Improving Anti-Breast Cancer Vaccines by Overcoming Tumour Induced Immunosuppressive Factors

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Abstract

Cancers are a rapidly increasing source of morbidity and mortality for Australians. In 2005, there were over 100,000 new cases of cancer diagnosed and this number is projected to grow by over 3,000 extra cases per year in 2006–2010. Although traditional therapies such as surgery, chemotherapy and radiation therapy may extend the life of many patients, the treatments are associated with severe toxicities and are rarely curative for disseminated cancers. The idea of harnessing the immune system for the treatment of cancers represents an attractive treatment modality which should effectively complement current treatment methods. Immunotherapies, including cancer vaccines, are designed to re-train the immune system to recognise and destroy cancer cells and tip the balance from tumour acceptance toward active tumour immunity. However, many cancer vaccine approaches have failed to live up to their potential. Cancers employ many immunosuppressive factors which contribute to nullifying anti-tumour immune responses during vaccination therapy. These immunosuppressive factors include galectin-1, regulatory T-cells (Tregs) and inhibitory molecules such as CTLA-4, which are potential targets in the enhancement of immunotherapeutic strategies. This thesis aimed to investigate the role of these tumour derived immunosuppressive factors and to enhance the immune response towards an allogeneic whole cell breast cancer vaccine using a murine model of breast cancer.

Chapter 3 outlines the development of a highly immunogenic murine breast cancer cell line used in vaccination protocols throughout this study. Moderate increases in survival and slower tumour growth rates were observed both in prophylactic and treatment settings. The results demonstrated that factors involved in tumourigenesis may overwhelm the anti-cancer immune responses generated by vaccination. In Chapter 4, the role of galectin-1 was studied for its effects on T-cell subpopulations and the induction of CTL responses against breast cancer cells. Certain disaccharides that block galectin-1 from interacting with other

carbohydrates and glycoproteins on cell surfaces were examined for their ability to enhance the potency of the cancer vaccine. These studies demonstrated that small carbohydrate molecules can be administered *in vivo* to inhibit the immunosuppressive activity of galectins and restore the immune environment, significantly inhibiting the growth of breast cancers to improve survival outcomes.

Chapter 5 investigated the role of Tregs and the CTLA-4 negative co-stimulatory molecule in inhibiting immune responses targeted toward murine breast tumours. Specifically, the administration of monoclonal anti-bodies (MAbs) which target Tregs was investigated. The results presented in this chapter clearly demonstrated that removing the immunosuppressive activity of Tregs by targeting their CTLA-4 surface receptor function is clearly preferable to broadly targeting Tregs through CD25 expression. This is demonstrated by enhanced the anti-tumour immune activity and increased survival in mice receiving anti-CTLA-4 MAb therapy. Furthermore, this chapter indicates that combining anti-cancer vaccination with CTLA-4 blockade induces increased $CD8^+$ TIL, thereby inhibiting tumour growth and increasing survival above that of either approach used alone.

Chapter 6 investigated the effect of triple combination immunotherapy which combined the agents trialled in previous chapters to form an ultimate cocktail immunotherapy. Taking into account inter-experimental variability and control populations which were consistently ran in parallel, results in this chapter demonstrated that combination immunotherapy consisting of a whole cell cancer vaccine, an anti-CTLA-4 mAb and an anti-galectin disaccharide, provides a superior approach for enhancing anti-tumour immune responses than the use of stand-alone therapies. The role of galectin-1 and CTLA-4 in the appropriation of the Treg phenotype was also investigated. It was shown that both galectin-1 and CTLA-4 expression on naive $CD4^+CD25^-$ T-cell contributes significantly to the conversion of cells to the $CD4^+CD25^+FoxP3^+$ Treg phenotype and that inhibiting these

molecules could significantly reduce this conversion with implications for altering the intratumoural ratio of $CD8^+$ effector T-cells to the immunosuppressive $CD4^+CD25^+FoxP3^+$ Tregs.

The results of this thesis highlight the fact that in order to improve current cancer therapies, including immunotherapies, a combinatorial approach must be used to combat as many evasion strategies as possible to tilt the balance back in favour of tumour elimination. This study demonstrates that a triple combination of a cancer vaccine with galectin-1 inhibition and the removal of negative co-stimulatory signals through CTLA-4 blockade can significantly improve outcomes for tumour challenged animals over any agent used alone, reinforcing the necessity for combination therapy to be applied in the current clinical setting.

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 thesis itself.

