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Stem Cell Therapies to Treat Muscular Dystrophy

Progress to Date

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Abstract

Muscular dystrophies are heritable, heterogeneous neuromuscular disorders and include Duchenne and Becker muscular dystrophies (DMD and BMD, respectively). DMD patients exhibit progressive muscle weakness and atrophy followed by exhaustion of muscular regenerative capacity, fibrosis, and eventually disruption of the muscle tissue architecture. In-frame mutations in the dystrophin gene lead to expression of a partially functional protein, resulting in the milder BMD. No effective therapies are available at present. Cell-based therapies have been attempted in an effort to promote muscle regeneration, with the hope that the host cells would repopulate the muscle and improve muscle function and pathology. Injection of adult myoblasts has led to the development of new muscle fibers, but several limitations have been identified, such as poor cell survival and limited migratory ability. As an alternative to myoblasts, stem cells were considered preferable for therapeutic applications because of their capacity for self-renewal and differentiation potential. In recent years, encouraging results have been obtained with adult stem cells to treat human diseases such as leukemia, Parkinson's disease, stroke, and muscular dystrophies. Embryonic stem cells (ESCs) can be derived from mammalian embryos in the blastocyst stage, and because they can differentiate into a wide range of specialized cells, they hold potential for use in treating almost all human diseases. Several ongoing studies focus on this possibility, evaluating differentiation of specific cell lines from human ESCs (hESCs) as well as the potential tumorigenicity of hESCs. The most important limitation with using hESCs is that it requires destruction of human blastocysts or embryos. Conversely, adult stem cells have been identified in various tissues, where they serve to maintain, generate, and replace terminally differentiated cells within their specific tissue as the need arises for cell turnover or from tissue injury. Moreover, these cells can participate in regeneration of more than just their specific tissue type. Here we describe multiple types of muscle- and fetal-derived myogenic stem cells, their characterization, and their possible use

in treating muscular dystrophies such as DMD and BMD. We also emphasize that the most promising possibility for the management and therapy of DMD and BMD is a combination of different approaches, such as gene and stem cell therapy.

Muscular dystrophies are heterogeneous neuromuscular disorders of inherited origin, including the X-linked recessive disorders Duchenne or Becker muscular dystrophy (DMD and BMD, respectively).[1] Typically, out-of-frame dystrophin gene mutations lead to a severe reduction or absence of dystrophin in the muscle, resulting in the DMD phenotype, whereas in-frame mutations lead to the expression of a partly functional truncated protein that results in the milder BMD. The frame-shift hypothesis holds true for over 90% of cases and is commonly used both for diagnosis and for differentiating DMD and BMD.[2] However, the frame-shift rule does have some exceptions: in-frame mutations in the gene coding for the crucial domains of the dystrophin protein (the actin- and dystroglycan-binding domains) may result in a DMD phenotype, whereas some out-of-frame mutations are associated with BMD because of different mechanisms, such as compensation by exon skipping or reinitiation codons located in the 5′ end of the gene.[2]

DMD is the most common of the two disorders, affecting one in every 3500 live-born males.[1] In the early phase of the disease, the skeletal muscle of DMD patients is characterized by an ongoing process of degeneration and regeneration that is followed by exhaustion of its regenerative capacity, fibrosis, and eventual disruption of the muscle tissue architecture. Clinically, DMD is characterized by progressive muscle weakness and atrophy, leading patients to be confined to a wheelchair before the age of 12 years and eventually to death from respiratory insufficiency.[1] No effective therapy is available at present. The spectrum of clinical presentation and severity of BMD is much broader than that of DMD. In general, most patients with BMD develop musculoskeletal symptoms at a much slower pace than those with DMD. The natural history of the disease varies considerably depending on the dystrophin gene mutation and the amount of dystrophin protein expressed in muscle. Many BMD patients remain ambulatory until the third or fourth decade or later. Despite the milder skeletal muscle involvement in BMD, the majority of BMD patients still develop dilated cardiomyopathy.[3]

Several studies over the last 2 decades have explored the potential of cell-based therapies to promote muscle regeneration. Initial efforts focused on the transplantation of adult myoblasts, which through cell fusion can lead to the development of new or hybrid muscle fibers.[4,5] However, this approach was hindered by the poor survival and limited migratory ability of injected myoblasts[6] and produced discouraging results in early clinical trials.[7,8] In contrast to myoblasts, stem cells, which are endowed with self-renewal and differentiation potential, may be preferable for therapeutic applications. Stem cells can replenish their numbers for long periods through cell division and, after receiving specific chemical signals, can produce through asymmetric cell division a progeny that can differentiate into multiple cell lineages with specific functions.[1]

In recent years, stem cells have received much attention because of their potential use in cell-based therapies for human diseases such as leukemia,[9] Parkinson's disease,[10] stroke,[11] and muscular dystrophies.[12,13] Embryonic stem cells (ESCs) are derived from mammalian embryos in the blastocyst stage; they have the potential to differentiate into a wide range of specialized cells,[14] leaving the possibility of their use in treating almost all human diseases. Stem cell lines are created by extracting the inner cell mass of blastocysts, with a focus on many investigative paths, including the differentiation of specific cell lines from human ESCs (hESCs) and their potential tumorigenicity. A major objection to hESC-based research arises from ethical considerations, given the requirement for the destruction of human embryos to obtain the cells.[15] Adult stem cells, on the other hand, are intrinsic to various tissues of the body and can maintain, generate, and replace terminally differentiated cells within their specific tissue to meet the needs of cell turnover or tissue injury. Adult stem cells have been found in most tissues, including bone marrow,[16] central nervous system,[17] skin,[18] cardiac muscle,[19] and skeletal muscle.[20] Recently, it has become evident that these adult stem cells can participate in regeneration of tissues other than their resident tissues.[21,22] In this article, we review multiple types of resident and circulating myogenic stem cells, their characterization, and possible use in treating muscular dystrophy. The identification of a stem cell population providing efficient muscle regeneration is critical for the progression of stem cell-based therapies for muscular diseases. We also show that the most promising possibility for the management and therapy of DMD and BMD is a combination of different approaches, such as gene and stem cell therapy.

1. Pluripotent Stem Cells

ESCs are derived from the inner cell mass of blastocyst stage embryos and are characterized by essentially unlimited

self-renewal and pluripotency, which is the ability to differentiate into all adult cell types. Differentiated somatic cells fall into specific classes or types, such as muscle, bone, or neurons, each having unique characteristics and functions. Because of these specializations, the cells are not interchangeable and most lose the ability to divide and create new cells. By contrast, ESCs can be induced to differentiate into virtually any cell type and can proliferate indefinitely in culture. These two characteristics give ESCs enormous potential in medicine and science: they carry the potential to repair damaged organs and replace cells that do not function properly. Since the first report of ESC derivation in mice was published in 1981,[23] various findings have emerged to explain the basic properties of ESCs. Recent advances in our understanding of ESC biology have included the identification of several master regulators of ESC pluripotency and differentiation. However, intensive study of ESC growth conditions has yet to produce a complete picture of the unique transcriptional and epigenetic state that is responsible for pluripotency and self-renewal in ESCs.

