineage differentiation (particularly into blood vessel cells), and their immune privilege—reveals, at least in part, the basis for the improvements observed after transplantation of these cells in skeletal muscle.[127] The use of such early myogenic progenitor cells might significantly improve the outcome of muscle cell–mediated therapies. It has been recently demonstrated that superior survival and proliferation occur after transplantation of myogenic cells derived from the pre-plate technique when the cells are obtained from adult mice, as opposed to newborn mice, suggesting that the age of the animals can influence the ability of myogenic cells to regenerate skeletal muscle.[131] Similarly, we have recently observed that the sex of the animals from which we derived MDSCs, as well as the sex of the host animals selected as recipients for cell transplantation, can influence the success of cell transplantation.[132] In fact, it was determined that female cells have a higher regeneration index than male cells and that female hosts are better recipients for optimal muscle regeneration after MDSC transplantation than male hosts.[132] The sex of the cells and the host may explain reported discrepancies in terms of regenerating potential of MDSCs obtained by different research groups where either male host animals were used[133] or male MDSCs were utilized because of the need to make use of fluorescent *in situ* hybridization to track male cells injected into female hosts.[134,135] These results indicate that both age and sex should be considered

as important variables that may influence the regenerating potential of a given cell population.

Early myogenic progenitor cells (MDSCs) isolated from mice engraft better than late myogenic progenitor cells (satellite cells) after intracardiac implantation in both cardiomyopathic and infarcted hearts of mice.[136,137] In fact, when compared with satellite cells, mouse MDSCs display superior engraftment in infarcted hearts because they survive better and favor angiogenesis, as observed in skeletal muscle.[127,136]

Myogenic cells in the walls of human blood vessels

The muscle-derived stem cells described in the above sections are but one example among several types of multipotent cells described in adult, developed, human and mouse organs. Without exception, and as described above in the case of MDSCs, all these multi-lineage cells have been isolated retrospectively from cultured adult tissues. Therefore the embryonic origin, identity, and, primarily, the anatomic localization of these stem cells within adult tissues remains unknown.

Some of us have recently attempted to characterize non-lineage-restricted adult human stem cells, assuming that the diversely designated mesenchymal stem cells, multipotent adult progenitor cells, and MDSCs are all derived, upon long-term culture, from the same original, omnipresent stem cell. The walls of blood vessels have emerged as a possible repository for such pan-organ stem cells, as almost all tissues are vascularized. As a subset of MDSCs express endothelial cell markers and promote neoangiogenesis through differentiation into endothelial cells via vascular endothelial growth factor secretion,[127,136,137] it was assumed that these cells are interrelated with endothelial cells. The existence of blood vessel–associated stem cells has also been supported by the description and characterization of mesoangioblasts, as previously described (see section entitled "Mesangioblasts as a potential source of stem cells to regenerate skeletal muscle"), and by the demonstration that definitive hematopoietic stem cells emerge in the embryo from a specialized subset of hemogenic vascular endothelial cells.[138,139]

Cells with high myogenic potential in human adult skeletal muscle are found within vascular endothelium. By multicolor immunostaining and confocal microscopy analysis of human skeletal muscle sections, it was observed that a subset of satellite cells co-express endothelial cell antigens. The existence of this novel population of myo-endothelial cells was further documented by flow cytometry. These cells, which represent less than 0.5% of the total skeletal muscle population, can be conveniently typified as $CD56^+/CD34^+/CD144^+/CD45^-$ and sorted accordingly to homogeneity. To assay the myogenic potential of this novel cell subset, these myo-endothelial cells and, in parallel, genuine $CD56^+CD34^-CD144^-CD45^-$ myogenic cells and $CD34^+/CD144^+/CD56^-/CD45^-$ endothelial cells were sorted and injected intramuscularly into SCID mouse skeletal muscles that had been injured previously by cardiotoxin injection. Our study revealed that both muscle endothelial cells and myo-endothelial cells are able, as is the case with conventional myogenic cells, to regenerate muscle fibers within the injured muscle. These experiments demonstrated that muscle vascular endothelial cells and, most notably, a novel subset of cells with an overlapping phenotype between myogenic and endothelial cells, are endowed with a regenerating potential in skeletal muscle to a level similar and even higher than myogenic cells (B. Zheng, B. Cao, M. Crisan, B. Sun, G. Li, A. Logar *et al*., manuscript submitted). These results have suggested the existence of a developmental relationship between vascular cells and myogenic cells. In support of this hypothesis, total muscle cells or sorted $CD56^+$ myogenic cells cultured under conditions that favor endothelial cell growth (endothelial cell growth medium-2, (Cambrex Corp., East Rutherford, NJ)) have yielded large numbers of cells co-expressing myogenic and endothelial cell markers (B. Zheng, B. Cao, M. Crisan, B. Sun, G. Li, A. Logar *et al*., manuscript submitted). Interestingly, myogenic potential does not seem to be restricted to muscle-derived endothelial cells, as vascular endothelial cells purified from adult human pancreas and adipose tissue can also support myofiber regeneration (M. Crisan, B. Sun, L. Casteilla, M. Gavina, S. Yap, C. Norotte *et al.,* manuscript submitted; S. Yap, M. Crisan, B. Sun, J. Huard, L. Casteilla, J.-P. Giacobino *et al.,* manuscript submitted).

Myogenic potential of human perivascular cells. Pericytes closely encircle endothelial cells in capillaries and microvessels.[140] These cells regulate microvessel contractility, and can inhibit the division of endothelial cells (reviewed in ref. 141). Pericytes are also suspected to include progenitors of chondrocytes, adipocytes, osteocytes, and odontoblasts.[142–144] However, these published differentiation experiments were performed only on pericyte-containing cultures and not on purified pericytes. Some of us aimed to determine whether pericytes are also endowed with myogenic potential, using an experimental strategy and tactics similar to those described above for endothelial cells. As a prerequisite to human pericyte sorting by flow cytometry, we determined, by immunohistochemistry, a relevant combination of markers for this elusive cell population. Expression of CD146 and NG2 typifies pericytes in all human tissues analyzed. In contrast, pericytes do not express endothelial cell antigens such as CD144 (VE-cadherin), von Willebrand factor, CD34, CD31, and the *Ulex europaeus* lectin ligand. $CD146^+/CD34^-/CD45^-/CD56^-$ pericytes were sorted by fluorescence-activated cell sorting from human skeletal muscle and confirmed, by reverse-transcriptase polymerase chain reaction analysis, not to include hematopoietic, endothelial, and regular myogenic cells.

