ABSTRACT

Introduction:. Duchenne disease (DMD) has been the hot topic for many years. For both fundamental and clinical research. Current knowledge allows us to focus on new therapeutic strategies to cure the genetic cause of the disease instead of symptomatic treatment. Due to the diversity of mutations in the DMD gene, a universal approach is being sought, applicable in unified therapy. The aim of this study was to develop mesoangioblast cell lines modified with a lentiviral construct containing the microdystrophin sequence (*μDYS*), which could potentialy improve skeletal muscle function in DMD patients with different genetic background.

Material and Methods: Induced pluripotent stem cells (iPSc), derived from dystrophic cells of boys with DMD (n=4, of mild and severe phenotype) and healthy person (control), were differentiated into mesenchymal-like cells (HIDEMs). A lentiviral construct containing the *μDYS* sequence was then used to transduce HI-DEMs cells, and the effects of transduction on the striated muscle activator RhoA (STARS) signaling pathway and oxidative stress parameters were observed as the second-order effects of DMD development. Transduced cells were administered to *mdx* mice (n=4), and the effect of the treatment on the distance traveled and the number of electrical shocks received by the animal during treadmill experiments was checked against a group of SHAM (n=4) and healthy (control, n=4) mice.

Results: The pluripotent nature of received iPSc cells was demonstrated, then the cultured *in vitro* cells from all the DMD patients and control were differentiated into HIDEMs cells, confirming their mesenchymal-like characteristics. Cells were then subjected to lentiviral transduction containing the *μDYS* sequence, achieving its overexpression in all the cell lines tested with exception of cells derived from tetraplegic patient. The expression levels of genes included in the STARS signaling pathway were checked. An increase in SRF expression was found only in patient 38 cells with mild DMD phenotype ($p < 0.001$). The activity levels of the enzymes catalase (CAT) and superoxide dismutase (SOD), as well as the level of total antioxidant capacity in homogenates of HIDEMs cells were also examined, and a decrease in CAT and TAC activities and an increase in SOD activity were found in patients 34 and 38 ($p < 0.001$). Transduced cells were transplanted into mice into the *tibialis anterior* muscle. Mice were subjected to treadmill tests, yielding a significant differences in distance covered by control mice versus *mdx* mice with HIDEMs cells with/without transduction with microdystrophin did not reveal differences in a treadmill reached distance, however significantly disminished the number of electric shocks in a group at *mdx* mice subjected to transduced HIDEMs cell intervention.

Conclusions: The results suggest that the use of cell therapy including HIDEMs transduction with a lentiviral construct containing the *μDYS* sequence may have the effect the level of oxidative stress and its regulatory STARS signaling pathway. However, the significant differences were found only in the cells derived from DMD patients with mild phenotype.