strated a significant increase of the muscle's strength in most of fibers of treated mice with a value similar to the normal C57BL. We speculated that the RTX treatment could modify the in vivo behaviour of pericyte-like cells which increase their number and participate at the vascular remodelling. Further experiments are necessary to demonstrate which pathway is involved in the differentiation of these progenitors and their role in the muscle's strength rescue in dystrophic mice.

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T.P.6.02

Aldehyde dehydrogenase activity identifies distinct populations of progenitors within human skeletal muscle

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Aldehyde dehydrogenase (ALDH) activity is one hallmark of human primitive progenitors presenting broad regeneration capacities in vivo. We investigated for the presence and putative roles of ALDH-expressing populations in human skeletal muscles. Immunohistofluorescence study on human muscle tissue sections highlighted rare endomysial cells presenting a positive labeling for ALDH1. Then, based on measurement of ALDH activity using the reliable fluorescent substrate Aldefluor®, we described well-defined sub-populations of human skeletal muscle SSClo/ALDHbr cells issued from bulk dissociated muscle biopsies, that were named SMALD cells. Two main sub-populations were discriminated according to CD34 expression, and termed SMALD/34− and SMALD/34+ cells. These sub-populations did not express endothelial (CD31), hematopoietic (CD45) and myogenic (CD56) markers, and could be partly discriminated by the variable expression of associated markers (CD44, CD90, CD105, CD140b). Upon sorting, SMALD cells were expanded and their differentiation abilities were evaluated and compared. While SMALD/34+ cells developed in vitro as an heterogeneous population of CD56− cells generating adipoblast-like cells, the SMALD/34− fraction developed in vitro as a highly enriched population of CD56+ myoblasts able to form myotubes. Moreover, SMALD/34− cells participated efficiently to muscle regeneration in vivo upon intramuscular transplantation in SCID mice, while SMALD/34+ cells did not. SMALD/34− population may thus become a new player in the field of muscle homeostasis or repair in therapeutic perspectives. Correlations between clinical or physiopathological status of human donors and the presence of these cells mandate large-scale studies.

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T.P.6.03

Restoring cell-basal lamina interaction to rescue tissue degeneration in congenital muscular dystrophy

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Congenital Muscular Dystrophy (CMD) is characterized by progressive wasting muscular dystrophy and dysmyelinating neuropathy, which may lead to severe disability in early childhood. The most frequent form is due to mutations of the LAMA2 gene encoding the laminin alpha2 chain, which forms merosin the predominant laminin isoform of muscle and nerve basement membrane. Although much is known about clinical aspects and genetic causes of CMD, and about the pathological mechanisms that lead to muscle and nerve degeneration, no useful therapy to arrest neuromuscular degeneration and to promote tissue repair is available to date. The overexpression of laminin2 or mini-agrin, a cross-linker molecule that allows reconnection of the basement membrane to the resident cells, showed amelioration of CMD in animal models. However, at present direct viral transduction of exogenous proteins into human tissues is not feasible. Cell therapy may instead constitute a promising tool to speed translation into clinical practice. Mesoangioblasts have shown promising results in terms of amelioration of muscular dystrophy phenotype and reconstitution of missing proteins in pre-clinical experiments. We infected mesoangioblasts with lentiviral vectors carrying a mouse mini-agrin gene. Mesoangioblasts synthesize and deliver mini-agrin in vitro and in vivo. When injected in CMD model, mesoangioblasts carrying the mini-agrin gene fuse into myotubes of mutant mice, which expressed the mini-agrin protein. These mice displayed amelioration of muscle histology and clinical phenotype.

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T.P.6.04

Comparison of skeletal muscle potential of mesenchymal stem-cells from different sources injected in *SJL* mice

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One of the aims of the HGRC-USP in Brazil is to compare the potential of stem cells from different sources to originate skeletal muscle. Limb-girdle muscular dystrophies (LGMD) are a heterogeneous group of disorders characterized by progressive degeneration of skeletal muscle caused by the absence or defective muscular proteins. The murine model for Limb-Girdle Muscular Dystrophy 2B (LGMD2B), the *SJL* mice, carries a deletion in the dysferlin gene that causes a reduction in the protein levels to 15% of normal. The mice show muscle weakness that begins at 4–6 weeks and is nearly complete by 8 months of age. The possibility to restore the defective muscle protein and improve muscular performance by cell therapy is a promising approach for the treatment of LGMD or other forms of progressive muscular dystrophies (PMD). Umbilical cord tissue and adipose tissue are routinely discarded and are rich sources of mesenchymal stem cells (MSCs), which have been widely investigated for cell-based therapy studies. We injected human or canine umbilical cord MSCs into the caudal vein of two different groups of *SJL* mice wich were compared with a third non injected control group. These animals received 10^6 cells for six months, weekly in the first month and then monthly, aiming to compare their muscle potential with the previous results obtained with human Adipose-derived Stem Cells (hADSC) in the *SJL* mice. We are evaluating their

ability to: engraft into recipient dystrophic muscle after systemic delivery; form chimeric muscle fibers; express human or canine muscle proteins in the dystrophic host and improve muscular performance. Together these data will provide evidences of which MSCs are more suitable for skeletal muscle regeneration in vivo in murine models. Further comparative preclinical studies are required in larger animals such as the Golden Retriever Muscular Dystrophy (GRMD) dog. Funding by: FAPESP, CNPq, INCT, FINEP and ABDIM.

