

The roles of the chemokines CXCL12 and CXCL16 in breast cancer

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Declaration

This work contains no material that has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge, contains no material previously published or written by another person, except where due reference is made in the text.

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September 2007

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I could write a volume equivalent to this thesis expressing my gratitude to everyone who has helped me to navigate my way to the end of my PhD studies, but for the sake of a forest somewhere, I'll keep it to a couple of pages.

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Table of Contents

Declaration	iii
Acknowledgments	iv
Table of Contents	vi
Table of Figures.....	x
List of tables	xii
Abbreviations	xiii
Publications arising from this work	xvi
Abstract	xvii
<u>CHAPTER 1: Introduction</u>	<u>3</u>
1.1 Overview	3
1.2 Breast cancer	4
1.2.1 Breast cancer progression.....	4
1.2.2 The immune response to breast cancer	8
1.2.2.1 Immunosurveillance.....	8
1.2.2.2 Immune evasion.....	9
1.2.2.3 Active Immunosuppression	10
1.2.2.4 Overcoming tumour-induced immunosuppression.....	12
1.3 The chemokine family	13
1.3.1 General properties of chemokines	13
1.3.2 Chemokine receptor signalling.....	14
1.3.3 Biological functions of chemokines.....	15
1.4 CXCL12/Stromal cell-derived factor (SDF)-1, CXCR4 and CXCR7.....	17
1.4.1 General properties of CXCL12 and its receptors	17
1.4.2 Physiological functions of CXCL12.....	19
1.4.2.1 Functions of CXCL12 in the haematopoietic system	19
1.4.2.2 Functions of CXCL12 in non-haematopoietic tissues	21
1.4.3 CXCL12 and CXCR4 in cancer	22
1.5 CXCL16 and CXCR6.....	25
1.5.1 General properties of CXCL16 and CXCR6.....	25
1.5.2 Physiological functions of CXCL16.....	26
1.5.3 CXCL16 and CXCR6 in cancer	28

1.6	The Research Project.....	29
CHAPTER 2: Materials & Methods		45
2.1	Subcloning of chemokine constructs	45
2.1.1	Chemokine DNA constructs	45
2.1.2	Cloning vector.....	45
2.1.3	Polymerase Chain Reaction.....	45
2.1.4	Subcloning	47
2.1.5	Identification of positive clones.....	47
2.2	Generation of transfected cell lines	48
2.2.1	Cells.....	48
2.2.2	Transfection	48
2.3	Mouse model	49
2.3.1	Animals.....	49
2.3.2	Tumour model.....	49
2.4	Analytical and functional assays	50
2.4.1	Flow cytometric staining	50
2.4.1.1	Cell preparation:.....	50
2.4.1.2	Staining procedure.....	51
2.4.2	Reverse-transcriptase PCR	51
2.4.3	Sandwich ELISA for detection of chemokines.....	52
2.4.4	Calcium mobilisation	53
2.4.5	In vitro proliferation assay.....	53
2.4.6	Soft agar assay	54
2.4.7	Haemoglobin assay	54
2.4.8	Immunohistochemical staining	54
2.4.9	In vitro cytotoxicity assay.....	55
2.4.10	IL-17 ELISPOT.....	56
2.4.11	Intracellular cytokine staining	57
2.4.12	Cytokine bead array	57
2.5	Statistical analysis	58
2.6	Solutions and buffers	58
2.6.1	Media.....	58
2.6.1.1	Alpha Medium (serum-free)	58

2.6.1.2	Alpha Medium (complete)	58
2.6.1.3	2x Iscove's Modified Dulbecco's Medium (complete)	59
2.6.1.4	RPMI (incomplete)	59
2.6.1.5	RPMI (minimal complete).....	59
2.6.1.6	RPMI (complete)	59
2.6.2	Other solutions and buffers	59
2.6.2.1	Buffer P1	59
2.6.2.2	Buffer P2	60
2.6.2.3	Buffer P3	60
2.6.2.4	Calcium Buffer	60
2.6.2.5	Collagenase Solution.....	60
2.6.2.6	Gill's Haematoxylin.....	60
2.6.2.7	10x Hank's Balanced Salt Solution	60
2.6.2.8	Mouse Red Cell Removal Buffer (MRCRB)	61
2.6.2.9	PBS/azide (PA).....	61
2.6.2.10	PBS/BSA/azide (PBA)	61

