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

Wei Xia

M.Sc.

Lung Research Laboratory, Hanson Institute

Department of Thoracic Medicine, Royal Adelaide Hospital

South Australia

Department of Medicine

University of Adelaide

South Australia

.....

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

May 2008

Table of Contents

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 killeg 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 *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. *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 *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.

The author acknowledges that copyright of published works contained within this thesis (as list below) resides with the copyright holder/s of those works.

Wei XIA

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

Xia W, Lake R, Takayama K, Holmes MD, Reynolds PN. (2005). Vascular Promoter-based Viral Therapy for Mesothelioma. Thoracic Society of Australia and New Zealand Annual Scientific Meetings, Perth, Australia. Respirology, **10** (suppl.) A72.

Xia W, Holmes MD, Lake R, Reynolds PN. (2006). Immunocompetent Murine Mesothelioma Model for the Evaluation of Replicative Adenovirus Therapy. Thoracic Society of Australia and New Zealand Annual Scientific Meetings, Canberra, Australia. Respirology, **11** (suppl. 2) A62.

Xia W, Holmes MD, Lake R, Reynolds PN. (**2007).** Combined Viral Therapy and Immunotherapy in the Immuno-competent Murine Model of Mesothelioma. Thoracic Society of Australia and New Zealand Annual Scientific Meetings, Auckland, New Zealand. Respirology, **12** (suppl. 1) A57.

Xia W, Aljahdhami I, Milic N, Holmes MD, Russell S, Reynolds PN. (2007). Attenuated Measles Virus as a Therapy for Thoracic Malignancies. Thoracic Society of Australia and New Zealand Annual Scientific Meetings, Auckland, New Zealand. Respirology, **12** (suppl. 1) A57.

Xia W, Holmes MD, Lake R, Reynolds PN. (2007). Combined FGK45 and Adenovirus in the Immuno-competent Murine Model of Mesothelioma. 12th Congress

of the Asian Pacific Society of Respirology, Gold Coast, Australia. Respirology, **12** (suppl. 4) A227.

Xia W, Aljahdhami I, Milic N, Holmes MD, Russell S, Reynolds PN. (2007). Measles Vaccine (MV-Edm) as a Therapy for Thoracic Malignancies. 12th Congress of the Asian Pacific Society of Respirology, Gold Coast, Australia. Respirology, **12** (suppl. 4) A226.

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

CaCl: 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

$MgCl_2$: 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 viruse

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: thyroidtranscription 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