

# **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

$^{\circ}\text{C}$	Degrees Celsius
$\mu\text{g}$	Microgram
$\mu\text{M}$	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
$\text{CO}_2$	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
<i>g</i>	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

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## Other Publications

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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.