GENE THERAPY FOR MESOTHELIOMA:
STUDIES OF CONDITIONALLY REPLICATIVE
ADENOVIRUSES AND MEASLES VIRUS

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A thesis submitted to the University of Adelaide in fulfilment of the
requirements for the degree of Doctor of Philosophy

May 2008
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Abstract

Malignant mesothelioma (MM) is an aggressive malignancy of the pleural and peritoneal surfaces. Australia has the highest reported national incidence of mesothelioma in the world, and rates are increasing (Leigh et al., 2002). The clinical outcome for patients with this disease is extremely poor, with median survival of 9 to 12 months (Rizzo et al., 2001; Carbone et al., 2002). The latest developments in chemotherapy, radiotherapy and radical surgery have done little to improve the overall survival rate (Kindler 2000; Zellos et al., 2002). New approaches to therapy are thus required (Nowak et al., 2002). Cancer therapy using conditionally replicative adenoviruses (CRAds) and attenuated measles virus (vaccine strain MV-Edm) are novel and promising approaches to cancer treatment. CRAds strategy relies on selective viral replication in tumour cells but not normal cells. Major efforts have been directed toward achieving selective replication by the deletion of viral functions dispensable in tumour cells or by the regulation of viral genes with tumour-specific promoters (Alemany et al., 2000). However, the major clinical limitation of viral therapy has been lack of efficacy rather than safety concerns.

In this study, I constructed CRAds in which tumour-specific promoter for Flt-1 (vascular endothelial growth factor receptor) control the essential E1 gene expression, and evaluated the cell-killing efficacy and specificity of CRAds driven by VEGF and Flt-1 promoters in the number of established mesothelioma cell lines and actual primary tumour cells from patients. CRAds with either VEGF or flt-1 promoters showed a strong killing effect on mesothelioma cells.
Co-delivery of CRAds with MMP-9 (matrix metalloproteinase-9) was assessed to determine whether therapeutic efficacy could be improved by reducing tumour-associated fibrosis thereby enhancing viral spread through a tumour mass. Combined therapy did result in greater suppression of tumour growth \textit{in vivo}.

I also identified an immuno-competent murine model of mesothelioma that was permissive for adenoviral replication. Combined viral therapy with immunotherapy (FGK45, an anti-CD40 antibody) in this model resulted in greater effect than Adwt or FGK45 alone and in greatest survival.

I evaluated the capacity of MV-Edm to infect human mesothelioma cells to form syncytia, and lead to apoptosis and cell death. I also assessed the mode of death by analysis of markers of apoptosis including caspase-3. \textit{In vivo} study showed that MV-Edm-GFP transduction could be detected in human xenografts in immune deficient mice. Further studies to evaluate the mechanisms and efficacy of anti-tumour immune stimulation induced by tumour cell killing with CRAds and MV-Edm will be discussed in this study. MV-Edm has good killing effect on mesothelioma cells \textit{in vitro}.

In summary the work presented herein provide new insights into stratgies to improve viral therapies for mesothelioma.
Declaration of Originality

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

I give my consent to this copy of my thesis being made available in the University Library.

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Acknowledgments

This work was supported by the International Postgraduate Research Scholarship (IPRS) and Adelaide University Scholarship. This research was in part supported by NH&MRC and Medical Research and Compensation Foundation.

Many thanks go to my supervisors, Associate Professor Paul Reynolds and Associate Professor Mark Holmes, for your financial support, your invaluable guidance and understanding, and giving me the opportunity to attend conferences throughout the course of this Ph.D.

To Dr Ann Reynolds, thank you very much for your patience and time with my project. You were always available whenever I had trouble with my project over the past years. A great thankyou to Dr Sandy Hodge and Dr Greg Hodge for your technical support and advice with my flow cytometry experiments and data analysis. To Dr Joseph Tan, Dr Geoff Matthews and Jessica Ahern for your questions raised and useful discussions.

To my husband Feng Feng, thank you very much for your support and useful discussions. A special thanks to my lovely daughter Krystal Feng, for teaching and correcting my English. To my parents and relatives, thank you all very much for your encouragement and utmost support during my studies.
Publications and Presentations


