Review TRENDS in Molecular Medicine Vol.10 No.10 October 2004

New therapies for muscular dystrophy: cautious optimism

Giulio Cossu[1,2,3] and Maurilio Sampaolesi[1]

[1]Stem Cell Research Institute, Dibit, H. San Raffaele, 58 Via Olgettina, 20132 Milan, Italy
[2]Department of Histology and Medical Embryology. University of Rome La Sapienza, 14 Via Scarpa, 00161 Rome, Italy
[3]Institute of Cell Biology and Tissue Engineering, San Raffaele Biomedical Park of Rome, 100 Via di Castel Romano, 00128 Rome, Italy

The quest for a therapy for muscular dystrophy has been the driving force behind the past 40 years of advances in this field. Numerous results, such as the identification of satellite cells and gene mutations that are responsible for most forms of dystrophies, advances in gene transfer and modification technology and, more recently, stem cells, have fueled hopes. However, administering corticosteroids still remains the only effective treatment available. Several recent advances have uncovered a diversity of possible therapeutic approaches, from pharmacological treatments to gene therapy (exon-skipping and adeno-associated viruses) and cell therapy with different types of newly identified stem cells. Importantly, a combination of these strategies might greatly enhance the possibility of successful therapy.

Muscular dystrophies are clinically and molecularly heterogeneous diseases that are characterized by the primary wasting of skeletal muscle, which compromises patient mobility. In the most severe case, Duchenne muscular dystrophy (DMD), respiratory and cardiac functions are affected, leading to wheelchair dependency, respiratory failure and premature death [1]. In many cases, the mutation affects the proteins that form a link between the cytoskeleton and the basal lamina. A mutation in one of these proteins often causes the disassembly of the whole complex, leading to increased fragility of the sarcolemma, especially during intense contractile activity. This, in turn, results in increased calcium entry (although the molecular mechanisms have not been elucidated in detail) and focal or diffuse damage to the fiber [2]. Damaged or dead fibers can be repaired or replaced by satellite cells [3]. These cells, which were originally identified as a result of their location between the basal lamina and the membrane of the muscle fiber, are now considered the resident 'stem-like' cells in skeletal muscle. They are responsible for muscle growth and regeneration in post-natal life [4]. However, the satellite cells of patients with muscular dystrophy share the same molecular defect and produce fibers that are also prone to degeneration. With time, the population of satellite cells is exhausted and the muscle tissue is progressively replaced by connective and adipose tissue.

Corresponding author: Giulio Cossu (cossu.giulio@hsr.it).
Available online 15 September 2004

Muscular dystrophies are among the most difficult diseases to treat, even though the underlying molecular defects are now known. This is due to the fact that skeletal muscle is the most abundant tissue of the body and is composed of large multinucleated fibers, the nuclei of which have permanently lost the ability to divide. Consequently, any cell or gene replacement must restore proper gene expression in hundreds of millions of post-mitotic nuclei, which are embedded in a highly structured cytoplasm and surrounded by a thick basal lamina. Similarly, most pharmacological trials must overcome the complex and partly unknown biochemical mechanism of fiber degeneration that involves pathways, such as calcium fluxes and protease activity, for which inhibitors are associated with high systemic toxicity. Nevertheless, the results that have been accumulated during the last few years have opened new perspectives for all these different approaches.

The pharmacological approach

Several pharmacological strategies have been attempted to counteract the consequences of the dystrophic process, including protease inhibitors, calcium blockers and drugs that act on protein and lipid metabolism [5]. Few of these have produced promising results in animal models (almost exclusively mice) and even fewer have entered clinical trials, with little further success. Fiber degeneration is accompanied by a chronic inflammation (mainly macrophages and lymphocytes) that leads to sclerosis and a reduction of the vascular supply. This starts a vicious circle that reduces oxygen supply and increases the likelihood of degeneration for surviving and regenerated fibers [1]. Therefore, anti-inflammatory molecules appeared to be the logical therapeutic strategy and, for more than a decade, corticosteroids have represented the only pharmacological therapy with relatively modest but consistent beneficial consequences [6]. Recent data indicate that corticosteroid treatment in DMD can delay the loss of independent ambulation by 2 to 4 years, significantly reduce the risk of developing skeletal defects and delay the onset of respiratory and cardiac failure. However, their use is associated with significant side effects, such as weight gain and osteoporosis with the risk of bone fractures [7].

