Characterisation of Caspase-2 Function in the DNA Damage Response and Tumour Suppression

A thesis submitted in total fulfilment of the requirements of the degree of Doctor of Philosophy

by

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Abstract

Caspases are a family of cysteine proteases that have essential functions in the regulation of apoptosis and inflammation. Despite being the most evolutionarily conserved caspase, the physiological functions of caspase-2 remain poorly defined. This is partly because *caspase-2* knockout (*Casp2*$^{-/-}$) mice show no overt phenotype and only limited, tissue-specific apoptotic defects. Previous work from our laboratory has provided the first direct evidence demonstrating a role for caspase-2 in tumour suppression and protection against cellular transformation. However, the molecular mechanisms by which caspase-2 exerts these functions were not clearly defined.

In order to characterise the tumour suppressor function of caspase-2, the processes and pathways disrupted in *caspase-2*-deficient cells were investigated. Analysis of serially-passaged mouse embryonic fibroblasts (MEFs) demonstrated that *caspase-2*-deficiency promoted escape from replicative senescence which coincided with impaired induction of cyclin-dependent kinase inhibitors. Consistent with the increased proliferation rate of primary *Casp2*$^{-/-}$ MEFs, spontaneously-immortalized *Casp2*$^{-/-}$ MEFs that had escaped replicative senescence also displayed an enhanced proliferative capacity compared to their wild type counterparts. These findings suggest that caspase-2 regulates cell proliferation which may contribute to its ability to protect against cellular transformation. Furthermore, serially-passaged *Casp2*$^{-/-}$ primary MEFs and Eμ-*Myc*/*Casp2*$^{-/-}$ lymphomas showed enhanced aneuploidy, demonstrating that loss of *caspase-2* promotes genomic instability (GIN). Treatment of *Casp2*$^{-/-}$ MEFs with ionizing radiation (IR) resulted in persistent DNA damage and defective cell cycle

checkpoint regulation, suggesting that *caspase-2*-deficient cells have an impaired ability to efficiently respond to and repair DNA damage. Further analysis revealed that c*aspase-2*-deficient MEFs and Eμ-*Myc* lymphomas displayed defective activation of p53 and its downstream targets following IR treatment. Therefore, an attenuated p53 response may contribute to defective DNA damage response (DDR) signalling and GIN in *caspase-2*-deficient cells.

In order to further investigate the extent and specificity of caspase-2 function in tumour suppression using an independent tumour model, $Atm^{+/-}$ and $Casp2^{-/-}$ mice were inter-crossed to generate $Atm^{-/-}Casp2^{-/-}$ mice. Initial characterization revealed that *caspase-2*-deficiency enhanced growth retardation and caused perinatal lethality in $Atm^{-/-}$ mice. A comparison of tumour susceptibility demonstrated that $Atm^{-/-}Casp2^{-/-}$ mice developed lymphomas with a dramatically increased onset and penetrance compared to $Atm^{-/-}$ mice, providing additional evidence supporting a tumour suppressor function for caspase-2. Furthermore, $Atm^{-/-}Casp2^{-/-}$ lymphomas showed an increased proliferation rate and enhanced oxidative damage compared to $Atm^{-/-}$ lymphomas. Moreover, lymphomas and pre-malignant lymphocytes derived from $Atm^{-/-}Casp2^{-/-}$ mice displayed enhanced aneuploidy, linking the function of caspase-2 in the maintenance of genomic stability to its tumour suppressive activity.

Overall, this thesis provides novel insights into the physiological functions of caspase-2, highlighting its roles in the regulation of cell proliferation, the DDR, maintenance of genomic stability and tumour suppression.

Declaration

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, 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. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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Joseph Puccini

May 2014

Publications

The following publications have resulted from work performed during the period of this candidature.

Publications included in thesis:

1. Dorstyn L, **Puccini J**, Wilson CH, Shalini S, Nicola M, Moore S, Kumar S. *Caspase-2* deficiency promotes aberrant DNA-damage response and genetic instability. *Cell Death and Differentiation* (2012) 19, 1288–1298.

2. **Puccini J**, Dorstyn L, Kumar S. Caspase-2 as a tumour suppressor (Review). *Cell Death & Differentiation* (2013) 20, 1133-1139**.**

3. **Puccini J**, Shalini S, Voss AK, Gatei M, Wilson CH, Hiwase DK, Lavin MF, Dorstyn L, Kumar S. Loss of *caspase-2* augments lymphomagenesis and enhances genomic instability in *Atm*-deficient mice. *Proceedings of the National Academy of Sciences of the USA* (2013). 110, 19920-5.

