



HAL
open science

Stem Cell Based Therapies to Treat Muscular Dystrophy

F.D. Price, K. Kuroda, M.A. Rudnicki

► **To cite this version:**

F.D. Price, K. Kuroda, M.A. Rudnicki. Stem Cell Based Therapies to Treat Muscular Dystrophy. *Biochimica et Biophysica Acta - Molecular Basis of Disease*, 2007, 1772 (2), pp.272. 10.1016/j.bbadis.2006.08.011 . hal-00562727

HAL Id: hal-00562727

<https://hal.science/hal-00562727>

Submitted on 4 Feb 2011

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Accepted Manuscript

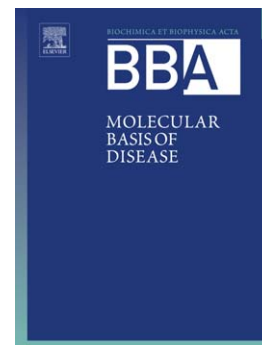
Stem Cell Based Therapies to Treat Muscular Dystrophy

F.D. Price, K. Kuroda, M.A. Rudnicki

PII: S0925-4439(06)00173-6
DOI: doi: [10.1016/j.bbadis.2006.08.011](https://doi.org/10.1016/j.bbadis.2006.08.011)
Reference: BBADIS 62629

To appear in: *BBA - Molecular Basis of Disease*

Received date: 27 July 2006
Accepted date: 30 August 2006



Please cite this article as: F.D. Price, K. Kuroda, M.A. Rudnicki, Stem Cell Based Therapies to Treat Muscular Dystrophy, *BBA - Molecular Basis of Disease* (2006), doi: [10.1016/j.bbadis.2006.08.011](https://doi.org/10.1016/j.bbadis.2006.08.011)

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Stem Cell Based Therapies to Treat Muscular Dystrophy

F.D. Price, K. Kuroda, M.A. Rudnicki

Molecular Medicine Program and Center for Stem Cell and Gene Therapy, Ottawa Health

Research Institute, 501 Smyth Rd, Ottawa Ontario, K1H 8L6, Canada

mrudnicki@ohri.ca

| | |
|--|--|
| Introduction | |
| Muscular Dystrophy | |
| The Function of Stem Cells in Development and Tissue Homeostasis | |
| Skeletal Muscle Regeneration | |
| Applications of Typical Muscle Stem Cells | |
| Transplantation of Satellite Cell derived Myoblasts | |
| Satellite Cell Transplantation | |
| Single Muscle Fiber | |
| Applications of Atypical Muscle Stem Cells..... | |
| Muscle Side Population Cells | |
| Bone Marrow Stem Cells | |
| Mesenchymal Stem Cells | |
| Muscle Derived Stem Cells (MDSCs) | |
| Vessel Associated Stem Cell Populations | |
| Embryonic Stem Cells | |
| Synergistic Methods to Improve Stem Cell Based Therapies | |
| Conclusions and Future Perspectives | |
| References | |

Abstract

Muscular dystrophies comprise a heterogeneous group of neuromuscular disorders, characterized by progressive muscle wasting, for which no satisfactory treatment exists. Multiple stem cell populations, both of adult or embryonic origin, display myogenic potential and have been assayed for their ability to correct the dystrophic phenotype. To date, many of these described methods have failed, underlying the need to identify the mechanisms controlling myogenic potential, homing of donor populations to the musculature, and avoidance of the immune response. Recent results focus on the fresh isolation of satellite cells and the use of multiple growth factors to promote mesangioblast migration, both of which promote muscle regeneration. Throughout this chapter, various stem cell based therapies will be introduced and evaluated based on their potential to treat muscular dystrophy in an effective and efficient manner.

Keywords Stem Cell - Muscular Dystrophy - Skeletal Muscle - Regenerative Medicine - Cell Therapy

1. Introduction

1.1 Muscular dystrophy

Numerous types of muscular dystrophy exist and differ depending on their degree of severity and the muscle types affected¹. Duchenne muscular dystrophy (DMD), the most common form of muscular dystrophy, is an X-linked genetic disorder that occurs at a rate of approximately 1 in 3500 male births². DMD arises due to either spontaneous mutations or inherited nonsense point mutations in the dystrophin gene^{3,4}, the result of which is progressive muscle wasting and weakness attributed to the loss of a functional dystrophin protein⁵. Dystrophin, an important cytoskeletal protein, and a major component of the dystrophin-glycoprotein complex (DGC), is responsible for the maintenance of cell integrity, mediation of cytoplasmic signaling and muscle cell function⁶. Without dystrophin, muscle cells cannot form the DGC and degenerate as a result of mechanical stress during contraction.

To test prospective therapeutic treatments for DMD numerous large and small animal models have been created; the most common being the mdx mouse, which parallels DMD defects seen in diaphragm muscle as a result of a genetic mutation causing premature termination of the dystrophin transcript^{7,8}. Although the mdx mouse lacks a functional dystrophin protein, it only displays a mild dystrophic phenotype, which is attributed to a greater degree of fiber regeneration and a reduction in endomysial fibrosis compared to DMD⁹. More recent mouse models include the utrophin/dystrophin null mouse^{10,11} and the dystrophin/a7-integrin double mutant mouse, both of which more closely resemble human DMD^{12,13}. Feline¹⁴, zebrafish¹⁵, and

the canine X-linked model of muscular dystrophy^{16,17} complement the mouse models and provide researchers with additional tools to study this disease

1.2 The Function of Stem Cells in Development and Tissue Homeostasis

Stem cells are defined by certain characteristics, primarily an ability for long term self renewal and the capacity to differentiate into multiple cell lineages. Stem cells are responsible for the development and maintenance of tissues and organs and self-renew or differentiate in response to a combination of biochemical signals and biomechanical stimuli provided by the stem cell niche. Stem cells can be isolated from either adult or embryonic tissue, and depending on a hierarchical state differ in their ability to give rise to multiple cell lineages. This hierarchy progresses from a state of totipotency through to unipotency; whereby, at each level the ability to differentiate into multiple cell types is progressively diminished (**Fig1**). Stem cell division can be either symmetric or asymmetric. An asymmetric division results in the formation of two non-identical daughter cells; one commits to a specialized fate while the other remains quiescent to maintain the stem cell pool. Conversely, differentiating daughter cells undergo symmetric divisions giving rise to a reservoir of precursor cells that contribute to tissue regeneration.

Small quantities of adult stem cells exist in most tissues throughout the body where they remain quiescent for long periods of time prior to being activated in response to disease or tissue injury. Adult stem cells can be isolated from cells of the hematopoietic¹⁸, neural¹⁹, dermal²⁰, muscle²¹⁻²³ and hepatic²⁴ systems. It is traditionally thought that adult stem cells give rise to the specialized cell types of the tissue from which they originated. However, some recent reports have indicated that adult stem cells can differentiate into lineages other than their tissue of origin, for example

transplanted bone marrow or enriched hematopoietic stem cells (HSCs) are reported to give rise to cells of the mesoderm²⁵⁻³⁰, endoderm^{31,32}, and ectoderm^{33,34}. Future experiments elaborating upon the origins and characteristics of adult stem cells are necessary in order to fully distinguish their potential from embryonic stem cells.

The embryonic stem cell (ESC) is defined by its origin - the inner cell mass of the blastocyst. ESCs traditionally differ from adult stem cells in that they are deemed pluripotent; meaning they can give rise to cells derived from all three germ layers³⁵. Gene expression patterns observed during the *in vitro* differentiation of ESCs mimic that seen *in vivo*; and these cells can give rise to numerous cell types *in vitro* including neurons³⁶, bone^{37,38}, pancreatic islets³⁹, and skeletal muscle⁴⁰.

In the past multiple stem cell populations have been assayed for their ability to treat muscular dystrophy, the majority of which have met with limited success. In order to correct the dystrophic phenotype, transplanted cells must fuse to existing, or form new, myotubes. Upon fusion, the contribution of genetically normal myonuclei to the muscle myofiber should result in the production of a functional dystrophin protein. Stem cell based therapies for the treatment of muscular dystrophy can progress via two strategies. The first involves cells from a patient afflicted with DMD and is termed autologous stem cell transfer. In this process cells from the patient are genetically altered *in vitro* to restore dystrophin expression and subsequently re-implanted (reviewed^{41,42}). In the second strategy, allogenic stem cell transfer, cells are isolated from an individual with functional dystrophin and subsequently transplanted into a dystrophic patient (reviewed⁴³). Both of these strategies have advantages and disadvantages. Autologous

cells are advantageous in that they are derived from the patient and therefore unlikely to elicit an immune response. However, the process of genetic alteration has in the past led to undesirable effects including transformation of donor cells and even death⁴⁴⁻⁴⁶. Allogenic cells, on the other hand, are not subject to genetic modification, making them ideal for functional muscle regeneration. However, the patient is at risk for immune rejection, raising the issues of donor compatibility and appropriate immunosuppressive regimes. In this chapter, multiple sources of stem cells with myogenic potential will be identified and their candidacy as cell sources to treat muscular dystrophy will be assessed. The identification of a stem cell population that provides efficient and effective muscle regeneration is critical for the progression of stem cell based therapies to treat muscular dystrophy

1.3 Skeletal Muscle Regeneration

Adult skeletal muscle is capable of a remarkable degree of regeneration, suggesting the presence of a stem cell population either resident within muscle or capable of migrating to muscle. The major component of adult skeletal muscle is the myofiber; a giant syncytial cell containing hundreds of myonuclei within a continuous cytoplasm. Under physiological conditions the ability of adult muscle to undergo regeneration is largely attributed to a distinct subpopulation of myogenic cells, termed satellite cells, located between the basal lamina and sarcolemma of mature skeletal muscle fibers^{47,48}. Despite the fact that satellite cells are multipotent in that they can give rise to osteogenic, chondrogenic and adipogenic cells under appropriate conditions⁴⁹, they are a distinct lineage of myogenic stem cells that remain mitotically quiescent under normal physiological conditions. Upon muscle damage or in a state of disease, satellite cells activate and proliferate giving rise to a population of cells that contribute to muscle regeneration via a process

of differentiation and fusion^{50,51}. Satellite cells can be characterized by a panel of cell surface markers including: m-Cadherin⁵², c-Met⁵³, Syndecan 3 and 4^{54,55}, CD-34⁵⁶, and nuclear markers Pax7⁵⁷, MNF⁵⁸, and Myf5⁵⁶. Patients afflicted with DMD rapidly exhaust their satellite cell reserves due to continuous cycles of muscle injury and regeneration⁵⁹, and as such lose their ability to regenerate, resulting in compromised muscle function and degeneration.

