Regulation of the Chemokine Receptors CXCR4, CXCR7, and the Androgen Receptor in Prostate Cancer

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The chemokine receptor CXCR4 contributes to tumour cell migration and invasion during the progression of prostate cancer. In particular, this pathway is central to the metastasis of prostate cancer to the bone marrow. Limited therapeutic options exist for prostate cancer patients who have progressed to advanced metastatic disease, and pharmacological interference of the chemokine network may serve to control tumour cell dissemination and the establishment of metastasis. A more detailed knowledge of the mechanisms regulating chemokine receptors is required, in order to further characterise and explore the capacity and effectiveness of targeting these pathways for therapeutic intervention in prostate cancer.

Here, the regulation of CXCR4 protein expression and function was investigated in relation to androgens and the extracellular matrix. Accumulating evidence of CXCR4 regulation by androgens and the androgen receptor have indicated that androgens not only promote the growth and development of prostate cancer, but may actively contribute to the metastatic progression of prostate through modulation of the chemokine network. In the current study, the endogenous protein expression and functionality of the androgen receptor were firstly characterised in the androgen-insensitive prostate cancer cell lines DU145 and PC3, using the androgen-sensitive LNCaP cells as a basis for comparison. Investigations were performed using two-dimensional culture in conjunction with the more physiologically relevant three-dimensional in vitro culture model. As expected, LNCaP cells expressed prostate-specific antigen and displayed androgen-sensitive growth regulation, indicative of a functional androgen receptor. The androgen-insensitive DU145 cell line remained androgen receptor-negative in both two-dimensional and three-dimensional culture conditions. Surprisingly, androgen receptor-negative PC3 cells displayed a clear induction of androgen receptor protein expression in three-dimensional culture. The growth of PC3 cells remained androgen-insensitive in three-dimensional culture, and although androgen receptor responded to treatment with androgens by undergoing nuclear translocation, no production of the androgen receptor-target gene, prostate-specific antigen, was detected. Furthermore, evidence of differential androgen receptor regulation by signalling pathway activity was observed between PC3 and LNCaP cells, revealing a divergence in androgen receptor regulation between androgen-sensitive LNCaP cells and androgen-insensitive PC3 cells.
Consistent with findings in the literature, androgen regulation of CXCR4 expression was demonstrated in LNCaP cells, although the functional consequences of this regulation were limited. Functional studies of ligand-induced signalling and LNCaP cell migration revealed that CXCR4 displayed limited functional responses in this cell line. The more invasive, androgen-insensitive DU145 and PC3 cell lines were found to express highly functional CXCR4, which mediated ligand-induced cell migration responses. The treatment of androgen receptor-positive three-dimensional PC3 cultures with androgens resulted in increased CXCR4 protein expression, similar to that observed in LNCaP; a response which was mediated by androgen receptor activity. However, the lack of prostate-specific antigen production in these PC3 cultures indicated limited androgen receptor transcriptional activity, despite nuclear translocation of the receptor in response to DHT. Further investigations indicated that androgen receptor signalling may contribute to CXCR4 regulation in PC3 cells, an effect mediated through differential pathways to that observed in LNCaP cells.

The alternative SDF-1α-binding receptor, CXCR7, has also been associated with prostate cancer progression via regulation of tumour growth and invasion. Studies of prostate cancer cell proliferation in two-dimensional culture revealed that CXCR7 was required to maintain the growth of LNCaP cells in depleted culturing conditions generated using charcoal-stripped FBS. Considering previous reports of a mutual regulation occurring between CXCR7 and CXCR4 in vitro, the regulation of these receptors were studied in three-dimensional culture. A marked up-regulation of both CXCR7 and CXCR4 protein was observed upon culturing PC3 cells in three dimensions. The expression of these proteins was found to co-localise at stellate projections, structures which penetrated into the surrounding matrix and were rich in matrix metalloproteinase protein expression. A crucial role for integrin β1 was demonstrated in the formation and maintenance of the PC3 stellate phenotype, as a mediator of cell-extracellular matrix interactions. Consistent with the close association between CXCR4 and CXCR7 protein expression with stellate projections, inhibition of integrin β1 resulted in reduced protein expression for both chemokine receptors. The results reported here indicated that the protein expression of CXCR7 and CXCR4 were linked with the more invasive, stellate phenotype of PC3 cells in three-dimensional culture in vitro. When considered in the context of chemokine receptors in the regulation of prostate cancer metastasis, these findings may have implications for inter-regulation between chemokine receptors, integrin β1, and the extracellular matrix.
potentially contributing to the progression of prostate cancer to the invasive, metastatic tumour cell phenotype characteristic of advanced disease.
STATEMENT OF ORIGINALITY