Takahashi and Yamanaka[24] found that the expression of four factors (OCT3/4, KLF4, SOX2, and MYC) is sufficient to produce cells similar to ESCs, called induced pluripotent stem cells (iPSCs), from somatic cells. The same factors serve to reprogram human fibroblasts to an ESC-like pluripotent state.[25,26] The most notable aspects of these findings are the small number of factors required to make an ESC-like cell and their conservation between species. However, the most important potential use of hESCs is in transplantation medicine to develop cell replacement therapies. Although the embryonic cells showed an enormous potential for developmental capacity, the ethical and moral concerns surrounding the resulting destruction of the embryo have made the derivation and use of hESCs highly controversial.[15,27]

1.1 Embryonic Stem Cell (ESC) Muscular Differentiation

A key step in establishing the medical potential of hESCs is the development of techniques for their conversion into tissue-restricted precursors suitable for transplantation. Recently, Barberi et al.[28] illustrated the developmental progression of hESCs toward mesenchymal fates through a transient early endodermal/mesodermal stage, by isolating a CD73+ subpopulation from hESCs. Moreover, from among these cells they isolated large numbers of skeletal myoblasts expressing myogenic differentiation factor 1 (MYOD1), which terminally differentiated and formed myotubes expressing myogenin and desmin. They also monitored the survival and integration of these myoblasts after injection into severe combined immunodeficient (SCID)/beige mice; the long-term persistence of donor-derived cells indicated that cells successfully integrated and probably engaged in self-renewal.[28] Sakurai et al.[29] demonstrated the contribution to muscular regeneration of an embryonic paraxial mesodermal-derived cellular subpopulation expressing platelet-derived growth factor receptor-α (PDGFRα) and vascular endothelial growth factor receptor 2 (VEGFR2; also known as KDR). When transplanted into nude mice, these cells were localized in the interstitial zone of muscles, in the area adjacent to the myofibers. This localization is consistent with the idea that ESC-derived mesodermal progenitors differentiate into satellite cells. Next, the investigators determined that the satellite cells derived from the PDGFRα+ population had the same potential as the host cells to grow into a mature myofiber, and that they expressed mesogenin and the transcription factor TBX6 but not PAX3 and PAX7.

Recently, Bhagavati and Xu[30] published a paper concerning the generation of skeletal muscles from transplanted embryonic cells in dystrophic mice. They observed the capacity of the ESCs to proliferate extensively and form skeletal muscle cells. Because the *in vitro* differentiation of ESCs is frequently disorganized, to obtain successful generation of skeletal muscle cells they suggested selective induction of the skeletal muscle lineage in cultures of ESCs by activating a specific developmental pathway. Following this idea, they co-cultured mouse ESCs with a preparation from mouse muscles enriched for myogenic stem cells and precursor cells and injected the treated cells into a mouse model of DMD (mdx) to prove their myogenic capacity. They occasionally obtained clusters of fibers positive for dystrophin and β-dystroglycan, and showed that blood vessels penetrated the entire length of the newly formed muscle fibers. Interestingly, they found no evidence of teratoma formation. This preliminary work could open the door to new insights into the treatment of these types of diseases.[30]

Kamochi et al.[31] reported that differentiated ESCs transfected with insulin-like growth factor II (IGF2) regenerated injured skeletal muscle more effectively than ESCs that were undifferentiated. ESCs genetically engineered to express IGF2 could represent another cell source for cell-based transplantation therapy to repair muscle damaged by injury or myopathy. In their studies with PAX3 and a dystrophic mouse model, Darabi et al.[32] noted that the paraxial mesoderm is not generated efficiently during ESC differentiation induced by conventional protocols, so they used direct induction by PAX3. They isolated a homogeneous population of proliferating myogenic progenitors by sorting for PDGFRα and the absence of FLK1. The cells were injected into a dystrophic mouse, engrafting efficiently without teratoma formation.[32] The

PAX3/ESC-derived population was considered a feasible cell therapy with long-term therapeutic potential, even though further studies are needed to better assess their contribution to the satellite cell compartment and their capacity to undergo self-renewal *in vivo*.[32]

Extracellular cytokines, signaling pathways, and transcriptional molecules are fundamental topics in the regulation of the differentiation of ESCs. Recently, Sakurai et al.[29] demonstrated that the addition of bone morphogenic protein 4 (BMP4) to serum-free ESC cultures can induce primitive streak-like mesodermal cells. They showed that these cells can differentiate both *in vitro* and *in vivo* into osteocytes and osteoblasts without forming teratomas. Alternatively, removal of BMP4 after only 3 days of differentiation and 4 days of exposure to lithium chloride under serum-free conditions induced the differentiation of ESCs into myogenic progenitors. These cells also could express the myogenic markers MYOD1 and myogenic factor 5 (MYF5) and differentiate into mature skeletal muscle cells both *in vivo* and *in vitro*.[33]

1.2 Human ESC Derivation

Normally, hESCs are derived by microsurgical removal of cells from the inner cell mass of a blastocyst stage embryo. To bypass the ethical issues related to embryo destruction, ESCs can be obtained from a single blastomere,[34] although whether these cells can be considered for clinical studies remains unclear. First used in 1996 for the creation of Dolly the sheep,[35] somatic cell nuclear transfer, also referred to as nuclear cloning, is another method used to obtain embryonic cells. It consists of the introduction of a nucleus from an adult donor cell into an enucleated oocyte to generate a cloned embryo.[36] Transferred to the uterus of a female recipient, this embryo has the potential to grow into a clone of the adult donor cell, a process termed 'reproductive cloning.' ESCs derived by nuclear transfer are genetically identical to the donor and thus potentially useful for therapeutic applications; unfortunately, reproductive cloning is a largely inefficient and error-prone process resulting in the failure of most clones during development, both because of the activation of inadequate pathways of early embryonic development[37] and suppression of pathways of differentiation.[38]

1.3 Induced Pluripotent Stem Cells

Recent reports have described the derivation of iPSCs from adult mouse and human cells[24-26,39,40] by the introduction of specific sets of genes encoding transcription factors expressed in undifferentiated ESCs (e.g. *OCT4*, *KLF4*, *SOX2*, and *MYC*) to reprogram the adult cells. Although initial studies indicated that these cells shared characteristics of 'true' ESCs,[41] more detailed work is needed to determine how closely they truly resemble ESCs.