2. Applications of Typical Muscle Stem Cells

2.1 Transplantation of Satellite Cell derived Myoblasts

Satellite cells are present at low quantities in adult muscle and account for 2-5% of sublamellar nuclei associated with myofibers⁶⁰. Due to their scarcity and the difficulties in isolating pure populations, freshly isolated satellite cells have been largely neglected as a source for cell therapy. The progeny of muscle satellite cells, upon culture and expansion *in vitro*, are termed primary myoblasts, these cells are highly proliferative and can be maintained in an undifferentiated state^{61,62}. Historically primary myoblasts have been the principal source of muscle progenitors for cell-based therapies aimed at treating muscular dystrophy. Myoblast transplantation (MT) involves the delivery of primary unmodified skeletal myoblasts to muscle typically via an intramuscular injection. This method is advantageous in that muscle biopsies are easily conducted on limb musculature, techniques for genetic modification of myoblasts are efficient, and large quantities of *in vitro* expanded myoblasts are easily achieved.

The potential of MT originates from initial experiments performed in mice which demonstrated the capacity of donor myogenic cells to regenerate recipient muscle⁶³⁻⁶⁵. Experiments conducted by Partridge et al. using the immortal C2C12 mouse myoblast cell line validated the ability of

exogenous myoblasts to induce synthesis of dystrophin in dystrophin-deficient mdx muscle fibers⁶⁶. Subsequent experiments confirmed and elaborated upon these results by using myoblasts from newborn⁶⁷ or adult mice^{68,69} in addition to human myoblasts^{70,71} as donor cells for transplant into mdx mice. These experiments demonstrated the ability to track the transplanted cells in the host through the use of LacZ staining, or in the case of human myoblast transfer antibodies specific for human dystrophin. MT was later tried in non-human primates in order to assess the regenerative capacity and immune response involved. Primate derived myoblasts successfully integrated into allogenic hosts when injected 1mm apart and in combination with the immunosuppressive FK506⁷²⁻⁷⁴. These experiments indicate that primate derived myoblasts could integrate into regenerating muscle and survive after one year, however none of these experiments provide any evidence as to whether the transplanted cells provide any physiological correction of the dystrophic phenotype.

On the basis of research conducted in mice and nonhuman primates, human clinical trials involving the transplantation of myoblasts were initiated in the early 1990s^{75,76}. Initial trials involved repetitive intramuscular injections of large quantities of myoblasts ($>10^6$ cells) distributed over multiple sites. Although reported as successful⁷⁶, functional evidence was elusive and plagued with false positives resulting from revertant fibers⁷⁷, which arise from a second mutation and occur due to either a somatic deletion or through splicing of further exons in the dystrophin gene. These events lead to the restoration of the reading frame allowing for the production of a truncated, yet partially functional dystrophin molecule⁷⁸. Later clinical trials involved techniques to distinguish dystrophin-positive fibers derived from donor DNA from host revertant fibers. These techniques eliminate confusion concerning the contribution of donor cells

to muscle regeneration and allow for a more confident assessment of physiological benefit post transplantation.

The majority of past experiments involving myoblast transfer to treat DMD failed to show substantial physiological correction of the dystrophic phenotype⁷⁹⁻⁸⁵. Although, recent clinical attempts show improvement in the areas of cell survival, migration, and evasion of the immune response, these issues remain at the forefront of myoblast transplantation^{86,87}. Since grafted myoblasts have limited migration, repeated local injections are required to treat a significant portion of the myofibers in any given muscle. Considering DMD patients succumb to heart and diaphragm failure, repeated injections 1 to 2mm apart would be required in these muscles to ensure patient survival, a technique that is currently beyond our grasp. In addition, transplanted myoblasts do not participate in long term muscle regeneration making them less than ideal for the treatment of DMD. In conclusion, while myoblast transfer provides transient delivery of dystrophin and improves the strength of injected dystrophic muscle it is considered an interim solution to ease the suffering of patients with muscular dystrophy. In order to be considered a viable widespread treatment option for DMD, myoblasts must contribute to multiple rounds of regeneration and be conducive to widespread distribution throughout the musculature.

2.2 Satellite Cell Transplantation

The ability to directly isolate a pure population of satellite cells from diaphragm muscle, by using a Pax3-GFP knock-in mouse⁸⁸, was recently accomplished. This Pax3-GFP mouse incorporates the green fluorescent protein (GFP) under the control of the Pax3 promoter allowing faithful recapitulation of Pax3 expression. The use of fluorescent activated cell sorting (FACS) permits the purification of a GFP positive population of Pax3⁺/CD34⁺/Pax7⁺ cells. Based on gene expression, these results suggest the isolation of a predominantly quiescent population of

satellite cells. When injected into dystrophic muscle, this population of cells is capable of restoring dystrophin expression three weeks post-transplantation. Importantly, the yield of dystrophin expressing muscle obtained when small numbers of isolated satellite cells were transplanted into irradiated muscle was significant. Freshly isolated satellite cells not only restored dystrophin expression in mdx mice but also formed roughly 17% of the satellite cell pool expressing both Pax7 and Pax3-GFP; an indication that donor cells were capable of contributing to the muscle satellite cell compartment. More over, approximately 25-fold more cells are needed to obtain similar levels of regeneration from donor cells isolated by enzymatic dissociation of whole adult muscles, as opposed to grafting Pax3-GFP sorted cells⁶².

However, the full potential of this approach is affected by several limitations. First, the cultivation of freshly isolated satellite cells in vitro significantly reduces their in vivo myogenic potential; therefore, whether or not sufficient numbers of donor satellite cells can be obtained is a key issue. The isolation of sufficient quantities of Pax3-GFP satellite cells is difficult because these cells can only be isolated from the diaphragm and body trunk muscles but not from limb muscles. In fact, the current absence of appropriate cell surface markers to identify a Pax3⁺/CD34⁺/Pax7⁺ population of satellite cells makes this isolation technique impossible in humans. Given that genetic manipulations generally require short-term cultivation in vitro, and in vitro culture decreases the regenerative potential of Pax3-GFP populations, then genetic correction of autologous sorted satellite cells does not appear to be a viable option. This is particularly important from a clinical standpoint since cell transplantation of autologous genetically corrected satellite cells to DMD patients is theoretically the ideal approach to minimize host immune rejection of donor cells. A clinically relevant approach to using fresh

satellite cells would involve their isolation from the peripheral musculature, based on a panel of cell surface markers, subsequent culture in vitro under conditions that promote the maintenance of their stem cell state, followed by gene therapy prior to transplantation. In the absence of cell surface markers to isolate quiescent satellite cells from the musculature this alternative is currently not an option; therefore, research into the identification of a feasible isolation strategy is of the utmost importance.

2.3 Single Muscle Fiber

Experiments conducted in the early 1980s involving the transplant of whole muscle indicated that resident satellite cells are capable of initiating regeneration^{89,90}. While, enzymatic dissociation and the subsequent transplantation of satellite cells from myofibers results in marginal muscle regeneration, the transplantation of satellite cells still associated with a single muscle fiber (containing as few as seven satellite cells) can generate in the range of 100 myofibers with thousands of corresponding myonuclei⁹¹. Interestingly, the satellite cells resident upon a transplanted myofiber will contribute to the host satellite cell compartment and be available for multiple rounds of regeneration. Transplanted satellite cells appear to migrate throughout the muscle in which the myofibers were implanted; however, no direct quantification of the migratory potential of donor satellite cells exists. The notion that single muscle fibers will be used to treat muscular dystrophy does not in itself present a realistic therapeutic approach. Questions regarding the procurement of donor muscle fibers is somewhat belied by the large regenerative potential of individual satellite cells. However, no evidence suggests donor satellite cells are able to populate neighboring muscles; indicating that this method of cell transplant would involve multiple transplantations.

