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Therapeutic potential of murine mesenchymal stem cells (MSC) from different origins in the treatment of muscular dystrophy

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As no effective treatment options are still available for muscular dystrophies, cell therapy is a strong hope. Mouse models for these diseases are an important tool for testing putative therapies. Mesenchymal cells derived from bone marrow (bMSC) and from adipose tissue (aMSC) are multipotent and can lead to other tissues such as bone, cartilage, connective and muscle tissues, *in vivo* and *in vitro*.

The main objective of this study was to evaluate the therapeutic potential of MSC from different origins in the treatment of muscular dystrophy in murine models: *mdx* (dystrophin deficient), *Lama2^{dy2/J}* (laminin- α 2 deficient) and *Large^{myd}* (defect in glycosylation of α -DG). We compared the results with injected normal mice to study the homing and behavior of these cells in non-dystrophic conditions. We isolated and characterized the bMSC and aMSC from eGFP mice by flow cytometry and by *in vitro* differentiation. Then, these cells were transplanted in the different strains by systemic injections repeated for 4 weeks.

We could find the cells in some of the treated animals, tracking the eGFP gene by PCR. We also were able to find the expression of eGFP protein in the muscle of some of the injected animals. The maintenance of the cells, however, was very variable among the treated animals, suggesting that the critical features to the success of the therapy remains unknown as individual variation. Comparative studies of the potential of different stem cells injected into different animal models are important to improve the effectiveness of stem cells in neuromuscular therapies. Financial support: FAPESP-CEPID, CNPq-INCT, FINEP, ABDIM.

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Therapeutic potential of murine mesenchymal stem cells (MSC) from adipose tissue in the treatment of muscular dystrophy in the new double mutant mouse model for the genes *Dystrophin* and *Large*

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Mesenchymal stem cells derived from adipose tissue (aMSC) are multipotent and can lead to bone, cartilage and muscle cells, *in vivo* and *in vitro*. Therefore, they may be a relevant source of cells for therapies for muscle diseases, mainly using animal models. Among them, two well known models are the *mdx* mouse with deficiency of dystrophin but a mild phenotype, and the *large^{myd}* mouse with defect of glycosylation and severe muscle weakness.

We recently generated in our lab a new dystrophic model, double mutant for dystrophin and large proteins. This *mdx/large^{myd}* mouse presents a significant weakness and deficiency of dystrophin in the muscle being therefore a very good model for testing cell therapies. The injected cells can be tracked both through DNA analysis for the wild allele of the *large* gene, as well as through the study of the presence of normal dystrophin protein in the muscle. Additionally, functional evaluation can show possible clinical benefit. We performed

the transplantation of normal murine adipose MSC, previously characterized, in this double mutant by systemic injections in the caudal vein, repeated for 3 weeks. In the fourth week, the animals were sacrificed, a necropsy was done, and several muscles and tissues were studied.

The DNA of the injected cells was found in heart, stomach, quadriceps and diaphragm of the injected animals, suggesting that these cells were properly engrafted to the muscles. Furthermore, we were able to identify the presence of traces of dystrophin in some of the muscles studied suggesting the ability of adipose derived mesenchymal stem cells to differentiate into muscle. However, additional studies are necessary to improve the amount of expressed muscle proteins leading to a better therapeutic effect. Financial support: FAPESP-CEPID, CNPq-INCT, FINEP, ABDIM.

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P3.25
Satellite cell dependent growth and regeneration of skeletal muscle requires BMP signalling

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Antagonistic gradients of Bone Morphogenetic Proteins (BMPs), a subfamily of diffusible morphogens of the TGF- β family of signalling molecules, and their antagonist Noggin have been implicated in determining the entry of embryonic myogenic precursors into muscle differentiation.

Here we demonstrate that the signalling system BMP/Noggin regulates the balance between the generation and differentiation of the resident stem cells of postnatal skeletal muscle, so-called satellite cells, thereby defining muscle growth and regeneration. Satellite cells from postnatal muscle expressed BMPs, the transmembrane receptor BMPRI1A as well as the BMP antagonists Noggin, Follistatin and Chordin. Abrogation of BMP signalling in cultures of isolated muscle fibres following treatment with recombinant Noggin, a specific BMP antagonist, caused a precocious differentiation of satellite cells. Such precocious differentiation was associated with a precocious loss in Pax7 expression, a strong decline in muscle precursor generation and a deficit in satellite cell self-renewal. Treatment of postnatal mice with AAV-Noggin retarded muscle growth and myofibres remained small and contained less myonuclei. Similarly, myofibre regeneration (following cryodamage and in dystrophic *mdx* muscle) was severely impaired following AAV-Noggin treatment and preliminary results show that regenerating muscle contained less muscle precursors. Treatment of adult mouse muscle with AAV-Noggin resulted in muscle fibre atrophy and in loss of quiescent satellite cells. These results show that BMP signalling is required for satellite cell dependent myofibre growth and regeneration by permitting for sufficient precursor generation and by delaying myogenic differentiation. Moreover, BMPs maintain the trophic state of myofibres and maintain the muscle stem cell reservoir. Our data suggest that suppressing the activity of BMP antagonists could provide novel therapeutic strategies to ameliorate regeneration of skeletal muscle.

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