Studies of the Ubiquitin-NFκβ pathway in women and baboons with endometriosis

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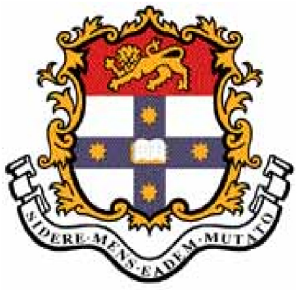

A thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy

FACULTY OF MEDICINE
THE UNIVERSITY OF SYDNEY

O Me! O Life!

“That you are here - that life exists and identity,

That the powerful play goes on, and you may

contribute a verse.”

(Walt Whitman)

To my Mother - Gloria Santos

You have sacrificed so much to get me where I am. Giving me love, support and strength, especially when I didn't have courage and belief. Unwavering in your love, even when I didn't deserve it. Thank you for always being there for me during the many times I have felt lonely and debilitated with self-doubt.

It is my solemn wish to be sincere, loving and be of use to you and to others, as gratitude to you for believing in me.

In memory of Uncle Jim

James Mervyn Greenslade

(Australian Defence Force)

Thank you for opening the door to opportunities that enabled me to develop the courage and the skills to walk through.

STATEMENT OF AUTHENTICATION

This thesis is submitted to the Faculty of Medicine at the University of Sydney, in fulfilment of the requirement for the Degree of Doctor of Philosophy (PhD) conducted between 2003-2009 as a part-time candidate.

This work was originally submitted under my maiden name Romina Santos Ilad, thus publications arising from this thesis are authored in my former name.

This PhD is a continuation of my Honours Degree, where certain texts included within the literature review remain the same but has been updated to correspond with present knowledge and mechanisms.

The work presented is original except as appropriately acknowledged, submitted in this final format whilst studying medicine - hence, the delay between original submission and final print.

Romina Santos Reyftmann 8th January 2012

PUBLICATIONS AND ABSTRACTS

Publications arising from this thesis

Ilad RS., Fleming SD., Murphy, CR and Fazleabas, A.T (2010) **Immunohistochemical study of the ubiquitin-nuclear factor-κβ pathway in the endometrium of the baboon (*Papio anubis)* with and without endometriosis.** *Reproduction, Fertility and Development*: 22(7): 1118-30.

Ilad RS., Fleming SD., Bebington, CR and Murphy, CR (2004). **Ubiquitin is associated with the survival of ectopic stromal cells in endometriosis.** *Reproductive Biology and Endocrinology:* 2(1): 69.

Abstracts arising from this thesis

- ***March 2008**
  10th World Congress on Endometriosis, Melbourne, VIC. **IKK-α, a likely kinase for NFκβ mediated ectopic endometrial cell survival in the baboon (*Papio anubis*).** *Ilad RS., Fleming SD., Murphy CR., Cooper MJ and Fazleabas AT.*

- ***September 2005**
  9th World Congress on Endometriosis, Maastricht, The Netherlands. **Ubiquitin is associated with the survival of ectopic stromal cells in endometriosis.** *Ilad RS., Fleming SD., Bebington, CR and Murphy, CR.*

- ***September 2004**
  College of Health Sciences 4th Biennial Research Conference From Cell to Society 4, Leura, NSW. **Ubiquitin is associated with the survival of ectopic stromal cells in endometriosis.** *Ilad RS., Fleming SD., Bebington, CR and Murphy, CR. (Oral presentation).*

*Presented by Ilad RS

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Life and its inevitable challenges and heartaches continued, whether or not I had a good day at the lab and I often had to navigate through many mental and emotional roadblocks. Some situations have tested my values to the point where I nearly lost who I was and what I stood for. However, I am glad that I went through them now, as it has highlighted what really mattered in my life. During those times when I felt I did not have the physical and mental strength to go on, when all I did and wanted to do was withdraw from the world, I discovered who truly mattered and who truly cared for me.

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ABSTRACT

Background: Our aim was to determine if changes in ubiquitin are correlated with proteins known to be affected directly or indirectly by the ubiquitin-proteasome pathway. For example, the intermediary kinase of the NFκβ pathway IKK is tagged directly by ubiquitin, aiding its degradation within the large, self-compartmentalised protease called the 26S proteasome. IKK degradation allows the release of NFκβ (an indirect consequence of ubiquitination) that then translocates into the nucleus to initiate the transcription of survival factors such as cIAP2, BCL-XL, BFL1, FLIP, IL-8, and TNF-α.

Methods: Lyophilised baboon endometrial tissues from the University of Illinois at Chicago (UIC) and from women undergoing laparoscopy from the Sydney South West Area of Health Service (SSWAHS) were processed for RNA extraction and analysis. Immunohistochemical studies were also conducted on formalin fixed baboon and human endometrial tissues from UIC and from the Sydney West Area Health Service (SWAHS).

