Journal of Cellular Biochemistry Supplement 38:80–87 (2002)

Recent Advances in and Therapeutic Potential of Muscle-Derived Stem Cells

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Abstract Over the past few years, issues related to the commitment and potential of reservoir precursor cells that reside in most tissues have been revisited. Many reports have documented either plasticity or de-differentiation of a number of precursor cells isolated from several tissues, including bone marrow, brain, and skeletal muscle. These findings have challenged the dogma that mononuclear cells derived from adult, post-mitotic tissues can differentiate and contribute only to the tissue from which they originate. Thus, much current research in stem cells is testing the therapeutic potential of these cells to deliver normal genes and their encoded proteins into damaged or injured tissues. This review will focus on muscle-derived precursor cells and their apparently heterogeneous nature and summarize some of the most recent findings and hypotheses on their characterization and practical use. J. Cell. Biochem. Suppl. 38: 80–87, 2002. © 2002 Wiley-Liss, Inc.

Key words: muscle stem cell; satellite cell; mesenchyme; myoblast transfer

Stem cells are cells capable of extreme proliferation that leads to self-renewal and gives rise to more differentiated progenitors. Research into these cells has grown at a tremendous rate, and stem cells have been isolated not only from early embryos but have now also been identified in fetal and adult tissues. Although adult skeletal muscle is considered a terminally differentiated organ, it contains populations of mononuclear cells that can undergo several cycles of proliferation and are able to terminally differentiate to become part of mature myofibers. This review will focus on muscle stem cells and their isolation, characterization, and therapeutic potential.

Transplantation of mononuclear muscle-derived cells, or myoblast transfer (MT), was initially attempted in an effort to introduce dystrophin, the protein mutated in Duchenne Muscular Dystrophy (DMD), to the skeletal muscle of *mdx* mice [Partridge et al., 1978, 1989; Karpati et al., 1989] and DMD patients [Law et al., 1991; Gussoni et al., 1992; Huard et al., 1992a; Karpati et al., 1993; Mendell et al., 1995; Morandi et al., 1995; Neumeyer et al., 1998], which lack full-length, functional dystrophin. Initial MT trials showed that while the treatment was safe, it was not effective in human subjects. The results of these studies led to further investigations aimed at improving the efficacy of MT, a number of which have focused on the isolation of skeletal muscle stem cells.

DEVELOPMENTAL ORIGINS OF SKELETAL MUSCLE

Skeletal muscle originates from the mesoderm and is the end result of a highly complex set of processes [Buckingham, 2001]. Cells migrate from the somites that flank the neural tube in early embryos and subsequently fuse to produce long, multi-nucleate myotubes, which further join to form myofibers [Hauschka, 1994; Buckingham, 2001]. In addition to the paraxial mesoderm, skeletal muscle precursors have also been identified in tissues of non-somite origin, such as the dorsal aorta [De Angelis et al., 1999]. Adult skeletal muscle is constituted by terminally differentiated multinucleated myofibers and by several populations of mononuclear cells (Fig. 1A). One such population consists of satellite cells, which are adjacent to skeletal muscle myofibers and lie underneath the basal

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Received 3 October 2001; Accepted 4 October 2001

Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/jcb.10051

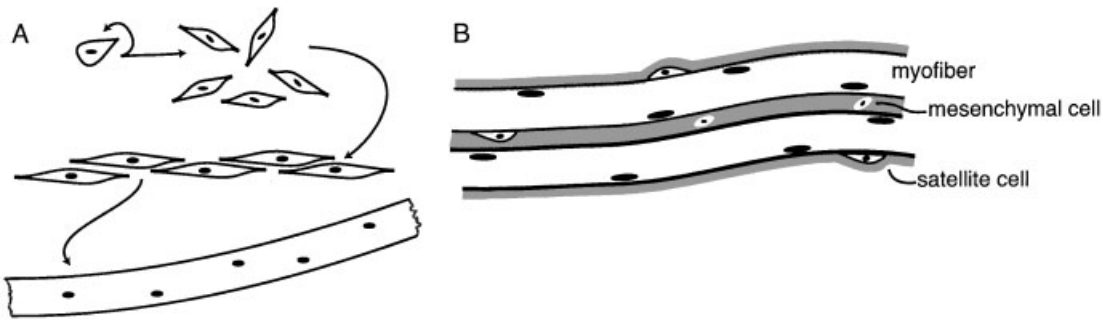

Fig. 1. **A**: Schematic representation of differentiation of a myoblast into myocytes which orient and then fuse to form a myotube. **B**: Schematic representation of the relative positions of myofibers, satellite cells, and mesenchymal cells. The basement membrane is represented in gray.

lamina (Fig. 1B) [Mauro, 1961; Bischoff, 1994]. There are also mononuclear cells within the connective tissue of healthy skeletal muscle, referred to as mesenchymal cells. Little is known about these cells at the present time, but there is preliminary evidence that a subpopulation may be extremely primitive.

