APPENDIX A

INSTRUMENTS

Instruments used in this study were listed as following:

- Analytical balance (Adventurer-ohaus AR 2130, USA)
- Blotting module (Amersham Biosciences, USA)
- CAMAG UV Lampe (CAMAG, German)
- Centrifuge (Kubota 5200, Japan)
- Electrophoresis module (Amersham Biosciences, USA)
- Eppendorf centrifuge 5417R (Eppendorf, Germany)
- Magnetic stirrer (Thermolyne Co., USA.)
- pH meter. Model 3560 (Beckman, USA)
- Power supply (Amersham Biosciences, USA)
- Roller mixer SRT1 (Stuart scientific, UK)
- Scan densitometer (BioRad, USA)
- Ultrasonic Processor UP50H (Hielscher, German)
- Vortex mixture, (Scientific Industries, New York, USA.)
- Water bath (Memmert)

APPENDIX B

CHEMICALS AND REAGENTS

All chemicals (essential) used were analytical grade:

- Acrylamide (Sigma Chemical Co., USA. No. A 8887)
- Amersham Full-Range Rainbow Molecular Weight Markers (GE Healthcare, UK. RPN800E)
- Ammonium persulfate (Bio-Rad Laboratories, No. 161-0700)
- Anti-GAPDH antibody [6C5] (Abcam, Japan. No. ab8245)
- BCA protein assay kit (PIERCE, USA. No.23227).
- Boric acid (Sigma Chemical Co., USA. No. B 7901)
- Brilliant blue R-250 (Biorad Chemical Company, USA.)
- Bromophenol blue (Sigma Chemical Co., USA. No. B5225)
- ECL Plus detection kit (Amersham Biosciences, UK.)
- Ethanol (Merck Darmstadt, Germany. No. K 32820483 402)
- Glycine (Sigma Chemical Co., USA. No. G8898)
- Hydrochoric acid (Merck Darmstadt, Germany)
- Lyophilized rabbit polyclonal antibody p53 protein (CM5) (Novocastra, UK. No. NCL-p53-CM5p)
- 2-mercaptoethanol (Sigma Chemical Co., USA. No. M4125)
- Methanol (Merck Darmstadt, Germany)

- Mouse Monoclonal Anti-GRP94 (2H3) (Santacruze, inc., USA. No. sc-53929)
- N,N'-Methylene-bis-acrylamide (sigma Chemical Co., USA. No. M 7256)
- N,N,N',N'-tetramethylethylenediamine: TEMED (Sigma Chemical Co., USA. No. T8133)
- p53 protein (DO-7) liquid mouse monoclonal antibody (Novocastra, UK. No. NCL-L-p53-DO7)
- Peroxidaase-Conjugated Rabbit Anti-Rat Immunoglobulins (DakoCytomation, Denmark. No. P0450)
- Polyclonal Goat Anti-Mouse Immunoglobulins/HRP (DakoCytomation, Denmark. No. P0447)
- Polyclonal Goat Anti-Rabbit Immunoglobulins/HRP (DakoCytomation, Denmark. No. P0448)
- Protease inhibitor cocktail tablets (Complete, Mini, EDTA-free, Roche Applied Science, Germany. Cat. No. 11836170001)
- Protein G Agarose (Pierce, USA. No. 20398)
- Rabbit polyclonal antibody Glucosidase IIβ (H-195) (Santacruze, inc., USA. No. sc-10774)
- Rabbit TrueBlot: Anti-Rabbit IgG HRP (eBioscience, inc., USA. Cat. No. 18-8816)
- Rat Monoclonal Anti-GRP78 (76-E6) (Santacruze, inc., USA. No. sc-13539)
- SuperSignal west pico chemiluminescent substrate (Thermo scientific, USA. No.34080)

- Tris [hydroxymethyl]-aminomethane hydrochloride (Merck, Darmstadt, Germany. No. 648311
- Tunicamycin from Streptomyces sp. (Sigma-aldrich, USA. No. T7765)
- Tween20 (Fluke, Germany. No. 93773)
- Other chemicals were analytical grade

APPENDIX C

REAGENT PREPARATIONS

I. Reagents for preparation of protein from tissues and cell lines

1. SDS lysis buffer

0.5 M Tris pH 6.8	2.5	ml
SDS	0.8	g
Glycerol	2	ml

Adjust volume to 10 ml with deionized distilled water and store at -20 ^{o}C.

2. Nuclear isolation buffer (NIB)

Sucrose	4.27	g
NP-40	0.125	g
EDTA	0.093	g
NaCl	0.29	g
Tris	0.061	g

Adjust to pH 7.5 with HCl.

Make up to 50 ml with deionized distilled water and stored at-20 ^{o}C.