In general the data presented in this section provides evidence that quiescent satellite cells maintained in their niche retain a large degree of regenerative potential. Once it is possible to simulate the satellite cell niche in vitro the real potential of satellite cells could be harnessed for therapeutic purposes. Experiments conducted with fresh satellite cells as well as intact muscle fibers allude to the necessity of identifying the molecular mechanisms responsible for satellite cell self renewal and differentiation. The drastic increase in regenerative potential from either freshly isolated satellite cells or intact myofibers suggests a link between the maintenance of the satellite cell niche and the efficiency of muscle regeneration. Future experiments to identify the components of the satellite cell niche that are responsible for the activation or maintenance of satellite cells in a quiescent state will be of great importance for the validation of satellite cell based therapies.

3. Applications of Atypical Muscle Stem Cells

3.1 Muscle Side Population Cells

Within muscle, in addition to satellite cells, there exists a population of stem cells that possess myogenic potential, termed side population (SP) cells. This stem cell population, isolated by fluorescent activated cell sorting (FACS) based on its exclusion of the Hoechst 33342 dye (via the ABC transporter Bcrp1/ABCG2), can be isolated from many adult tissues⁹². SP cells isolated from bone marrow (bmSP) or muscle (mSP) on their own are unable to undergo myogenic differentiation in vitro, yet upon intramuscular transplantation can give rise to both myocytes and satellite cells^{25,93}. The mSP population when isolated from a Pax7^{-/-} background, where satellite

cells are absent, and co-cultured with myoblasts or forced to express MyoD undergo muscle specification. These data suggest mSP cells and satellite cells constitute distinct populations that progress along different myogenic pathways⁵⁷. Studies directly comparing muscle regeneration after intravenous injection of bone marrow side population bmSP and mSP cells indicated a reduced ability of the mSP fraction to reconstitute the hematopoietic compartment in lethally irradiated mice; however, both populations regenerate muscle to a similar degree²⁵.

In contrast to satellite cells or primary myoblasts, mSP cells are able to migrate from the blood stream into muscle, a desirable feature for widespread distribution of a therapeutic cell type. Intravenous transplantation of mSP cells typically yields at most a 1% engraftment rate, however upon delivery into noninjured, nonirradiated mdx mice via femoral artery catheterization mSP cells engraftment into muscle at rates approaching 5-8% in select muscles^{94,95}. These results provide evidence that, under physiological conditions, the mSP population can provide dystrophin to diseased muscle via arterial transplantation.

One aspect of mSP transplantation is puzzling, if mSP cells can take up the satellite cell position, and this is reported in numerous articles^{25,93,95}, why do they not appear to contribute to long term muscle regeneration? Perhaps mSP give rise to committed myogenic Pax7 expressing cells but not to satellite stem cells. Further advances in the field of mSP transplantation must address the following issues: low levels of integration following arterial or intramuscular transplantation, an inability to partake in long term regeneration, and achieving physiological improvements to dystrophic muscle. Prior to these issues being resolved mSP cells currently do not constitute a viable cell source to treat DMD.

3.2 Bone Marrow cells

Bone marrow transplantation (BMT) or hematopoietic stem cell transplantation (HSCT) involves the transplantation of hematopoietic stem cells (HSC) in order to produce new blood cells and repopulate the bone marrow⁹⁶. Evidence of a population of circulating cells with myogenic potential present in the bone marrow was identified in the late 1960s. Ferrari et al. (1998) later confirmed that BM-derived cells can, at very low levels, undergo myogenic differentiation and participate in muscle repair after injury²⁶. This research presented the idea of delivering donor cells via the circulation to take part in skeletal muscle regeneration; a potentially powerful development considering the daunting task of injecting donor cells into individual muscle masses. The following year studies involving the transplantation of BM-derived cells conducted in the mdx mouse partially restored dystrophin expression²⁵. Bone marrow derived cells persist in the musculature for long periods of time and maintain their dystrophin expression, however quantitatively the amount of muscle generated after a BM transplant does not comprise a therapeutically relevant amount when only 0.5% of regenerating fibers contain donor cells^{97,98}.

A clear mechanism detailing the process by which cells in the bone marrow contribute to muscle regeneration remains elusive. Experiments conducted by LaBarge et al. attempted to elaborate on the process by which bone marrow cells contribute to muscle regeneration by the transplantation of bone marrow derived cells (BMDCs) into irradiated SCID mice⁹⁹. BMDCs appear to contribute following muscle irradiation to the satellite cell niche and further exercise induced damage led to the incorporation of BMDCs into multinucleated myofibers at a frequency approaching 3.5%. These initial experiments have been elaborated upon using exercise as

opposed to muscle irradiation leading to the conclusion that BMDC can incorporate into muscle under physiological conditions¹⁰⁰. However, the question remains whether integration of cells from the bone marrow into muscle is a physiologically relevant process. Experiments by Camargo *et al* and Corbel *et al.* (2003) analyzed the ability of bone marrow derived HSCs to participate in muscle regeneration; and while both studies found HSC progeny could incorporate into muscle, this ability is more likely attributed to fusion rather than the existence of a myogenic HSC^{101,102}. Other hematopoietic stem cell populations exist including the CD45⁺/Sca-1⁺ population, which following muscle injury undergoes a 30 fold expansion in regenerating muscle and readily undergoes myogenic differentiation *in vitro*¹⁰³. These experiments further concluded that Wnt signaling molecules play a role in augmenting the myogenic specification of CD45⁺/Sca1⁺ cells. Although, later experiments would lead to the conclusion that under physiological conditions bone marrow, and the HSCs contained within, play a minor role in muscle regeneration¹⁰⁴ this technique in combination with appropriate growth factors and suitable methods for transplantation may eventually serve as a method to treat DMD.

Although the major stem cell component of bone marrow is that of the HSC, and the contribution of bone marrow derived cells to the physiological process of muscle regeneration is considered by some to be trivial, the contribution of cells in the bone marrow may differ significantly between mice and humans. AC133, a human cell surface marker for the hematopoietic/endothelial lineages was recently used to isolate a population of cells from human blood that can, upon *in vitro* co-culture with myogenic cells or exposure to Wnt-producing cells, undergo a degree of myogenic conversion¹⁰⁵. This finding has reopened the debate on a blood born population with myogenic potential. These AC133⁺ cells, when co-cultured or exposed to

Wnts, display an mRNA pattern reminiscent of that found in satellite cells including: M-Cadherin, Pax7, CD34 and Myf5. Whether AC133⁺ cells constitute a true myogenic progenitor is difficult to determine considering these cells do not undergo myogenic differentiation spontaneously when myogenesis was induced with low serum levels. Nevertheless, limited myogenic conversion and dystrophin expression is observed upon intra-arterial injection or intramuscular injection of AC133⁺ cells. This method offers certain advantages over other HSC populations including: engraftment of donor cells under physiological conditions exceeds that shown previously for bone marrow, HSCs or mSP cells, functional tests of injected muscles revealed a substantial recovery of force after treatment. Considering these qualities this method to treat DMD warrants further analysis regarding the process by which the AC133⁺ population contributes to myogenic regeneration, the localization of AC133⁺ cells within the circulatory system, and the ability to expand ex vivo these cells prior to transplantation.

3.3 Mesenchymal Stem cells

Multipotent mesenchymal stem cells (MSCs), first derived from bone-forming progenitor cells resident in the bone marrow, are capable of producing skeletal muscle in addition to osteoblasts, chondroblasts, and adipocytes¹⁰⁶⁻¹⁰⁸. Typical methods for MSC isolation involve percoll fractionation and subsequent culture with varying growth factors¹⁰⁹. The debate surrounding the pluripotent nature of MSCs continues; indeed reports suggest a population of cells co-purified with MSCs can, in vitro, differentiate into visceral mesoderm, neuroectoderm and endoderm¹¹⁰. These pluripotent stem cells are termed multipotent adult progenitor cells or MAPCs. MAPCs appear to reside in the brain, skeletal muscle, and bone marrow of human and mouse tissues¹¹¹. Being adult derived; MAPCs avoid many ethical and immunological hurdles associated with

embryonic stem cells facilitating their therapeutic application. Continuing research must focus on the origins of MAPCs and the molecular mechanisms that govern their development as well as the ability to induce myogenesis from these cells prior to them being a potential therapeutic source to treat DMD.

Other MSC populations exist and can be isolated via enzymatic digestion and serial passaging of cells from adult human synovial membrane (hSM-MSCs)¹¹². These hSM-MSCs possess multilineage potential *in vitro* and recapitulate the temporal gene expression typical of embryonic myogenesis when directly injected into injured TA muscles of immuno-suppressed mdx mice¹¹³. hSM-MSCs can engraft into regenerating muscle, express dystrophin, and give rise to putative satellite cells that persist for 6 months after transplantation. However, the gene expression profile of hSM-MSC derived satellite cells must be clarified considering neither Mcadherin, Pax7, or CD34 are shown to be expressed in these cells. A method to resolve this issue would be to conduct single cell clonal analysis to confirm the ability of hSM-MSC derived satellite cells to proliferate and give rise to multi-nucleate myotubes. At this point in time additional research into the physiological benefits of hSM-MSCs post transplantation, as well as the confirmation of satellite cell characteristics are necessary in order to progress therapeutically with this technique.