Publications non included in thesis:

1. Shalini S, Dorstyn L, Wilson C, **Puccini J**, Ho L, Kumar S. Impaired antioxidant defence and accumulation of oxidative stress in *caspase-2*-deficient mice. *Cell Death & Differentiation* (2012) 19, 1370-1380.

2. **Puccini J**, Dorstyn L, Kumar S. Genetic background and tumour susceptibility in mouse models. *Cell Death & Differentiation* (2013) 20, 964.

Awards

Best Poster Prize at ANZSCDB Meeting

(Adelaide, 2011)

National Travel Award

(South Australian Pathology Medical Staff Specialist Fund, 2011)

Conference Travel Scholarship to attend the 20th Euroconference on Apoptosis

(European Cell Death Organization, 2012)

Faculty of Health Sciences Postgraduate Travelling Fellowship

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Best Student Oral Presentation at ANZSCDB Meeting

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The 1st Australian Workshop on Cell Death (Oral Presentation)

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ANZSCDB Meeting (Poster)

Adelaide, South Australia (2011)

Faculty of Health Sciences Postgraduate Research Conference (Poster)

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Abbreviations

53BP1	p53 binding protein 1
8-OHdG	8-hydroxy-2'-deoxyguanosine
ALL	acute lymphoblastic leukaemia
AML	acute myelogenous leukaemia
AP	alkaline phosphatase
APAF-1	apoptotic protease activating factor 1
APS	ammonium persulphate
AT	ataxia telangiectasia
ATM	ataxia telangiectasia mutated
ATR	ataxia telangiectasia and Rad3-related
BAK	Bcl-2 homologous antagonist/killer
BAX	Bcl-2-associated protein X
BCL	B cell lymphoma
BH3	Bcl-2 homology 3
BID	BH3-interactingdomain death agonist
BN	binucleated
bp	base pair
BRCA1	breast cancer 1
BSA	bovine serum albumin
BUB1	budding uninhibited by benzimidazoles 1
C	Celsius
CARD	caspase activation and recruitment domain
Cat	catalase
CBMN	cytokinesis-block micronucleus assay
CDC25	cell division cycle 25
Cdk	cyclin-dependent kinase
cDNA	complementary DNA
CHK	checkpoint kinase
cm	centimetre(s)
Cy	cyanine
Cys	cysteine
Cyt c	cytochrome c

DAB	diaminobenzidine
DAPI	4’,6’-diamidino-2-phenylindole
DD	death domain
DDR	DNA damage response
DED	death effector domain
DEPC	diethylpyrocarbonate
DGR	dorsal root ganglion
DIABLO	direct IAP-binding protein with low PI
DMEM	Dulbecco's Modified Eagles Medium
DNA	deoxyribonucleic acid
DSB	double-strand break
dsDNA	double-stranded DNA
DTT	dithiothreitol
dUDP	deoxyuridine diphosphate
E	embryonic day
ECF	enhanced chemifluorescence
ECL	enhanced chemiluminescence
ECM	epithelial-mesenchymal transition
EDTA	ethylenediaminetetraacetic acid
EGFR	epidermal growth factor receptor
ELISA	enzyme-linked immunosorbent assay
EMT	extracellular matrix
FADD	FAS-associated protein with death domain
FASL	FAS-ligand
FBS	foetal bovine serum
FC	flow cytometry
FISH	fluorescence in situ hybridisation
g	grams(s)
G6PD	glucose-6-phosphate-dehydrogenase
GADD45	growth arrest and DNA damage 45
gDNA	genomic DNA
GIN	genomic instability
Gy	gray
h	hour(s)
HDM2	human double minute 2

HEPES	N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid
HRP	horseradish peroxidase
IAP	inhibitor of apoptosis
IB	immunoblot
ICC	immunocytochemistry
IgG	immunoglobulin G
IHC	immunohistochemistry
IL	interleukin
iMEF	immortalised mouse embryonic fibroblast
IR	ionizing radiation
kDa	kilodalton(s)
L	litre(s)
M	molar
MAD2	mitotic arrest deficient 2
mA	milliamperes(s)
MCL1	myeloid cell leukaemia 1
MDC1	mediator of DNA damage checkpoint 1
MDM2	mouse double minute 2
MEF	mouse embryonic fibroblast
mg	milligram(s)
min	minute(s)
mL	millilitre(s)
MLH1	MutL homolog 1
mM	millimolar
mmol	millimole(s)
MMP	matrix metalloproteinase
MMTV	mouse mammary tumour virus
MN	micronucleus
MOMP	mitochondrial outer membrane permeabilisation
MRN	MRE11-RAD50-NBS1
mRNA	messenger RNA
NADPH	nicotinamide adenine dinucleotide phosphate
NGF	nerve growth factor
NLS	nuclear localisation sequence
nM	nanomolar

