ADAMTS1 IS A PROMOTER OF METASTATIC CELL BEHAVIOUR IN MAMMARY CANCER CELLS

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“The ones crazy enough to think they can change the world are the ones that do”

- Steve Jobs

“It always seem impossible until it’s done”

-Nelson Mandela

“Everything is okay in the end. If its not okay, its not the end”

- John Lennon

Abstract

Metastatic disease is the primary cause of mortality in breast cancer. It is characterised by the dissemination of cancer cells from the primary site, infiltration into vessel networks and the establishment of new tumour growth in secondary tissues. Several events are required for metastasis to occur, including enhancement of cell-matrix adherence, augmented motility and invasiveness. The extracellular matrix (ECM) environment plays a vital role in the processes involved in metastatic progression and undergoes aberrant remodelling to permit and support the metastatic cascade.

Metalloproteinases are a group of enzymes that play a major role in ECM remodelling. The ADAMTS metalloproteinase family has been implicated in the re-organisation of the tumour microenvironment associated with cancer development and metastatic disease progression. Of the 19 ADAMTS proteases, considerable attention has been devoted to the role of its first member ADAMTS1 in cancer metastasis.

Both exogenous overexpression and upregulation of the endogenous *ADAMTS1* gene have been strongly associated with metastatic disease in breast cancer. The MMTV-PyMT transgenic breast cancer model recapitulates *in vivo* metastasis and ablation of *Adamts1* impeded the aggressive advancement and growth of pulmonary metastases. The signalling pathways and mechanistic events through which ADAMTS1 mediates its pro-metastatic effects are currently unknown. The aim of this present study is to therefore identify the causal events imposed by ADAMTS1 to promote breast cancer metastasis, with much focus on its role in matrix adhesion, cell migration and invasion.

Using isolated primary mammary carcinoma cells PyMT/*Adamts1*$^{+/+}$, PyMT/*Adamts1*$^{+/-}$ and PyMT/*Adamts1*$^{-/-}$ mice, I performed real-time assessment of cell-matrix adhesion, motility and invasion and found diminished capacity of PyMT/*Adamts1*$^{-/-}$ cells to adhere to matrigel and migrate towards a chemoattractive environment. Consistent with the reciprocal approach, introduction of *Adamts1* into the MCF10A breast cell line induced the inverse effect, promoting cell adhesion and motility in cells overexpressing *Adamts1*. Cell-matrix adhesion is a major cue for the determination of front-rear polarity necessary in cell migration and hence, the influence of ADAMTS1 on cell-matrix adhesion underpinned its effects on breast cancer cell migration. Breast cancer cell invasion was unaffected by loss or gain of *Adamts1*, suggesting a redundant role for ADAMTS1 in this process.

To unravel the transcriptional differences and mechanistic pathways induced by ADAMTS1, microarray analysis was undertaken with PyMT/*Adamts1*$^{+/+}$ and PyMT/*Adamts1*$^{-/-}$ mammary tumours. Remarkably, only 2 differentially regulated genes were identified from our analysis. Further investigation of the most dysregulated gene, BC018473, revealed a non-homologous inheritance of this strain specific gene, which unfortunately prevented conclusions being drawn on the underlying genetic effects attributable to *Adamts1* ablation.

This study was the first to present a novel role for ADAMTS1 in the promotion of breast cancer cell adhesion to the ECM. This capacity to dynamically modulate adhesion through ADAMTS1 is important in cell migration and highlights a potential mechanism by which ADAMTS1 promotes breast cancer metastasis.

Declaration

This thesis contains no material, which has been accepted for the award of any other degree or diploma in other university or tertiary institution. The content of this thesis is an original body of work and does not contain any published material written by another person, except where due reference has been made.

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1. de Arao Tan I, Ricciardelli C & Russell DL (2013). "The metalloproteinase ADAMTS1: A comprehensive review of its role in tumorigenic and metastatic pathways." International Journal Cancer.133(10):2263-76

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Publications arising from thesis

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2. de Arao Tan I, Frewin K, Ricciardelli C & Russell DL. **"ADAMTS1 promotes the adhesion of mammary cancer cells to structural proteins that make up the extracellular matrix and basement membrane that in turn promotes cancer cell migration**". *In preparation*

Abstracts arising from thesis

1. **de Arao Tan I**, Frewin K, Williams ED, Opeskin K, Pritchard MA, Ingman W, Ricciardelli C & Russell DL. *"Role of the protease Adamts1 in breast cancer growth and metastasis"* Australian Society for Medical Research, SA conference, Adelaide, SA, June 2009

2. **de Arao Tan I**, Frewin K, Williams ED, Opeskin K, Pritchard MA, Ingman W, Ricciardelli C & Russell DL. *"Role of the protease Adamts1 in breast cancer growth and metastasis"* Society for Reproductive Biology, Adelaide, SA, 2009.