In addition to the MSC types stated above there exists a method to convert MSCs to the skeletal muscle lineage¹¹⁴ via infection with activated Notch in the presence of various cytokines. Notch is a transmembrane protein, which upon binding with its ligands delta or jagged, undergoes cleavage to release an intracellular domain (NICD) in order to effect downstream signaling. The

Notch signaling pathway is involved in embryonic tissue morphogenesis¹¹⁵, adult cell fate selection¹¹⁶, and has been linked to satellite cell activation and muscle differentiation, making it a candidate molecule for the myogenic induction of MSCs¹¹⁷. Experiments conducted by Dezawa et al (2005) demonstrate the ability to generate skeletal muscle progenitors from human and rat MSCs. Single cell clonal analysis confirms the ability of isolated muscle-MSCs (M-MSCs) to form multi-nucleate myotubes following treatment with the NICD and culture in low concentrations of horse serum to induce fusion. Intravenous injection of M-MSCs in immunosuppressed rats, pretreated with cardiotoxin in the gastrocnemius muscle, resulted in their incorporation into newly formed myofibers, and not bone, heart, liver, kidney or undamaged muscle. M-MSCs localize to the sublaminar portion of myofibers, express Pax7 and c-Met via immunofluorescence of separate cellular fields and upon multiple rounds of regeneration contribute to muscle repair.

Questions remain regarding the contribution of M-MSCs under physiological conditions to the functional amelioration of the dystrophic phenotype. Interestingly, M-MSCs express myogenin (a terminal marker of muscle differentiation) at high levels along with MyoD, early markers of muscle formation Six1, and Six4 along with Pax7 a satellite cell marker. Considering their gene expression profile M-MSCs appear to be a heterogeneous population of undifferentiated and partially differentiated myogenic progenitors. Further characterization of M-MSCs and the in vivo derived satellite cells is required to answer these questions. In addition, the use of Notch to induce myogenic specification is a unique approach more commonly associated with the maintenance of quiescent satellite cells or the prevention of terminal muscle differentiation. This is not to say the use of Notch to promote myogenic specification is improper, however without a

clear molecular mechanism governing the role of Notch in the myogenic commitment of MSCs nor a basis for converting MSCs to the myogenic lineage without over-expressing the NICD this current route is not practical for the treatment of patients with DMD. This method offers the ability to derive myogenic cells that can be easily obtained and expanded from patients, genetically modified in vitro, and re-introduced via the circulation all beneficial therapeutic characteristics.

In summary, multiple different types of MSCs can be isolated and serve as potential cell types for therapy, however in the absence of a defined group of cell surface markers a reproducible system to isolate cells with myogenic potential from MSCs becomes difficult. The reproducible isolation of pure MSC populations and their downstream differentiation into the muscle lineage holds tremendous potential for the treatment of neuromuscular disorders. Given the rapid progress in the areas of cell purification and characterization therapeutic relevancy may not be far off.

3.4 Muscle Derived Stem Cells (MDSCs)

Muscle derived stem cells (MDSCs) are a distinct population of stem cells resident in adult skeletal muscle. MDSCs are thought to reside upstream of satellite cells in the terms of their potency, and are not restricted to either the myogenic or mesenchymal lineages^{118,119}. Numerous muscle derived stem cell populations have been shown to contain hematopoietic potential^{93,120,121} the most prominent being that derived via the preplate technique²². These MDSCs represent a heterogeneous population of cells in terms of their high expression of either Sca1 or CD34. While the physiological location of MDSCs remains unknown they often express MNF and the

myogenic regulatory factor MyoD and share certain characteristics with a population of hematopoietic stem cells expressing CD34 and Sca1¹²². When injected into the muscle or into the circulation of mdx mice MDSCs contribute to muscle regeneration and express dystrophin^{123,124}. In comparison to transplantations conducted with primary myoblasts, MDSCs show a 10 fold increase in dystrophin expression and incorporate into vessels and surrounding nerves²².

Although certain characteristics of MDSCs including their ease of proliferation in vitro, ability to migrate through the vasculature, and their multipotentiality are amenable to therapeutic applications, a lack of physiological improvement to the dystrophic condition coupled with long term self renewal resulting in their transformation mar the use of MDSCs to treat DMD^{125,126}. In an attempt to determine the physiological location of MDSCs, some reports claim they originate in the bone marrow and reside in the musculature¹²⁰ while others claim they are muscle derived^{126,127}. Further research into the origins of MDSCs is required prior to the clinical use of these cells.

3.5 Vessel Associated Stem Cell Populations

Cells with endothelial and myogenic properties exist and can be isolated at embryonic, fetal¹²⁸, and postnatal stages¹²⁹ (3 weeks) of development yet the identification of a bona fide adult vessel derived stem cell remains elusive. In 1999 De Angelis et al discovered a stem cell population resident in the embryonic dorsal aorta having both endothelial and myogenic markers¹³⁰.

Although, explant cultures of the dorsal aorta do not initially express any myogenic markers; upon differentiation in culture they gain both endothelial and myogenic markers known to be present on adult muscle satellite cells. Surprisingly, the quantity of satellite cells produced in

vitro from the dorsal aorta is in the range of 50 fold more than that derived from somitic explants¹³⁰. Dorsal aorta derived myogenic cells can in vitro fuse with primary myoblasts during differentiation and when transplanted into TA muscles contribute their nuclei to muscle regeneration¹³⁰.

Further work on vessel associated stem cells isolated from the dorsal aorta demonstrated their multi-potentiality leading to their classification as *mesangioblasts*¹³¹. Studies conducted by Sampolesi *et al* using immunocompetent α -sarcoglycan null mice which serve as a model system to study limb-girdle muscular dystrophy indicated that the intra-arterial injection of mesangioblasts results in their migration throughout the vasculature, giving rise to both morphological and functional correction of the dystrophic phenotype, a quality absent in other myogenic stem cell populations¹³². This method was further improved by the treatment of mesangioblasts with either stromal-derived factor (SDF) 1 or tumor necrosis factor (TNF) α which resulted in enhanced transmigration in vitro and migration into dystrophic muscle in vivo¹³³. The combination of pretreatment with TNF α and SDF-1 and the expression of α -4 integrin lead to remarkable (~50%) incorporation of arterially transplanted mesangioblasts into α -sarcoglycan deficient muscle. Long term survival of pre-treated mesangioblasts is observed in muscle masses after 4 months, with α -sarcoglycan mice expressing ~60% of the α -sarcoglycan detected in a wild type mouse. Although these reports are extremely promising, confirmation of the human equivalent of mesangioblasts that can be isolated from adult tissue is of great importance.

Pre-treated mesangioblast can migrate through the vasculature, engraft at therapeutic levels in muscle, persist and contribute to long term regeneration, and upon genetic modification of autologous cells do not elicit an immune response. Due to these qualities mesangioblasts constitute a potential therapeutic cell type to treat DMD. Prior to their use in clinical trials effective methods to isolate mesangioblasts from adult human tissue must be established, information regarding their long term contribution to muscle function, and the effects of unwanted penetration into non desired cell types must be resolved.

Research supporting the vascular origins of myogenic stem cell populations provides therapeutic potential for the systematic delivery of myogenic progenitors. Whether or not these populations (embryonic, fetal or post natal) of vessel associated stem cells are unique, or derived from an upstream stem cell remains to be determined. Considering these cells are associated with the vasculature it is not a stretch to imagine stem cell populations with myogenic potential residing in multiple adult tissues. While the exact characteristics of vessel associated stem cells remains a mystery their ability to migrate throughout the vasculature coupled with their myogenic potential make them an attractive source for use in cell therapy.

4. Embryonic Stem cells

Small quantities of skeletal muscle were first derived from murine embryonic stem (ES) cells nearly 20 years ago⁴⁰, however since then few advances have been made to improve the efficiency of the process. First reports regarding gene expression patterns, functional properties, and morphology of ES derived skeletal muscle parallel observations in vivo^{40,134}. Gene targeting in ES cells is often used to assess gene function, and as a result numerous ES cell lines with

modifications to genes important in myogenesis exist. Typically, created to study embryonic development in the mouse these ES cell lines were analyzed during in vitro differentiation to study the molecular mechanisms involved in embryonic myogenesis¹³⁵⁻¹³⁹. Over time limited inhibitors¹⁴⁰ and activators¹⁴¹ of skeletal muscle formation from ES cells were identified along with strategies to harness their potential for regenerative medicine¹⁴².

One study conducted by Barberi *et al* and published in 2005 shows therapeutic potential and involves the derivation of skeletal muscle by way of hES derived mesenchymal stem cells¹⁴³. This method offers the ability to derive unlimited numbers of pure MSCs from hESCs, and their subsequent differentiation into the skeletal muscle lineage. In order to obtain skeletal muscle from hES derived MSCs (hESMPCs) either co-culture or conditioned medium from the murine myoblast cell line C₂C₁₂ or the addition of 5-AzaC as a demethylating agent is required. Currently these experiments do not address the ability of hESMPCs to give rise to Pax7 positive cells nor their ability to contribute to muscle regeneration in vivo.

ES cells to date have not had a significant impact on the development of cell-based therapies to treat muscular dystrophy. Currently one study exists using ES cells to treat muscular dystrophy¹⁴⁴. In this study differentiated ES cells (3 days) were co-cultured with dissociated skeletal muscle fibers for four days prior to transplantation into *mdx* muscle. After 2 weeks dystrophin expression was detected in select regions over and above that which would occur naturally from revertant fibers. These experiments represent the preliminary steps for the use of ES cells to treat DMD and currently do not demonstrate any indication of the long-term regenerative capacity of transplanted cells. Questions remain as to the mechanism by which ES

cells, co-cultured in the presence of adult skeletal muscle, contribute to the process of muscle regeneration.

