



**COLLEGE of
BIOLOGICAL SCIENCE**

DEPARTMENT OF MOLECULAR
AND CELLULAR BIOLOGY

Announcement:

*All interested members of the university community are invited to attend the Final Oral Examination for the degree of **Doctor of Philosophy** of*

OLIVIA GRAFINGER

on Tuesday, September 1, 2020 at 1:30 p.m. (online)

Thesis Title: **The role of beta-1 integrin signaling in regulating the phosphorylation and recycling of MT1-MMP at invadopodia**

Examination Committee:

Dr. Ray Lu, Molecular and Cellular Biology (Exam Chair)
Dr. Marc Coppolino, Dept. of Molecular and Cellular Biology
Dr. Nina Jones, Dept. of Molecular and Cellular Biology
Dr. Alicia Vilorio-Petit, Dept. of Biomedical Sciences
Dr. Hon Leong, Dept. of Medical Biophysics, Sunnybrook
Research Institute (External Examiner)

Advisory Committee:

Dr. Marc Coppolino (Adv.)
Dr. Jim Uniacke
Dr. Nina Jones

Abstract: Malignant cancer cells can invade extracellular matrix (ECM) through the formation of F-actin-rich subcellular structures, termed invadopodia. ECM degradation at invadopodia is mediated by matrix metalloproteinases (MMPs), and recent findings indicate that membrane-anchored membrane type-1 matrix metalloproteinase (MT1-MMP) has a primary role in this process. Maintenance of an invasive phenotype is dependent on internalization of MT1-MMP from the plasma membrane and its recycling to sites of ECM remodeling, which requires phosphorylation of the enzyme's cytoplasmic domain. In addition to ECM degradation, invadopodia are sites where ECM adhesion is upregulated through increased localization of integrin receptors. The expression of integrins containing the $\beta 1$ subunit has been found to be upregulated in numerous invasive cancer cells, and downstream signaling from integrins has been found to play a role in cellular behaviours such as migration, proliferation, and survival. ECM adhesion and degradation at invadopodia are tightly regulated processes which must be balanced for cells to efficiently invade and migrate. The goal of this study was to identify and characterize a signaling pathway that links $\beta 1$ integrin and MT1-MMP at sites of invadopodia in invasive cancer cells. We utilized an antibody-mediated approach, both to activate and inhibit $\beta 1$ integrin, and monitored the resulting changes in MT1-MMP activity and invadopodia formation. The activation of $\beta 1$ integrin was found to be crucial for MT1-MMP phosphorylation which facilitated the internalization of the enzyme from the cell surface. It was also found that MT1-MMP was recycled in Rab5-positive early endosomes and Rab7-positive late

endosomes prior to delivery to newly forming invadopodia at the migration front. The activation of focal adhesion kinase (FAK), Src, and the epidermal growth factor receptor (EGFR), in response to β 1 integrin activation, was also observed and it was determined that these signaling molecules act in a pathway to promote phosphorylation of MT1-MMP on Thr⁵⁶⁷. Furthermore, inhibition of β 1 integrin did not result in the phosphorylation of MT1-MMP, instead causing its accumulation at the cell surface and a loss of long-term invasiveness. Together, these results define the importance of a β 1 integrin signaling axis in the regulation of MT1-MMP activity at invadopodia.

Curriculum Vitae: Olivia obtained her Bachelor of Science (Honours) Life Sciences, at McMaster University in 2015. In fall of the same year, she began her M.Sc. graduate studies under the supervision of Dr. Marc Copolino, and then later transferred to the Ph.D. program, in the same lab.

Awards:

Queen Elizabeth II Graduate Scholarship in Science and Technology, 2019-2020
Ontario Graduate Scholarship, Provincial, 2018-2019
Pharmacia Graduate Prize, University of Guelph, 2018

Publications:

Grafinger, O.R., Gorshtein, G., Stirling, T., Brasher, M.I., Copolino, M.G. (2020) β 1 integrin-mediated signaling regulates MT1-MMP phosphorylation to promote tumour cell invasion. *Journal of Cell Science*. 133(9).

Brasher, M.I., Martynowicz, D.M., **Grafinger, O.R.**, Hucik, A., Shanks-Skinner, E., Uniacke, J., Copolino, M.G. (2017) Interaction of Munc18c and Syntaxin4 facilitates invadopodium formation and extracellular matrix invasion of tumour cells. *Journal of Biological Chemistry*. 292(39): 16199-16210