LABORATORY OF TISSUE BIOLOGY AND THERAPEUTIC ENGINEERING
CNRS-UMR5305

M2- CANDIDATE

Mechanoregulated genes in articular chondrocytes and mechanically-driven pathogenesis of osteoarthritis

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Background:
Biomechanical factors are essential for the regulation of the musculoskeletal system in both normal and pathological situations. For example, in conditions such as osteoarthritis (OA), tendinopathies, muscle atrophy and intervertebral disc degeneration, the extracellular matrix (ECM) microenvironment transmits abnormal mechanical signals to cells in these load-bearing connective tissues.
The main function of articular cartilage (AC) is to facilitate joint movement and absorb shocks. Thus, under physiological conditions, articular surfaces are subjected to mechanical stimuli, of which compression, fluid shear stress, hydrostatic and osmotic pressure are highly represented. Chondrocytes are surrounded by the pericellular matrix (PCM), which transmits chemical and biomechanical signals to the cells. Abnormal mechanical load of the PCM triggers metabolic changes in chondrocytes causing ECM loss and tissue degeneration, eventually leading to OA[1, 2]. To date, it is still not fully understood how chondrocytes respond to mechanical stimuli in relation to cell signaling, a cellular mechanism known as mechanotransduction[3].
Project: this project aims to better understand how chondrocytes respond to mechanical forces. Although recent studies indicate mechanosensitive ion channels such as TRP vaniloid-1, Piezo-1 and -2 as crucial responders for mechanical stress, conditional ablation of the encoding genes does not significantly change the severity of OA in a mechanical murine model induced by destabilization of the medial meniscus (DMM). As an attempt to identify additional players of cartilage mechanobiology, we will investigate for the first time the role of integrin α10β1, an integrin receptor most abundantly expressed in cartilage[4].
Through *in vitro* approach, we developed a cellular model to investigate the conversion of mechanical forces into biochemical signaling in 3D chondrocyte cultures. Mouse chondrocytes embedded in agarose disks are submitted to dynamic compression in a compression bioreactor (Flexcell system). This protocol allows us to analyze the effect of compression on mRNA level by genome-wide gene expression analysis, and on the phosphorylation state of signaling molecules[5,6] by proteomic approaches. This mechanosensitive system will be characterized through live-imaging and spatial omics analyses.

These studies will pave the road for further identification of novel mechanosensitive genes and will reveal intracellular signaling activated by dynamic compression in chondrocytes and will preclude the *in vivo* part of the project.

Missions and tasks: the student will have to isolate chondrocytes from knee joint or rib cages of WT/genetically modified mice for their further use as primary culture in 3D. Dynamic compression will be applied to these organoid-like structures. Following cell infection with several lentiviral constructs, we will measure the activation of mechanosensitive signaling such as YAP-TAZ pathways and investigate spatial gene expression. This approach will then be applied to cartilage explants and compared with joints under normal/abnormal mechanical load.

Skills: the master student will be able/taught to culture cell, including primary culture and 3D culture technique following cartilage dissection. He/she will analyse bulk and single cell gene expression (qPCR, smFISH), perform protein detection (confocal/light sheet microscopy), as well as various cell function assays (matrix deposition, metabolic activity).

References

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