Recently, an old idea has been reconsidered on the basis of new results; muscle trophy is the balance between

 1471-4914/$ - see front matter doi:10.1016/j.molmed.2004.08.007

anabolic processes (the synthesis of contractile proteins and, to a minor extent, the proliferation and recruitment of progenitor cells) and catabolic processes (protein degradation due to calcium leakiness and the activation of proteases). It was reasoned that stimulating anabolic processes would counteract, or at least delay, muscle wasting. This was true for insulin growth factor 1 (IGF-1), which is a known muscle growth factor, and myostatin, a negative regulator of muscle growth. When mice over-expressing IGF-1 where crossed to mdx mice (a model for DMD), dystrophy was attenuated [8]. Similarly, when neutralizing antibodies against myostatin were systemically delivered to mdx mice, dystrophic mice showed a dramatic delay of muscle wasting [9]. Moreover, several other 'booster' proteins were identified that stimulated muscle regeneration and ameliorated dystrophy, such as integrin α7, acetyl-N-galactosamine transferase, and A disintegrin and metalloprotease 12 (ADAM12) (reviewed in [10]). Special attention was attracted by utrophin (a protein that is related to dystrophin), for which increased expression in mdx mice prevented the occurrence of pathology [11]. However the utrophin gene is large and, as is dystrophin, difficult to transfer. As an alternative approach, a high-throughput screen for small molecules that can upregulate utrophin synthesis was carried out but, so far, no new candidate drugs have been identified.

Blocking the proteasome in mdx mice causes the re-appearance at the membrane of many proteins of the dystrophin complex (except dystrophin itself), suggesting another method to counteract the muscle protein degradation [12]. Finally, the expression of nitric oxide synthase increased angiogenesis and also resulted in the amelioration of the mdx phenotype [13] by counteracting the reduced vascular supply that accompanies fiber degeneration, inflammation and sclerosis. Because muscular dystrophy defects result from the premature degradation of proteins that are important for muscle function and maintenance, these pharmacological agents might be invaluable for slowing down the progression of the disease, ameliorating the quality of life of the patient and, at the same time, increasing the chance of success for gene or cell therapy. It remains to be seen whether these strategies can be applied as a treatment, because the toxicity of these molecules must be assessed.

Gene therapy

The task of replacing a missing gene in all, or at least in a good proportion, of the post-mitotic nuclei of skeletal muscle is daunting. Furthermore, for DMD, the form of dystrophy that most needs a therapy, the gene to be replaced is the largest known, with a cDNA of 14 Kb [2]. Two successive waves of enthusiasm were generated by the use of adenoviral vectors that were successful in delivering dystrophin to a very large fraction of muscle fibers in newborn dystrophic mice [14]. The first generation of treatments, however, caused a strong immune reaction against the vector (which was not apparent in newborn animals, which were tolerant). The second generation of treatment, 'gutted' vectors, can accommodate the full-length cDNA for dystrophin, but do not carry genes encoding viral proteins. These induce a much weaker immune reaction, but have to cross the basal lamina of muscle fibers and the efficiency of transduction is greatly reduced in juvenile and adult animals [15–16].

Adeno-associated viral (AAV) vectors are derived from a non-pathogenic replication-deficient virus with a small (~4.7-kb) single-stranded DNA genome. They appear to be more efficient for transducing adult fibers (owing to their smaller size) [17], especially if delivered systemically together with factors that increase vascular permeability [18–19]; a clinical trial using these vectors is ongoing. They cannot accommodate the full-length dystrophin cDNA, but a truncated version (micro-dystrophin) that gives good functional rescue when replacing dystrophin in transgenic mdx mice [20]. Moreover, they can accommodate the full-length sarcoglycan cDNAs, the proteins that are mutated in several forms of limb girdle muscular dystrophies [1].

