Role of MicroRNA in Early Life Placental Programming of Insulin Resistance and Metabolic Health

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'I love fools' experiment, I am always making them' – Charles Darwin

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STATEMENT OF ORIGINALITY AND AUTHETICITY

I certify that this work contains no material which has been accepted 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 in text. In addition, I certify that no part of this work will, in the future, be used in a submission 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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Signed,

Himawan Harryanto

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TABLE OF ABBREVIATIONS AND BIOCHEMICAL NAMES

Acox1	Acyl-CoA oxidase 1, palmitoyl
AdipoR2	Adiponectin Receptor 2
AMC	Adelaide Microarray Centre
ANOVA	Analysis of Variance
BMI	Body Mass Index
BUVL	Bilateral uterine artery vessel ligation
cDNA	complementary DNA
Cbl	E3 ubiquitin-protein ligase
Ccdc88a	Coiled-coil domain containing 88A
CSIRO	Commonwealth Scientific and Industrial Research Organisation
DMR	Differentially Methylated Region
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DNMT	DNA methyltransferase
dNTP	Deoxyribonucleotide triphosphate
FFA	Free Fatty Acid
Foxo1	Forkhead box protein O1
Foxo3	Forkhead box protein O3
HEC	Hyperinsulinaemic euglycaemic clamp
Hk2	Hexokinase 2
IGF-1	Insulin-like Growth Factor 1

IPA	Ingenuity Pathway Analysis
IPGTT	Intraperitoneal glucose tolerance test
IR	Insulin Receptor
Irs1	Insulin receptor substrate 1
Irs2	Insulin receptor substrate 2
IVGTT	In Vitro Glucose Tolerance Test
IUGR	Intrauterine Growth Restriction/Retardation
LNA	Locked Nucleic Acid
μg	microgram
microRNA/miRNA/miR	Micro ribonucleic acid
Mon2	MON2 homolog
mRNA	Messenger ribose nucleic acid
p110β	PI-3-kinase, catalytic, beta polypeptide
p85α	PI-3-kinase, regulatory subunit 1
PASW	Predictive Analytics SoftWare
PI	Ponderal Index or Placental Insufficiency
Pparα	Peroxisome proliferative activated receptor, alpha
PR	Placental Restriction or placentally restricted
RNA	Ribonucleic acid
RT	reverse transcribed
RT-PCR	Real Time Polymerase Chain Reaction
SA	South Australia
SEM	Standard error of mean

Slc2a4	Solute carrier family 2, member 4
T2DM	Type 2 Diabetes Mellitus
UNICEF	United Nations Children's Fund
WEHI	Walter and Eliza Hall Institute of Medical Research
WHO	World Health Organization

CONFERENCE PRESENTATIONS ARISING FROM THIS THESIS

'Placental Restriction Alters Insulin Actions and microRNAs Expression in Insulin Sensitive Tissues of Adult Offspring in the Rat'
H. Harryanto, E. M. Jamil, B. L. Summers-Pearce, P. A. Grant, M. J. De Blasio, S. Moretta, W-C. Kong, K. L. Gatford, M. Dziadek, M. E. Wlodek, J. A. Owens

August 2012
ESA-SRB Annual Scientific Meeting 2012
Oral presentation on ESA Orals: Diabetes

'Role of Hepatic miR-142-3p and miR-144 in Development of Non-Alcoholic Fatty Liver Disease in Placentally Restricted Adult Sheep Offspring'
Himawan Harryanto, Miles J. De Blasio, Patricia A. Grant, Kathryn L. Gatford, Julie A. Owens

July 2011
2nd RNAi Australia Meeting
Oral presentation

'Placental Restriction Alters MicroRNAs Expression in Skeletal Muscle, Liver and Adipose Tissue Young and Adult Offspring in the Sheep'
H. Harryanto, M. J. De Blasio, P. A. Grant, K. L. Gatford, J. S. Robinson, J. A. Owens

August 2010
ESA-SRB Annual Scientific Meeting 2010
Oral presentation on ESA Orals: Early life programming & IUGR