CHAPTER 3: Generation of Chemokine Constructs & Cell Lines 69

3.1	Overview	69
3.2	Generation of chemokine constructs.....	69
3.2.1	Chemokine constructs used in this study	69
3.2.2	Cloning of chemokine constructs	70
3.3	The 4T1.2 model system	72
3.4	<i>In vitro</i> generation and characterisation of chemokine construct-expressing cell lines	74
3.4.1	Generation of chemokine construct-expressing cell lines.....	74
3.4.2	In vitro characterisation of chemokine construct-expressing cell lines	75
3.5	Summary of findings and preliminary discussion	76

CHAPTER 4: The Effect of Chemokine expression on breast cancer *in*

***vivo* 105**

4.1	Overview	105
4.2	Effects of exogenous chemokine expression on primary tumour growth ...	105
4.3	Effects of CXCL12 construct expression on metastasis	106

4.4	Effects of CXCL16 construct expression on metastasis.....	108
4.5	Summary and preliminary discussion.....	108
CHAPTER 5: The Effect of CXCL12 expression on the anti-tumour		
immune response		125
5.1	Overview.....	125
5.2	Identification of leukocyte subsets important for the anti-tumour effect of CXCL12.....	125
5.2.1	The role of T cells	125
5.2.2	The role of dendritic cells	127
5.2.3	The role of NKT cells.....	127
5.3	The role of cell-mediated immunity	128
5.4	The role of cytokines	130
5.5	Summary of findings and preliminary discussion	133
CHAPTER 6: Discussion		163
6.1	Introduction	163
6.2	The effects of CXCL12 overexpression on breast tumour progression	164
6.2.1	The effects of CXCL12 on tumour cells and the tumour vasculature.....	164
6.2.2	The importance of T cells and dendritic cells.....	165
6.2.3	The role of cell-mediated cytotoxicity	168
6.2.4	The role of cytokines.....	171
6.2.5	The potential effects of CXCL12 on suppressor cells.....	172
6.2.6	Other potential mediators of the effects of CXCL12	173
6.2.7	Summary and future directions.....	175
6.3	The effects of CXCL12 _(P2G) overexpression on breast tumour progression	177
6.4	The effects of CXCL16 construct overexpression on breast tumour progression	181
6.5	Perspectives and concluding remarks	183
CHAPTER 7: References		193

Amendments

- p. xv “phospholipases C” should read “phospholipase C”
- p. 35 In Table 1.4, the heading of the second column, “Role of CXCL16CXCR6” should read, “Role of CXCL16/CXCR6”
- p. 53 In Section 2.4.4, the concentration of CXCL12 and CXCL16 used for stimulation of the cells should read “300ng/ml” and not “300µg/ml”
- p. 107 In the first line, “angiogenesis with the tumour” should read “angiogenesis within the tumour”
- p. 129 In the second line of the second paragraph, “4TX12 1 tumour-bearing were” should read “4TX12 1 tumour-bearing mice were”
- p. 131 In the third-to-last line, “Type I IFN- γ -producing also” should read “Type I IFN- γ -producing T cells also”