(Signed)_____

Acknowledgement of published papers included in this thesis

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Included in this thesis is 1 published paper in *Chapter 4* which is co-authored with other researchers. My contribution to the co-authored paper is outlined at the front of the relevant chapter. The bibliographic details for this paper are:

Chapter 4: Stannard, K. A., P. M. Collins, et al. (2010). "Galectin inhibitory disaccharides promote tumour immunity in a breast cancer model." Cancer Lett **299**(2): 95-110.

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

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Supervisor: Name of Supervisor

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Abbreviations

2-ME	2-mercaptoethanol
Ag	Antigen
AICD	Activation induced cell death
Amax	Absorbance maximum
APC	Antigen presenting cell
APC-conjugated	Allophycocyanin-conjugated
APM	Antigen processing machinery
BATDA	bis (acetoxymethyl) 2,2':6',2"- terpyridine-6,6"- dicarboxylate
BCG	Bacille Calmette-Guerin
BCL-2	B-cell lymphoma protein-2
BRACA1/2	Breast cancer gene 1/2
BSA	Bovine serum albumin
CAM	Cellular adhesion molecule
cAMP	Cyclic adenosine monophosphate
CCD	Charge-coupled device
CML	Chronic myeloid leukaemia
CRD	Carbohydrate recognition domain
CSC	Cancer stem cell
CTL	Cytotoxic T-lymphocyte
CTLA-4	Cytotoxic T-lymphocyte antigen-4
DC	Dendritic cell
DFS	Disease free survival
DMEM	Dulbeccos's modified eagle medium
DMSO	Dimethyl sulfoxide
dNTP	Deoxynucleotide Triphosphate
DOL	Degree of labelling
DR-5	Death receptor-5
DSMB	Data safety monitoring board
EDTA	Ethylenediaminetetraacetic acid
EGFR	Epidermal Growth Factor Receptor

ELISA	Enzyme linked immunosorbant assay
Erk	Extracellular Signal-Regulated Kinase
E:T	Effector:Target
FACS	Fluorescent activated cell sorting
FCS	Foetal calf serum
FDA	Food and drug administration
FITC	Fluorescein isothiocyanate
FI	Fold increase
Gal-1	Galectin-1
GE	Gastroesophageal
GITR	Glucocorticoid-induced tumor necrosis factor receptor
GM-CSF	Granulocyte Macrophage Colony Stimulating Factor
HER-2	Human epidermal growth factor receptor 2
HNC	Head and neck cancer
IBD	Inflammatory bowel disease
IC50	Inhibitory concentration of 50%
ICAM	Intracellular adhesion molecule
IDO	Indolamine 2,3-dioxygenase
IFN	Interferon
Ig	Immunoglobulin
IL	Interleukin
I.P.	Intra-peritoneal
I.T.	Intra-tumoural
I.V.	Intra-venous
LAK	Lymphokine-Activated Killer Cell
LN	Lymph node
MAb	Monoclonal antibody
MAPK	Mitogen-Activated Protein Kinases
MCP	Modified citrus pectin
MFI	Mean fluorescence intensity
MHC	Major histocompatibility class I/II

MLC	Mixed lymphocyte culture
MMP	Matrix metalloprotease
MMTV	Mouse Mammary Tumour Virus Long Terminal Repeat Promoter
mRNA	messenger ribonucleic acid
NBCS	New bourn calf serum
NKT	Natural killer T-cell
OTC	Optimal cutting temperature
OS	Overall survival
PBMC	Peripheral blood mononucleocyte
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PD-1	Programmed cell death-1
pDC	Plasmocytoid dendritic cell
PE	Phycoerythrin
PI	Propidium iodide
PI3K	Phosphatidylinositol-3-Kinase
PTLD	Post Transplant Lymphoproliferative Disorder
rhGal-1	Recombinant human Galectin-1
rpm	Revolutions per minute
S.C.	Subcutaneous
SEM	Standard error of the mean
SPF	Specific pathogen free
TAA	Tumour associated antigen
TAP1/2	Transporter Associated with Antigen Processing ½
TCR	T-cell receptor
TDG	Thiodigalactoside
TGF-β	Transforming growth factor-beta
Th	T-helper cell
TIL	Tumour infiltrating lymphocyte
TNF	Tumour necrosis factor

TRAIL	Tumour necrosis factor-related apoptosis-inducing ligand
Treg	Regulatory T-cell
UV	Ultraviolet
Vb	Vinblastine
VEGF	Vascular Endothelial Growth Factor

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