PhD Project
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Deciphering the role of NuMA1 in the formation & maintenance of skeletal muscle

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 (*ie* ability to regulate mRNA expression) is directly determined by the number of myonuclei in myofibers[2]. How each myonucleus reaches its optimal localization and shape along myofibers is a long-term process[3]. We and others have previously shown that an interplay between molecular motors, Microtubule-Associated Proteins (MAPs), and actin/microtubule network integrity is involved in myonuclei localization along muscle formation and maturation[4–6]. **However, the consequences of myonuclei positioning alterations in myopathies remains poorly understood.**

Preliminary data: Using a large siRNA screen on developing myotubes to find new candidates involved in the long-term organization of myonuclei spreading, we identified NuMA1 (Nuclear Mitotic Apparatus protein 1) as an essential new regulator of myonuclear spreading/anchoring in both developing and in mature myofibers. So far, NuMA1 was known to play an important role in dividing cells during mitotic phase[7]. In muscle fiber, we confirmed that NuMA1 is mainly localized inside myonuclei of myotubes/myofibers, but to our surprise, we discovered that it progressively accumulates in the cytoplasmic compartment where it regulates the microtubule network organization and thus, myonuclei positionning. **Our data point the activity of AMPK in the control of this NuMA1 cytoplasmic retention and attribute for the first time a specific cytoplasmic role for NuMA1 in post-mitotic myofiber formation and maintenance**[8] .We thus developed of new murine models where invalidation of NuMA1 (thanks to our $Numa1^{F/F}$ mouse model) is timely restricted to myofibers and confirmed the implication of NuMA1 on myofiber behaviors *in vivo* and highlighted various contribution regarding muscle fibers type and age.

Proposed project developed during the PhD

Deciphering the role of NuMA1 in the control of gene expression in myofibers. Although the cytoplasmic role of NuMA1 during mitosis event is well documented, its role inside the nucleus remains to be determined, especially in post-mitotic cells. We will thus analyze the transcriptomic profile of myofiber formation and maturation using the muscle-specific Numa1-cKO mouse model. We will perform single nucleus RNA-seq (snRNA-seq) to detect the specific transcriptome of a single myonucleus and will conduct a comparative gene expression analysis in muscle extracts obtained from $Numa1^{F/F;\ HSACRE}$ and $Numa1^{F/+;\ HSACRE}$ mouse. **This approach will allow to (i) decipher the impact of NuMA1 on mature muscle fiber transcriptional profile at the single nucleus level and (ii) will allow the comparison of myonuclei transcriptomes according to NuMA1 presence in myonuclei**.

Validation of detected differently expressed genes in myofibers. Cellular functions of mis-regulated genes will be addressed using *in vitro* formed myotubes/myofibers strategies. We will develop an RNA interference screen approach on a small set of selected NuMA1 putative target gene candidates and test their involvement in the early and late steps of myofiber formation, using *in vitro* primary myofiber differentiation set-up. As NuMA1 is closely linked to cytoskeleton and myonuclei organization, we will preferentially examine myonuclei architecture/localization and microtubule network in si/shRNA-mediated knockdown of selected "hits" in developing cultured myotubes. Analysis of myotubes/myofibers will consist in extracting parameters regarding (i) myonuclei spreading and shape, (ii) microtubule network organization and (iii) localization of proteins related to late maturation steps such as sarcomeric structures and t-tubule-related proteins organization. From this small screen, we will focus on relevant "hits" affecting extracted parameters along myotube/ myofiber maturation. Different tools are available in the team to analyze microtubule dynamics/patterning such as EB3-GFP, SiR-tubulin® and lamin-Chromobody®, to assess myonuclei shape/motility alteration in developing muscle fibers. **These methods, coupled with live video-microscopy will allow the quantification of different parameters related to microtubule/actin/myonuclei dynamics under the control of NuMA1.**

Implication of NuMA1 in MuSC activation. Muscle stem cells (MuSCs) are required for skeletal muscle maintenance, growth, and repair. Following MuSC activation, several factors drive asymmetric cell division to generate a stem cell and a proliferative progenitor that will contribute to form new muscle. The balance between symmetric self-renewal and asymmetric division significantly impacts the efficiency of regeneration. Using $NuMA1^{F/F}$; $PAX7^{CRE\text{-}ERT2}$ mice, NuMA1 will be timely and specifically invalidate in adult MuSC. **We will determine to what extent NuMA1 is important in muscle stem cell fate regulation (*i.e.* activation, proliferation, differentiation and self-renewal).**

Bibliography (red is publication of the team)

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