

## TABLE OF CONTENTS

	Page
<b>ACKNOWLEDGMENT</b>	iii
<b>ABSTRACT</b>	iv
<b>LIST OF TABLES</b>	xi
<b>LIST OF ILLUSTRATIONS</b>	xii
<b>ABBREVIATIONS</b>	xiv
<b>I. INTRODUCTION</b>	1
<b>II. LITERATURE REVIEWS</b>	5
1. Anatomy and histology of the stomach and large bowel	5
2. Stomach cancer	7
3. Risks factors for stomach cancer	8
4. Colorectal cancer	10
5. Risk factors for colorectal cancer	13
6. Staging of stomach and colorectal cancer	15
7. Cyclooxygenase isoenzymes	16
8. Physiological and pathological functions of COX-1 and COX-2	18
9. Regulations of COX-1 and COX-2 expression	22
9.1 Regulation of COX-1 expression	22
9.2 Regulation of COX-2 expression	23
10. Expression of COX-2 in stomach and colorectal cancer	25

<b>III. METERIALS AND METHODS</b>	27
1. Tissue collection and storage	27
2. Preparation of tissue homogenate	27
3. Estimation of protein concentration of tissue homogenate by the bicinchoninic acid (BCA) assay kit	28
3.1 Preparation of BSA (bovine serum albumin) standard	28
3.2 Preparation of BCA working reagent	28
3.3 Protein measurement by using microplate	29
4. Assessment of COX protein expression by Western blotting	29
4.1 Separation of protein by SDS-PAGE	30
4.1.1 Preparation of running gel (10%)	31
4.1.2 Preparation of stacking gel (4%)	31
4.1.3 Preparation of the sample and electrophoresis	32
4.2 Transfer proteins	32
4.3 Immunodetection	33
4.4 Visualization of detected protein band	33
4.5 Stripping and reprobing membrane	34
5. Classification of pathological features	34
6. Investigation of the relationship between COX protein expression to the malignant potential and stage grouping by statistical analysis	34
7. Statistical analysis	35
<b>IV. RESULTS</b>	36
1. Quality controls of the study	36
1.1 Controls for protein quantification	36
1.2 Control for protein loading	36
1.3 Control for the specificity of antibodies	38
2. Study population of the patients	39
3. Pathological characteristics of colorectal and stomach cancers	40

4. Expression of COX proteins in colorectal and stomach cancers	41
5. COX expression in relation to classification of pathological features	45
<b>V. DISCUSSION</b>	57
<b>VI. CONCLUSION</b>	62
<b>REFERENCES</b>	63
<b>APPENDICES</b>	82
Appendix A: List of the chemicals and materials.	83
Appendix B: List of instruments.	85
Appendix C: Reagents and buffers preparation.	86
<b>CURRICULUM VITAE</b>	90

## LIST OF TABLES

Table	Page
1 The staging guidelines of the stomach cancer.	19
2 The comparison of staging systems for colorectal cancer.	20
3 Clinical characteristics of the cancer patients.	40
4 Pathological characteristics of the cancer patients.	41
5 Comparison of COX expression in tumor with adjacent normal tissues of the cancer patients.	45
6 Summary of relationship between COX-2 expression and pathological features in colorectal and stomach cancers	55
7 Summary of relationship between COX-1 expression and pathological features in colorectal and stomach cancers	56

## LIST OF ILLUSTRATIONS

Figure	Page
1 The position diagram of the stomach and large bowel.	6
2 Layers of the gastrointestinal tract wall.	6
3 Molecular model for the progression of colorectal cancer through the adenoma-carcinoma sequence.	12
4 Regulation of COX-2 in cancer.	24
5 An example of standard curve obtained from diluting BSA to various concentrations and detected by BCA protein assay.	37
6 The quality control chart of protein concentration of the homogenate determined by BCA assay.	37
7 The Representative specificity and cross-reactivity testing of COX-2 and COX-1 antibodies with the protein standards using Western blotting.	39
8 Protein expression for COX-2, COX-1, and $\beta$ -actin assessed by Western blotting in colorectal cancer tissues and the corresponding adjacent normal tissues.	43
9 Protein expression for COX-2, COX-1, and $\beta$ -actin as assessed by Western blotting in stomach cancer tissues and the corresponding adjacent normal tissues.	44
10 Distribution of COX-2 overexpression and COX-1 alteration in relation to sizes of the primary colorectal tumors.	46
11 Distribution of COX-2 overexpression and COX-1 alteration in relation to histological grading of the colorectal tumors.	47
12 Distribution of COX-2 overexpression and COX-1 alteration in relation to lymph node metastasis of the colorectal tumors.	48
13 Distribution of COX-2 overexpressio and COX-1 alteration in relation to	

stage of the colorectal tumors.	49
14 Distribution of COX-2 overexpression and COX-1 alteration in relation to malignant potential of the colorectal tumors.	50
15 Distribution of COX-2 overexpression and COX-1 alteration in relation to sizes of the primary stomach tumors.	51
16 Distribution of COX-2 overexpression and COX-1 alteration in relation to histological grading of the stomach tumors.	52
17 Distribution of COX-2 overexpression and COX-1 alteration in relation to lymph node metastasis of the stomach tumors.	53
18 Distribution of COX-2 overexpression and COX-1 alteration in relation to stage of the stomach tumors.	54

## ABBREVIATIONS

%	percentage
°C	degree celsius
$\alpha$	alpha
$\mu\text{g}$	microgram
BCA	bicinchoninic acid
$\beta$	beta
BSA	bovine serum albumin
cm	centimetre
CV	coefficient of variation
ddH <sub>2</sub> O	deionized water
dH <sub>2</sub> O	distilled water
DNA	deoxyribonucleic acid
ECL	enhanced chemiluminescence
gm	gram
HCl	hydrochloric acid
hr	hour
HRP	hoseradish peroxidase
IgG	immunoglobulin G
Kb	kilobase pair
Kda	kilodalton
M	molar
mg	milligram
min	minute
ml	millilitre
mRNA	messenger ribonucleic acid

ng	nanogram
nm	nanometre
OCV	optimal condition of variances
RCV-K	routine condition of variances-known values
RT	room temperature
SD	standard deviation
SDS-PAGE	sodium dodecyl sulfate-polyacrylamide gel electrophoresis
TMPD	<i>N, N, N', N'</i> -tetramethylethylenediamine



ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่  
Copyright© by Chiang Mai University  
All rights reserved