Erasmus Traineeship - Detailed Program

Trainee: Pablo Ovejero Romero

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

Project Title:
Generation of an inducible Yap/Taz knockout in immortalized murine lung epithelial cells using CRISPR technology

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. New ways to approach treatment of this disease are needed. Fibrotic lung tissue has been shown to have increased stiffness. We have shown that the mechanical signalling pathway, Hippo signalling, and its main effectors, Yap/Taz, are dysregulated in IPF. However, the exact roles are not widely understood. 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). We have previously explored the role of Yap/Taz through silencing RNA methods. However, Yap/Taz are required for cell survival and changes in their activity occurs quickly, i.e. within minutes. Therefore, a conditional system is required to study the role of Yap/Taz in lung epithelial cells. The aim of this project is to generate a stable cell line with an inducible Yap/Taz knock-out. CRISPR-cas9 has been shown to produce stable modifications to many biological systems making it the perfect choice to achieve this aim.

Approach:
Commercially available immortalized mouse lung epithelial cells (MLE-12) will be used to create the inducible knockout line. This project will be carried out in 3 major sub-aims:

1. *Generation of an inducible GFP reporter using CRISP*. To achieve this goal, first the system must be validated by using a fluorescence reporter that can be activated using a stimulus such as doxycycline.
2. *Generation of an inducible Yap/Taz knockout*. The knockout will be generated using the approach implemented in sub-aim1.
3. *Validation of the inducible knockout in a fibrotic environment.* To validate the resulting cell line, the new cells will be treated with a fibrotic stimulus (Transforming Growth Factor Beta (TGF-β)) and cellular response will be evaluated with/out induction of the knockout.

Expected outcome/Objectives:

The trainee will be taught several techniques including:

- Cell culture techniques.
- CRISPR
- Imaging techniques: Brightfield imaging, Fluorescence Imaging, high content Imaging
- Molecular techniques: Western-blotting, qPCR, Luciferase reporter assays.

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				