Characterisation of the *Fasciola hepatica* miRNome and an evaluation of its role in the host-parasite relationship

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Doctor of Philosophy

under the supervision of

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Certificate of original authorship

I, Alison Mae Ricafrente declare that this thesis, is submitted in fulfilment of the requirements for the award of Doctor of Philosophy, in the School of Life Sciences, 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.

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Statement & list of papers and conferences

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Research articles:

Tran N, Ricafrente A, To J, Lund M, Marques TM, Gama-Carvalho M, Cwiklinski K, Dalton JP, Donnelly S. *Fasciola hepatica* hijacks host macrophage miRNA machinery to modulate early innate immune responses. ***Scientific Reports***. 2021 Mar 24;11(1):1-1.

Ricafrente, A., Cwiklinski, K., Nguyen, H., Dalton J.P., Tran, N. & Donnelly, S. Stage-specific miRNAs regulate gene expression associated with growth, development and parasite-host interaction during the intra-mammalian migration of the zoonotic helminth parasite *Fasciola hepatica*. ***BMC Genomics***. 2022 Dec;23(1):1-9.

Conferences:

Ricafrente A, Tran N, Marques TM, To J, Gama-Carvalho M, Cwiklinski K, Dalton JP, Donnelly S. Understanding how the miRNAs of *Fasciola hepatica* contribute to the host-parasite relationship. ***Parasitic Helminths - New Perspectives in Biology and Infection Conference***. 2019 Hydra, Greece.

Ricafrente A, Tran N, Marques TM, To J, Gama-Carvalho M, Cwiklinski K, Dalton JP, Donnelly S. Elucidating the role of microRNAs in the development of *Fasciola hepatica*. ***Irish Society of Parasitology Meeting***. 2019 Belfast, UK.

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Supplementary Material

can be accessed at https://doi.org/10.26195/zbnf-8253

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Abbreviations

Abbreviation	Term
AcCoA	Acetyl-CoA
Ad	Adult
AFBI	Agri-Food Biosciences Institute
AGO	Argonaute
ANOVA	Analysis of variance
ASCT	Acetate:succinate CoA transferase
ATP	Adenosine triphosphate
BAN	4-Bromoanisole
BCL10	B-cell lymphoma/leukemia 10 signaling adaptor
BMDM	Bone marrow-derived macrophages
CCR5	C-C chemokine receptor type 5
CD	Cluster of differentiation
cDNA	Complimentary DNA
CITR	Citrate
CoA	Coenzyme A
CPM	Counts per million
CREB1	CAMP responsive element binding protein 1
DAVID	Database for Annotation, Visualization and Integrated Discovery
DC	Dendritic cell
DGCR8	DiGeorge syndrome critical region 8 gene
DNA	Deoxyribonucleic acid
dNTP	Deoxynucleoside triphosphate
dpi	Days post-infection
ELISA	Enzyme linked immunosorbent assay
EV	Extracellular vesicle
EZH2	Enhancer of zeste 2 polycomb repressive complex 2 subunit
FBP	Fructose 1,6-bisphosphate
FBS	Foetal bovine serum
FC	Fold change
FEST	Fluke egg sedimentation test
FhCL	Fasciola cathepsin
FP6	Fructose 6-phosphate
FRD	Fumarate reductase
FUM	Fumarate
G1P	Glucose 1-phosphate
GM-CSF	Granulocyte macrophage colony stimulating factor receptor
GO	Gene ontology
GP6	Glucose 6-phosphate
h	Hour(s)
HDAC	Histone deacetylase

Abbreviation	Term
HSP	Heat shock protein
HSPA4	HSP Family A Member 4
IFN	Interferon
IKZF3	Ikaros Family Zinc Finger 3
IL	Interleukin
ILC	Innate lymphoid cell
iNOS	Inducible nitric oxide synthase
IRF	Interferon regulatory factor
ITS2	Internal transcribed spacer 2
JAK1	Janus kinase 1
JUV	Juvenile/Immature
KEGG	Kyoto encyclopaedia of genes and genomes
LCP1	Lymphocyte cytosolic protein 1
LPS	Lipopolysaccharide
MAL	Malate
MAPK	Mitogen-activated protein kinase
M-CSF	Macrophage colony stimulating factor
Methymal-CoA	Methylmalonyl-CoA
MFE	Minimum free energy
MHC	Major histocompatibility complex
miRISC	miRNA induced silencing complex
miRNA	MicroRNA
mRNA	Messenger RNA
NEJ	Newly excysted juvenile
NET	Neutrophil extracellular trap
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells.
NO	Nitric oxide
NOD	Non-obese diabetic
nt	Nucleotide
NTC	Non- template control
OXAC	Oxaloacetate
PBS	Phosphate buffer solution
PCA	Principal components analysis
PCR	Polymerase chain reaction
PEP	phosphoenolpyruvate
pi	Post-infection
PoC	Point-of-care
PRDM1	Positive regulatory domain I-binding factor 1
pre-miRNA	Precursor miRNA
pri-miRNA	Primary miRNA
PRKCB	Protein kinase C beta
PROP	Propionate