An alternative strategy for gene therapy involves 'exon skipping'. This molecular strategy prevents the transcription of the exon containing the mutation. Skipping can be achieved through oligonucleotides or by small RNAs that hybridize with the donor and/or acceptor sites of the mutated exon, causing its exclusion from the otherwise intact transcript. Because the skipped exon usually does not encode a functionally essential domain, the resulting protein is shorter but functional. Despite the fact that the oligonucleotides appear to function for only a short period *in vitro*, they are much more stable in muscle fibers *in vivo* and, recently, long-term correction of dystrophy in mdx mice was achieved by a single injection of oligonucleotides [21]. Based on this finding, several clinical trials in patients have been planned, and one in UK has now been funded. Other gene-therapy approaches, such as plasmid DNA injection or DNA–RNA chimeric oligonucleotides, which currently appear to be less efficient, have been reviewed previously [22–23].

Cell therapy

The identification of satellite cells in 1961 [3] offered the first hope for treating muscular dystrophy with cells that can make new muscle. Since the beginning, two alternatives appeared: (i) using cells obtained from a healthy donor, which express the normal copy of the mutated gene but induce an immune rejection unless the patient is permanently immune suppressed; or (ii) using cells obtained from the patient, which do not require immune suppression but must be 'genetically corrected' *in vitro* (to restore the expression of the mutated protein). This latter task was made possible (although far from easy) by the cloning of the genes that result in muscular dystrophy. Satellite cells and cell lines derived from them have been used since the late 1970s, mainly through intra-muscular injection. A first pivotal study involved the injection of wild-type myoblasts (from the immortal myogenic cell line C2C12) into mdx mice and resulted in the conversion of muscles from dystrophin-negative to dystrophin-positive [24]. This study led to several clinical trials in the early 1980 s that failed for several reasons, the most important of which were the poor survival and the very limited migratory capacity of injected donor cells, together with the immune response of the patient that was not

suppressed at that time. Myogenic cell transplantation was continued and optimized in few laboratories in preclinical studies. Very recently, a novel clinical trial with partially matched donor cells and immune suppression showed the reconstitution of up to 11% of dystrophin-positive fibers in the area of injection [25].

The major problem still faced by this approach is the lack of dispersion of donor cells, which remain in the area of injection, making it difficult to reach an even distribution within the whole muscle. This might be overcome by using blood-borne stem or progenitor cells. This perspective became theoretically possible in the late 1990s, with the demonstration of cells in the bone marrow that could contribute to muscle regeneration following bone marrow transplantation [26]. The possible systemic delivery of circulating cells was the obvious choice over satellite cells that cannot cross the endothelial layer. It was subsequently reported that a fraction of bone marrow cells, the SP (side population, which are characterized by the ability to exclude dye as a result of the presence of a multi-drug exclusion pump that is typical of stem cells) would give rise to dystrophin-positive fibers in the mdx mouse following bone marrow transplantation [27]; in both studies, the extent of colonization by donor cells was very small, far from any hope of clinical benefit. During the following years, many reports appeared describing the trans-differentiation of bone marrow cells into embryologically unrelated cell types, such as hepatocytes, neurons, cardiomyocytes and epithelia. Controversy soon arose on the significance of these data, which have been interpreted as methodological artifacts or rare events of cell fusion rather than signal-mediated changes in cell fate (for a thoughtful review, see [28]). Leaving aside the possibility of artifacts, cell fusion would result in the exposure of the donor-cell nucleus to the host-cell transcription factors and, for muscle, the dominant effect of MyoD would soon activate muscle genes in the donor cells. Although this might not be a major problem in terms of future clinical applications (especially considering that muscle cells are multinucleated) the rarity of the event and our ignorance on the underlying mechanism make this process a distant future clinical application.

During the last two years, however, several reports have convincingly demonstrated that bone marrow SP cells can be recruited to dystrophic or regenerating muscle and can differentiate into skeletal muscle cells upon exposure to differentiating muscle cells or in response to Wnt molecules that are secreted by recruiting cells; moreover, a fraction of SP cells localizes to a position (between the basal lamina and the sarcolemma) that is typical of satellite cells and expresses markers of satellite cells [29–31]. Two independent groups showed that the progeny of a single SP cell can reconstitute the hematopoietic system of a mouse following bone marrow transplant and can also contribute cells to regenerating muscle [32,33]. Finally, a recent paper showed that it is possible to transduce SP cells from a dystrophic mouse with a lentiviral vector expressing human micro-dystrophin, and reconstitute a few dystrophic fibers with the human protein following intra-vascular delivery [34]. Interestingly, cells expressing the hematopoietic marker AC133 that can differentiate into dystrophin-positive fibers *in vivo* are present in the human circulation, suggesting that strategies developed in murine models might later be transferred to patients [35].