Now that embryonic tissue is no longer required to produce a pluripotent cell, investigators can create tissue-based models of human disease based on cells derived from individual patients. This technology has the potential to herald a new era of patient-specific, cell-based medicine; however, given the oncogenic potential of undifferentiated iPSCs arising from the unsafe reintroduction of these genes,[24] the safety of these cells has to be tested accurately before any therapies are attempted. Continuous overexpression of transcription factors, especially the MYC oncogene, may be associated with tumorigenesis.[24] Even though work has shown that promoters of these viral vectors can be silenced by endogenous gene expression during reprogramming, chimeric mice derived from iPSCs were still more prone to tumor formation.

Following some improvements in iPSC technology, Nakagawa et al.[42] generated pluripotent stem cells without MYC overexpression both from mouse and human fibroblasts, although with lower efficiency. In addition, chimeric mice created from these non-MYC iPSCs did not form tumors at an elevated rate. Recently, Chuang et al.[43] proposed the use of baculoviral systems as a new gene delivery vector for stem cell engineering, and in particular for transgenic expression in hESCs. These vectors can be used for large segments, more than 30 kb, that do not fit into adenoviral or lentiviral vectors and could limit the risk derived from the substantial immunogenicity of adenoviral vectors. Because the most critical step in obtaining iPSCs was the use of viral vectors, which risks activation of endogenous oncogenes, new methods have proven to be effective in safely reprogramming differentiated cells into iPSCs. As recently reviewed by several groups,[44,45] Okita et al.[46] obtained virus vector-free iPSCs by repeated transfections into mouse fibroblasts of expression plasmids containing the complementary DNA of the four pluripotency-associated genes. Alternatively, it is possible to use chemical factors. Shi et al.[47] used BIX 01284, a specific inhibitor of histone methyltransferases, together with two transcription factors to enable mouse fetal neural progenitor reprogramming into iPSCs. Another molecule, the histone deacetylase inhibitor valproic acid, enabled pluripotency reprogramming of human fibroblasts with two pluripotency-associated transcription factors.[48] To decrease the number of virus particles introduced into host cells, other groups have tested self-cleaving peptides,[49] doxycycline-inducible transcription factors,[50] and Cre-recombinase excisable viruses.[51] Finally, Zhou et al.[52] reported the

protein-induced reprogramming of mouse embryonic fibroblasts without genetic modification by using repeated transductions of different recombinant reprogramming proteins together with valproic acid treatment.

2. Adult Stem Cells

The human body has a relatively small number of lineage-committed cells. These adult stem cells can differentiate into a defined range of cell types. For example, hematopoietic stem cells can change into a number of different types of specialized blood cells. In recent years, different types of stem cells have been explored for the treatment of muscular dystrophy, in parallel with new perspectives for different approaches with gene and cell therapies together with all of their advantages and limitations.[53] Stem cell populations suitable for clinical studies were found to derive from multiple regions of the body at various stages of development. Several papers have been published concerning adult muscle side-population cells,[54] bone marrow-derived stem cells,[55] muscle-derived stem cells,[56] mesoangioblasts,[57] blood-[58] and muscle-derived CD133+ stem cells,[59] and pericytes.[60] However, adult stem cells have limitations. First, they are limited in terms of the types of cells into which they can differentiate (e.g. blood stem cells cannot form bone). Second, only some of these cells can migrate through the vasculature,[58,59,61] and efforts have been made to increase the migratory ability of these cells; for example, identifying cell surface markers such as adhesion molecules and appropriate growth factors.[62,63] Moreover, unlike ESCs, adult stem cells do not appear to have the same capacity to grow and divide indefinitely, and they are difficult to isolate as well as to grow in the laboratory.

Because mesoangioblasts have been extensively tested in mouse models of disease, and more recently even in dystrophic dogs,[64] it is reasonable to assume that they are at the top of the list in terms of therapeutic potential. However, the fact that the derivation and use of these cells has not yet been replicated by other labs suggests caution. Because of their ease of accessibility, CD133+ cells isolated from either muscle or blood can be considered suitable, especially as vehicles for gene therapy.[59,65] Despite their potential, satellite cell preparations are not feasible for therapeutic applications because it is impossible to expand them without losing their regenerative potential.

2.1 Mesoangioblasts

First isolated by Cossu and Bianco[66] from dorsal aorta in avian and mammalian species, these progenitor cells are a novel class of stem cells that can differentiate into various mesodermal phenotypes. They express several early endothelial markers, such as FLK1, stem cell antigen 1, CD34, and vascular endothelial cadherin, but do not express late ones, such as von Willebrand factor.[67] Moreover, they lack many of the leukocyte molecules implicated in transmigration.[66] Sampaolesi et al.[68] demonstrated that the injection of wild-type mesoangioblasts morphologically and functionally corrected the dystrophic phenotype of muscles in adult immunocompetent α-sarcoglycan null mice, a model organism for limb-girdle muscular dystrophy. Despite these results, the authors treated the human mesoangioblasts with tumor necrosis factor (TNF)-α and transfected them with either α4 integrin or L-selectin to increase their migration to dystrophic muscles. These cells were injected into the skeletal muscles of immunodepressed dystrophic mice (mdx-SCID) and, under these conditions, the migration was more efficient than for corresponding untreated cells. Treated mesoangioblasts led to massive colonization followed by reconstitution of the majority of α-sarcoglycan-expressing fibers (5-fold more than with control cells).[68] These findings suggested that pre-treatment of mesoangioblasts with TNFα and the presence of α4 integrin are required for the efficient delivery of stem cells to injured muscles in the course of muscular dystrophy. Further work may still be needed to optimize human mesoangioblast migration to skeletal muscle.

Furthermore, Sampaolesi et al.[64] treated some dystrophic dogs with heterologous and autologous approaches using mesoangioblasts transduced with a lentiviral vector expressing human micro-dystrophin. The dogs that received heterologous cells were treated with a combination of immunosuppressive drugs and corticosteroids. In the transplanted dogs, an improvement was observed in muscle function and mobility together with increasing dystrophin expression in the dystrophic animals.[64] Because these positive results could depend on a transient effect of the immunosuppressive drugs,[69] Cossu and Sampaloesi[53] emphasized that the treated dogs expressed dystrophin in the majority of the muscle fibers, which were characterized by normal force contraction and amelioration of defective mobility.

Among the various cells that participate in the architecture of blood vessels, rather than vascular endothelium[70] and mesoangioblasts, pericytes have been found closely encircling endothelial cells in capillaries and microvessels.[71] According to various studies,[72-74] mesoangioblasts could be related to perivascular cells, and muscle-derived stem cells could be related to other multilineage progenitor cells such as mesenchymal stem cells and multipotent adult progenitor cells. In particular, mesoangioblasts and pericytes share various

markers such as alkaline phosphatase, CD49B, CD13, and CD44. Dellavalle et al.[75] found that pericytes sorted from human skeletal muscle by alkaline phosphatase expression regenerated skeletal myofibers in dystrophic immunodeficient mice. Furthermore, Péault and collaborators[76] showed that the pericytes also isolated from non-muscular tissues had myogenic properties. Until now, intra-arterial delivery of mesoangioblasts[66] has appeared to be one of the most promising cell therapy protocols for muscular dystrophy because of a partial but significant restoration of muscle structure and function. However, before clinical trials are performed, many problems need to be solved including efficient delivery to the target tissue.

