Mesenchymal Stem Cell Homing to Advanced and Metastatic Prostate Cancer

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UTS

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Certificate of original authorship

I certify that the work in this thesis has not previously been submitted for a degree nor has it been submitted as part of requirements for a degree except as part of the collaborative doctoral degree and/or fully acknowledged within the text.

I also certify that the thesis has been written by me. Any help that I have received in my research work and the preparation of the thesis itself has been acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

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Abbreviations

AAT	Androgen ablation therapy
ADT	Androgen deprivation therapy
Adv	Adenovirus
AFMS	Anterior fibromuscular stroma
α-MEM	alpha-Minimum Essential Medium
ANOVA	Analysis of variance
APC	Antigen presenting cells
AR	Androgen receptor
BFGF	Basic fibroblast growth factor
bFGF receptor	Basic fibroblast growth factor receptor
BM	Bone marrow
BMSC	Bone marrow-derived mesenchymal stem cells
BPH	Benign prostatic hyperplasia
CAR	Chimeric antigen receptor
CC	C-C motif
CCR	C-C motif receptor
CCD	Charge-coupled device
CD	Cytosine deaminase
CD:UPRT/Fcy:Fur	Cytosine deaminase uracil phosphoribosyl transferase
CEA	Carcinoembryonic antigen
CLZ	Coelenterazine
CM	Conditioned medium
CMV	Cytomegalovirus
CrAD	Conditionally replicating oncolytic Adv
CRPC	Castration-resistant PCa

CT	Threshold cycle
CTL	Cytotoxic T-lymphocyte
CXC	C-X-C motif
CXCR	C-X-C motif receptor
D-Luc	D-Luciferin
DMEM	Dulbecco's Modified Eagle's medium
D-PBS	Dulbecco's-Phosphate-Buffered Saline
DRE	Digital rectal examination
EBRT	External beam radiation therapy
EGF	Epidermal growth factor
EGFR	Epidermal growth factor receptor
ELISA	Enzyme-Linked Immunosorbent Assay
FACS	Fluorescence-activated cell sorting
FAK	Focal adhesion kinase
FC	Fluorocytosine
FCS	Foetal calf serum
FDA	Food and Drug Administration
Fl	Firefly luciferase
FdUDP	Fluorodeoxyuridine diphosphate
FdUMP	Fluorodeoxyuridine monophosphate
FdUTP	Fluorodeoxyuridine triphosphate
FGF	Fibroblast growth factor
FU	Fluorouracil
FUMP	fluorouridine monophosphate
GDEPT	Gene directed enzyme prodrug therapy
GFP	Green fluorescent protein
GITR	Glucocorticoid induced tumour necrosis factor receptor

GMP	Good manufacturing practice
GRO	Growth-regulated oncogene
GVHD	Graft failure and graft-versus-host disease
GVT	Graft-versus-tumour
HBSS	Hank's Balanced Salt Solution
HCELL	hematopoietic cell E-selectin/L-selectin ligand
HGF	Human growth factor
HGPIN	High-grade prostatic intraepithelial neoplasia
HLA	Human Leukocyte Antigen
HPF	High Powered Field of View
HSC	Haematopoietic stem cells
HSCT	HSC transplantations
HTLV	Human T-cell Leukaemia Virus
HUVEC	Human umbilical vein endothelial cells
IA	Intraarterial
IC	Intracorporeal
ICAM-1	Intracellular adhesion molecule-1
IFN	Interferon
IGF	Interleukin growth factor
IGFR	Interleukin growth factor
IL	Interleukin
IM	Intramyocardial
IP	Intraperitoneal
IRES	Internal ribosome entry sequence
IRMT	Intensity modulated radiotherapy
ISCT	International Society for Cellular Therapy
IT	Intratumoral

IV	Intravenously
IVC	Individual ventilated cage
IVIS	*In vivo* Imaging System
LacZ	β-galactosidase
LFA-1	lymphocyte function-associated antigen 1
LPAM-1	Integrin $\alpha_4\,\beta_7$
MAC-1	Macrophage-1 antigen
MAdCAM-1	Mucosal vascular addressin cell adhesion molecule 1
mCRPC	Metastatic castrate-resistant prostate cancer
MHC	Major Histocompatibility Complex
MES-SDS	2-[N-morpholino]ethanesulfonic acid-Sodium dodecyl sulfate polyacrylamide
MO	Medium only
MRI	Magnetic resonance imaging
MSC	Mesenchymal stem cells
N.S	Not significant
NCCN	National comprehensive cancer network
NDV	Newcastle disease virus
NK	Natural killer
PAP	Prostate acid phosphatase
PBMC	Peripheral blood mononuclear cell
PBSC	Peripheral blood stem cell
PCa	Prostate cancer
PDGF	Platelet-derived growth factor
PET	Positron emission topography
PIN	Prostatic intraepithelial neoplasia
PNP	Purine nucleoside phosphorylase