3. **de Arao Tan I**, Frewin K, Williams ED, Opeskin K, Pritchard MA, Ingman W, Ricciardelli C & Russell DL. *"The role of Adamts1 in breast cancer progression and metastasis"* Matrix Biology Society of Australia and New Zealand, Adelaide, SA, 2009.

4. **de Arao Tan I**, Frewin K, Ricciardelli C & Russell DL. "*The metalloproteinase Adamts1 increases the adhesive capacity of mammary epithelial cancer cells*" 7th International Conference on Proteoglycans/Matrix Biology Society of Australia and New Zealand Annual Meeting, Manly, NSW, October 2011

5. **de Arao Tan I**, Frewin K, Ricciardelli C & Russell DL. "*The metalloproteinase Adamts1 increases the capacity of mammary cancer cells to adhere to extracellular components*". Faculty of Health Sciences Postgraduate Conference, Adelaide, SA, August 2011

6. **de Arao Tan I**, Frewin K, Ricciardelli C & Russell DL. "*The metalloproteinase Adamts1 increases the capacity of mammary cancer cells to adhere to extracellular components*". Research Centre for Reproductive Health and Centre for Cancer Stem Cell Research Conference. Adelaide, SA, November 2011

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Abbreviations

1°mMCC	primary mouse mammary cancer cell
ADAMTS1	a disintegrin and metalloproteinase with thrombospondin motifs 1
Adamts1$^{-/-}$	*Adamts1* knockout
Adamts1$^{+/-}$	*Adamts1* heterozygous
Adamts1$^{+/+}$	*Adamts1* wild type
ADPC	androgen dependent prostate cancer
ANOVA	analysis of variance
AR	androgen receptor
bp	base pair
C-terminal	carboxyl terminal
cDNA	complemtary deoxyribonucleic acid
CHO	chinese hamster ovary
cm	centimetre
CRC	colorectal carcinoma
CRPC	castrate resistant prostate cancer
DMEM	dulbecco's minimum essential medium
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
dNTP	deoxyribonucleic triphosphate
ECD	glutamic acid-cysteine-aspartic acid
ECM	extracellular matrix
EDTA	ethlyenediaminetetraacetic acid
EGF	epidermal growth factor
ER	estrogen receptor
FBS	fetal bovine serum
GFP	green fluorescent protein
GSE	genomic spatial event
h	hour

HA	hyaluronan
HB-EGF	heparin binding epidermal growth factor
HCC	hepatocellular carcinoma
HCC	hepatocellular carcinoma
HS	heparan sulphate
HSC	hepatic stellate cells
HSPG	heparan sulphate proteoglycans
IRES	internal ribosomal entry site
LSD	least significant difference
mg	milligram
min	minute
ml	millilitre
mm	millimetre
mM	millimolar
MMP	matrix metalloproteinase
MMTV	mouse mammary tumour virus
MTV	mammary tumour virus
N-terminal	amino terminal
Na_2HPO_4	disodium hydrogen phosphate
NaCl	sodium chloride
NaH_2PO_4	sodium dihydrogen phosphate
ng	nanogram
No.	number
NSCLC	non-small cell lung cancer
^oC	degrees Celsius
OPG	osteoprotegrin
PBS	phosphate buffer saline
PCR	polymerase chain reaction
PSF	penicillin/streptomycin/fungizone
PPAR-γ	peroxisome proliferator-activated receptor gamma
PyMT	polyoma middle-T
qRT-PCR	quantitative reverse transcription polymerase chain reaction
R^2	coefficient of determination

RANKL	nuclear kappa B
RGD	arginine-glycine-asparagine
RNA	ribonucleic acid
rpm	revolutions per minute
sec	second
SEM	standard error of mean
shRNA	short hairpin ribonucleic acid
TGF-β	transforming growth factor beta
TNFα	tumour necrosis factor alpha
TSP	thrombospondin
U/ml	units per ml
v/v	volume per volume percentage solution
VEGF	vascular endothelial growth factor
w/v	weight per volume percentage solution
wt	wild type
µg	microgram
µl	microlitre