Currently, two questions remain unanswered: (i) whether SP cells are recruited to myogenesis through signal-induced myogenic commitment (including differentiation into satellite cells) or through the fusion of a myeloid intermediate cell with host muscle fibers (or eventually both) and (ii) whether SP cells are the only stem or progenitor cell associated with the hemo-vascular system that posses myogenic potential. In favor of signal-mediated commitment is the induction by Wnt molecules, similar to what happens in embryonic myogenesis, and the identification of donor SP-derived cells expressing satellite cell markers in a location typical of satellite cells [29–32]; in favor of fusion is the expression of a myelomonocitic marker in the population with myogenic capacity and the failure to detect donor-derived satellite cells *in vivo* and in culture [33]. Evidence that SP cells are not the only mesoderm progenitor that can differentiate into skeletal muscle comes from several studies showing that different CD45 (a pan-myeloid marker) negative cells, such as multipotent adult progenitors (MAPs) [36], mesoangioblasts [37] or muscle-derived stem cells (MDSCs) [38], can differentiate into skeletal myotubes *in vitro* and *in vivo* when delivered to regenerating or dystrophic muscle (Figure 1). The expression of CD45 in SP cells clearly defines the hematopoietic nature of these cells; the lack of CD45 in these other types of stem cells, which are generally associated with the vascular niche in bone marrow, skeletal muscle [38,39] or other tissues [40], identifies them as non-hematopoietic and probably belonging to the endothelial or pericyte lineages [41].

Notably, in at least one case in which wild-type or dystrophic genetically corrected mesoangioblasts were delivered intra-arterially to dystrophic muscle of α-sarcoglycan knockout mice (a model for limb girdle muscular dystrophy), it resulted in a dramatic functional amelioration of the dystrophic phenotype [42]. In principle, to optimize the efficacy of stem-cell therapy for muscular dystrophy, it will be necessary: (i) to isolate cells from an easily accessible anatomical site; (ii) to expand them *in vitro* without the loss of stem-cell properties; (iii) to efficiently transduce them with viral vectors (such as lentiviral vectors, which are by far the most efficient integrating vectors but have not yet been approved for use in patients); and (iv) to facilitate homing to diseased muscle through circulatory routes by using molecules that can recruit them, such as HMGB1 (high-mobility group B1) [43]. SP cells are still difficult to expand *in vitro* but recent advances for the expansion *in vitro* of hematopoietic stem cells should hopefully solve this question [44]. CD45-negative stem cells have not been systematically tested for their ability to rescue dystrophic muscle by intra-vascular delivery (Table 1), with the exception of mouse mesoangioblasts, for which the human counterparts are still being characterized.

Concluding remarks

The current advances that have been made in pre-clinical research in the field of muscular dystrophy justify a

