UTS UNIVERSITY OF TECHNOLOGY SYDNEY

MicroRNA to microRNA Interactions and their role in Head and Neck Squamous Cell Carcinoma

by Meredith Kate Hill

Thesis submitted in fulfilment of the requirements for the degree of

Doctor of Philosophy

under the supervision of:
Associate Professor Nham Tran and
Associate Professor Valerie Gay

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Certificate of Original Authorship

I, Meredith Hill declare that this thesis, is submitted in fulfilment of the requirements for the award of Doctor of Philosophy, in the School of Biomedical Engineering, Faculty of Engineering and IT 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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COVID-19 Impact Statement

Due to the COVID-19 pandemic, I experienced two extended periods of suspended experimental work. The first was during the NSW-wide lockdown from March 2020 to July 2020. The second was for the period of the Greater Sydney lockdown from July 2021 to November 2021. I was also under stronger COVID-19-related restrictions from August 2021 due to high transmission rates in my area of residence.

During these two lockdown periods it was highly recommended that all research activities be conducted from home. Additionally, during the 2021 lockdown, laboratory access was restricted to those with highly time-sensitive research that was unable to be postponed or reorganised. As my research did not fit within this category and I was under strict travel rules, I was unable to conduct experiments during this time. This meant that a proportion of the experiments within this thesis were delayed, and biological triplicates were not achieved for all cell lines. These issues particularly affected Chapters 5 and 6 of this thesis.

Additionally, the decrease in air freight and the associated increase in boat freight due to COVID-19 prolonged the time for essential reagents to arrive. In some cases the shipment delay was greater than three months. The delivery of RNA samples for miRNA sequencing was also postponed due to travel restrictions and shipment hindrances. The sequencing service provider was also changed due to these reasons. As the results for the sequencing were received within the last four months of candidature, the differentially expressed miRNAs were not followed up with confirmatory RT-qPCR. I also aimed to conduct luciferase assays with a MIR17HG-psiCHECK-2 construct, available from a laboratory group in Wuhan, China. Acquisition of this plasmid was significantly delayed, and was ultimately not pursued, due to the COVID-19 pandemic. Again, these complications mainly impacted Chapters 5 and 6.

The aims of this thesis had to be slightly adjusted to account for changes to the experimental timeline and the resources available. Ultimately, the time delays, whether due to the two lockdowns or shipment setbacks, were mitigated by

redistributing focus towards the more computational heavy chapters or the synthesis of this thesis, primarily Chapters 3, 4, and 7.

Thesis Structure and Contributing Works

This thesis includes published works featured in:

Literature Review Article:

Hill, M. & Tran, N. miRNA interplay: mechanisms and consequences in cancer. Disease Models & Mechanisms 14, dmm047662 (2021).

Forum Review articles:

Hill, M. & Tran, N. MicroRNAs regulating microRNAs in cancer. *Trends in cancer* **4**, 465-468 (2018).

Hill, M. & Tran, N. Global miRNA to miRNA Interactions: Impacts for miR-21. *Trends in cell biology* **31**, 3-5 (2021).

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Disease Models and Mechanisms

Part of Chapter 1 has been published as a Literature Review Article in Disease Models and Mechanisms.

Hill, M. & Tran, N. miRNA interplay: mechanisms and consequences in cancer. Disease Models & Mechanisms 14, dmm047662 (2021).

The text presented in Section 1.8 is the accepted version of the manuscript. Numbering of sections, referencing style, and the numbering of tables and figures were altered to align with the thesis format.

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Author Contribution:

Authors: Meredith Hill, Nham Tran

Meredith Hill (graduate student) is the first author of this Literature Review Article. Her contribution was as follows:

Meredith researched the field and collated information that pertained to miRNA:miRNA interactions. She created the preliminary forms of Figure 1.5, 1.6, and 1.7, which were altered by the journal to align with the editorial style. She was highly involved in manuscript writing and subsequent editing, communication with the editor of the journal, and responding to reviewers queries.

Nham Tran provided the initial idea, advice and feedback on draft versions of the article, and aided in the revision of the manuscript and submission to the journal.

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Trends in Cancer

Part of Chapter 3 has been published as a Forum Article in Trends in Cancer.

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Authors: Meredith Hill, Nham Tran

Meredith Hill (graduate student) is the first author of this article and her contribution is as follows:

Meredith researched the field and collated information on miRNA:miRNA interactions and their relationship to cancer. She was highly involved in manuscript writing and subsequent editing, and responding to reviewers queries.

Nham Tran provided the initial idea, contacted the editor, created Figure 3.1 and provided feedback on draft versions of the article. He also communicated with the editor and aided in the revision of the manuscript for final publication.

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Meredith Hill (graduate student) is the first author of this article and her contribution is as follows:

Meredith researched the field and collated information on miRNA:miRNA interactions as they pertained to miR-21. She created the preliminary and final form of Figure 5.1. She was highly involved in manuscript writing and subsequent editing, communicating with the editor of the journal, and responding to reviewers queries.

