Effect of *C1q* Null Mutation on Mammary Gland Development and Breast Cancer Susceptibility

A Thesis Submitted for the Degree of Doctor of Philosophy by

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In loving memory of my mother, whose role in my life was, and remains, immense.

I dedicate this thesis to you, Mak.

FATIMAH ABDULLAH

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Summary

The complement protein C1q promotes rapid macrophage-mediated clearance of dying cells and tolerance to self antigens. mRNA encoding C1q is a key gene upregulated during mammary gland regression, and we hypothesise that C1q complement protein promotes mammary gland tumourigenesis through induction of tolerance to tumour antigens. We have investigated the role of C1q in normal mammary gland development, hormone-mediated regression, and in tumour development using two different mammary tumour mouse models together with *C1q* $^{-/-}$ mice. In the absence of C1q, mammary gland development proceeds normally during puberty, with a similar abundance of terminal end buds and rate of epithelial cell proliferation at 6 weeks of age compared to wildtype *C1q* $^{+/+}$ mice. However, deficiency in C1q perturbed mammary gland regression, with 45% increased number of ductal branch points 24 hours following progesterone receptor antagonist RU486 (also known as mifepristone)-induced epithelial cell apoptosis in *C1q* $^{-/-}$ mice compared to *C1q* $^{+/+}$ mice (p=0.027, n=7-11). There was a reduction of macrophage abundance (p=0.002, n=6-7) and a 3.5-fold increase of TUNEL positive apoptotic cells (p=0.011, n=5-6) in the ductal epithelium of *C1q* $^{-/-}$ mice compared to *C1q* $^{+/+}$ mice 24 hours following RU486 administration. To investigate the role of C1q in mammary tumour development, MMTV-PyMT transgenic mice were crossed with *C1q* $^{-/-}$ mice and monitored weekly from 6 weeks by palpation to determine tumour latency, and mammary tumours dissected to assess total tumour burden. Lack of C1q did not affect development of mammary hyperplasia at 10 weeks, however development of palpable tumours was increased by 1 week in *PyMT*$^{+}$*/C1q* $^{-/-}$ mice compared to *PyMT*$^{+}$*/C1q* $^{+/+}$ mice (p=0.012, n=43-45). The number of tumours was significantly reduced in 15-week old *PyMT*$^{+}$*/C1q* $^{-/-}$ mice compared to *PyMT*$^{+}$*/C1q* $^{+/+}$ mice (p=0.028, n=15-16). There was also a significant reduction in the total tumour burden in *PyMT*$^{+}$*/C1q* $^{-/-}$ mice compared to *PyMT*$^{+}$*/C1q* $^{+/+}$ mice at 15 weeks of age (p=0.036, n=15-16). The frequency of tumours that had progressed to carcinoma stage was also reduced in *PyMT*$^{+}$*/C1q* $^{-/-}$ mice at 15 weeks of age (p=0.05, n=11-15) and 18 weeks of age (p=0.003, n=28-29). Carcinogen-induced mammary tumourigenesis was also investigated in mice administered 7,12-dimethylbenz[α]anthracene (DMBA) by oral gavage for 6 weeks. DMBA-treated *C1q* $^{-/-}$ mice were highly resistant to mammary tumourigenesis, with tumours detected in only 2 of 20 mice over the 30 week monitoring period, compared to 10 of 20 DMBA-treated *C1q* $^{+/+}$ mice (p=0.02). T lymphocytes were skewed to the CD4^{+} subset, with a significant increase in IFNG-producing CD4^{+} T cells in the mammary gland draining lymph nodes in DMBA-treated *C1q* $^{-/-}$ mice (p=0.014, n=6-10). These findings suggest that C1q promotes tissue remodeling and clearance of dying cells during mammary gland regression, and increases mammary cancer susceptibility and tumour progression.

Declaration

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name 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 the text.

