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EARLY-DIFFERENTIATED C2C12 MYOTUBES RELEASE A PRO-TROPHIC SECRETOME THAT MODULATES NSC-34 MATURATION: IMPLICATIONS FOR NEUROMUSCULAR CROSSTALK IN AMYOTROPHIC LATERAL SCLEROSIS

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Skeletal muscle is increasingly recognized as an active participant in motoneuron health, acting as a key modulator of neuromuscular communication and stability by the soluble factors and extracellular vesicles, which constitute its secretome. In Amyotrophic Lateral Sclerosis (ALS), the breakdown of this crosstalk may exacerbate motoneuron vulnerability. In this study, we investigated the influence of conditioned medium (CM) from C2C12 myotubes at different stages of differentiation, early, after 3 days, and late, after 5 days, on the differentiation of NSC-34 cells, transfected with m-SOD1-wt or the aggressive mutant m-SOD1-G85R.

Morphological analysis, performed by confocal microscopy and scanning electron microscopy, shows that conditioned medium harvested at 3 days significantly promoted neurite extension and neuronal complexity in NSC-34 mSOD1 G85R cells compared to controls; instead, the conditioned medium from 5-day-differentiated cultures had a weaker effect. At the molecular level, Western blot analysis and real-time PCR revealed a significant increase in key markers associated with cholinergic and synaptic differentiation, including synaptophysin, ChAT, VAChT, and AChE, in NSC-34 cells exposed to the 3-day CM. Conversely, CM from 5-day-differentiated cultures exhibited a markedly reduced pro-differentiative impact, suggesting a dynamic temporal remodeling of the muscle secretome. The biochemical analysis of C2C12 lysates indicated that AKT/pAKT signaling modulation provides a potential intracellular basis for the observed trophic shift.

In conclusion, our results demonstrate that the muscle secretome in an early differentiation phase exerts a stronger pro-differentiative and pro-trophic effect on MN-like cells compared to more advanced stages, suggesting that the temporal dynamics of muscle-to-MN signaling are critical for neuromuscular homeostasis. Overall, this study is consistent with the view that alterations in the muscle secretome may affect motor neuron differentiation and contribute to the dysregulation of neuromuscular crosstalk in ALS.