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*

OLIVIA BEBENEK

on April 25th, 2024, at 1:00pm

Thesis Title: **The RNA helicase DDX28 acts as a tumour suppressor in breast cancer and influences bioenergetics in hypoxia**

Examination Committee:

Dr. Krassimir Yankulov, Molecular and Cellular Biology (Exam Chair)
Dr. Jim Uniacke, Dept. of Molecular and Cellular Biology
Dr. Marc Coppolino. Dept. of Molecular and Cellular Biology
Dr. Shaun Sanders, Dept. of Molecular and Cellular Biology
Dr. Natoya Peart, Dept. of Biology, University of Waterloo (External Examiner)

Advisory Committee:
Dr. Jim Uniakce (Adv)
Dr. Marc Coppolino
Dr. Jasmin Lalonde
Dr. Paul Spagnuolo

Abstract: Hypoxia refers to a deficiency in oxygen delivery to tissues and has been associated with many pathophysiological processes, including cancer. As a characteristic component of the tumour microenvironment, hypoxia leads to increased metastasis, poorer patient prognosis and resistance to treatments. The master regulators of oxygen homeostasis in mammalian cells are the hypoxia-inducible transcription factors (HIF) 1α and 2α. HIF-2α has been implicated not only in transcription, but also as a member of the eukaryotic initiation factor 4E2 (eIF4E2)-directed hypoxic translation machinery. Oxygen is required for oxidative phosphorylation (OXPHOS), which is used by eukaryotic cells to generate high levels of ATP, therefore in hypoxia, cells must switch to the less efficient glycolytic pathway. That said, a growing body of evidence demonstrates that the balance between glycolysis and OXPHOS utilization differs by cell type. Previously, we have seen an increase in cancer hallmarks such as the translation of eIF4E2 target mRNAs, HIF-2α protein levels, cell proliferation and viability upon DDX28 depletion in hypoxia. This led us to suggest that DDX28 acts as a tumour suppressor in hypoxia, but the mechanism behind how it was doing so was unknown. Here, we hypothesize that the dysregulation of DDX28 levels in hypoxia leads to an increase in cancerous phenotypes. We show that many cancerous cell lines have decreased DDX28 levels compared to a non-cancerous cell line. Subsequently, we show that cell viability, migration, growth and invasion are influenced by DDX28 overexpression (OE) in U-87 MG human glioblastoma and MDA-MB-231 breast cancer cell lines and 3-D tumour models. Additionally, we examine these same cancer hallmarks after treatment with OXPHOS and glycolysis inhibitors. By measuring the abundance of glycolysis and OXPHOS-associated mRNAs and the ratio of mitochondrial versus glycolytic ATP production, we reveal that DDX28 OE alters cellular bioenergetics. After

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consistently observing opposite effects of DDX28 overexpression in either cell line, we conclude that DDX28 acts as a tumour suppressor in MDA-MB-231, but not U-87 MG cells. Overall, this research defines previously uncharacterized functions of DDX28, contributes to the ongoing body of research on the complexities underlying how cell bioenergetics affects cancer, and provides fertile ground for future research into how this could be leveraged for treatment strategies.

Curriculum Vitae: Olivia obtained her Bachelor of Science (Honours) in Molecular Biology and Genetics at the University of Guelph in 2019. In the fall of 2019, she entered into the MSc program under the supervision of Dr. Jim Uniacke and transferred to the Molecular and Cellular Biology PhD program in the fall of 2020.

Awards: Ontario Graduate Scholarship (2022-2023); Teaching and Career Development Fellowship (2022); Graduate Tuition Scholarship (2019-2021)

Publications: Evagelou SL, Bebenek O, Specker EJ, Uniacke J. 2020. DEAD-box protein family member DDX28 is a negative regulator of HIF-2α and eIF4E2-directed hypoxic translation. Mol Cell Biol. 40:e00610-19. DOI: 10.1128/MCB.00610-19.