SKELETAL MUSCLE STEM CELLS

Many tissues of the body have reservoirs of reparative, regenerative stem cells. Satellite cells are thought to be skeletal muscle stem cells due to their proliferative and regenerative capacity. Satellite cells have been primarily defined by their position, directly opposed to the sarcolemma of muscle fibers, but covered by the same basement membrane as the myofiber [Mauro, 1961; Bischoff, 1994]. They are normally quiescent, but are activated by various forms of muscle damage, including exercise, and physical and chemical trauma. Once activated, satellite cells divide asymmetrically to generate another satellite cell and multiple activated myogenic precursor cells (MPCs). MPCs undergo several rounds of division before fusing to pre-existing or new myofibers.

More recent studies have demonstrated the existence of primitive cells within skeletal muscle that have the capacity to regenerate not only muscle but bone [Lee et al., 2000] and blood as well [Gussoni et al., 1999; Jackson et al., 1999; Torrente et al., 2001]. The precise relation between satellite cells and these other muscle-derived precursor cells (MDPCs) is unclear. MDPCs can be isolated by a variety of different methods, and have also been shown to express different subsets of markers, suggesting that they either are heterogeneous in nature or that depending on isolation and culture procedures, the markers they express differ. Broadly defined, the populations of MDPCs could be said to contain cells that are already committed to the muscle lineage, cells that are committed but can be induced to de-differentiate by appropriate molecular signals and/or primitive cells which can differentiate into muscle as well as a variety of other tissues.

PURIFICATION OF MDPCS

In an effort to identify skeletal muscle stem cells, various groups have endeavored to transfer knowledge of other tissue stem cells, such as hematopoietic stem cells (HSCs), to mononuclear muscle cells. As no MDPC-specific markers were known, enrichment techniques have relied upon other predicted physical properties of the cells.

Fluorescence Activated Cell Sorting (FACS)

Very primitive hematopoietic stem cells have been shown to eliminate vital DNA dyes, such as Hoechst 33342, via a multi-drug-resistance (MDR)-like pump [Goodell et al., 1996]. These cells have been named side population (SP) cells and appear to lose their ability to exclude Hoechst 33342 when stained in the presence of verapamil. Recent findings have demonstrated that the pump responsible for Hoechst dye exclusion in SP cells is the ABC transporter Bcrp1/ABCG2 [Zhou et al., 2001]. The potency of SP cells is apparent, since injection of as few as 150 bone marrow SP cells into the circulation of lethally irradiated host mice is sufficient for radioprotection, with the bone marrow and blood cell lineages being reconstituted by introduced donor cells [Goodell et al., 1996]. This

FACS-based technique has been gradually optimized to identify primitive cells in other tissues. In skeletal muscle, at significantly higher concentrations of Hoechst 33342 than for bone marrow, a population of SP cells has been identified (Fig. 2) [Gussoni et al., 1999]. In FACS profiles, the SP cells are located in the lower left hand corner, while the main population (MP), which does not exclude the DNA dyes as efficiently, is in the center of the profile (Fig. 2A). Importantly, in the presence of verapamil muscle, SP cells are unable to exclude Hoechst 33342 (Fig. 2B). Attempts to culture SP cells in vitro have shown that these cells settle on laminin-coated tissue culture plasticware after 1 week. Immunofluorescence staining of cultured muscle SP cells using an antibody to desmin, an intermediate filament cytoskeletal protein expressed by myoblasts but not fibroblasts, has indicated that SP cells are a mixture of desmin-positive and negative cells [Gussoni et al., 1999].

Pre-Plating Technique

Given that more primitive cells display delayed settling in in vitro culture conditions, a technique consisting of serial plating of muscle-derived mononuclear cells has been developed [Qu et al., 1998]. This pre-plating technique passages unsettled, floating cells onto new tissue culture plates first at short intervals (1 and 2 h after the first plating) to eliminate contaminating fibroblasts which settle rapidly [Rando and Blau, 1994; Qu et al., 1998], followed by longer incubations of 24 h for approximately 6 days [Qu et al., 1998; Torrente et al., 2001]. This method enriches for small cells, which are referred to as PP6 cells.

