SENSITISING HUMAN MELANOMA CELLS TO TRAIL-INDUCED APOPTOSIS

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Hsin-Yi Tseng

DEDICATION

I dedicate this thesis to all my family members and relatives, especially my parents Tai Yuan Tseng and Shu Hui Wang, my siblings, Hsin-Hui Tseng and Hsuan Chih Tseng, who have been so supportive throughout my PhD candidature. I also dedicate this thesis to my partner Kwang Hong Tay who keeps me going during depressed and stressful moments.

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Publications Arising from Work in this Thesis

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➔ Poster Presentation (Lorne Cancer Conference 2012)

➔ Poster Presentation (AACR 2012)

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SYNOPSIS

Melanoma is a skin cancer that remains a major public health problem in Australia because of its high incidence and the high morbidity and high mortality associated with the disease. Melanoma has proven largely resistant to many chemotherapeutic and biological agents. The introduction of a member of Tumour Necrosis Factor (TNF) family named TNF-Related Apoptosis Inducing Ligand (TRAIL) seemed to be a promising candidate due to its differential sensitivity to cancer and normal cells. Although many studies have reported approaches for sensitising cancer cells to TRAIL-induced apoptosis via up-regulation of its death receptors, TRAIL-R1 and TRAIL-R2, little was known about the regulation of these receptors. Previously, reports from our laboratory have shown that the sensitivity of melanoma cells to TRAIL-induced apoptosis is in general correlated with the levels of the cell surface expression of TRAIL-R2. Therefore, the general aim of this thesis was to understand the underlying mechanism by which TRAIL-R2 is regulated and to provide more information in identifying new therapeutic approaches for increasing the sensitivity of melanoma cells to apoptosis by TRAIL.

In Chapter Three, we identified the Murine Double Minute 2 (MDM2) antagonist, Nutlin-3, could enhance TRAIL-induced apoptosis as a result of p53-mediated up-regulation of TRAIL-R2. Unexpectedly, Nutlin-3 up-regulated Myeloid-Cell Leukaemia Sequence 1 (Mcl-1) and inhibited apoptosis induced by the microtubule-targeting drug docetaxel. The contrasting effects of Nutlin-3 on TRAIL- and docetaxel-induced apoptosis demonstrated that Nutlin-3 may be a useful agent in improving the therapeutic efficacy of TRAIL in melanoma but could have unexpected adverse effects in combination with other chemotherapeutic drugs such as docetaxel.

The MAGE proteins have been demonstrated to impinge on cell survival, proliferation and apoptosis in cancer. Studies in Chapter Four demonstrated that one of the MAGE proteins, MAGE-D2, plays an important role in protecting melanoma cells from TRAIL-induced apoptosis by suppressing TRAIL-R2 expression. We determined that MAGE-D2 is generally expressed at high levels in melanoma cells compared to melanocytes. Although its inhibition by small interfering RNA (siRNA) did not cause

cell death, it rendered melanoma cells more sensitive to TRAIL-induced apoptosis which was associated with enhanced formation of Death-Inducing Signalling Complexes (DISC) and up-regulation of TRAIL-R2. Regulation of TRAIL-R2 by Melanoma-associated Antigen D2 (MAGE-D2) also appeared to be mediated by p53. We have shown that MAGE-D2 plays a role in repressing p53 expression in melanoma cells, as knockdown of MAGE-D2 resulted in up-regulation of p53 activity which in turn leads to the up-regulation of TRAIL-R2 protein expression. This up-regulation is not observed in p53-null or mutant p53 melanoma cells with MAGE-D2 knocked down, suggesting the dependency of p53 in regulating TRAIL-R2. Altogether, this suggests that targeting MAGE-D2 may be a useful strategy in improving the therapeutic efficacy of TRAIL in melanoma.