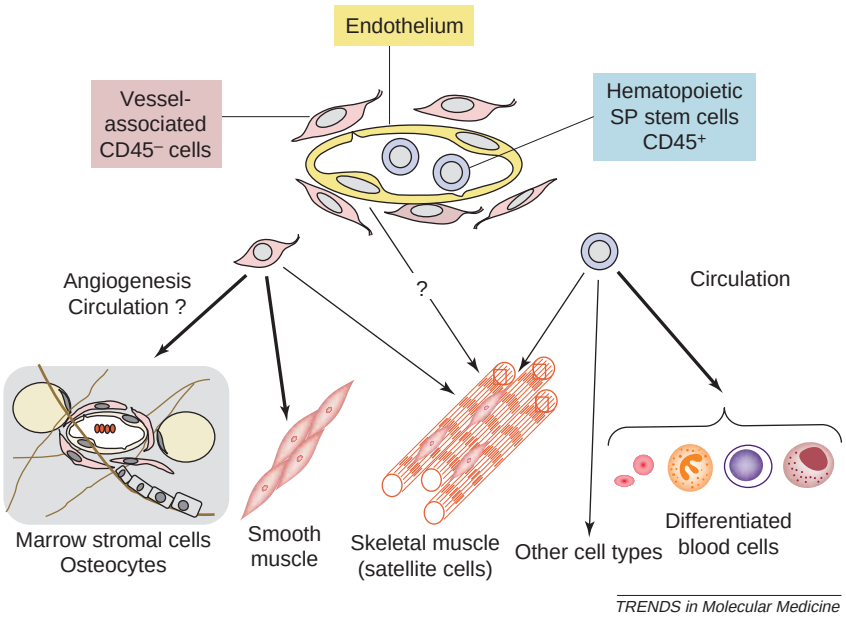

Figure 1. Two different progenitors with myogenic potential. The diagram summarizes the possible anatomical origin of $CD45^+$ hematopoietic (SP) stem cells and of CD45-vessel-associated progenitors ($CD45^-$). SP cells (pale blue) primarily differentiate into blood cells, but might be recruited via the circulation to other tissues, including skeletal muscle. Alternatively, $CD45^-$ vessel-associated progenitors (pink) are ill-defined, probably heterogeneous cells that primarily form pericytes, vessel smooth muscle layer and bone marrow stromal cells. They might undergo skeletal myogenic differentiation, probably during the angiogenic process that accompanies muscle regeneration. Endothelial cells (yellow) might also undergo skeletal myogenesis during regeneration as they do during embryogenesis [46,47]. The possible embryological origin of these cells is discussed in [41].

Table 1. The phenotype of different stem cells and their potential to contribute to muscle regeneration in dystrophic muscle

Stem cell	Source	Growth *in vitro*	Homing to diseased muscle?	Myogenic differentiation induced by:	Effect in primary myopathy	Refs
Hematopoietic stem cells (SP)	Bone marrow	Limited	Yes	Co-culture with myogenic cells; Wnts	Modest (<1%)	[27,29–33]
Multipotent adult progenitors	Bone marrow	Yes	Unknown	5′-Aza-cytidine	Unknown	[36]
Mesoangioblasts	Small vessels	Yes	Yes	Co-culture with myogenic cells	Good (~30% of muscles down-stream injected artery)	[37]
Endothelial myo-genic progenitors	Muscle vessels	Limited	Unknown	Co-culture with myogenic cells	Unknown	[39]
Muscle-derived stem cells	Skeletal muscle	Yes	Yes	Low serum; standard myo-genic culture	Good (>90% in injected area)	[38]
Sinovial muscle stem cells	Sinovial membranes	Yes	Yes	5′-Aza-cytidine	Modest (<1%)	[40]

cautious optimism, more so than only a few years ago [45]. Results from clinical trials will be available in the coming months: it would not be wise to expect spectacular results. It is, however, realistic to expect that some beneficial effect will be observed in some of the ongoing studies and this ought to be possible without any major (at least short term) toxicity. This would justify the planning of further trials that could incorporate the predicted results from ongoing experimental work. New generations of viral vectors, improved methods for efficient and long-lasting exon skipping, and increasing knowledge about the various types of stem cells (resulting in more efficient ways to manipulate them) should be the basis for the next generation of trials, all of which might benefit from the design of combined pharmacological therapies. In turn, these will need to be tested on patients independently from gene or cell therapy to select the most efficacious and least toxic for successive combined therapy. It is probable that muscular dystrophies, which present such a complex pathogenesis, will only be defeated by a combined effort that is aimed to replace or correct the mutated gene product and simultaneously counteract the devastating consequences of the primary mutation on muscle structure and function.

Acknowledgements

Our work is supported by Telethon, European Community, Duchenne Parent Project, Association Francoise contra les Myopathies and the Italian Ministries of Health and of Research. We thank Francesco Muntoni for helpful suggestions and critical reading of the manuscript.

