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Generation of Induced Pluripotent Stem Cells from Patients with Duchenne Muscular Dystrophy and their induction to Neurons

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Duchenne muscular dystrophy (DMD) is an X-linked recessive disease characterized by deficient expression of the cytoskeletal protein dystrophin. DMD has been associated with intellectual disability and mental retardation (MR) and is present in about a third of all patients. Loss of Dp71, the major dystrophin-gene product in brain, and the dystrophin associated proteins (DAPs) are thought to contribute to severity of MR, but the specific function of the neural dystrophin proteins are poorly understood for a limited access to DMD patients brain tissue (1). Differentiation of induced Pluripotent Stem Cells (iPSCs) provides an opportunity to generate an unlimited supply of living neurons genetically identical to those present in patients.

In this study we obtained DMD-iPSCs from peripheral blood mononuclear cells of DMD patients with cognitive impairment and we performed morphological (fluorescence and electron microscopy), molecular (Western Blot and Real Time PCR) and functional (electrophysiology) characterization both of iPSC-derived Neural Stem Cells (NSCs) and the differentiated neurons. Preliminary data showed a reduction of Dp71 and DAPs proteins, including the AQP4, potassium channel Kir4.1, α- and β-dystroglycan (α/βDG) and α-syntrophin (αSyn), both at transcriptional and traductional level, coupled with membrane dys-arrangment in DMD-iPSCs compared with healthy iPSCs. Moreover, we demonstrated that the neurons obtained from the differentiation of iPSCs derived from DMD patient showed after confocal analysis, altered cytoskeleton and reduction in Dp71expression, and by single-cell imaging experiments and electrophysiology, altered intracellular calcium homeostasis, in analogy with what shown in the dystrophic mdx mouse neurons (2). Overall these results showed that the Dp71 and DAPs alterations affect also the neural precursor as well as the differentiated neurons in DMD patients, so suggesting a key role in the pathogenesis of neurocognitive deficits in DMD disease.

References

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Keywords
Duchenne Muscular Dystrophy, Induced Stem Cells