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ALS Skeletal Muscle: Victim or Culprit

Agnes Badu-Mensah, M.Sc.[1,2], **Xiufang Guo, Ph. D.**[1,2], **James J. Hickman, Ph. D.**[1,2,*]

[1]University of Central Florida College of Medicine, Burnett School of Biomedical Sciences, Orlando, Florida, USA

[2]Hickman Hybrid Systems Laboratory NanoScience Technology Center University of Central Florida Orlando, Florida, USA

Aside its function in locomotion, posture maintenance and respiration, the human skeletal muscle (hSKM) is reported to be a critical metabolic regulator [1]. The hSKM is acknowledged as the primary site of glucose metabolism and storage [1]. Additionally, it serves as a reservoir for amino acids [1,2]. In recently times, the hSKM has also been described as an endocrine organ. It is known to secrete a plethora of myokines that systemically affect other organs including the liver, pancreas, and immune system among others [3,4]. Proper function of the hSKM is therefore critical for maintaining whole body metabolic homeostasis. As such, perturbations in skeletal muscle resulting in metabolic and functional changes has deleterious consequences on the overall health of an organism. Unsurprisingly, muscle health decline is associated with poor disease prognosis in numerous conditions [2]. Thus, paying attention to muscle health may be pertinent to improving disease outcomes and overall wellbeing of an organism.

The specific progressive loss of upper and lower motoneurons remains a mystery in Amyotrophic Lateral Sclerosis (ALS) pathology and has inspired many controversies particularly concerning the site of disease onset. Corticomotoneuron glutamate-induced hyper-excitability was initially thought to induce ALS onset by causing lower motoneuron degeneration [5–8]. Earlier researchers were of this view because of clinical reports indicating that motoneurons with no corticomotoneuron synapses, such as oculomotor and abducens, were spared in ALS pathology [5,6]. Thus, initial research efforts were geared towards curbing excitotoxity. In fact, Riluzole, the first FDA-approved drug was designed for this purpose. However, it was soon realized that just mitigation of glutamate hyperexcitability does not prevent disease progression and eventual death [9].

More recent studies have now shown that ALS is non-cell autonomous and may involve other tissue types such as astrocytes, microglia, Schwann cells, and skeletal muscle. Among these tissue types, findings have reported pathological involvement of the hSKM in ALS onset and progression. A prior study reported changes in activity of Cyclin dependent

*Author for correspondence: jhickman@ucf.edu.

Conflicts of Interest

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kinase-5 (Cdk5), a kinase implicated in myogenesis and AChR clustering, prior to symptom onset [10]. Another showed that Nogo-A, an axonal guidance protein secreted by the hSKM and known to repel axons when aberrantly expressed, is upregulated in both patients and transgenic models before early symptom onset [11]. That same report demonstrated a positive correlation between Nogo-A upregulation and disease severity. Furthermore, studies on hSKM-specific expression of mutant SOD1 genes (G93A and G37R) in mice models demonstrated altered muscle morphology and metabolism [12,13]. However, they had differing results on the effect of the diseased muscle on neuromuscular junctions (NMJ), thus leaving the specific role of the hSKM debatable. Nonetheless, findings from transgenic models were called into question. Some researchers raised concerns on whether observations made in transgenic models could be a consequence of the excessively high copy levels of the mutant human SOD1 gene in the primary transgenic models and not necessarily impairments representative of the disease condition in humans [14,15]. This necessitated the utilization of human tissue-derived models in ALS research. However, initial efforts to specifically investigate patient hSKM *in vitro* faced challenges in the ability to culture the primary skeletal muscle cells [16,17]. It was observed that patient tissue-derived myoblasts could not be reliably expanded over several passages before becoming senescent [16,17]. In essence, patient biopsy-derived myoblasts had a very limited culture span compared to myoblasts obtained from healthy patients. Notwithstanding the point, the studies indirectly validated the claim that the ALS muscle has inherent defects that may negatively affect regeneration.

The availability of patient derived induced pluripotent stem cells (iPSCs) created an opportunity to study human ALS hSKM directly. By ectopically expressing inducible MyOD, Lenzi et al. generated and studied mutant FUSed in Sarcoma (FUS) and Tar Binding Protein-43 (TDP-43) myotubes [18]. They demonstrated differences in response to acetylcholine stimulation between healthy and FUS and TDP-43 mutant myotubes [18]. Another study by Pichiarelli et al. demonstrated deficits in endplate maturation specifically, ACh receptor (AChR) clustering in FUS 1 and FUS 2 in myotubes generated from patient iPSCs [19]. Interestingly, endplate maturation showed no improvement even in the presence of healthy motoneurons [19]. While the human reports suggest there are some inherent deficits in the ALS hSKM that may contribute to ALS pathology, no study directly assessed the function of ALS hSKM. Thus, the study by Badu-Mensah et al. aimed to investigate the regenerative and functional deficits of the ALS skeletal muscle. Using a previously published small molecule-directed differentiation protocol, ALS myoblasts were generated from patient-derived iPSCs (ALS-iPSCs) harboring mutations in the SOD1 gene [20]. Resultant ALS myoblasts were morphologically and quantitatively assessed via phase-contrast microscopy, immunocytochemistry, and flow cytometry. The authors noted that although ALS myoblasts were proliferative and expressed myogenic markers at levels comparable to healthy controls, they were flat and irregularly shaped as previously described by Pradat et al. [16] for tissue samples, additionally, ALS myoblasts were found to have reduced regeneration capability. Resultant ALS myotubes were shorter, thinner and had reduced AChR expression compared to healthy myotubes. These findings indicate that there are intrinsic deficits in the ALS hSKM that have deleterious effects on hSKM regeneration.

Furthermore, the authors assessed the contractile function of the aneural ALS hSKM models generated. Skeletal muscle weakness is an established ALS hallmark [21,22]. However, while muscle weakness has been solely attributed to axonal retraction, existing evidence suggests that the ALS hSKM may inherently contribute to observed weakness [18]. Thus, contraction fidelity (i.e., the ability to respond to stimulus), contractile force and time-to-peak (TTP) (time between stimulation and muscle contraction peak) of the iPSC-derived ALS hSKM were assessed. Compared to healthy hSKM, ALS hSKM had significantly reduced contraction fidelity, force and delayed TTP. Mitochondrial analysis to gain clues for the observed contractile deficits revealed reduced inner mitochondrial membrane potential and metabolic plasticity.

Collectively, the authors generated ALS hSKM models from patient-derived iPSCs that reliably recapitulated patient conditions and can now be used as a platform for ALS research and drug discovery purposes. With the models, they demonstrated that the human skeletal muscle is a target for mutant SOD1 toxicity. Additionally, the authors showed that the ALS hSKM has inherent deficits that negatively affect its regeneration. Also, evidence was presented that these deleterious alterations in the ALS hSKM affect its function independent of axonal denervation. Some hSKM deficits were shown to contribute to NMJ dysfunction. These findings demonstrate that the ALS hSKM is not a mere victim of motoneuron denervation but is an active participant in NMJ disruption during ALS progression.

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