PSA	Prostate specific antigen
P/S, glu	Penicillin/streptomycin, glutamine
PSGL1	P‑selectin glycoprotein ligand 1
PSMA	Prostate specific membrane antigen
PZ	Peripheral zone
REIC	Reduced expression in immortalized cells
Rl	Renilla luciferase
ROI	Regions of interest
RR	Ribonucleotide reductase
RT	Room temperature
SC	Subcutaneous
SGE	Super gene expression
sLe$_X$	sialyl Lewis X
SPECT	Single-photon emission computed tomography
TAA	Tumour-associated antigens
TBE	Tris/Brate/Ethylenediaminetetraacetic
TCR	T cell receptor
TK	Thymidine kinase
TRAIL	Tumour necrosis factor-related apoptosis-inducing ligand
TZ	Transition zone
UBMC	Unsorted bone marrow cells
UCMSC	Umbilical cord mesenchymal stem cell
VCAM	Vascular cell adhesion molecule
VEGF	Vascular endothelial growth factor
VLA	Very late antigen

Abstract

Prostate cancer (PCa) is the most common cancer affecting men worldwide. Current treatment strategies to combat advanced and metastatic disease are ineffective and this has created a need to explore novel therapies, such as cell and gene therapies. A promising strategy involves capitalising on the innate ability of bone marrow-derived mesenchymal stem cells (BMSCs) to home to sites of cancer and release a genetic payload. BMSCs have the added benefit of being immune evasive, which is a major problem for other cell and gene therapy protocols.

BMSCs used in this study had been previously stably nucleofected to express a cell tracking reporter gene firefly luciferase (fl) and the yeast fusion suicide gene cytosine deaminase and uracil phosphoribosyltransferase (BMSC-Fcy:Fur). RM1 murine PCa cells were gene modified with the cell tracking reporter gene renilla luciferase (rl). An immune intact B6 albino mouse model was developed to investigate BMSC-Fcy:Fur homing to RM1 lung pseudometastases, and therapeutic effect. Using bioluminescence imaging (BLI) it was discovered that BMSC-Fcy:Fur showed greater persistence in the lungs of mice with RM1 tumours at 3 hours post-injection compared to their cancer free counterparts, however the BMSCs did not persist for longer than 24 hours in the lungs likely because of their advanced passage. By delivering prodrug when BMSC-Fcy:Fur were largely present in the lungs, a significant decrease lung RM1 colonies and a 25% improvement in survival was achieved. Importantly, BMSC-Fcy:Fur treatment was associated with no adverse events and did not promote PCa growth, confirming their safety for allogenic use in the treatment of PCa.

Previous findings have led to investigations into why BMSCs are attracted to PCa and how to best capitalise BMSC-PCa tropism for the development of novel therapies for metastatic PCa. It was anticipated that a greater understanding of the molecular events governing BMSC tropism for cancer may permit improved therapeutic BMSC targeting to metastatic PCa. Towards this aim, I isolated and characterised a subpopulation of BMSCs from B6 albino mouse BM. Cells were sorted from early passage following dual colour staining with antibodies against a stem cell antigen-1 (Sca-1) and a haematopoietic marker (CD45). Specifically, when compared to unsorted BM cells (UBMCs), the subpopulation showed: typical BMSC

phenotype: $Sca-1^+$, $CD44^+$, $CD90^+$, $CD106^+$, $CD31^-$, $CD34^-$ and CD45; enhanced adipogenic ability; similar osteogenic ability; and similar colony formation ability. Subsequently, these BMSCs were used to understand the signals mediating migration towards PCa. BMSC migration to RM1 derived conditioned medium (CM) was assessed using Transwell migration assays. These assays revealed that migration was due to soluble chemokines. Cytokine arrays and genome wide microarrays identified candidate chemokine receptors CCR2, CXCR2, CCR1, and CCR5 as potential mediators of BMSC migration towards RM1 CM. Inhibition of individual chemokine receptors led to a reduction in migration of BMSCs to RM1 CM, however not to level observed with pertussis toxin alone suggesting multiple or other chemoattractant receptors (chemokine receptors or growth factor receptors) may be involved in migration. Importantly, it was found that overexpression of CCR2 and CXCR2 on the surface of BMSC-Fcy:Fur significantly improved their migration to RM1 PCa CM.

In this thesis, I have demonstrated that systemic delivery of allogenic BMSCs followed by GDPET activation at a time when BMSCs are most localised to the tumour, is a safe and efficacious method to combat late stage PCa. Moreover, I showed for the first time that BMSCs can be enriched from B6 albino mice using Sca-1 and CD45 cell sorting. Lastly, I showed that BMSCs migration is facilitated by various chemokine receptors and overexpression of CCR2

and CXCR2 promotes migration to RM1 CM. These findings provide new insights into the

signals mediating migration to PCa and may be exploited in future to improve on migration

of BMSCs to PCa.