BCHM 421/422 – 2021-2022

Project Outline:

Supervisor: Mark Ormiston

Project Title: Manipulating blood vessel growth in the lung using bone morphogenetic proteins

Project Goals:

The bone morphogenetic proteins (BMP) are a family of growth factors that regulate many cellular processes. One member of this family, BMP9, is known to circulate in the blood and influence endothelial function, vascular homeostasis, and angiogenesis[1,2]. Research in the Ormiston lab is focused on the contribution of BMP9 signaling to pulmonary arterial hypertension (PAH), a deadly disease of obstructive vascular overgrowth that is associated with mutations in the gene encoding type II BMP receptor (*BMPR2*). Previous work in our lab has shown that *BMPR2* loss results in a shift in the endothelial BMP9 response, from the maintenance of vascular stability to the promotion of excessive proliferation[3]. Ongoing work in the lab is focused on understanding the mechanisms underlying this shift, as a means to create molecularly tailored therapies that target dysregulated blood vessel growth. This work is relevant to both PAH and other diseases of uncontrolled vascular growth, such as the vascularization of lung tumors.

The 421/422 student will explore the impact of *BMPR2* loss on downstream BMP9 signaling in the pulmonary endothelium. Recent studies have suggested that the treatment of human pulmonary artery endothelial cells (HPAECs) with BMP9 induces oscillatory signaling via the SMAD family of transcriptional mediators[4]. More importantly, the manipulation of contributors to this signaling pathway can change the frequency of oscillations, resulting in altered angiogenic responses.

The student will use primary endothelial cell culture models and siRNA silencing to study the impact of *BMPR2* loss on the oscillatory response of HPAECs to BMP9 treatment. Immunoblotting and qPCR will be used to examine changes in downstream signaling at the gene and protein level to determine the key regulators of this feedback mechanism. Subsequent work will involve the creation of a bimolecular fluorescence complementation (BiFC) construct to monitor signal oscillations in real time[5]. Briefly, the construct will encode C- and N-terminal fragments of the VENUS fluorescent protein, tethered to discreet SMAD intracellular mediators. Introduction of this construct into endothelial cells will allow for the induction of transient fluorescence exclusively when the SMAD subunits interact and signaling is activated. This reporter will enable the real-time monitoring of signaling kinetics in live cells, providing critical information on the impact of specific cellular manipulations on the regulation of vascular growth.

Experimental Approaches:

qPCR, immunoblotting, siRNA and molecular cloning to create the BiFC reporter construct

References:

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