PhD Project
(Team MNCA/Supervisors: Dr. Sébastien & Dr. Gache) **Graduate Initiative MuSkLE**
(Muriel.sebastien@inserm.fr & Vincent.gache@inserm.fr)

Deciphering the role of tubulin isotypes in the formation & maintenance of skeletal muscle

Scientific context

Microtubules in myofibers: Microtubules are assemblies of αβ-tubulin heterodimers, that are needed all along a cell's life to support its morphology, intracellular transport, division or migration[1]. These cellular functions are all possible via the same polymer, thanks to a perfectly balanced regulatory pathway involving Microtubule-Associated Proteins (MAPs), and Tubulin Post-Translational Modifications (PTMs), known as the "Tubulin code"[2]. **How the Tubulin code balances all its players to drive specific microtubule patterns remains elusive.** In addition, these patterns could be controlled by the intrinsic properties of the microtubule's building blocks themselves: the tubulin heterodimers. In fact, several tubulin isotypes exist and are differently expressed in tissues and developmental stages[3]. The idea is now rising that microtubules actually support the sarcolemma and participate in mechanotransduction, notably thanks to their orthogonal grid organization[4]. **How the Tubulin isotypes composition drive muscle formation and functionnality is still pending.**

Preliminary data: A recent hypothesis suggests that microtubule lattice spacing (expanded or compacted) is an integral member of the Tubulin code. Indeed, the competitive behaviors of the expander drug paclitaxel and the compactor MAP Doublecortin (DCX) were observed and quantified in reconstitution assays and in cell lines[5]. In both model systems, the competitor that saturates the lattice, specifies the lattice spacing. In this way, the "winner" controls the general organization of the network: rectilinear when compacted or curved when expanded. **This work highlighted that tubulin heterodimers can produce different lattice spacing and therefore specify the regulation of the microtubule network.**

Proposed project to develop during the PhD

Deciphering the role of tubulin isotypes in the control of myofibers formation. We will characterize a mouse model in which a specific tubulin isotype is invalidated (sTubI-KO mice, already available in the Lab). First, we will evaluate histological differences of sTubI-KO and WT muscle fibers at different times of development: 1 month (muscle growing phase), 2 months (end of the muscle growing phase) and 4 months (muscle maturation phase). Specifically, we will monitor the establishment of myonuclei positioning in myofibers (myonuclei delocalization, centralization and spreading) using immunofluorescence on cross-sections and single extracted myofibers from muscles that differ in fiber type composition (i.e. *Tibialis Anterior, Soleus, Extensor Digitorum longus*, and *Gastrocnemius*), as previously described[6]. We will focus on several morphometric parameters reflecting myofiber homeostasis: i) fiber number, ii) fiber size (cross-sectional area (CSA)), iii) fiber typing (fast and slow fibers) and iv) fiber metabolism (e.g. succinate dehydrogenase staining). **These experiments will evaluate the impact of this specific isotype in the control of muscle fibers formation.**

Deciphering the role of tubulin isotypes in the control of myofibers functionality. We hypothesize that either the excitation-contraction coupling (ECC) process or the neuromuscular junction (NMJ) integrity will be affected. Indeed, in skeletal muscle, the ECC takes place in a subdomain of the sarcoplasmic reticulum (SR) that is physically linked to the microtubule network. As efficient ECC depends on a properly organized microtubule network[7], we will monitor voltage-activated SR Ca^{2+} release in *FDB* myofibers isolated from sTubI KO mice. The NMJ integrity is, in part, maintained through the local recruitment of microtubule subsets. We will thus investigate the potential implication of this specific tubulin isotype in NMJ integrity by immunofluorescence using super-resolution confocal microscopy. The physiological impact on force production will be measured on whole hindlimb muscles, using a non-invasive experimental setup. **Together, these experiments will evaluate the global impact of this specific tubulin isotype depletion on the establishment of functional myofibers and muscles.**

Implication of tubulin isotypes in MuSCs activation. Muscle stem cells (MuSCs) are required for skeletal muscle maintenance, growth, and repair. Following MuSC activation, several factors drive cell division to generate a stem cell and a proliferative progenitor that will contribute to form new muscle. Using sTubI KO mice, **we will determine to what extent this specific tubulin isotype is important in muscle stem cell fate regulation (*i.e.* activation, proliferation, differentiation and self-renewal) using *in vitro*[6,8,9] , *ex-vivo*[10,11] and *in vivo*[6] approaches to myofiber formation (*e.g.* induction of muscle regeneration process *in vivo* through cardiotoxin injection).**

Bibliography (In red are publications from team members)

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