Table of Figures

Figure 1.1 The hallmarks of breast cancer.	37
Figure 1.2 The steps of breast cancer metastasis.	38
Figure 1.3 Immunosuppressive networks induced by breast tumours.	40
Figure 1.4 Chemokine signalling.	41
Figure 2.1 The pEF-IRES-puro6 (pEF) mammalian expression vector.	65
Figure 3.1 Amino acid sequences of chemokine constructs.	80
Figure 3.2 Cloning of chemokine constructs.	82
Figure 3.3 Generation of the CXCL16 ₍₉₋₂₂₀₎ -His construct by overlap extension PCR.	84
Figure 3.4 Vector maps of chemokine constructs.	86
Figure 3.5 Expression of CXCL12 by 4T1.2 cells.	87
Figure 3.6 Expression of CXCL16 by 4T1.2 cells.	89
Figure 3.7 Expression of CXCR4 and CXCR7 by 4T1.2 cells.	90
Figure 3.8 Expression of CXCR6 by 4T1.2 cells.	92
Figure 3.9 Expression of chemokine constructs by transfected 4T1.2 cell populations.	94
Figure 3.10 Level of CXCL12 construct production by individual clones from transfected 4T1.2 populations.	95
Figure 3.11 Level of CXCL16 construct production by individual clones from transfected 4T1.2 populations.	96
Figure 3.12 CXCL12 construct expression by cell lines derived from pooled clones.	97
Figure 3.13 CXCL16 construct expression by cell lines derived from pooled clones.	98
Figure 3.14 In vitro proliferation rates of CXCL12-expressing 4T1.2 cell lines.	100
Figure 3.15 In vitro proliferation rates of CXCL16-expressing 4T1.2 cell lines.	101
Figure 3.16 In vitro tumorigenicity of chemokine construct-expressing cell lines.	102
Figure 4.1 Comparison of the growth of CXCL12 construct-expressing 4T1.2 mammary tumours and parental 4T1.2 tumours in vivo.	110
Figure 4.2 Comparison of the growth of CXCL12 construct-expressing 4T1.2 mammary tumours and control 4T12Ala tumours in vivo.	111
Figure 4.3 Growth of CXCL12-expressing 4T1.2 mammary tumours in vivo.	112
Figure 4.4 Growth of CXCL12 _(P2G) -expressing 4T1.2 mammary tumours in vivo.	113
Figure 4.5 Growth of CXCL16-expressing 4T1.2 mammary tumours in vivo.	114

Figure 4.6 Spontaneous metastasis to the lungs of 4TX12 1 and 4T12P2G tumour-bearing mice.....	115
Figure 4.7 Haemoglobin content of CXCL12-construct expressing tumours.	116
Figure 4.8 Vascularisation of CXCL12 construct-expressing tumours.....	117
Figure 4.9 Lymphangiogenesis within CXCL12 construct-expressing tumours.....	119
Figure 4.10 Experimental metastasis of CXCL12-expressing tumour cell lines to the lungs of Balb/c mice.	121
Figure 4.11 Metastases in the lungs of 4T16 and 4TΔ16 tumour-bearing mice.....	122
Figure 5.1 Growth of CXCL12-expressing tumours in SCID mice.....	138
Figure 5.2 Growth of CXCL12-expressing tumours cells in nude mice.	139
Figure 5.3 Correlation of the number and proportion of splenic T cells subsets with tumour weight.....	140
Figure 5.4 Characterisation of T cell subsets from early stage tumours and tumour draining lymph nodes.	142
Figure 5.5 T cell infiltration of CXCL12-expressing 4T1.2 tumours at early time points.	143
Figure 5.6 CD4 ⁺ T cell infiltration of late stage CXCL12-expressing 4T1.2 tumours. ...	145
Figure 5.7 CD8 ⁺ T cell infiltration of late stage CXCL12-expressing 4T1.2 tumours. ...	147
Figure 5.8 Accumulation of CD11c ⁺ cells in the tumour-draining lymph nodes.	149
Figure 5.9 CD11c ⁺ cells within CXCL12-expressing tumours.....	151
Figure 5.10 Growth of CXCL12-expressing tumours in invariant NKT cell-deficient mice.	153
Figure 5.11 Cytotoxic activity of lymphocytes against 4T1.2 tumour cells in vitro.....	154
Figure 5.12 Growth of CXCL12-expressing tumours in perforin- and TRAIL-deficient mice.....	155
Figure 5.13 Growth of CXCL12-expressing tumours in IFN-γ knockout mice.	156
Figure 5.14 Quantitation of IL-17-producing cells in tumour-draining lymph nodes by ELISPOT assay.	157
Figure 5.15 Intracellular cytokine staining of lymphocytes isolated from tumour-draining lymph nodes.	158
Figure 5.16 Cytokine production by cells isolated from tumour-draining lymph nodes.	159
Figure 6.1 Working model of CXCL12 action in primary mammary tumours.	186
Figure 6.2 Working model of CXCL12 _(P2G) action in mammary tumour metastasis.....	188

