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PROTEOMIC BIOMARKER DISCOVERY FOR PREECLAMPSIA

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A thesis submitted in partial fulfillment of the requirements
for the degree of Doctor of Philosophy in Biological Sciences,
The University of Auckland, 2008.

ABSTRACT

Preeclampsia is a serious multisystem complication of late pregnancy with adverse effects for mothers and babies. Currently this disorder is diagnosed from clinical observations occurring late in the disease process. Unknown factors in the maternal circulation, possibly released by the preeclamptic placenta, have been linked to the pathophysiological changes characteristic of the disorder. The research in this thesis used proteomic techniques to identify putative preeclampsia biomarkers from two sources: secreted from a placental cell line undergoing differentiation, and directly sampled from the serum and plasma of women with late-onset preeclampsia.

The first part of this research examined the secreted proteome of a placental choriocarcinoma cell line (BeWo) undergoing forskolin-mediated differentiation. Development of serum-free culture techniques enabled analysis of these secreted proteins by two-dimensional gel electrophoresis (2DE). Statistical testing revealed the significant involvement of seven spots during this differentiation model, with VE-cadherin and matrix metalloproteinase 2 among the proteins identified.

In the second part of this research, maternal serum and plasma proteins were compared from women with preeclampsia and healthy pregnant women. Serum samples were analyzed using 2DE, and plasma was subjected to difference gel electrophoresis (DIGE). Bioinformatic analysis of both datasets identified multiple spot clusters able to classify samples according to disease state. Five of these serum proteins were differentially regulated in preeclampsia, including two isoforms of apolipoprotein E whose isoform-specific expression was confirmed using western blots. Analysis of plasma from preeclamptic women identified six proteins, again including apolipoprotein E. Proteins from both studies are linked to preeclampsia pathophysiology through lipid transport, complement, and retinol transport systems.

The culture methods and secreted proteomic techniques developed in this work have uncovered proteins in a placental cell line and maternal serum and plasma that are associated with preeclampsia. These methods can be extended to any system where secreted proteins are of interest. The differentially regulated proteins found in this study provide an important first step towards developing effective biomarkers for diagnosing and/or predicting preeclampsia.

ACKNOWLEDGEMENTS

First, and foremost, I thank my supervisors, Garth Cooper and Robyn North, for allowing me the opportunity to pursue this PhD on a project with real importance. Marion Blumenstein, my advisor, was a huge help from the start of the project to its completion and I am proud to call her my colleague and friend. These three very busy people invested a significant amount of time in my education, and I am truly grateful for their guidance.

I was fortunate enough to learn a little about bioinformatics and statistics from Mik Black, a kind and very patient kindred technophile. Steven Wu was my compatriot in bioinformatics in SCOPE and never shied away from lending me a hand with analyses I always seemed to need at the last minute. Mik, Steven, Nik Kasabov, Peter Hwang, and Ronald Kates did great work not only in their analyses of my data, but in helping me to understand what it all meant. Thank you for teaching me about your fields.

A 2-D gel proteomicist is nothing without a mass spectrometrist, and I was fortunate enough to work with several very talented ones: Martin Middleditch, Dave Greenwood, Christina Buchanan, and Janine Cooney were responsible for all of my protein identifications. Thank you all for your tireless work, help, and advice.

Members of the Cooper lab, past and present, contributed in untold ways to my research. Hong Wang gifted me precious donkey serum for countless western blots, for which Denise Greenwood was a huge help. Debbie Hay gave excellent feedback on cell culture and written thesis drafts. I still miss regular discussions about music and protein biology with Leo Payne. Friends and officemates, including Anna Brooks, Catherine Lloyd, Lena Ostrovsky, Haylyn Wong, Sarah Glyn-Jones, Bronwen Jongbloed, Tina Lowe, and Gabriel James put up with a lot and formed a great network for support, advice, and friendship. Other SBS members were just as essential in this process, including Jo Dodd, Julie Hill, and Sarah Grieg.

Cynthia Tse deserves an extra-special mention for being the best "enabler" I have ever known – without her, I would have ground to a halt at nearly every turn, particularly at the spectacularly-organized end! The world (and the lab) is a much better place with you in it, Cynthia, and thank you so much again for all you have done for me. I literally could not have finished this PhD without you.

Members of the SCOPE clinical team have had a profound effect on my research, for without them, my work would not exist: Rennae Taylor, Lesley McCowan, Iris Shin, Desley Minahan, and the SCOPE midwives made this work possible, thanks in turn to the generosity of the mothers who participated in the New Zealand portion of the project.

My colleagues at HortResearch, particularly John Ingram, Kim Lo, and Roneel Prakash, met me during the end of my thesis writing period and successfully resisted running away from me at high speed. Thank you for being patient with me and allowing me time to finish what I had started.

My family never stopped believing that I would actually finish at university one of these days, and I'm proud to not have disappointed them. Mom and Dad, you'll have to call me Doctor Kelly now! Thanks for your love and support even from afar. My in-laws here in New Zealand were just as supportive though they probably didn't realize that I haven't had a break from "school" for 25 years!

At so many points during this research, particularly during the preparation of this thesis, I thought about giving up and doing something else. My husband, Ross, not only kept me going, but his scientific input strengthened my work just as his love strengthens my life. I can't express how thankful I am for his support, guidance, cooking skills, and boundless enthusiasm for science and for life.

