UTS UNIVERSITY OF TECHNOLOGY SYDNEY

Investigation of miR-652 in Host Immunity Against Intracellular Bacterial Pathogens

by Maxwell T. Stevens

Thesis submitted in fulfilment of the requirements for the degree of

Doctor of Philosophy

under the supervision of Associate Professor Bernadette Saunders and Dr Matthew Padula

University of Technology Sydney
Faculty of Science

July 2022

CERTIFICATE OF ORIGINAL AUTHORSHIP

I, Maxwell Stevens, declare that this thesis is submitted in fulfilment of the requirements for the award of Doctor of Philosophy, in the Faculty of Science at the University of Technology Sydney.

This thesis is wholly my own work unless otherwise referenced 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.

Signature: Production Note:
Signature removed prior to publication.

Date: 30 July 2022

Acknowledgments

The last 4 years have been an endurance event, and there are many people who I need to thank for helping me through.

Firstly, I would like to acknowledge and thank my supervisor Associate Professor Bernadette Saunders and my co-supervisor Dr Matthew Padula. Your support and guidance throughout my PhD have instilled me with the ethical and scientific base I needed to really delve into my study, continually learning and developing as a research scientist. You provided sound advice when I needed it most, but also gave me the reigns to drive my project and make the most of this opportunity. Also, thank you to Dr Nilesh Bokil for the technical introduction to kick off the whole project.

A big thank you to everyone who has been a part of the Saunders lab with me, with special thanks to Dr Jessica Pedersen and Giang Le. Your physical assistance and effervescent characters were the essential ingredients to get through arduous days in the PC3, toiling for hours through flow cytometry data, or carefully deciphering my most perplexing results. So much of this work would not have been accomplished without you all.

Thank you to Professor Warwick Britton and all the members of the mycobacteria group at the Centenary Institute. The technical expertise you've all shared has been amazing, and the opportunity to regularly present my work to you all has been invaluable. Presentation opportunities have been few and far between these 2 years, and I am very grateful. Your fresh perspectives and weekly discussion fostered my scientific thinking, which really made this thesis what it has finally become.

My parents, family, and friends have been a constant, not just through my PhD, but through all my work and education. Thank you so much for all your encouragement and support. To Matthew Hamlyn, thank you for your constant positivity, it is a very welcome light when things feel rough. To Dr Marley Pulbrook, thanks for your wit, humour, and encouragement as we've both been slogging through what feels like endless study.

And most importantly, thank you to my wife Emily. These years have been hard and I could not have done it without you. There were many months where you were literally the only person I saw, which is lucky because you are the best support I could wish for. Your love, friendship, and encouragement have kept me sane through long nights in the lab and prolonged weeks writing. Thank you so, so much; I love you!

Thesis format

This is a thesis by compilation, consisting of two published peer-reviewed papers, two results chapters, and a general discussion chapter.

Publications associated with this thesis

Stevens, M. T., B. D. Nagaria, W. J. Britton, and B. M. Saunders. 2021. Macrophages of different tissue origin exhibit distinct inflammatory responses to mycobacterial infection. *Immunol. Cell Biol.* 99: 1085-1092.

Stevens, M. T., and B. M. Saunders. 2021. Targets and regulation of microRNA-652-3p in homoeostasis and disease. *J. Mol. Med.* 99: 755-769.

Table of Contents

List of figures

Chapter 1

Chapter 2

Chapter 3

Chapter 4

Chapter 5

Appendices

List of tables

Chapter 3

Chapter 5

Chapter 6

Abbreviations

3'UTR	3' untranslated region
ADC	Albumin dextrose catalase
ANOVA	Analysis of variance
BMDM	Bone marrow-derived macrophage
BSA	Bovine serum albumin
CBA	Cytometric bead array
cDNA	Complementary deoxyribonucleic acid
CFU	Colony forming unit
cGAMP	Cyclic GMP-AMP
CNS	Central nervous system
CVD	Cardiovascular disease
DC	Dendritic cell
DNA	Deoxyribonucleic acid
dsDNA	Double stranded deoxyribonucleic acid
DTT	Dithiothreitol
EDTA	Ethylenediaminetetraacetic acid
ET	Ethical threshold
FBS	Foetal bovine serum
LB	Lysogeny broth
LPS	Lipopolysaccharide
MDR TB	Multidrug-resistant
miRNA	microRNA
MLN	Mediastinal lymph node
MOI	Multiplicity of infection
NK cell	Natural killer cell
NP	Nanoparticle
OADC	Oleic acid albumin dextrose catalase
PAMP	Pathogen-associated molecular pattern