MARKERS EXPRESSED BY SATELLITE CELLS AND MDPCS

Satellite Cells

Numerous studies have focused on identifying myogenic markers on satellite cells and cultured myoblasts [Seale and Rudnicki, 2000]. Both satellite cells and cultured myoblasts appear to be fully committed to the myogenic line and express myogenic markers as they differentiate. When initiating division, satellite cells express either myf-5 or Myo-D. Subsequently, these two transcription factors are simultaneously expressed followed by two other transcription factors, MRF4 and myogenin [Seale and Rudnicki, 2000].

Satellite cells have also been shown to express a number of other proteins including desmin, c-met, M-cadherin, Pax7, and Bcl-2. Desmin is an early marker for myogenic cells, expressed both in culture and in vivo. It is present in replicating myoblasts and also has an important function in stabilizing the mature myofiber. In fact, desmin-null ($des^{-/-}$) mice have normal muscle development, but an impaired repair function similar to that seen in the *mdx* mouse [Li et al., 1994, 1997]. These results

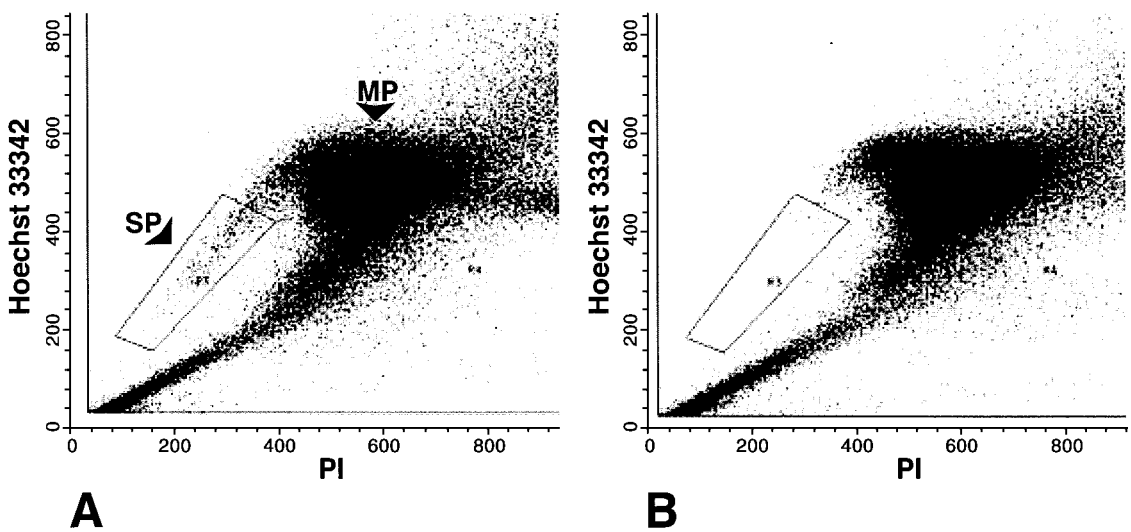

Fig. 2. FACS analysis of skeletal muscle mononuclear cells stained with Hoechst 33342 in the absence (**A**) or in the presence (**B**) of verapamil. SP cells are only visible in (A) since they appear to be affected by verapamil.

suggest that although desmin is expressed prior to other muscle markers, it should be thought of as an indicator of myogenic commitment, not a determinant [Li et al., 1994, 1997]. c-Met, a growth factor surface receptor, is expressed both on myoblasts in vitro and on satellite cells in vivo and is required both for migration of myoblast precursors during development and activation of satellite cells [Cornelison and Wold, 1997]. M-cadherin is a cell adhesion molecule expressed during fetal myogenesis, which has also been found on quiescent satellite cells [Irintchev et al., 1994; Beauchamp et al., 2000]. A comparative study of the expression of c-met and M-cadherin on satellite cells has indicated that M-cadherin is present on less than 20% of quiescent satellite cells [Cornelison and Wold, 1997]. This study proposed that M-cadherin is only expressed on cells that are preparing to enter the regenerative phase and that M-cadherin$^+$ c-met$^+$ cells represent a small, distinct population of activated satellite cells [Cornelison and Wold, 1997]. The paired box transcription factor, Pax7, was identified as a satellite cell-derived myoblast-specific gene based on subtractive hybridization studies [Seale et al., 2000]. Interestingly, the skeletal muscle of *Pax7*$^{-/-}$ mice shows a complete absence of satellite cells, but maintains SP cells in Hoechst FACS analysis [Seale et al., 2000]. These results imply that Pax7 could be an important factor promoting the differentiation of SP cells into satellite cells [Seale et al., 2000]. The apoptosis inhibiting protein, Bcl-2, is expressed by cells that subsequently express Myf-5 and/or MyoD. Bcl-2 marks the beginning of the transition to myogenic commitment and is required for clonal expansion of proliferating cells [Dominov et al., 1998]. Although the identity has not been proven definitively, cells positive for Bcl-2 by immunohistochemical analysis of skeletal muscle sections greatly resemble satellite cells [Dominov et al., 1998].