II. Reagents for electrophoresis on polyacrylamide gel

1. Separating gel buffer stock (1.5 M Tris-HCl pH 8.8)

Tris	36.3	g
ddH_2O	150	ml

Adjust to pH 8.8 with HCl.

Make up to 200 ml with deionized distilled water and store at 4 ^oC.

2. Stacking gel buffer stock (0.5 M Tris-HCl pH 6.8)

Tris	6	g
ddH_2O	60	ml

Adjust to pH 6.8 with HCl.

Make up to 100 ml with deionized distilled water and stored at 4 ^oC.

3. 10% APS

Ammonium persulfate	20	mg
ddH_2O	200	µl

Mix together. (Prepared before using)

4. 2X Sample buffer

SDS	0.8	g
0.5 M tris-HCL, pH 6.8	2.5	ml
Glycerol	2	ml
0.5% bromophenol blue	2	mg

Make up to 10 ml with deionized water and stored at -20 ^oC

5. 30% Acrylamide stock solution

Acrylamide	150	g
Bis-acrylamide	4.0	g

Total volume 500 ml with deionized water mix and store at 4 ^{o}C

III. Reagents for SDS-PAGE and western blot analysis

1. 7% Separating gel solution

ddH_2O	7.5	ml
30% Acrylamide stock solution	3.5	ml
Separating gel buffer stock	3.75	ml
10% SDS	150	µl
10% ammonium persulfate	100	µl
TEMED	20	µl

Swirl gently to mix and pour the solution into the gel cassette.

2. 4% Stacking gel solution

ddH_2O	3	ml
30% Acrylamide stock solution	665	µl
Stacking gel buffer stock	1.25	ml
10% SDS	50	µl
10% ammonium persulfate	50	µl
TEMED	10	µl

Swirl gently to mix and pour the solution into the gel cassette.

3. 10X Running buffer

Tris-base	30.3	g
Glycien	144	g
SDS	10	g

Make up to 1L with deionized water and stored at room temperature.

4. 1X Running buffer

10X Running buffer	100	ml

Make up to 1L with deionized distilled water.

5. 10X Transfer buffer

Tris-base	30.3	g
Glycien	144	g

Make up to 1L with deionized distilled water and stored at room temperature.

6. 1X Transfer buffer

10X Transfer buffer	100	ml
Methanol	200	ml

Make up to 1L with deionized distilled water.

7. Washing buffer (0.05% TBS-Tween)

Tris	6	g
NaCl	9	g
dH_2O	600	ml

Adjust to pH 7.5 with HCl.

Tween 20	0.5	ml

Make up to 1L with deionized distilled water and stored at room temperature.

8. Stripping buffer

Tris	3.78	g
SDS	10	g
dH_2O	400	ml

Adjust to pH 6.7 with HCl.

Make up to 500 ml with deionized distilled water and stored at room temperature.

Add 2-mercaptoethanol (2-ME) 7 µl/1 ml before using.

CURRICULUM VITAE

Name Miss Benjamart Suradej

Date of Birth April 21, 1986

Institution attended

- Kowittamrong Chiangmai School, March 1997

  : Elementary Certificate

- Doisaketwittayakom School, March 2000

  : Primary high school Certificate

- Yupparajwittayalai School, March 2003

  : High school Certificate

- Chiang Mai university, March 2007

  : B. Sc (Medical Technology)

- Faculty of Associated Medical Sciences, Chiang Mai university, May 2009 – present

  : M. Sc Student in Medical Technology

Poster presentation

- Suradej B., Leartprasertsuke N., Cressey R. The Association of matrix metalloproteinase-9 (MMP-9) gene polymorphism and cigarette smoking on the risk of lung cancer in Northern Thai population. Graduate school, Chiang Mai University, 2007

Oral presentation/Proceeding

- Suradej B., Leartprasertsuke N., Kasinrerk W., Cressey R. Overexpression of endoplasmic reticulum glucosidase II in non-small lung cancer: a novel biomarker for diagnosis. The 1st Asean Plus Three Graduate Research Congress (AGRC 2012), the Empress Hotel, Chiang Mai, Thailand 1-2 March 2012

Publication

- Suradej B., Pata S., Lertprasertsuk N., Kasinrerk W and Cressey R. Similarity of structure and behavior in response to endoplasmic reticulum (ER) stress and UV irradiation of glucosidase II to p53 tumor suppressor: a potential novel cancer biomarker (2011). **Cancer Investigation**, under review

Honors and Awards

2012	Excellent Oral Presentation at The 1st ASEAN Plus Three Graduate Research Congress (AGRC 2012) at The Empress Hotel, Chiang Mai, Thailand
2007	Good Poster Presentation at Faculty of Associated Medical Sciences, Chiang Mai University