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Development of an *in vitro* co-culture models between motoneurons and muscle fibers to enhance muscle fibers maturation

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Research project:

The building block of skeletal muscle is the post-mitotic muscle fiber (myofibers). Myofiber is formed by the fusion of hundreds of specialized mononucleated cells (myoblasts/myocytes), which shape syncytial cells (myotubes). Myotubes are immature myofibers in which **positioning of nuclei (*i.e.* myonuclei), referred as myonuclei localization and shape**, is finely regulated[1]. During muscle development, myonuclei actively spread within myofibers. Myonuclei finally adopt a specific localization in the mature myofiber, regularly positioned at its periphery.
Myonuclei are located between the plasma membrane of myofibers and myofibril structures[2]. This peripheral localization of myonuclei induces drastic changes in their shape, mainly due to forces applied on their nuclear envelope. This conformational adaptation of myonuclei is believed to stabilize internal and external mechanical forces, and consequently, to constrain chromatin organization and gene expression[3]. This myonuclei organization, set by an interplay between the various cytoskeletons (microtubule, actin and intermediate filaments) is thought to

guarantee a spatial coordination of the transcriptomic activity, that ultimately contributes to myofiber functional integrity[4].

We recently show that MACF1 controls MTs architecture and dynamics along myofiber's maturation, specifically around myonuclei, and, as a consequence, governs myonuclei motion. Our *in vivo* studies show that MACF1 deficiency is mainly associated with alteration in extra-synaptic myonuclei positioning and microtubules network organization, both preceding neuromuscular junction (NMJ) fragmentation[5-7].

This project will aim to develop an *in vitro* co-culture models between primary motoneurons extracted from rat and primary muscle fibers formed from extracted myoblasts from mice to decipher how this method enhance muscle fibers maturation, with a special focus on organelles trafficking.

Models and techniques:

- Culture of primary cells (rat/mouse)
- Differentiation
- Immunofluorescence
- Real-time imaging (by confocal microscopy)

References:

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