

Vessel-associated stem cells from skeletal muscle: From biology to future uses in cell therapy

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Abstract

Over the last years, the existence of different stem cells with myogenic potential has been widely investigated. Besides the classical skeletal muscle progenitors represented by satellite cells, numerous multipotent and embryologically unrelated progenitors with a potential role in muscle differentiation and repair have been identified. In order to conceive a therapeutic approach for degenerative muscle disorders, it is of primary importance to identify an ideal stem cell endowed with all the features for a possible use *in vivo*. Among all emerging populations, vessel-associated stem cells are a novel and promising class of multipotent progenitors of mesodermal origin and with high myogenic potential which seem to best fit all the re-

quirements for a possible cell therapy. *In vitro* and *in vivo* studies have already tested the effectiveness and safety of vessel-associated stem cells in animal models. This leads to the concrete possibility in the future to start pilot human clinical trials, hopefully opening the way to a turning point in the treatment of genetic and acquired muscle disorders.

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INTRODUCTION

Adult skeletal muscle is a highly dynamic tissue capable of self renewal and repair during adult life in response to increased workload, stress conditions or damage. Satellite cells (SCs) represent the main stem cell population responsible for this regenerative capacity due to their self-renewal properties and their ability to secure normal growth and maintenance of skeletal muscle^[1,2].

However, in the last years, the paradigm that SCs are the only stem cells capable of undergoing muscle differentiation

has been challenged by numerous studies. A growing number of observations demonstrates that a variety of cell types from different adult tissues have the ability to differentiate into skeletal muscle *in vitro* and *in vivo* (Table 1)^[3-7]. This editorial will mainly focus on mesoangioblasts, vessel-associated progenitors of mesodermal origin, able to reconstitute damaged skeletal muscle^[8-10].

VESSEL-ASSOCIATED MULTIPOTENT STEM CELLS

In recent years, the discovery of myogenic precursors within the bone marrow (BM) and the identification of a novel class of mesodermal multipotent progenitors from the dorsal aorta of mouse embryos termed “mesoangioblasts”^[8] have promoted the development of novel and puzzling hypothesis on nature, embryonic origin, identity, plasticity and mutual relationship between various stem cells populations.

The observation that several adult tissues contain progenitors that under specific conditions may give rise to embryologically unrelated tissues encouraged scientists to identify a possible primitive ancestor for the different stem cells. A stem cell associated with the blood vessel wall is the most conceivable candidate as a possible source of pan-organ, multilineage stem cells. So far, a number of stem cells associated with the vasculature system have been identified and characterized: hematopoietic stem cells (HSCs)^[11], hemangioblasts^[12], endothelial progenitor cells (EPCs)^[13], mesenchymal stem cells (MSCs), mesoderm adult progenitors (MAPs) and, more recently, mesoangioblasts^[14-16].

The demonstration that mesoangioblasts derive from the dorsal aorta antagonizes the classical theory of a somitic origin for all myogenic precursors. Jackson *et al.*^[17] reported that adult skeletal muscle contains progenitors able to repopulate murine hematopoietic system. Therefore, a possible relationship seems to exist between different progenitors with an apparent common root in the hemangioblastic system.

Embryonic origin and developmental migration of vessel-associated multipotent cells

A cartoon summarizing the possible embryonic origin of myogenic stem cells is represented in Figure 1. According to the hypothesis of a unique origin of multilineage stem cells^[17-19], it has been postulated that Pax3-expressing cells, able to migrate from the paraxial mesoderm to the dorsal aorta, use angiogenesis as a route to all mesoderm tissues during fetal histogenesis. As soon as a blood vessel penetrates into a muscle during development, vessel-associated progenitors can adopt a satellite cell fate, but some of them may remain undifferentiated, keeping their multipotency during postnatal growth or muscle regeneration. Therefore, angiogenesis represents a path for these cells not only

during histogenesis but possibly also during adult life (i.e. during active phase of angiogenesis such as skeletal muscle inflammation/damage/neoplasia). Both endothelial cells and pericytes can detach from the vessel wall toward the growing tissues and also toward the blood flux, circulating and reaching areas of angiogenesis/damage where differentiation into local tissue eventually occurs.

The possible lineage model for the common ancestor is represented in Figure 1, also showing a critical role of VEGF signalling and its receptor Flk1b^[20,21]. The sheer mesodermal progenitors are supposed to remain associated with endothelial cells in an abluminal position (being sheer hematopoietic and sheer endothelial progenitors in luminal and parietal position respectively) and remain quiescent in post-natal microvascular niches.

