Microparticles Mediate Trait Dominance in Cancer

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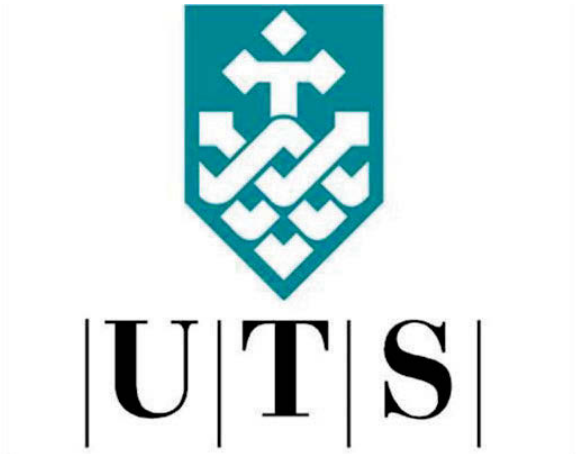
A thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy



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CERTIFICATE OF ORIGINAL AUTHORSHIP

I, Jamie Lu, certify that the work in this thesis has not previously been submitted for a degree nor has it been submitted as part of requirements for a degree except as fully acknowledged within the text.

I also certify that the thesis has been written by me. Any help that I have received in my research work and the preparation of the thesis itself has been acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

Jamie Lu

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List of Abbreviations

^0C	Degrees Celsius
µg	Microgram
µM	Micromole
ABC	ATP-binding cassette
ACN	Acetonitrile
ATP	Adenosine triphosphate
cDNA	Complementary deoxyribonucleic acid
BSA	Bovine Serum Albumin
C-terminus	Carboxyl-terminus
CD44	Cluster of differentiation 44
CO_2	Carbon Dioxide
CsA	Cyclosporin A
DNA	Deoxyribonucleic acid
DNR	Daunorubicin
DTT	Dithiothreitol
EDTA	Ethylenediamine tetraacetic acid
FCM	Flow cytometry
FCS	Fetal calf serum
FITC	Fluorescein isothiocyanate
g	acceleration due to gravity
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
GSH	Glutathione
GST	Glutathione *S*-Transferase
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HRP	Horseradish peroxidase
HSP	Heat shock protein

IgG	Immunoglobulin-G
kDa	Kilo Dalton
LC	Liquid chromatography
mAb	Monoclonal antibody
MDR	Multidrug resistance
MFI	Mean fluorescence intensity
miRNA	micro ribonucleic acid
lncRNA	long non-coding ribonucleic acid
MPs	Microparticles
MRP	Multidrug resistance associated protein
mRNA	Messenger Ribonucleic Acid
MS	Mass spectrometry
MS/MS	Tandem mass spectrometry
N-terminus	Amino-terminus
ng	Nanogram
PAGE	Polyacrylamide gel electrophoresis
PBC	Probenecid
PBS	Phosphate buffered saline
PE	Phosphatidylethanolamine
P-gp	P-glycoprotein
pmole	picomole
ppm	Parts per million
PS	Phosphatidylserine
PVDF	Polyvinylidene fluoride
qRT-PCR	quantitative Real-Time Polymerase Chain Reaction
RRTA	Rabbit Reticulocyte Translation Assay
RNA	Ribonucleic acid
rpm	Revolutions per minute

S.E.M	Standard Error of the Mean
SEM	Scanning Electron Microscopy
SDS	Sodium Dodecyl Sulphate
TCA	Trichloroacetic acid
TBS	Tris-buffered saline
TBST	Tris-buffered saline-Tween 20
TMD	Transmembrane Domain
Tween-20	Polyoxyethylenesorbitan monolaurate
UTR	Untranslated Region

Publications arising from this work

Jamie F. Lu; Deep Pokharel; Mary Bebawy. "**MRP1 and its role in anticancer drug resistance",** *Drug metabolism reviews* 2015, DOI: 10.3109/03602532.2015.1105253

Jamie F. Lu; Frederick Luk; Joyce Gong; Ritu Jaiswal; Georges E.R. Grau, Mary Bebawy, **"Microparticles mediate MRP1 intercellular transfer and the re-templating of intrinsic resistance pathways"**, *Pharmacological Research* 2013, DOI: 10.1016/j.phrs.2013.07.009

Jamie F. Lu; Deep Pokharel; Matthew P. Padula; Mary Bebawy. **"A novel method to detect translation of membrane proteins following microvesicle intercellular transfer of nucleic acids**", *Journal of Biochemistry* 2016; doi: 10.1093/jb/mvw033

Jamie F. Lu; Deep Pokharel; Frederick Luk; Mary Bebawy. "**Functional translation of total RNA packaged in microparticles shed from multidrug resistant cancer cells". [abstract]**. *Proceedings of the AACR Precision Medicine Series: Drug Sensitivity and Resistance: Improving Cancer Therapy*; Jun 18-21, 2014; Orlando, FL. Philadelphia (PA): AACR; Clin Cancer Res 2015;21(4 Suppl): Abstract nr B19.

