PASTEUR PARIS UNIVERSITE (PPU) INTERNATIONAL DOCTORAL PROGRAM 2020

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PROJECT

- **FILE #09**
- **ACRONYM:** OASIS
- **TITLE:** Understanding FGF signaling to treat spinal defects

LABORATORY

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LABORATORY PRESENTATION AND RESEARCH TOPICS

- **SUPERVISOR HDR:** Legeai-Mallet Laurence, laurence.legeai-mallet@inserm.fr

- **SPECIFY THE TEAM NAME:** Molecular and physiopathological bases of osteochondrodysplasia

- **WEBSITE OF THE TEAM:** https://www.institutimagine.org/fr/186

DESCRIPTION OF THE PROPOSED PROJECT

- **KEYWORDS:** Axial skeleton, Intervertebral disc, Fibroblast Growth Factor Receptor 3, dwarfism

- **ABSTRACT**

The goal of this project is to provide an in-depth understanding of FGFR3 (Fibroblast Growth Factor Receptor 3) function in axial skeleton formation, in the context of a FGFR3-related dwarfism, hypochondroplasia (HCH). We will focus on intervertebral disc (IVD) changes and vertebral canal stenosis. Recently, in the first *Hch* mouse model (*Fgfr3*$^{N534K/+}$) generated, defects in the structure of the vertebral bodies and intervertebral discs (IVD) were observed (unpublished data). First, we will characterize the cellular defects in the spine of our *Hch* mouse model. We will perform molecular analysis of key markers expressed in the IVD during development and growth on *Fgfr3*$^{N534K/+}$ to decipher the IVD changes and spinal stenosis in relation to abnormal FGFR3 signaling. We will quantify the quality of the bone using uCT analysis and MRI. Secondly, we will decipher the regulatory network of FGFR3 function in the spine in health and disease. To gain a global understanding of the changes in gene and protein expressions, we will perform single cell, RNA-sequencing of the different zones of cartilage endplate and the IVD of *Hch* mice. We will use the 10x Genomics Chromium platform to generate high-throughput single cell RNA-seq data. Then, we will determine the role of endplate hypertrophic chondrocytes in intervertebral disc pathogenesis for HCH patients. We will assess the impact on the transition and fate of hypertrophic chondrocytes in the IVD using specific *Hch* mouse models, and we will study the impact on disc biology with a quantitative assessment of the changes. Cell fate mapping using an inducible Col10a1-CreERT2 mouse will allow an assessment of the hypertrophic chondrocytes to annulus fibrosus cells. It will be critical to understand the impact in the context of excessive FGFR3 signaling at this region and the cellular transition from hypertrophic chondrocytes to annulus fibrosus cells. Finally, we will perform preclinical studies of drug efficacy for treating spine defects in *Hch* mouse model. To evaluate the benefit effects of drugs (e.g. tyrosine kinase inhibitors, CNP etc..) on axial

skeleton, we will treat young and adult animals during short and long period. The efficacy will be evaluated during the development using radiography for *in vivo* imaging to monitor the improvement of scoliosis, stenosis and other aspects of the vertebrae during the treatment.

- **DESCRIPTION OF THE PROJECT**

The goal of this PhD International project is to provide an in-depth understanding of FGFR3 function in axial skeleton formation, in the context of a FGFR3-related dwarfism, hypochondroplasia (HCH). Impaired growth of long bones due to excessive activation of FGFR3 signaling are well studied but not for spinal defects such as intervertebral disc (IVD) changes and vertebral canal stenosis. Recently, in the first *Hch* mouse model (*Fgfr3*$^{N534K/+}$) generated defects in the structure of the vertebral bodies and intervertebral discs (IVD) were observed (unpublished data).

We have four different objectives. 1) **To characterize the cellular defects in the spine of our *Hch* mouse model**. A molecular analysis of key markers expressed in the IVD during development and growth will be key in deciphering the IVD changes and spinal stenosis in relation to abnormal FGFR3 signaling. Heterozygous *Fgfr3*$^{N534K/+}$ embryos at E11.5-E18.5 will be analyzed to study the impact during early IVD formation, focusing on histological changes with molecular markers for the cartilage endplate, nucleus pulposus and annulus fibrosus cells; postnatally, we will perform similar histological and cell marker analyses, focusing on the progression of lumbar lordosis during development and aging. As hypertrophic chondrocytes can also transit to osteoblasts in endochondral ossification, it will be important to focus on the mineralization of the vertebral bodies. We will perform histological analysis on undecalcified bones in to study the micro-architecture of bone. We will quantify the quality of the bone using uCT and MRI analysis.

2) **To decipher the regulatory network of FGFR3 function in the spine in health and disease**. To gain a global understanding of the changes in gene and protein expressions, we will perform single cell, RNA-sequencing of the different zones of cartilage endplate and the IVD of *Hch* mice. We will use the 10x Genomics platform to generate high-throughput single cell RNA-seq data.

3**) To determine the role of endplate hypertrophic chondrocytes in intervertebral disc pathogenesis for HCH patients**. We will assess the impact on the transition and fate of hypertrophic chondrocytes in the IVD using specific *Hch* mouse models, and we will study the impact on disc biology with a quantitative assessment of the changes. Cell fate mapping using an inducible Col10a1-CreERT2 mouse will allow an assessment of the epithelial to mesenchymal transition (EMT) process in the transition of hypertrophic chondrocytes to annulus fibrosus cells. It will be critical to understand the impact in the context of excessive FGFR3 signaling at this region and the cellular transition from hypertrophic chondrocytes to annulus fibrosus cells.

4) **To perform preclinical studies of drug efficacy for treating spine defects in *Hch* mouse model.** To evaluate the benefit effects of drugs (e.g. tyrosine kinase inhibitors, CNP etc..) on axial skeleton, we will treat young and adult animals during short and long period. The efficacy will be evaluated during the development using radiography for *in vivo* imaging to monitor the improvement of scoliosis, stenosis and other aspects of the vertebrae during the treatment. We will also incorporate a newly developed ELISA for the serum collagen type X marker (CXM) for

monitoring the growth response and timing of the treatment. This will provide vital comparison of the therapeutic outcomes of the drugs for spine treatments.

The significant anomalies of the 2 major components of IVD observed in HCH will contribute to decipher the fine mechanism controlling the IVD homeostasis. Degenerative disc disease is significant component of spine disorders that now afflicts nearly 1/3 of the adult population, improving patient care has become a priority for all physicians. This project could ultimately decipher the complete biological pathogenesis process and provide better treatments. Importantly, we will gain an understanding of the spine/IVD response to potential drug treatments that are developing or in clinical trials.

- **REFERENCES**

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EXPECTED PROFILE OF THE CANDIDATE

- **EXPERIENCE REQUIRED**
  Cellular biology, biology molecular, mouse model experiment