Muscle-Derived Precursor Cells

Muscle SP cells have been analyzed for the expression of several cell surface markers, some of which are known to be expressed by hematopoietic stem cells (CD34, c-kit, CD43, and CD45) (Table I) [Gussoni et al., 1999]. Like HSCs, over 80% of muscle SP cells are Sca-1$^+$ [Gussoni et al., 1999; Jackson et al., 1999]. CD34 has also been detected by FACS analysis, but the percentage of CD34$^+$ SP cells across preparations is not constant. Whether this variation is due to donor age variation or other factors is unclear. Unlike HSCs, muscle SP cells are CD45$^-$ and CD43$^-$ [Gussoni et al., 1999]. Differences have been reported in the expression of c-kit, with one study finding that 90% of muscle SP cells are negative for expression of c-kit [Gussoni et al., 1999], and another reporting that 75% of muscle SP cells are c-kit$^+$ [Jackson et al., 1999]. These variations may be the result of differences in cell isolation, in vitro culturing conditions or analysis protocols. FACS analysis of these cells indicates that M-cadherin is not expressed (Table I).

The PP6 population, isolated from muscle using the pre-plating technique, has been found to contain a high proportion of Sca-1$^+$ CD34$^+$ cells (Table I) [Lee et al., 2000; Torrente et al., 2001]. More detailed analysis of these markers by flow cytometry has shown that 60% of PP6 cells initially express Sca-1, but that this declines while CD34 expression increases after 5 days in culture [Jankowski et al., 2001]. Discrepancies in the expression of desmin and M-cadherin in the PP6 cells have also been reported (Table I).

The heterogeneity of MDPCs and variation in the expression of surface markers and

TABLE I. Analysis of Cell-Surface and Myogenic Markers Expressed by Muscle-Derived Precursor Cells

	Sca-1	CD34	c-kit	CD45	M-cadherin	Desmin	Reference
PP6	58[a]–95[a,b]	25[a]; > 95[b]	0[a]	0[a]	5–30[b]	94[b]	Lee et al. [2000]
PP6	> 90[a]	74[a]			< 1[b]	10[b]	Torrente et al. [2001]
WMC[c]	20[a]		10[a]	13[a]			Jackson et al. [1999]
Muscle SP	80[a]		75[a]	2[a]			Jackson et al. [1999]
Muscle SP	> 80[a]	30–70[a]	< 10[a]	< 2[a]	0[a]		Gussoni et al. [1999]

[a]Flow-cytometry analysis.
[b]Immunofluorescence analysis.
[c]Whole muscle cells.

transcription factors has led to a failure to define a marker or set of markers specific to these cells. The only marker on which there is agreement is Sca-1, which is not expressed exclusively by SP or PP6 cells, as it is also present on MP cells. The identification of Bcrp1/ABCG2 as a transporter expressed by SP cells from bone marrow, skeletal muscle, and embryonic stem cells has raised the possibility of it being a useful marker for routine purification of SP cells, as well as a tool for studying their location within the tissue of origin [Zhou et al., 2001]. It will be particularly useful in examining the relation of skeletal muscle SP cells to myofibers, satellite cells, and the mesenchyme.

ABILITY OF SKELETAL MUSCLE STEM CELLS TO REGENERATE TISSUES IN VIVO

As mentioned earlier, in an effort to deliver normal dystrophin to murine and human dystrophic muscle, wild-type myoblasts were introduced via intramuscular injections. In these studies, although introduced donor cells had fused with host myofibers around the site of injection and produced normal dystrophin, the amount of detected wild-type dystrophin was not therapeutically significant. Donor cells may have suffered from poor spread from the injection site, a low survival rate, and possibly immune rejection by the host system [Huard et al., 1992b; Gussoni et al., 1997; Qu et al., 1998; Beauchamp et al., 1999].