References

1 Emery, A.E. (2002) The muscular dystrophies. *Lancet* 359, 687–695
2 Blake, D.J. *et al.* (2002) Function and genetics of dystrophin and dystrophin-related proteins in muscle. *Physiol. Rev.* 82, 291–329
3 Mauro, A. (1961) Satellite cell of skeletal muscle fibers. *J. Biohys. Biochem. Cytol.* 9, 493–495

4 Zammit, P. and Beauchamp, J. (2001) The skeletal muscle satellite cell: stem cell or son of stem cell? *Differentiation* 68, 193–204
5 Satoyoshi, E. (2000) Therapeutic trials on progressive muscular dystrophy. *Eur. Neurol.* 43, 127–132
6 Campbell, C. and Jacob, P. (2003) Deflazacort for the treatment of Duchenne dystrophy: a systematic review. *BMC Neurol.* 3, 7
7 Manzur, A.Y. *et al.* (2004) Glucocorticoid corticosteroids for Duchenne muscular dystrophy (Cochrane Review). In: *The Cochrane Library*, Issue 2, CD003725, John Wiley & Sons Ltd.
8 Barton, E.R. *et al*. (2002) Muscle-specific expression of insulin-like growth factor I counters muscle decline in *mdx* mice. *J. Cell Biol.* 157, 137–148
9 Bogdanovich, S. *et al*. (2002) Functional improvement of dystrophic muscle by myostatin blockade. *Nature* 420, 418–421
10 Engvall, E. and Wewer, U.M. (2003) The new frontier in muscular dystrophy research: booster genes. *FASEB J.* 17, 1579–1584
11 Deconinck, N. *et al*. (1997) Expression of truncated utrophin leads to major functional improvements in dystrophin-deficient muscles of mice. *Nat. Med.* 3, 1216–1221
12 Bonuccelli, G. *et al*. (2003) Proteasome inhibitor (MG-132) treatment of *mdx* mice rescues the expression and membrane localization of dystrophin and dystrophin-associated proteins. *Am. J. Pathol.* 163, 1663–1675
13 Wehling, M. *et al*. (2001) A nitric oxide synthase transgene ameliorates muscular dystrophy in *mdx* mice. *J. Cell Biol.* 155, 123–131
14 Ragot, T. *et al*. (1993) Efficient adenovirus-mediated transfer of a human minidystrophin gene to skeletal muscle of mdx mice. *Nature* 361, 647–650
15 Chen, H.H. *et al*. (1997) Persistence in muscle of an adenoviral vector that lacks all viral genes. *Proc. Natl. Acad. Sci. U. S. A.* 94, 1645–1650
16 Dello Russo, C. *et al*. (2002) Functional correction of adult mdx mouse muscle using gutted adenoviral vectors expressing full-length dystrophin. *Proc. Natl. Acad. Sci. U. S. A.* 99, 12979–12984
17 Herzog, R.W. (2004) AAV-mediated gene transfer to skeletal muscle. *Methods Mol. Biol.* 246, 179–194
18 Greelish, J.P. *et al*. (1999) Stable restoration of the sarcoglycan complex in dystrophic muscle perfused with histamine and a recombinant adeno-associated viral vector. *Nat. Med.* 5, 439–443
19 Gregorevic, P. *et al*. (2004) Systemic delivery of genes to striated muscles using adeno-associated viral vectors. *Nat. Med.* 10, 828–834
20 Harper, S.Q. *et al*. (2002) Modular flexibility of dystrophin: implications for gene therapy of Duchenne muscular dystrophy. *Nat. Med.* 8, 253–261
21 Lu, Q.L. *et al*. (2003) Functional amounts of dystrophin produced by skipping the mutated exon in the *mdx* dystrophic mouse. *Nat. Med.* 9, 1009–1014
22 Burton, E.A. and Davies, K.E. (2002) Muscular dystrophy – reason for optimism? *Cell* 108, 5–8
23 Van Deutekom, J.C.T. and Van Ommen, G-J. (2003) Advances in Duchenne Muscular Dystrophy gene therapy. *Nat. Rev. Genet.* 4, 774–783
24 Partridge, T.A. *et al*. (1989) Conversion of *mdx* myofibres from dystrophin negative to positive by injection of normal myoblasts. *Nature* 337, 176–179
25 Ikezawa, M. *et al*. (2003) Dystrophin delivery in dystrophin-deficient DMD*mdx* skeletal muscle by isogenic muscle-derived stem cell transplantation. *Hum. Gene Ther.* 14, 1535–1546
