OMICS Group Conferences
Accelerating Scientific Discovery

2nd International Conference and Exhibition on
Orthopedics & Rheumatology

August 19-21, 2013 Embassy Suites Las Vegas, NV, USA

Myogenesis in gene corrected induced pluripotent stem cells

Darko Bosnakovski
Goce Delcev University-Stip,

Patient specific induced pluripotent stem (IPS) cells are powerful tool for autologous cell therapy and tissue engineering. IPS cells can be generated from different tissue sources, including skin, muscle and fat. 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. In addition, provides suitable *in vitro* model for testing different therapeutic approaches like drug and gene therapies. 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 atrophy of facial, shoulder and upper arm muscles. Within each D4Z4 repeat is embedded a double homeobox gene (DUX4, double homeobox, chromosome 4). DUX4 is specifically detected in biopsy sample from FSHD patients. Furthermore, it was shown to be expressed in FSHD cultured myoblasts, but not in the cells from healthy donors. We showed that DUX4 expressed in high levels induced rapid cell death and in the low levels interfere with cell cycle. Furthermore missregulates myogenesis by blocking terminal differentiation of myotubes. We generated IPS from myoblasts from seven FSHD patients and controls using four reprogramming factors, Sox2, Klf4, Oct4 and c-Myc. Generated IPS line were characterized by expression of pluripotent markers and formation of teratomas. To have adequate cells for study the mechanism of FSHD and tissue engendering, we corrected the affected locus by removing 4qA161 allele. We targeted FSHD-iPS cells using zinc finger nucleases and linear targeting vector bearing a single homology arm, neo cassette and human artificial telomeric repeat. Expression analyses using RT-PCR revealed that selected neo resistant IPS clones did not transcript DUX4 as original FSHD-IPS cells. To derivate myoblast from IPS cells we had to recapitulate myogenesis during embryogenesis. Firstly, we generated mesenchymal cells using EB culturing system and cocktail of mesoderm specific growth factors. Mesenchymal stem cells (named IPS-MSC) were characterized by surface markers CD73 and CD105, and ability for adipogenic and osteogenic differentiation. Myoblasts were generated from IPS-MSC by transduction with Myf5. Stably transfected cells in proliferation medium were able to expand more than 20 passages. They had typical myoblast surface marker and gene expression profile. Under specific conditions myoblast generated from IPS cells were able to fuse in terminally differentiated myotubes.

darko.bosnakovski@ugd.edu.mk