Sorted pericytes cultured in muscle proliferation medium, and then in muscle fusion medium, developed into multinucleated myotubes expressing myosin heavy chain. Furthermore, sorted pericytes ($CD146^+/CD45^-/CD34^-/CD144^-/CD56^-$), myoblasts ($CD146^-/CD45^-/CD34^-/CD144^-/CD56^+$), and unseparated muscle cells all regenerated muscle fibers after injection into the cardiotoxin-injured skeletal muscles of SCID-non-obese diabetic mice, indicating a muscle-regenerating potential for pericytes (M. Crisan, B. Sun, L. Casteilla, M. Gavina, S. Yap, C. Norotte *et al.,* manuscript submitted).

Strikingly, pericytes sorted from adult human adipose tissue or pancreas exhibited similar potential to skeletal muscle pericytes for generating myotubes in culture and myofibers in SCID-non-obese diabetic mouse muscles (S. Yap, M. Crisan, B. Sun, J. Huard, L. Casteilla, J.-P. Giacobino *et al*., manuscript submitted). Hypothetically, blood vessel walls would harbor a mostly dormant reserve of multi-lineage stem cells that could

be recruited in emergency situations, when professional tissue-specific progenitors have been exhausted.

Altogether, the results reported above point to the existence of a myogenic potential within cells that make up the walls of blood vessels, *i.e.*, endothelial cells and pericytes. A rare subset of myo-endothelial cells has been additionally identified in skeletal muscle that exhibits myogenic potential and may represent a developmental intermediate between both lineages. Under normal-life conditions, the existence of a physiological role for vascular cells in the development and/or regeneration of human skeletal muscle remains to be demonstrated, especially in light of the fact that a similar myogenic potential is present within pericytes and endothelial cells purified from pancreas, fat, and, possibly, other tissues. Rather than a restricted myogenic potential, pericytes purified from human tissues have exhibited a broader ability to differentiate into bone, cartilage, and adipocytes (data not shown), in agreement with published preliminary results.[142–144] The quasi-omnipresence of pericytes in the organism suggests a pan-organ dissemination of multi-lineage stem cells, which may have been the origin of mesenchymal stem cells, multipotent adult progenitor cells, MDSCs, and other adult stem cells immortalized in culture. It should not be assumed, however, that the sharing of surface markers between cell populations indicates that the cells are related, as additional behavioral testing should be performed to determine whether a relationship truly exists between these various populations of cells. From a practical perspective, these novel myogenic progenitors derived from the walls of blood vessels appear to be amenable to biotechnological processing. In fact, these cells can be sorted to homogeneity by flow cytometry from skeletal muscle and even more accessible sources such as adipose tissue. Therefore, the transplantation of autologous blood vessel–related progenitors could potentially be envisioned as a therapy for skeletal muscle diseases.

CONCLUSION

Our understanding of tissue regeneration and repair at adult stages remains largely inspired by the advanced knowledge gained on the hematopoietic system, in which a hierarchy of increasingly committed blood cell progenitors is dominated by a small subset of multipotent stem cells. In contrast, although the ability of skeletal muscle, at any stage of post-natal life, to regenerate upon sustained physical activity or injury is well documented, intramuscular myogenic cells have long been assumed to be restricted to a population of late, committed muscle progenitors, namely the satellite cells. Considerable progress has been made recently in understanding the biology of satellite cells, the ontogeny of which has been traced back to paraxial mesoderm, under a molecular control that has been largely deciphered. However, although satellite cells remain recognized as the primary cells responsible for the regeneration of post-natal skeletal muscle, research over the past few years has shown that several other cell types, including MDSCs, side-population cells, and mesoangioblasts, can display similar behaviors in experimental conditions. The (possibly common) origin of these stem cell populations and their relationship to satellite cells remain largely unknown. Most recent results suggest that at least some of these stem cells originate in the walls of muscle and non-muscle blood vessels, in which subsets of prospectively sorted endothelial and pericyte cell lineage cells are endowed with myogenic potential. It remains completely unknown whether these novel myogenic cells, which have the potential to give rise to other mesodermal cell lineages, play any role in muscle renewal in steady-state conditions, or are only recruited in conditions of emergency. Although major developments are being made to improve the success of myoblast transplantation in DMD patients, we believe that such novel cells endowed with strong myogenic potential, whatever their normal function *in situ*, should also be considered for the cell-mediated therapy of muscle diseases. Although this review has focused primarily on post-natal stem cells, we do not exclude the idea that embryonic stem cells, and perhaps their progeny, may also be used for muscle regeneration and repair. In fact, it has been recently reported that differentiated embryonic stem cells transfected with insulin-like growth factor II regenerate injured skeletal muscle in a more effective manner than undifferentiated embryonic stem cells.[145] This study illustrates the idea that embryonic stem cells that are genetically engineered to express insulin-like growth factor II may represent another cell source for cell-based transplantation therapy to repair muscle damaged by injury or myopathy, but additional experiments are required to further evaluate their muscle-regenerating potential.