Currently no methods exist to generate large quantities of skeletal muscle from ES cells and for this reason the ES cell model system remains better suited as a tool to study embryonic myogenesis. Potential benefits of using ES cells to treat muscular dystrophy focus on the isolation of large quantities of myogenic stem cells, their ease of genetic modification in vitro, and the ability to derive immune matched cell lineages for transplant. Although the creation of patient specific hES cell lines remains mired in controversy, the principle retains a high degree of feasibility¹⁴⁵ and in the interim progress on the mechanisms involved in myogenic induction and differentiation continue.

5. Synergistic methods to improve stem cell based therapies

In this chapter we have presented numerous stem cell types with varying degrees of myogenic potential. Irrespective of their source, these stem cell populations share certain hurdles to overcome prior to their therapeutic use. Survival and the subsequent migration from the site of injection remains suboptimal for many of the cell populations outlined previously and from a clinical standpoint the immune response generated upon introduction of a foreign cell type, or in the case of DMD a foreign protein, is a concern. As the mechanisms surrounding the survival and proliferation of myogenic cells post transplant are unraveled^{146,147} and appropriate growth factors¹⁴⁸⁻¹⁵⁰ are identified the success of cell based therapies to treat muscular dystrophy will improve. Mechanisms involved in the migration of donor cells to skeletal muscle and the satellite cell niche are still poorly understood. Although some stem cell types, namely mesangioblasts,

mSP cells, M-MSCs, have the ability to migrate through the vasculature, most do not. Potential future avenues to increase the migratory ability of stem cell populations include the identification of cell surface markers¹⁵¹ (eg L-selectin⁺ cells) and appropriate growth factors¹⁵²⁻¹⁵⁵. As is observed in the case of mesangioblasts, pre-treatment with the growth factors TNF α and SDF-1 led to a substantial improvement in their migratory abilities.

Cell transplantation often elicits an immune response, and in addition to immunosuppressive drugs, there exist methods to overcome immune rejection. One such method involves the establishment of central immune tolerance through a process of mixed hematopoietic chimerism¹⁵⁶. The mechanism surrounding mixed hematopoietic chimerism is not fully understood yet the procedure is well established in animal models and pursued in the clinic¹⁵⁷. Matching the genetic background of the various stem cell derived myogenic precursors with hematopoietic stem cells could potentially allow the tolerisation of patients to myogenic transplants

Irrespective of the stem cell population chosen to treat muscular dystrophy the above-mentioned characteristics (survival, localization, and immunogenicity) remain. In order for the chosen cell type to be successful it must be optimized to deal with these issues. The identification of suitable growth factors, appropriate surface markers, and methods to escape immune detection will be of great importance for the progression of this field.

6. Conclusions and Future Perspectives

Stem cell therapy is an attractive method to treat muscular dystrophy because in theory only a small number of cells, together with a stimulatory signal for expansion, are required to elicit a therapeutic effect. In order to achieve clinical relevance candidate stem cell populations must be easily obtained, upon isolation remain capable of efficient myogenic conversion, and when transplanted must integrate into the musculature leading to the functional correction of the dystrophic phenotype.

Stem cell populations with myogenic potential can be derived from multiple regions of the body at various stages of development (**Fig2**). Many questions linger regarding the mechanisms by which atypical, or non satellite cell derived precursors, participate in muscle regeneration. A general consensus in the field identifies satellite cells as the primary, if not only, physiologically relevant population that contributes significantly to muscle regeneration. However, because satellite cells are not currently amenable for distribution throughout the vasculature they do not constitute a viable option to treat DMD. A superficial comparison of the atypical myogenic stem cell populations reveals a common theme whereby a cell often linked to the circulatory system is able to migrate to regenerating muscle and contribute in a limited way to this process. The localization of adult mSP, MDSCs, Mesangioblasts, Bone Marrow derived HSCs and HSC populations expressing the cell surface marker AC133 remains unknown. The idea that myogenic potential resides in cells associated with the vasculature is not novel, in fact pericytes which line the capillaries appear to possess qualities of multipotent mesenchymal progenitors^{158,159}. If indeed pericytes take part in myogenic regeneration this could explain the widespread distribution of atypical stem cell populations with myogenic potential.

In conclusion, upon comparing the prospective stem cell populations with myogenic potential, the cell type that fulfills the most criteria for use in the treatment of DMD is the mesangioblast. Mesangioblasts serve as a paradigm for wide spread distribution, and upon growth factor pre-treatment are able to correct significantly the dystrophic phenotype. Perhaps the application of growth factor pre-treatment to other myogenic stem cell populations may improve their ability to treat muscular dystrophy, however for now mesangioblasts serve as a beacon of hope for patients suffering from various muscular dystrophies.

.

,

Reference List

1. Emery,A.E. The muscular dystrophies. *Lancet* **359**, 687-695 (2002).
2. Emery,A.E. Population frequencies of inherited neuromuscular diseases--a world survey. *Neuromuscul. Disord.* **1**, 19-29 (1991).
3. Kunkel,L.M., Monaco,A.P., Middlesworth,W., Ochs,H.D. & Latt,S.A. Specific cloning of DNA fragments absent from the DNA of a male patient with an X chromosome deletion. *Proc. Natl. Acad. Sci. U. S. A* **82**, 4778-4782 (1985).
4. Ray,P.N. *et al.* Cloning of the breakpoint of an X;21 translocation associated with Duchenne muscular dystrophy. *Nature* **318**, 672-675 (1985).
5. Hoffman,E.P., Brown,R.H., Jr. & Kunkel,L.M. Dystrophin: the protein product of the Duchenne muscular dystrophy locus. *Cell* **51**, 919-928 (1987).
6. Matsumura,K. *et al.* The role of the dystrophin-glycoprotein complex in the molecular pathogenesis of muscular dystrophies. *Neuromuscul. Disord.* **3**, 533-535 (1993).
7. Sicinski,P. *et al.* The molecular basis of muscular dystrophy in the mdx mouse: a point mutation. *Science* **244**, 1578-1580 (1989).
8. Ryder-Cook,A.S. *et al.* Localization of the mdx mutation within the mouse dystrophin gene. *EMBO J.* **7**, 3017-3021 (1988).
9. Dangain,J. & Vrbova,G. Muscle development in mdx mutant mice. *Muscle Nerve* **7**, 700-704 (1984).

10. Grady,R.M. *et al.* Skeletal and cardiac myopathies in mice lacking utrophin and dystrophin: a model for Duchenne muscular dystrophy. *Cell* **90**, 729-738 (1997).
11. Deconinck,A.E. *et al.* Utrophin-dystrophin-deficient mice as a model for Duchenne muscular dystrophy. *Cell* **90**, 717-727 (1997).
12. Guo,C. *et al.* Absence of alpha7 integrin in dystrophin-deficient mice causes a myopathy similar to Duchenne muscular dystrophy. *Hum. Mol. Genet.* **15**, 989-998 (2006).
13. Rooney,J.E. *et al.* Severe muscular dystrophy in mice that lack dystrophin and {alpha}7 integrin. *J. Cell Sci.* **119**, 2185-2195 (2006).
14. Gaschen,F.P. *et al.* Dystrophin deficiency causes lethal muscle hypertrophy in cats. *J. Neurol. Sci.* **110**, 149-159 (1992).
15. Guyon,J.R. *et al.* The dystrophin associated protein complex in zebrafish. *Hum. Mol. Genet.* **12**, 601-615 (2003).
16. Cooper,B.J. *et al.* The homologue of the Duchenne locus is defective in X-linked muscular dystrophy of dogs. *Nature* **334**, 154-156 (1988).
17. Valentine,B.A., Cooper,B.J., de Lahunta,A., O'Quinn,R. & Blue,J.T. Canine X-linked muscular dystrophy. An animal model of Duchenne muscular dystrophy: clinical studies. *J. Neurol. Sci.* **88**, 69-81 (1988).
18. Osawa,M., Hanada,K., Hamada,H. & Nakauchi,H. Long-term lymphohematopoietic reconstitution by a single CD34-low/negative hematopoietic stem cell. *Science* **273**, 242-245 (1996).

19. Galli,R. *et al.* Skeletal myogenic potential of human and mouse neural stem cells. *Nat. Neurosci.* **3**, 986-991 (2000).
20. Toma,J.G. *et al.* Isolation of multipotent adult stem cells from the dermis of mammalian skin. *Nat. Cell Biol.* **3**, 778-784 (2001).
21. Asakura,A., Komaki,M. & Rudnicki,M. Muscle satellite cells are multipotential stem cells that exhibit myogenic, osteogenic, and adipogenic differentiation. *Differentiation* **68**, 245-253 (2001).
22. Qu-Petersen,Z. *et al.* Identification of a novel population of muscle stem cells in mice: potential for muscle regeneration. *J. Cell Biol.* **157**, 851-864 (2002).
23. Young,H.E. *et al.* Human reserve pluripotent mesenchymal stem cells are present in the connective tissues of skeletal muscle and dermis derived from fetal, adult, and geriatric donors. *Anat. Rec.* **264**, 51-62 (2001).
24. Shafritz,D.A., Oertel,M., Menthena,A., Nierhoff,D. & Dabeva,M.D. Liver stem cells and prospects for liver reconstitution by transplanted cells. *Hepatology* **43**, S89-S98 (2006).
25. Gussoni,E. *et al.* Dystrophin expression in the mdx mouse restored by stem cell transplantation. *Nature* **401**, 390-394 (1999).
26. Ferrari,G. *et al.* Muscle regeneration by bone marrow-derived myogenic progenitors. *Science* **279**, 1528-1530 (1998).
27. Orlic,D. *et al.* Bone marrow cells regenerate infarcted myocardium. *Nature* **410**, 701-705 (2001).