Results: IKKα immunostaining were elevated in the cytoplasm of glands during late follicular phase and stromal cells during menses in the baboon, whilst proteasome was elevated in the nucleus of glands and stroma at menses, as well as within the glandular cytoplasm of endometriotic tissues. Comparable levels of Iκβα exists throughout the menstrual cycle of the eutopic endometrium, whilst a reduced glandular nuclear Iκβα was seen in endometriotic tissues of the animal model. TNF-α was increased within stromal cells at menses but was equivalent between the eutopic and ectopic

endometrium. Ubiquitin was similar between LF and mid luteal phase of eutopic glands and stroma, as well as between eutopic and ectopic endometrium at mid luteal. IKKβ and NFκβ levels were alike within eutopic and ectopic tissues throughout the menstrual cycle. In women however, IKKα, IKKβ, Iκβα and NFκβ had comparable levels within all cell types and cycle phases, whilst a greater proteasome immunostaining was seen in the nucleus of eutopic stromal cells at secretory phase, as well as in the nucleus of ectopic stromal cells at proliferative phase. However, the nucleus of stromal cells and glandular cytoplasm of endometriotic tissues both had lower proteasome levels during the secretory phase.

Conclusions: In this study, IKKα had a greater potential to activate the p65 subunit of NFκβ in ectopic stromal cells than IKKβ and TNF-α did not seem to be correlated with greater free ubiquitin expression in endometriosis in the baboon. This suggests that IKKα is a likely candidate for ectopic stromal cell survival in endometriosis in the animal model but this is unlikely to be mediated by the ubiquitin NFκβ pathway. Similarly, in women with endometriosis a different pathway to NFκβ is potentially responsible for a greater ectopic cell survival potential, however an increased patient cohort is required to definitively ascertain the involvement of IKKα in the future.

LIST OF ABBREVIATIONS

19S	19S proteasome
20S	20S proteasome
26S	26S proteasome
ABComplex/HRP	Avidin biotin complex/horse radish diaminobenzidine
ANG	Angiogenin, ribonuclease, RNAse A family, 5
ANGIS	Australian National Genomic Information Service
AP-1	Activation protein-1
A.S.R.M	American Society of Reproductive Medicine
ATP	Adenosine triphosphate
BAFF	B cell activating factor from the tumour necrosis factor family
Bax	B-cell lymphoma 2 family-associated X protein
bFGF	Basic fibroblast growth factor
BLAST	Basic Local Alignment Search Tool
BCL2	B-cell lymphoma 2 family
Bcl-2	B-cell leukemia/lymphoma-2
Bcl-3	B-cell CLL/lymphoma-3
BCL-X_L	B-cell leukaemia/lymphoma – X longer transcript
BFL1	B-cell leukaemia/lymphoma 2 related protein A1
c-Rel	c-v-rel reticuloendotheliosis viral oncogene homolog
CA-125	Cancer antigen-125
cDNA	Complementary deoxyribonucleic acid
CHM	Chinese herbal medicine
CHUK	Conserved helix-loop-helix ubiquitous kinase
cIAP2	Baculoviral inhibitor of apoptosis protein repeat containing 2
COC	Combined oral contraceptive
COL1A1	Collagen type I alpha 1
COX-2	Cyclooxygenase-2
Ct	Cycle threshold

DAB	Diaminobenzidine
DEPC	Diethyl pyrocarbonate
DNA	Deoxyribonucleic acid
dNTP	Deoxyribonucleotide triphosphate
DTT	Dithiothreitol
E1	Enzyme-1 (ubiquitin activating enzyme)
E2	Enzyme-2 (ubiquitin carrier protein /conjugating enzyme)
E_2	Oestradiol
E3	Enzyme-3 (ubiquitin protein ligase)
EAOC	Endometriosis-associated ovarian cancer,
ECM	Extracellular matrix
EGF	Epidermal growth factor
ER-α	17β-Oestradiol receptor-alpha
FADD	Fas associated death domain
Fas	TNF receptor superfamily member
FLICE	Fas associated death domain like interleukin 1 β-converting enzyme
FLIP	FLICE inhibitory protein
FSH	Follicle stimulating hormone
GDF15	Growth differentiation factor 15
GnRHa	Gonadotrophin-releasing hormone agonist
H&E	Haematoxylin and eosin
hCG	Human chorionic gonadotrophin
HOXA10	Homeobox protein A10
HSD17B2	17β-hydroxysteroid dehydrogenase type 2
IAP	Inhibitor of apoptosis protein
IBD	Inflammatory bowel disease
ICAM-1	Inter-cellular adhesion molecule 1
IGF	Insulin growth factor
IGFBP1	Insulin like growth factor binding protein 1
IgG	Immunoglobulin G
IκB	Inhibitor of kappa B