Abbreviations

293: human embryonic kidney cell line
5-FC: 5-fluorocytosine
5-FU: 5-fluorouracil
7-AAD: 7-amino-actinomycin-D
AAV: Adeno-associated virus
Ac-DEVD-AMC: Caspase-3 substrate II, Fluorogenic
Ad: Adenovirus
Ad5: serotype 5 adenovirus
Ad3: serotype 3 adenovirus
Ad5/3: Ad5 containing a chimeric fibre protein possessing the Ad3 knob
AEC: airway epithelial cell
AFP: α-fetoprotein promoter
APC: antigen-presenting cell
APS: ammonium persulfate
BEAS-2B: human bronchial epithelial cell
BES: N, N-bis[2-hydroxyethyl]-2-aminoethanesulphonic acid
CaCl2: calcium chloride
CAR: coxsackie adenoviral receptor
CD: cytosine deaminase
cDNA: complementary DNA
CEA: carcinoembryonic antigen
CF: cystic fibrosis
CHO: chinese hamster ovary
CPE: cytopathic effect
CRAd: conditionally replicative adenovirus
CsCl: caesium chloride
CT: computer tomographic
CTL: cytotoxic T-lymphocyte
DC: dendritic cell
DEPC: diethy1-pyrocarbonate
DMEM: Dulbecco’s modified Eagles medium
dNTP: deoxynucleotide triphosphate
ECM: extracellular matrix
EC: endothelial cell
E.coli: Escherichia coli
EDTA: ethylenedinitrilotetraacetic acid
EGF: epidermal growth factor
EMA: epithelial membrane antigen
EORTC: European Organisation for Research and Treatment of Cancer
EPP: extrapleural pneumonectomy
ERK: extracellular signal-regulated kinase
F: fusion (protein)
FBS: foetal bovine serum
FGF: fibroblast growth factor
Flt-1: vascular endothelial growth factor receptor
FVC: forced vital capacity
GCV: ganciclovir
GFP: green fluorescent protein
GM-CSF: granulocyte-macrophage colony-stimulating factor
H: hemagglutinin (protein)
HBME-1: human mesothelial cell 1
H&E: haematoxylin and eosin
HeLa cell: cervical cancer cell line
HCl: hydrochloric acid
HEPES: N-(2-hydroxyethyl) piperazine-N’-(2-ethanesulphonic acid)
HMVEC-LB1: lung-derived normal human microvascular blood vessel endothelial cell
TERT: telomerase reverse transcriptase
HIV: human immunodeficiency virus
HRE: hypoxia responsive element
HSV: herpes simplex virus
HUVEC: human umbilical vein endothelial cell
IFN-α, -β,-γ: interferons-alpha, interferons-beta, interferons-gamma
IGF-1: insulin-like growth factor-1
IL-2: interleukin-2
IMRT: intensity-modulated radiation therapy
ITR: inverted terminal repeat
Kb: kilobases
kDa: kilo Dalton
KDR: vascular endothelial growth factor receptor
L: large (protein)
LAK: lymphokine-activated killer
LB: lysogeny broth
LTR: long terminal repeat
Luc: firefly luciferase
M: matrix (protein)
MAP: mitogen-activated protein
MAPK: mitogen-activated protein kinase
MgCl₂: magnesium chloride
MHCI: major histocompatibility complex I
Min: minute
MK: midkine
MM: malignant mesothelioma
MMP: matrix metalloproteinase
MMPI: matrix metalloproteinase inhibitor
MOI: multiplicity of infection
MoMuLV: moloney murine leukaemia virus
MPM: malignant pleural mesothelioma
MT-MMP: membrane type matrix metalloproteinase
MRI: magnetic resonance imaging
mRNA: messenger RNA
MuLV: murine leukaemia virus
MV: measles virus
MV-CEA: MV-Edm expressing carcinoembryonic antigen
MV-Edm: live attenuated Edmonston B strain of measles virus
MV-ERV: Echistatin-targeted measles virus vector
MV-GFP: MV-Edm expressing green fluorescent protein
MV-Luc: MV-Edm expressing firefly luciferase
MV-NIS: MV-Edm coding for the thyroidal sodium iodide symporter
MV-NSE: anti-genomic MV-Edm
MuLV: murine leukaemia viruses
NaCl: sodium chloride
N: nucleocapsid (protein)
NDV: Newcastle disease virus
NF2: neurofibromatosis type 2
NIS: thyroidal sodium iodide symporter
NK: natural killer
P: phospho- (protein)
PASMC: pulmonary artery smooth muscle cell
PBMC: peripheral blood mononuclear cell
PBS: phosphate buffered saline
PCR: polymerase chain reaction
P/D: pleurectomy/decortication
PER.C6: human embryonic retinoblast cell line
PET: positron emission tomography
PFU: plaque forming units
PRR: pattern recognition receptor
PSA: prostate specific antigen
QOL: quality of life
RAM: rat anti mouse
Rb: retinoblastoma gene
RBC: red blood cells
RGD: Arg-Gly-Asp
RNA: ribonucleic acid
RT-PCR: reverse transcription-polymerase chain reaction

rpm: revolutions per minute

SAGE: serial analysis of gene expression

SD: standard deviation

SDS: sodium dodecyl sulphate

SE: standard error

Sec: second

SEM: standard error of mean

s-Flt-1: the soluble fragment of Flt-1

SLAM: signalling lymphocyte activation molecule (also called CD150)

SMRP: soluble mesothelin-related protein

siRNA: small interfering RNA

SSPE: subacute sclerosing panencephalitis

SV40: simian virus 40

TBS: Tris buffered saline

TBS-T: Tris buffered saline with TWEEN-20

TCID$_{50}$: tissue culture infectious dose

TEMED: N,N,N’,N’-tetramethylethylenediamine

TERT: telomerase reverse transcriptase

Th1: T-helper type 1

TIMP: tissue inhibitor of matrix metalloproteinases

TK: thymidine kinase

TLR: toll-like receptor

TNF: tumour necrosis factor

TNF-α: tumour necrosis factor-α
TRAIL: TNF-related apoptosis inducing ligand

TTF-1: thyroid transcription factor-1

TUNEL: terminal uridine deoxynucleotidyl transferase dUTP nick end labelling

Tyr: tyrosinase

UV: ultra violet

VATS: video-assisted thoracoscopy

VEGF: vascular endothelial growth factor

Vero: African green monkey kidney

VP: viral particle

Wt: wild type

WT1: Wilms’ tumour 1 antigen