CHARACTERIZATION OF VESSEL-ASSOCIATED STEM CELLS

Skeletal muscle progenitors from mouse embryonic dorsal aorta

De Angelis *et al.*^[8] for the first time isolated a population of cells able to undergo skeletal muscle differentiation from embryonic dorsal aorta. The authors demonstrated that these cells were endowed with myogenic potential *in vivo* and participated in postnatal growth and muscle regeneration. Indeed, aorta-derived cells were able to incorporate into newly formed muscle fibers after direct intramuscular injection into the regenerating tibialis anterior of immune-deficient mice. Signals from the surrounding developing skeletal muscle seemed to be essential in the process of myogenesis and muscular differentiation. The detection of cells contralaterally to the site of injection confirmed their ability to circulate as predictable by their vascular origin. Aorta-derived cells clearly co-expressed early myogenic markers (M-cadherin, MyoD, Myf5, c-Met, desmin) as well as endothelial-vascular markers (VE-cadherin, Flk1, α M-integrin, β 3integrin, P-selectin, α -SMA, and CD31) strongly in accordance with the hypothesis of their origin from true endothelial cells or from a common precursor. Endothelial progenitors typically express CD34 while aorta-derived myogenic clones only expressed CD34 at the beginning of clonal expansion, suggesting early loss of this antigen while entering the myogenic differentiation pathway. While the positivity for endothelial markers was not detected on fetal myoblasts, the authors also demonstrated the expression of endothelial markers in a subset of SCs, in accordance with the idea that the main muscle resident population, classically considered of somitic origin, could, at least in part, derive from a common vascular-mesodermal progenitor.

In vitro studies showed a multilineage potential of aorta-derived stem cells^[14]: in fact, they were able to differentiate into skeletal muscle upon co-culture with myoblasts; they expressed osteoblasts markers (ALP, bone sialoprotein and

Table 1 Origin and main characteristics of possible muscular progenitors

Satellite cells		
Myoblasts/SCs	Ref. 43-48	Results Dystrophin restoration in nude/mdx mice after transplantation In clinical trials: either negative or poor results; main problems: Inability to cross vascular barrier and limited intramuscular migration/need of multiple intramuscular injections Immunosuppression needed to prevent cells rejection and apoptosis Poor efficiency of cells graft: very high number of transplanted cells needed DMD clinical trial, best result: restoration of dystrophin expression: 26%-30%
BMSCs/circulating AC133+ cells		
	49-52	Ability to undergo myogenic differentiation but only minimal effects <i>in vivo</i> Possibility of intra-arterial injection Activation induced by muscle inflammation/damage
HSC	53	Purification of CD45 ⁺ Sca1 ⁺ c-Kit ⁺ Lin ⁻ fraction with myogenic potential
MSC	54,55	Myogenic potential of MSC but poor muscle recovery <i>in vivo</i>
bmSP	5	Isolated through Hoechst 33342 exclusion Purification of CD45 ⁺ c-Met ⁺ CD43 ⁺ Lin ⁻ fraction Poor muscle incorporation rates
AC133+ cells	56,57	Circulating human hematopoietic/endothelial progenitors endowed with myogenic potential (upon co-culture with myoblasts) Advantage: circulating ability Ability to incorporate in SCc niche and participate in muscle regeneration to a poor extent
Other skeletal muscle progenitors		
MDSCs	58-63	Population of mesodermal origin, isolated from skeletal muscle based on temporal differences in binding collagen pre-coated plates Identification of CD34 ⁺ /Bcl ⁺ Sca1 ⁺ fraction with myogenic potential and ability to bind to muscular capillary network after intra-arterial injection Expression of L-selectin (ligand for MAdCAM-1) by CD34 ⁺ fraction probably accounting for their homing ability Migration and regenerative properties influenced by pathology, age and sex
mSP	5-7,64,65	Isolated from muscle through Hoechst 33342 dye exclusion Differences with bmSP: Both Sca1+Lin- but mSP are c-Kit- and CD43- mSP: ability to enter SCs niche Differences with SCs: Expression of Sca1/class of Sca-1 positive cells associated with blood vessels with high degree of plasticity Presence in Pax7null mice Ability to flow through small vessels 2 different fractions: CD45 ⁺ : hematopoietic origin and preferential differentiation CD45 ⁻ : somitic origin, greater myogenic potential (in co-culture with myoblasts/under stimulation of Wnt pathway)
Possible muscular progenitors of other origin		
ADSCs	66-68	Close relationship with myogenic cells (same mesodermal origin, inverse relation skeletal muscle/adipose tissue size, myoblasts/SCs capable to convert into adipose tissue) CD13 ⁺ CD44 ⁺ CD73 ⁺ CD90 ⁺ and stromal vascular fraction: <i>in vivo</i> and <i>in vitro</i> myogenic potential; myogenic potential enhanced by forced MyoD expression Advantages: easy availability, immune-privileged behavior, strong expansion <i>ex vivo</i>
EPCs	3,25,26,28	Identification of CD34 ⁺ CD144 ⁺ Flk1 ⁺ CD45 ⁻ CD56 ⁻ fraction with myogenic potential (contribution to muscle regeneration <i>in vivo</i>)

SCs: Satellite cells; BMSCs: Bone marrow stem cells; HSCs: Hematopoietic stem cells; MSCs: Mesenchymal stem cells; bmSP: Bone marrow side population; mSP: Muscle side population; MDSCs: Muscle derived stem cells; ADSCs: Adipose-derived stem cells; EPCs: Endothelial progenitor cells.

core-binding factor $\alpha 1$) when exposed to BMP2; when treated with dexamethasone they acquired a typical adipocyte morphology; after treatment with 1,25 (OH)₂ vitamin D they expressed the osteoclastic markers tartrate resistant acid phosphatase (TRAP) and calcitonin receptor with occasional multinucleated osteoclast-like morphology. Moreover, the whole cell population contained a small fraction (3%-5%) of CD45-positive cells in steady state but, when co-cultured with freshly isolated total BM cells, more

than 70% of cells expressed CD45 and a lower but significant percentage expressed macrophage-monocyte markers (Mac3 and CD11b).

