Stem cells to treat muscular dystrophies – Where are we?

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Abstract

The muscular dystrophies are inherited disorders characterised by progressive muscle wasting and weakness. Stem cell therapy is considered to be one of the most promising strategies for treating muscular dystrophies. In this review, we first examine the evidence that a stem cell could be used to treat muscular dystrophies, and then discuss the criteria that an ideal stem cell should meet. We also highlight the importance of standard operation procedures to be followed for ensuring the consistent and reproducible efficacy of a particular stem cell. While at the moment the scientific community is looking for an ideal stem cell to treat muscular dystrophies, it is clear that in order for this field to benefit from therapeutic stem cell applications, additional careful investigations are required.

Keywords: Stem cell; Muscular dystrophies; Myoblast; Muscle-derived stem cell; Mesoangioblast; AC133+ cell; ES cell; Induced pluripotent stem cell; Satellite cell

1. Stem cells and skeletal muscle

A stem cell is defined as a cell that can self-renew and differentiate into one or more specialized cell types [1,2]. A stem cell may be (i) pluripotent, able to give rise to the endodermal, ectodermal, and mesodermal lineages, e.g. embryonic stem cells; (ii) multipotent, able to give rise to all cells in a particular lineage, e.g. the haematopoietic stem cell and neural stem cell; or (iii) unipotent, being able to give rise to only one cell type, e.g. keratinocytes [3].

Skeletal muscle, a largely post-mitotic tissue, is capable of growth, repair and regeneration. This is mediated by satellite cells, at least some of which are muscle stem cells, capable of regenerating muscle fibres and reconstituting the satellite cell pool with functional satellite cells [4]. Satellite cells are quiescent cells, located under the basal lamina of muscle fibres [5–8] that can be activated to give a pool of progeny muscle precursor cells, or myoblasts [9–11]. Myoblasts can be expanded in tissue culture and contribute to limited muscle regeneration following direct intra-muscular transplantation [12–18].

2. Stem cell therapy for muscular dystrophies

Conceptually, stem cell therapy should be an ideal treatment for recessive muscular dystrophies in which muscle fibres are lost as a result of a genetic mutation [19–22]. The majority of muscular dystrophies are caused by mutations in genes coding for proteins either associated with the muscle cell membrane, such as the dystrophin–glycoprotein complex (DGC) [23–25] or the extracellular matrix, such as laminin 2 [26,27] and collagen VI [28], or the nuclear membrane such as lamin A/C or emerin [29–31]. A lack of these proteins, e.g. dystrophin in DMD, causes mechanical fragility and contraction-induced damage of the muscle fibres [32,33], which leads to infiltration of inflammatory cells into the muscle, as well as activation of satellite cells [34] that take part in muscle regeneration. A prominent feature of dystrophic muscle is cycles of muscle fibre degeneration and regeneration, until, in the late stages of the disease, the endogenous satellite cell pool becomes exhausted and muscle fibres are replaced by fibrotic and adipose tissues, compromising normal muscle function [35]. In addition, the loss of muscle fibres interferes with the ability of therapies based on RNA repair, such as exon skipping, to be effective.
To either prevent or break the cycles of degeneration and regeneration in DMD, dystrophin must be restored to the muscle fibre membrane. This could be achieved by stem cell treatment, with donor cells either being taken from a normal individual (allograft), or from the patient himself, and engineered to express dystrophin (autograft). We will take DMD as a model of a muscular dystrophy and critically examine the evidence that it can be ameliorated by stem cell treatment.

