The Biogenic Pathways of Malignant and Non-Malignant Microvesicles

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Thesis submitted in fulfilment of the requirements for the degree of

Doctor of Philosophy

The University of Technology Sydney

GRADUATE SCHOOL OF HEALTH

2019

CERTIFICATE OF ORIGINAL AUTHORSHIP

I, Jack Taylor declare that this thesis, is submitted in fulfilment of the requirements for the award of Doctor of Philosophy, in the Graduate School of Health at the University of Technology Sydney.

This thesis is wholly my own work unless otherwise reference or acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

This document has not been submitted for qualifications at any other academic institution.

This research is supported by the Australian Government Research Training Program.

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Jack Taylor

8/11/19

Date

Acknowledgements

First and foremost I would like to thank Professor Mary Bebawy. Thank you for being so generous with your time, knowledge, and advice – I have learnt more than I thought possible thanks to you. For me, the most satisfying part of this journey and the research we have produced is that we have always worked as a team.

To my other supervisors, Dr Ritu Jaiswal, Professor Gregory Monteith, and Dr Michael Johnson, thank you for your support, expertise, and ideas. You have been a source of inspiration throughout my PhD.

I have also been lucky to have worked with The Two Christians: Christian Loebbe who is an absolute master on the AFM, and Christian Evenhuis the image analysis wizard.

To my fellow doctoral candidates in the Graduate School of Hacks. Thanks for the bi-weekly long lunches, coffee runs, beers, and for just being all round legends. Special mentions go to The American, Carmenelocita, and The White Horse – great effort from you guys. Keep it up!

To my UTS-unaffiliated friends, I am so lucky to have you all. The weekends surf trips, early morning surfing session, guitar groups, lunches, catch ups, etc. etc. etc. balanced my life perfectly. You all helped me immensely and I will be forever grateful.

I'd like to thank my most favourite family Emma, Mum, and Dad for the infinite love and support they give me. Mum, I know you would have brought me dinner every night if you could. I love you guys.

Finally, I'd like to thank my sunshine, Aims. Coming home to that smile every day is the best. Thanks for organising our adventures (as I am incapable), infinite laughs, and love, and support. You're unreal, I love you.

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List of Abbreviations

Ab	Antibody
AFM	Atomic force microscope
μM	Micro molar
ABC	ATP-binding cassette
ADP	Adenosine triphosphate
ALLM	N-Acetyl-L-leucyl-L-leucyl-L-methioninal
BAPTA-AM	1,2-Bis(2-aminophenoxy)ethane-N,N,N′,N′-tetraacetic acid tetrakis(acetoxymethyl ester)
BCRP	Breast cancer resistance protein
BSA	Bovine serum albumin
Ca2+	Calcium ion
DAPI	4′,6-diamidino-2-phenylindole
EBM-2	Endothelial Cell Basal Medium-2
ER	Endoplasm reticulum
ESCRT	Endosomal sorting complexes required for transport
EV	Extracellular vesicle
FAK	Focal adhesion kinase
FBS	Foetal bovine serum
GPG	Gycophorin C
ILV	Intraluminal vesicle
kPa	kilo Pascal
MDR	Multidrug resistance
MEG-1	Mammary Epithelial Cell Growth Medium-1
MP	Microparticle

MRP1	Multidrug Resistance-Associated Protein 1
MV	Microvesicle
MVB	Multivesicular body
NA	Numerical aperture
NBD	Nonbinding domains
nM	nanomolar
nMase	Neutral Sphingomyelinase
PBS	Phosphate buffered saline
PC	phosphatidylcholine
PCH	Pombe Cdc15 homology
PE	phosphatidylethanolamine
PFA	Paraformaldehyde
P-pg	P-glycoprotein
PS	phosphatidylserine
PSS	physiological salt solution
Ptdlns(4,5)P2	Phosphatidylinositol 4,5-bisphosphate
RBC	Red blood cell
RMS	Root mean square
ROCK1	Rho-associated, coiled-coil containing protein kinase 1
ROS	Reactive oxygen species
RPMI	Roswell park media
SD	Standard deviation
SERCA	Sarco/endoplasmic reticulum calcium ATPase
SM	sphingomyelin
SOCE	Store-operated calcium entry

TG	Thapsigargin
TMD	Transmembrane domains
TRPC5	Transient receptor potential channel
YM58483	*N*-[4-[3,5-*Bis*(trifluoromethyl)-1*H*-pyrazol-1-yl]phenyl]-4-methyl-1,2,3-thiadiazole-5-carboxamide

Publications arising from this work

Taylor, J., Jaiswal, R. & Bebawy, M. 2017. Calcium-calpain Dependent Pathways Regulate Vesiculation in Malignant Breast Cells. Current Cancer Drug Targets, 17, 486-494.