Manuscripts Submitted for publication

Jamie F. Lu; Deep Pokharel; Mary Bebawy; "***ABCB1* **is a transcriptional regulator of MRP1 expression in multidrug resistant leukaemia cells**", *Under review in Journal of Biological Chemistry* (Submitted December 2015)

Conference Presentations

Jamie F. Lu, Ritu Jaiswal, Frederick Luk, Georges E.R. Grau, Mary Bebawy, "**Intercellular Transfer Of Functional MRP1 And The Re-templating Of Intrinsic Resistance Pathways Via Microparticles**", Australian Society of Biophysics: Membrane Transporter Satellite Meeting; 2nd December 2012; UNSW, Australia

Jamie F. Lu, Ritu Jaiswal, Frederick Luk, Georges E.R. Grau, Mary Bebawy, "**Intercellular Transfer Of Functional MRP1 And The Re-templating Of Intrinsic Resistance Pathways Via Microparticles**", *International society of extracellular vesicles*, April 17-20, 2013; Boston, MA, USA

Jamie F. Lu, Deep Pokharel, Frederick Luk, Mary Bebawy, "**Functional translation of total RNA packaged in microparticles shed from multidrug resistant cancer cells**," *AACR Precision medicine series: Drug sensitivity and resistance: Improving cancer therapy*; 18-21st June, 2014; Orlando, FL, USA.

Other Publications

Deep Pokharel; Matthew P. Padula; **Jamie F. Lu**; Jessica Tacchi; Frederick Luk; Steven P. Djordjevic; Mary Bebawy. "**Proteome analysis of multidrug-resistant, breast cancer-derived microparticles**," *Journal of extracellular vesicles* 2014, DOI: 10.3402/jev.v3.24384.

Deep Pokharel; Matthew P. Padula; **Jamie F. Lu**; Ritu Jaiswal; Steven P. Djordjevic; Mary Bebawy. "**The functional role of CD44 and ERM in conferring multidrug resistance in breast cancer**," *Molecules* 2016, doi:10.3390/molecules21030290

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Abstract

Multidrug resistance (MDR) persists to be a major hindrance to the successful treatment in clinical oncology and is the cause of over 90% of treatment failure in cancer. The two main membrane spanning proteins, P-glycoprotein (*ABCB1*/P-gp) and Multidrug Resistance-associated protein 1 (*ABCC1*/MRP1) are responsible for the efflux of a plethora of unrelated anti-cancer drugs out of cells, resulting in MDR. Cancer cells overexpressing these efflux proteins are insensitive to chemotherapeutic treatments by maintaining sub-lethal intracellular cytotoxic drug concentrations. Given their enormous substrate profile, of which there is significant overlap, the expression of either efflux protein would result in a poor prognosis. In some cancers, the overexpression of these proteins is correlated with clinical stage, with early stage tumours expressing one efflux transporter and substituted by another transporter at an advanced stage. Our group has established the transfer and dissemination of ABC-transporter mediated MDR via a subset of extracellular vesicles known as microparticles (MPs). This study investigates the molecular mechanisms governing the alteration and acquisition of MDR traits in cancer cell populations via MPs.

Spontaneously shed MPs from cancer cells represent a prominent modality for intercellular communication by virtue of their capacity to transport and disseminate bioactive cargo through the vasculature. Their ability to carry large membrane spanning proteins and nucleic acids, imparts their capacity to confer MDR among otherwise drug sensitive tumour cells. Herein, the study validates the MP-transfer and functionality of MRP1 in drug sensitive acute leukaemia cells. The study also introduces

MP-mediated trait dominance and demonstrate the re-templating of a pre-existing MDR phenotype in recipient cells. To validate the transfer and translation of MP packaged nucleic acids, a novel methodology was developed, abolishing the requirement for labelled probes and interspecies models. Using, surface peptide shaving, detection of MP packaged P-gp was removed and showed transcript translation of transferred *ABCB1* in drug sensitive recipient cells after more than 24 h. Finally, the study identifies transcript suppression mechanisms involved in MP-mediated trait dominance and identify a novel relationship between the function of miRNA with a non-target mRNA transcript. Specifically, the presence of a rival transcript *ABCB1* facilitates the *ABCC1* suppression by miR-326. These findings substantially advance our understanding on the molecular mechanisms leading to the alteration of MDR traits and can be translated into clinical oncology by providing prognostic information and additional therapeutic targets.