3. Assessment of the contribution of donor cells to regenerated muscle fibres and reconstitution of the satellite cell pool

In order to assess the efficacy of a particular stem cell type to treat dystrophic muscle, markers of either muscle fibres, or satellite cells, of donor origin are needed. When grafting cells from a normal donor into a dystrophin-deficient host, dystrophin is the obvious marker of muscle fibres of donor origin. However, the presence of revertant muscle fibres in DMD patients and animal models of DMD [36,37], may give rise to false positive results, particularly in the mdx mouse, in which the number of revertant fibres increases with age [37]. A second marker is therefore used to confirm that the muscle fibre is indeed of donor origin. When grafting donor cells from the genetically-modified 3F-nLacZ-2E mouse, in which muscle fibre nuclei express beta-galactosidase, myonuclei of donor origin express beta-galactosidase [4,38]. Alternatively, cells from donor mice expressing a marker gene, e.g. GFP [39], or cells retrovirally-marked with a marker gene [40,41] may be used to detect cells, including muscle fibres, of donor origin. However, consideration must be paid to limitations of genetic markers, for example, promoters may switch off in vivo, or donor-derived proteins may spread along a multinucleated fibre away from the myonuclei that made them [42].

When grafting human cells into host mice, human specific dystrophin or spectrin antibodies may be used to detect muscle fibres of donor origin [43,44] and antibodies that detect human but not mouse nuclei (e.g. human lamin a/c) may be used to confirm that the muscle fibres are indeed of human origin [43–45].

The Myf5nLacZ/+ mouse has been used to determine if donor mouse cells have given rise to satellite cells [4,46], but in the case of cells of human origin, a human-specific antibody (e.g. human lamin a/c) has to be combined with a satellite-cell specific antibody (e.g. Pax7).

As well as forming large amounts of muscle, contribution to functional satellite cells would be required to treat a chronic condition such as DMD. Unless dystrophin is expressed along the length of each fibre in every muscle of the body after the initial stem cell application, either entire fibres, or dystrophin-negative segments of fibres, are likely to undergo necrosis in the future. Functional donor-derived satellite cells should repair and replace muscle fibres throughout life, although even in normal individuals, these processes become less efficient with time, leading to sarcopenia in old age [47,48]. Although many stem cells seem to give rise to at least some satellite cells after transplantation [39,49,50], few studies have shown that these satellite cells are either numerous [4,46] or functional [44,51], and indeed some are clearly not functional [44]. To test whether cells grafted into mouse models of DMD are functional muscle stem cells, cells are grafted, given time to contribute to regeneration and then grafted muscles are injured in such a way as to destroy muscle fibres, but spare endogenous stem cells (e.g. by injection of the snake venom notexin [46,51,52]). The finding of newly-regenerated muscle fibres of donor origin a week after injury is evidence that at least some of the originally grafted cells were indeed functional muscle stem cells, able to regenerate muscle fibres following muscle damage. But for long-term effect, these stem cells of donor origin do not have to be satellite cells, as long as they are capable of being retained within the host muscle and contributing to muscle regeneration.

4. Use of autologous rather than heterologous stem cells

For treatment of DMD, stem cells derived from normal donors would give rise to muscle fibres expressing dystrophin, but the treated individuals would need immunosuppression to prevent immunological rejection of donor muscle fibres. Obviously, autologous stem cells would not rectify the dystrophin deficiency, although they might contribute to muscle regeneration and reconstitute the satellite cell pool without eliciting immunological rejection. To treat patients, it would be necessary to use autologous cells after they have been genetically-modified with either a mini-dystrophin gene [53] or a gene designed to skip mutated dystrophin exons [54], to give rise to a shorter, but still functional, dystrophin [55].

Lentiviruses are ideal to introduce genes into stem cells, as they infect post-mitotic or quiescent cells and become integrated in the host genome, resulting in stable long-term gene expression both in vitro and in vivo [56–58]. But they have the drawbacks of a relatively small gene insert size [59,60] and possible insertional mutagenesis in the host genome [61,62]. The choice of an appropriate promoter, so that the gene of interest is only switched on in muscle fibres [63,64] and maintains long-term expression in vivo [65,66] is also fundamental.