Intra-arterial myoblast injections have also been tested in the hope of more widespread dissemination of donor cells to multiple muscles via the circulation [Neumeyer et al., 1992]. More recently, intra-arterial delivery of PP6 cells and intravenous delivery of muscle SP cells have also been tested in *mdx* mice. Intra-arterial injection of $CD34^+$ $Sca\text{-}1^+$ PP6 cells followed by vital microscopy analysis indicated that the donor cells adhere to the muscle endothelium prior to fusing with host myofibers [Torrente et al., 2001]. When damage was induced in *mdx* muscles prior to cell injection, a higher proportion of donor cells were detected in the recipient muscle after intra-arterial delivery [Torrente et al., 2001]. Thus, it is possible that damaged tissues secrete signals that guide injected cells to the injured site and trigger tissue repair. Similarly, upon intravenous injection of skeletal muscle SP cells in *mdx* mice, donor-derived cells were found in multiple recipient muscles including quadriceps, tibialis anterior, and diaphragm [Gussoni et al., 1999]. Donor cells were detected both fused to pre-existing fibers expressing dystrophin (Fig. 3A) and adjacent to myofibers in a position suggestive of, but not proven to be, satellite cells

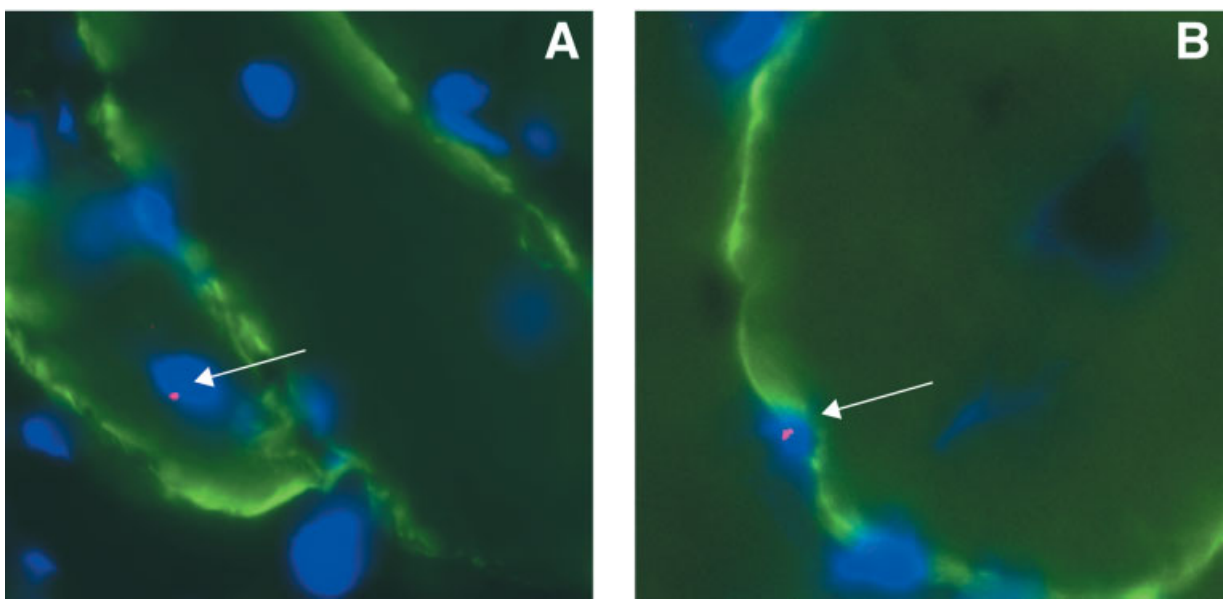

Fig. 3. Fluorescence in-situ hybridization detection of the Y chromosome of normal donor cells fused into (**A**) or associated with (**B**) *mdx* host myofibers. Nuclei are stained in blue by DAPI, the Y-chromosome hybridization signal is shown in red and dystrophin is visualized as the sarcolemmal staining in green.

(Fig. 3B). Although these preliminary results suggest a promising use of SP cells for therapy of muscle diseases, the number of donor-derived dystrophin positive myofibers still remains too low, and much work is needed to study ways of increasing the percentage of engrafted cells within dystrophic muscle. Identification of signals released by injured myofibers that induce stem cell recruitment and engraftment will contribute greatly.