nm	nanometer(s)
nmole	nanomole(s)
OD	optical density
P	passage
PAGE	polyacrylamide gel electrophoresis
PBS	phosphate buffered saline
PBS-T	phosphate buffered saline-Tween-20
PCD	programmed cell death
PCNA	proliferating cell nuclear antigen
PCR	polymerase chain reaction
PE	phycoerythrin
PFA	paraformaldehyde
pg	picogram(s)
pH3	phospho-histone H3
PI	propidium iodide
PI3K	phosphoinositide 3-kinase
PPP	pentose phosphate pathway
Prdx3	peroxiredoxin 3
PTEN	phosphatase and tensin homolog
PUMA	p53-upregulated modulator of apoptosis
PVDF	polyvinylidene fluoride
qPCR	quantitative PCR
RB	retinoblastoma
RNA	ribonucleic acid
RNase	ribonuclease
RO	reverse osmosis
ROS	reactive oxygen species
RPMI	Roswell Park Memorial Institute medium
RT	room temperature
SA-β-gal	senescence-associated β-galactosidase
SDS	sodium dodecyl sulphate
sec	second(s)
SEM	standard error of the mean
Ser	serine
SESN	sestrin

SFE	signal-free end
siRNA	small interfering RNA
SMAC	second mitochondria-derived activator of caspases
SOD2	superoxide dismutase 2
SSB	single-strand break
SV40	simian virus 40
TAE	Tris-acetic acid-EDTA
TBS	Tris-buffered saline
TBS-T	Tris-buffered saline/Tween-20
TdT	terminal deoxynucleotidyl transferase
TEMED	tetramethylethylenediamine
TNFSF	tumour necrosis factor superfamily
TP53	tumour protein 53
TRAIL	tumour necrosis factor-related apoptosis-inducing ligand
TRP53	transformation-related factor protein 53
TUNEL	terminal deoxynucleotidyl transferase dUTP nick end labeling
UV	ultraviolet
v/v	volume per volume
VEGFA	vascular endothelial growth factor A
wk	week(s)
w/v	weight per volume
WT	wild type
x g	times the force of gravity
X-gal	5-bromo-4-chloro-3-indolyl-β-D-galactosidase
yr	year(s)
°	degrees
µg	micrograms
µL	microlitre
µM	micromolar

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Thesis Structure

This thesis incorporates both published and unpublished work which is structured as follows:

Chapter 1

Sections 1.1 – 1.4 are unpublished and provide a review of the literature relevant to the themes of this thesis. Section 1.5 provides a general introduction to caspase-2 which is comprised of part of a published review article:

Puccini J, Dorstyn L, Kumar S. Caspase-2 as a tumour suppressor (Review). *Cell Death & Differentiation* (2013) 20, 1133-1139**.**

Chapter 2

This chapter details the experimental procedures conducted throughout this thesis and expands on the materials and methods from the published articles presented in chapters 3 and 4.

Chapter 3

This chapter includes unpublished data as well as work published in:

Dorstyn L, **Puccini J**, Wilson CH, Shalini S, Nicola M, Moore S, Kumar S. *Caspase-2* deficiency promotes aberrant DNA-damage response and genetic instability. *Cell Death and Differentiation* (2012) 19, 1288–1298).

Chapter 4

This chapter is comprised of work published in:

Puccini J, Shalini S, Voss AK, Gatei M, Wilson CH, Hiwase DK, Lavin MF, Dorstyn L, Kumar S. Loss of *caspase-2* augments lymphomagenesis and enhances genomic instability in *Atm*-deficient mice. *Proceedings of the National Academy of Sciences of the USA* (2013). 110, 19920-5.

Chapter 5

This chapter is comprised of unpublished work that provides an overall discussion linking the findings from chapters 3 and 4 and also incorporates parts of a published review article:

Puccini J, Dorstyn L, Kumar S. Caspase-2 as a tumour suppressor (Review). *Cell Death & Differentiation* (2013) 20, 1133-1139**.**