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ABSTRACT

Human in vitro models to reconstruct pathogenic mechanisms in ALS

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Induced Pluripotent Stem Cells (iPSCs) offer an opportunity to model *in vitro* neurodegenerative diseases such as Amyotrophic Lateral Sclerosis (ALS), a fatal condition caused by loss of upper and lower motoneurons (MNs). Numerous ALS-linked genes have been lately emerged. In the case of the RNA-binding protein FUS, most *in vitro* studies rely on cells in which the mutated protein is overexpressed; therefore the patient settings, the complexity of the MN and its surroundings are not recapitulated. Hence, we generated human iPSC lines by reprogramming keratinocytes from one late onset and two juvenile ALS patients respectively (FUS^{R521C}, FUS2 ^{R495QfsX527} FUS3 ^{D502TfS*27}). As controls we used gender-aged-matched healthy subjects or the corresponding isogenic control generated via Crispr/Cas9. Thus, iPSC-derived MNs provided an *in vitro* model to study the behavior of the mutant proteins in the appropriate cellular and genetic background.

In addition to MNs, the pathophysiology in ALS may also involve the skeletal muscle. In a motor unit, the MN connects to the muscle via the neuromuscular junction (NMJ) and during development MNs are potent inducers of acetylcholine receptor (AchR) clustering to form mature NMJs. To that end, we examined synaptic contacts in single neuronal or myotube cultures and in neuron-muscle co-cultures derived from our iPSC lines. First, we demonstrated that aberrant cytoplasmic localization of mutated FUS, a hallmark of ALS-FUS pathology, was recapitulated in iPSC-derived MNs. Moreover, FUS which was present at the pre-synapse in CNTL cells aberrantly accumulated at synapses in the patient cells. In myotube cultures and motor neuron-myotube co-cultures we were able to detect from FUS-ALS patients that the endplate maturation was impaired and AChR expression reduced. Co-cultures showed intrinsic toxicity of ALS-mutant FUS in both motor neurons and myotubes. Therefore, the iPSC system presented here represents a suitable model for investigating the role of FUS mutations in ALS etiopathogenesis.