Contents

	Page
บทคัด ย่อ	
(3)	
Abstract	(5)
Acknowledgement	(7)
Contents	(9)
List of Tables	(10)
List of Figures	(11)
Abbreviations and Symbols	(14)
1. Introduction	1
2. Literature reviews	5
3. Objectives	77
4. Materials and Methods	78
5. Results	94
6. Discussion	136
7. Conclusion	148
8. References	152
Appendix A	193
Appendix B	205
Appendix C	217
Vitae	223
	(9)

List of Tables

Tab	Table	
1.	Comparison of apoptosis and necrosis	15
2.	Bcl-2 family proteins	34
3.	Alternative names and active-sites of human caspases	46
4.	Substrates and function of caspases	47
5.	The sequences of the optimal primer used for reverse transcription	81
	PCR analysis (RT-PCR)	
6.	The target genes and sequences of siRNA	82
7.	Preparation of SDS-polyacrylamide gel	202
8.	The restriction endonuclease enzymes, recognition sequences and	203
	optimal temperature	
9.	Properties of U2OS cell	220
10.	Abbreviations and molecular weight for Amino acids	223

List of Figures

Figu	gure	
1.	Diagrammatic representation of cell fate, showing commonly	16
	observed morphological features	
2.	Apoptosis process	17
3.	Engulfment of apoptotic cells	18
4.	Simplified scheme of major apoptosis pathway	22
5.	Extrinsic death receptor pathway	23
6.	A model for p53-mediated apoptosis	24
7.	Schematic diagram of representative Bcl-2 family proteins	35
8.	Models for the intrinsic cell death pathways in C. elegans, Drosophila,	36
	and mammals	
9.	Three-dimensional structure of Bcl-2 family proteins	37
10.	Pathways of apoptosis	38
11.	Molecular organization of Mcl-1	40
12.	Regulation of Apoptosis by Mcl-1	42
13.	Apoptotic caspases in mammals, fruitflies and nematodes	56
14.	A conserved apoptotic pathway in nematodes, mammals and fruit flies	58
15.	Caspase structure and activation	60
16.	The activation of caspase-9	61
17.	Regulation and functional importance of TCTP	68

List of Figures (Continued)

Figu	Figure P	
18.	Sequence conservation, 3D structure and functional mapping of the	69
	TCTP protein	
19.	Schematic representation of four-step gene silencing pathway	75
20.	Non-specific responses to dsRNA in mammalian systems	76
21.	Expression of Pm-TCTP/fortilin specific mRNA in response to viral	95
	infection	
22.	Overexpressing of Pm-TCTP/fortilin and Human-fortilin prevent cell from	98
	undergoing cell death	
23.	Protection of U2OS cells overexpressing Pm-TCTP/fortilin from	99
	5-FU-induced cytotoxicity	
24.	Protection from 5-FU induced cytotoxicity in U2OS cells overexpressing	101
	fortilin	
25.	Time course of MCL1 and Human-fortilin silencing by siRNAs	104
26.	Dose response of MCL1 and Human-fortilin silencing by siRNAs	105
27.	Double silencing of MCL1 and Human-fortilin silencing by siRNAs	106
28.	MCL1 and Human-fortilin prevent 5-FU-induced cell death	109
29.	MCL1 and Human-fortilin prevent 5-FU-induced cell death	111
30.	Immunocytochemical analysis	115
31.	Subcellular fractionation and Western blot analysis	117

List of Figures (Continued)

Figu	ire I	age
32.	Protection from 5-FU-induced cytotoxicity in U2OS cells overexpressing	121
	Human-fortilin	
33.	Dose-dependent protection of 5-FU-challenged U2OS cells by	122
	Human-fortilin in the absence of MCL1	
34.	Protection from 5-FU-induced cytotoxicity in U2OS cells overexpressing	126
	MCL1	
35.	Dose-dependent protection of 5-FU-challenged U2OS cells by MCL1 in the	127
	absence of Human-fortilin	
36.	Human-fortilin protects cells from undergoing cell death in the absence of	132
	its protein partner, MCL1, as shown in studies using polyclonal cell populat	ion
37.	MCL1 protects cells from undergoing cell death in the absence of its	134
	protein partner, Human-fortilin, as shown in studies using polyclonal	
	cell population	
38.	Comparison of the amino acid sequences from Pm-TCTP/Fortilin and	140
	Human-Fortilin	
39.	Conclusion of expression of Pm-fortilin/TCTP (Part I)	152
40	Conclusion of expression of human-fortilin/TCTP (Part II)	153

Abbreviations and Symbols

5-FU = 5-Fluorouracil

ATP = Adenosine triphosphate

Bcl-2 = B-cell lymphoma leukemia-2

BSA = Bovine serum albumin

bp = Base pair (s)

°C = Degree celcius

cDNA = Complementary DNA

DEPC = Diethyl pyrocarbonate

DNA = Deoxyribonucleic acid

dNTP = dATP, dCTP, dGTP and dTTP

DTT = Dithiothreitol

E. coli = Escherichia coli

EDTA = Ethylenediamine tetraacetic acid

EtBr = Ethidium bromide

et al. = And others

g = gram

h = hour(s)

HA = Haemaglutinin

His = Histidine tag

i.e. = id. est, for example

Abbreviations and Symbols (Continued)

kb = Kilobase(s)

kDa = Kilodalton(s)

LB = Luria-Bertani (medium)

M = Molarity

MCL1 = Myeloid cell leukemia 1

mA = milliampare

mM = millimolar

mg = milligram

 \min = \min (s)

ml = milliliter

 $\mu g = microgram$

 μl = microliter

 $\mu M = micromolar$

ng = nanogram

nm = nanometer

nmole = nanomole

nt = nucleotide (s)

OD = Optical density

ORF = Open reading frame

PAGE = Polyacrylamide gel electrophoresis

PBS = Phosphate buffer saline

Abbreviations and Symbols (Continued)

PCR = Polymerase chain reaction

pmole = picomole

Pm = Penaeus monodon

PMSF = Phenyl methyl sulfonyl fluoride

ppt = part per thousand

RNA = Ribonucleic acid

RNase = Ribonuclease

rpm = revolutions per minute

RT-PCR = Reverse transcription polymerase chain reaction

SDS = Sodium dodecyl sulfate

sec = second

siRNA = small interfering ribonucleic acid

STET = Sodium choride-Tris-EDTA-Triton

TAE = Tris-acetate-EDTA

TBE = Tris-borate-EDTA

TCTP = Translationally controlled tumor protein

TEMED = N,N,N',N'-tetramethyl-ethylenediamine

Tm = melting temperature

Tris = Tris (hydroxymethyl) aminoethane

Tris-HCl = Tris (hydroxymethyl) aminoethane hydrochloric acid

TNF = Tumor necrosis factor

Abbreviations and Symbols (Continued)

U = unit(s)

UV = ultraviolet

V = volt

v/v = volume per volume

w/v = weight per volume

WSSV = White spot syndrome virus