MUSCLE-DERIVED PRECURSOR CELL PLASTICITY

Another interesting aspect of MDPCs is their differentiation potential and presumed plasticity. For example, skeletal muscle SP cells are able to radioprotect lethally irradiated animals and differentiate into bone marrow cells [Gussoni et al., 1999]. These observations have been confirmed by another study that demonstrated the ability of skeletal muscle-derived mononuclear cells to repopulate the bone marrow even when co-injected with unfractionated bone marrow cells [Jackson et al., 1999]. Furthermore, serial transplantation using bone marrow-derived cells from a chimeric lethally irradiated animal rescued using primary muscle cells indicated the primitive nature of muscle-derived cells and their ability to differentiate into multiple lineages in vivo [Jackson et al., 1999].

Similarly, MDPCs isolated by the pre-plating technique (PP6, Bcl-2^+, $CD34^+$) have been shown to differentiate not only into mature skeletal muscle [Lee et al., 2000] and smooth muscle [Yokoyama et al., 2001] but also into osteogenic cells when exposed to BMP-2 in culture or when genetically engineered to express BMP-2 [Lee et al., 2000, 2001].

The de-differentiation capacity and plasticity of muscle-derived cells has also been demonstrated by exposure of in vitro differentiated C2C12 myotubes to msx1, a homeobox transcription factor that is involved in the cellular de-differentiation of urodele amphibians [Odelberg et al., 2000]. Ectopic expression of msx1 induces cleavage of differentiated C2C12 myotubes into smaller myotubes and proliferating, mononuclear cells. These msx1-induced mononuclear cells are able to re-differentiate in vitro into cells of myogenic, osteogenic, chondrogenic, and adipogenic lineages thus demonstrating pluripotency.

CONCLUSIONS AND FUTURE DIRECTIONS

Skeletal muscle contains several types of mononuclear cells, including defined satellite cells and undefined muscle-derived precursor cells. These cells, regardless of the method utilized for their isolation or purification, appear to be heterogeneous in nature. In fact, phenotypic analysis of satellite cells by single fiber culture indicates the presence of multiple populations of quiescent satellite cells, the majority of which, but not all, are $CD34^+$ $Myf5^+$ [Beauchamp et al., 2000]. Similarly, analysis of MDPCs isolated by FACS or pre-plating techniques has shown heterogeneity in these cells for the expression of several markers, including CD34, desmin, and c-kit (Table I). Thus, it is important to optimize protocols that will standardize satellite and MDP cell isolations as a starting point that will enable us to compare markers present or absent in a given population. Concordance in the methods and protocols utilized for the isolation and expansion of muscle precursor cells is essential prior to comparing expression and marker studies from different groups. As new markers for satellite cells and MDPCs become available, it may be possible to determine how these various cells function, what their roles are in mature skeletal muscle, and how they are related.

From a therapeutic standpoint, although satellite cells and MDPCs have shown plasticity and, therefore, great potential, much work is still needed. Some of the most crucial questions leading current and future muscle stem cell research include determining ways to expand these cells while maintaining their primitive characteristics and plasticity, and understanding how and why these cells are recruited into multiple tissues with the goal of directing this phenomenon into specific target organs. Finally, muscle may not be the only source of these stem cells. For example, bone marrow contains muscle-precursor cells that are capable of contributing to the repair of myofibers [Ferrari et al., 1998].

Research into stem cells from muscle and other tissues is now facing numerous challenges. Although much hope is being placed on stem cell therapy, successful implementation will likely require optimization of several steps, including cell preparation, recipient conditioning, and even multiple cell infusion/mobilization protocols before significant improvements

are observed. Many of us know that this will be a very arduous task; nonetheless, it needs to be explored.

REFERENCES

Beauchamp JR, Morgan JE, Pagel CN, Partridge TA. 1999. Dynamics of myoblast transplantation reveal a discrete minority of precursors with stem cell-like properties as the myogenic source. J Cell Biol 144:1113–1122.

Beauchamp JR, Heslop L, Yu DS, Tajbakhsh S, Kelly RG, Wernig A, Buckingham ME, Partridge TA, Zammit PS. 2000. Expression of CD34 and Myf5 defines the majority of quiescent adult skeletal muscle satellite cells. J Cell Biol 151:1221–1234.