Although it is well-known that TRAIL-R2 can be up-regulated by p53, the study in Chapter Five showed that up-regulation of TRAIL-R2 by 2-Deoxy-D-Glucose (2-DG) was independent of p53. Instead, X-box Binding Protein 1 (XBP1) in the endoplasmic reticulum (ER) stress pathway was responsible for this up-regulation. Results in this chapter demonstrated that p53-null and mutant p53 melanoma cells displayed increased levels of TRAIL-R2 expression upon 2-DG treatment and that inhibiting p53 expression in p53 wild-type melanoma cell lines did not impact on the up-regulation of TRAIL-R2 by 2-DG.

In Chapter Six, we further demonstrated that conditional induction of p53 expression did not regulate TRAIL-R2 protein expression in melanoma cells and that other mechanisms may be involved. In particular, enhancing p53 levels in p53-inducible cell lines did not impact on the level of TRAIL-R2 expression or sensitise melanoma cells to TRAIL-induced apoptosis. Interestingly, we determined that Cisplatin (CDDP), a DNA-damaging drug that activates p53, could up-regulate TRAIL-R2 mRNA but did not up-regulate TRAIL-R2 protein levels. This evidence pointed to regulation by translational mechanisms. The results were further supported by studies in TRAIL-selected cells where it was found that exposure to TRAIL for a prolonged period of time resulted in the down-regulation of cell surface TRAIL-R2 but not its mRNA. The precise mechanism of translational control remains to be defined but appears to involve cap-independent mechanisms.

List of Abbreviations

2-DG:	2-Deoxy-D-Glucose
2-ME:	ß-Mercaptoethanol
4E-BP:	eIF4E-Binding Protein
5-FU:	Fluorouracil
AJCC:	American Joint Commission on Cancer
α-MSH:	α-Melanocyte-Stimulating Hormone
APP:	Amyloid Precursor Protein
APS:	Ammonium Persulphate
ATM:	Ataxia Telangiectasia Mutated
BAD:	Bcl-2-Antagonist of Cell Death
BAK:	Bcl-2 Antagonist/Killer
BAX:	Bcl-2-Associated X Protein
Bcl-2:	B Cell Lymphoma Gene 2
B-CLL:	B Chronic Lymphocytic Leukaemia
BH:	Bcl-2 Homology
BID:	BH3-Interacting-Domain Death Agonist
BIK:	Bcl-2-Interacting Killer
BIM:	Bcl-2-Interacting Mediator of Cell Death
BMF:	Bcl-2 Modifying Factor
BOK:	Bcl-2-Related Ovarian Killer
BSA:	Bovine Serum Albumin
CARD:	Caspase-Recruitment Domain
CCCP:	Carboxyl Cyanide 3-Chlorophenylhydrazone
CDDP:	Cisplatin
CDK:	Cyclin Dependent Kinase
CDS:	Coding Sequence
Chk:	Checkpoint Kinase
CHX:	Cycloheximide
CLL:	Chronic Lymphocytic Leukaemia
CTL:	Cytotoxic T Lymphocyte
DAXX:	Death Domain-Associated Protein
DcR:	Decoy Receptor

DD:	Death Domain
DED:	Death Effector Domain
DISC:	Death-Inducing Signalling Complexes
DMEM:	Dulbecco's Modified Eagle's Medium
DNA:	Deoxyribonucleic Acid
DR:	Death Receptor
E1:	Ubiquitin-Activating Enzyme
E2:	Ubiquitin-Conjugating Enzyme
E3:	Ubiquitin Ligase
EDAR:	Ectodysplasin A Receptor
EDTA:	Ethylenediaminetetraacetic Acid
EGTA:	Ethylene Glycol-bis(ß-amino-ethyl ether)N, N'-tetraacetic Acid
EF:	Elongation Factor
EGF:	Epidermal Growth Factor
eIF:	Eukaryotic Initiation Factor
ER:	Endoplasmic Reticulum
ERK:	Extracellular Signal-Regulated Kinase
FADD:	Fas-Associated Death Domain
FCS:	Foetal Calf Serum
FDA:	Food and Drug Administration
FITC:	Fluorescein Isothiocyanate
FLICE:	FADD-like Interleukin 1β-Converting Enzyme
FLIP:	FLICE-Inhibitory Protein
GDP:	Guanosine Diphosphate
GTP:	Guanosine Triphosphate
HDAC:	Histone Deacetylase
Hrk:	Harakiri
IAP:	Inhibitor of Apoptosis
ICE:	Interleukin-1-ß-Converting Enzyme
IF:	Initiation Factor
IFN:	Interferon
IL:	Interleukin
IRES:	Internal Ribosome Entry Site
ITAF:	IRES-Transacting Factors