PBMC	Peripheral blood mononuclear cell
PBS	Phosphate buffered saline
PC3	Physical containment level 3
PEG	Polyethylene glycol
PEI	Polyethylenimine
Pre-miR	Pre-microRNA
Pri-miR	Primary microRNA
RISC	RNA-induced silencing complex
RNA	Ribonucleic acid
ROC curve	Receiver operating characteristic curve
RT-qPCR	Real time quantitative polymerase chain reaction
SD	Standard deviation
SDS-PAGE	Sodium dodecyl sulphate polyacrylamide gel electrophoresis
SEM	Standard error of the mean
siRNA	Small interfering ribonucleic acid
SNP	Single nucleotide polymorphism
TBST	Tris-buffered saline Tween 20
TCA cycle	Tricarboxylic acid cycle
TCEP	Tris(2-carboxyethyl)phosphine
TFA	Trifluoroacetic acid
TLR	Toll-like receptor
TP	Time point

Abstract

Tuberculosis (TB) is an infectious respiratory disease caused by the bacterial pathogen *Mycobacterium tuberculosis*. Each year, 1.5 million deaths are attributable to TB and survivors are prone to increased all-cause mortality, due to excessive TB-associated pulmonary inflammation. Recent literature indicated the microRNA hsa-miR-652-3p (miR-652) was downregulated in plasma of Chinese TB patients, and further decreased in patients who failed to clear the bacteria after antibiotic therapy. This thesis investigated the activities of miR-652 during *in vitro* and *in vivo* infections with intracellular bacterial pathogens, with a special focus on the macrophage response to infection.

My initial study aimed to characterise the phenotypic differences between murine alveolar (AMJ2-C11) and peritoneal (IC-21) macrophage cell lines during *in vitro* mycobacterial infections, in order to illustrate the influence of tissue origin on macrophage function. Both cell lines were able to control *M. bovis* BCG and *M. tuberculosis* H37Rv bacterial loads. However, AMJ2-C11 cells exhibited a more inflammatory phenotype, with significantly increased cytokine release and nitric oxide generation. Additionally, expression of inflammatory cell surface markers was increased on AMJ2-C11 cells relative to IC-21 cells. These data suggest that whilst tissue origin can influence macrophage phenotype, cell plasticity ensures diverse macrophages can respond to invading pathogens.

Chapter 4 investigated the impact of miR-652 on the murine immune response to *M. tuberculosis.* Bone marrow macrophages from miR-652$^{-/-}$ C57BL/6 mice were able to control bacterial growth over 6 days *in vitro*, though IL-6, TNF, MIP-1α, and KC expression was significantly lower than in their wild type counterparts. Western blot results indicated AKT and mTOR activation was attenuated in miR-652$^{-/-}$ macrophages. miR-652$^{-/-}$ mice infected aerogenically with *M. tuberculosis* were able to control the bacterial load in the lungs and spleen equal to wild type mice over 13 weeks. Leukocyte populations were comparable between mouse strains, however, early CD8^{+} effector T cell numbers were elevated in the lung and lymph node miR-652$^{-/-}$ mice, suggesting miR-652 may have some impact on T cell differentiation during bacterial infection.

Chapter 5 investigated this question in a $CD8^+$ T cell-focused infection model; intraperitoneal *Listeria monocytogenes* infection. miR-$652^{-/-}$ mice were highly susceptible to a low-dose infection of 2000 CFU/mouse, exhibiting significantly increased weight loss and high morbidity. The early onset of morbidity indicated a deficiency in the innate immune response. Highly necrotic liver lesions in miR-$652^{-/-}$ mice displayed intense recruitment of neutrophils and macrophages, but bacterial load was uncontrolled in these mice. To investigate the antimicrobial phenotype of miR-$652^{-/-}$ macrophages, primary peritoneal macrophages were infected with *L. monocytogenes in vitro*. A proteomic analysis highlighted dysregulation of key immune pathways, including the lysosome pathway and the pentose phosphate pathway. Also downregulated was the *in silico*-predicted miR-652 target CAPZB. Transfection experiments using luciferase reporter constructs indicated miR-652 does not target a predicted sequence in the CAPZB 3'UTR. Further, CAPZB mRNA and protein were unaffected by transfection with a miR-652 mimic in IC-21 mouse peritoneal macrophage cells, indicating CAPZB expression is unaffected by miR-652.

This thesis demonstrates miR-652 plays clear roles in the proper innate immune response to acute infection with an intracellular bacterial pathogen. The pathways impacted in miR-$652^{-/-}$ macrophages position miR-652 as an important regulator of immune function, potentially regulating inflammation and cell metabolism. Host-directed therapies possess amazing potential as a complement to existing antimicrobial drugs. microRNA-based therapeutics for infectious diseases are progressing well through clinical trials. Analysis of the genes validated as targets for miR-652 underscores the promise for a miR-652 mimic as a therapeutic in chronic TB, particular when administered with a cell-targeted delivery mechanism. Additional holistic research is needed to evaluate the impacts of miR-652 in macrophages to realise the potential of miR-652 as a therapeutic miRNA.