'IUGR Alters rno-miR-16, -18a and -142-3p in Day 260 Omental Fat BUVL Rat Offspring Tissue'
H. Harryanto, B. L. Summers-Pearce, M. J. De Blasio, S. Moretta, T. A. How, P. A. Grant, J. A. Owens

August 2010
ESA-SRB Annual Scientific Meeting 2010
Poster presentation

'Role of microRNAs in Placental Programming of Skeletal Muscle Insulin Resistance'

H. Harryanto, M. L. Harland, P. A. Grant, M. J. De Blasio, K. L. Gatford, J. S. Robinson, J. A. Owens

Presented on:

October 2009
IFPA Conference 2009, Adelaide
Poster Presentation
September 2009
Faculty of Health Sciences Research Expo 2009
Poster Presentation

August 2009
ESA-SRB Annual Scientific Meeting 2009
Oral presentation on ESA Orals: Metabolism & Obesity

June 2009
ASMR Medical Research Week®
SA Scientific Meeting 2009
Poster Presentation

ABSTRACT

Intrauterine growth restriction (IUGR) is associated with insulin resistance and diabetes, particularly later in adult life. Placental restriction is a common cause of IUGR, this condition induces insulin resistance and/ or insulin deficiency and consequently, impaired glucose tolerance in offspring, in the sheep and rat. Reduced expression of insulin signalling genes and that of their key metabolic targets in insulin sensitive tissues and of some molecular determinants of pancreatic β-cell insulin secretion and mass, contributes to impaired insulin action in offspring, in experimental and human IUGR. However, the underlying molecular mechanisms whereby IUGR alters the molecular profile of insulin sensitive and secreting tissues in later life are largely unknown. The studies described in this thesis examine the potential role of microRNAs (miRNAs) in the developmental programming of impaired insulin action in IUGR offspring. MiRNAs are short single-stranded RNAs (22 nucleotide in length), which are able to reduce the translation and/or abundance of mRNA and protein of targets. Each miRNA is predicted to regulate the abundance of many targets in co-ordinated networks to modify function, providing a potentially powerful pathway for developmental programming to influence later phenotype.

Here, IUGR was induced by restricting placental growth and development surgically in sheep (pre-conception removal of most implantation sites) or in the rat (ligation of uterine blood vessels in late gestation). In each species, the effect of placental restriction and IUGR on miRNA expression and expression of key predicted targets, including that of insulin signalling and key metabolic genes in the insulin sensitive tissues: liver, skeletal muscle and adipose (perirenal in sheep and omental in rat), in adult offspring were characterised. The effect of placental restriction on pancreatic miRNA expression in adult offspring was also determined in the sheep.

Placental restriction and IUGR mostly increased miRNA expression in insulin sensitive tissues of the adult sheep and in a sex specific manner, suggesting the potential for increased repression of the translation or abundance of their molecular targets and related functions. The liver, followed by skeletal muscle and adipose tissue, showed the greatest susceptibility in terms of numbers of miRNAs with altered expression following placental restriction. In males, placental restriction increased hepatic expression of eight miRNAs by ~1.5-3.5-fold, with differential expression of four independently confirmed by qPCR (hsa-miR-1, hsa-miR-21, hsa-miR-142-3p and hsa-miR-144). Each of these four miRNAs was predicted to target molecules involved in insulin signalling, metabolism and hepatic disease. The latter included *p85α, Pparα, Igf1, Foxo3* and *Acox1*, all exhibiting reduced hepatic expression (~2.3-4.0 fold) following placental restriction in males, with the abundance of hsa-miR-1, hsa-miR-142-3p and hsa-miR-144 correlating negatively with *Acox1* expression. Thus, placental restriction co-ordinately alters hepatic expression of miRNAs and predicted targets related to non-alcoholic fatty liver disease (NAFLD) in adult male offspring in sheep. Reduced hepatic expression of *Pparα* (regulates lipid catabolism), and *Acox1* (peroxisomal fatty acid β-oxidation) is characterised to promote the development of NAFLD, increasingly common following fetal growth restriction in humans, and miRNAs may partly mediate this prenatal programming of NAFLD.

In the sheep, placental restriction increased vastus lateralis expression of seven miRNAs by ~1.23-2.04 fold, with differential expression of two independently confirmed by qPCR (hsa-miR-17-5p and hsa-miR-376b). Both of these two miRNAs were predicted to regulate *Pparα* expression, which tended to negative correlate with that of hsa-miR-376b (r = -0.617, *P*-value: 0.052) and hsa-miR-17-5p (r = -0.533, *P*-value: 0.087), in placentally restricted female offspring. Placental restriction also increased perirenal fat hsa-miR-451 expression in female offspring. This miRNA is predicted to regulate a network that is involved in lipid metabolism, molecular transport and small molecule biochemistry in adipose tissue. Furthermore, perirenal fat expression of hsa-miR-451 was correlated positively with *in vivo* insulin sensitivity of free fatty acids in control offspring (r = 0.687, *P* = 0.020, n = 11), but not in placentally restricted offspring.

In the rat, placental restriction impaired insulin secretion in adult offspring, while insulin sensitivity was enhanced in young adult offspring, which then disappeared with aging, particularly in females. Placental restriction and IUGR also mostly increased miRNA expression in the insulin sensitive tissues of older adult offspring and usually in a sex specific manner, with omental fat the most affected, followed by skeletal muscle and liver. Placental restriction and IUGR reduced hepatic expression of the insulin signalling molecule, *p110β* in female offspring and that of related molecules, *Slc2a2* and *Igf1*, in male offspring. Placental restriction also increased hepatic rno-miR-126 expression, in female offspring and is predicted to target molecules involved in lipid metabolism, molecular transport and small molecule biochemistry. Placental restriction and IUGR reduced omental fat expression of *Irs1*, *Irs2* and *Slc2a4* in male offspring and increased that of rno-miR-18a, rno-miR-142-3p, rno-miR-19b, rno-miR-21, rno-miR-20b and mmu-miR-106a. The latter are predicted to target insulin signalling but also small molecule biochemistry, lipid

metabolism and its regulation, including similar pathways to those targeted by IUGR altered miRNAs in liver of adult offspring in sheep. Expression of omental fat rno-miR-18a was found to be negatively correlated with *Slc2a4* in placentally restricted offspring overall (r = -0.451, *P* = 0.040).

Placental restriction also alters the pancreatic expression of three miRNAs in adult sheep offspring, hsa-miR-339-5p in males, rno-miR-331* in females and hsa-miR-513a-3p in both sexes. Furthermore, the predicted molecular and functional targets of these differentially expressed miRNAs and predicted functional outcomes following placental restriction mirror the previously reported sex differences in *β*-cell insulin secretory function and mass in the placentally restricted adult sheep. We found that hsa-miR-339-5p was predicted to regulate PLEKHH1 and PAK6, proteins which are essential to maintain the development of the pancreas and/or differentiation of islets. Down-regulation of both rno-miR-331* and hsa-miR-513a-3p in the pancreas of placentally restricted female offspring would be expected to indirectly up-regulate *Pdx1* expression, and potentially contribute to the increased number of *β*-cells per islet they exhibit.

Overall, placental restriction and IUGR mostly increase abundance of miRNAs in key insulin sensitive tissues of adult offspring in both sheep and the rat, with sex-specific and tissue-specific differences. Nevertheless, the networks targeted by miRNAs differentially expressed following IUGR in such tissues, share common functions and pathways, both across tissues and species, including small molecule biochemistry, lipid metabolism, carbohydrate metabolism and molecular transport. Of interest, regardless of the tissues and species, placental restriction generally increased

expression of miR-142-3p, miR-1, miR-21 and miR-17-5p. Of note, expression of hsa-miR-1 and hsa-miR-21 were each up-regulated in both liver and vastus lateralis in sheep offspring. These up-regulations of common miRNAs expression could be due to alteration of epigenetic mechanism affected by placental restriction, such as DNA methylation, or common systemic regulations of their expression that has been ‘programmed’ due to placental restriction.

Therefore, placental restriction of fetal growth does alter expression of miRNAs and their networks involving insulin signalling and metabolism in key insulin sensitive tissues in the adult. The mechanism underlying this and the extent to which they contribute to overall developmental programming of metabolic dysfunction warrant for future investigation.