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Abbreviations

Ago	Argonaute
ASO	Antisense Oligonucleotide
AUC	Area Under the Curve
BMA	Bayesian Modelling Analysis
BRCA	Breast Cancer gene
CEBPB	CCAAT Enhancer Binding Protein Beta
cDNA	complementary DNA
DGCR8	Di George Critical Region 8
DNA	Deoxyribonucleic Acid
DOI	Depth of Invasion
EGFR	Epidermal Growth Factor Receptor
EMT	Epithelial Mesenchymal Transition
ERBB2	Erb-B2 Receptor Tyrosine Kinase
GLM	Generalised Linear Model
GO	Gene Ontology
HNSCC	Head and Neck Squamous Cell Carcinoma
HPSCC	Hypopharyngeal Squamous Cell Carcinoma
HPV	Human Papilloma Virus
HR	Hazard Ratio
Hsp	Heat shock protein
KEGG	Kyoto Encyclopedia of Genes and Genomes
LSCC	Laryngeal Squamous Cell Carcinoma
MAPK	Mitogen-Activated Protein Kinase
MCC	Maximal Clique Centrality
MDM2	Mouse Double Minute 2
miRNA	microRNA
mRNA	messenger RNA
MYC	MYC Proto-Oncogene
ncRNA	non-coding RNA
NGS	Next Generation Signalling
NPSCC	Nasopharyngeal Squamous Cell Carcinoma
ONC	Oncogene
OPSCC	Oropharyngeal Squamous Cell Carcinoma
OSCC	Oral Squamous Cell Carcinoma
PCR	Polymerase Chain Reaction

PDCD4	Programmed Cell Death 4
PI3K-Akt	phosphatidylinositol 3-kinase - protein kinase B
Pre-miRNA	Precursor microRNA
Pri-miRNA	Primary microRNA
PTEN	Phosphatase and Tensin Homolog
RISC	RNA Induced Silencing Complex
RMND5A	Required for Meiotic Nuclear Division 5 Homolog A
RNA	Ribonucleic Acid
RNA pol II	RNA polymerase II
RNase	Ribonuclease
ROC	Receiver Operating Characteristic
RPM	Reads Per Million
RT-qPCR	Real-Time quantitative PCR
SCC	Squamous Cell Carcinoma
SP1	Transcription Factor Sp1
STAT3	Signal Transducer and Activator of Transcription 3
TCGA	The Cancer Genome Atlas
TF	Transcription Factor
TGFB	Transforming Growth Factor Beta
TNRC6A	Trinucleotide Repeat Containing Adaptor 6A
TP53/p53	Tumour Protein P53
TRBP	Transactivation response element-binding protein
TSG	Tumour Suppressor Gene
UTR	Untranslated Region
XPO-5	Exportin-5

Abstract

Head and Neck Squamous Cell Carcinoma (HNSCC) is rising in incidence worldwide, hence there is an increased need to elucidate the molecular pathways responsible for this disease. MicroRNAs (miRNAs) are of great interest due to their role in the initiation and progression of several cancers, including HNSCC. These are a class of small non-coding RNAs that typically perform post-transcriptional regulation of messenger RNA (mRNA) through the recognition of complementary sequences within the 3´ untranslated region (UTR). However, several studies have demonstrated that miRNAs regulate other miRNAs, known as a miRNA-miRNA interaction. This is a relatively new area of research and no information exists pertaining to the impact of these interactions in HNSCC. This thesis focused on the discovery of miRNA-miRNA interactions in HNSCC that are initiated by the oncogenic miRNA, miR-21, and expanded upon a model for direct miRNA-miRNA regulation.

To investigate miRNA-miRNA interactions in HNSCC, miR-21 was overexpressed in HNSCC cells and the changes in miRNA expression were evaluated using a TaqMan™ OpenArray. Fold change analysis determined that 10 miRNAs were upregulated and 150 miRNAs were downregulated in response to miR-21. The top-most dysregulated miRNAs and their gene targets were integrated into a series of networks to determine the cellular impact of miRNA-miRNA interactions. The Cancer Genome Atlas (TCGA) HNSCC cohort was used to evaluate the changes in this set of miRNAs in patients. Of note from this analysis was miR-92a, which is a member of the miR-17~92a cluster. *In vitro* experimentation and miRNA sequencing confirmed that the overexpression of miR-21 resulted in the downregulation of members of the miR-17~92a cluster, as well as two other miRNAs, miR-30c and miR-375. In exploring possible mechanisms for the observed changes in the miR-17~92a cluster, its host gene, MIR17HG, was found to contain several putative miR-21 binding sites. To expand on this mode of miRNA regulation, a novel bioinformatics workflow was developed to identify predicted miRNA binding sites within pri-miRNAs. This analysis uncovered that miRNA binding sites are

abundant within pri-miRNAs and were enriched compared to sites in random sequences.

By using an explorative approach, this thesis collectively identified novel potential miRNA-miRNA interactions of miR-21 in HNSCC, and provided evidence that the direct binding of a miRNA to a pri-miRNA may be a widespread, albeit underexplored, mechanism for miRNA regulation. This has implications on the development of miRNA therapeutics and the use of miRNA-based biomarkers for prognostic and treatment purposes in HNSCC.