2.2 Blood- and Muscle-Derived CD133+ Cells

We previously demonstrated the stem cell characteristics of circulating human CD133+ cells and their ability to restore dystrophin expression and eventually regenerate the satellite cell pool in dystrophic SCID/mdx mice after intramuscular and intra-arterial delivery.[59,77] In 2003, Stamm et al.[78] demonstrated that autologous bone marrow-derived CD133+ cells can induce angiogenesis and restore myocardial tissue viability after infarction. Recently, we isolated CD133+ cells from normal and dystrophic biopsies and showed that the muscle-derived CD133+ cells were present in both normal and dystrophic muscles, although pathological muscle biopsies of young patients (aged 5–14 years) contained a higher number of these cells than muscle biopsies of healthy donors matched for age.[65] The expression of CD45 antigen (approximately 4%) in both normal and dystrophic muscle-derived CD133+ cells confirmed their hematopoietic commitment. Moreover, the expression of several muscular markers confirmed by real-time polymerase chain reaction and the cells' ability to differentiate into multinucleated myotubes expressing myosin heavy chains demonstrated their myogenic commitment.[65]

Based on these characteristics, we investigated the safety of the injection of muscle-derived CD133+ cells in DMD muscles and showed that intramuscular transplantation of muscle-derived CD133+ cells appeared to be a safe and feasible procedure.[65] As published by Benchaouir et al.,[59] we compared the behavior of two distinct dystrophic CD133+ cell populations, one from blood and one from skeletal muscle, after transduction with a lentivirus carrying a construct designed to skip exon 51.[79] These cells expressed an exon-skipped version of human dystrophin and participated in muscle regeneration after *in vivo* transplantation into SCID/mdx mice.[59] Furthermore, we demonstrated that the muscle-derived CD133+ stem cells had the potential to differentiate towards both the muscle lineage and the endothelial lineage. In particular, dystrophic muscle-derived CD133+ cells were more efficient than their blood counterparts in ameliorating morphology and restoring skeletal muscle function toward normal in dystrophic murine muscles.[59] Unfortunately, several factors require amelioration before the blood- and muscle-derived CD133+ cells can be considered suitable for cellular therapy. We need to enhance the rate of proliferation of blood-derived CD133+ cells in culture and their storage for repeated treatments; we also have to understand why blood-derived CD133+ cells are less efficient than muscle-derived CD133+ cells in terms of contributions of muscle nuclei. Finally, we must identify a strategy for delivering myogenic cells over the long term to the various sites of sporadic regeneration that occur in muscular dystrophies.

2.3 Satellite Cells

Satellite cells are small progenitor cells that lie between the basement membrane and sarcolemma of muscle fibers. They are normally quiescent and become activated and undergo cell division only after oxidative stress or specific stimuli. The survival and expansion of satellite cells depends on PAX3 and PAX7; in particular, PAX7 is required only in the juvenile period when progenitor cells make the transition into quiescence.[80] Because they possess the ability to differentiate as skeletal myoblasts and subsequently activate myogenic differentiation to form new myofibers, satellite cells could be considered good candidates for cellular therapy. Recently, Montarras et al.[81] isolated a pure population of satellite cells from diaphragm muscle of a PAX3-GFP (green fluorescent protein) knock-in mouse. When transplanted into a dystrophic dog model, these cells restored dystrophin expression 3 weeks post-transplantation, while in irradiated dystrophic mice, they formed a small amount of the satellite cell pool that expressed both PAX7 and PAX3.[81] Notably, these cells showed a remarkably efficient level of muscular regeneration compared with results obtained after the injection of human cells isolated from adult muscle.[82]

Recently, Cerletti et al.[83] isolated a novel subset of skeletal muscle satellite cells having similar properties to adult muscle stem cells by direct cell sorting using a distinct combination of cell-surface markers. To examine the therapeutic potential of skeletal muscle progenitors, they transplanted them into mdx mice and determined that they were responsible for regenerating mature muscle fibers, restoring dystrophin expression, reducing muscle inflammation and fibrosis, and improving physiologic muscle function. Unfortunately, the growth of

freshly isolated satellite cells *in vitro* significantly reduced their *in vivo* myogenic potential, and obtaining a sufficient quantity of these cells was not possible.

Although transplantation of autologous genetically corrected satellite cells into DMD patients could theoretically be the ideal approach to minimizing host immune rejection of donor cells, methodological limitations have not allowed this technique.[84] Nevertheless, the results obtained in the mouse model led to testing of satellite cell/myoblast injection in DMD patients in phase I clinical trials. Donor satellite cells/myoblasts isolated from muscle biopsies and grown in culture[85,86] were injected into the muscles of the DMD patients via multiple injections. Unfortunately, these trials demonstrated that the efficiency of the dystrophin production in muscle fibers of DMD patients is too low (~1%) and identified no functional or clinical improvement in the children.[85,86]

3. Progress to Date

Several questions remain to be answered before any of the previously described cell preparations can be moved into clinical trials, although great advances have been made in the generation of cell populations. Based on their unique characteristics and their *in vivo* skeletal muscle regeneration potential, adult stem cell populations discussed in this review are excellent clinical candidates. As shown previously, mesoangioblasts[66] and blood-derived CD133+ cells[58] can migrate through the vasculature, but most do not. Potential future methods to increase the migratory ability of stem cell populations include the identification of cell surface markers, such as adhesion molecules[62] and appropriate growth factors.[62,87]

Mesoangioblasts serve as a paradigm for widespread distribution, and after treatment with growth factors can efficiently correct the dystrophic phenotype. For now, the intra-arterial injection of mesoangioblasts represents hope for patients affected by various muscular dystrophies.

Satellite cells were among the first cell types used in cell-based therapy of muscular dystrophy. Expanded satellite cells or myoblasts were isolated from wild-type mice and intramuscularly injected into mdx mice;[88,89] unfortunately, myoblast transplantation proved to be an inefficient technique because of the low efficiency of dystrophin production in muscle fibers of DMD patients and no functional or clinical improvement in children.[86]

ESC-derived progenitors have excellent self-renewal and regenerative potential, but the research on these cells is in the initial stages. Recently, Jaenisch and Young[90] reported that adult cells can behave as unipotent and multipotent stem cells, such as hematopoietic stem cells, even if totipotent and pluripotent cells are restricted to the early embryo.

Although possessing decreased developmental potential, the nuclei of most adult cells maintain the nuclear plasticity required to reset to an embryonic state. It is possible to enhance this process by exposing the oocyte to specific factors through nuclear transfer or exposing the cells to pluripotent cell-specific factors by driving overexpression of defined transcription factors. With publication of the work of Takahashi and Yamanaka[24] concerning the reprogramming of cells to a state of pluripotency, a new frontier in the field of regenerative medicine opened. In fact, for the first time, reprogramming of cells provided a realistic way to generate sufficient numbers of patient-specific pluripotent stem cells.