This work has not previously been submitted for a degree or diploma in any university. To the best of my knowledge and belief, the thesis contains no material previously published or written by another person except where due reference is made in the dissertation itself.

_____________________________

Debra L. Kiss
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## List of Abbreviations

2D = Two-dimensional  
3D = Three-dimensional  
ADT = Androgen deprivation therapy  
AMACR = α-Methylacyl-coA racemase  
ANOVA = Analysis of variance  
APS = Ammonium persulphate  
AR = Androgen receptor  
ARE = Androgen responsive element  
ATCC = American type culture collection  
BCa = Breast cancer  
Bcl-2 = B-cell lymphoma 2  
BPH = Benign prostatic hyperplasia  
BSA = Bovine serum albumin  
Cat. No. = Catalogue Number  
CCR1 = CC chemokine receptor 1  
CCR5 = CC chemokine receptor 5  
CCR6 = CC chemokine receptor 6  
CCR7 = CC chemokine receptor 7  
cDNA = Complementary DNA  
CNS = Central nervous system  
CRPC = Castration-resistant prostate cancer  
CRS = Cell recovery solution  
CS-FBS = Charcoal-stripped FBS  
CT = Computed tomography  
CTC = Circulating tumour cell  
CTD = Carboxy-terminal domain  
CXCR1 = CXC chemokine receptor 1  
CXCR2 = CXC chemokine receptor 2  
CXCR3 = CXC chemokine receptor 3  
CXCR4 = CXC chemokine receptor 4  
CXCR7 = CXC chemokine receptor 7  
CYP17 = Cytochrome P450 17α-hydroxylase/17,20-lyase  
DAPI = 4’,6-diamidino-2-phenylindole dihydrochloride  
DBD = DNA-binding domain  
DC = Dendritic cell  
DHT = Di-hydroxytestosterone  
DIC = Differential interference contrast microscopy  
DNA = Deoxyribonucleic acid  
dNTP = Deoxyribonucleotide triphosphate  
DRE = Digital rectal exam  
DS = Double stranded  
ECM = Extracellular matrix  
EGF = Epidermal growth factor  
EHS = Engelbreth-holm-swarm (EHS)  
ELISA = Enzyme-linked immunosorbent assay  
EMT = Epithelial-to-mesenchymal transition  
EPCA = Early PCa antigen  
ERG = Avian V-ETS erythroblastic virus E26 oncogene homolog
ERK 1/2 = Extracellular Signal-regulated kinase 1/2
ETS = E-twenty six
E.coli = Escherichia coli
FAK = Focal adhesion kinase
FBS = Fetal bovine serum
fPSA = Free prostate specific antigen
GAPDH = Glyceraldehyde 3-phosphate dehydrogenase
GCSF = Granulocyte colony stimulating factor
GEM = Genetically engineered mouse
GnRH = Gonadotropin-releasing hormone
GPCR = G protein-coupled receptor
HEK = Human embryonic kidney
Her2 = Human epidermal growth factor receptor 2
HIV = Human immunodeficiency virus
hK2 = Human kallikrein 2
HRP = Horseradish peroxidase
HSP = Heat shock protein
IGF = Insulin-like growth factor
IL-6 = Interleukin 6
IL-8 = Interleukin 8
ITAC = Interferon-inducible T cell alpha chemoattractant
JAK-STAT = Janus activated kinase-signal transducer and activator of transcription
KLF5 = Krüppel-like factor 5
LBD = Ligand binding domain
LH = Leutenising hormone
LHRH = Leutenising hormone releasing hormone
MAPK = Mitogen-activated protein kinase
mAR = Membrane androgen receptor
MIP-1α = Macrophage inflammatory protein-1 alpha
MMP = Matrix metalloproteinase
MMP-11 = Matrix metalloproteinase 11
MMP-9 = Matrix metalloproteinase 9
MRI = Magnetic resonance imaging
mTOR = Mammalian target of rapamycin
MTT = 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MW = Molecular weight
N-Cadherin = Neural-cadherin
NCBI = National Centre for Biotechnology Information
NEB = New England Biolabs
NK = Natural killer
NTD = N (amino)-terminal domain
PAGE = Polyacrylamide gel electrophoresis
PBS = Phosphate-buffered saline
PCa = Prostate cancer
PCA3 = PCa antigen 3
PCR = Polymerase chain reaction
PFA = Paraformaldehyde
PGA = Polyglycolide
PI3K = Phosphoinositide 3-Kinase
PICP = C-Terminal pro-peptide of pro-collagen type 1
PIN = Prostatic intraepithelial neoplasia
PINP = N-Terminal pro-peptide of pro-collagen type 1
PLA = Polylactide
PLC = Phospholipase C
PLG/PLGA = Poly(lactide-co-glycolide)
PSA = Prostate specific antigen
PTEN = Phosphatase and tensin homolog
PVDF = Polyvinylidene fluoride
Rb = Retinoblastoma
RIPA = Radio-immunoprecipitation assay
RNA = Ribonucleic acid
ROI = Region of interest
RPMI = Roswell Park Memorial Institute
RT = Room temperature
RT-PCR = Reverse transcriptase polymerase chain reaction
SDF-1α = Stromal-derived factor-1 alpha
SDS = Sodium dodecyl sulphate
SDS-PAGE = Sodium dodecyl sulphate-polyacrylamide gel electrophoresis
S.E.M = Standard error of the mean
SFM = Serum-free culture medium
SIV = Simian immunodeficiency virus
TBS = Tris-buffered saline
TBST = Tris-buffered saline-tween 20
TC = Tissue culture
TEMED = Tetramethylethylenediamine
TGFβ = Transforming growth factor beta
TIMP = Tissue inhibitor of metalloproteinase
TIMP-2 = Tissue inhibitor of metalloproteinase 2
TMPRSS2 = Transmembrane protease, serine 2
TNM = Tumour, node and metastasis
TRAMP = Transgenic adenocarcinoma of the mouse prostate
uPA = Urokinase plasminogen activation axis
V = Volts
VEGF = Vascular endothelial growth factor
v/v = Volume per volume
w/v = Weight per volume
DECLARATION BY AUTHOR