Abbreviation	Term
Prop-CoA	Propionyl-CoA
PTEN	Phosphatase and tensin homolog
PYR	pyruvate
RAD50	Double strand break repair protein
RELA	REL proto-oncogene, NF-κB subunit
RMPI	Roswell Park Memorial Institute
RNA	Ribonucleic acid
RT	Reverse transcription
RT-qPCR	Reverse transcription-quantitative PCR
RXRA	Retinoid X Receptor Alpha
SAC	Spindle assembly check point
SAP	Sin3A Associated Protein
SD	Standard deviation
SDH	succinate dehydrogenase
SOAP	Short oligonucleotide alignment program
SP1	Specificity protein 1
STAT	Signal transducer and activator of transcription
SUCC	Succinate
Succ-CoA	Succinyl-CoA
TCA	The citric acid
TCBZ	Triclabendazole
TDE	Thermodynamic ensemble
Th1/2	T helper 1 /2
TLR	Toll like receptor
TNF	Tumor necrosis factor
TPM	Transcripts per million
UDP-G	Uridine biphosphate glucose
Uninf	Uninfected
UTR	Untranslated region
UTS	University of Technology Sydney
w	Week(s)
WHO	World Health Organisation
wpi	Weeks post-infection
XPO	Exportin

Abstract

The liver fluke, *Fasciola hepatica*, is recognised as one of the most successful parasites worldwide due to its remarkable capacity to infect every mammal it encounters. For this reason, liver fluke disease, or fasciolosis, has the widest geographical spread of any parasite disease and contributes to significant animal loss, particularly within the agricultural sector. Since the discovery of the post-transcriptional regulation of genes by micro(mi)RNA in the free-living worm *Caenorhabditis elegans*, a myriad of processes within worm biology are now linked to miRNAs. These concepts have catalysed interest in the contribution that miRNAs have on the dynamic shifts of the *F. hepatica* transcriptome and parasite survival within the host.

In Chapter 1, the miRnome assemblies of early miRNA discovery projects were compared to determine knowledge to date. Examination of 38 miRbase miRNAs revealed that the revised miRNome was highly associated to the regulation of inflammatory events and innate mechanisms of pathogen recognition and expulsion by the host. These preliminary explorations were experimentally challenged in Chapter 2. Sequencing of miRNAs isolated from the peritoneal macrophages of *F. hepatica* infected mice revealed that specific Fasciola miRNAs were internalised by host macrophages. In particular, fhe-miR-125b was uncovered as a potent immune regulator due to its capacity to suppress the expression of a central signal transduction molecule Traf6 within the host, after functionalisation by mammalian Ago.

The realisation of the complete *F. hepatica* miRnome in Chapter 3 expanded the number of *F. hepatica* miRNAs to 124 within intra-mammalian life stages; newly excysted juveniles (NEJs), immature and adult fluke, exposing a wider collection of isomiRs, life stage specific novel miRNAs and genomic clustering. By integrating the life stage miRnomes with their predicted targets within the transcriptomes of each life stage identified the key biological processes in metabolism, parasitism, and growth that were systematically targeted during parasite development within the host. With the expanded miRnome, the utility of parasite miRNAs as biomarkers of fasciolosis was explored, with diagnostic capabilities examined through RT-qPCR analysis of sera from infected sheep (Chapter 4).

The collective outcomes of this research project have fostered new perspectives in *F. hepatica* research. These include, evolving methods of miRNA discovery; re-thinking the biogenesis of microRNAs; mapping the molecular events of parasite development; unveiling new mechanisms of host-parasite interplay, and advancing diagnostic techniques.