CO-CULTURE OF HUMAN iPSC DERIVED MOTOR NEURONS AND SKELETAL MUSCLES PROVIDES A PHYSIOLOGICAL NEUROMUSCULAR JUNCTION MODEL IN-A-DISH

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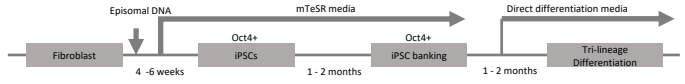
Abstract: The Neuromuscular junction (NMJ) is a highly organized synapse which is formed between the axon of a motor neuron and a muscle fiber. Motor neurons transmit efferent signals to control the muscle fiber contraction. The functional integrity of NMJ is tightly associated with the etiology, pathophysiology and progression of neuromuscular diseases. NMJ models have been well established using rodent cells, however, there is still a need to establish a human model due to the physiological differences between mouse and humans. Here, we have established an NMJ model by co-culturing human motor neurons and skeletal muscles differentiated from the same human iPSC line. The formation of functional NMJ connections are confirmed by imaging for muscle fiber calcium transients and the contractions in response to glutamate-induced motor neuron firing. In the future, the application of this model using patient-derived iPSC cells as well as high throughput small molecule screen assay will deepen our research on the etiology and provide targeting molecule candidates that can improve the physiological function of NMJ.

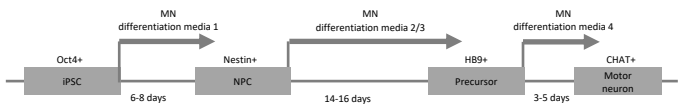
Materials & Methods:

iPSC Derivation & Characterization:



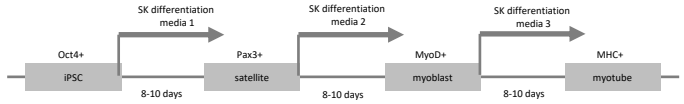
The iPSCs (ASE-9211) were derived by using the Episomal Reprogramming System. The individual clones were expanded on Matrigel-coated plates in mTeSR1 media before banking. The cells have been fully characterized by pluripotency marker staining, G-banding karyotyping & tri-lineage differentiation.

Motor Neuron Differentiation:



The iPSCs (ASE-9211) were differentiated into CHAT+ motor neurons by using motor neuron (MN) differentiation media 1-4 (formulated in-house) in 23-29 days. NPC: neural precursor cells

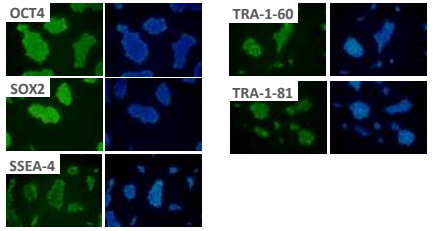
Skeletal Muscle Differentiation:



The iPSCs (ASE-9211) were differentiated into MHC+ skeletal muscles by using a skeletal muscle (SK) differentiation media 1-3 (formulated in-house) in 24-30 days.

iPSC Derivation & Characterization:

Pluripotency Markers:



Tri-lineage Differentiation:

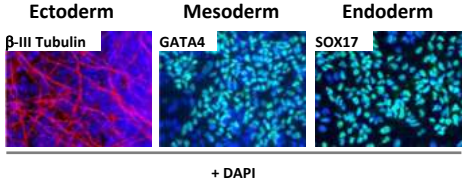


Figure 1. iPSCs (ASE-9211) were derived from a healthy donor and the cells were maintained in mTeSR plus media for ~10 passages. The cells were determined to be pluripotent (as ascertained by pluripotency marker staining for OCT4, SOX2, SSEA4, TRA-1-60, and TRA-1-81), demonstrated normal karyotype (data not shown), and have been successfully derived into multiple lineages including the directed differentiation to the three germ layers: ectoderm (β-III Tubulin), mesoderm (GATA4) and endoderm (SOX17). DAPI was used to stain the nucleus counterstain (blue).

Motor Neurons Derived from iPSCs:

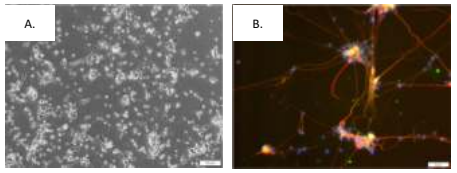


Figure 2. Motor neuron were derived from iPSC line, ASE- 9211. A. Bright-field image B. Antibody staining images (Red: Tuj1; Green: CHAT; Blue: DAPI).

Skeletal Muscles Derived from iPSCs:

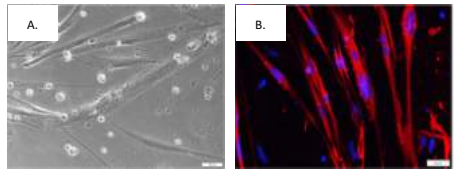


Figure 3. Skeletal muscles were derived from iPSC line, ASE-9211. A. Bright-field images of the myotubes with elongated structures. B Antibody staining for cells expressing MHC and containing multiple nuclei. These skeletal muscles show spontaneous contraction (data not shown).

Neuromuscular Junction (NMJ) Modeling:

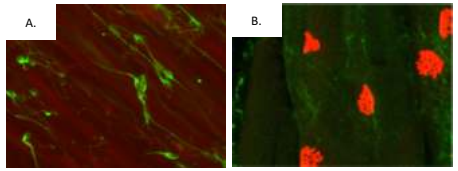


Figure 4. Co-culture of skeletal muscles and motor neurons to generate neuromuscular junctions. A. Skeletal muscles (red) and motor neuron (green) were co-cultured at 1:3-5 ratio. B. The neuromuscular junction was detected by Bungarotoxin (red).

Conclusions:

1. We have generated hundreds of iPSC lines from healthy/diseased donors using integration-free reprogramming methods (Episomal, mRNA, viral), as well as isogenic lines by using genome editing technology.
2. The iPSCs show pluripotency toward multiple lineages including neurons, astrocytes, cardiomyocytes, hepatocytes, etc.
3. In this study, we have established a NMJ model by co-culturing human motor neurons and skeletal muscles differentiated from the same human iPSC line, ASE-9211.
4. In the future, the application of this model using patient derived iPSC cells as well as high throughput small molecule screen assay will deepen our research on the etiology of neuromuscular disorders and provide targeting molecule candidates that can improve the physiological function of NMJ.

References:

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