28. Jackson, K.A. *et al.* Regeneration of ischemic cardiac muscle and vascular endothelium by adult stem cells. *J. Clin. Invest* **107**, 1395-1402 (2001).
29. Lin, Y., Weisdorf, D.J., Solovey, A. & Hebbel, R.P. Origins of circulating endothelial cells and endothelial outgrowth from blood. *J. Clin. Invest* **105**, 71-77 (2000).
30. Asahara, T. *et al.* Bone marrow origin of endothelial progenitor cells responsible for postnatal vasculogenesis in physiological and pathological neovascularization. *Circ. Res.* **85**, 221-228 (1999).
31. Petersen, B.E. *et al.* Bone marrow as a potential source of hepatic oval cells. *Science* **284**, 1168-1170 (1999).
32. Theise, N.D. *et al.* Derivation of hepatocytes from bone marrow cells in mice after radiation-induced myeloablation. *Hepatology* **31**, 235-240 (2000).
33. Brazelton, T.R., Rossi, F.M., Keshet, G.I. & Blau, H.M. From marrow to brain: expression of neuronal phenotypes in adult mice. *Science* **290**, 1775-1779 (2000).
34. Mezey, E., Chandross, K.J., Harta, G., Maki, R.A. & McKercher, S.R. Turning blood into brain: cells bearing neuronal antigens generated in vivo from bone marrow. *Science* **290**, 1779-1782 (2000).
35. Evans, M.J. & Kaufman, M.H. Establishment in culture of pluripotential cells from mouse embryos. *Nature* **292**, 154-156 (1981).

36. Lee,S.H., Lumelsky,N., Studer,L., Auerbach,J.M. & McKay,R.D. Efficient generation of midbrain and hindbrain neurons from mouse embryonic stem cells. *Nat. Biotechnol.* **18**, 675-679 (2000).
37. Buttery,L.D. *et al.* Differentiation of osteoblasts and in vitro bone formation from murine embryonic stem cells. *Tissue Eng* **7**, 89-99 (2001).
38. zur Nieden,N.I., Kempka,G. & Ahr,H.J. In vitro differentiation of embryonic stem cells into mineralized osteoblasts. *Differentiation* **71**, 18-27 (2003).
39. Lumelsky,N. *et al.* Differentiation of embryonic stem cells to insulin-secreting structures similar to pancreatic islets. *Science* **292**, 1389-1394 (2001).
40. Rohwedel,J. *et al.* Muscle cell differentiation of embryonic stem cells reflects myogenesis in vivo: developmentally regulated expression of myogenic determination genes and functional expression of ionic currents. *Dev. Biol.* **164**, 87-101 (1994).
41. Mendell,J.R. & Clark,K.R. Challenges for gene therapy for muscular dystrophy. *Curr. Neurol. Neurosci. Rep.* **6**, 47-56 (2006).
42. Chakkalakal,J.V., Thompson,J., Parks,R.J. & Jasmin,B.J. Molecular, cellular, and pharmacological therapies for Duchenne/Becker muscular dystrophies. *FASEB J.* **19**, 880-891 (2005).
43. Partridge,T.A. Stem cell therapies for neuromuscular diseases. *Acta Neurol. Belg.* **104**, 141-147 (2004).

44. Assessment of adenoviral vector safety and toxicity: report of the National Institutes of Health Recombinant DNA Advisory Committee. *Hum. Gene Ther.* **13**, 3-13 (2002).
45. Marshall,E. Gene therapy death prompts review of adenovirus vector. *Science* **286**, 2244-2245 (1999).
46. Hacein-Bey-Abina,S. *et al.* A serious adverse event after successful gene therapy for X-linked severe combined immunodeficiency. *N. Engl. J. Med.* **348**, 255-256 (2003).
47. Goldring,K., Partridge,T. & Watt,D. Muscle stem cells. *J. Pathol.* **197**, 457-467 (2002).
48. Partridge,T.A. Cells that participate in regeneration of skeletal muscle. *Gene Ther.* **9**, 752-753 (2002).
49. Seale,P., Asakura,A. & Rudnicki,M.A. The potential of muscle stem cells. *Dev. Cell* **1**, 333-342 (2001).
50. Moss,F.P. & Leblond,C.P. Satellite cells as the source of nuclei in muscles of growing rats. *Anat. Rec.* **170**, 421-435 (1971).
51. Snow,M.H. An autoradiographic study of satellite cell differentiation into regenerating myotubes following transplantation of muscles in young rats. *Cell Tissue Res.* **186**, 535-540 (1978).
52. Irintchev,A., Zeschnigk,M., Starzinski-Powitz,A. & Wernig,A. Expression pattern of M-cadherin in normal, denervated, and regenerating mouse muscles. *Dev. Dyn.* **199**, 326-337 (1994).

53. Cornelison,D.D. & Wold,B.J. Single-cell analysis of regulatory gene expression in quiescent and activated mouse skeletal muscle satellite cells. *Dev. Biol.* **191**, 270-283 (1997).
54. Cornelison,D.D., Filla,M.S., Stanley,H.M., Rapraeger,A.C. & Olwin,B.B. Syndecan-3 and syndecan-4 specifically mark skeletal muscle satellite cells and are implicated in satellite cell maintenance and muscle regeneration. *Dev. Biol.* **239**, 79-94 (2001).
55. Cornelison,D.D., Olwin,B.B., Rudnicki,M.A. & Wold,B.J. MyoD(-/-) satellite cells in single-fiber culture are differentiation defective and MRF4 deficient. *Dev. Biol.* **224**, 122-137 (2000).
56. Beauchamp,J.R. *et al.* Expression of CD34 and Myf5 defines the majority of quiescent adult skeletal muscle satellite cells. *J. Cell Biol.* **151**, 1221-1234 (2000).
57. Seale,P. *et al.* Pax7 is required for the specification of myogenic satellite cells. *Cell* **102**, 777-786 (2000).
58. Garry,D.J., Yang,Q., Bassel-Duby,R. & Williams,R.S. Persistent expression of MNF identifies myogenic stem cells in postnatal muscles. *Dev. Biol.* **188**, 280-294 (1997).
59. Heslop,L., Morgan,J.E. & Partridge,T.A. Evidence for a myogenic stem cell that is exhausted in dystrophic muscle. *J. Cell Sci.* **113** (Pt 12), 2299-2308 (2000).
60. Bischoff,R. The Satellite cell and muscle regeneration. In Myogenesis. Engle,A.G. & Franzini-Armstrong,C. (eds.), pp. 97-118 (McGraw-Hill, New York,1994).

61. Morgan, J.E., Pagel, C.N., Sherratt, T. & Partridge, T.A. Long-term persistence and migration of myogenic cells injected into pre-irradiated muscles of mdx mice. *J. Neurol. Sci.* **115**, 191-200 (1993).
62. Morgan, J.E., Fletcher, R.M. & Partridge, T.A. Yields of muscle from myogenic cells implanted into young and old mdx hosts. *Muscle Nerve* **19**, 132-139 (1996).
63. Morgan, J.E., Watt, D.J., Sloper, J.C. & Partridge, T.A. Partial correction of an inherited biochemical defect of skeletal muscle by grafts of normal muscle precursor cells. *J. Neurol. Sci.* **86**, 137-147 (1988).
64. Watt, D.J., Lambert, K., Morgan, J.E., Partridge, T.A. & Sloper, J.C. Incorporation of donor muscle precursor cells into an area of muscle regeneration in the host mouse. *J. Neurol. Sci.* **57**, 319-331 (1982).
65. Watt, D.J. Factors which affect the fusion of allogeneic muscle precursor cells in vivo. *Neuropathol. Appl. Neurobiol.* **8**, 135-147 (1982).
66. Partridge, T.A., Morgan, J.E., Coulton, G.R., Hoffman, E.P. & Kunkel, L.M. Conversion of mdx myofibres from dystrophin-negative to -positive by injection of normal myoblasts. *Nature* **337**, 176-179 (1989).
67. Morgan, J.E., Hoffman, E.P. & Partridge, T.A. Normal myogenic cells from newborn mice restore normal histology to degenerating muscles of the mdx mouse. *J. Cell Biol.* **111**, 2437-2449 (1990).

