Regulation of the uteroplacental renin–angiotensin system in human pregnancy

By

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Bachelor of Medical Science (Hons)

A thesis submitted in fulfilment of the requirements for the degree of
Doctorate of Philosophy

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on April 2013

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DECLARATION

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STATEMENT OF CONTRIBUTION TO JOINT PUBLICATIONS

I, Yu Wang, attest that I have made a primary and original contribution to the publications, and manuscripts awaiting publication, included in this thesis, as detailed below and endorsed by my supervisors.

Chapter	Title	Status	Contribution
2	Regulation of the Renin–Angiotensin System (RAS) in BeWo and HTR–8/SVneo trophoblast cell lines.	Published	Experimental design and procedures Data analysis Manuscript preparation
3	Fetal sex affects expression of Renin–Angiotensin System components in term human decidua.	Published	Experimental design and procedures Data analysis Manuscript preparation
4	The effects of cyclic AMP, sex steroids and global hypomethylation on the expression of genes controlling the activity of the Renin–Angiotensin System in placental cell lines.	Published	Experimental design and procedures Data analysis Manuscript preparation
5	Regulation of Renin–Angiotensin System (RAS) pathways in the Human Decidua	Submitted	Experimental design and procedures Data analysis Manuscript preparation

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STATEMENT OF CONTRIBUTION

I attest that Research Higher Degree candidate **Yu Wang** contributed to experimental design and procedures, data analysis and manuscript preparation to the paper/publication entitled "*Regulation of the Renin–Angiotensin System (RAS) in BeWo and HTR–8/SVneo trophoblast cell lines*"

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STATEMENT OF CONTRIBUTION

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ACKNOWLEDGEMENTS

I would like to take this opportunity to acknowledge the help and support of my dear supervisors, Eugenie Lumbers, Kirsty Pringle and Tamas Zakar, without whom none of this would be possible. Beyond their guidance and support with all things scientific, they have also had a great and positive impact on the person that I am now. Eugenie, I feel deeply inspired each time I see you, for I see boundless energy and passion. Kirsty, your patience and knowledge, has made each and every problem and puzzle seem less daunting. Tamas, I am very grateful for all of your help and guidance.

I would like to acknowledge the head of the Mothers and Babies research centre, Professor Roger Smith and the University of Newcastle for giving me the opportunity to do a PhD in Newcastle. In addition, note that this PhD was supported by the NHMRC and HMRI.

To all my friends and colleagues at the Mothers and Babies research centre, you have made everyday much more enjoyable and frustrating moments bearable. I am especially grateful to Annie and all those on the collection roster for organising and collecting samples.

To all my friends, thank you for putting up with me, I love you all, what more can I say. To my mum and dad, I am thankful everyday that I have been bless with such wonderful and loving parents.

TABLE OF CONTENT

ABSTRACT

A renin–angiotensin system (RAS) exists within the decidua and the placenta and has been shown to play a significant role in the regulation of trophoblast proliferation, invasion and migration, angiogenesis and the modulation of blood flow.

As the RAS is crucial for the normal progression of pregnancy, it naturally follows then; that disruptions to the uteroplacental RAS during pregnancy may contribute to pregnancy complications such as intrauterine growth restriction (IUGR) and preeclampsia. Although many studies have linked changes in the RAS to these pathologies, our understanding of how the RAS is involved in these physiological changes is lacking, as are adequate medical interventions. This thesis attempts to address how the RAS is regulated within the uteroplacental unit.

We explored the RAS in two trophoblast (*i.e.,* placental) cell lines, HTR–8/SVneo and BeWo cells, to determine how they express the genes of the RAS and their proteins, in order to determine their value as models of the placental RAS. We found however, that HTR–8/SVneo cells expressed only the Angiotensin II (Ang II)/type 1 Ang II receptor (AT_1R) pathway, while the BeWo cells expressed only the Angiotensin 1–7 (Ang 1–7)/Mas receptor pathway. Therefore these cell lines are not good models for placental RAS, but they are useful for exploring the regulation of RAS pathways within the placenta. Our aim was then to determine if we could induce the RAS pathways not expressed, in those cell lines that lacked them.

We were also interested in the maternal decidua, as it is the main site of production of renin in the intrauterine tissues during human pregnancy and it plays a critical role in regulation of trophoblast invasion and placentation. We found that the

expression of certain RAS genes within the decidua was sex specific. Decidua is a maternal tissue, yet the sex of the fetus determines the level of genes expression of the RAS pathway. This is extremely interesting since fetal sex is a major determinant of pregnancy outcome.

We then showed that the sex specific differences in (pro)renin gene expression (*REN*) was not due to maternal sex steroid exposure. In fact, *ex vivo*, prorenin protein, along with several other RAS genes were expressed in a sex specific manner. Based on these observations, we were interested in determining how the sex of the fetus could affect RAS gene expression of a maternal tissue. In addition, we wanted to show whether this sex difference could be attenuated with cAMP stimulation.