Of particular interest is the expression of α -SMA (commonly expressed by skeletal myoblasts, smooth muscle precursors and pericytes) in a variable percentage of aorta-derived cells (5%-30%)^[13]. Cells positive for this marker divided less frequently than the general population and remained in a constant proportion. This seems to be a di-

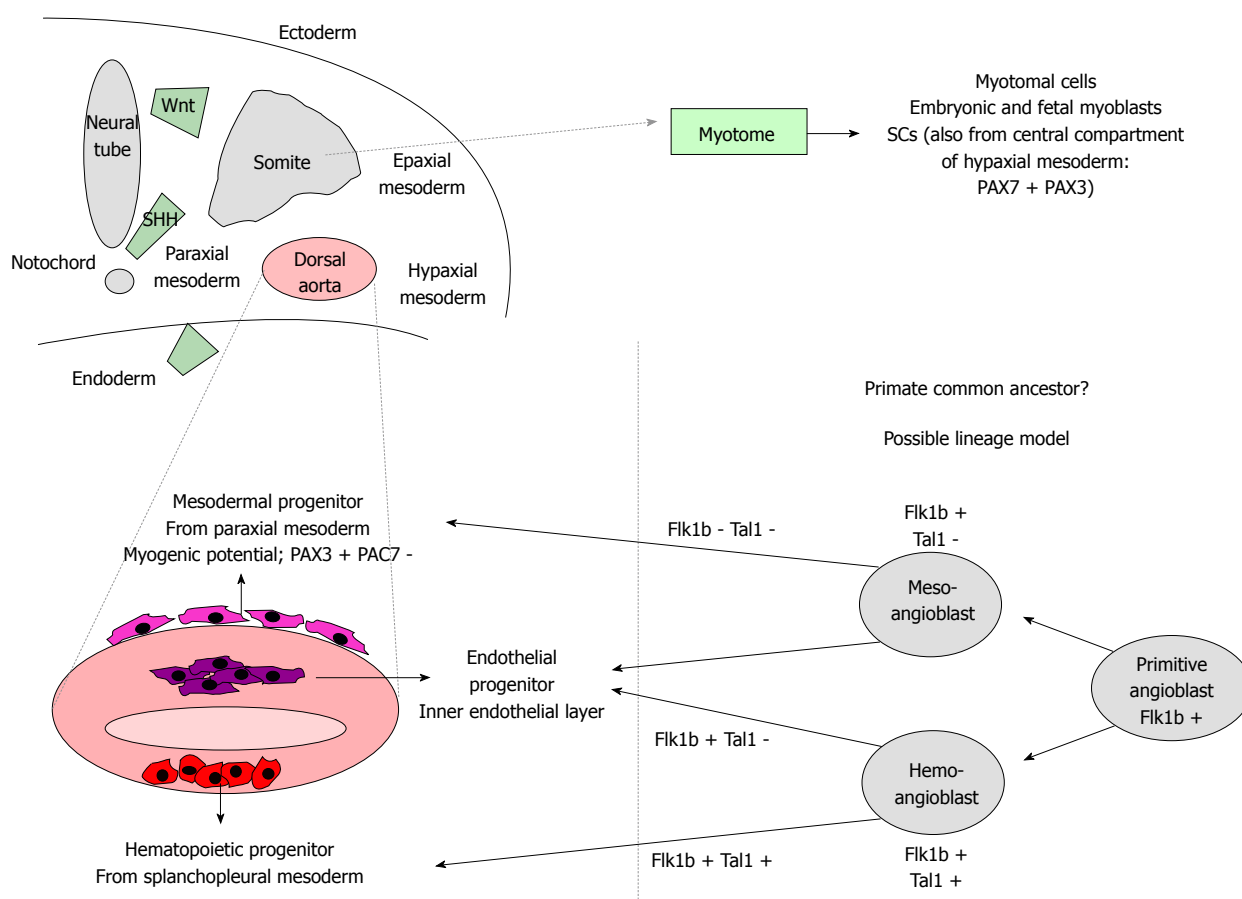


Figure 1 Embryonic origin of muscular progenitors. *Upper panel.* Schematic representation of the somitic origin throughout the myotome of classical muscular progenitors-myotomal cells, myoblasts, SCs. A fraction of SCs derives from a population of undifferentiated PAX7⁺PAX3⁺ stem cells located in the central compartment of hypaxial mesoderm. *Lower panel. Left.* Origin of various multipotent progenitors from different areas of dorsal aorta (mesodermal progenitors endowed with the highest myogenic potential, endothelial, hematopoietic progenitors). Wnt, sonic hedgehog and endoderm signalings can influence commitment. *Right.* Possible lineage model of mesodermal progenitors which could derive from a unique common primitive ancestor (angioblast) (partially developed from Cossu *et al.*^[15]). Flk1b: Flt ligand kinase 1b; Tal1: T acute leukemia 1; SHH: Sonic hedgehog.