Iκβ	Inhibitor of kappa beta
Iκβα	I-kappa-B-alpha
IκBβ	I-kappa-B-beta
IκBγ	I-kappa-B-gamma
IκBKα	Inhibitor of kappa light polypeptide gene enhancer in B cells, kinase of alpha
IKBKB	Inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase beta
IKBKG	Inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase of gamma
IKK	Inhibitor of kappa beta kinase
IKK1	I-kappa-B kinase 1
IKK2	I-kappa-B kinase 2
IKKα	I-kappa-B-kinase-alpha
IKKβ	I-kappa-B-kinase-beta
IKKγ	I-kappa-B-kinase-gamma
IL-1β	Interleukin-1 beta
IL-1	Interleukin-1
IL-8	Interleukin-8
ISG15	Interferon stimulated gene 15
JNK	c-Jun N-terminal kinase
K48	Lys-48 linked
K63	Lys-63 linked
LDL	Low density lipoprotein
LF	Late Follicular
LH	Luteinizing hormone
LT	Lymphotoxin
MAPK	Mitogen activated protein kinase
MCP-1	Monocyte chemotactic peptide-1
MHC-1	Major histocompatibility complex-1
MIF	Migration inhibitory factor
miRNA	MicroRNAs

ML	Mid-luteal
MMLV-RT	Moloney Murine Leukaemia Virus Reverse Transcriptase
MMP	Matrix metalloproteinases
MRI	Magnetic resonance imaging
NEMO	Nuclear factor kappa beta essential modifier
NCBI	National Centre for Biotechnology Information
NFκβ	Nuclear factor kappa beta
NFκβ1	p50
NFκβ2	p52
NFκBIKα	Nuclear factor of kappa light chain gene enhancer in B cells inhibitor, kinase of alpha
NFκBIKβ	Nuclear factor of kappa light chain gene enhancer in B cells inhibitor, kinase of beta
NIK	Nuclear factor kappa beta inducing kinase
NLS	Nuclear localisation signal
NPC	Nuclear pore complex
NSAID	Non steroidal anti-inflammatory drugs
p50	Nuclear factor kappa beta 1
p52	Nuclear factor kappa beta 2
p65	v-rel reticuloendotheliosis viral oncogene homolog A, nuclear factor of kappa
p105	Nuclear factor kappa beta 1 precursor
p100	Nuclear factor kappa beta 2 precursor
PDAR	Pre-developed assay reaction
PDGF	Platelet-derived growth factor
PDTC	Pyrrolidine dithiocarbonate
PEST	P = proline; E = glutamic acid; S =serine and T =threonine
PGE_2	Prostaglandin E_2
PLAU	Plasminogen activator, urokinase
PMA	Phorbol ester
PMC	Peritoneal mesothelial cells
PR	Progesterone receptor
qRT-PCR	Quantitative real-time polymerase chain reaction
RANTES	Regulated on activation normal T cell expressed and secreted protein

RelA	p65 or v-rel reticuloendotheliosis viral oncogene homolog A, nuclear factor of kappa
RelB	v-rel reticuloendotheliosis viral oncogene homolog B, nuclear factor of kappa
RIP	Receptor interacting protein
RIN	RNA integrity number
RNA	Ribonucleic acid
ROS	Reactive oxygen species
RT	Reverse transcribed
S	Sedimentation coefficient, or the Svedberg coefficient
SF-1	Steroidogenic factor-1
SPRMs	Selective progesterone receptor modulators
SSWAHS	Sydney South West Area Health Service
SWAHS	Sydney West Area Health Service
TBS	Tris buffer saline
TCDD	2,3,7,8-tetracholorodibenzo-*p*-dioxin or dioxin
TGF-α	Transforming growth factor-alpha
TGF-β	Transforming growth factor beta
Thal	Thalidomide
TIMP1	Tissue inhibitor of metalloproteinase 1
TNF	Tumour necrosis factor
TNF-R1	Tumour necrosis factor receptor 1
TNF-R2	Tumour necrosis factor receptor 2
TNF-α	Tumour necrosis factor-alpha
TNFAIP2	Tumour necrosis factor-alpha induced protein 2
TRADD	Tumour necrosis factor receptor associated death domain
TRAF2	Tumour necrosis factor receptor associated factor 2
TUNEL	Terminal deoxynucleotidyl transferase
UCRP	Ubiquitin cross reactive protein
VEGF	Vascular endothelial growth factor
Wnt	Wingless int
XIAP	X-linked inhibitor of apoptosis protein