This thesis is composed of my original work, and contains no material previously published or written by another person except where due reference has been made in the text. I have clearly stated the contribution by others to jointly-authored works that I have included in my thesis.

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Debra L. Kiss
PUBLISHED WORKS BY THE AUTHOR INCORPORATED INTO THE THESIS

Included in this thesis are two published paper, the first of which has been incorporated into Chapters 3 and 4, and was co-authored with other researchers. My contribution to the co-authored paper was in undertaking Western blots and immunocytochemistry, and participating in scientific discussion relating to the manuscript.

The bibliographic details for these papers are as follows:-


Additionally, another manuscript has been published based on the results presented in Chapter 6. As first author, my contribution to this manuscript consisted of performing western blots, immunocytochemistry, image analysis, data interpretation and preparation of the manuscript. The details for this manuscript are as follows:-


Appropriate acknowledgements of those who contributed to the research but did not qualify as authors are included in the published paper.

__________________________________________

Debra L. Kiss

__________________________________________

Professor Vicky M. Avery
Integrin regulation of EMT markers in tumour-stromal co-cultures of prostate cancer. Windus, L.C. Glover, T. Kiss, D.L. Avery, V.M. Currently under review at *Molecular Cancer*.
CONTRIBUTIONS OF OTHERS TO THE THESIS

Professor Vicky Avery has assisted with discussion and establishment of thesis aims, research hypotheses, choice of methods, critical analysis of experimental results, scientific discussions and in the structuring of the thesis. Professor Avery also performed editing and proofreading of the thesis itself.

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- Chapter 3: Figures 3.1, 3.3 - 3.8, and 3.10 - 3.12
- Chapter 4: Figures 4.1, 4.13 - 4.17, and 4.21 - 4.22
- Chapter 6: Figures 6.2, and 6.15 - 6.17

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