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)

February, 2019

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This research was supported by an Australian Governments Research Training (RTP) Scholarship

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.

Eugenie R. Lumbers	04.02.2019
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Sarah J. Delforce	04.02.2019
Andrea Mathe	04.02.2019
Brian J. Morris	04.02.2019
Hannah Drury	04.02.2019
Rikki Quinn	04.02.2019

Rebecca Lim	04.02.2019
Melissa A. Tadros	04.02.2019
Sarah Robertson	04.02.2019
Celine Corbisier de Meaultsart	04.02.2019
John Schjenken	04.02.2019

Acknowledgements

My PhD has made me some amazing friends, taken me some gorgeous places, made me cry (a lot) and made me fall in love with biochemistry all over again. I know I wouldn't have been able to do it without so many incredible people. First, I need to thank Kirsty, my incredible lab mum / serial draft-reader for giving me a chance when I came out of nowhere and asked to be a part of her lab. I can't thank you enough for all the support, the cheering up when I cried in your office, the guidance and the mentoring. I have no doubt that the combination of you and Eugenie was the reason I loved my project so much. Eugenie, you are the smartest Wonder Woman I've had the privilege of meeting, and I 100% want to be you when I grow up one day. Thank you for your guidance, the many, many proof-reading hours you have spent on my work, and the exciting debates about miRNAs. I am so grateful to have had amazing, strong and inspiring supervisors.

Thank you to my lab buddies who have always been there for a coffee, or a karaoke R&B Friday session. In only your seat order; Saijey, Yu Qi, Jules, Sam, Celine, Gabby, Hannah, Mark, Sarah, Sonia and Jason, you make me smile and I'm so glad to know you the way I do. Thank you in particular to those of you who have put up with a meltdown on occasion, or dragged me to boot camp at 5am, or water aerobics, or had a good coffee and a gossip. You are forever welcome in my spare bedroom and I intend to drop in on you whenever I have the option. A huge thank you to Andrea, who tirelessly was kind and helpful to me as I asked her the same questions over and over again. Thank you as well to Nish, for the extremely kind little notes or presents you have left me over the years, and I apologise for the month-long period I spent leaving anonymous notes as your creepy admirer.

Mike, you were the best Honours supervisor, and I'm grateful to call you my friend. Thank you for our coffee catch-ups, for trusting me with the knowledge of Dylan before others, for the

little pep talks when you still managed to catch me crying, even though we are on separate floors. Thank you for the jokes and the amazing references which you insist were ‘slagging me off’ to future employers.

Celeste, I consider you my accidental best friend. You started off as someone I wanted to keep in touch with and somehow became the person I tell everything to. Thank you for not charging me for your excellent counselling services; I would be broke. You have been an incredible friend and I know I’m not going to let you go, regardless of the distance.

I’m not really sure how to thank you Jamie. You know me better than I know myself and have somehow always supported me while making me feel independent and strong. I know I am the luckiest person to have you. Thank you for always listening to me, whether I was blabbering animatedly about some new thing I read that I thought was amazing, or some pretty graph that I made, or I was complaining or crying. Thank you for not judging me when I came home claiming that life was awful and I was having potato gems for dinner. I have changed since you met me, and a lot of that has happened in the last three years. Thank you for helping me change for the better. I love you.

Deb, Jamie, Sam; you’re amazing. Thank you for always listening to me ramble about things you don’t need to hear about. Thank you for supporting me whenever I was down and didn’t want to chat. Thank you for all the dinners that made me feel loved, regardless of what was going on. I’m incredibly lucky to have gained the most lovely people as my family.

And, to my family; Zali, Illy, Yuri, I don’t know better siblings. I love you all. Thank you for your love and humour and happiness. Mama and Papa; I think I would like to dedicate this thesis to you. Not only because of the million things you have done for me, or for raising me, or for supporting me, or for loving me, but because you never let me believe that I couldn’t do it.

Publications arising from this thesis

Wang Y, Lumbers E**, Arthurs AL**, Corbisier de Meaultsart C, Mathe A, Avery-Kiejda KA, Roberts CT, Broughton Pipkin F, Marques FZ, Morris BJ, Pringle KG.

Regulation of the human placental (pro)renin receptor-prorenin-angiotensin system by microRNAs. Molecular Human Reproduction (2018). **24**(9): 453-464

Arthurs AL, Lumbers ER, Delforce SJ, Mathe A, Morris BJ, Pringle KG.

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.

Oxygen-induced regulation of placental microRNA and renin-angiotensin system expression in first-trimester chorionic villi.

Submitted to Reproduction, under review.

*These authors contributed equally to this study.

Arthurs AL, Lumbers ER, Pringle KG.

MicroRNA mimics that target the placental renin-angiotensin system inhibit trophoblast proliferation. Molecular Human Reproduction (2019) **25**(4): 218–227

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Arthurs AL, Lumbers ER, Corbisier de Meaultsart C, Robertson S, Schjenken J, Pringle KG. *The role of miR-155 in placentation.*

Prepared for submission to Cell.

Abstracts arising from this thesis

Anya L. Arthurs, Yu Wang, Eugenie R. Lumbers, Kirsty G. Pringle. 2017

Oxygen regulation of placental miRNA expression**.** *Fetal Neonatal Workshop, Canberra.*

Anya L. Arthurs, Andrea Mathe, Eugenie R. Lumbers, Kirsty G. Pringle. 2017

miRNAs targeting the placental renin-angiotensin system and their regulation by oxygen. *Australian Society for Medical Research Conference, Newcastle.*

Anya L. Arthurs, Andrea Mathe, Eugenie R. Lumbers, Kirsty G. Pringle. 2017

Placental miRNA expression and renin-angiotensin system activity are regulated by oxygen. *Society of Reproductive Biology Conference, Perth.*

Anya L. Arthurs, Eugenie R. Lumbers, Kirsty G. Pringle. 2018

Placental miRNAs that target the renin-angiotensin system, and their effect on trophoblast proliferation. *Australian Society for Medical Research Conference, Newcastle.*

Anya L. Arthurs, Eugenie R. Lumbers, Kirsty G. Pringle. 2018

The effects of placental miRNAs predicted to target the renin-angiotensin system on trophoblast proliferation. *Society of Reproductive Biology Conference, Adelaide.*

Anya L. Arthurs, Eugenie R. Lumbers, Kirsty G. Pringle. 2018

Placental miRNAs, the renin-angiotensin system and proliferation. *International Federations of Placental Associations conference, Tokyo, Japan.*

Anya L. Arthurs, Eugenie R. Lumbers, John Schjenken, Sarah Robertson, Kirsty G. Pringle. 2018 (Invited speaker).

miR-155 and the renin-angiotensin system in placentation. *NSW Reproduction Forum, Sydney.*

Anya L. Arthurs, Eugenie R. Lumbers, Kirsty G. Pringle. 2019 (Invited speaker).

The role of miRNAs and the renin-angiotensin system in placental development. *Erasmus University, Rotterdam, The Netherlands.*

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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_XR	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-κB	Nuclear factor κ-light-chain-enhancer of activated B cells
p85α-PI3K	p85α-phosphoinositol 3-kinase
PAI	Plasminogen activator inhibitor
pc	Post-coital
PCR	Polymerised chain reaction
PE	Preeclampsia
PLAP	Placental alkaline phosphotase
PPAR-γ	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-β	Transforming growth factor β
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$^{-/-}$ mouse model revealed the consequences of miR-155 deletion. Placentae from miR-155$^{-/-}$ 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_1R), mRNA and protein was measured and was significantly increased in miR-155$^{-/-}$ 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.