26 Ferrari, G. *et al*. (1998) Skeletal muscle regeneration by bone marrow derived myogenic progenitors. *Science* 279, 1528–1530
27 Gussoni, E. *et al*. (1999) Dystrophin expression in the *mdx* mouse restored by stem cell transplantation. *Nature* 401, 390–394
28 Daley, G.Q. (2002) Prospects for stem cell therapeutics: myths and medicines. *Curr. Opin. Genet. Dev.* 12, 607–613
29 Asakura, A. *et al*. (2002) Myogenic specification of side population cells in skeletal muscle. *J. Cell Biol.* 159, 123–134
30 LaBarge, M.A. and Blau, H.M. (2002) Biological progression from adult bone marrow to mononucleate muscle stem cell to multinucleate muscle fiber in response to injury. *Cell* 111, 589–601
31 Polesskaya, A. *et al*. (2003) Wnt signaling induces the myogenic specification of resident $CD45^+$ adult stem cells during muscle regeneration. *Cell* 113, 841–852
32 Camargo, F.D. *et al*. (2003) Single hematopoietic stem cells generate skeletal muscle through myeloid intermediates. *Nat. Med.* 9, 1520–1527
33 Corbel, S.Y. *et al*. (2003) Contribution of hematopoietic stem cells to skeletal muscle. *Nat. Med.* 9, 1528–1532
34 Bachrach, E. *et al*. (2004) Systemic delivery of human microdystrophin to regenerating mouse dystrophic muscle by muscle progenitor cells. *Proc. Natl. Acad. Sci. U. S. A.* 101, 3581–3586
35 Torrente, Y. *et al*. (2004) Human circulating $AC133^+$ stem cells restore dystrophin expression and ameliorate function in dystrophic skeletal muscle. *J. Clin. Invest.* 114, 182–195
36 Reyes, M. *et al*. (2001) Purification and *ex vivo* expansion of postnatal human marrow mesodermal progenitor cells. *Blood* 98, 2615–2625
37 Minasi, M.G. *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
38 Cao, B. *et al*. (2003) Muscle stem cells differentiate into hematopoietic lineages but retain myogenic potential. *Nat. Cell Biol.* 5, 640–646
39 Tamaki, T. *et al*. (2002) Identification of myogenic-endothelial progenitor cells in the interstitial spaces of skeletal muscle. *J. Cell Biol.* 157, 571–577
40 De Bari, C. *et al*. (2003) Skeletal muscle repair by adult human mesenchymal stem cells from synovial membrane. *J. Cell Biol.* 160, 909–918
41 Cossu, G. and Bianco, P. (2003) Mesoangioblasts, vascular progenitors for extra-vascular mesoderm. *Curr. Opin. Genet. Dev.* 13, 537–542
42 Sampaolesi, M. *et al*. (2003) Cell therapy of α sarcoglycan null dystrophic mice through intra-arterial delivery of mesoangioblasts. *Science* 301, 487–492
43 Palumbo, R. *et al*. (2004) Extracellular HMGB1, a signal of tissue damage, induces mesoangioblast migration and proliferation. *J. Cell Biol.* 164, 441–449
44 Piacibello, W. *et al*. (2002) Lentiviral gene transfer and *ex vivo* expansion of human primitive stem cells capable of primary, secondary, and tertiary multilineage repopulation in NOD/SCID mice. *Blood* 100, 4391–4400
45 Cossu, G. and Mavilio, F. (2000) Myogenic stem cells for the therapy of primary myopathies: wishful thinking or therapeutic perspective? *J. Clin. Invest.* 105, 1669–1674
46 De Angelis, L. *et al*. (1999) Skeletal myogenic progenitors originating from embryonic dorsal aorta co-express endothelial and myogenic markers and contribute to post-natal muscle growth and regeneration. *J. Cell Biol.* 147, 869–878
47 Kardon, G. *et al*. (2002) Local extrinsic signals determine muscle and endothelial cell fate and patterning in the vertebrate limb stereotyped composition of slow and fast myotubes. *Dev. Cell* 3, 533–545