On this basis, iPSCs have been efficiently used in murine models of sickle cell anemia[91] and Parkinson's disease.[92] However, even if these cells prove suitable for cell therapy, it has yet to be demonstrated that human iPSCs can be generated without the introduction of DNA into the genome to enhance the efficiency of manipulation of human iPSCs and the capacity to obtain any desired cell type. In spite of the genomic integrity and the normal karyotype of human and murine iPSCs, a few abnormalities have been found in the chromosomes.[93] In hepatocyte and stomach cell-derived iPSCs no common insertion sites are present, but it is not possible to affirm that genetic changes might or might not occur during reprogramming processes with retroviral and lentiviral vectors.[94]

Before there are any possible future clinical applications of hESCs, their engraftment must be improved upon. At the same time, it is necessary to develop a protocol to obtain similar populations of muscle precursors from hESCs. Looking toward clinical applications, adult stem cells purified from DMD patients could be engineered *ex vivo* and reinjected into the initial donor intra-arterially to allow distribution of the cells to the whole body musculature. In this way, it could be possible to treat severely affected patients who have reduced body mass.

One of the most important problems to solve for future clinical therapies is the amelioration of safety procedures for genetic modifications. A reliable method for DMD therapy seems to be the exon-skipping approach mediated by antisense oligonucleotides or molecules such as PTC124.

The use and practical applications of ESCs in cell replacement therapy are still in the preliminary stages, and require more investigation and clinical trials before they can be accepted as ideal for the treatment of neuromuscular diseases. Nevertheless, the rapid increase in experimental findings reinforces the hope that ESCs will prove to be a versatile source of

renewable cells for application in cell replacement therapy. There is sufficient optimism among researchers that ESC-based therapies may offer a reliable and cost-effective therapeutic substitute for treatment of severe degenerative disorders in the near future.[15] The therapeutic potential of adult stem cells appears to be much lower than that of ESCs, and the large majority of adult stem cells are difficult to isolate and hard to propagate in culture.[38] They can be genetically manipulated only through the introduction of retroviral transgenes,[59] which could cause overexpression of genes at variable levels and cancer.[95] Moreover, the differentiation potential of adult stem cells seemed to be restricted, although several groups have demonstrated that bone marrow-derived cells could enter sites of muscle regeneration and also contribute myonuclei to the newly formed muscle fibers.[96,97] Conversely, resident muscular cells could reconstitute the bone marrow and via this route re-enter and contribute to the regeneration of skeletal muscle.[98,99] Recently, Jiang et al.[100] demonstrated the pluripotency of mesenchymal stem cells derived from adult marrow, which showed their differentiation into cells of all three germ layers both *in vitro* and *in vivo* after being injected into blastocysts. Unfortunately, they did not assess the ability of these cells to correct a disease phenotype into either humans or mouse animal models.

ESCs can become any type of cell through the use of specific culture conditions or genetic manipulation. To avoid the ethical and practical limitations of therapeutic cloning mentioned previously, it would be useful to reprogram somatic cells directly into ESCs without the use of oocytes. Achieving this goal requires a deep understanding of the role of several molecular factors in establishing and maintaining pluripotency. An example is OCT4[101] and OCT4-null embryos, which cannot form a pluripotent inner cell mass; consequently, their development is arrested. To circumvent the need for human oocytes, it may be possible to modify the expression of OCT4 and its related genes in somatic cells to reprogram their nuclei to an embryonic state.

4. Conclusions

Several studies have exploited the potential of cell-based therapies to promote muscle regeneration with the hope that the host cells will fuse and repopulate the muscle, thus improving muscle function and pathology. However, many obstacles have appeared in the course of this research. First, myoblast transplantation was hindered by the poor survival and limited migratory ability of transplanted cells. In two papers, Tremblay and co-workers[102,103] demonstrated that DMD patients injected with myogenic cells obtained from skeletal muscle biopsies of normal donors or from their parents induced the expression of donor-derived dystrophin. However, this expression was restricted to the sites of muscle precursor cell injection and sometimes resulted in cases of acute immune rejection.[104,105] Other adult stem cells, such as side-population cells,[54] muscle-derived stem cells,[56] and satellite cells,[83] have regenerative potential. Although the large majority of these cells have produced significant engraftment when transplanted into dystrophic mice, only the mesoangioblasts and CD133+ cells aided functional recovery of treated muscle.[59,68]

ESC-based therapy would have many advantages: it could allow the transplantation of a more primitive cell with greater replicative potential, and patient-specific ESCs could be induced from adult somatic cells. The development of several ESC-based technologies, such as genetic manipulation tools, has accelerated the application of these cells in clinical therapy, even if ethical, logistic, and economic concerns require attention. In particular, the ability to generate cells in culture with *in vivo* muscle regenerative potential and systemically transfer them to recipients is an important step towards the therapeutic application of ESC-derived cells. Unfortunately, there still is no reproducible method for generating ESC-derived myogenic progenitors for skeletal muscle regeneration. In addition, the derivation of an ESC-derived myogenic population with proliferative and regenerative potential has not been accomplished. Only two papers have described some evidence for engraftment following transplantation of an ESC-derived population, but they were limited to qualitative detection of donor-derived cells in recipient muscle.[30,31]

The PAX3 ESC-derived population exhibited good potential for skeletal muscle regeneration, but their capacity to replenish the satellite cell niche should be studied.[32] The revolutionary work of Takahashi et al.[106] allowed the use of the patient's own reprogrammed cells to eliminate the main risks of allogeneic transplantation, specifically immune rejection of the grafted tissue. The combination of iPSC technology with the lineage-specific reprogramming using PAX3 could be ideal for the generation of patient-specific myogenic progenitors for musculoskeletal diseases.[107] However, before this goal can be accomplished, many problems must be overcome, such as development of a protocol to obtain similar populations of muscle precursors from hESCs, optimization of engraftment so that the satellite cell compartment can be significantly repopulated, and activation of the PAX3 regulatory cascade possibly without genetic manipulation.[107] Although their

potential is enormous, iPSCs frequently give rise to teratomas if transplanted into nude mice.

In recent years, several groups have published innovative work in which adult stem cells and ESCs were genetically modified by *ex vivo* introduction of corrective genes and then transplanted into recipient dystrophic animal models.[31,59] However, evidence is lacking about the potential for migration through the vessels after systemic administration of ESCs or iPSCs.

