

ห้องสมุดงานวิจัย สำนักงานคณะกรรมการวิจัยแห่งชาติ



E42115



**DETERMINATION OF CHOLANGIOCARCINOMA ASSOCIATED
SERUM PROTEINS BY PROTEOMIC APPROACH**

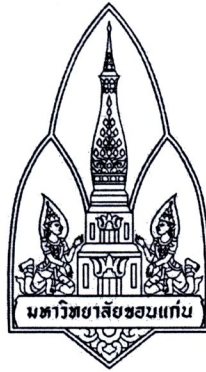
MR. ARTHIT TOLEK

**A THESIS FOR THE DEGREE OF MASTER OF SCIENCE
KHON KAEN UNIVERSITY**

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MR. ARTHIT TOLEK

**A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE
REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE
IN MEDICAL BIOCHEMISTRY
GRADUATE SCHOOL KHON KAEN UNIVERSITY**

2011



**THESIS APPROVAL
KHON KAEN UNIVERSITY
FOR
MASTER OF SCIENCE
IN MEDICAL BIOCHEMISTRY**

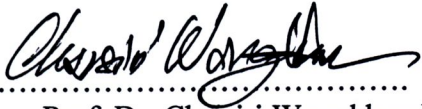
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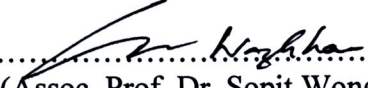
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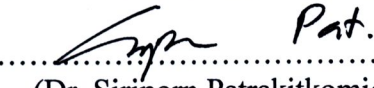
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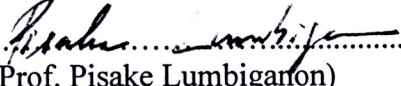
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อาทิตย์ โตเล็ก. 2554. การตรวจวัดโปรตีนที่มีความสัมพันธ์กับการเป็นโรคมะเร็งท่อน้ำดีโดยเทคนิคด้าน
โปรตีนโอมิก. วิทยานิพนธ์ปริญญาวิทยาศาสตรมหาบัณฑิต สาขาชีวเคมีทางการแพทย์ บัณฑิตวิทยาลัย
มหาวิทยาลัยขอนแก่น.

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บทคัดย่อ

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แบบแผนโปรตีนของซีรัมจากผู้ป่วยโรคมะเร็งท่อน้ำดีก่อนและหลังผ่าตัดเปรียบเทียบกับแบบแผนที่ได้
จากคนปกติ ซึ่งได้ผ่านการกำจัดอัลบูมินและอิมมูโนโกลบูลินก่อนแยกโปรตีนแบบสองมิติ (two-dimensional
gel electrophoresis, 2-DE) โดยแยกโปรตีนด้วย isoelectric focusing ในมิติแรกและ polyacrylamide gel
electrophoresis ในมิติที่สอง และย้อมด้วยสี colloidal coomassie และ silver stain วิเคราะห์ภาพถ่ายแบบแผน
โปรตีนจากซีรัมของผู้ป่วยโรคมะเร็งท่อน้ำดีก่อนการผ่าตัดและหลังผ่าตัดจำนวน 6 ราย (invasive papillary type 3
รายและ well-differentiated type 3 ราย) และซีรัมจากคนปกติ 10 ราย โดยใช้โปรแกรมทางคอมพิวเตอร์
ImageMaster 2D platinum 7.0