List of tables

Table 1.1 Chemokine nomenclature.....	31
Table 1.2 The chemokine receptors and their biological functions.	33
Table 1.3 The biological functions of CXCL12 and CXCR4.....	34
Table 1.4 The biological functions of CXCL16 and CXCR6.....	35
Table 2.1 Primers used in this study.....	62
Table 2.2 Antibodies used in this study.	63
Table 2.3 Other reagents used in this study.	64
Table 3.1 Chemokine constructs and their functions.	79
Table 5.1 Comparison of tumour growth inhibition by CXCL12 in wild-type and nude mice.	137
Table 5.2 Correlation of splenic T cell populations with final tumour weight.....	137

Abbreviations

300.19	a pre-B lymphocyte cell line
4T12Ala	4T1.2 cells transfected with the CXCL12 _(Ala) ::pEF DNA construct
4T12P2G	4T1.2 cells transfected with the CXCL12 _(P2G) ::pEF DNA construct
4TX12	4T1.2 cells transfected with the CXCL12::pEF DNA construct
4T16	4T1.2 cells transfected with the CXCL16::pEF DNA construct
4TΔ16	4T1.2 cells transfected with the CXCL16 ₍₉₋₂₂₀₎ ::pEF DNA construct
ADAM10	a disintegrin and metalloproteinase 10
ADCC	antibody-dependent cell-mediated cytotoxicity
APC	antigen presenting cell
APC-Cy7	allophycocyanin-cytochrome 7
ASMC	aortic smooth muscle cell
bFGF	basic fibroblast growth factor
BCIP/NBT	5-bromo-4-chloro-3' indolylphosphate p-toluidine salt/Nitro Blue tetrazolium
BCX6	300.19 cells transfected with a CXCR6::pEF DNA construct
BSA	bovine serum albumin
CAM	cell adhesion molecule
CCL	CC chemokine
CCR	CC chemokine receptor
CTL	cytotoxic T lymphocyte
CXCL	CXC chemokine
CXCR	CXC chemokine receptor
DAB	3,3'-diaminobenzidine
DC	dendritic cell
DLN	draining lymph node
EC	endothelial cell
ECM	extracellular matrix
EF-1α	elongation factor-1α
EGF	epidermal growth factor
ELISA	enzyme-linked immunosorbent assay
ELISPOT	enzyme-linked immunosorbent spot assay
EMMPRIN	extracellular matrix protease inducer
EPC	endothelial progenitor cell
ER	oestrogen receptor
ERK	extracellular signal-regulated kinase

FCS	foetal calf serum
FITC	fluorescein isothiocyanate
GAPDH	glutaraldehyde 3-phosphate dehydrogenase
GM-CSF	granulocyte-macrophage colony stimulating factor
GRK	G protein-coupled receptor kinase
HBSS	Hank's balanced salt solution
HD	Hodgkin's disease
HER2	human epidermal growth factor receptor 2
HIF-1 α	hypoxia-inducible factor-1 α
HIV	human immunodeficiency virus
HRP	horseradish peroxidase
HSC	haematopoietic stem cell
HUVEC	human umbilical vascular endothelial cell
IEL	intraepithelial lymphocyte
IFN- γ	interferon- γ
IKDC	interferon-producing killer dendritic cell
IL	interleukin
iNOS	inducible nitric oxide synthase
IRES	internal ribosome entry site
JAK	janus kinase
LDL	low density lipoprotein
LN	lymph node
MAPK	mitogen activated protein kinase
MHC	major histocompatibility complex
MMP	matrix metalloproteinase
MQ H ₂ O	MilliQ H ₂ O
MRCRB	mouse red cell removal buffer
MSC	myeloid suppressor cell
NK cell	natural killer cell
NO	nitric oxide
NPC	nasopharyngeal carcinoma
oxLDL	oxidised low density lipoprotein
PA	PBS/Azide
PBA	PBS/BSA/azide
PBL	peripheral blood leukocyte
PBS	phosphate buffered saline

