The role of the atypical chemokine receptor CCX-CKR in progression and metastasis of cancer

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DECLARATION

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TABLE OF CONTENTS

TABLE OF FIGURES

LIST OF TABLES

ABBREVIATIONS

αMEM	Minimum Essential Medium Alpha
APC	antigen presenting cell
BSA	bovine serum albumin
CCX-CKR	Chemocentryx chemokine receptor
CNS	central nervous system
CTLA	cytotoxic T-lymphocyte antigen
DARC	Duffy antigen receptor for chemokine
DC	dendritic cell
DEPC	diethylpyrocarbonate
DLN	draining lymph node
DMEM	Dulbecco's Modified Eagle Medium
DR	death receptor
DTT	DL-Dithiothreitol
E/F PBS	endotoxin-free phosphate buffered saline
EAE	experimental autoimmune encephalomyelitis
ECM	extracellular matrix
EDTA	ethylenediaminetetraacetic acid
EGFR	epidermal growth factor receptor
ELISA	enzyme-linked immunosorbent assay
EMT	epithelial-mesenchymal transition
FBS	foetal bovine serum
FCS	forward scatter
GAPDH	glyceraldehyde-3-phosphate dehydrogenase
GFP	green fluorescent protein
GOI	gene of interest
GPCR	G-protein coupled receptor
GRK	G-protein coupled receptor kinase
HLA	human leukocyte antigen
HRP	horseradish peroxidase
ICCS	intracellular cytokine staining
IDO	indoleamine 2,3-dioxygenase
IF	immunofluorescence
IFN	interferon
IGF-1R	Insulin-like growth factor-1 receptor
IL	interleukin
IMDM	Iscove’s Modified Dulbecco’s Medium
KO mice	knockout mice
LMP	low-molecular-weight protein
LN	lymph node
LPS	lipopolysaccharide
mAB	monoclonal antibody
MAPK	mitogen activated protein kinase
MDSC	myeloid derived suppressor cell
MET	mesenchymal epithelial transition
MFI	mean fluorescent intensity
MHC	major histocompatability complex
MIC	MHC Class I chain-related molecules

MMP	matrix metalloproteinase
MRCRB	mouse red cell removal buffer
MΦ	macrophage
NK cell	natural killer cell
NKT	natural killer T cell
NF-κB	nuclear factor-κB
NO	nitric oxide
PARP	poly (ADP-ribose) polymerase
PBS	phosphate buffered saline
PDGF	platelet-derived growth factor
PFA	paraformaldehyde
PI	propidium iodide
PI3K	phosphoinositol 3-kinases
PKC	protein kinase C
PLC	phospholipase C
PMA	phorbol 12-myristate 13-acetate
PMS	N-methyl dibenzopyrazine methyl sulphate
PMSF	phenylmethanesulphonyl fluoride
PNAd	peripheral node addressin
qPCR	quantitative polymerase chain reaction
RAG	recombination activating gene
RG	reference gene
RNAi	RNA interference
ROI	reactive oxygen intermediate
RPLP0	ribosomal protein large P0
SCID	severe combined immunodeficiency
SDS	sodium dodecyl sulphate
SEM	standard error of the mean
shRNA	short-hairpin RNA
SNP	single nucleotide polymorphism
SSC	side scatter
TAM	tumour associated macrophage
TAP	transporter associated with antigen processing
TBS	Tris buffered saline
T_C	cytotoxic T cell
TCR	T cell receptor
TGF	transforming growth factor
T_H	helper T cell
TIL	tumour infiltrating leukocyte
TMBS	tumour-bearing mouse serum
TNF	tumour necrosis factor
TRAIL	tumour necrosis factor-related apoptosis-inducing ligand
T_{reg}	regulatory T cell
TSP	thrombospondin
VEGF	vascular endothelial growth factor
wt	wildtype
XTT	2,3-Bis(2-methoxy-4-nitro-5-sulphophenyl)-2H-tetrazolium-5-carboxanilideinner salt
ZO	zona occudens

PUBLICATIONS ARISING FROM THIS WORK

Manuscripts in preparation:

Harata-Lee Y., Comerford I., Brazzatti J.A., and McColl S.R., The atypical chemokine receptor CCX-CKR accelerates the epithelial-mesenchymal transition of mammary carcinoma.