การเปรียบเทียบแบบแผนโปรตีนที่ได้จากการทำสามซ้ำของซีรัมผู้ป่วยโรคมะเร็งท่อน้ำดีแต่ละรายและ
สองซ้ำที่ได้จากกลุ่มคนปกติ พบโปรตีน 129 จุดที่มีปริมาณแตกต่างกันอย่างมีนัยสำคัญ ซึ่งได้นำไปวิเคราะห์หาค่า
ด้วย mass spectrometer และสืบค้นชนิดของโปรตีนจากฐานข้อมูล NCBI โดยใช้โปรแกรม MASCOT ผลการ
วิเคราะห์พบว่า โปรตีน 77 จุดมีปริมาณสูงขึ้นและ 52 จุดมีปริมาณลดลงเมื่อเปรียบเทียบแบบแผนซีรัมก่อนผ่าตัด
ของผู้ป่วยมะเร็งท่อน้ำดีกับแบบแผนซีรัมหลังผ่าตัดและที่ได้จากคนปกติ โดยพบ Apolipoprotein M และ retinol
binding protein (RBP) มีปริมาณเพิ่มขึ้น apolipoprotein A-IV มีปริมาณลดลง นอกจากนี้พบจำนวนจุดโปรตีนที่
แตกต่างกันมากขึ้นเมื่อเปรียบเทียบแบบแผนซีรัมก่อนผ่าตัดของผู้ป่วยมะเร็งท่อน้ำดีกับของคนปกติ พบการ
เพิ่มขึ้นของ Alpha-1-B-glycoprotein (A1BG), serpin protease, และบางจุดของ alpha-1-antitrypsin, antithrombin
และ apolipoprotein A-I ในซีรัมผู้ป่วยมะเร็งท่อน้ำดี ในขณะที่ afamin (AFM), apolipoprotein A-IV, plasma
glutathione peroxidase, alpha-2-macroglobulin, serum transferrin, interleukin 1-beta, regulatory subunit B56,
และ alpha-2-HS-glycoprotein มีปริมาณลดลง นอกจากนี้ยังพบความแตกต่างของระดับ haptoglobin (HP) และ
จำนวน Zinc-alpha-2-glycoprotein variants ที่แตกต่างกันเมื่อเปรียบเทียบแบบแผนของผู้ป่วยมะเร็งท่อน้ำดีกับ
คนปกติ

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เพื่อทำการยืนยันผลที่ได้จากการวิเคราะห์ 2-DE และ mass spectrometry ควรทำการยืนยันผลของโปรตีนที่สนใจโดยใช้แอนติบอดีที่มีความจำเพาะต่อโปรตีนนั้น (western blot analysis) และใช้จำนวนตัวอย่างที่มากขึ้น โดย AFM และ A1BG เป็นโปรตีนที่ควรเลือกมาศึกษาต่อเพื่อทดสอบศักยภาพในการวินิจฉัยและประยุกต์ใช้ทางคลินิกสำหรับผู้ป่วยมะเร็งท่อน้ำดีต่อไป

Arthit Tolek. 2010. **Determination of Cholangiocarcinoma Associated Serum Proteins by Proteomic Approach.** Master of Science Thesis in Medical Biochemistry, Graduate School, Khon Kaen University.

Thesis Advisors: Assoc. Prof. Dr. Chaisiri Wongkham,
Assoc. Prof. Dr. Sopit Wongkham,
Dr. Siriporn Patrakitkomjorn

ABSTRACT

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Protein patterns of pathological proven cholangiocarcinoma (CCA) subjects were created and compared between pre- and post-operative sera and with those of healthy subjects. Albumin and immunoglobulin depleted sera were separated using two-dimensional gel electrophoresis (2-DE): isoelectric focusing and sodium dodecyl sulfate polyacrylamide gel electrophoresis. High sensitive visualization of colloidal coomassie and silver staining were used. Serum protein patterns of 2-DE from 6 mass forming CCA subjects (3 each of invasive papillary type and well-differentiated type), and 10 healthy subjects were analyzed using 2-DE software (ImageMaster 2D platinum 7.0, GE Healthcare) via recorded pictures and compared to each other.