Bischoff R. 1994. The satellite cell and muscle regeneration. In: Engel AG, Franzini-Armstrong C, editors. Myology. New York: Mc Graw Hill. p 97–119.

Buckingham M. 2001. Skeletal muscle formation in vertebrates. Curr Opin Genet Dev 11:440–448.

Cornelison DD, Wold BJ. 1997. Single-cell analysis of regulatory gene expression in quiescent and activated mouse skeletal muscle satellite cells. Dev Biol 191:270–283.

De Angelis L, Berghella L, Coletta M, Lattanzi L, Zanchi M, Cusella-De Angelis MG, Ponzetto C, Cossu G. 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.

Dominov JA, Dunn JJ, Miller JB. 1998. Bcl-2 expression identifies an early stage of myogenesis and promotes clonal expansion of muscle cells. J Cell Biol 142:537–544.

Ferrari G, Cusella-De Angelis G, Coletta M, Paolucci E, Stornaiuolo A, Cossu G, Mavilio F. 1998. Muscle regeneration by bone marrow-derived myogenic progenitors. Science 279:1528–1530.

Goodell MA, Brose K, Paradis G, Conner AS, Mulligan RC. 1996. Isolation and functional properties of murine hematopoietic stem cells that are replicating in vivo. J Exp Med 183:1797–1806.

Gussoni E, Pavlath GK, Lanctot AM, Sharma K, Miller RG, Steinman L, Blau HM. 1992. Normal dystrophin transcripts detected in Duchenne muscular dystrophy patients after myoblast transplantation. Nature 356:435–438.

Gussoni E, Blau HM, Kunkel LM. 1997. The fate of individual myoblasts after transplantation into muscles of DMD patients. Nat Med 3:970–977.

Gussoni E, Soneoka Y, Strickland C, Buzney E, Khan M, Flint A, Kunkel L, Mulligan R. 1999. Dystrophin expression in the mdx mouse restored by stem cell transplantation. Nature 401:390–394.

Hauschka SD. 1994. The embryonic origin of muscle. In: Engel AG, Franzini-Armstrong C, editors. Myology. New York: Mc Graw Hill. p 3–73.

Huard J, Bouchard J-P, Roy R, Malouin F, Danserau G, Labrecque C, Albert N, Richards CL, Lemieux B, Tremblay JP. 1992a. Human myoblast transplantation: preliminary results of 4 cases. Muscle Nerve 15:550–560.

Huard J, Roy R, Bouchard JP, Malouin F, Richards CL, Tremblay JP. 1992b. Human myoblast transplantation between immunohistocompatible donors and recipients produces immune reactions. Transplant Proc 24:3049–3051.

Irintchev A, Zeschnigk M, Starzinski-Powitz A, Wernig A. 1994. Expression pattern of M-cadherin in normal, denervated, and regenerating mouse muscles. Dev Dyn 199:326–337.

Jackson KA, Mi T, Goodell MA. 1999. Hematopoietic potential of stem cells isolated from murine skeletal muscle [In Process Citation]. Proc Natl Acad Sci USA 96:14482–14486.

Jankowski RJ, Haluszczak C, Trucco M, Huard J. 2001. Flow cytometric characterization of myogenic cell populations obtained via the preplate technique: potential for rapid isolation of muscle- derived stem cells. Hum Gene Ther 12:619–628.

Karpati G, Pouilot Y, Zubrzycka-Gaarn E, Carpenter S, Ray PN, Worton RG, Holland P. 1989. Dystrophin is expressed in mdx skeletal muscle fibers after normal myoblast implantation. Am J Pathol 135:27–32.

Karpati G, Ajdukovic D, Arnold D, Gledhill RB, Guttmann R, Holland P, Koch PA, Shoubridge E, Spence D, Vanasse M, Watters GV, Abrahamowicz M, Duff C, Worton RG. 1993. Myoblast transfer in Duchenne muscular dystrophy. Ann Neurol 34:8–17.

Law PK, Goodwin TG, Fang QW, Chen M, Li HJ, Florendo JA, Kirby DS. 1991. Myoblast transfer therapy for Duchenne muscular dystrophy. Acta Paediatr Jpn 33:206–215.

Lee JY, Qu-Petersen Z, Cao B, Kimura S, Jankowski R, Cummins J, Usas A, Gates C, Robbins P, Wernig A, Huard J. 2000. Clonal isolation of muscle-derived cells capable of enhancing muscle regeneration and bone healing. J Cell Biol 150:1085–1100.