REFERENCES

1. Mauro, A (1961). Satellite cell of skeletal muscle fibers. *J Biophys Biochem* **9**: 493–495.
2. Sherwood, RI, Christensen, JL, Conboy, IM, Conboy, MJ, Rando, TA, Weissman, IL *et al.* (2004). Isolation of adult mouse myogenic progenitors: functional heterogeneity of cells within and engrafting skeletal muscle. *Cell* **119**: 543–554.
3. Collins, CA, Olsen, I, Zammit, PS, Heslop, L, Petrie, A, Partridge, TA *et al.* (2005). Stem cell function, self-renewal, and behavioral heterogeneity of cells from the adult muscle satellite cell niche. *Cell* **122**: 289–301.
4. Zammit, PS, Partridge, TA and Yablonka-Reuveni, Z (2006). The skeletal muscle satellite cell: the stem cell that came in from the cold. *J Histochem Cytochem* **54**: 1177–1191.
5. Beresford, JN, Bennett, JH, Devlin, C, Leboy, PS and Owen, ME (1992). Evidence for an inverse relationship between the differentiation of adipocytic and osteogenic cells in rat marrow stromal cell cultures. *J Cell Sci* **102**: 341–351.
6. Pittenger, MF, Mackay, AM, Beck, SC, Jaiswal, RK, Douglas, R, Mosca, JD *et al.* (1999). Multilineage potential of adult human mesenchymal stem cells. *Science* **284**: 143–147.
7. Prockop, DJ (1997). Marrow stromal cells as stem cells for nonhematopoietic tissues. *Science* **276**: 71–74.
8. Toma, C, Pittenger, MF, Cahill, KS, Byrne, BJ and Kessler, PD (2002). Human mesenchymal stem cells differentiate to a cardiomyocyte phenotype in the adult murine heart. *Circulation* **105**: 93–98.
9. Krause, DS, Theise, ND, Collector, MI, Henegariu, O, Hwang, S, Gardner, R *et al.* (2001). Multi-organ, multi-lineage engraftment by a single bone marrow-derived stem cell. *Cell* **105**: 369–377.
10. Mezey, E, Chandross, KJ, Harta, G, Maki, RA and McKercher, SR (2000). Turning blood into brain: cells bearing neuronal antigens generated *in vivo* from bone marrow. *Science* **290**: 1779–1782.
11. Petersen, BE, Bowen, WC, Patrene, KD, Mars, WM, Sullivan, AK, Murase, N *et al.* (1999). Bone marrow as a potential source of hepatic oval cells. *Science* **284**: 1168–1170.
12. Theise, ND, Badve, S, Saxena, R, Henegariu, O, Sell, S, Crawford, JM *et al.* (2000). Derivation of hepatocytes from bone marrow cells in mice after radiation-induced myeloablation. *Hepatology* **31**: 235–240.
13. Jiang, Y, Vaessen, B, Lenvik, T, Blackstad, M, Reyes, M and Verfaillie, CM (2002). Multipotent progenitor cells can be isolated from postnatal murine bone marrow, muscle, and brain. *Exp Hematol* **30**: 896–904.
14. Mitchell, KE, Weiss, ML, Mitchell, BM, Martin, P, Davis, D, Morales, L *et al.* (2003). Matrix cells from Wharton's jelly form neurons and glia. *Stem Cells* **21**: 50–60.
15. Wang, HS, Hung, SC, Peng, ST, Huang, CC, Wei, HM, Guo, YJ *et al.* (2004). Mesenchymal stem cells in the Wharton's jelly of the human umbilical cord. *Stem Cells* **22**: 1330–1337.
16. Seaberg, RM, Smukler, SR, Kieffer, TJ, Enikolopov, G, Asghar, Z, Wheeler, MB *et al.* (2004). Clonal identification of multipotent precursors from adult mouse pancreas that generate neural and pancreatic lineages. *Nat Biotechnol* **22**: 1115–1124.
17. Toma, JG, Akhavan, M, Fernandes, KJ, Barnabe-Heider, F, Sadikot, A, Kaplan, DR *et al.* (2001). Isolation of multipotent adult stem cells from the dermis of mammalian skin. *Nat Cell Biol* **3**: 778–784.
18. Shih, DT, Lee, DC, Chen, SC, Tsai, RY, Huang, CT, Tsai, CC *et al.* (2005). Isolation and characterization of neurogenic mesenchymal stem cells in human scalp tissue. *Stem Cells* **23**: 1012–1020.
19. Zuk, PA, Zhu, M, Mizuno, H, Huang, J, Futrell, JW, Katz, AJ *et al.* (2001). Multilineage cells from human adipose tissue: implications for cell-based therapies. *Tissue Eng* **7**: 211–228.
20. Bischoff, R. (1994). The satellite cell and muscle regeneration. In: Engel, AG and Franszini-Armstrong, C (eds). *Myogenesis*. McGraw-Hill: New York. pp. 97–118.