68. Kinoshita, I. *et al.* Very efficient myoblast allotransplantation in mice under FK506 immunosuppression. *Muscle Nerve* **17**, 1407-1415 (1994).
69. Kinoshita, I., Huard, J. & Tremblay, J.P. Utilization of myoblasts from transgenic mice to evaluate the efficacy of myoblast transplantation. *Muscle Nerve* **17**, 975-980 (1994).
70. Huard, J., Tremblay, G., Verreault, S., Labrecque, C. & Tremblay, J.P. Utilization of an antibody specific for human dystrophin to follow myoblast transplantation in nude mice. *Cell Transplant.* **2**, 113-118 (1993).
71. Huard, J., Verreault, S., Roy, R., Tremblay, M. & Tremblay, J.P. High efficiency of muscle regeneration after human myoblast clone transplantation in SCID mice. *J. Clin. Invest* **93**, 586-599 (1994).
72. Kinoshita, I. *et al.* Myoblast transplantation in monkeys: control of immune response by FK506. *J. Neuropathol. Exp. Neurol.* **55**, 687-697 (1996).
73. Skuk, D., Goulet, M., Roy, B. & Tremblay, J.P. Myoblast transplantation in whole muscle of nonhuman primates. *J. Neuropathol. Exp. Neurol.* **59**, 197-206 (2000).
74. Skuk, D., Goulet, M., Roy, B. & Tremblay, J.P. Efficacy of myoblast transplantation in nonhuman primates following simple intramuscular cell injections: toward defining strategies applicable to humans. *Exp. Neurol.* **175**, 112-126 (2002).
75. Law, P.K. *et al.* Dystrophin production induced by myoblast transfer therapy in Duchenne muscular dystrophy. *Lancet* **336**, 114-115 (1990).

76. Law,P.K. *et al.* First human myoblast transfer therapy continues to show dystrophin after 6 years. *Cell Transplant.* **6**, 95-100 (1997).
77. Hoffman,E.P., Morgan,J.E., Watkins,S.C. & Partridge,T.A. Somatic reversion/suppression of the mouse mdx phenotype in vivo. *J. Neurol. Sci.* **99**, 9-25 (1990).
78. Thanh,L.T., Nguyen,T.M., Helliwell,T.R. & Morris,G.E. Characterization of revertant muscle fibers in Duchenne muscular dystrophy, using exon-specific monoclonal antibodies against dystrophin. *Am. J. Hum. Genet.* **56**, 725-731 (1995).
79. Law,P.K. *et al.* Myoblast transfer therapy for Duchenne muscular dystrophy. *Acta Paediatr. Jpn.* **33**, 206-215 (1991).
80. Gussoni,E. *et al.* Normal dystrophin transcripts detected in Duchenne muscular dystrophy patients after myoblast transplantation. *Nature* **356**, 435-438 (1992).
81. Huard,J. *et al.* Human myoblast transplantation: preliminary results of 4 cases. *Muscle Nerve* **15**, 550-560 (1992).
82. Karpati,G. *et al.* Myoblast transfer in Duchenne muscular dystrophy. *Ann. Neurol.* **34**, 8-17 (1993).
83. Morandi,L. *et al.* Lack of mRNA and dystrophin expression in DMD patients three months after myoblast transfer. *Neuromuscul. Disord.* **5**, 291-295 (1995).
84. Neumeyer,A.M. *et al.* Pilot study of myoblast transfer in the treatment of Becker muscular dystrophy. *Neurology* **51**, 589-592 (1998).

85. Miller,R.G. *et al.* Myoblast implantation in Duchenne muscular dystrophy: the San Francisco study. *Muscle Nerve* **20**, 469-478 (1997).
86. Skuk,D. *et al.* Dystrophin expression in muscles of duchenne muscular dystrophy patients after high-density injections of normal myogenic cells. *J. Neuropathol. Exp. Neurol.* **65**, 371-386 (2006).
87. Skuk,D. *et al.* Dystrophin expression in myofibers of Duchenne muscular dystrophy patients following intramuscular injections of normal myogenic cells. *Mol. Ther.* **9**, 475-482 (2004).
88. Montarras,D. *et al.* Direct isolation of satellite cells for skeletal muscle regeneration. *Science* **309**, 2064-2067 (2005).
89. Roberts,P., McGeachie,J.K., Grounds,M.D. & Smith,E.R. Initiation and duration of myogenic precursor cell replication in transplants of intact skeletal muscles: an autoradiographic study in mice. *Anat. Rec.* **224**, 1-6 (1989).
90. Hansen-Smith,F.M. & Carlson,B.M. Cellular responses to free grafting of the extensor digitorum longus muscle of the rat. *J. Neurol. Sci.* **41**, 149-173 (1979).
91. Collins,C.A. *et al.* Stem cell function, self-renewal, and behavioral heterogeneity of cells from the adult muscle satellite cell niche. *Cell* **122**, 289-301 (2005).
92. Zhou,S. *et al.* 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 (2001).

93. Asakura,A., Seale,P., Girgis-Gabardo,A. & Rudnicki,M.A. Myogenic specification of side population cells in skeletal muscle. *J. Cell Biol.* **159**, 123-134 (2002).
94. Bachrach,E. *et al.* Systemic delivery of human microdystrophin to regenerating mouse dystrophic muscle by muscle progenitor cells. *Proc. Natl. Acad. Sci. U. S. A* **101**, 3581-3586 (2004).
95. Bachrach,E. *et al.* Muscle engraftment of myogenic progenitor cells following intraarterial transplantation. *Muscle Nerve* (2006).
96. Blume,K.G. & Thomas,E.D. A review of autologous hematopoietic cell transplantation. *Biol. Blood Marrow Transplant.* **6**, 1-12 (2000).
97. Gussoni,E. *et al.* Long-term persistence of donor nuclei in a Duchenne muscular dystrophy patient receiving bone marrow transplantation. *J. Clin. Invest* **110**, 807-814 (2002).
98. Ferrari,G. & Mavilio,F. Myogenic stem cells from the bone marrow: a therapeutic alternative for muscular dystrophy? *Neuromuscul. Disord.* **12 Suppl 1**, S7-10 (2002).
99. LaBarge,M.A. & Blau,H.M. Biological progression from adult bone marrow to mononucleate muscle stem cell to multinucleate muscle fiber in response to injury. *Cell* **111**, 589-601 (2002).
100. Palermo,A.T., LaBarge,M.A., Doyonnas,R., Pomerantz,J. & Blau,H.M. Bone marrow contribution to skeletal muscle: a physiological response to stress. *Dev. Biol.* **279**, 336-344 (2005).

101. Camargo,F.D., Green,R., Capetanaki,Y., Jackson,K.A. & Goodell,M.A. Single hematopoietic stem cells generate skeletal muscle through myeloid intermediates. *Nat. Med.* **9**, 1520-1527 (2003).
102. Corbel,S.Y. *et al.* Contribution of hematopoietic stem cells to skeletal muscle. *Nat. Med.* **9**, 1528-1532 (2003).
103. Polesskaya,A., Seale,P. & Rudnicki,M.A. Wnt signaling induces the myogenic specification of resident CD45+ adult stem cells during muscle regeneration. *Cell* **113**, 841-852 (2003).
104. Sherwood,R.I., Christensen,J.L., Weissman,I.L. & Wagers,A.J. Determinants of skeletal muscle contributions from circulating cells, bone marrow cells, and hematopoietic stem cells. *Stem Cells* **22**, 1292-1304 (2004).
105. Torrente,Y. *et al.* Human circulating AC133(+) stem cells restore dystrophin expression and ameliorate function in dystrophic skeletal muscle. *J. Clin. Invest* **114**, 182-195 (2004).
106. Caplan,A.I. Mesenchymal stem cells. *J. Orthop. Res.* **9**, 641-650 (1991).
107. Prockop,D.J. Marrow stromal cells as stem cells for nonhematopoietic tissues. *Science* **276**, 71-74 (1997).
108. Friedenstein,A.J., Piatetzky-Shapiro,I.I. & Petrakova,K.V. Osteogenesis in transplants of bone marrow cells. *J. Embryol. Exp. Morphol.* **16**, 381-390 (1966).

109. Barry,F.P. & Murphy,J.M. Mesenchymal stem cells: clinical applications and biological characterization. *Int. J. Biochem. Cell Biol.* **36**, 568-584 (2004).
110. Jiang,Y. *et al.* Pluripotency of mesenchymal stem cells derived from adult marrow. *Nature* **418**, 41-49 (2002).
111. Jiang,Y. *et al.* Multipotent progenitor cells can be isolated from postnatal murine bone marrow, muscle, and brain. *Exp. Hematol.* **30**, 896-904 (2002).
112. De Bari,C., Dell'Accio,F., Tylzanowski,P. & Luyten,F.P. Multipotent mesenchymal stem cells from adult human synovial membrane. *Arthritis Rheum.* **44**, 1928-1942 (2001).
113. De Bari,C. *et al.* Skeletal muscle repair by adult human mesenchymal stem cells from synovial membrane. *J. Cell Biol.* **160**, 909-918 (2003).
114. Dezawa,M. *et al.* Bone marrow stromal cells generate muscle cells and repair muscle degeneration. *Science* **309**, 314-317 (2005).
115. Artavanis-Tsakonas,S., Rand,M.D. & Lake,R.J. Notch signaling: cell fate control and signal integration in development. *Science* **284**, 770-776 (1999).
116. Milner,L.A. & Bigas,A. Notch as a mediator of cell fate determination in hematopoiesis: evidence and speculation. *Blood* **93**, 2431-2448 (1999).
117. Conboy,I.M. & Rando,T.A. The regulation of Notch signaling controls satellite cell activation and cell fate determination in postnatal myogenesis. *Dev. Cell* **3**, 397-409 (2002).

