PhD Project
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Deciphering the role of new candidates in the control of skeletal muscle formation

Scientific context

Myonuclei positioning in myofibers: Muscle fibers, or myofibers, are syncytia composed of hundreds of post-mitotic myonuclei that share the same cytoplasm. The precise organization of myonuclei in fibers suggested the existence of MyoNuclear Domains (MNDs), where each myonucleus is responsible for gene expression in its surrounding cytoplasm and guarantees functional muscle integrity[1]. Myonuclear transcriptional flexibility (*i.e.* ability to regulate mRNA expression) is directly determined by the number of myonuclei in myofibers[2]. **The myonuclei organization thus guarantees a spatial coordination of the transcriptomic activity and is set by an interplay between various cytoskeleton proteins in which the microtubules (MTs) network appears as a key factor**[3]. In this context, the MT network is an essential actor in myofiber formation, elongation, maturation and function[4].

Preliminary data: We previously conducted a biochemical approach to decipher the proteome related to the microtubule network[5]. We already screened for more than 400 candidates using siRNA technique and isolate a few candidates that impact/control myonuclei alignment in developing muscle fibers such as MACF1[6], SH3KBP1[7] and NuMA1[8]. In this framework, 10 additional candidates still need to be characterized regarding their role in myofibers development. This will be the main subject of the PhD project.

Proposed project developed during the PhD

Validation and selection of candidates that impact microtubule-related pathways in muscle fibers. We already obtained the DNA constructs for the 10 candidates and their respective shRNA constructs (lentiviruses & plasmids). The first aim will be to decipher their roles along muscle fiber formation, using si/shRNA strategies to downregulate specifically the expression of the candidates. The impact of their depletion on key features related the microtubule network will be monitored. We will use immunofluorescence followed by confocal microscopy to evaluate roles on (1) myoblast fusion, (2) myonuclear domain characteristics, (3) microtubule network patterning and (4) Golgi apparatus & mitochondria spatial distribution. Microtubule assembly emanates mainly from the membrane of myonuclei in myotubes, a process that requires the relocalization of microtubule nucleator proteins together with the remodeling of the primary cilium from mononucleated myoblasts. We will thus investigate on the timely evolution of the localization of various compounds of the nuclear membrane and on the primary cilium (Pericentrin, gamma-Tubulin, AKAP450, Dynein motors, Nesprins, ARL13b, acetylated Tubulin). This approach will be complemented by an over-expression of the candidates during *in vitro* muscle fiber formation, with molecular tools allowing to track the localization of proteins (GFP tagged constructs). ***In fine*, this part of the project will allow the selection of a few candidates that impact muscle fiber formation.**

Interplay of candidates with organelles dynamics. According to the immunofluorescence approach results, we will select a limited number of candidates to evaluate their respective roles on the dynamics of organelles along muscle fiber formation using time-lapse video microscopy to track (1) myonuclear motility, (2) microtubule dynamics, (3) Golgi apparatus remodeling and (4) mitochondria dynamics. We will test how the overexpression of each candidate will locally affect the integrity of the myonuclei and microtubule dynamics during myofiber maturation, by using fluorescent reporters, such as lamin-Chromobody® for nuclei and EB1 (End Binding Protein 1) or CLIP170 (CAP-GLY domain containing linker protein 1) for microtubule. Golgi apparatus or mitochondria movement, kinetics and directionality will be tracked using specific dyes such as "SPY650-Golgi™" or "mitochondria-GFP CellLight™". This analysis will be performed at selected stages of Golgi apparatus/mitochondria/myonuclei co-maturation in fibers, in order to gather a picture of organelle dynamic changes subsequent to myonuclei maturation/microtubule alteration.

A role for specific candidate in muscle formation in vivo. For the most promising candidates defined *in vitro* (regarding potential implication in myonuclei architecture and microtubule-related function), their roles will be further addressed *in vivo* in WT mice by injecting AAV expressing either shRNA or overexpressing cDNA intramuscularly. shRNA sequences will be based on si/shRNA validated previously. AAV vectors (serotype1) constructs will be injected in the *Tibialis Anterior* muscle (muscle homeostasis and function analysis) or *Flexor Digitalis Brevis* muscle (myofiber coupling excitation/contraction analysis). Muscle parameters will be then analyzed to investigate on myofiber homeostasis and function.

Bibliography (red is publication of the team)

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