Role of TGFβ signalling in connective tissue progenitors during musculoskeletal assembly

The coordinated development of skeletal muscles and their associated connective tissues (skeleton, tendons, fascias) is essential for the formation of an integrated and functional musculoskeletal system. Muscle-associated connective tissue (MCT) progenitors have emerged as key regulators of muscle patterning, differentiation, and integration within the body axis. Defects in interaction between MCT and muscle progenitors have been implicated in a range of congenital human disorders affecting the musculoskeletal system[1]. However, the cellular and molecular processes underlying these interactions remain elusive.

MCT progenitors originate from distinct embryonic populations along the body axis. In the head, most MCT progenitors derive from the neural crest, while deriving from the somitic mesoderm in the trunk and from the lateral plate mesoderm in the limb[2]. The neck constitutes a transition zone of hybrid origin at the interface of head, trunk and limb fields[3]. In both head and limb regions, previous studies have demonstrated that TGFβ signalling pathway is required within MCT progenitors for the proper formation of the musculoskeletal system[4,5]. Whether TGFβ signalling acts in a comparable manner in MCT progenitors derived from distinct embryonic sources along the axis, and how this pathway contributes to muscle morphogenesis in distinct anatomical contexts, remain open questions.

This PhD project aims to investigate the role of TGFβ signalling in MCT progenitors during embryonic and foetal musculoskeletal development using the mouse as a model system. The project will rely on Cre–loxP–based genetic approaches to perform lineage tracing and conditional inactivation of TGFβ signalling in targeted embryonic lineages.

First, the spatiotemporal organization of musculoskeletal progenitors will be characterized through genetic fate-mapping analyses, with particular attention to the relative positioning and spatial interactions between connective tissue and muscle progenitors during specification and differentiation. In a second phase, the functional consequences of disrupting TGFβ signalling in MCT progenitors of distinct embryonic origins will be assessed through conditional deletion of the TGFβ receptor 2 (TgfβR2) in connective tissue progenitors of neural crest or lateral plate mesoderm origin. Mutant embryos and fetuses will be analyzed at tissue, cellular, and molecular levels to determine how altered TGFβ signalling impacts muscle patterning, connective tissue organization, and overall musculoskeletal assembly.

To identify gene regulatory networks downstream of TGFβ signalling during musculoskeletal development, the project will integrate high-throughput single-cell multiomic approaches. Single-cell RNA sequencing and chromatin accessibility profiling (scRNA-seq + ATAC-seq) will be combined to characterize transcriptional programs and regulatory landscapes in connective tissue and muscle progenitors. Integrative analyses will allow the identification of candidate downstream effectors mediating TGFβ-dependent crosstalk between these cell populations. Candidate genes will be validated *in vivo* through spatiotemporal expression analyses and *ex vivo* using gain- and loss-of-function experiments in co-culture systems combining primary connective tissue cells and myoblasts.

Together, these approaches will provide mechanistic insights into how TGFβ signalling coordinates connective tissue–muscle interactions during mammalian musculoskeletal morphogenesis in different anatomical contexts. The results are expected to improve our understanding of the developmental origins of congenital connective tissue disorders associated with dysregulation of the TGFβ pathway, including Klippel–Feil, Marfan, Loeys–Dietz, and Myhre syndromes.

The doctoral candidate will be integrated into a stimulating interdisciplinary and international research environment and will benefit from the complementary expertise and state-of-the-art technological platforms available within the host team and institute, including high-resolution imaging (confocal, light-sheet, and micro-CT scan), single-cell multiomics facilities, and dedicated bioinformatics support.

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References. [1] Sefton, E. M. & Kardon, G. *Current topics in developmental biology* 132, 137-176 (2019). [2] Nassari, S. *et al. Frontiers in cell and developmental biology* 5, 22 (2017). [3] Heude, E. *et al. eLife* 7 (2018). [4] Blitz, E. *et al. Development* 140, 2680-2690 (2013). [5] Hosokawa, R. *et al. Developmental biology* 341, 186-195 (2010)

mouse embryo

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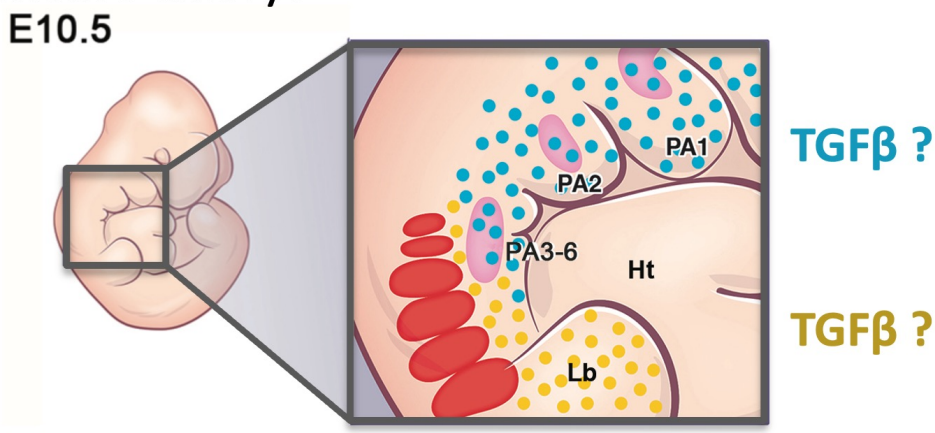

TGFβ ?

TGFβ ?

cardiopharyngeal mesoderm (CPM)

somitic mesoderm (SM)

lateral plate mesoderm (LPM)

cranial neural crest (CNC)