TABLE OF CONTENTS

	Page
ACKNOWLEDGEMENTS	iii
ABSTRACT (in English)	v
ABSTRACT (in Thai)	vii
TABLE OF CONTENTS	ix
LIST OF TABLES	xiv
LIST OF FIGUES	xv
ABBREVIATIONS	xviii
CHAPTER I INTRODUCTION	1
CHAPTER II LITERATURE REVIEWS	6
1. Survivin gene	7
2. Expression characteristics of survivin	7
3. Survivin as a tumor marker	8
4. Livin gene	9
5. Expression characteristics of livin	10
6. Livin as a tumor marker	10

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

Page

7. Auto-antibody: a potential ideal tumor marker 11

CHAPTER III OBJECTIVES 14

CHAPTER IV MATERIALS AND METHODS 15

1. Detection of expression level of livin and survivin in tumor tissues with Western blot Analysis 15

1.1 Population of the study 15

1.2 Preparation of cell lysate 15

1.3 Protein assay 16

1.4 Separation of protein by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) 17

1.5 Electrotransfer of separated proteins from the gel into the membrane 18

1.6 Immunoblotting process 18

2. Detection of autoantibodies against livin and survivin in lung cancer patients 19

2.1 Production of recombinant livin and survivin tumor antigens 19

2.1.1 Construction of expression vector 19

2.1.1.1 Preparation of livin and survivin encoding DNA for insertion into pET-15b vector 23

2.1.1.2 Digestion of pET-15b vector and NdeI-livin-BamHI PCR product, NdeI-survivin-BamHI PCR product with restriction enzymes 25

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright © by Chiang Mai University
All rights reserved

Page

2.1.1.3 Ligation 26

2.1.1.4 Transformation of competent cells 26

2.1.1.5 Positive clones screening 27

2.1.1.6 Growth of the correct transformant in liquid media and storage 27

2.1.2 Expression of livin and survivin recombinant proteins in bacterial host cells 28

2.1.2.1 Determination of the level of expressed protein 28

2.2 Optimization of ELISA 29

2.2.1 Finding optimal antigen concentration 30

2.2.2 Finding optimal coating buffer 31

2.2.3 Finding optimal blocking agent 31

2.2.4 Finding optimal serum dilution 31

2.3 Evaluation of the established ELISA 32

2.3.1 Reproducibility testing of the established ELISA 32

2.3.2 Verification of autoantibody specificity by pre-absorption experiment 33

2.3.2.1 Purification of $(His)_6$-survivin protein by His-Bind Resin chromatography 33

2.3.2.2 Pre-absorption of samples with recombinant survivin 35

2.4 Detection of livin and survivin specific autoantibodies in lung cancer patient serum using the generated $(His)_6$-livin and survivin proteins 35

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

Page

2.5 Statistical analysis 35

CHAPTER V RESULTS 36

1. Detection of expression level of livin and survivin in tumor tissues and normal tissues with Western blot analysis 36

2. Detection of autoantibodies against livin and survivin in lung cancer patients 39

2.1 Production of recombinant livin and survivin tumor antigens 39

2.1.1 Construction of expression vectors (pET-15b-livin and pET-15b-survivin) 39

2.1.2 Expression of livin and survivin recombinant proteins in bacterial host cells 46

2.2 Optimization of ELISA 49

2.2.1 Optimization of antigen concentration 49

2.2.2 Optimization of coating buffer for coating $(His)_6$-livin and $(His)_6$-survivin antigens onto a microtiter plate 52

2.2.3 Optimization of blocking agent 55

2.2.4 Optimization of serum dilution 58

2.3 Evaluation of the established ELISA 60

2.3.1 Reproducibility study of the established ELISA 60

2.3.2 Verification of autoantibody specificity by pre-absorption experiment 63

2.3.2.1 Purification of $(His)_6$-survivin protein 63

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

Page

2.3.2.2 Pre-absorption of anti-survivin antibody with purified $(His)_6$-survivin protein 65

2.4 Detection of livin and survivin autoantibodies in lung cancer patient serum using the generated $(His)_6$-livin and $(His)_6$-survivin proteins 67