PCR	polymerase chain reaction
pDC	plasmacytoid dendritic cell
PDGF	platelet-derived growth factor
PE	phycoerythrin
PE-Cy7	phycoerythrin-cytochrome 7
pEF	pEF-IRES-puro6
pfp	perforin
PGC	primordial germ cell
PI3K	phosphoinositol 3-kinase
PIP3	phosphatidylinositol 3,4,5-phosphate
PKC	protein kinase C
PLC	phospholipase C
PMA	phorbol 12-myristate 13-acetate
PMS	N-methyl dibenzopyrazine methyl sulfate
PTEN	phosphatase and tensin homologue deleted on chromosome 10
RNAi	ribonucleic acid interference
RT-PCR	reverse transcription polymerase chain reaction
SCID	severe combined immunodeficient
SDF-1	stromal cell-derived factor-1
siRNA	small interfering ribonucleic acid
STAT	signal transducer and activator of transcription
TAA	tumour-associated antigen
TAM	tumour-associated macrophage
TCR	T cell receptor
TGF- β	transforming growth factor- β
Th	T helper
TLR	Toll-like receptor
TNF- α	tumour necrosis factor- α
Tr, T _{reg}	T regulatory
TRAIL	tumour necrosis factor-related apoptosis-inducing ligand
uPA	urokinase-type plasminogen activator
VCAM-1	vascular cell adhesion molecule-1
VEGF	vascular endothelial growth factor
VLA-4	very late antigen-4
XTT	sodium 3'-[1-(phenylaminocarbonyl)-3,4-tetrazolium]-bis (4-methoxy-6-nitro) benzene sulfonic acid hydrate

Publications arising from this work

Manuscripts

Sharon A. Hampton-Smith & Shaun R. McColl. ‘Overexpression of CXCL12 in an orthotopic model of breast cancer improves the efficacy of the anti-tumour immune response.’ *Manuscript in preparation.*

Conference Proceedings

Adelaide Breast Development and Cancer Meeting, 2006 (Adelaide, Australia).

Title: Chemokines in breast cancer: whose side are they on anyway?

Oral Presentation

Adelaide Immunology Retreat II, 2006 (Adelaide, Australia).

Title: The role of the chemokine CXCL12 in breast cancer.

Oral presentation

Abstract

A growing body of work implicates chemokines and their receptors in the progression of various types of cancer, including breast cancer. However, as potent chemotactic factors for leukocytes, chemokines also have the potential to enhance anti-cancer immunity. Evidence suggests that the chemokine CXCL12 and its receptors may be important in a number of aspects of breast cancer progression and site-specific metastasis. Another chemokine, CXCL16, has been identified as a specific chemotactic factor for Type I-polarised T lymphocytes, which are major effectors of cell-mediated immunity and hence efficacious anti-tumour immune responses. The aim of this study, therefore, was to further elucidate the roles of CXCL12 and CXCL16 in breast cancer development and metastasis. To achieve this, wild-type CXCL12 and CXCL16 and antagonists of CXCL12 and CXCL16 activity, CXCL12_(P2G) and CXCL16₍₉₋₂₂₀₎ respectively, were overexpressed in the 4T1.2 mouse model of breast carcinoma. Overexpression of wild-type CXCL12 potently inhibited both primary tumour growth and metastasis in this model. This was attributed to the induction of an anti-tumour response dependent, in part, on T cells, interferon- γ and the cytotoxic mediators perforin and TRAIL. This response was characterised by increased numbers of CD11c⁺ cells in the tumour-draining lymph nodes and enhanced cytolytic activity of lymph node-derived effector cells against tumour cells. Unexpectedly, CXCL12_(P2G) inhibited metastasis of tumour cells to the lungs of tumour-bearing mice, without affecting primary tumour growth. Intravenous injection of tumour cells revealed that CXCL12_(P2G) expression could block metastatic steps occurring post tumour cell escape from the primary tumour, though a role for CXCL12_(P2G) at earlier metastatic steps could not be ruled out. Further work is needed to clarify the precise stages of metastasis at which CXCL12_(P2G) exerts its effects. No obvious effects on primary breast tumour growth were observed when CXCL16 or CXCL16₍₉₋₂₂₀₎ were overexpressed in tumour cells. Interestingly, CXCL16₍₉₋₂₂₀₎ expression inhibited experimental metastasis but not spontaneous metastasis. The findings of this study begin to shed light on the roles of CXCL12 and CXCL16 in breast cancer progression and also highlight the potential therapeutic applications of CXCL12, CXCL16 and/or their antagonists in the treatment of breast cancer and breast cancer metastasis.