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LIST OF ABBREVIATIONS

SI unit prefixes are not included in the list below.

°C	degrees Celsius
1-D	one-dimensional
2-D	two-dimensional
2DE	two-dimensional gel electrophoresis
A	ampere(s)
A1BG	α-1B-glycoprotein
ANOVA	analysis of variation
ApoC2	apolipoprotein C-II
ApoE	apolipoprotein E
$ApoE_A$	apolipoprotein E, acidic isoform
$ApoE_B$	apolipoprotein E, basic isoform
BCA	bicinchoninic acid
BFA	brefeldin A
BSA	bovine serum albumin
C7BzO	3-(4-heptyl)phenyl-3-hydroxypropyl)dimethylammoniopropanesulfonate
cAMP	cyclic adenosine monophosphate
CHAPS	3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate
CHRP2	complement factor H-related protein 2
CI	confidence interval
CTB	cytotrophoblast
CV	coefficient of variation
Da	Dalton(s)
DIGE	difference gel electrophoresis
DLOO	double leave-one-out
DMEM	Dulbecco/Vogt Modified Eagle's Minimal Essential Medium
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DTT	dithiothreitol
E-cadherin	epithelial cadherin
EDTA	ethylenediaminetetraacetic acid
ESI	electrospray ionization
EVT	extravillous cytotrophoblast
FDR	false discovery rate
FSK	forskolin
FT-ICR	Fourier transform ion cyclotron resonance
g	gram(s)
GalNAc	*N*-acetyl galactosamine
Glc	glucose
GO	Gene Ontology
h	hour(s)
hCG	human chorionic gonadotropin
HCl	hydrochloric acid

HDL	high density lipoprotein
HELLP	hemolytic anemia, elevated liver enzyme activity, low platelet count syndrome
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HLA	human leukocyte antigen
HPLC	high-performance liquid chromatography
HUPO	Human Proteome Organization
IAA	iodoacetamide
IATI	inter-α-trypsin inhibitor
ICAM	intercellular cell adhesion molecule
IFN	interferon γ
IgA	immunoglobulin A
IgG	immunoglobulin G
IHRP	inter-α-trypsin inhibitor family heavy chain-related protein
IMP5	ImageMaster™ 2D Platinum version 5.0
IMP6	ImageMaster™ 2D Platinum version 6.0.1
IPG	immobilized pH gradient
IS	internal standard
kDa	kilodaltons(s)
KIR	killer immunoglobulin-like receptor
L	liter(s)
LC	liquid chromatography
LC-MS	liquid chromatrography coupled with mass spectrometry
LC-MS/MS	liquid chromatography coupled with tandem mass spectrometry
LDH	lactate dehydrogenase
LDL	low density lipoprotein
MALDI	matrix-assisted laser desorption/ionization
MARS	Multiple Affinity Removal System
MBP	mannose-binding protein C
MES	2-(N-morpholino)ethanesulfonic acid
min	minute(s)
MMP	matrix metalloproteinase
mol	mole(s)
MRM	multiple reaction monitoring
MS	mass spectrometry
MS/MS	tandem mass spectrometry
MudPIT	multidimensional protein identification technology
MW	molecular weight
NCBI	National Center for Biotechnology Information
NK	natural killer cell
NL	nonlinear
NSC	nearest shrunken centroids
PAGE	polyacrylamide gel electrophoresis
PAI-1	plasminogen activator inhibitor-1
PAI-2	plasminogen activator inhibitor-2
PAPP-A	pregnancy-associated plasma protein A
PBS	phosphate-buffered saline

PBST	phosphate-buffered saline + Tween 20
PES	polyethersulfone
pI	isoelectric point
PlGF	placental growth factor
PLTP	phospholipid transfer protein
PP13	placental protein 13
PPP	Plasma Proteome Project
PSA	prostate-specific antigen
PVDF	polyvinylidene fluoride
RBP	retinol binding protein
RFE	recursive feature elimination
RNA	ribonucleic acid
s	second(s)
SALSA	Scoring Algorithm for Spectral Analysis
SAP	serum amyloid P-component
SCOPE	SCreening for Pregnancy Endpoints
SD	standard deviation
SDS	sodium dodecyl sulfate
SDS-PAGE	SDS polyacrylamide gel electrophoresis
SELDI	surface-enhanced laser desorption/ionization
sFlt1	soluble fms-like tyrosine kinase 1
SFM	serum-free media
SGA	small for gestational age
SILAC	stable isotope labeling with amino acids in cell culture
SNP	single nucleotide polymorphism
STB	syncytiotrophoblast
STBM	syncytiotrophoblast microvillous membranes
TG	triglyceride
TGF-β	transforming growth factor-β
TIMP	tissue inhibitor of metalloproteinases
TNF-α	tumor necrosis factor-α
TOF	time-of-flight
Tris	2-amino-2-(hydroxymethyl)propane-1,3-diol
V	volts
V·h	volt hours
VCAM	vascular cell adhesion molecule
VE-cadherin	vascular epithelial cadherin
VEGF	vascular endothelial growth factor
VLDL	very low density lipoprotein
W	watts