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*

SARA TIMPANO

on Friday, November 16, 2018 at 9:30 a.m. in SSC 2315

Thesis Title: **An investigation of the molecular and cellular biology of human physioxic cell culture.**

Examination Committee:

Dr. A. Nassuth, Dept. of Molecular and Cellular Biology (Exam Chair)
Dr. J. Uniacke, Dept. of Molecular and Cellular Biology
Dr. J. Yankulov, Dept. of Molecular and Cellular Biology
Dr. A. Bendall, Dept. of Molecular and Cellular Biology
Dr. N. Thakor, University of Lethbridge (External Examiner)

Advisory Committee:

Dr. J. Uniacke (Adv)
Dr. J. Yankulov
Dr. R. Lu
Dr. J. Kirkland

Abstract: Researchers routinely culture cells under controlled temperature (37 °C) and CO_2 (5%) conditions to mimic the temperature and pH levels of the human body. Oxygen is an essential parameter that is often neglected in the majority cell culture and experimental procedures. Though human cells require oxygen, the atmospheric oxygen levels (21% O_2; 160 mmHg; normoxia) are much higher relative to the oxygen conditions experienced *in vivo* (~12 – 1% O_2; ~92 mmHg – 7 mmHg; physioxia). We have not only found that basic cellular functions operate differently in physioxia, but both cancerous and primary cells benefit from being cultured under physioxic conditions compared to normoxic conditions. Under physioxia, human cells utilize two cap-dependent protein synthesis machineries that act upon two discreet pools of mRNA, strongly suggesting that physioxia cultured cells have a different proteome compared to normoxia cultured cells. Additionally, under physioxia cells display increased viability, growth, and metabolism and decreased oxidative damage, oxidative stress response, DNA breaks, and mitochondrial morphology abnormalities relative to normoxia cultured cells. Each cell line was unique in its response to the different oxygen conditions, but in general all cell lines benefited from being cultured under 8% - 5% O2.. These data also indicate that standard cell culture practice subjects cells to avoidable levels of oxidative damage and stress the importance of oxygen as a cell culture parameter when attempting to obtain physiologically relevant results.

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Curriculum Vitae: Sara obtained her Bachelor of Science (Honours) at the University of Guelph in 2013. In the winter of 2014, she began her M.Sc. graduate work with advisor Dr. Jim Uniacke, and then transferred directly to the Ph.D. program in the summer of 2015.

Awards:

(2014) RiboClub Travel Fellowship
(2014-2015) Queen Elizabeth II Graduate Scholarship in Science & Technology
(2016-2017) Ontario Graduate Fellowship
(2017) Donald R. Phillips Scholarship
(2017-2018) Ontario Graduate Scholarship

Publications:

Timpano, S., B. D. Guild, E. J. Specker, G. Melanson, P. J. Medeiros, S. L. Sproul, and J. Uniacke. 2018. Physioxic human cell culture improves viability, metabolism and mitochondrial morphology while reducing DNA damage. *Manuscript submitted and under review*

*Melanson, G., *S. Timpano, and J. Uniacke. The eIF4E2-directed hypoxic cap-dependent translation machinery reveals novel therapeutic potential for cancer treatment. *Oxid Med Cell Longev,* 2017:6098107. doi:10.1155/2017/6098107 (2017) (*joint first author)

Timpano, S. and J. Uniacke. Human cells cultured under physiological oxygen utilize two cap-binding proteins to recruit distinct mRNAs for translation. *Journal of Biological Chemistry*, 291, 10772-10782 (2016)

Timpano, S., G. Melanson, S. L. Evagelou, B. D. Guild, E.J. Specker, and J. Uniacke. Analysis of Cap-binding Proteins in Human Cells Exposed to Physiological Oxygen Conditions. *Journal of Visualised Experiments,* 118, e55112, doi:10.3791/55112 (2016)

Ho, J.J., M. Wong, T. E. Audas, D. Kwon, S. K. Carlsson, S. Timpano, S. L. Evagelou, S. Brothers, M. L. Gonzalgo, J. R. Krieger, S. Chen, J. Uniacke, S. Lee. 2016. Systematic Reprogramming of Translational Efficiencies. *Cell Reports*. 14(6): 1293-1300 (2016)