rect reminiscence of the behavior of post-natal pericytes and could be considered as evidence that dorsal aorta progenitors which express an immature endothelial phenotype can also give rise to pericyte-like progeny.

The *in vivo* experiments on chick embryos confirmed the multilineage potential of aorta-derived stem cells: both endothelial (Flk⁺ cells) and subendothelial (pericytes, Flk⁻) subsets initially appeared associated to the vasculature of the host, generating chimeric microvascular districts in a broad range of tissues including muscle, teguments and skeleton^[14].

The majority of genes preferentially expressed by aorta-derived cells are expressed in the mesoderm^[16]. Among the most represented pathways were the TGFβ/BMP one, implicated in cell survival and efficiently protecting stem cells from programmed cell death^[22,23], and the Wnt signalling pathway, involved in early angiogenesis and in skeletal myogenesis in somites. Of particular importance is the high and selective expression of so called “inflammatory genes” (i.e. E-selectin, β7integrin, ALCAM, several cytokines receptors and CD44) thus confirming the role of aorta-derived cells in tissue regeneration.

Moreover, aorta-derived cells also constitutively express inducible Heat Shock Proteins (HSP 70.1 and HSP 70.3) in absence of cellular stress and this may represent a protection from sudden cellular stress on areas of necrosis and inflammation^[24].

Aorta-derived cells fulfil all the requested criteria for true stem cells^[14]: they are endowed with self-renewal ability and are able to continuously generate one or more types of differentiated cells. *Ex vivo* expansion of the progeny of a single founder cell generates large clones that become independent from the feeder, grow *in vitro* for more than one year and are resistant to retroviral transduction. Upon continuous sub-culturing, all the clones retain the same morphology, the expression of “hemangioblastic” markers such as CD34, Flk1 and c-Kit and especially the capacity to differentiate into most mesoderm cell types including hematopoietic and mesodermal tissue lineages both *in vitro* and *in vivo*.

Cossu *et al.*^[15] termed this novel stem cell “mesoangioblast” in parallel with the existence of “hemangioblast” with vascular and hematopoietic potential. Differently from hemangioblasts which represent progenitors for a tissue