Harata-Lee Y., Comerford I., Bunting M.D., Li M., Bastow C., Smyth M.J., and McColl S.R., shRNA-mediated knockdown of atypical chemokine receptor, CCX-CKR leads to melanoma rejection through enhanced recruitment of anti-melanoma leukocytes.

Conference Proceedings:

The 37th Annual Scientific Meeting of the Australasian Society for Immunology (2007): Oral and Poster Presentation entitled "The Atypical Chemokine receptor CCX-CKR suppresses the progression of mammary carcinoma."

Australian Society for Medical Research South Australian Meeting (2009): Poster Presentation entitled "The Atypical Chemokine receptor CCX-CKR suppresses the progression of murine melanoma."

ABSTRACT

The significance of chemokine receptors CCR7, CCR9 and their ligands CCL19, CCL21, and CCL25 in various types of cancer including mammary carcinoma and melanoma has been highlighted over the last decade. The atypical chemokine receptor CCK-CKR is a high affinity receptor for these chemokine ligands but rather than inducing classical downstream signalling events promoting migration, it instead sequesters and targets its ligands for degradation. Therefore, CCX-CKR has been proposed to regulate chemokine bioavailability *in vivo*. This putative function of CCX-CKR to regulate the levels of pro-tumourigenic chemokines initially led to the hypothesis that local and systemic regulation of chemokine levels by CCX-CKR influences tumour growth and metastasis *in vivo*, and ultimately, targeting of CCX-CKR could be an effective cancer therapy. Three broad approaches were taken to investigate the role of CCX-CKR in tumour progression and metastasis including overexpression of the receptor on tumour cells, deletion from the mouse host and receptor expression knockdown in tumour cells. The results revealed that overexpression of CCX-CKR on 4T1.2 mouse mammary carcinoma cells inhibits orthotopic tumour growth. However, this effect could not be correlated with chemokine scavenging *in vivo* and was not attributed to host adaptive immunity from experiments performed during the course of the current study. On the other hand, overexpression of CCX-CKR on 4T1.2 cells also resulted in enhanced spontaneous metastasis and haematogenous metastasis *in vivo*. *In vitro* characterisation of tumourigenicity of 4T1.2 cells revealed that overexpression of CCX-CKR rendered them more invasive, less adherent to the ECM and to each other and more resistant to anoikis. These are established characteristics of cells which have undergone EMT and indeed, CCX-CKR overexpressing cells showed a typical expression pattern of EMT markers. In contrast, when endogenous expression of CCX-CKR is deleted in the mouse host, growth and metastasis of E0771 mammary carcinoma and B16 melanoma are inhibited, which is accompanied by elevated

levels of CCX-CKR ligands in tumours and relevant naïve tissues from CCX-CKR-deleted mice. Similarly, shRNA-mediated knockdown of endogenous CCX-CKR from B16 melanoma cells leads to the rejection of primary and secondary tumours. This effect is attributed to elevated levels of CCX-CKR ligands and $CCR7^{+}$ and $CCR9^{+}$ leukocytes in tumour tissues, which resulted in an overall enhancement of the host anti-tumour immune response. Consistent with these observations, growth of CCX-CKR knockdown tumours was comparable to that of control tumours in CCR7-deleted mice indicating host CCR7 dependency of CCX-CKR-mediated rejection of B16 melanoma. Together, findings from this study revealed important insights into the complex role of CCX-CKR in cancer progression and highlights CCX-CKR as a novel target for the development of more effective anti-melanoma therapies and potentially for the treatment of other types of cancer which affect millions of people worldwide.