In addition to viral vectors, recently developed non-viral vectors such as transposons [67,68] and human artificial chromosomes (HACs) are possible alternative gene delivery tools for future clinical applications. A transposon-based vector has the capacity of both stable integration of target genes into the host genome and high gene expression level over long period of time in cells such as hematopoietic stem cells [69], mesenchymal stem cells, muscle progenitor cells and iPS cells [70]; while HACs enable a stable episomal maintenance that avoids insertional mutations and in addition, have the ability to carry large gene inserts including regulatory elements [71]. The use of HACs to introduce a full length dystrophin gene into iPS cells
derived from DMD patients has been reported [72]; autologous muscle stem cells carrying a HAC expressing dystrophin may be a promising future therapy for DMD.

5. The ideal stem cell for treating DMD

Theoretically, stem cells to treat DMD should fulfil the following criteria:

1. Be expandable in vitro without losing stem cell properties.
2. Be immuno-privileged.
3. Be systemically-deliverable, as the majority of the skeletal muscles in the body are affected.
4. Survive, proliferate and migrate upon arrival within host muscle, in order to reach the maximum area of each muscle.
5. Differentiate into muscle fibres either to repair damaged fibres, or to replace fibres that have already been lost.
6. Reconstitute the satellite cell pool with functional stem cells, so that when a fibre, or part of a fibre, undergoes necrosis in the future, satellite cells capable of producing dystrophin are present to repair and maintain the fibre.
7. Be capable of expressing dystrophin once they have contributed to muscle.
8. Lead to improvement in muscle strength, so that the treated individual has an improved quality of life.

Stem or precursor cells have long been considered as possibilities to treat muscular dystrophies [73,74]. The availability of both mouse [75–78] and dog models of DMD [79–81], particularly immunodeficient mouse models [4,18,46,55,82] allowed the ability of exogenous stem cells to contribute to regenerated muscle fibres to be tested. The advantages and disadvantages of different stem cells as a treatment for DMD are summarized in Table 1 and discussed in detail below.

5.1. Myoblasts

Quiescent satellite cells can efficiently regenerate skeletal muscle and functionally reconstitute the satellite cell compartment [4]. But although they can be grafted in an experimental setting [4,46], transplanting freshly-isolated, quiescent donor satellite cells would not be feasible for treatment. The first cell type to be considered for treatment of DMD was therefore the myoblast, the progeny of satellite cells. Myoblasts can be expanded in culture [83,84] and form muscle following intra-muscular injection into mdx mice [18,85], but they have several disadvantages [86,87], including the fact that they regenerate skeletal muscle far less efficiently than freshly-isolated satellite cells [88]. In addition, myoblasts do not seem to be systemically-deliverable [82,89] and have a limited capacity for migration even after intra-muscular injection [90–94]. Although attempts have been made to improve the migration of myoblasts from an injection site within skeletal muscle, none have been particularly successful [95–99]. Clinical trials of myoblasts to treat DMD were disappointing [100–103] and despite recent promising data [104–106], interest in myoblasts to treat muscular dystrophies waned.

5.2. Other stem cells within the skeletal muscle

There are stem cells other than satellite cells present within muscle, such as muscle-derived stem cells [77,107,108], muscle side population cells [109], myogenic endothelial cells [110] that can contribute to muscle regeneration, although the origin and precise identity of these cells and the extent to which they act as muscle stem cells within normal, healthy muscles, are unclear. The most promising cells that are systemically-deliverable to skeletal muscle are mesoangioblasts (or pericytes) [82,89,111,112] and cells derived from muscle that express the stem cell marker CD133 [55,113]. Mesoangioblasts and pericytes are blood-vessel associated stem cells from embryonic [114] or postnatal [82] stages respectively [115]. The ability of these cells to contribute to muscle regeneration has been tested in both dystrophic mouse (α-sarcoglycan null mice and SCID-mdx mice) and dog models (dystrophin-deficient GRMD dog), using cells derived from mouse [89,111], dog [112] and man [82]. There is also evidence that donor pericytes may reconstitute, to a limited extent, the satellite cell pool after systemic delivery [82], although it is unclear whether they reconstitute the pericyte compartment. Based on the promising transplantation efficiency observed, a phase I clinical trial on human mesoangioblast transplantation is at the advanced planning stage [115].