According to current findings, the most promising possibility for the therapy of DMD is a combination of different approaches to obtain the beneficial effects of multiple strategies combined into a single approach, such as cellular therapy associated with gene therapy or pharmacologic treatments. One of the most used approaches is autologous transfer, in which a patient's own cells are genetically corrected *in vitro* using lentiviral vectors and then reimplanted to allow the re-expression of functional dystrophin protein. The 'exon-skipping' approach is an alternative strategy for gene therapy and is achieved using antisense oligonucleotides that hybridize with the donor and/or acceptor sites of the mutated exon, causing its exclusion from the intact transcript. On the other side, allogeneic transfer involves injecting cells isolated from an individual with functional dystrophin into the patient, which can lead to problems associated with immunorejection or requiring administration of specific immunosuppressive drugs.

Several problems exist with these approaches and must be overcome, including the low efficiency with which stem cells enter muscle via the vasculature, difficulties with enhancing proliferation of stem cells in culture, time required in culture for autologous cells prior to implantation back into the patient, longevity of the transplanted muscle nuclei *in vivo*, and development of tumors as a consequence of hazardous integration of the provirus. Success with the clinical application of adult stem cells or ESCs will ultimately require the capacity for large-scale production of a desired cell type with appropriate functionality, identification of the optimal number of cells for transplant, a modification to less-invasive delivery systems, and techniques to label cells for transplant and subsequent tracking of cell fate.

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References

1. Emery AE. The muscular dystrophies. Lancet 2002; 359: 687-95
2. Manzur AY, Muntoni F. Diagnosis and new treatments in muscular dystrophies. J Neurol Neurosurg Psychiatry 2009; 80: 706-14
3. Nigro G, Politano L, Passamano L, et al. Cardiac treatment in neuromuscular diseases. Acta Myol 2006; 25 (3): 119-23
4. Brussee V, Tardif F, Roy B, et al. Successful myoblast transplantation in fibrotic muscles: no increased impairment by the connective tissue. Transplantation 2002; 67: 1618-22
5. Gussoni E, Blau HM, Kunkel LM. The fate of individual myoblasts after transplantation into muscles of DMD patients. Nat Med 1997; 3: 970-7
6. Qu Z, Balkir L, van Deutekom JC, et al. Development of approaches to improve cell survival in myoblast transfer therapy. J Cell Biol 1998; 142: 1267
7. Partridge T, Lu QL, Morris G, et al. Is myoblast transplantation effective? Nat Med 1998; 4: 1208-9
8. Tremblay JP, Malouin F, Roy R, et al. Results of a triple blind clinical study of myoblast transplantations without immunosuppressive treatment in young boys with Duchenne muscular dystrophy. Cell Transplant 1993; 2: 99-112
9. Owonikoko T, Agha M, Balassanian R, et al. Gemtuzumab therapy for isolated extramedullary AML relapse following allogeneic stem-cell transplant. Nat Clin Pract Oncol 2007; 4: 491-5
10. Singh N, Pillay V, Choonara YE. Advances in the treatment of Parkinson's disease. Prog Neurobiol 2007; 81: 29-44
11. Gilman S. Time course and outcome of recovery from stroke: relevance to stem cell treatment. Exp Neurol 2006; 199: 37-41
12. Nowak KJ, Davies KE. Duchenne muscular dystrophy and dystrophin: pathogenesis and opportunities for treatment. EMBO Rep 2004; 5: 872-6
13. Endo T. Stem cells and plasticity of skeletal muscle cell differentiation: potential application to cell therapy for degenerative muscular diseases. Regen Med 2007; 2: 243-56
14. Chen S, Borowiak M, Fox JL. A small molecule that directs differentiation of human ESCs into the pancreatic lineage. Nat Chem Biol 2009; 5 (4): 258-65
15. Jung KW. Perspectives on human stem cell research. J Cell Physiol 2009; 220 (3): 535-7
16. Jiang Y, Vaessen B, Lenvik T, et al. Multipotent progenitor cells can be isolated from postnatal murine bone marrow, muscle, and brain. Exp Hematol 2002; 30: 896-904
17. Mitchell KE, Weiss ML, Mitchell BM, et al. Matrix cells from Wharton's jelly form neurons and glia. Stem Cells 2003; 21: 50-60
18. Toma JG, Akhavan M, Fernandes KJ, et al. Isolation of multipotent adult stem cells from the dermis of mammalian skin. Nat Cell Biol 2001; 3: 778-84
19. Zuk PA, Zhu M, Mizuno H, et al. Multilineage cells from human adipose tissue: implications for cell-based therapies. Tissue Eng 2001; 7: 211-28
20. Bischoff R. The satellite cell and muscle regeneration. In: Engel AG, Franszini-Armstrong C. Myogenesis. New York: McGraw-Hill, 2007: 97-118
21. Grounds MD, White JD, Rosenthal N, et al. The role of stem cells in skeletal and cardiac muscle repair. J Histochem Cytochem 2002; 50 (5): 589-610
22. Kataoka K, Medina RJ, Kageyama T, et al. Participation of adult mouse bone marrow cells in reconstitution of skin. Am J Pathol 2003; 163 (4): 1227-31
23. Evans M, Kaufman M. Establishment in culture of pluripotential cells from mouse embryos. Nature 1981; 292:154-156
24. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 2006; 126 (4): 663-76
25. Park IH, Zhao R, West JA. Reprogramming of human somatic cells to pluripotency with defined factors. Nature 2008; 451 (7175): 141-6