21. Charge, SB and Rudnicki, MA (2004). Cellular and molecular regulation of muscle regeneration. *Physiol Rev* **84**: 209–238.
22. Hughes, SM and Blau, HM (1990). Migration of myoblasts across basal lamina during skeletal muscle development. *Nature* **345**: 350–353.
23. Yokota, T, Lu, QL, Morgan, JE, Davies, KE, Fisher, R, Takeda, S *et al.* (2006). Expansion of revertant fibers in dystrophic mdx muscles reflects activity of muscle precursor cells and serves as an index of muscle regeneration. *J Cell Sci* **119**: 2679–2687.
24. Seale, P, Sabourin, LA, Girgis-Gabardo, A, Mansouri, A, Gruss, P and Rudnicki, MA (2000). Pax7 is required for the specification of myogenic satellite cells. *Cell* **102**: 777–786.
25. Irintchev, A, Zeschnigk, M, Starzinski-Powitz, A and Wernig, A (1994). Expression pattern of M-cadherin in normal, denervated, and regenerating mouse muscles. *Dev Dyn* **199**: 326–337.
26. Cornelison, DD and Wold, BJ (1997). Single-cell analysis of regulatory gene expression in quiescent and activated mouse skeletal muscle satellite cells. *Dev Biol* **191**: 270–283.
27. Beauchamp, JR, Heslop, L, Yu, DS, Tajbakhsh, S, Kelly, RG, Wernig, A *et al.* (2000). Expression of CD34 and myf5 defines the majority of quiescent adult skeletal muscle satellite cells. *J Cell Biol* **151**: 1221–1234.
28. Oustanina, S, Hause, G and Braun, T (2004). Pax7 directs postnatal renewal and propagation of myogenic satellite cells but not their specification. *EMBO J* **23**: 3430–3439.
29. Relaix, F, Montarras, D, Zaffran, S, Gayraud-Morel, B, Rocancourt, D, Tajbakhsh, S *et al.* (2006). Pax3 and Pax7 have distinct and overlapping functions in adult muscle progenitor cells. *J Cell Biol* **172**: 91–102.
30. Rosen, GD, Sanes, JR, LaChance, R, Cunningham, JM, Roman, J and Dean, DC (1992). Roles for the integrin VLA-4 and its counter receptor VCAM-1 in myogenesis. *Cell* **69**: 1107–1119.
31. Garry, DJ, Yang, Q, Bassel-Duby, R and Williams, RS (1997). Persistent expression of MNF identifies myogenic stem cells in postnatal muscles. *Dev Biol* **188**: 280–294.
32. Cornelison, DD, Filla, MS, Stanley, HM, Rapraeger, AC and Olwin, BB (2001). Syndecan-3 and syndecan-4 specifically mark skeletal muscle satellite cells and are implicated in satellite cell maintenance and muscle regeneration. *Dev Biol* **239**: 79–94.
33. Seale, P, Ishibashi, J, Holterman, C and Rudnicki, MA (2004). Muscle satellite cell-specific genes identified by genetic profiling of MyoD-deficient myogenic cell. *Dev Biol* **275**: 287–300.
34. Jankowski, RJ, Deasy, BM and Huard, J (2002). Muscle-derived stem cells. *Gene Ther* **9**: 642–647.
35. Thornell, LE, Lindstrom, M, Renault, V, Mouly, V and Butler-Browne, GS (2003). Satellite cells and training in the elderly. *Scand J Med Sci Sports* **13**: 48–55.
36. Armand, O, Boutineau, AM, Mauger, A, Pautou, MP and Kieny, M (1983). Origin of satellite cells in avian skeletal muscles. *Arch Anat Microsc Morphol Exp* **72**: 163–181.
37. Kassar-Duchossoy, L, Giacone, E, Gayraud-Morel, B, Jory, A, Gomes, D and Tajbakhsh, S (2005). Pax3/Pax7 mark a novel population of primitive myogenic cells during development. *Genes Dev* **19**: 1426–1431.
38. Relaix, F, Rocancourt, D, Mansouri, A and Buckingham, M (2005). A Pax3/Pax7-dependent population of skeletal muscle progenitor cells. *Nature* **435**: 948–953.
39. De Angelis, L, Berghella, L, Coletta, M, Lattanzi, L, Zanchi, M, Cusella-De Angelis, MG *et al.* (1999). Skeletal myogenic progenitors originating from embryonic dorsal aorta coexpress endothelial and myogenic markers and contribute to postnatal muscle growth and regeneration. *J Cell Biol* **147**: 869–878.
40. Asakura, A, Seale, P, Girgis-Gabardo, A and Rudnicki, MA (2002). Myogenic specification of side population cells in skeletal muscle. *J Cell Biol* **159**: 123–134.
41. LaBarge, MA and Blau, HM (2002). Biological progression from adult bone marrow to mononucleate muscle stem cell to multinucleate muscle fiber in response to injury. *Cell* **111**: 589–601.
42. Polesskaya, A, Seale, P and Rudnicki, MA (2003). Wnt signaling induces the myogenic specification of resident $CD45^+$ adult stem cells during muscle regeneration. *Cell* **113**: 841–852.
43. Conboy, IM and Rando, TA (2002). The regulation of Notch signaling controls satellite cell activation and cell fate determination in postnatal myogenesis. *Dev Cell* **3**: 397–409.
44. Zammit, PS, Golding, JP, Nagata, Y, Hudon, V, Partridge, TA and Beauchamp, JR (2004). Muscle satellite cells adopt divergent fates: a mechanism for self-renewal? *J Cell Biol* **166**: 347–357.
45. Mansouri, A, Goudreau, G and Gruss, P (1999). Pax genes and their role in organogenesis. *Cancer Res* **59**: 1707s–1709s; discussion 1709s–1710s.
46. Goulding, MD, Chalepakis, G, Deutsch, U, Erselius, JR and Gruss, P (1991). Pax-3, a novel murine DNA binding protein expressed during early neurogenesis. *EMBO J* **10**: 1135–1147.
47. Jostes, B, Walther, C and Gruss, P (1990). The murine paired box gene, Pax7, is expressed specifically during the development of the nervous and muscular system. *Mech Dev* **33**: 27–37.
48. Maroto, M, Reshef, R, Munsterberg, AE, Koester, S, Goulding, M and Lassar, AB (1997). Ectopic Pax-3 activates MyoD and Myf-5 expression in embryonic mesoderm and neural tissue. *Cell* **89**: 139–148.
49. Tajbakhsh, S, Rocancourt, D, Cossu, G and Buckingham, M (1997). Redefining the genetic hierarchies controlling skeletal myogenesis: Pax-3 and Myf-5 act upstream of MyoD. *Cell* **89**: 127–138.
50. Schafer, BW, Czerny, T, Bernasconi, M, Genini, M and Busslinger, M (1994). Molecular cloning and characterization of a human PAX-7 cDNA expressed in normal and neoplastic myocytes. *Nucleic Acids Res* **22**: 4574–4582.
51. Bennicelli, JL, Advani, S, Schafer, BW and Barr, FG (1999). PAX3 and PAX7 exhibit conserved cis-acting transcription repression domains and utilize a common gain of function mechanism in alveolar rhabdomyosarcoma. *Oncogene* **18**: 4348–4356.
52. Conway, SJ, Henderson, DJ and Copp, AJ (1997). Pax3 is required for cardiac neural crest migration in the mouse: evidence from the splotch (Sp2H) mutant. *Development* **124**: 505–514.
53. Tremblay, P, Dietrich, S, Mericskay, M, Schubert, FR, Li, Z and Paulin, D (1998). A crucial role for Pax3 in the development of the hypaxial musculature and the long-range migration of muscle precursors. *Dev Biol* **203**: 49–61.