kb:	Kilobase
kDa:	Kilodalton
JNK:	c-Jun N-terminal Kinase
LARD:	Lymphocyte-Associated Receptor of Death
LB:	Lysogeny Broth
LT-α:	Lymphotoxin-α
m^7G:	7-Methyl Guanosine
MAGE:	Melanoma-Associated Antigen
MAPK:	Mitogen-Activating Protein Kinase
MC1R:	Melanocortin Receptor 1
Mcl-1:	Myeloid-Cell Leukaemia Sequence 1
MDM2:	Mouse Double Minute 2
MEK:	Mitogen-Activated Protein Kinase Kinase
MFI:	Mean Fluorescence Intensity
MHD:	MAGE Homology Domain
miR:	MicroRNA
mRNA:	Messenger RNA
MTS:	3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium
NF-κB:	Nuclear Factor-Kappa B
NGF:	Nerve Growth Factor
NGFR:	Nerve Growth Factor Receptor
NHPA:	National Health Priority Area
NK:	Natural Killer
NSCLC:	Non-Small Cell Lung Cancer
OPG:	Osteoprotegrin
PARP:	Poly(ADP-Ribose) Polymerase
PBS:	Phosphate Buffered Saline
PCD:	Programmed Cell Death
PCR:	Polymerase Chain Reaction
PD-1:	Programmed Death Receptor 1
PDK:	Phosphoinositide-Dependent Kinase
PE:	R-Phycoerythrin
PI:	Propidium Iodide

PI3K:	Phosphatidylinositol 3-Kinase
PIB5PA:	Phosphatidylinositol-4,5-biphosphate 5-Phosphatase A
PKA:	Protein Kinase A
PP2A:	Protein Phosphatase 2A
PTEN:	Phosphatase and Tensin Homologue Deleted on Chromosome 10
PTM:	Post-Translational Modification
PUMA:	p53-Upregulated Modulator of Apoptosis
qPCR:	Quantitative Polymerase Chain Reaction
RF:	Release Factor
RGP:	Radial Growth Phase
RHD:	Rel Homology Domain
RIP:	Receptor Interacting Protein
RNA:	Ribonucleic Acid
ROS:	Reactive Oxygen Species
rRNA:	Ribosome RNA
RTK:	Receptor Tyrosine Kinase
SAPK:	Stress-Activated Protein Kinase
SDS-PAGE:	Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis
shRNA:	Short Hairpin RNA
siRNA:	Small Interfering RNA
SNB:	Sentinel Node Biopsy
SOC:	Super-Optimal broth with Catabolite repression
SODD:	Silencer of Death Domain
TAA:	Tumour-Associated Antigens
TBE:	Tris Borate EDTA
TBS:	Tris-Buffered Saline
TBS-T:	TBS-Tween 20
TEMED:	N, N, N’, N’-Tetramethylethylenediamine
TGA:	Therapeutic Goods Administration
TM:	Tunicamycin
TNF:	Tumour Necrosis Factor
TNFR:	Tumour Necrosis Factor Receptor
TNFRSF:	Tumour Necrosis Factor Receptor Super Family
TRADD:	TNFR-Associated Death Domain Protein

TRAIL:	TNF-Related Apoptosis-Inducing Ligand
TRAMP:	TNFR-Related Apoptosis-Mediating Protein
Trk:	Tropomyosin-Related Kinase
tRNA:	Transfer RNA
TPY:	Threonine-Proline-Tyrosine
UTR:	Untranslated Region
UV:	Ultraviolet
VGP:	Vertical Growth Phase
XBP1:	X-box Binding Protein 1
XEDAR:	X-linked Ectodermal Dysplasia Receptor
XIAP:	X-linked Inhibitor of Apoptosis

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