Skeletal muscle-derived AC133+ cells, that express the glycosylated epitope of the human CD133 antigen, are also a promising stem cell for treating muscular dystrophies [55]. However, since these cells only occupy very small fraction of mononucleated cells within the skeletal muscle, their application is limited by the technical difficulties of isolating pure AC133+ cells from muscle and then expanding them in culture.

Recently a cell population called PW1+/Pax7– interstitial cells (PICs) was identified [39] within the interstitial space of mouse skeletal muscle which could differentiate into both skeletal and smooth muscle lineages in vitro and contribute to skeletal muscle regeneration as well as give rise to both satellite cell and PICs after intra-muscular transplantation. However, their systemic deliverability has not yet been tested.

Skeletal muscle therefore contains many different cells that appear to fulfil the definition of a muscle stem cell, but, as it is extremely difficult to prepare pure populations of cells for study, it is possible that one cell population may contain a fraction of another stem cell type.

5.3. Bone marrow and blood-derived stem cells

A stem cell that is more easily accessible than from skeletal muscle (which requires a muscle biopsy) and which has
neither been deleteriously affected by the dystrophic muscle environment, nor depleted by contributing to continuous rounds of muscle regeneration, would be ideal for treatment of muscular dystrophies. Bone marrow- [38,116–119] or blood-derived stem cells [120–122] are easily accessible and mostly are well characterised. Although the original publications using very sensitive markers demonstrated that they gave rise to muscle fibres on transplantation into mouse models, the amount of muscle was very small [38,116,123–125] and the muscle fibres containing donor nuclei may not express muscle-specific proteins [126] e.g. dystrophin [127] or α-sarcoglycan [128]. In addition, although bone-marrow-derived stem cells may give rise to cells in the satellite cell position, they may not all be functional satellite cells [129].

5.4. Mesenchymal stem cells from other origins

Mesenchymal stem cells, derived from non-muscle tissues e.g. synovial membrane [130] do give rise to regenerated muscle fibres, but not to any appreciable extent. Even fibroblasts, e.g. dermal fibroblasts [131–133] or skin fibroblasts that have been forced down the myogenic lineage by expression of the muscle regulatory factor MyoD [134,135], do regenerate muscle fibres, albeit with low efficiency, in mouse models. However even if they do not con-
tribute significantly to muscle regeneration, mesenchymal cells may still have a therapeutic effect by either producing extracellular matrix molecules [45], or reducing inflammation in DMD [136].

5.5. Pluripotent stem cells

Recent interest has focused on the contribution of pluripotent stem cells, e.g. embryonic stem (ES) cells, to muscle regeneration. The main problem in their use for muscle regeneration is selecting for non-tumorigenic cells [137] and highly myogenic sub-populations. Nevertheless, ES cells that were induced to differentiate down the skeletal muscle lineage prior to transplantation [138], survived and did not make tumors after intra-muscular grafting in host mice [139,140]. It was reported that by combining the induction of pax3 with selection of a PDGFαR⁺/Flik⁺ cell population, mouse ES cells could contribute to muscle formation in mouse models [141,142] without tumor formation. The use of human ES cells has been hampered due to ethical issues, but has been overcome by the establishment of induced pluripotent stem (iPS) cells [143]. iPS cells are artificial pluripotent stem cells generated from somatic cells [144–146], which are very similar in behaviour to ES cells [147,148]. The contribution of a satellite cell-like sub-fraction of iPS to skeletal muscle regeneration has been reported [50] but these experiments are difficult to reproduce, as the SM/C-2.6 antibody that was used to select the satellite-like cells, that recognises an unidentified cell surface antigen on satellite cells that is neither c-met nor M-cadherin [149], is not commercially available.

