

# **The role of microRNAs that target the renin-angiotensin system in placental development and function**

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Bachelor of Biomedical Science (Honours Class I)

*A thesis submitted in fulfilment of the requirements for the  
degree of Doctorate of Philosophy (Experimental Pharmacology)*

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# Declaration

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I hereby certify that the work embodied in the thesis is my own work, conducted under normal supervision. The thesis contains no material which has been accepted, or is being examined, for the award of any other degree or diploma 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. I give consent to the final version of my thesis being made available worldwide when deposited in the University's Digital Repository, subject to the provisions of the Copyright Act 1968 and any approved embargo.

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By signing below, I confirm that Anya Arthurs contributed to the papers and publications included in this thesis. Their contribution is outlined within individual chapters.

Signed: Kirsty G Pringle

# Statements of contribution

By signing below, I confirm that Anya Arthurs contributed upward of 50% towards data collection/analysis and manuscript preparation for all the publications included in this thesis for which I am a co-author.

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*The role of oxygen in regulating microRNAs in control of the placental renin-angiotensin system.* Molecular Human Reproduction (2019). **25**(4): 206-217

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**Arthurs AL\***, Delforce SJ\*, Drury HR, Quinn RK, Lim R, Tadros MA, Lumbers ER, Pringle KG.

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\*These authors contributed equally to this study.

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# Abbreviations

ACE	Angiotensin converting enzyme
Ago2	Argonaute 2
AGT	Angiotensinogen
Ang	Angiotensin
ANGPT	Angiopoietin
ANOVA	Analysis of variance
ATP	Adenosine triphosphate
AT <sub>x</sub> R	Angiotensin II Type x Receptor
AVP	Arginine vasopressin
BMI	Body mass index
C14MC	Chromosome 14 cluster
C19MC	Chromosome 19 cluster
CTB	Cytotrophoblast
CVE	Chorionic villus explant
CYR61	Cysteine-rich protein 61
DMEM	Dulbecco's modified eagle media
DNA	Deoxyribonucleic acid
EGF	Epidermal growth factor
ELISA	Enzyme-linked immunosorbent assay
ERK	Extracellular signal-related kinase
EV	Extracellular vesicle

EVT	Extravillous trophoblast
g-ASCF	Glycerol substituted artificial cerebrospinal fluid
hCG	Human chorionic gonadotrophin
hCS	Human chorionic somatomammotrophin
HIF	Hypoxia-inducible factor
HPL	Human placental lactogen
HRP	Horseradish peroxidase
IGF	Insulin-like growth factor
IUGR	Intrauterine Growth Restriction
LNA	Locked nucleic acid
MAPK	Mitogen activated protein kinase
miRNA	microRNA
mRNA	Messenger RNA
nc-RNA	Non-coding RNA
NF- $\kappa$ B	Nuclear factor $\kappa$ -light-chain-enhancer of activated B cells
p85 $\alpha$ -PI3K	p85 $\alpha$ -phosphoinositol 3-kinase
PAI	Plasminogen activator inhibitor
pc	Post-coital
PCR	Polymerised chain reaction
PE	Preeclampsia
PLAP	Placental alkaline phosphatase
PPAR- $\gamma$	Peroxisome proliferator-activated receptor
pre-miRNA	Precursor miRNA
pri-miRNA	Primary miRNA
(P)RR	(Pro)renin receptor

PTB	Preterm birth
PTEN	Phosphatase and tensin homolog
PVDF	Polyvinylidene difluoride
RAS	Renin-angiotensin system
RISC	RNA-induced silencing complex
RNA	Ribonucleic acid
SEM	Standard error of the mean
SGA	Small for Gestational Age
STB	Syncytiotrophoblast
TBS	Tris buffered saline
TGF- $\beta$	Transforming growth factor $\beta$
TLR	Toll-like receptor
UTR	Untranslated region
V-ATPase	Vacuolar-ATPase
VEGF	Vascular endothelial growth factor

# Abstract

This thesis explores the role of microRNAs (miRNAs) that target the placental renin-angiotensin system (RAS) in placental development and function. The placental RAS contributes to trophoblast proliferation, migration and invasion, as well as vasoconstriction and angiogenesis. As such, the placental RAS is essential for adequate placental development and function. miRNAs targeting RAS mRNAs are present in the developing placenta, and their expression varies across gestation, along with RAS expression. Furthermore, changes in expression of these miRNAs are seen in pregnancy complications. This research investigated:

- the effects of low oxygen tension, such as the low oxygen environment of the first trimester placenta, on expression of miRNAs targeting the placental RAS
- the effect of miRNAs targeting the placental RAS on the functional ability of trophoblasts to proliferate, and
- the *in vitro* and *in vivo* roles of miR-155 in the placenta.

I found that in HTR-8/SVneo cells (an immortalised human extravillous trophoblast cell line) cultured in low oxygen tension (~1%), there was suppressed expression of ten miRNAs predicted to target the RAS. Furthermore, this allowed increased expression of two critical RAS components. This would suggest that a low oxygen environment encourages placentation by suppressing miRNA expression to allow RAS activity. These experiments were then repeated in first trimester chorionic villus explants (CVE) obtained from primary tissue, where the expression of only four of the tested miRNAs was suppressed by low oxygen tension. The RAS components that were increased in this low oxygen milieu were also different to those increased in the cell line experiments. These experiments taken together illustrate the differing roles of miRNAs and the RAS in the extravillous trophoblasts and the chorionic villi.

Investigation into the effect of miRNAs predicted to target the RAS on trophoblast proliferation showed that treatment with mimics of these miRNAs suppressed, or completely inhibited, trophoblast proliferation. In the case of many miRNAs tested, a dose-dependent response was observed, with higher mimic concentrations leading to lower proliferation of the cells. The effect of these miRNAs on their intended RAS targets was also assessed, with 7 of the 9 miRNAs suppressing mRNA expression of their RAS targets. These experiments demonstrated the functional effect of miRNA dysregulation of placental development through trophoblast proliferation. This has particular implications for a number of pregnancy complications that arise from poor placental development such as fetal growth restriction and preeclampsia.

Finally, the role of miR-155 in placentation was observed, as a miR-155<sup>-/-</sup> mouse model revealed the consequences of miR-155 deletion. Placentae from miR-155<sup>-/-</sup> dams had significantly larger labyrinthine zones (responsible for substrate transfer to the fetus), but no change in placental weight, indicating an increase in the labyrinth zone to placental area ratio that would suggest that the placenta has improved efficiency of substrate transfer. These dams also had larger fetuses, possibly as a consequence of the changes in placental morphology. As miR-155 is known to target the angiotensin II type 1 receptor (AT<sub>1</sub>R), mRNA and protein was measured and was significantly increased in miR-155<sup>-/-</sup> placentae. Additionally, *in vitro* investigation into the functional effects of miR-155, utilising a miR-155 mimic, showed that treatment with this miRNA decreases trophoblast proliferation in a dose-dependent manner. Altogether, this study clarified the importance of appropriate miR-155 regulation during pregnancy. This was particularly important as miR-155 upregulation is seen in preeclampsia (PE), a dangerous pregnancy complication.

Therefore, through utilising a cell line, primary tissue explants and a murine model, I have been the first to demonstrate the effects of various miRNAs targeting the placental RAS on

RAS expression, both in and out of a low oxygen milieu, trophoblast proliferation, placental morphology and fetal growth.