The roles of the chemokines CXCL12 and CXCL16 in breast cancer

Sharon Hampton-Smith, B.Sc. (Hons.)

Discipline of Microbiology & Immunology
School of Molecular & Biomedical Science
University of Adelaide

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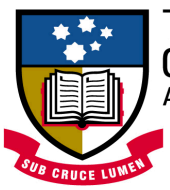

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Declaration

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Acknowledgments

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.

First, a big thank you to my supervisor, Shaun McColl. You conceived my project to begin with and provided me with the guidance I needed to get it up and running. But you were also brave enough to step back and let me try new things and develop new skills. You let me find the independence and confidence to conduct scientific research that can only come from getting in there and doing it. And of course, of late I have deeply appreciated your proofreading of innumerable drafts in amongst all the other responsibilities you have to take care of! Thank you also to my co-supervisor, Robin Anderson. You have provided invaluable cancer and metastasis expertise, as well as more specific advice regarding the 4T1.2 model that was central to this project. I appreciate all the practical tips and the conceptual assistance you have provided along the way and the time you took to meet with me when you made the trip from Melbourne to Adelaide.

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bringing life to the lab; and KK, thank you so much for all the girly days, chick flicks and culinary adventures – your friendship has been such a blessing!

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Of course, to my Beautiful One: You alone have seen every high and low and cared more deeply than I can imagine about each. Yet long after the pages of this thesis have crumbled to dust, You will go on. Thank You that such love will never fade.

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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 l IFN-γ-producing also" should read "Type I IFN-γ-producing T cells also"

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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-1α	hypoxia-inducible factor-1α
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_2O	MilliQ H_2O
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-phospate
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 $CXCL16_{(9\text{-}220)}$ 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 $CXCL16_{(9\text{-}220)}$ were overexpressed in tumour cells. Interestingly, $CXCL16_{(9\text{-}220)}$ 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.