Triplicate gels of sera from individual CCA subject and duplicate gel of sera from individual healthy subject were obtained and compared. Total 129 protein spots that had statistically different expression ($P < 0.01$) were further identified by mass spectrometry. Then, mass spectrum of each spot was searched against the NCBI database by MASCOT program. Overall, 77 spots were elevated and 52 spots were decreased in pre-operative CCA sera when compared between post-operative sera and healthy subjects. Apolipoprotein M and retinol binding protein (RBP) were elevated and apolipoprotein A-IV was reduced in pre-operative group. In addition, there were more spots different between the pre-operative sera (CCA) and the healthy sera. Alpha-1-B-glycoprotein (A1BG), serpin protease, some spots of alpha-1-antitrypsin, antithrombin and apolipoprotein A-I were elevated in CCA group, but afamin (AFM), apolipoprotein A-IV, plasma glutathione peroxidase, alpha-2-macroglobulin, serum

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more spots different between the pre-operative sera (CCA) and the healthy sera. Alpha-1-B-glycoprotein (A1BG), serpin protease, some spots of alpha-1-antitrypsin, antithrombin and apolipoprotein A-I were elevated in CCA group, but afamin (AFM), apolipoprotein A-IV, plasma glutathione peroxidase, alpha-2-macroglobulin, serum transferrin, interleukin 1-beta, regulatory subunit B56, alpha-2-HS-glycoprotein were decreased. Besides, different expression levels of haptoglobin (HP) and Zinc-alpha-2-glycoprotein variants were found in CCA sera comparing to those of healthy subjects.

To confirm the result of 2-DE and mass spectrometry analysis, western blot analysis of the candidate proteins must be validated in a larger sample size. AFM and A1BG are the suggested candidate proteins to validate and explore for diagnostic values and the possibility of clinical application.

**Goodness portion to the present thesis is dedicated for my advisors,
cholangiocarcinoma patients for their valuable sample,
the entire teaching staff and my family.**

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I would like to express my deepest and sincere gratitude to my advisor, Associate Professor Dr.Chaisiri Wongkham for his kindly providing me a good opportunity to study in this field, supervision, the laboratory facilities, encouragement, valuable suggestions, kindness, forbearance, entire criticism throughout the course of study. I deeply appreciate the time he spared during the preparation of this thesis.

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Arthit Tolek

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LIST OF ABBREVIATIONS

2-DE	two dimensional gel electrophoresis
µg	microgram
µl	microliter
/	per
%	percent
ALB	albumin
ANOVA	analysis of variance
Bis	N, N'-methylene-bis-acrylamine
BSA	Bovine serum albumin
CA19-9	carbohydrate antigen 19-9
CA125	carbohydrate antigen 125
CCA	cholangiocarcinoma
CHAPS	(3-[3-cholamidopropyl]dimethylammonio]-1-propanesulfonate
CEA	carcinoembryonic antigen
cm	centimeter
CT	computed tomography
CV	coefficients of variation
°C	degree celcius
DTT	dithiothreitol
g	gram
IEF	isoelectric focusing
IgG	immunoglobulin G
kDa	kilo-Daltons
h	hour
L	liter
LC	liquid chromatography
LMW	low molecular weight
M	molarity

LIST OF ABBREVIATIONS (Cont.)

MASCOT	Modeling, Analysis, and Simulation of Computer and Telecommunication Systems
mA	milliampere
mM	millimolar
mL	milliliter
MRCP	magnetic resonance cholangiopancreatography
MRI	magnetic resonance imaging
MS	mass spectrometry
MW	molecular weight
MWCO	molecular weight cut off
N	normality
no.	number
<i>P</i>	probability value
pH	potential of hydrogen
pI	isoelectric point
PSC	primary sclerosing cholangitis
PTMs	posttranslational modifications
SDS	sodium dodecyl sulphate
SDS-PAGE	sodium dodecyl sulphate-polyacrylamide gel electrophoresis
TEMED	N,N,N',N'-tetramethyl ethylenediamine
Tris	Tris-(hydroxymethyl) aminomethane
W	watt
w/v	weight per volume
x g (RFC)	relative centrifugal force