26. Keller G, Snodgrass HR. Human embryonic stem cells: the future is now. Nat Med 1999; 5 (2): 151-2
27. de Wert G, Mummery C. Human embryonic stem cells: research, ethics and policy. Hum Reprod 2003; 18 (4): 672-82
28. Barberi T, Bradbury M, Dincer Z, et al. Derivation of engraftable skeletal myoblasts from human embryonic stem cells. Nat Med 2007; 13 (5): 642-8
29. Sakurai H, Inami Y, Tamamura Y, et al. Bidirectional induction toward paraxial mesodermal derivatives from mouse ES cells in chemically defined medium. Stem Cell Res 2009; 3: 157-69
30. Bhagavati S, Xu W. Generation of skeletal muscle from transplanted embryonic stem cells in dystrophic mice. Biochem Biophys Res Commun 2005; 333 (2): 644-9
31. Kamochi H, Kurokawa MS, Yoshikawa H, et al. Transplantation of myocyte precursors derived from embryonic stem cells transfected with IGFII gene in a mouse model of muscle injury. Transplantation 2006; 82 (4): 516-26
32. Darabi R, Gehlbach K, Bachoo RM, et al. Functional skeletal muscle regeneration from differentiating embryonic stem cells. Nat Med 2008; 14 (2): 134-43
33. Chargé SB, Rudnicki MA. Cellular and molecular regulation of muscle regeneration. Physiol Rev 2004; 84 (1): 209-38
34. Teramura T, Takehara T, Kishi N, et al. A mouse and embryonic stem cell derived from a single embryo. Cloning Stem Cells 2007; 9 (4): 485-94
35. Campbell KH, McWhir J, Ritchie WA, et al. Sheep cloned by nuclear transfer from a cultured cell line. Nature 1996; 380 (6569): 64-6
36. Wilmut I, Beaujean N, de Sousa PA, et al. Somatic cell nuclear transfer. Nature 2002; 419 (6907): 583-6
37. Byrne JA, Pedersen DA, Clepper LL, et al. Producing primate embryonic stem cells by somatic cell nuclear transfer. Nature 2007; 450 (7169): 497-502
38. Rideout 3rd WM, Hochedlinger K, Kyba M, et al. Correction of a genetic defect by nuclear transplantation and combined cell and gene therapy. Cell 2002; 109 (1): 17-27
39. Kim JB, Zaehres H, Araúzo-Bravo MJ, et al. Generation of induced pluripotent stem cells from neural stem cells. Nat Protoc 2009; 4 (10): 1464-70
40. Chang CW, Lai YS, Pawlik KM, et al. Polycistronic lentiviral vector for "hit and run" reprogramming of adult skin fibroblasts to induced pluripotent stem cells. Stem Cells 2009; 27 (5): 1042-9
41. Liu SV. iPS cells: a more critical review. Stem Cells Dev 2008; 17 (3): 391-7
42. Nakagawa M, Koyanagi M, Tanabe K, et al. Generation of induced pluripotent stem cells without Myc from mouse and human fibroblasts. Nat Biotechnol 2008; 26 (1): 101-6
43. Chuang CK, Sung LY, Hwang SM, et al. Baculovirus as a new gene delivery vector for stem cell engineering and bone tissue engineering. Gene Ther 2007; 14 (19): 1417-24
44. Amabile G, Meissner A. Induced pluripotent stem cells: current progress and potential for regenerative medicine. Trends Mol Med 2009; 15 (2): 59-68
45. Rolletschek A, Wobus AM. Induced human pluripotent stem cells: promises and open questions. Biol Chem 2009; 390: 845-9
46. Okita K, Nakagawa M, Hyenjong H, et al. Generation of mouse induced pluripotent stem cells without viral vectors. Science 2008; 322: 949-53
47. Shi Y, Desponts C, Do JT, et al. Induction of pluripotent stem cells from mouse embryonic fibroblasts by Oct-4 and Klf4 with small-molecule compounds. Cell Stem Cell 2008; 3: 568-74
48. Huangfu D, Osafune K, Maehr R, et al. Induction of pluripotent stem cells from primary human fibroblasts with only Oct4 and Sox2. Nat Biotec 2008; 26: 1269-75
49. Carey BW, Markoulaki S, Hanna J, et al. Reprogramming of murine and human somatic cells using a single polycistronic vector. PNAS 2009; 106: 157-62
50. Woltjen K, Micheal IP, Mohseni P, et al. Piggyback transposition reprograms fibroblasts to induced pluripotent stem cells. Nature 2009; 458: 266-70
51. Soldner F, Hockemeyer D, Beard C, et al. Parkinson's disease patient-derived induced pluripotent stem cells free of viral reprogramming factors. Cell 2009; 964-77
52. Zhou H, Wu S, Joo JY, et al. Generation of induced pluripotent stem cells using recombinant proteins. Cell Stem Cell 2009; 4 (5): 381-4
53. Cossu G, Sampaolesi M. New therapies for Duchenne muscular dystrophy: challenges, prospects and clinical trials. Trends Mol Med 2007; 13 (12): 520-6
54. Asakura A, Rudnicki MA. Side population cells from diverse adult tissues are capable of in vitro hematopoietic differentiation. Exp Hematol 2002; 30: 1339-45
55. Dezawa M, Ishikawa H, Itokazu Y, et al. Bone marrow stromal cells generate muscle cells and repair muscle degeneration. Science 2005; 309: 314-7
56. Qu-Petersen Z, Deasy B, Jankowski R, et al. Identification of a novel population of muscle stem cells in mice: potential for muscle regeneration. J Cell Biol 2002; 157: 851-64
57. Palumbo R, Sampaolesi M, De Marchis F, et al. Extracellular HMGB1, a signal of tissue damage, induces mesoangioblasts migration and proliferation. J Cell Biol 2004; 164 (3): 441-9
58. Gavina M, Belicchi M, Rossi B, et al. VCAM-1 expression on dystrophic muscle vessels has a critical role in the recruitment of human blood-derived CD133+ stem cells after intra-arterial transplantation. Blood 2006; 108: 2857-66
59. Benchaouir R, Meregalli M, Farini A, et al. Restoration of human dystrophin following transplantation of exon-skipping-engineered DMD patient stem cells into dystrophic mice. Cell Stem Cell 2007; 13: 646-57
60. Doherty MJ, Ashton BA, Walsh S, et al. Vascular pericytes express osteogenic potential in vitro and in vivo. J Bone Miner Res 1998; 13: 28-38
61. Galvez BG, Sampaolesi M, Brunelli S, et al. Complete repair of dystrophic skeletal muscle by mesoangioblasts with enhanced migration ability. J Cell Biol 2006; 174 (2): 231-43
62. Torrente Y, El Fahime E, Caron NJ, et al. Tumor necrosis factor-alpha. [TNF-alpha] stimulates chemotactic response in mouse myogenic cells. Cell Transplant 2003; 12: 91-100
63. Lafreniere JF, Mills P, Bouchentouf M, et al. Interlukin-4 improves the migration of human myogenic precursor cells in vitro and in vivo. Exp Cell Res 2006; 312 (7): 1127-41
64. Sampaolesi M, Blot S, D'Antona G, et al. Mesoangioblast stem cells ameliorate muscle function in dystrophic dogs. Nature 2006; 444 (7119): 574-9
65. Torrente Y, Belicchi M, Marchesi C, et al. Autologous transplantation of muscle-derived CD133+ stem cells in Duchenne muscle patients. Cell Transplant 2007; 16 (6): 563-77
66. Cossu G, Bianco P. Mesoangioblasts-vascular progenitors for extravascular mesodermal tissues. Curr Opin Genet Dev 2003; 13 (5): 537-42
67. Tagliafico E, Brunelli S, Bergamaschi A. TGFbeta/BMP activate the smooth muscle/bone differentiation programs in mesoangioblasts. J Cell Sci 2004; 117 (Pt 19): 4377-88
68. Sampaolesi M, Torrente Y, Innocenzi A, et al. Cell therapy of alpha-sarcoglycan null dystrophic mice through intra-arterial delivery of mesoangioblasts. Science 2003; 301 (5632): 487-92
69. Davies KE, Grounds MD. Treating muscular dystrophy with stem cells? Cell 2006 29; 127 (7): 1304-6
70. Zambidis ET, Oberlin E, Tavian M, et al. Blood-forming endothelium in human ontogeny: lessons from in utero development and embryonic stem cell culture. Trends Cardiovasc Med 2006; 16: 95-101
71. Andreeva ER, Pugach IM, Gordon D, et al. Continuous subendothelial network formed by pericyte-like cells in human vascular bed. Tissue Cell 1998; 30: 127-35