6. Pre-clinical testing of stem cells for DMD

Although some stem cells do hold promise for treatment of DMD, there is no cell that has been validated by different laboratories to fulfil all the requirements detailed above. Different research groups use different protocols for cell preparation and maintenance and different animal models and methods to analyse and quantify their data, making it impossible to compare work done by different laboratories. For example, although human synovial stem cells were shown to be able to contribute to muscle regeneration in immunodeficient host mice [130], these findings were not replicated by another group [45]. However, there are many differences between the two studies that might account for these discrepancies, including the different sources of the host cells, different host mice, and different methods used to injure the host muscle prior to grafting (discussed in [45]). To overcome the problems, standard operating procedures should be validated across different laboratories and made available to the scientific community to allow consistent and reproducible identification, preparation and purification of cells and maintenance of their stem cell characteristics on expansion in vitro and analysis of their muscle regenerative capacity in vivo. Ideally, cells prepared by one laboratory should be tested by another laboratory and control and grafted muscles analysed in a blinded fashion, to verify that they give the same results in different hands. The most rigorous testing would be for two different laboratories to independently prepare and expand one type of stem cell (e.g. muscle-derived AC133⁺ cells) and each laboratory to test both cell types in their own and each other’s preferred host mouse strain (e.g. mdx nu/nu compared to mdx SCID), following, for example, intra-arterial injection. At least 6 mice of each strain should be grafted with each cell type by both laboratories, to give statistical significance. Each laboratory should then analyse half of each grafted muscle in a blinded manner, using the same antibodies to detect muscle fibres of donor origin. Such a procedure would determine whether a particular stem cell preparation is indeed worthy of further consideration for therapeutic purposes.

A robust stem cell should be functional in different dystrophic animal models that might have different muscle pathologies (e.g. inflammation, fibrosis, muscle fibre necrosis, ongoing regeneration) – such a cell would likely to be functional in human muscles. However, it should be borne in mind that differences in the immunological status of the host mice, as well as the pathological milieu of the host muscle, might have a profound effect on a donor muscle stem cell.

When the stem cell of choice has been identified, it will have to be extracted and expanded in GMP conditions, characterised with cell specific markers, tested for safety and cryopreserved in such a way to maintain its function. The animal for pre-clinical testing of the efficacy of a particular cell preparation also needs consideration. How relevant are data from mouse cells grafted into mdx mice? Would it be more informative to graft human cells into immunodeficient mdx mice? Or would one have to use a larger animal model? And how would one define a “successful” cell preparation? Arbitrarily, one might think that 30–50% of the fibres of downstream muscles being dystrophin-positive and sufficient satellite cells of donor origin to enable regeneration of at least the same amount of donor muscle fibres after injury would be suitable to progress the donor cell preparation to a clinical trial. However, no laboratory has yet shown such results.

In order to answer at least some of the questions summarized above, a clinical trial to assess the safety and efficacy of human stem cells to treat DMD is being planned. Limited studies performed so far suggest that both intramuscular injection of myoblasts [150], although controversial [151,152], and muscle-derived AC133⁺ cells appear to be safe [153], but as yet, there has been no trial of systemically-delivered stem cells to treat DMD.

While several groups are looking for the holy grail of an ideal stem cell that will work when administered systemically, the isolation of a stem cell that might be capable of either treating individual vital muscles after direct injection, or improving the host muscle environment to enhance fibre survival or endogenous stem cell function, might still be beneficial.
Whatever strategy or cell is used, it is clear that any clinical trial should be carefully considered and only performed if rigorous pre-clinical assays give replicable evidence of efficacy.

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References

[8] Zammit PS. All muscle satellite cells are equal, but are some more equal than others? J Cell Sci 2008;121:2975–82.