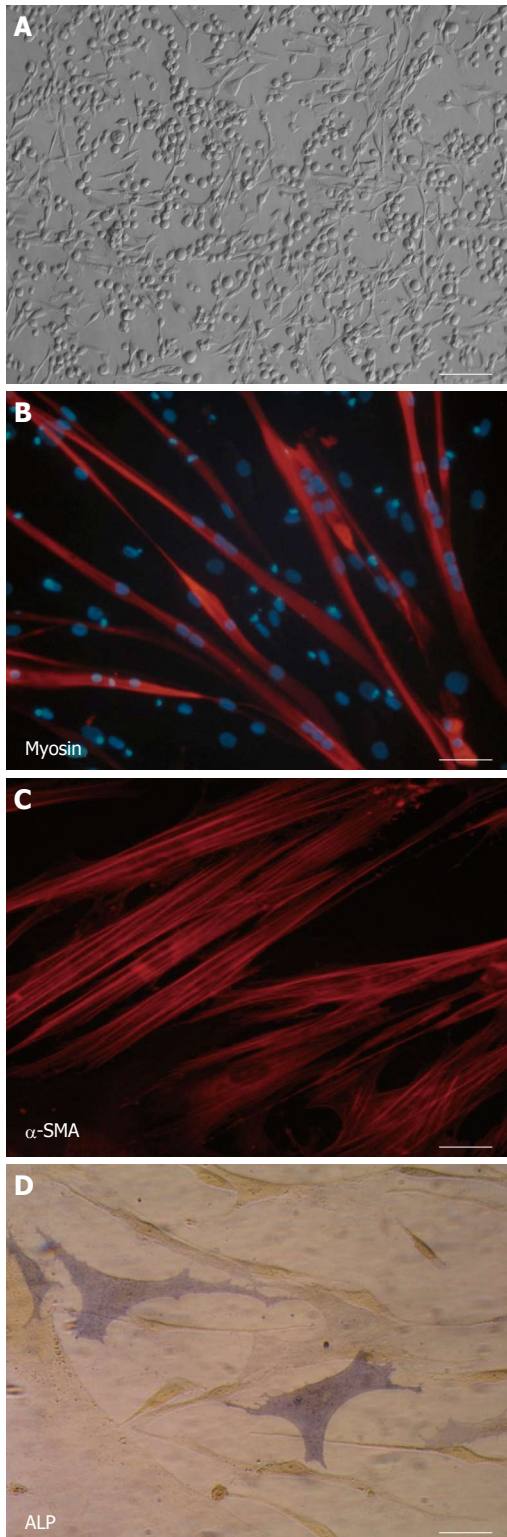


Figure 2 Cell morphology and multilineage differentiation of human mesoangioblasts. **A:** Phase contrast image showing human proliferating mesoangioblasts with refractive triangular, adherent and round loosely adherent/floating components. Scale bar: 40 μ m; **B:** Skeletal muscle differentiation. After exposure to normal human myoblasts-conditioned medium and subsequently to differentiation medium, human mesoangioblasts efficiently fuse into myosin-positive fully differentiated myotubes. Scale bar: 40 μ m; **C:** Smooth-muscle differentiation. After treatment with TGF β human mesoangioblasts stained with anti- α -SMA antibody show approximately 80% positivity. Scale bar: 10 μ m; **D:** Osteoblasts differentiation. After treatment with BMP2 human mesoangioblasts differentiate into strongly ALP-positive osteoblast-like cells. Scale bar: 40 μ m.

with rapid turnover (hematopoietic cells), mesoangioblasts represent only an additional source of mesodermal derivatives mainly generated from embryonic progenitors, which can represent a useful reservoir of possible progenitors during pathological conditions in the course of adult life.

Human vessel-derived stem cells: the adult counterpart of embryonic murine mesoangioblasts

Recently, we and others^[9,10] isolated the human counterpart of murine mesoangioblasts from fragments of diagnostic skeletal muscle biopsies. These cells displayed a high proliferative rate (approximately 20 population doublings independent of donor's age), maintained a normal diploid karyotype and a limited lifespan, differently from mouse mesoangioblasts, after a certain number of population doublings underwent senescence.

After appropriate stimuli and culture conditions, human mesoangioblasts were able to differentiate into smooth and skeletal muscle, osteoblasts or adipocytes (Figure 2). When co-cultured with murine myogenic cells, exposed to muscle-differentiation medium^[10] or cultured in normal human myoblasts-conditioned medium to facilitate their commitment^[9], a large proportion of cells differentiated into multinucleated myotubes.

Human mesoangioblasts expressed pericytes markers (annexin V, ALP, desmin, α -SMA, vimentin and PDGF receptor β), while, at variance with their murine counterpart, they did not express typical endothelial markers (CD31, CD34 and VEGF receptor 2/KDR) and M-cadherin, NCAM, cytokeratins or neurofilaments. Differently from SCs, they did not constitutively express myogenic markers (MyoD, Myf5, Myogenin, Pax7). The expression of surface antigens was as follows: strongly positive for CD13 and CD44, weakly positive for CD49b and uniformly negative, among others, for CD31, CD34, CD45, CD133, CD56^[9,10]. Immunophenotyping confirmed a strong positivity for ALP. It is remarkable to note that in adult skeletal muscle only vessels-derived cells are known to be strongly positive for ALP and cells co-expressing ALP and CD31 are located adjacent to the endothelium underneath the vessel basal lamina as typical pericytes. The quasi-omnipresence of pericytes in the organism, according to the hypothesis of pan-organ dissemination of multi-lineage stem cells, suggests a relationship between human mesoangioblasts and pericytes: embryonic mesoangioblasts occupy an endothelial position (also confirmed by the expression of endothelial markers in murine aorta-derived mesoangioblasts) and during development they may move to a more external perithelial position, progressively switching from an "endothelium-like" to a "pericyte-like" phenotype. Therefore such a population of interstitial, pericyte-derived cells might, most likely, represent the progeny of prenatal mesoangioblasts. With regard to this, we previously described the presence of numerous strongly ALP-positive round cells with a MyoD-positive nucleus in the endomysium of skeletal muscle thus demonstrating the commitment toward a myogenic fate of part of

the ALP-positive cells. In the same areas we observed SCs expressing Pax7 while ALP-positive cells in the interstitium were Pax7-negative. Since ALP is not expressed in SCs and myoblasts, the presence of cells expressing both ALP and myogenic markers suggests recruitment of pericytes derived cells into the myogenic lineage^[9].

The myogenic potential of pericyte-derived stem cells does not seem to be restricted to muscle-derived endothelial cells: vascular endothelial cells with mesodermal characteristics and myogenic potency *in vitro* and *in vivo* have also been purified from adult human pancreas and adipose tissue^[25,26]. Moreover, vessel-associated stem cells can also secrete multiple paracrine growth factors/cytokines which likely strengthen their strong regenerative potential^[27].