In conclusion, this thesis shows the RAS pathways within two trophoblast cell lines, establishes a decidual explant model that expresses the RAS and demonstrates that the decidual RAS is sexually dimorphic and finally discusses how these findings may contribute to our understanding of the role fetal sex plays in determining pregnancy outcome.

LIST OF FIGURES

LIST OF PUBLICATIONS INCLUDED AS PART OF THIS THESIS

Wang Y, Pringle KG, Sykes SD, Marques FZ, Morris BJ, Zakar T, Lumbers ER, 2012

Fetal sex affects expression of Renin–Angiotensin System components in term human decidua. *Endocrinology* 153:462-468

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Regulation of Renin–Angiotensin System (RAS) pathways in the Human Decidua (Submitted to *Endocrinology*)

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Sykes SD, Mitchell C, Pringle KG, Wang Y, Zakar T, Lumbers ER

Transgestational DNA methylation in the regulation of the human intrauterine Renin–Angiotensin System and prorenin processing enzymes (Submitted to *Human Reproduction*)

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LIST OF ABBREVIATIONS

µL	Microlitre
µg	Microgram
(P)RR	(pro)renin receptor
ACE	Angiotensin converting enzyme
ACE1	Angiotensin converting enzyme gene
ACE2	Angiotensin converting enzyme 2
ACE2	Angiotensin converting enzyme 2 gene
AGT	Angiotensinogen
AGT	Angiotensinogen gene
AGTR1	Angiotensin II type 1 receptor gene
AGTR2	Angiotensin II type 2 receptor gene
Ang 1–7	Angiotensin 1–7
Ang 1–9	Angiotensin 1–9
Ang I	Angiotensin I
Ang II	Angiotensin II
Ang III	Angiotensin III
Ang IV	Angiotensin IV
ANOVA	Analysis of variance
APA	Aminopeptidase A
ARBs	Angiotensin receptor blockers
AT_1R	Angiotensin II type 1 receptor
AT_2R	Angiotensin II type 2 receptor
ATP6AP2	(Pro)renin receptor gene
AZA	5'–aza–2'–deoxycytidine
BeWo	Choriocarcinoma derived cell line
c.p.m	Counts per minute
cAMP	Cyclic adenosine monophosphate
CRE	cAMP response element
CREB	cAMP response element–binding protein
CRH	Corticotropin releasing hormone
DNA	Deoxyribonucleic acid
DNMT	DNA methyltransferase
E_2	Estradiol–17β
ECM	Extracellular matrix
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme linked immunosorbent assay
eNOS	Endothelial nitric oxide synthase
ERK1	Extracellular signal–related protein kinase 1
ERK2	Extracellular signal–related protein kinase 2

EVT	Extravillous trophoblast cell
h	Hour
hCG	Human chorionic gonadotropin
HEPES	4– (2–hydroxyethyl) –1–piperazineethanesulfonic acid
HESCs	Human endometrial stromal cells
HSP	Heat shock protein
HTR–8/SVneo	Transformed first trimester extravillous trophoblast cell line
IGFBP–1	Insulin-like growth factor binding protein–1 gene
IGFBP–1	Insulin-like growth factor binding protein–1
JG	Juxtaglomerular
JNK	c–jun N terminal kinase
KAI1	Gene for the transmembrane glycoprotein of the tetraspanin family
LDH	Lactate dehydrogenase
LH	Luteinizing hormone
MAPK	Mitogen-activated protein kinase
MAS1	Protooncogene receptor gene
MMP	Metalloproteinase
MPA	Medroxyprogesterone acetate
Mas	Protooncogene receptor
mL	Millilitre
mRNA	Messenger RNA
mg	Milligram
miRNA	Micro RNA
$NaHCO_3$	Sodium bicarbonate
NEP	Neutral endopeptidase
ng	Nanogram
nm	Nanometre
NSP	Nephrectomised sheep plasma
OD	Optical density
PAI	Plasminogen activator inhibitor
PEP	Prolylendopeptidase
pg	Picogram
PGHS–2	Prostaglandin H synthase–2
PI3K	Phosphatidylinositol-3 kinase
Pit–1	Positive transcription factor 1
PLZF	Promyelocytic Leukaemia zinc finger protein
PMSF	Phenylmethylsulfonyl fluoride
PRL	Prolactin
qRT PCR	Real-time quantitative reverse transcription polymerase chain reaction

RAS	Renin-Angiotensin System
REN	Renin gene
RIA	Radioimmunoassay
RNA	Ribonucleic Acid
SPSS	Software package for statistical analysis
SRY	Sex determining region Y gene
TGF–β_1	Transforming growth factor beta 1
TIMP	Tissue inhibitors of metalloproteinase
TMB	3,3,5,5–tetramethylbenzidine
V–ATPase	Vacuolar H^+–adenosine triphosphatase
VEGF	Vascular endothelial growth factor
VSMC	Vascular smooth muscle cells

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