54. Mansouri, A, Stoykova, A, Torres, M and Gruss, P (1996). Dysgenesis of cephalic neural crest derivatives in $Pax7^{-/-}$ mutant mice. *Development* **122**: 831–838.
55. Seale, P, Ishibashi, J, Scime, A and Rudnicki, MA (2004). Pax7 is necessary and sufficient for the myogenic specification of $CD45^+$:$Sca1^+$ stem cells from injured muscle. *PLoS Biol* **2**: E130.
56. Daston, G, Lamar, E, Olivier, M and Goulding, M (1996). Pax-3 is necessary for migration but not differentiation of limb muscle precursors in the mouse. *Development* **122**: 1017–1027.
57. Epstein, JA, Lam, P, Jepeal, L, Maas, RL and Shapiro, DN (1995). Pax3 inhibits myogenic differentiation of cultured myoblast cells. *J Biol Chem* **270**: 11719–11722.
58. Williams, BA and Ordahl, CP (1994). Pax-3 expression in segmental mesoderm marks early stages in myogenic cell specification. *Development* **120**: 785–796.
59. Borycki, AG and Emerson, CP (1997). Muscle determination: another key player in myogenesis? *Curr Biol* **7**: R620–R623.
60. Khan, J, Bittner, ML, Saal, LH, Teichmann, U, Azorsa, DO, Gooden, GC *et al.* (1999). cDNA microarrays detect activation of a myogenic transcription program by the PAX3-FKHR fusion oncogene. *Proc Natl Acad Sci USA* **96**: 13264–13269.
61. Kuang, S, Charge, SB, Seale, P, Huh, M and Rudnicki, MA (2006). Distinct roles for Pax7 and Pax3 in adult regenerative myogenesis. *J Cell Biol* **172**: 103–113.
62. McKinnell, IW, Parise, G and Rudnicki, MA (2005). Muscle stem cells and regenerative myogenesis. *Curr Top Dev Biol* **71**: 113–130.
63. Partridge, TA, Morgan, JE, Coulton, GR, Hoffman, EP and Kunkel, LM (1989). Conversion of mdx myofibres from dystrophin-negative to -positive by injection of normal myoblasts. *Nature* **337**: 176–179.
64. Huard, J, Bouchard, JP, Roy, R, Labrecque, C, Dansereau, G, Lemieux, B *et al.* (1991). Myoblast transplantation produced dystrophin-positive muscle fibres in a 16-year-old patient with Duchenne muscular dystrophy. *Clin Sci* (*Lond*) **81**: 287–288.
65. Huard, J, Bouchard, JP, Roy, R, Malouin, F, Dansereau, G, Labrecque, C *et al.* (1992). Human myoblast transplantation: preliminary results of 4 cases. *Muscle Nerve* **15**: 550–560.
66. Tremblay, JP, Bouchard, JP, Malouin, F, Theau, D, Cottrell, F, Collin, H *et al.* (1993). Myoblast transplantation between monozygotic twin girl carriers of Duchenne muscular dystrophy. *Neuromuscul Disord* **3**: 583–592.
67. Tremblay, JP, Malouin, F, Roy, R, Huard, J, Bouchard, JP, Satoh, A *et al.* (1993). Results of a triple blind clinical study of myoblast transplantations without immunosuppressive treatment in young boys with Duchenne muscular dystrophy. *Cell Transplant* **2**: 99–112.
68. Karpati, G, Ajdukovic, D, Arnold, D, Gledhill, RB, Guttmann, R, Holland, P *et al.* (1993). Myoblast transfer in Duchenne muscular dystrophy. *Ann Neurol* **34**: 8–17.
69. Gussoni, E, Pavlath, GK, Lanctot, AM, Sharma, KR, Miller, RG, Steinman, L *et al.* (1992). Normal dystrophin transcripts detected in Duchenne muscular dystrophy patients after myoblast transplantation. *Nature* **356**: 435–438.
70. Miller, RG, Sharma, KR, Pavlath, GK, Gussoni, E, Mynhier, M, Lanctot, AM *et al.* (1997). Myoblast implantation in Duchenne muscular dystrophy: the San Francisco study. *Muscle Nerve* **20**: 469–478.
71. Mendell, JR, Kissel, JT, Amato, AA, King, W, Signore, L, Prior, TW *et al.* (1995). Myoblast transfer in the treatment of Duchenne's muscular dystrophy. *N Engl J Med* **333**: 832–838.
72. Vilquin, JT, Kinoshita, I, Roy, R and Tremblay, JP (1995). Cyclophosphamide immunosuppression does not permit successful myoblast allotransplantation in mouse. *Neuromuscul Disord* **5**: 511–517.
73. Guerette, B, Asselin, I, Skuk, D, Entman, M and Tremblay, JP (1997). Control of inflammatory damage by anti-LFA-1: increase success of myoblast transplantation. *Cell Transplant* **6**: 101–107.
74. Huard, J, Acsadi, G, Jani, A, Massie, B and Karpati, G (1994). Gene transfer into skeletal muscles by isogenic myoblasts. *Hum Gene Ther* **5**: 949–958.
75. Fan, Y, Maley, M, Beilharz, M and Grounds, M (1996). Rapid death of injected myoblasts in myoblast transfer therapy. *Muscle Nerve* **19**: 853–860.
76. Skuk, D, Roy, B, Goulet, M and Tremblay, JP (1999). Successful myoblast transplantation in primates depends on appropriate cell delivery and induction of regeneration in the host muscle. *Exp Neurol* **155**: 22–30.
77. Guerette, B, Asselin, I, Vilquin, JT, Roy, R and Tremblay, JP (1994). Lymphocyte infiltration following allo- and xenomyoblast transplantation in mice. *Transplant Proc* **26**: 3461–3462.
78. Hardiman, O, Sklar, RM and Brown, RH Jr. (1993). Direct effects of cyclosporin A and cyclophosphamide on differentiation of normal human myoblasts in culture. *Neurology* **43**: 1432–1434.
79. Hong, F, Lee, J, Song, JW, Lee, SJ, Ahn, H, Cho, JJ *et al.* (2002). Cyclosporin A blocks muscle differentiation by inducing oxidative stress and inhibiting the peptidyl-prolyl-*cis*-*trans* isomerase activity of cyclophilin A: cyclophilin A protects myoblasts from cyclosporin A-induced cytotoxicity. *FASEB J* **16**: 1633–1635.
80. Kinoshita, I, Roy, R, Dugre, FJ, Gravel, C, Roy, B, Goulet, M *et al.* (1996). Myoblast transplantation in monkeys: control of immune response by FK506. *J Neuropathol Exp Neurol* **55**: 687–697.
81. Kinoshita, I, Vilquin, JT, Guerette, B, Asselin, I, Roy, R and Tremblay, JP (1994). Very efficient myoblast allotransplantation in mice under FK506 immunosuppression. *Muscle Nerve* **17**: 1407–1415.
82. Smythe, GM and Grounds, MD (2001). Absence of MyoD increases donor myoblast migration into host muscle. *Exp Cell Res* **267**: 267–274.
83. El Fahime, E, Torrente, Y, Caron, NJ, Bresolin, MD and Tremblay, JP (2000). *In vivo* migration of transplanted myoblasts requires matrix metalloproteinase activity. *Exp Cell Res* **258**: 279–287.
84. Cousins, JC, Woodward, KJ, Gross, JG, Partridge, TA and Morgan, JE (2004). Regeneration of skeletal muscle from transplanted immortalised myoblasts is oligoclonal. *J Cell Sci* **117**: 3259–3269.
85. Skuk, D, Roy, B, Goulet, M, Chapdelaine, P, Bouchard, JP, Roy, R *et al.* (2004). Dystrophin expression in myofibers of Duchenne muscular dystrophy patients following intramuscular injections of normal myogenic cells. *Mol Ther* **9**: 475–482.