2.5 Correlations between anti-livin, anti-survivin antibodies positivity and clinicopathologic features 69

CHAPTER VI DISCUSSION 70

CHAPTER VII CONCLUSION 76

REFERENCES 78

APPENDICES 90

CURRICULUM VITAE 99

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

LIST OF TABLES

Table		Page
1	Clinicopathologic features of lung tumor samples investigated in this study (n=52)	37
2	Correlations of anti-livin and anti-survivin antibodies levels upon diagnosis with clinicopathological parameters in all patients (n=42)	69

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

LIST OF FIGURES

Figure		Page
1	Representative western blot analysis of cell lysates (T = tumor tissue and N = normal tissue) from lung cancer patients.	38
2	Agarose gel analysis showing the obtained livin PCR products using NdeIlivin and livinBamHI as primers.	42
3	Agarose gel analysis showing the obtained survivin PCR products using NdeIsurvivin and survivinBamHI as primers.	42
4	An example of agarose gel analysis of PCR products to verify the appropriate ligation between pET-15b vector and livin DNA in ligation mixture.	43
5	An example of agarose gel analysis of PCR products to verify the appropriate ligation between pET-15b vector and suvivin DNA in ligation mixture.	43
6	Agarose gel analysis of antibiotic resistant colonies screening for pET-15b-livin vector uptaking clone by PCR.	44
7	Agarose gel analysis of antibiotic resistant colonies screening for pET-15b-survivin vector uptaking clone by PCR.	44
8	Agarose gel analysis of restriction enzyme digested products to confirm the obtained positive colony of livin.	45

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright © by Chiang Mai University
All rights reserved

Figure **Page**

9 Agarose gel analysis of restriction enzyme digested products to confirmthe obtained positive colonies of survivin. 45

10 Western blot analysis of hexahistidine-livin fusion protein express in *E.coli* DE3 and DE3pLysS cells using anti-histidine mAb and anti-livin mAb. 47

11 Western blot analysis of hexahistidine-survivin fusion protein express in *E.coli* DE3 and DE3pLysS cells using anti-histidine mAb and anti-survivin mAb. 48

12 Optimization of the concentration of His_6-livin antigen to be coated onto an ELISA plate. 50

13 Optimization of the concentration of His_6-survivin antigen to be coated onto an ELISA plate. 51

14 Finding the most suitable buffer for coating $(His)_6$-livin antigen onto microtiter plate. 53

15 Finding the most suitable buffer for coating $(His)_6$-survivin antigen onto microtiter plate. 54

16 Finding the most suitable blocking agent for detection of anti-livin autoantibody. 56

17 Finding the most suitable blocking agent for detection of anti-survivin autoantibody. 57

18 Finding the most suitable serum dilution for detection of anti-livin and anti-survivin antibodies. 59

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

Figure **Page**

19 The quality control chart of livin's OD of the control serum by established ELISA. 61

20 The quality control chart of survivin's OD of the control serum by established ELISA. 62

21 Coomassie blue stain of polyacrylamide gel showing preliminary purification process of His_6-survivin fusion protein using affinity chromatography. 64

22 Pre-absorption of anti-survivin mAb and sera from lung cancer patients with purified recombinant survivin protein. 66

23 Detection of antibodies response to livin and survivin by established ELISA. 68

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

ABBREVIATIONS AND SYMBOLS

bp	base pair
cDNA	complementary DNA
^{o}C	degree Celsius
CV	coefficient of variation
DNA	deoxyribonucleic acid
ECL	enhanced chemiluminescence
g	gram
HCl	hydrochloride
His	histidine
$(His)_6$-livin	hexahistidine tagged livin
$(His)_6$-survivin	hexahistidine tagged survivin
IgG	immunoglobulin G
kb	kilobase
kDa	kilo-Dalton
l	litre
ml	mililitre
mM	millimolar
mRNA	messenger RNA
M	molar

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

MW	molecular weight

nm	nanometer
NaCl	sodium chloride
NaOH	sodium hydroxide
OD	optical density
PBS	phosphate-buffered saline
rpm	revolution per minute
RNA	ribonucleic acid
SD	standard deviation
SDS	sodium dodesyl sulfate
SDS-PAGE	sodium dodesyl sulfate-polyacrylamide gel electrophoresis
TEMED	N,N,N',N'-tetramethylethyllene diamine
Tris	tris(hydroxymethyl) aminomethane
Tris-HCl	tris(hydroxymethyl) aminomethane hydrochloride
µl	microlitre
µg	microgram
α	alpha
β	beta
(v/v)	volume: volume ratio

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

(w/v) weight: volume ratio

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved