THE PIVOTAL ROLE OF INSULIN-LIKE GROWTH FACTORS IN PREGNANCY SUCCESS

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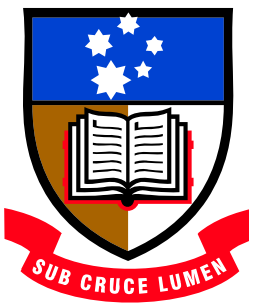

“Opportunity is missed by most people because it is dressed in overalls and looks like work.” Thomas Edison

Abstract

Appropriate placental development in early gestation is essential for subsequent placental function and hence optimal fetal growth and pregnancy outcome. Placental insufficiency has been implicated in common disorders of pregnancy, which result in fetal and maternal mortality or morbidity, and also increase the risk of poor health in adult offspring.

Prior to the onset of maternal blood flow to the placenta at ~10 weeks of gestation, placentation occurs in a relatively hypoxic environment, which is essential for healthy pregnancy. IGF-II is abundantly expressed by the invasive trophoblast and may interact with oxygen to regulate placentation. Additionally, maternally-derived IGFs may act on the placenta and the mother to regulate fetal growth. This thesis investigated the role and interaction of oxygen and IGF-II on human placental outgrowth during early pregnancy *in vitro*. Furthermore, the impact of maternal IGF treatment during early to mid pregnancy, on placental development and substrate transfer, nutrient partitioning between the mother and fetus, and fetal growth, were also determined in mid and late gestation in guinea pigs.

We have demonstrated, using human early first trimester placental villous explants, that IGF-II mediates the effect of hypoxia on placental outgrowth. Culture of placental explants in hypoxia, or with exogenous IGF-II, enhanced trophoblast outgrowth and inhibited TGF-β1 activation, a negative regulator of trophoblast function. In addition, culture of explants in hypoxia induced *Igf2* gene expression in outgrowing trophoblast, without altering *Upar*, *Igf1r, Igf2r* or *Tgfβ1* transcription. We propose that this novel interaction of oxygen, IGF-II and TGF-β1 during pregnancy is an important determinant of placental development. Furthermore, we showed that exogenous IGF-II stimulates villous explant trophoblast outgrowth in placenta from >10 weeks gestation, suggesting that IGF-II may be a potential therapeutic agent to enhance placental growth.

In guinea pigs, maternal treatment with IGF-I or IGF-II, in early to mid pregnancy, has sustained anabolic effects on fetal growth, enhanced fetal survival and increased placental delivery, and fetal and maternal utilization of, glucose and amino acids near term. These effects were also evident by mid gestation following earlier IGF-I treatment. Despite these similar pregnancy outcomes, there were IGF specific effects on the placenta and mother, suggesting that IGFs may mediate some of their effects via different pathways. IGF-I administration severely reduced maternal adiposity in late pregnancy,

suggesting persistent diversion of nutrients to the fetus. In contrast, IGF-II elicited its effects by substantially improving development of the placental exchange region, which correlated with placental function.

We have suggested that the discrete effects of IGF-I and IGF-II stem from distinct interactions of the IGFs with various receptors. Maternal administration of an analogue of IGF-II that selectively interacts with IGF2R (Leu^{27}-IGF-II), revealed that many of the effects of IGF-II treatment, were mediated by IGF2R, while IGF-I presumably acts through IGF1R.

Together, this work has highlighted the major and somewhat complementary roles of maternal IGFs during the first half of pregnancy, in regulating placental development, fetal growth and pregnancy success. Importantly, it indicates the potential use of maternal IGFs in diagnostic and therapeutic approaches to pregnancy complications.

Declaration

This work is original and has not been accepted for the award of any other degree or diploma in any university or other tertiary institution. To the best of my knowledge, this thesis does not contain material previously written or published by another, except where due reference in the text has been given.

I give consent to the University of Adelaide to make this thesis available for loan and photocopying after it has been accepted for the degree.

Amanda Nancy Sferruzzi-Perri
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24. Roberts CT, Sferruzzi-Perri AN, Donaldson AC, Grant PA *IGF-II regulates activation of TGFβ1 at the cell surface and thereby controls differentiation of the placenta*. National Australian Society for Medical Research Conference, Adelaide, Australia. Abstract 57.

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Abbreviations

A	Adenosine
AA	Amino acids
Ang2	Angiopoietin 2
AIB	Methyl [^{14}C]-amino-isobutyric acid
ALS	Acid liable subunit
BM	Basal membrane
ARNT	Arylhydrocarbon receptor nuclear transferase
BM	Basal syncytiotrophoblast membrane
bp	Base pairs
BSA	Bovine serum albumin
C	Cytosine
°C	Degrees Celsius
cDNA	Complimentary DNA
Chol	Cholesterol
Ct	Cycle threshold
CTB	Cytotrophoblast
Cpm	Counts per minute
DAB	3,3-diaminobenzidine
DNA	Deoxyribonucleic acid
DPM	Disintegrations per minute
EVT	Extravillous cytotrophoblast
FAK	Focal adhesion kinase
FC	Fetal capillary
FFA	Free fatty acids
fwd	Forward primer
hCG	Human chorionic gonadotrophin
hPL	Human placental lactogen
HPLC	High pressure liquid chromatography
HIF	Hypoxia inducible factor
HLA	Human leukocyte antigen

HRE	Hypoxia response element
HRP	Horse radish peroxidise
G	Guanine
Gluc	Glucose
Glut	Glucose transporter
IGF	Insulin-like growth factor
IGFBP	Insulin-like growth factor binding protein
IGF1R	Type 1 IGF receptor
IGF2R	Type 2 IGF receptor
InsR	Insulin receptor
IUGR	Intrauterine growth restriction
IVS	Intervillous space
kDa	Kilo Daltons
LAP	Latency associated peptide
Leu^{27}-IGF-II	[Leu^{27}]-IGF-II
mA	Milli Amps
MBS	Maternal blood space
MG	[^{3}H]-methyl-D-glucose
MHC	Major compatibility complex
MMP	Matrix metalloproteinase
MVM	Microvillous apical syncytiotrophoblast membrane
M6P	Mannose 6 phosphate
mRNA	Messenger RNA
mmHg	Millimetres of mercury
PAI	Plasminogen activator inhibitor
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PFA	Paraformaldehyde
PIGF	Placental growth factor
rev	Reverse primer
RGD	Arg-Gly-Leu
RIA	Radioimmunoassay
RNA	Ribonucleic acid

Rpm	Revolutions per minute
RT-PCR	Real time polymerase chain reaction
SDS	Sodium dodecyl sulphate
STB	Syncytiotrophoblast
T	Thymine
TIMP	Tissue inhibitors of matrix metalloproteinase
TGF	Transforming growth factor
TNF	Tumour necrosis factor
Trig	Triglycerides
Troph	Trophoblast
TβR-V	Type V transforming growth factor-β receptor
TGFβIR	TGFβ type 1 receptor
TGFβIIR	TGFβ type 2 receptor
uPA	Urokinase plasminogen activator
uPAR	Urokinase plasminogen activator receptor
V	Volts
VEGF	Vascular endothelial growth factor
VIA	Video image analysis