86. Skuk, D, Goulet, M, Roy, B, Chapdelaine, P, Bouchard, JP, Roy, R *et al.* (2006). Dystrophin expression in muscles of duchenne muscular dystrophy patients after high-density injections of normal myogenic cells. *J Neuropathol Exp Neurol* **65**: 371–386.
87. Skuk, D, Goulet, M, Roy, B, Piette, V, Cote, CH, Chapdelaine, P *et al.* (2007). First test of a "high-density injection" protocol for myogenic cell transplantation throughout large volumes of muscles in a Duchenne muscular dystrophy patient: eighteen months follow-up. *Neuromuscul Disord* **17**: 38–46.
88. Camirand, G, Rousseau, J, Ducharme, ME, Rothstein, DM and Tremblay, JP (2004). Novel Duchenne muscular dystrophy treatment through myoblast transplantation tolerance with anti-CD45RB, anti-CD154 and mixed chimerism. *Am J Transplant* **4**: 1255–1265.
89. Stephan, L, Pichavant, C, Bouchentouf, M, Mills, P, Camirand, G, Tagmouti, S *et al.* Induction of tolerance across fully mismatched barriers by a non-myeloablative treatment excluding antibodies and irradiation. *Cell Transplant* **15**: 835–846.
90. Quenneville, SP, Chapdelaine, P, Skuk, D, Paradis, M, Goulet, M, Rousseau, J *et al.* Autologous transplantation of muscle precursor cells genetically modified with a lentivirus for muscular dystrophy: human cells and primates models. *Mol Ther* **15**: 431–438.
91. Goncalves, MA, Holkers, M, Cudre-Mauroux, C, van Nierop, GP, Knaan-Shanzer, S, van der Velde, I *et al.* (2006). Transduction of myogenic cells by retargeted dual high-capacity hybrid viral vectors: robust dystrophin synthesis in duchenne muscular dystrophy muscle cells. *Mol Ther* **13**: 976–986.
92. Floyd, SS Jr., Clemens, PR, Ontell, MR, Kochanek, S, Day, CS, Yang, J *et al.* (1998). *Ex vivo* gene transfer using adenovirus-mediated full-length dystrophin delivery to dystrophic muscles. *Gene ther* **5:** 19–30.
93. Di Rocco, G, Iachininoto, MG, Tritarelli, A, Straino, S, Zacheo, A, Germani, A *et al.* (2006). Myogenic potential of adipose-tissue-derived cells. *J Cell Sci* **119**: 2945–2952.
94. El Fahime, E, Mills, P, Lafreniere, JF, Torrente, Y and Tremblay, JP (2002). The urokinase plasminogen activator: an interesting way to improve myoblast migration following their transplantation. *Exp Cell Res* **280**: 169–178.
95. Lafreniere, JF, Mills, P, Tremblay, JP and Fahime, EE (2004). Growth factors improve the *in vivo* migration of human skeletal myoblasts by modulating their endogenous proteolytic activity. *Transplantation* **77**: 1741–1747.
96. Gussoni, E, Soneoka, Y, Strickland, CD, Buzney, EA, Khan, MK, Flint, AF *et al.* (1999). Dystrophin expression in the mdx mouse restored by stem cell transplantation. *Nature* **401**: 390–394.
97. Jackson, KA, Mi, T and Goodell, MA (1999). Hematopoietic potential of stem cells isolated from murine skeletal muscle. *Proc Natl Acad Sci USA* **96**: 14482–14486.
98. Goodell, MA, Brose, K, Paradis, G, Conner, AS and Mulligan, RC (1996). Isolation and functional properties of murine hematopoietic stem cells that are replicating *in vivo*. *J Exp Med* **183**: 1797–1806.
99. Montanaro, F, Liadaki, K, Volinski, J, Flint, A and Kunkel, LM (2003). Skeletal muscle engraftment potential of adult mouse skin side population cells. *Proc Natl Acad Sci USA* **100**: 9336–9341.
100. Montanaro, F, Liadaki, K, Schienda, J, Flint, A, Gussoni, E and Kunkel, LM (2004). Demystifying SP cell purification: viability, yield, and phenotype are defined by isolation parameters. *Exp Cell Res* **298**: 144–154.
101. Liadaki, K, Kho, AT, Sanoudou, D, Schienda, J, Flint, A, Beggs, AH *et al.* (2005). Side population cells isolated from different tissues share transcriptome signatures and express tissue-specific markers. *Exp Cell Res* **303**: 360–374.
102. Bachrach, E, Li, S, Perez, AL, Schienda, J, Liadaki, K, Volinski, J, Flint, A *et al.* (2004). Systemic delivery of human microdystrophin to regenerating mouse dystrophic muscle by muscle progenitor cells. *Proc Natl Acad Sci USA* **101**: 3581–3586.
103. McKinney-Freeman, SL, Jackson, KA, Camargo, FD, Ferrari, G, Mavilio, F and Goodell, MA (2002). Muscle-derived hematopoietic stem cells are hematopoietic in origin. *Proc Natl Acad Sci USA* **99**: 1341–1346.
104. McKinney-Freeman, SL, Majka, SM, Jackson, KA, Norwood, K, Hirschi, KK and Goodell, MA (2003). Altered phenotype and reduced function of muscle-derived hematopoietic stem cells. *Exp Hematol* **31**: 806–814.
