Erasmus Traineeship - Detailed Program

Trainee: Laura Martínez Rábade

Supervisors: Mr. Hani N. Alsafadi and Dr. Darcy E. Wagner

Project Title:

Identification of Yap/Taz co-transcriptional factor binding partners using state of the art bioinformatics tools

Rationale: Idiopathic pulmonary fibrosis (IPF) is a lethal lung disease that is characterised by repetitive injury resulting in scaring of the distal lung tissue and the lung epithelium is the major site of injury. IPF has no cure and available therapies can only slow down the disease. We and others have previously shown that the mechanical signalling pathway, Hippo signalling, and its main effectors, Yap/Taz, are dysregulated in IPF. Yap/Taz are co-transcription factors that do not bind directly to DNA but exert their target gene expression though binding to other transcription factors (TFs). Hippo-Yap/Taz control a wide range of homeostatic biological processes such as differentiation, proliferation, migration, and organ size. While there is increased activity of Yap/Taz, the exact TF binding partners are not known in the context of pulmonary fibrosis. To address this, we have performed chromatin immunoprecipitation sequencing (ChIP-Seq) in several mouse lung epithelial cell types to identify exact target gene motifs that are activated. The aim for this traineeship project is to identify the TFs bound to the identified motifs using state-of-the-arts bioinformatics tools. The resulting TFs will be validated against publicly available single cell dataset to confirm their role in a cell specific context. Currently, there are several hallmark single cell datasets obtained from human normal and fibrotic cells. Results from this study will aid in defining disease causing TF interaction that could be therapeutically targeted.

Approach:

ChIPseq data is obtained from freshly isolated primary alveolar and tracheal epithelial cells from normal and fibrotic mice. The proposed analysis will be performed through the following sub-aims:

1. Transcription factor (TF) identification using motif analysis using CiiiDER and TRANSFAC databases. CiiiDer is a recently developed tool used to identify potential binding sites of provided sequences; TRANSFAC is the largest TF curated database that has motif specific information.
2. Validation of identified transcription factors. TFs will be validated by imaging of epithelial cells and lung tissues to confirm the co-expression of the TFs and Yap/Taz.
3. Establish workflows for TF cell specific signature using single cell datasets.

Expected outcome/Objectives:

The trainee will be taught several techniques including:

- Cell culture techniques; primary cell isolation from mouse lungs using newly developed techniques.
- Imaging techniques: Brightfield imaging, Fluorescence Imaging, high content Imaging
- Bioinformatics:
    - The use of high-powered computing clusters (HPC)
    - Single cell data analysis
    - Transcription factor Motif analysis
    - R statistical and graphing packages.

At the end of the traineeship, the trainee is expected to show sufficient knowledge of the performance and troubleshooting of these techniques.

Preliminary timeline:

	February 2021	March 2021	April 2021	May 2021
Introduction/training				
Sub-aim 1				
Sub-aim 2				
Sub-aim 3				
Writing