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T.P.6.05

Poor maintenance of eGFP-mesenchymal stem cells in the dystrophic muscle

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Muscular dystrophies (MD) are heterogeneous genetic muscle disorders, still devoid of efficient treatment, and the mouse models for these diseases are an important tool for testing putative therapies. With a view to developing treatments for muscular dystrophy, we are testing the myogenic potential of murine bone marrow mesenchymal stem cells (bMSC) following transplantation into dystrophic mice. The bMSC were isolated from transgenic mice carrying a gene expressing enhanced green fluorescent protein (bMSC-eGFP), which constitutes a good marker for tracking them in the injected organism. In our previous studies, we observed that after 30 days post-injection of these cells, treated muscles from *mdx* mice showed no sign of muscle regeneration, nor any evidence of dystrophin expression. In contrast, non-GFP murine embryonic stem cells were retained. Additionally, human MSCs from adipose tissue were also retained after injection into *SJL* mouse muscles. To verify if eGFP expressing cells are less tolerated by the dystrophic muscle, a single dose of about 10^6 bMSC-eGFP cells was injected intra-muscularly into *mdx* and normal C57Bl animals, and tracked through PCR using primers for the DNA sequence related to the eGFP gene. We detected the presence of eGFP up to 2 days post-injection, in both affected and normal animals. After this time, no traces of the eGFP DNA from transplanted cells were detected, suggesting that the cells had been effectively eliminated from the muscles. These results suggest that, in addition to a possibly adverse environment offered by the dystrophic muscle for the homing and maintenance of injected bMSC stem cells, cells expressing eGFP are poorly tolerated, and rapidly eliminated. 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. Funding: FAPESP,CNPq-INCT,FINEP, ABDIM.

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T.P.6.06

Real-time monitoring of cell transplantation in mouse dystrophic muscles by a secreted alkaline phosphatase reporter gene

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Transplantation of muscle precursor cells (MPCs) is a promising approach for the treatment of muscular dystrophies. However, preclinical and clinical results have shown that the technology is not yet efficient enough for most therapeutic applications. Among the problems that remain unsolved are the low cellular survival, poor proliferation and the lack of migration of the transplanted cells. One major technical hurdle for the optimisation of transplantation protocols is how to follow precisely the fate of the cells after transplantation. In the present work, we examined the use of a secreted form of the mouse alkaline phosphatase (mSeAP) enzyme as reporter system transduced into MPCs using a retroviral vector. We show that circulating mSeAP could be detected in the serum of the transplanted mice at different time points after MPC transplantation. We also found that the level of circulating mSeAP is highly correlated with the number of transplanted cells and that mSeAP is an excellent histological marker. Further, studying the levels of circulating mSeAP compared to the number of muscle fibers positive to mSeAP and to dystrophin, enabled detailed analyses of bottleneck steps for successful transplantation. Taken together, our results demonstrate that mSeAP is an excellent quantitative "real-time" reporter gene for cell therapy preclinical studies.

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T.P.6.07

Lin(-) bone marrow stem cells transplantation repairs structure of mdx mice neuromuscular junctions

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Mdx mice are a model of Duchenne myodystrophy caused by deficiency of dystrophin. Muscles of mdx mice are characterized by a high levels of death of striated muscle fibers and accordingly by a high level of regeneration. Moreover, neuromuscular junctions (NMJ) in mdx mouse altered. To study the influence of stem cells (SC) therapy for muscle differentiation we transplanted C57BL/6 Lin(−) bone marrow (BM) SCs to mdx mice M. quadriceps femoris. Lin(−) BM SC were prepared by exhaustion of the C57BL/6 BM cell suspension with set of antibodies to differentiated antigens (Caltag, USA) with help of Dynal (Norway) magnetic system under cytometry control. To localize NMJs we used tetramethylrhodamine-α-bungarotoxin (Biotium, USA) with help of LSM 5 PASCAL (Carl Zeiss) confocal microscope. The study was made at 4, 8, 16 and 24 weeks after SCs transplantation. Single Lin(−) BM SCs transplantation positively influence mdx mice striated muscle differentiation. Death level of striated muscle fibers was decreased beginning 4 weeks. Accumulation of muscle fibers without central nuclei began from 8 weeks. Dystrophin synthesis increased at 16, 24 weeks. On cross sections of mdx muscle the square of NMJs increased significantly from $78.4 \pm 5.1\ \mu m^2$ for control mice up to $106.9 \pm 3.4\ \mu m^2$ in 4 weeks after SCs transplantation. There is a good accord with NMJs square of C57Bl/6 mice, $102.8 \pm 3.0\ \mu m^2$. On longitudinal section of mdx muscle we observed a decrease quantity of acetylcholine receptors (AChR) clusters in NMJs from 4.7 ± 0.3 to 3.7 ± 0.1 and increase AchR clusters squire from 58.0 ± 3.9 to $80.0 \pm 4.9\ \mu m^2$ at 4 weeks after transplantation. Results show that single intramuscular transplantation of C57BL/6 Lin(−) BM SCs increase differentiation of mdx mice striated muscle fibers. First of all BM SC transplantation influence for death level and structure of NMJs. Reparation of structure of NMJs after stem cells therapy is showing of success of cell therapy of muscle fibers.

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