In addition, I certify that no part of this work will, in the future, be used in a submission in my name 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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Siti Mariam Noor Din

December 2017

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Presentations at Scientific Meetings

2014

Siti M Noor Din, Danielle J Glynn, Leigh J Hodson, Sarah A Robertson, Wendy V Ingman. *"Null mutation in C1q impairs tumourigenesis in MMTV-PyMT and DMBA-induced mammary cancer mouse models"*, Robinson Institute Research Symposium, 6th November 2014, National Wine Centre, Adelaide, South Australia, Australia. **(Poster)**

Siti M Noor Din, Danielle J Glynn, Leigh J Hodson, Sarah A Robertson, Wendy V Ingman. *"Null mutation in C1q impairs tumourigenesis in MMTV-PyMT and DMBA-induced mammary cancer mouse models"*, 29th International Association for Breast Cancer Research Conference, 14th-17th September 2014, Novotel Sydney Manly Pacific Hotel, Manly, New South Wales, Australia. **(Poster)**

2013

Siti M Noor Din, Danielle J Glynn, Leigh J Hodson, Sarah A Robertson, Wendy V Ingman. *"Null mutation in C1q impairs tumour development in MMTV-PyMT mice"*, Robinson Institute Research Symposium, 4th November 2013, National Wine Centre, Adelaide, South Australia, Australia. **(Poster)**

Siti M Noor Din, Danielle J Glynn, Leigh J Hodson, Sarah A Robertson, Wendy V Ingman. *"Null mutation in C1q impairs tumour development in MMTV-PyMT mice"*, Gordon Research Conference on Mammary Gland Biology, 8th-14th June 2013, Stoweflake Resort and Conference Centre, Stowe, Vermont, United States. **(Poster)**

2012

Siti M Noor Din, Danielle J. Glynn, Sarah A Robertson, Wendy V Ingman. *"Null mutation in C1q impairs tumour development in MMTV-PyMT mice"*, 42nd Annual Scientific Meeting of the Australasian Society for Immunology, 2nd-6th December 2012, Melbourne Exhibition and Convention Centre, Melbourne, Victoria, Australia. **(Poster)**

Siti M Noor Din, Lachlan M Moldenhauer, Mark DeNichilo, Sarah A Robertson, Wendy V Ingman. *"The carcinogen DMBA increases the proportion of IL10-producing CD4 and CD8 T cells in the mammary gland draining lymph node"*, The Queen Elizabeth Hospital Research Day Conference, Adelaide, Australia, 12th October 2012, Basil Hetzel Institute, Adelaide, South Australia, Australia. **(Poster)**

Siti M Noor Din, Lachlan M Moldenhauer, Mark DeNichilo, Sarah A Robertson, Wendy V Ingman. *"The carcinogen DMBA increases the proportion of IL10-producing CD4 and CD8 T cells in the mammary gland draining lymph node"*, South Australia Breast Cancer Research Showcase, 24th May 2012, National Wine Centre, Adelaide, South Australia, Australia. **(Poster)**

2011

Siti M Noor Din, Lachlan M Moldenhauer, Mark DeNichilo, Sarah A Robertson, Wendy V Ingman. *"The carcinogen DMBA increases the proportion of IL10-producing CD4 and CD8 T cells in the mammary gland draining lymph node",* 41st Annual Scientific Meeting of the Australasian Society for Immunology, 11th-15th December 2011, Adelaide Exhibition and Convention Centre, Adelaide, South Australia, Australia. **(Poster)**

Siti M Noor Din, Lachlan M Moldenhauer, Sarah A Robertson, Wendy V Ingman. *"Defining the immune response to carcinogen challenge in the mammary gland"*, The Queen Elizabeth Hospital Research Day Conference, 14th October 2011, Basil Hetzel Institute, Adelaide, South Australia, Australia. **(Poster)**

Siti M Noor Din, Lachlan M Moldenhauer, Sarah A Robertson, Wendy V Ingman. *"Defining the immune response to carcinogen challenge in the mammary gland"*, Faculty of Health Sciences Postgraduate Research Conference, 25th August 2011, National Wine Centre, Adelaide, South Australia, Australia. **(Poster)**

Siti M Noor Din, Lachlan M Moldenhauer, Sarah A Robertson, Wendy V Ingman. *"Defining the immune response to carcinogen challenge in the mammary gland"*, Australian Society for Medical Research (ASMR) Scientific Meeting, 8th June 2011, Adelaide Exhibition and Convention Centre, Adelaide, South Australia, Australia. **(Poster)**

