BCHM 421/422 – 2020/2021

Project Outline:

Pulmonary arterial hypertension (PAH) is a fatal disease affecting the blood vessels of the lung. The disease is marked by the excessive growth of pulmonary vascular cells (1), leading to obstruction of the lung microvasculature, increased resistance to pulmonary blood flow and eventual death due to right-sided heart failure. PAH is strongly linked to mutations in *BMPR2*, the gene encoding the type II bone morphogenetic protein receptor (BMPR-II) (2). Work in the Ormiston lab is focused on how the therapeutic delivery of specific proteins that bind this receptor, such as bone morphogenetic protein 9 (BMP9), can be used to treat PAH by enhancing BMPR-II signaling in the endothelial cells that line the inner surface of the blood vessels in the lungs (3).

Previous studies have shown that BMP9 therapy selectively inhibits the proliferation of healthy endothelial cells and reverses disease in established rodent models of PAH. However, recent work in endothelial cells from PAH patients has shown that the BMP9 response is reversed in these cells, resulting in excessive proliferation instead of growth suppression (4). We have shown that this shift is caused by reduced BMPR-II expression, which diverts the signaling of BMP9 through an alternative receptor, activin receptor IIb (ActR-IIb).

The project will focus on designing a biased form of BMP9 that signals through BMPR-II, but not ActR-IIb. This work will use a peptide array-based screen to identify the specific segments of the BMP9 protein that are critical to binding ActR-IIb. These segments will then be modified through selective amino acid substitution, with the ultimate goal of eliminating the affinity of the peptide for ActR-IIb, with minimal disruption to any interaction with BMPR-II, should such an interaction exist in the native peptide sequence. The discoveries gained from this project will be used to design an engineered form of full-length BMP9 protein that selectively binds BMPR-II. This modified protein will then be tested as an experimental PAH therapeutic that improves upon the proven efficacy of native BMP9 in the treatment of animal models of the disease.

Supervisor: Mark Ormiston

Project Title: Examining the contribution of Activin Type II Receptors to the pathological endothelial cell phenotype in pulmonary arterial hypertension

Project Goals:

1) Acquire proficiency with the array printer system.
2) Screen initial arrays for interactions with the extracellular domains of ActR-IIb and BMPR-II.
3) Perform iterative modification of candidate peptide sequences to selectively eliminate the binding affinity of the peptide for ActR-IIb.

4) Validate results from the array system using both modified and native peptide sequences by surface plasmon resonance.

Experimental Approaches:

1) Operation of the Intavis MultiPep CF Cellu-SPOT peptide array printer
2) Design and screening of custom peptide arrays, based on the sequence of BMP9
3) Logical design of modified peptide sequences, intended to disrupt BMP9-ActR-IIb interactions
4) Use of surface plasmon resonance to assess and quantify protein-protein interactions

References:

1. Paulin, R. & Michelakis, E.D. The metabolic theory of pulmonary arterial hypertension. *Circ Res* 115, 148-164 (2014).
2. Machado, R.D*., et al.* Mutations of the TGF-beta type II receptor BMPR2 in pulmonary arterial hypertension. *Hum Mutat* **27**, 121-132 (2006).
3. Long, L., Ormiston, M.L*., et al.* Selective enhancement of endothelial BMPR-II with BMP9 reverses pulmonary arterial hypertension. *Nat Med* 21, 777-785 (2015).
4. Theilmann A.L. *et al.* Endothelial *BMPR2* loss drives a proliferative response to BMP9 in pulmonary arterial hypertension. *ATVB*. In revision