118. Huard,J., Cao,B. & Qu-Petersen,Z. Muscle-derived stem cells: potential for muscle regeneration. *Birth Defects Res. C. Embryo. Today* **69**, 230-237 (2003).
119. Cao,B. *et al.* Muscle stem cells differentiate into haematopoietic lineages but retain myogenic potential. *Nat. Cell Biol.* **5**, 640-646 (2003).
120. Issarachai,S., Priestley,G.V., Nakamoto,B. & Papayannopoulou,T. Cells with hemopoietic potential residing in muscle are itinerant bone marrow-derived cells. *Exp. Hematol.* **30**, 366-373 (2002).
121. Asakura,A. & Rudnicki,M.A. Side population cells from diverse adult tissues are capable of in vitro hematopoietic differentiation. *Exp. Hematol.* **30**, 1339-1345 (2002).
122. Jackson,K.A., Mi,T. & Goodell,M.A. Hematopoietic potential of stem cells isolated from murine skeletal muscle. *Proc. Natl. Acad. Sci. U. S. A* **96**, 14482-14486 (1999).
123. Torrente,Y. *et al.* Intraarterial injection of muscle-derived CD34(+)Sca-1(+) stem cells restores dystrophin in mdx mice. *J. Cell Biol.* **152**, 335-348 (2001).
124. Payne,T.R. *et al.* Regeneration of dystrophin-expressing myocytes in the mdx heart by skeletal muscle stem cells. *Gene Ther.* **12**, 1264-1274 (2005).
125. Mueller,G.M., O'Day,T., Watchko,J.F. & Ontell,M. Effect of injecting primary myoblasts versus putative muscle-derived stem cells on mass and force generation in mdx mice. *Hum. Gene Ther.* **13**, 1081-1090 (2002).
126. Deasy,B.M. *et al.* Long-term self-renewal of postnatal muscle-derived stem cells. *Mol. Biol. Cell* **16**, 3323-3333 (2005).

127. Deasy,B.M., Jankowski,R.J. & Huard,J. Muscle-derived stem cells: characterization and potential for cell-mediated therapy. *Blood Cells Mol. Dis.* **27**, 924-933 (2001).
128. Le Grand,F. *et al.* Endothelial cells within embryonic skeletal muscles: a potential source of myogenic progenitors. *Exp. Cell Res.* **301**, 232-241 (2004).
129. Tamaki,T. *et al.* Identification of myogenic-endothelial progenitor cells in the interstitial spaces of skeletal muscle. *J. Cell Biol.* **157**, 571-577 (2002).
130. De Angelis,L. *et al.* 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 (1999).
131. Minasi,M.G. *et al.* The meso-angioblast: a multipotent, self-renewing cell that originates from the dorsal aorta and differentiates into most mesodermal tissues. *Development* **129**, 2773-2783 (2002).
132. Sampaolesi,M. *et al.* Cell therapy of alpha-sarcoglycan null dystrophic mice through intra-arterial delivery of mesoangioblasts. *Science* **301**, 487-492 (2003).
133. Galvez,B.G. *et al.* Complete repair of dystrophic skeletal muscle by mesoangioblasts with enhanced migration ability. *J. Cell Biol.* **174**, 231-243 (2006).
134. Rose,O. *et al.* Expression of M-cadherin protein in myogenic cells during prenatal mouse development and differentiation of embryonic stem cells in culture. *Dev. Dyn.* **201**, 245-259 (1994).

135. Myer,A., Olson,E.N. & Klein,W.H. MyoD cannot compensate for the absence of myogenin during skeletal muscle differentiation in murine embryonic stem cells. *Dev. Biol.* **229**, 340-350 (2001).
136. Sumariwalla,V.M. & Klein,W.H. Similar myogenic functions for myogenin and MRF4 but not MyoD in differentiated murine embryonic stem cells. *Genesis.* **30**, 239-249 (2001).
137. Braun,T. & Arnold,H.H. ES-cells carrying two inactivated myf-5 alleles form skeletal muscle cells: activation of an alternative myf-5-independent differentiation pathway. *Dev. Biol.* **164**, 24-36 (1994).
138. Rohwedel,J. *et al.* Loss of beta1 integrin function results in a retardation of myogenic, but an acceleration of neuronal, differentiation of embryonic stem cells in vitro. *Dev. Biol.* **201**, 167-184 (1998).
139. Weitzer,G., Milner,D.J., Kim,J.U., Bradley,A. & Capetanaki,Y. Cytoskeletal control of myogenesis: a desmin null mutation blocks the myogenic pathway during embryonic stem cell differentiation. *Dev. Biol.* **172**, 422-439 (1995).
140. Rohwedel,J., Horak,V., Hebrok,M., Fuchtbauer,E.M. & Wobus,A.M. M-twist expression inhibits mouse embryonic stem cell-derived myogenic differentiation in vitro. *Exp. Cell Res.* **220**, 92-100 (1995).
141. Prella,K., Wobus,A.M., Krebs,O., Blum,W.F. & Wolf,E. Overexpression of insulin-like growth factor-II in mouse embryonic stem cells promotes myogenic differentiation. *Biochem. Biophys. Res. Commun.* **277**, 631-638 (2000).

142. Dinsmore,J. *et al.* Embryonic stem cells differentiated in vitro as a novel source of cells for transplantation. *Cell Transplant.* **5**, 131-143 (1996).
143. Barberi,T., Willis,L.M., Socci,N.D. & Studer,L. Derivation of multipotent mesenchymal precursors from human embryonic stem cells. *PLoS. Med.* **2**, e161 (2005).
144. Bhagavati,S. & Xu,W. Generation of skeletal muscle from transplanted embryonic stem cells in dystrophic mice. *Biochem. Biophys. Res. Commun.* **333**, 644-649 (2005).
145. Hwang,W.S. *et al.* Patient-specific embryonic stem cells derived from human SCNT blastocysts [Retracted Dec, 2005]. *Science* **308**, 1777-1783 (2005).
146. Benabdallah,B.F., Bouchentouf,M. & Tremblay,J.P. Improved success of myoblast transplantation in mdx mice by blocking the myostatin signal. *Transplantation* **79**, 1696-1702 (2005).
147. Bouchentouf,M., Benabdallah,B.F. & Tremblay,J.P. Myoblast survival enhancement and transplantation success improvement by heat-shock treatment in mdx mice. *Transplantation* **77**, 1349-1356 (2004).
148. Lavasani,M., Lu,A., Peng,H., Cummins,J. & Huard,J. Nerve growth factor improves the muscle regeneration capacity of muscle stem cells in dystrophic muscle. *Hum. Gene Ther.* **17**, 180-192 (2006).
149. Spencer,M.J. & Mellgren,R.L. Overexpression of a calpastatin transgene in mdx muscle reduces dystrophic pathology. *Hum. Mol. Genet.* **11**, 2645-2655 (2002).

150. Shavlakadze,T., White,J., Hoh,J.F., Rosenthal,N. & Grounds,M.D. Targeted expression of insulin-like growth factor-I reduces early myofiber necrosis in dystrophic mdx mice. *Mol. Ther.* **10**, 829-843 (2004).
151. Torrente,Y. *et al.* Identification of a putative pathway for the muscle homing of stem cells in a muscular dystrophy model. *J. Cell Biol.* **162**, 511-520 (2003).
152. Torrente,Y. *et al.* Tumor necrosis factor-alpha (TNF-alpha) stimulates chemotactic response in mouse myogenic cells. *Cell Transplant.* **12**, 91-100 (2003).
153. Horsley,V., Jansen,K.M., Mills,S.T. & Pavlath,G.K. IL-4 acts as a myoblast recruitment factor during mammalian muscle growth. *Cell* **113**, 483-494 (2003).
154. Lafreniere,J.F., Mills,P., Bouchentouf,M. & Tremblay,J.P. Interleukin-4 improves the migration of human myogenic precursor cells in vitro and in vivo. *Exp. Cell Res.* **312**, 1127-1141 (2006).
155. Schulze,M., Belema-Bedada,F., Technau,A. & Braun,T. Mesenchymal stem cells are recruited to striated muscle by NFAT/IL-4-mediated cell fusion. *Genes Dev.* **19**, 1787-1798 (2005).
156. Wekerle,T. & Sykes,M. Mixed chimerism as an approach for the induction of transplantation tolerance. *Transplantation* **68**, 459-467 (1999).
157. Fehr,T. & Sykes,M. Tolerance induction in clinical transplantation. *Transpl. Immunol.* **13**, 117-130 (2004).

158. Schor,A.M., Canfield,A.E., Sutton,A.B., Arciniegas,E. & Allen,T.D. Pericyte differentiation. *Clin. Orthop. Relat Res.* 81-91 (1995).
159. Doherty,M.J. *et al.* Vascular pericytes express osteogenic potential in vitro and in vivo. *J. Bone Miner. Res.* **13**, 828-838 (1998).

Figure Legends

Figure 1. Stem Cell Hierarchy. Progression of stem cells during development from a state of totipotency through to terminal differentiation with intermediate stem cell types highlighting the divergence of cell lineages.

Figure 2. Diversity of Myogenic Stem Cell Populations. The identification of various stem cell populations with myogenic potential along with their known or potential localization within the body. Abbreviations mSP (Muscle Side Population), MDSC (Muscle Derived Stem Cell), HSC (Hematopoietic Stem Cell)

Figure 1

[Click here to download high resolution image](#)