More recently, a population of cells termed myo-endothelial was isolated from skeletal muscle. These cells accounted for less than 0.5% of the total skeletal muscle population, co-expressed CD56, CD34 and CD144 and were able to regenerate muscle fibers within the injured muscle^[28]. These cells were identified in the interstitial spaces of murine skeletal muscle using CD34 antigen (Sca34 cells: CD34⁺CD45⁻)^[29], displayed the potential to differentiate into adipocytes, endothelial cells and, under appropriate culture conditions, expressed all myogenic markers and were able to feed the satellite niche *in vivo*.

Cardiac mesoangioblasts

Recently, mesoangioblasts from adult mouse cardiac muscle were isolated^[30]. These cells were clonogenic and self-renewable and co-expressed endothelial, pericyte and cardiac markers (i.e. Nkx2.5, GATA-4). Cardiac mesoangioblasts were able to spontaneously differentiate into contracting cardiomyocytes which in part displayed pacemaker/conduction ability (sino-atrial like myocytes) that correlated with the expression of specific markers (GATA-6, connexins, HCN channels). The same authors isolated human cardiac mesoangioblasts expressing markers and cardiac genes similar to their murine counterpart^[31]. Human cardiac mesoangioblasts have limited self-renewing and clonogenic ability and, despite their commitment to cardiomyogenesis, do not contract spontaneously and fail to efficiently regenerate cardiac muscle *in vivo*.

VESSEL-ASSOCIATED STEM CELLS AND *IN VIVO* STUDIES: FUTURE PERSPECTIVE FOR CELL THERAPY AND APPLICABILITY IN HUMAN PATHOLOGY

Murine mesoangioblasts: first evidence of therapeutic potential in dystrophic mice

The therapeutic potential of murine mesoangioblasts was first tested in α SG null mice^[32]. Allogenic wild type (wt) mesoangioblasts injected systemically into the femoral artery were able to flow through blood circulation and migrate into downstream skeletal muscles, mainly reaching areas

where degeneration and regeneration were occurring. In particular, injected mesoangioblasts were detected either at the periphery of fibers where they expressed SCs markers (M-cadherin, c-Met) or in several vessels near areas of regeneration where they expressed α -SMA or platelet endothelial cell adhesion molecule, suggesting that they can incorporate into regenerating fibers (feeding the SCs pool) or express markers of smooth muscle or endothelium. Muscle fibers showed a restored expression of α SG and the other members of the dystrophin-glycoprotein complex with a pattern comparable to wt muscles. Multiple transplantations led to a better muscle morphology with increased number of apparently normal fibers, reduction of necrotic areas and cellular infiltrates, preserved integrity of the sarcolemma and marked reduction of fibrosis. This was accompanied by a concomitant recovery of global muscle function. The effects of autologous, genetically corrected mesoangioblasts transplantation were comparable.

In a different study, the ability of mesoangioblasts injected intramuscularly to promote the expression of muscle-specific proteins and contribute to the muscle SCs compartment in regenerated fibers of *mdx/utrn*^{-/-} mice was confirmed^[33].

Homing and migration ability of mesoangioblasts

Homing to skeletal muscle *in vivo* is a complex process which depends on several factors; so far, only a small percentage of mesoangioblasts injected intra-arterially reaches downstream skeletal muscles (10% in downstream muscles and 1% in contralateral muscles)^[10]. Indeed, most cells pass through the capillary network toward the venous circulation and are finally trapped into filter organs (lung, liver, spleen). All the mechanisms by which mesoangioblasts can reach skeletal muscle are only partially mimicked by *in vitro* assays and can be influenced by several factors (i.e. amount of dystrophic fibers, age). Only vessels present in dystrophic muscles of young-aged mice express adhesion molecules necessary for efficient extravasation of cells, while with progression of the disease, muscle fibers display reduced regeneration ability and are replaced by scarring and fat with altered microcirculation, thus hampering stem cell migration.

The most important factor that regulates stem cells migration is represented by inflammation. Inflammatory cells create a first line of defence against the pathological process but at the same time cause local damage to the inflamed tissue, killing resident cells. This “dual” process leads to the creation of a sort of niche where stem cells can engraft and participate in tissue regeneration due to their ability to be attracted by all the signals resulting from local damage within the inflammatory infiltrate. Other factors related to microenvironment are fundamental for stem cells recruitment including the extracellular matrix, the activation of local endothelia which becomes more permissive to extravasation, the interplay between the differentiated cells in the tissue and the generation of signals which favor stem cells

survival and differentiation. Among the molecules responsible for mesoangioblasts migration is HMGB1, a chromatin-binding protein, with a crucial role in transcription and with the extracellular role of proinflammatory cytokine^[34,35]. As signal of tissue damage, HMGB1 is also passively released by necrotic cells, triggering inflammation^[35-39].

More recently other factors have been involved in mesoangioblasts migration. Mesoangioblasts express high levels of CXCR-4 and TNF-receptor^[16] and this observation led to hypothesize a major role of SDF-1 and TNF α in their migration^[35].