72. DeAngelis L, Berghella L, Coletta M, 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 1999; 147: 869-78
73. Caplan AI. Mesenchymal stem cells. J Orthop Res 1991; 9: 641-50
74. Pittenger MF, MacKay AM, Beck SC, et al. Multilineage potential of adult human mesenchymal stem cells. Science 1999; 284: 143-7
75. Dellavalle A, Sampaolesi M, Tonlorenzi R, et al. Pericytes of human skeletal muscle are myogenic precursors distinct from satellite cells. Nat Cell Biol 2007; 9: 255-67
76. Crisan M, Yap S, Casteilla L, et al. A perivascular origin for mesenchymal stem cells in multiple human organs. Cell Stem Cell 2008; 3: 301-13
77. Torrente Y, Belicchi M, Sampaolesi M, et al. Human circulating AC133[+] stem cells restore dystrophin expression and ameliorate function in dystrophic skeletal muscle. J Clin Invest 2004; 114 (2): 182-95
78. Stamm C, Friehs I, Choi YH, et al. Cytosolic calcium in the ischemic rabbit heart: assessment by pH- and temperature-adjusted rhod-2 spectrofluorometry. Cardiovasc Res 2003; 59 (3): 695-704
79. Goyenvalle A, Vulin A, Fougerousse F, et al. Rescue of dystrophic muscle through U7 snRNA-mediated exon skipping. Science 2004; 306 (5702): 1796-9
80. Lepper C, Conway SJ, Fan CM. Adult satellite cells and embryonic muscle progenitors have distinct genetic requirements. Nature 2009; 460: 627-31
81. Montarras D, Morgan J, Collins C, et al. Direct isolation of satellite cells for skeletal muscle regeneration. Science 2005; 309: 2064-7
82. Morgan JE, Fletcher RM, Partridge TA. Yields of muscle from myogenic cells implanted into young and old mdx hosts. Muscle Nerve 1996; 19: 132-9
83. Cerletti M, Jurga S, Witczak CA, et al. Highly efficient, functional engraftment of skeletal muscle stem cells in dystrophic muscles. Cell 2008; 134: 37-47
84. Price FD, Kuroda K, Rudnicki MA. Stem cell based therapies to treat muscular dystrophy. Biochim Biophys Acta 2007; 1772 (2): 272-83
85. Seale P, Ishibashi J, Scime R, et al. Pax7 is necessary and sufficient for the myogenic specification of CD45+: Sca1+ stem cells from injured muscle. PLoS Biol 2003; 2: E130
86. Péault B, Rudnicki M, Torrente Y, et al. Stem and progenitor cells in skeletal muscle development, maintenance, and therapy. Mol Ther 2007; 15 (5): 867-77
87. Horsley V, Jansen KM, Mills ST, et al. IL-4 acts as a myoblast recruitment factor during mammalian muscle growth. Cell 2003; 113: 483-94
88. Conway SJ, Henderson DJ, Copp AJ. Pax3 is required for cardiac neural crest migration in the mouse: evidence from the splotch (Sp2H) mutant. Development 1997; 124: 505-14
89. Tremblay P, Dietrich S, Mericskay M, et al. A crucial role for Pax3 in the development of the hypaxial musculature and the long-range migration of muscle precursors. Dev Biol 1998; 203: 49-61
90. Jaenisch R, Young R. Stem cells, the molecular circuitry of pluripotency and nuclear reprogramming. Cell 2008; 132 (4): 567-82
91. Hanna J, Wernig M, Markoulaki S, et al. Treatment of sickle cell anemia mouse model with iPS cells generated from autologous skin. Science 2007; 318 (5858): 1920-3
92. Wernig M, Lengner CJ, Hanna J, et al. A drug-inducible transgenic system for direct reprogramming of multiple somatic cell types. Nat Biotechnol 2008; 26 (8): 916-24
93. Aasen T, Raya A, Barrero MJ, et al. Efficient and rapid generation of induced pluripotent stem cells from human keratinocytes. Nat Biotechnol 2008; 26 (11): 1276-84
94. Aoi T, Yae K, Nakagawa M, et al. Generation of pluripotent stem cells from adult mouse liver and stomach cells. Science 2008; 321 (5889): 699-702. Erratum in: Science 2008; 321 (5889): 641
95. Check E. Gene regulation: RNA to the rescue? Nature 2003; 425 (6953): 10-2
96. Ferrari G, Cusella-De Angelis G, Coletta M, et al. Muscle regeneration by bone marrow-derived myogenic progenitors. Science 1998; 279 (5356): 1528-30. Erratum in: Science 1998; 281 (5379): 923
97. Ferrari G, Stornaiuolo A, Mavilio F. Failure to correct murine muscular dystrophy. Nature 2001; 411 (6841): 1014-5
98. Gussoni E, Pavlath GK, Lanctot AM, et al. Normal dystrophin transcripts detected in Duchenne muscular dystrophy patients after myoblast transplantation. Nature 1992; 356: 435-8
99. Jackson KA, Snyder DS, Goodell MA. Skeletal muscle fiber-specific green autofluorescence: potential for stem cell engraftment artefacts. Stem Cells 2004; 22 (2): 180-7
100. Jiang Y, Jahagirdar BN, Reinhardt RL, et al. Pluripotency of mesenchymal stem cells derived from adult marrow. Nature 2002; 418 (6893): 41-9
101. Niwa H, Masui S, Chambers I, et al. Phenotypic complementation establishes requirements for specific POU domain and generic transactivation function of Oct-3/4 in embryonic stem cells. Mol Cell Biol 2002; 22 (5): 1526-36
102. Law PK, Goodwin TG, Li HJ. Histoincompatible myoblast injection improves muscle structure and function of dystrophic mice. Transplant Proc 1998; 20: 1114-9
103. Law PK, Goodwin TG, Wang MG. Normal myoblast injections provide genetic treatment for murine dystrophy. Muscle Nerve 1988; 11: 525-33
104. Skuk D, Goulet M, Roy B, et al. Dystrophin expression in muscles of Duchenne muscular dystrophy patients after high-density injections of normal myogenic cells. J Neuropathol Exp Neurol 2006; 65 (4): 371-86
105. Skuk D, Roy B, Goulet M, et al. Dystrophin expression in myofibers of Duchenne muscular dystrophy patients following intramuscular injections of normal myogenic cells. Mol Ther 2004; 9 (3): 475-82
106. Takahashi K, Okita K, Nakagawa M, et al. Induction of pluripotent stem cells from fibroblast cultures. Nat Protoc 2007; 2 (12): 3081-9
107. Darabi R, Santos FN, Perlingeiro RC. The therapeutic potential of embryonic and adult stem cells for skeletal muscle regeneration. Stem Cell Rev 2008; 4 (3): 217-25

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