Lee JY, Musgrave D, Pelinkovic D, Fukushima K, Cummins J, Usas A, Robbins P, Fu FH, Huard J. 2001. Effect of bone morphogenetic protein-2-expressing muscle-derived cells on healing of critical-sized bone defects in mice. J Bone Joint Surg Am 83-A:1032–1039.

Li H, Choudhary SK, Milner DJ, Munir MI, Kuisk IR, Capetanaki Y. 1994. Inhibition of desmin expression blocks myoblast fusion and interferes with the myogenic regulators MyoD and myogenin. J Cell Biol 124:827–841.

Li Z, Mericskay M, Agbulut O, Butler-Browne G, Carlsson L, Thornell LE, Babinet C, Paulin D. 1997. Desmin is essential for the tensile strength and integrity of myofibrils but not for myogenic commitment, differentiation, and fusion of skeletal muscle. J Cell Biol 139:129–144.

Mauro A. 1961. Satellite cells of skeletal muscle. J Biophys Biochem Cytol 9:493–495.

Mendell JR, Kissel JT, Amato AA, King W, Signore L, Prior TW, Sahenk Z, Benson S, McAndrew PE, Rice R, Nagaraja H, Stephens R, Lantry L, Morris GE, Burghes AHM. 1995. Myoblast transfer in the treatment of Duchenne's muscular dystrophy. N Engl J Med 333:832–838.

Morandi L, Bernasconi P, Gebbia M, Mora M, Crosti F, Mantegazza R, Cornelio F. 1995. Lack of mRNA and dystrophin expression in DMD patients three months after myoblast transfer. Neuromuscul Disord 5:291–295.

Neumeyer AM, Di Gregorio DM, Brown RH. 1992. Arterial delivery of myoblasts to skeletal muscle. Neurology 42:2258–2262.

Neumeyer AM, Cros D, McKenna-Yasek D, Zawadzka A, Hoffman EP, Pegoraro E, Hunter RG, Munsat TL, Brown RH. 1998. Pilot study of myoblast transfer in the treatment of Becker muscular dystrophy. Neurology 51:589–592.

Odelberg SJ, Kollhoff A, Keating MT. 2000. Dedifferentiation of mammalian myotubes induced by msx1. Cell 103:1099–1109.

Partridge TA, Grounds M, Sloper JC. 1978. Evidence of fusion between host and donor myoblasts in muscle grafts. Nature 273:306–308.

Partridge TA, Morgan JE, Coulton GR, Hoffman EP, Kunkel LM. 1989. Conversion of mdx myofibers from dystrophin negative to positive by injection of normal myoblasts. Nature 337:176–179.

Qu Z, Balkir L, van Deutekom JC, Robbins PD, Pruchnic R, Huard J. 1998. Development of approaches to improve cell survival in myoblast transfer therapy. J Cell Biol 142:1257–1267.

Rando TA, Blau HM. 1994. Primary mouse myoblast purification, characterization, and transplantation for cell-mediated gene therapy. J Cell Biol 125:1275–1287.

Seale P, Rudnicki MA. 2000. A new look at the origin, function, and "stem-cell" status of muscle satellite cells. Dev Biol 218:115–124.

Seale P, Sabourin LA, Girgis-Gabardo A, Mansouri A, Gruss P, Rudnicki MA. 2000. Pax7 is required for the specification of myogenic satellite cells. Cell 102:777–786.

Torrente Y, Tremblay JP, Pisati F, Belicchi M, Rossi B, Sironi M, Fortunato F, El Fahime M, D'Angelo MG, Caron NJ, Constantin G, Paulin D, Scarlato G, Bresolin N. 2001. Intraarterial injection of muscle-derived CD34(+)Sca-1(+) stem cells restores dystrophin in mdx mice. J Cell Biol 152:335–348.

Yokoyama T, Huard J, Pruchnic R, Yoshimura N, Qu Z, Cao B, de Groat WC, Kumon H, Chancellor MB. 2001. Muscle-derived cell transplantation and differentiation into lower urinary tract smooth muscle. Urology 57:826–831.

Zhou S, Schuetz JD, Bunting KD, Colapietro AM, Sampath J, Morris JJ, Lagutina I, Grosveld GC, Osawa M, Nakauchi H, Sorrentino BP. 2001. The ABC transporter Bcrp1/ABCG2 is expressed in a wide variety of stem cells and is a molecular determinant of the side-population phenotype. Nat Med 7:1028–1034.