Spring 2023 Seminar Abstract

Kori Kruegel

Master of Biology – Cell and Molecular Concentration

The G-protein, Rap1b, plays a crucial role in mediating the survival and function of megakaryocytes. Its involvement in integrin activation, which facilitates adhesion and migration, as well as its ability to activate ERK, a transcription factor crucial for proliferation and differentiation, underscores its significance. The phosphorylation of Rap1b by cAMP-dependent protein kinase (PKA) determines its intracellular localization, with unphosphorylated Rap1b associating with the cell membrane and phosphorylated Rap1b diffusing throughout the cytosol. Our hypothesis is that unphosphorylated Rap1b is involved in cell adhesion, while phosphorylated Rap1b is crucial for cell proliferation and differentiation.

Looking at a broader scope, we aim to investigate link between diabetes and cardiovascular events and the connection to Rap1b. In doing so, we focus on the presence of PGE2 in the bloodstream of diabetics. PGE2 binds to EP3 receptors found in platelets and megakaryocytes, potentially activating Rap1b and increasing platelet production, which could contribute to cardiovascular events. To test this hypothesis, we utilize the EP3 agonist sulprostone, examining its effect on Rap1b activation using the Rap1b-Fluorescent Activity REporter (Rap1b-FLARE) in DAMI megakaryocytic cells. Through confocal microscopy, flow cytometry, and biochemical pull-down assays, we assess the localization and extent of Rap1b activation in response to sulprostone treatment. Additionally, we investigate downstream effects by examining phosphorylated ERK levels and conducting proliferation assays.

By elucidating the signaling pathways involving Rap1b, we aim to gain a deeper understanding of its role in megakaryocyte function.