

Myogenic differentiation of FSHD patient specific induced pluripotent stem cells

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Human induced pluripotent stem (IPS) cells overcome several disadvantages of human embryonic stem cells, including host specificity and ethical issues. Patient-specific IPS cells can be generated from every donor by using different cell types, making them a suitable tool for autologous cell therapy and tissue engineering. IPS cells generated from patients with genetic disorders capture the disease genotype in the cell, making them a good model for studying the pathology of the disease, especially during development, and testing different therapeutic approaches. FSHD is one of the most common inherited myopathies, caused by a contraction within a subtelomeric array of D4Z4 repeats 4q35.2. It is characterized by uneven and progressive weakness and atrophy of facial, shoulder and upper arm muscles. The D4Z4 repeat contains an intronless double homeobox gene named DUX4 (double homeobox, chromosome 4) which was recently shown to be expressed specifically in FSHD myoblasts, but not in unaffected control cells. DUX4 expressed in high levels induced rapid cell death and in the low levels interferes with myogenesis by misregulating MyoD and Myf5. Myoblasts expressing DUX4 failed to fuse and form terminally differentiated myotubes. We generated IPS from myoblasts and fibroblast from seven FSHD patients and five controls using four reprogramming factors, Sox2, Klf4, Oct4 and c-Myc. IPS cells were characterized by analyzing expression of pluripotent markers and formation of teratomas. From IPS cells we generated mesenchymal stem cells using EB culturing system and mesoderm inducing growth factors. Mesenchymal stem cells express specific surface markers including CD73 and CD105, and under specific condition were able for adipogenic and osteogenic differentiation. To induce myogenesis IPS-MSC we transfected with MyoD or Myf5. Stably transfected cells were able to expand as myoblasts in myogenic proliferation medium. Generated myoblasts express specific marker CD56 and under differentiation condition in growth factor deprived medium were able to fuse and terminally differentiate in myotubes. Myoblasts generated from IPS cells transplanted in MDX mice were able to engraft and restore dystrophin expression.

Biography

Darko Bosnakovski finished School of Veterinary Medicine at University "St. Cyril and Methodius" in Skopje, Macedonia, completed his Ph.D at Hokkaido University, Sapporo, Japan and his postdoctoral training at UTSW at Medical Center in Dallas, USA. He is Ass. Professor of Pharmacogenetics and biotechnology at Faculty of Medical Sciences, University Goce Delcev, Stip, R. Macedonia.

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