105. Asakura, A and Rudnicki, MA (2002). Side population cells from diverse adult tissues are capable of *in vitro* hematopoietic differentiation. *Exp Hematol* **30**: 1339–1345.
106. Schienda, J, Engleka, KA, Jun, S, Hansen, MS, Epstein, JA, Tabin, CJ *et al.* (2006). Somitic origin of limb muscle satellite and side population cells. *Proc Natl Acad Sci USA* **103**: 945–950.
107. Rivier, F, Alkan, O, Flint, AF, Muskiewicz, K, Allen, PD, Leboulch, P *et al.* (2004). Role of bone marrow cell trafficking in replenishing skeletal muscle SP and MP cell populations. *J Cell Sci* **117**: 1979–1988.
108. Bachrach, E, Perez, AL, Choi, YH, Illigens, BM, Jun, SJ, del Nido, P *et al.* (2006). Muscle engraftment of myogenic progenitor cells following intraarterial transplantation. *Muscle Nerve* **34**: 44–52.
109. Pavlath, GK and Gussoni, E (2005). Human myoblasts and muscle-derived SP cells. *Methods Mol Med* **107**: 97–110.
110. Frank, NY, Kho, AT, Schatton, T, Murphy, GF, Molloy, MJ, Zhan, Q *et al.* (2006). Regulation of myogenic progenitor proliferation in human skeletal muscle by BMP 4 and its antagonist gremlin. *J Cell Biol* **175**: 99–110.
111. Cossu, G and Biressi, S (2005). Satellite cells, myoblasts and other occasional myogenic progenitors: Possible origin, phenotypic features and role in muscle regeneration. *Semin Cell Dev Biol* **16**: 623–631.
112. Cao, B and Huard, J (2004). Muscle-derived stem cells. *Cell Cycle* **3**: 104–107.
113. Minasi, MG, Riminucci, M, De Angelis, L, Borello, U, Berarducci, B, Innocenzi, A *et al.* (2002). The meso-angioblast: a multipotent, self-renewing cell that originates from the dorsal aorta and differentiates into most mesodermal tissues. *Development* **129**: 2773–2783.
114. Sampaolesi, M, Torrente, Y, Innocenzi, A, Tonlorenzi, R, D'Antona, G, Pellegrino, MA *et al.* (2003). Cell therapy of alpha-sarcoglycan null dystrophic mice through intra-arterial delivery of mesoangioblasts. *Science* **301**: 487–492.
115. Galvez, BG, Sampaolesi, M, Brunelli, S, Covarello, D, Gavina, M, Rossi, B *et al.* (2006). Complete repair of dystrophic skeletal muscle by mesoangioblasts with enhanced migration ability. *J Cell Biol* **174**: 231–243.
116. Dellavalle, A, Sampaolesi, M, Tonlorenzi, R, Tagliafico, E, Sacchetti, B, Perani, L, *et al.* (2007). Pericytes of human skeletal muscle are myogenic precursors distinct from satellite cells. *Nat cell Biol* **9**: 255–267.
117. Sampaolesi, M, Blot, S, D'Antona, G, Granger, N, Tonlorenzi, R, Innocenzi, A *et al.* (2006). Mesoangioblast stem cells ameliorate muscle function in dystrophic dogs. *Nature* **444**: 574–579.
118. Gavina, M, Belicchi, M, Rossi, B, Ottoboni, L, Colombo, F, Meregalli, M *et al.* (2006). VCAM-1 expression on dystrophic muscle vessels has a critical role in the recruitment of human blood-derived CD133$^+$ stem cells after intra-arterial transplantation. *Blood* **108**: 2857–2866.
119. Torrente, Y, Belicchi, M, Sampaolesi, M, Pisati, F, Meregalli, M, D'Antona, G *et al.* (2004). Human circulating AC133$^+$ stem cells restore dystrophin expression and ameliorate function in dystrophic skeletal muscle. *J Clin Invest* **114**: 182–195.
120. Allen, DL, Teitelbaum, DH and Kurachi, K (2003). Growth factor stimulation of matrix metalloproteinase expression and myoblast migration and invasion *in vitro*. *Am J Physiol Cell Physiol* **284**: C805–C815.
121. Corti, S, Salani, S, Del Bo, R, Sironi, M, Strazzer, S, D'Angelo, MG *et al.* (2001). Chemotactic factors enhance myogenic cell migration across an endothelial monolayer. *Exp Cell Res* **268**: 36–44.
122. Lafreniere, JF, Mills, P, Bouchentouf, M and Tremblay, JP (2006). Interleukin-4 improves the migration of human myogenic precursor cells *in vitro* and *in vivo*. *Exp Cell Res* **312**: 1127–1141.
123. Torrente, Y, El Fahime, E, Caron, NJ, Del Bo, R, Belicchi, M, Pisati, F *et al.* (2003). Tumor necrosis factor-α (TNF-α) stimulates chemotactic response in mouse myogenic cells. *Cell Transplant* **12**: 91–100.
124. Ferrari, G, Cusella-De Angelis, G, Coletta, M, Paolucci, E, Stornaiuolo, A, Cossu, G *et al.* (1998). Muscle regeneration by bone marrow-derived myogenic progenitors. *Science* **279**: 1528–1530.
