Internship offer
M2 Musculo-Skeletal system, Locomotion, Exercise (MuSkLE)

Title of the Internship: **Role of TGF-beta signalling in connective tissue precursors for skeletal muscle patterning**

Laboratory : **Institut de Génomique Fonctionnelle de Lyon (IGFL / ENS Lyon), CNRS UMR 5242**

https://igfl.ens-lyon.fr/

Research team : **"Developmental and Evolutionary Histories of Vertebrates" (DEHV)**

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Project description

Muscle-associated connective tissue (MCT) progenitors have been identified as key regulators of skeletal muscle patterning (Nassari et al., 2017; Sefton & Kardon, 2019). Defects in MCT communication with adjacent muscle progenitors have been suggested to underlie some human congenital disorders affecting the musculoskeletal system (Sefton & Kardon, 2019). The morphogenetic determinants implicated in the interaction between MCT and muscle progenitors are still to be investigated. It has been shown that the MCT originates from different embryonic populations throughout the body plan (Heude et al., 2018; Nassari et al., 2017). At head and limb levels, studies have reported that TGFβ signalling is required in MCT progenitors for proper muscle formation (Blitz et al., 2013; Hosokawa et al., 2010). However, it is unclear whether TGFβ signalling pathway and MCT progenitors act equivalently along the axis for muscle patterning during development.

The present project aims to characterize the cellular and molecular determinants engaged in the communication between MCT and muscle progenitors for proper skeletal muscle patterning, that could be defective in TGFβ-related connective tissue-associated pathologies.

To this end, we will make use of the genetic *Cre-lox* tool available in the mouse to perform conditional invalidation of TGFβ signalling in MCT progenitors of different embryonic origins. Embryos and fetuses will be collected at key stages of muscle development for phenotypic and molecular analyses. Molecular analyses will be completed in both muscle and MCT progenitors and derivatives to study the morphogenetic defects resulting from TGFβ invalidation. Specific markers of muscle and MCT specification and differentiation will be investigated by single molecule fluorescence *in situ* hybridization (smFISH) and immunofluorescence stainings *in toto* (for early embryonic stages) and on serial sections (for later fetal stages). The phenotype of perinatal foetuses will be investigated by classical histological and immunostaining analyses on serial sections. Given the role of MCT derivatives and of TGFβ in controlling myofiber type (Hosokawa et al., 2010; Mathew et al., 2011), we will investigate how TGFβ inactivation affect the content in slow and fast muscle fibers during fetal muscle development, together with muscle and MCT extracellular matrix proteins (eg. Laminin, Sox9, Tcf4, Tnc, Scx, Collagens). Analyses will be then acquired by high-resolution epifluorescence, confocal fluorescence or lightsheet imaging depending on applications at the IGFL & ENS Imaging Platforms on site.

Understanding the function of TGFβ signalling pathway in coordinating musculoskeletal development in our mouse models will be crucial to elucidate the morphogenetic processes underlying TGFβ-related human congenital disorders such as Klippel-Feil, Marfan, Loeys-Dietz, or Myhre syndromes.

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References:

Blitz, E., Sharir, A., Akiyama, H., & Zelzer, E. (2013). Tendon-bone attachment unit is formed modularly by a distinct pool of Scx- and Sox9-positive progenitors. *Development*, *140*(13), 2680-2690. https://doi.org/10.1242/dev.093906

Heude, E., Tesarova, M., Sefton, E. M., Jullian, E., Adachi, N., Grimaldi, A., Zikmund, T., Kaiser, J., Kardon, G., Kelly, R. G., & Tajbakhsh, S. (2018). Unique morphogenetic signatures define mammalian neck muscles and associated connective tissues. *Elife*, *7*. https://doi.org/10.7554/eLife.40179

Hosokawa, R., Oka, K., Yamaza, T., Iwata, J., Urata, M., Xu, X., Bringas, P., Jr., Nonaka, K., & Chai, Y. (2010). TGF-beta mediated FGF10 signaling in cranial neural crest cells controls development of myogenic progenitor cells through tissue-tissue interactions during tongue morphogenesis. *Dev Biol*, *341*(1), 186-195. https://doi.org/10.1016/j.ydbio.2010.02.030

Mathew, S. J., Hansen, J. M., Merrell, A. J., Murphy, M. M., Lawson, J. A., Hutcheson, D. A., Hansen, M. S., Angus-Hill, M., & Kardon, G. (2011). Connective tissue fibroblasts and Tcf4 regulate myogenesis. *Development*, *138*(2), 371-384. https://doi.org/10.1242/dev.057463

Nassari, S., Duprez, D., & Fournier-Thibault, C. (2017). Non-myogenic Contribution to Muscle Development and Homeostasis: The Role of Connective Tissues [Review]. *Front Cell Dev Biol*, *5*, 22. https://doi.org/10.3389/fcell.2017.00022

Sefton, E. M., & Kardon, G. (2019). Connecting muscle development, birth defects, and evolution: An essential role for muscle connective tissue. *Curr Top Dev Biol*, *132*, 137-176. https://doi.org/10.1016/bs.ctdb.2018.12.004

Skills required:

- Background in Developmental Biology
- Knowledge in mouse genetics
- Skills in classical histology and molecular biology
- High resolution fluorescence imaging