BCHM 421/422 – 2018/2019

Project Outline: Pulmonary arterial hypertension (PAH) is a disease of occlusive vascular remodeling in the pulmonary circulation that causes right heart failure and death within 3-5 years of diagnosis[1]. Mutations in *BMPR2*, the gene encoding the bone morphogenetic protein (BMP) type II receptor (BMPR-II) account for 70-80% of heritable PAH and roughly 20% of seemingly idiopathic cases of disease[2]. Previous work by our group has demonstrated that signaling of the endothelial-selective[3] BMP ligand, BMP9, is impaired in PAH patients exhibiting reduced BMPR-II expression, resulting in excessive endothelial cell proliferation. We hypothesize that this proliferative response is the result of BMP9 signaling through an alternative type II receptor, namely the Activin type II receptor (ActR-II), in the absence of BMPR-II.

The SSP student will be responsible for testing this hypothesis using the siRNA-mediated knockdown of ActR-II in cultured pulmonary endothelial cells. ActR-II knockdown will be confirmed by qPCR and western blotting. The proliferative response to BMP9 will be assessed in endothelial cells following treatment with siRNAs targeting ActR-II or non-targeting siRNA controls, with or without concomitant siRNA-mediated knockdown of BMPR-II. If this initial set of experiments validates a role for BMP9 signaling via ActR-II in endothelial hyperproliferation, subsequent studies will involve the screening of inhibitory peptides (~15 amino acids, based on segments of the full-length BMP9 protein) that selectively block the interaction of BMP9 with ActR-II, but leave BMP9-BMPR-II interactions unaltered. Peptides selected from this screen will be tested in vitro for their ability to block BMP9-induced endothelial proliferation in the context of siRNA-mediated BMPR-II silencing.

This work will be conducted using infrastructure in the Ormiston lab, as well as the peptide arrayer housed in the Queen's Cardiopulmonary Unit (Q-CPU), a new, $10-million facility for translational research in diseases of the heart, lung and circulatory system.

Supervisor: Mark Ormiston

Project Title: High throughput screening of inhibitory peptides targeting the interaction of BMP9 with the Activin type II receptor

Keywords (3-5):

1. **Vascular biology**
2. **Bone morphogenetic protein signaling**
3. **High throughput peptide array screening**
4. **Animal models**
5. **Cell culture**

Project Goals:

1. Confirm ActR-II as the receptor responsible for the hyperproliferative effect of BMP9 in endothelial cells lacking BMPR-II.
2. Screen an array of peptides for targets that can selectively inhibit interactions of BMP9 with ActR-II, but not BMPR-II-BMP9 interactions.

3. Validate the inhibitory effect of selected peptides on endothelial cell growth in endothelial cells lacking BMPR-II.

Experimental Approaches: Primary endothelial cell culture, siRNA knockdown, qPCR, western blotting, proliferation assays, peptide array screening

References:

1. Benza RL, Miller DP, Barst RJ, Badesch DB, Frost AE, McGoon MD. An evaluation of long-term survival from time of diagnosis in pulmonary arterial hypertension from the reveal registry. *Chest*. 2012;142(2):448-456. doi:10.1378/chest.11-1460.
2. Machado RD, Aldred MA, James V, *et al*. Mutations of the TGF-β type II receptor BMPR2 in pulmonary arterial hypertension. *Hum Mutat*. 2006;27(2):121-132. doi:10.1002/humu.20285
3. Long L, Ormiston ML, Yang X, *et al*. Selective enhancement of endothelial BMPR2 with BMP9 reverses pulmonary arterial hypertension. *Nat Med*. 2015;21(7):777-785. doi:10.1038/nm.3877.