125. Berenson, RJ, Andrews, RG, Bensinger, WI, Kalamasz, D, Knitter, G, Buckner, CD *et al.* (1988). Antigen CD34$^+$ marrow cells engraft lethally irradiated baboons. *J Clin Invest* **81**: 951–955.
126. Torrente, Y, Camirand, G, Pisati, F, Belicchi, M, Rossi, B, Colombo, F *et al.* (2003). Identification of a putative pathway for the muscle homing of stem cells in a muscular dystrophy model. *J Cell Biol* **162**: 511–520.
127. Qu-Petersen, Z, Deasy, B, Jankowski, R, Ikezawa, M, Cummins, J, Pruchnic, R *et al.* (2002). Identification of a novel population of muscle stem cells in mice: potential for muscle regeneration. *J Cell Biol* **157**: 851–864.
128. Nomura, T, Ashihara, E, Tateishi, K, Asada, S, Ueyama, T, Takahashi, T *et al.* (2007). Skeletal myosphere-derived progenitor cell transplantation promotes neovascularization in delta-sarcoglycan knockdown cardiomyopathy. *Biochem Biophys Res Common* **352**: 668–674.
129. Sarig, R, Baruchi, Z, Fuchs, O, Nudel, U and Yaffe, D (2006). Regeneration and transdifferentiation potential of muscle-derived stem cells propagated as myospheres. Stem cells **24**: 1769–1778.
130. Winitsky, SO, Gopal, TV, Hassanzadehl, S, Takahashi, H, Gryder, D, Rogawski, MA *et al.* (2005). Adult murine skeletal muscle contains cells that can differentiate into beating cardiomyocytes *in vitro*. *PLoS Biol* **3**: e87.
131. Lee-Pullen, TF, Bennett, AL, Beilharz, MW, Grounds, MD and Sammels, LM (2004). Superior survival and proliferation after transplantation of myoblasts obtained from adult mice compared with neonatal mice. *Transplantation* **78**: 1172–1176.
132. Deasy, B, Lu, A, Tebbets, JC, Feduska, JM, Schugar, RC, Pollett, JB *et al.* A role for cell sex in stem cell mediated skeletal muscle regeneration: female cells have higher muscle regeneration efficiency *J Cell Biol* (in press).
133. Mueller, GM, O'Day, T, Watchko, JF and Ontell, M (2002). Effect of injecting primary myoblasts versus putative muscle-derived stem cells on mass and force generation in mdx mice. *Hum Gene Ther* **13**: 1081–1090.
134. Jankowski, RJ, Deasy, BM, Cao, B, Gates, C and Huard, J (2002). The role of CD34 expression and cellular fusion in the regeneration capacity of myogenic progenitor cells. *J Cell Sci* **115**: 4361–4374.
135. Jankowski, RJ and Huard, J (2004). Myogenic cellular transplantation and regeneration: sorting through progenitor heterogeneity. *Panminerva Med* **46**: 81–91.
136. Oshima, H, Payne, TR, Urish, KL, Sakai, T, Ling, Y, Gharaibeh, B *et al.* (2005). Differential myocardial infarct repair with muscle stem cells compared to myoblasts. *Mol Ther* **12**: 1130–1141.
137. Payne, TR, Oshima, H, Sakai, T, Ling, Y, Gharaibeh, B, Cummins, J *et al.* (2005). Regeneration of dystrophin-expressing myocytes in the mdx heart by skeletal muscle stem cells. *Gene Ther* **12**: 1264–1274.
138. Oberlin, E, Tavian, M, Blazsek, I and Peault, B (2002). Blood-forming potential of vascular endothelium in the human embryo. *Development* **129**: 4147–4157.
139. Zambidis, ET, Oberlin, E, Tavian, M and Peault, B (2006). Blood-forming endothelium in human ontogeny: lessons from in utero development and embryonic stem cell culture. *Trends Cardiovasc Med* **16**: 95–101.
140. Andreeva, ER, Pugach, IM, Gordon, D and Orekhov, AN (1998). Continuous subendothelial network formed by pericyte-like cells in human vascular bed. *Tissue Cell* **30**: 127–135.
141. Betsholtz, C, Lindblom, P and Gerhardt, H (2005). Role of pericytes in vascular morphogenesis. *Exs* 115–125.
142. Alliot-Licht, B, Bluteau, G, Magne, D, Lopez-Cazaux, S, Lieubeau, B, Daculsi, G *et al.* (2005). Dexamethasone stimulates differentiation of odontoblast-like cells in human dental pulp cultures. *Cell Tissue Res* **321**: 391–400.
143. Collett, GD and Canfield, AE (2005). Angiogenesis and pericytes in the initiation of ectopic calcification. *Circ Res* **96**: 930–938.
144. Farrington-Rock, C, Crofts, NJ, Doherty, MJ, Ashton, BA, Griffin-Jones, C and Canfield, AE (2004). Chondrogenic and adipogenic potential of microvascular pericytes. *Circulation* **110**: 2226–2232.
145. Kamochi, H, Kurokawa, MS, Yoshikawa, H, Ueda, Y, Masuda, C, Takada, E *et al.* (2006). Transplantation of myocyte precursors derived from embryonic stem cells transfected with IGFII gene in a mouse model of muscle injury. *Transplantation.* **82**: 516–526.