Mesoangioblasts do not constitutively express L-selectin, $\alpha 4$ -integrin and $\beta 2$ -integrin^[16]. *Ex vivo* pre-treatment of mesoangioblasts with cytokines and forced expression of certain adhesion molecules (L-selectin and $\alpha 4$ integrin) dramatically improved their migration ability *in vitro* and *in vivo*^[38]. Moreover, combined strategies (pre-treatment with TNF- α of mesoangioblasts expressing $\alpha 4$ integrin) resulted in even more significant increase of cell migration. Subsequent studies demonstrated that pre-treatment with NO was able to further ameliorate mesoangioblasts migration triggered by other cytokines and growth factors (TNF α , TGF β , VEGF) and to protect them from apoptogenic dystrophic muscle environment^[40]. Due to this intriguing dual role of inflammatory reaction within the site of damage, the role of macrophages in stem cells recruitment was subsequently investigated^[39]. In particular, “inflammatory” macrophages actively secreting TNF α and HMGB1 and generating substantial amounts of NO appear to initiate mesoangioblasts recruitment while the interaction between mesoangioblasts and “alternative” macrophages is relevant to complete the differentiation process.

Amelioration of migratory ability of mesoangioblasts would allow a better cells engraftment into the target tissue and a more efficient cell homing would reduce the amount of cells which normally remain trapped within the filter organs (spleen, lung, liver), hence minimizing the risk for potential harmful effects.

Canine mesoangioblasts in golden retriever DMD model

The therapeutic value of mesoangioblasts in large animal models was recently demonstrated for the first time in a canine model of DMD^[41]. Dystrophic dogs received either allogenic wt mesoangioblasts or autologous mesoangioblasts transduced *in vitro* with a lentiviral vector expressing human microdystrophin. Mesoangioblasts transplantation led to extensive reconstitution of fibers expressing dystrophin with improvement in the contraction force and, in many cases, preservation of walking ability. Donor wt mesoangioblasts seemed to be more efficient than autologous genetically corrected ones. This study showed that mesoangioblasts transplantation is a safe and effective procedure in large animals, opening the path for possible future clinical trials in muscular disorders.

Mesoangioblasts in human muscle pathology

The evidence of great myogenic potential of human me-

soangioblasts *in vitro* along with the demonstration of a possible therapeutic value in animal models have raised expectations for a possible use of these vessel-associated stem cells in inherited or acquired muscle disorders.

Human mesoangioblasts isolated from patients affected by DMD are indistinguishable from cells derived from normal muscle^[10] with the exception of a possible up-regulation of some inflammatory genes. Both wt and mini-dystrophin-transduced mesoangioblasts are able to colonize downstream muscles when injected intra-arterially into *mdx* mice, reconstituting muscle fibers with partial, but significant, recovery of motility^[10]. These observations are an essential issue for the possibility to use transduced autologous cells in genetic muscle disorders (first of all DMD).

The therapeutic potential of mesoangioblasts in adult muscle disorders has also been extensively explored by our group over the last few years. In particular, we focused our studies on idiopathic inflammatory myopathies (IM)^[9] and on facioscapulohumeral muscle dystrophy (FSHD)^[42].

IM are characterized by mononuclear cells infiltration of skeletal muscle and include three major forms: dermatomyositis (DM), polymyositis (PM) and inclusion body myositis (IBM). Despite mechanisms of CD8-mediated myocytotoxicity playing a major role in both PM and IBM, IBM responds poorly or not at all to immunosuppressive therapies. In PM and DM, a complement-mediated microangiopathy of skeletal muscle, current immunotherapies are usually effective with efficient muscle regeneration and recovery of muscle strength and trophism. On the contrary, IBM muscle is characterized by the presence of unique degenerative features and inefficient regenerative properties. For the first time, we characterized mesoangioblasts from patients with IM and demonstrated that cells obtained from IBM patients display defective differentiation in skeletal muscle and that this block can be corrected *in vitro* by transient MyoD transfection or by silencing the myogenesis inhibitor basic helix loop helix B3 (BHLHB3)^[9]. Of clinical relevance is the possibility of isolating myogenic stem cells from small muscle biopsies, to expand and commit them down skeletal muscle differentiation pathway and subsequently inoculate them intra-arterially by targeting selective muscle groups. In this manner, the characteristic early and severe atrophy of quadriceps in IBM could be treated by infusing mesoangioblasts with selective catheter-mediated delivery into the iliac arteries. Moreover, the use of autologous cells would eliminate the obligatory need of immune suppression.

More recently, we demonstrated that mesoangioblasts can be efficiently isolated from FSHD muscles and expanded to an amount of cells sufficient to transplant an adult patient^[42]. However, the ability of FSHD mesoangioblasts to differentiate into skeletal muscle was variably impaired and this defect correlated with the overall disease severity and the degree of histopathologic abnormalities of the muscle of origin. A remarkable differentiation defect was observed in mesoangioblasts from all mildly to severely affected muscles

whereas mesoangioblasts from morphologically normal FSHD muscles showed no myogenic differentiation block. This study would suggest the possibility of limiting muscle damage in FSHD patients using autologous mesoangioblasts from unaffected muscles without requiring immune suppression or genetic correction *in vitro*.

