UTS UNIVERSITY OF TECHNOLOGY SYDNEY

# Investigating the miRNA pathways contribution to intra-tumour heterogeneity in glioblastoma and RNA binding of isoforms of the miRNA effector protein Argonaute

**by Christopher Michael Smith**

Thesis submitted in fulfilment of the requirements for the degree of

**Doctor of Philosophy**

under the supervision of Gyorgy Hutvagner, Daniel Catchpoole, and Jinyan Li

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December 2022

# Certificate of Original Authorship

I, Christopher Smith, 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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Date: 2/12/2022

# Acknowledgements

First and foremost, I would like to express my sincerest gratitude to my supervisors Gyorgy Hutvagner, Daniel Catchpoole, and Jinyan Li for their mentoring and continuous support throughout this degree. This PhD would not have been possible without you all.

A special thanks to Sarah Bajan for all her wet lab work, which generated much of the data used in this thesis, as well as her mentoring throughout my early years as a student. Thanks to Hanjie Wu, Chaowang Lan, and all other members of the Hutvagner lab for their suggestions and help in the lab and with bioinformatics. In addition, thanks to Diego Fajardon for setting me in the right direction during my first year when I was learning bioinformatics.

Over at the Children's Hospital at Westmead I would like to thank the members of the Tumour Bank, including Aysen Yuksel and Li Zhou, who helped me find my way around the lab and make sure I had everything I needed there. Lawrence Cantrell for his assistance with training so I could work with equipment in The Westmead Institute for Medical Research.

I would also like to thank Dan Carter and Dominik Beck for their insightful feedback in each of my candidature assessments.

Last but certainly not least, a special thanks to my family, including my partner Vee who was by my side through every moment during these years, and my parents for raising and supporting me so I could reach this point in my life.

# Impact of the COVID-19 Pandemic on this Thesis

Our original plan to investigate our first hypothesis included an experiment to generate single cell RNA and small RNA data in a heterogeneous cancer model such as glioblastoma or neuroblastoma. This data was to be used to address both aims by enabling a direct comparison between miRNA and RNA expression profiles in potential cancer subpopulations. However, several months into the COVID-19 pandemic we decided to exclude this experiment, which we deemed high risk due to the scale and ambition of the project as well as the challenges in collaborating cross-institution during this period. We had concerns that access to researchers and laboratories would be impaired and make completion of this project unfeasible. In response, we retained our original aims but had to rely exclusively on public data. We chose glioblastoma as a heterogenous cancer model as it is one of the most extensively sequenced cancers with expression data available for RNAs and small RNAs in bulk tumours and single cells.

We also planned and initiated an experiment in 2021, approximately one year prior to my end of candidature. This involved collaboration with the Tumour Bank, part of The Children's Cancer Research Unit (CCRU) at The Children's Hospital at Westmead. This experiment intended to biologically validate observations of heterogeneity with miRNAs from the Dlk1-Dio3 locus in glioblastoma, by using a fluorescent in-situ hybridization assay (miRNAscope) for detection of miRNAs in formalin-fixed paraffin embedded glioblastoma samples. The project at the Tumour Bank was initially planned to begin late March in 2021. However, due to supplier errors and delays in shipping we were not able to receive all the required reagents to proceed with the experiment until June 2021, right before the second lockdown was instated in Sydney. The proximity of the outbreaks to Westmead hospital and the challenges in coordinating work with other lab members for assistance, access to facilities, and equipment installation, meant I was forced to delay this project until September 2021 with only a few months remaining before my final candidature assessment. While our preliminary results indicated that we were able to detect RNA using the control probes, the staining was inconsistent across cells, and we were unable to resolve this issue through optimization in the available timeframe.

We also had planned a second experiment in 2021, at the University of Technology, Sydney, to sequence Argonaute 2 using Nanopore sequencing for full length isoforms. This was intended to support the findings in chapter 4 where we used Capture-seq and short read sequencing to detect multiple isoforms of Argonaute 2 in human tissues and cell lines. To make this project feasible, we developed a modified library preparation protocol which could generate Argonaute 2 cDNA through targeted amplification. However, we were met with significant challenges with generating full length

amplicons of this gene, and because of constant delays throughout 2021 our timeline for both experiments clashed, and we chose to prioritise the miRNAscope project at the Tumour Bank.

Despite the challenges imposed by COVID-19 I was able to publish 3 first author papers – 1 review and 2 research papers.

**Publications**

Smith, C. M., Catchpoole, D. & Hutvagner, G. Non-Coding RNAs in Pediatric Solid Tumors. *Front. Genet.* **10**, 1–18 (2019).

Smith, C. *et al.* Cataloguing the small RNA content of honey using next generation sequencing. *Food Chem. Mol. Sci.* **2**, 100014 (2021).

Smith, C. M. & Hutvagner, G. A comparative analysis of single cell small RNA sequencing data reveals heterogeneous isomiR expression and regulation. *Sci. Rep.* **12**, 2834 (2022).

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# Abstract

miRNAs are highly abundant small non-coding RNAs that are essential for post-transcriptional gene regulation. Many of the mechanisms which regulate miRNA expression and function are poorly understood. Their dysregulation is documented extensively in many diseases including cancer.

Glioblastoma is a highly aggressive and heterogeneous brain cancer that affects patients of all ages. Intra-tumoural heterogeneity describes the existence of genomically distinct subpopulations of tumour cells which can lead to differences in growth rate, metastatic potential, or vulnerability to certain treatments. The first part of my thesis investigates how miRNAs and miRNA variants (isomiRs) may be involved in intra-tumoural heterogeneity by applying bioinformatics analyses to single cell small RNA and RNA sequencing data generated from previous studies. This work identified two miRNA clusters, the Dlk1-Dio3 locus and miR-224/452, as potential contributors to intra-tumour heterogeneity in glioblastoma and may be involved in cell state regulation. Additionally, we found evidence of cell autonomous regulation and function of isomiRs, highlighting another regulatory mechanism that may play a role in heterogenous cancers. These miRNAs may have utility as cancer biomarkers and implicate a novel set of targets for therapeutic research.

The second part of my thesis investigates splice variants (isoforms) of Argonaute, an essential protein in the miRNA pathway that mediates their regulatory effects. Numerous Argonaute isoforms have been previously annotated, with alterations in protein domains critical for miRNA binding and function. However, current studies base their assumptions of miRNA activity through a single variant of Argonaute and the consequence of these alterations and their biological relevance has not been investigated yet. We identified two variants of Argonaute with altered miRNA binding characteristics that are variably expressed in normal and cancerous cells, revealing a novel form of miRNA regulation that could also have implications in cancer research.