



THE UNIVERSITY  
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**Micro-RNAs in cancer: novel origins and  
sequence variation**

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## Overview

Non-coding RNAs have become a hot topic of cancer research in recent decades, with miRNAs being probably the most active area of research. However, the functions of short non-coding RNA (ncRNA) fragments commonly existing in cells remains less understood. Herein we explore the novel ncRNAs which play a role in cancer progression.

The first part of the thesis focuses on miRNAs processed from novel sources that have a role in p53 regulation. p53 is a master tumour repressor that participates in vast regulatory networks, including feedback loops involving microRNAs (miRNAs) that regulate p53 and that themselves are direct p53 transcriptional targets. A group of polycistronic miRNA-like non-coding RNAs is derived from small nucleolar RNAs (sno-miRNAs) that are transcriptionally repressed by p53 through their host gene, *SNHG1*. Among them, sno-miR-28 is the most abundant sno-miRNA bound to the AGO (Argonaute) proteins and it directly targets TAF9B, thereby de-stabilizing p53. Therefore, a positive a regulatory loop was observed comprising p53, *SNHG1*, sno-miR-28 and TAF9B, which influences p53 stability and downstream p53-regulated pathways. In addition, *SNHG1*, *SNORD28* and sno-miR-28 are all significantly upregulated in breast tumours and the overexpression of sno-miR-28 promotes breast

epithelial cell proliferation and colony formation. This research has broadened our knowledge of the crosstalk between small non-coding RNA pathways and p53 regulation.

The second part of the thesis investigates naturally existing isoforms of miR-222 that play pro-apoptotic roles. Alternative processing at the 3' end of miRNAs has been broadly observed, producing variable lengths of miRNA mature forms. Deep-sequencing of various tissues and tumours, combined with sequencing of AGO-bound miRNAs from cell lines, indicates a variable proportion of endogenous miR-222 that is extended by one to five nucleotides at the 3' end. We demonstrated that the 3' heterogeneity of miR-222 possesses dramatic implications for the phenotype of miR-222 transfected cells, with longer isoforms driving apoptosis in addition to the proliferation inhibition bestowed by both the short (canonical) and longer forms. Further investigation revealed intrinsic apoptotic events exhibiting a positive correlation to the length of miR-222 isoforms, but not the specific 3' sequence. However, the apoptosis failed to be correlated to interferon immunoresponse, and the longer miR-222 isoform exhibits identical targeting activity as the canonical miR-222. Widespread disruption of the expression of key PI3K-AKT components was observed upon miR-222CUCU transfection. A PI3K regulatory subunit, PIK3R3, was of particular interest, as siPIK3R3 phenocopied miR-222CUCU in terms of

apoptotic effects and inhibition of PI3K-AKT gene expression. Given the high prevalence of 3' variance in many other miRNAs, the functional impact of miR-222 isoforms reveals another layer of miRNA regulation that has implications for cancer therapy.

Taken together, the existence of miRNAs processed from novel sources and isomiRs has added to the complexity of our knowledge about miRNA regulation. These areas are much less explored than conventional miRNA processing and regulation, but their profound molecular biological and physiological implications suggest an unexplored layer of miRNA biology and may shed light to the contemporary research of cancer progression.

## Publications

### Original Research Papers

- Feng Yu, Cameron P. Bracken, Katherine A. Pillman, David M. Lawrence, Gregory J. Goodall, David F. Callen, Paul M. Neilsen. p53 represses the oncogenic sno-miR-28 derived from a snoRNA. PLoS One. 2015; 10(6): e0129190.
- Sun D, Yu F, Ma Y, Zhao R, Chen X, Zhu J, Zhang CY, Chen J, Zhang J. "Sun D and Yu F contributed equally to this work." MicroRNA-31 activates Ras pathway and functions as an oncogenic microRNA in human colorectal cancer by repressing RAS p21 GTPase activating protein 1 (RASA1). Journal of Biological Chemistry. 2013 Mar 29; 288(13):9508-18.

### Presentations

- Poster: Naturally existing isoforms of miR-222 play distinct pro-apoptotic roles. The 7th Barossa Meeting on cell signalling in cancer biology and therapy, 18-21 Nov. 2015
- Poster: Naturally existing isoforms of miR-222 play distinct pro-apoptotic roles. ASMR SA Annual Scientific Meeting, 3 June 2015.
- Poster: p53 represses the oncogenic sno-miR-28 derived from a snoRNA. Florey International Postgraduate Research Conference, 25 September 2014.

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## **Declaration**

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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