CONCLUSION

Stem cell therapy for muscle disorders: myoblast transplantation

Due to the evidence that myoblasts can be transplanted into dystrophic mice and partially reconstitute dystrophin expression, several attempts have been made using SCs-derived myoblasts intramuscular transplantation as a therapeutic approach for degenerative muscle diseases^[43-48]. SCs are safe, can repopulate the stem cells niche and have the higher myogenic differentiation ability among all the myogenic stem cell populations identified so far. However, the failure of *in vivo* studies was mainly due to the poor survival and the very limited migratory capacity of these muscle progenitors. In addition, SCs are unable to cross vessel walls thus requiring multiple intramuscular injections and making the development of new strategies to improve the graft success necessary.

Alternative myogenic stem cells for cell therapy

Several other types of stem cells with myogenic potential have been considered in the last years, including blood-borne progenitors which, with their circulating ability, could better distribute within the whole muscle. Hematopoietic stem cells, BM side population and circulating AC133⁺ cells have therefore been proposed for a cell therapy. The major limiting problem of this approach is represented by the poor extent of skeletal muscle colonization by hematopoietic cells.

Within the BM and associated with vascular niche, different non-hematopoietic stem cells of common mesodermal origin (probably belonging to the endothelial or pericyte lineages) have been recently identified (multipotent adult progenitors, muscle-derived stem cells, mesoangioblasts). This results in a complex scenario, that most likely will further expand in the future so that it will be eventually possible to choose between a variety of myogenic progenitors with different properties but similarly able to efficiently reconstitute damaged muscle.

Are mesoangioblasts the ideal stem cells for skeletal muscle diseases?

Mesoangioblasts apparently meet all the requirements needed for cell therapy *in vivo*. In fact, these stem cells used so far for systemic delivery in dystrophic mice and dogs have the ability to cross the vessel wall and reach specific areas of degeneration/regeneration of skeletal muscle in response to cytokines. Other essential and favourable characteristics of mesoangioblasts are their safety as a therapeutic

tool as demonstrated in large animal models and their good availability, being easily obtainable from accessible postnatal tissues. A phase I clinical trial with donor-derived mesoangioblasts has already been planned for the end of 2010 in DMD^[69]. Indeed, they can be easily and efficiently isolated from muscle specimens including vitally-frozen diagnostic biopsies. This is of particular relevance in pathologies often misdiagnosed (i.e. IBM versus PM) because it makes possible to go back to stored muscles to isolate and grow mesoangioblasts. Mesoangioblasts can be extensively (though not indefinitely) expanded *in vitro* maintaining their myogenic potential to an amount of cells suitable for an *in vivo* treatment. In addition, they can be manipulated *in vitro* being easily transducible with viral vectors carrying therapeutic genes (with satisfying efficiency, safety, long term expression), thus overcoming differentiation defects. If engineered cells with inclusion of a relatively small cDNA in a viral vector could represent a valuable therapeutic strategy for human genetic dystrophies, on the other hand, in non genetic muscle disorders (i.e. IM, in particular IBM), autologous cells would be able to participate to skeletal muscle regeneration without a stable viral transduction and the need of immunotherapy. Obviously, an ideal muscle stem cell should also present the ability to differentiate *in vivo* in new muscle fibers with high efficiency and give rise to physiologically normal muscle cells. So far, mesoangioblasts have shown to have the greater myogenic potential among the various proposed mesodermal stem cells.

A fundamental issue for a possibly effective cell therapy of skeletal muscle disorders remains the enhancement of stem cells homing within the damaged areas, also limiting the accumulation of cells in filter organs. In this regard, several studies have demonstrated that various feasible *ex vivo* treatments with cytokines are able to optimize human mesoangioblasts migration to skeletal muscle. Several factors seem to influence mesoangioblasts homing and differentiation, above all age and condition of the muscle in which they should home, so a transplant strategy is likely to be more effective in younger and less compromised patients. Although in diseased human muscle mesoangioblasts appear to be greatly activated by muscle damage and inflammation, their quantitative contribution to muscle regeneration in physiological and specific pathological conditions remains to be fully elucidated. Moreover, because similar myogenic potential is present ubiquitously within pericytes and endothelial cells purified from many organs (pancreas, fat and possibly other tissues) it is very difficult to fully understand the physiological role of such widespread populations.

Future perspectives

In order to design clinical trials tailored to specific muscle diseases, many issues remain to be solved, mainly concerning the requirement (in most pathologies) of functional correction of large muscles, the need to produce homogeneous cell populations as well as a number of species-specific

reagents and the high variability among different patients. Pharmacological and biomolecular strategies are strongly needed to optimize survival, migration and myogenic capabilities of stem cells, especially in myopathies in which a muscle differentiation block exists. Equally important is the analysis of putative environmental cues acting in the pathological muscle milieu to minimize the detrimental effects on the survival and the effectiveness of the transplanted stem cells.

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