UNIVERSITY of GUELPH

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*

MEGAN BRASHER

on Wednesday, April 29, 2020 at 9:30 a.m.

Thesis Title: **The regulation of Syntaxin4 and VAMP2 is required for invadopodium formation and extracellular matrix invasion in tumor cells**

Examination Committee:

Dr. R. Lu. Molecular and Cellular Biology (Exam Chair)
Dr. N. Jones, Dept. of Molecular and Cellular Biology
Dr. J. Uniacke. Dept. of Molecular and Cellular Biology
Dr. W. Zhang. Dept. of Molecular and Cellular Biology
Dr. S. Damjanovski, Dept. of Biology, Western University (External Examiner)

Advisory Committee:
Dr. M. Coppolino (Adv)
Dr. N. Jones
Dr. J. Uniacke
Dr. A. Viloria-Petit

Abstract: Tumor cell invasion involves targeted localization of proteins required for interactions with the extracellular matrix and proteolysis. The localization of many proteins during these cell-extracellular matrix interactions relies on membrane trafficking mediated in part by SNAREs. A few SNARE proteins are involved in the formation of invasive structures called invadopodia; however, it is unclear how these SNARE proteins are regulated during tumor cell invasion. Munc18c is known to regulate the plasma membrane SNARE Stx4, and here it is shown that Munc18c is required for Stx4-mediated invadopodium formation and cell invasion. Biochemical and microscopic analysis revealed a physical association between Munc18c and Stx4, which was later shown to occur through residues 1 through 29 (N-term), or 1 through 15 (1-15) of Stx4. Invadopodium formation, gelatin degradation, cell invasion and cell surface levels of MT1-MMP and EGFR were found to be reliant on interaction between Syntaxin4 and Munc18c, as expression of residues 1 through 29 or 1 through 15 of Stx4 led to decreases in these processes. Munc18c function was found to contribute to SNARE complex formation between Stx4, SNAP23 and VAMP2, which led us to investigate VAMP2's role in invadopodium formation. Knockdown and inhibition of endogenous VAMP2-containing SNARE complexes led to decreased invadopodium formation, gelatin degradation, cell migration and cell invasion. Cdc42 has been characterized as a regulator of VAMP2 function and was previously found to interact with residues 1 through 28 (1-28) of VAMP2. Expression of VAMP2-1-28 led to a decrease in invadopodium formation and gelatin degradation, suggesting that regulation of VAMP2 by Cdc42 is required for cell invasion. Overall, these findings highlight the importance of SNARE regulation during tumor cell invasion.

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Curriculum Vitae: Megan obtained her Bachelor of Science (Honours) at the University of Guelph in 2014. In the fall of the same year, she began her M.Sc. graduate program in the lab of Dr. Marc Coppolino, then later transferred to the doctoral program in the summer of 2016.

Awards: CIHR Travel Award April 2018; Ontario Graduate Fellowship 2016-2017

Publications: Olivia R. Grafinger, Genya Gorshtein, Tyler Stirling, Megan I. Brasher, Marc G. Coppolino. β1 integrin-mediated signaling regulates MT1-MMP phosphorylation to promote tumour cell invasion. Journal of Cell Science (March 2020).

Megan I. Brasher, David M. Martynowicz, Olivia R. Grafinger, Andrea Hucik, Emma Shanks-Skinner, James Uniacke and Marc G. Coppolino. Interaction of Munc18c and syntaxin4 facilitates invadopodium formation and extracellular matrix invasion of tumor cells. Journal of Biological Chemistry (Sept. 2017).

Megan I. Brasher, Liliana Surmacz, Bryan Leong, Jocelyn Pitcher, Ewa Swiezewska, Eran Pichersky and Tariq A. Akhtar. A two-component enzyme complex is required for dolichol biosynthesis in tomato. The Plant Journal (June 2015).