Abbreviations

APC	allophycocyanin
APC-Cy7	allophycocyanin-Cy7
APAF1	apoptotic protease activating factor 1
ASR	age-standardised rate
BFA	Brefeldin A
BSA	bovine serum albumin
BrdU	bromodeoxyuridine
BSA	bovine serum albumin
Ca_{2+}	calcium
CLN	cervical lymph nodes
CO_2	carbon dioxide
CRP	C-reactive protein
CR2	complement receptor 2
CSF-1	macrophage colony-stimulating factor-1
CTLs	cytotoxic T lymphocytes
CTLA-4	cytotoxic T lymphocyte-associated protein 4
DAB	3, 3'-diaminobenzidine
DAMPs	damage-associated molecular patterns
DAPI	4', 6-diamidino-2-phenylindole
DECs	decidual endothelial cells
DMBA	7,12-dimethylbenz[α]anthracene
DNA	deoxyribonucleic acid
DNP	2,4-dinitrophenyl
DR	death receptor
E_2	estradiol
ECM	extracellular matrix
EDTA	ethylenediaminetetraacetic acid
FACS	fluorescence-activated cell sorting
FasL	Fas ligand
FADD	Fas-associated death domain
FLIP	FADD-like apoptosis regulator
FDCs	follicular dendritic cells
FITC	fluorescein isothiocyanate
H_2O_2	hydrogen peroxidase
HI-FBS	heat inactivated-fetal bovine serum
HMGB1	high mobility group box chromosomal protein 1
h	hour
HRP	horseradish peroxidase
ICAM-1	intercellular adhesion molecule 1
IFNA	interferon alpha
IFNG	interferon gamma
Ig	immunoglobulin
IgG	immunoglobulin G
IL4	interleukin-4
IL6	interleukin-6
IL10	interleukin-10
IL12	interleukin-12
IL13	interleukin-13

iNOS	nitric oxide synthase
KLH	keyhole limpet hemocyanin
LPS	lipopolysaccharide
LRP1	low-density lipoprotein receptor-related protein 1
lysoPC	lipid lysophosphatidylcholine
MAC	membrane attack complex
MASPs	ficolin-C1s serine proteases complex
MBL	mannose-binding lectin
MCA	methylcholanthrene
MDSCs	myeloid-derived suppressor cells
MG DLN	mammary gland draining lymph nodes
MHCI	major histocompatibility complex class I
MHC Class II	major histocompatibility complex class II
MLKL	mixed lineage kinase domain-like
MMS	methyl methanesulfonate
MMTV	mouse mammary tumour virus
MOMP	mitochondrial outer membrane permeabilization
MTORC1	mechanistic target of rapamycin complex 1
NaCl	sodium chloride
NBF	neutral buffered formalin
NK cells	natural killer cells
NKT cells	natural killer T cells
NMU	N-methyl-N-nitrosourea
OVA	ovalbumin
PAH	polycyclic aromatic hydrocarbon
PALN	para-aortic lymph nodes
PAMPs	pathogen-associated molecular patterns
pDCs	plasmacytoid dendritic cells
PE	phycoethrin
PerCP	peridinin-chlorophyll-protein complex
PerCP-Cy5.5	peridinin-chlorophyll-protein complex Cy5.5
P_4	progesterone
PBS	phosphate buffered solution
PCR	polymerase chain reaction
PMA	phorbol 12-myristate 13-acetate
PMN-MDSCs	neutrophil-like myeloid-derived suppressor cells
PRRs	pattern recognition receptors
PtdSer	phosphatidylserine
PTX3	pentraxin
P/S	penicillin/streptomycin
PyMT	polyomavirus middle T antigen
RBC	red blood cell
RIPK1	receptor-interacting serine/threonine protein kinase 1
ROS	reactive oxygen species
rpm	revolutions per minute
RU486	progesterone receptor antagonist mifepristone
S1P	sphingosine-1-phosphate
SEM	standard error of mean
SLE	systemic lupus erythematosus
SRP	serum amyloid P-component

T1D	type 1 diabetes mellitus
TAE	tris-acetate-EDTA
TAMs	tumour-associated macrophages
TCR	T cell receptor
TdT	terminal deoxynucleotidyl transferase
TEB	terminal end bud
T_H	T helper
Th_1	T helper 1
Th_2	T helper 2
TNF	tumour necrosis factor
TNFA	tumour necrosis factor alpha
TLR	Toll-like receptor
TRADD	TNFR-associated death domain
TRAIL	TNF-related apoptosis-inducing ligand
T_{reg}	T regulatory cells
TUNEL	terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick end labelling
TWEAK	TNF-related weak inducer of apoptosis
V(D)J	variable (V), diversity (D), and joining (J) gene segments