Taylor, J. & Bebawy, M. 2019. Proteins Regulating Microvesicle Biogenesis and Multidrug Resistance in Cancer. Proteomics, 19, 1615-9861.

Taylor J., Azimi, I., Monteith, G., & Bebawy, M. 2020. Ca^{2+} mediates extracellular vesicle biogenesis through alternate pathways in malignancy, Journal of Extracellular Vesicles, 9:1, DOI: 10.1080/20013078.2020.1734326

Manuscripts under review

Taylor, J., Patio, K., Morris, M., Evenhuis, C., Johnson, M., Bebawy, M. 2019. Membrane to Cytosol Redistribution of αII-Spectrin Drives Extracellular Vesicle Biogenesis in Malignant cells. (Pre-submission inquiry)

Scholarships

Research

Australian Government Research Training Program Stipend

Travel

Vice-Chancellor's Postgraduate Research Student Conference Fund

Conference Presentations

Sarco/endoplasmic reticulum ATPase inhibition activates calcium signalling pathways for microvesicle biogenesis. International Society of Extracellular Vesicles. May 2nd – May 6th, 2018, Barcelona, Spain.

Sarco/endoplasmic reticulum ATPase inhibition activates calcium signalling pathways for microvesicle biogenesis. Australasian Extracellular Vesicles Conference. 14th – 16th November, 2018, Sydney, Australia.

Abstract

Plasma membrane-derived extracellular vesicles (EV) are released for most cells types and are important mediators of intercellular communication. In the context of cancer, EVs mediate the intercellular transfer of deleterious traits including multidrug resistance (MDR), enhanced metastatic capacity, evasion of immune surveillance, and altered tissue biomechanics. The biogenesis of plasma membrane EVs is characterised by an increase in intracellular calcium followed by successive membrane and cytoskeletal changes. However, the precise biogenic mediators and differences between malignant and non-malignant plasma membrane-derived EV biogenesis remain undefined. Uncovering discrete pathways of biogenesis in malignant and non-malignant cells holds potential in the search for new strategies to circumvent the acquisition and transfer of plasma membrane EV-meditated deleterious traits in cancer.

In this thesis, the molecular pathways that regulate plasma membrane EV biogenesis in malignant and non-malignant cells are investigated.

This study evidences that discrete EV biogenic pathways exist in malignant and non-malignant cells. At rest, biogenesis of cancer EVs is regulated by a calcium-calpain dependent pathway whereas non-malignant biogenesis is driven by alternative pathways. Comparing the surface topography of malignant and non-malignant cells using atomic force microscopy demonstrates malignant cells have intrinsically higher vesiculation at rest and this is shown to be driven by high basal calcium mobilisation. Interrogating the calcium signalling pathways that regulate biogenesis with pharmacological modulators of calcium revealed increases in free cytosolic Ca^{2+} via endoplasmic reticulum (ER) Ca^{2+} store depletion with thapsigargin increases EV biogenesis in both malignant and non-malignant cells. Evidencing a role for the ER in plasma membrane EV biogenesis for the first time. Furthermore, the store-operated calcium entry

(SOCE) plays an essential role in the maintenance of plasma membrane EV biogenesis after store depletion.

The calcium-calpain pathway is also a known regulator of αII-spectrin structural dynamics and this work evidences that it plays a role in increased vesiculation in malignant cells. High resolution confocal microscopy in combination with a custom designed, automated image analysis plugin, provided an unbiased platform to show that the subcellular localisation of spectrin is distinctly different in malignant and non-malignant cells at rest. Non-malignant cells display prominent αII-spectrin borders and are low vesiculators. It is the redistribution of αII-spectrin from the membrane to the cytoplasm that drives plasma membrane-derived EV biogenesis in malignant cells.

These findings provide new insight into the biogenic pathways regulating EV biogenesis in both malignant and non-malignant cells and identifies strategies for the selective modulation of plasma membrane EV biogenesis in malignancy.