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APPENDICES

Reagents for laboratory experiments

I. Reagents for cell culture

1. Complete RPMI-1640 medium

RPMI-1640 (Gibco BRL)		1 pack
$NaHCO_3$		1.176 g
Adjust pH to 7.4 by HCl		
Distilled water	to	1 liter
Filter through 0.22-μm filter		
Filtered RPMI-1640		100 ml
Fetal bovine serum		10 ml
Penicillin (200,000 U/ml)		50 μl
Streptomicin (200,000 μg/ml)		50 μl
Store at 4°C.		

2. Antibiotics

a. Penicillin 200,000 U/ml

Penicillin		1,000,000 U
Sterile distilled water	to	5 ml
Store at -20°C.		

b. Streptomycin (200,000 μg/ml)

Streptomycin		1 g
Sterile distilled water	to	5 ml
Store at -20°C.		

3. 0.25% Trypsin-EDTA

2.5% Trypsin solution (Gibco BRL)	2 ml
Versine EDTA (Gibco BRL)	50 ml
Filter through 0.22-μm filter.	
Store at 4°C.	

4. **Phosphate buffered saline (PBS) pH 7.4 for cell culture**

NaCl	8.0 g
KCl	0.2 g
KH_2PO_4	0.2 g
Na_2HPO_4	1.15 g

Note: Adjust pH to 7.4 by NaOH, add distilled water to final volume of 1 liter. Sterilized by autoclaving at 121°C, 15 pounds per square inch for 15 min and store at room temperature.

II. **Reagents for Proteomics**

1. **Sample preparation solution with urea and thiourea**

Urea	10.5 g
Thiourea	3.8 g
CHAPS[a]	1 g
IPG Buffer[b]	500 µl
DTT	154 mg
Double-distilled water to	25 ml

Note: [a]Other neutral or zwitterionic detergents may be used at concentrations up to 2% (w/v). [b]Use IPG Buffer in the pH range corresponding to the pH range of the IEF separation to be performed.

2. **Thiourea rehydration stock solution**

Urea	10.5 g
Thiourea	3.8 g
CHAPS	0.5 g
IPG Buffer	125 µl
1% Bromophenol blue stock solution	50 µl
Double-distilled water to	25 ml

Note: DTT is added just prior to use: add 7 mg DTT per 2.5-ml aliquot of rehydration stock solution. Store in 2.5-ml aliquots at -20°C.

3. **SDS equilibration buffer solution**

Urea		72.1 g
Tris-HCL, pH 8.8		10 ml
Glycerol (87% w/w)		69 ml
SDS		4 g
Bromophenol blue stock solution		400 µl
Double-distilled water	to	200 ml

Note: This is a stock solution. Just prior to use, add DTT or iodoacetamide as described in the protocol.

4. **10X Laemmli SDS electrophoresis buffer**

Trisbase		303 g
Glycine		1441 g
SDS		100 g
Distilled water	to	10 liters

Note: The pH of this solution should not be adjusted.

5. **30% T, 2.6% C monomer stock solution**

Acrylamide		300 g
N,N'-methylenebisacreylamide		8 g
Distilled water	to	1 liter

Note: Filter solution through a 0.45-µm filter. Store at 4°C in the dark.

6. **4X resolving gel buffer solution**

Tris base		181.7 g
Distilled water	to	750 ml
HCL		adjust to pH 8.8
Distilled water	to	1 liter

Note: Filter solution through a 0.45-µm filter. Store at 4°C.

7. **Bromophenol blue stock solution**

Bromophenol blue		100 mg
Tris-base		60 mg
Distilled water	to	10 ml

8. **10% SDS**

SDS, electrophoresis grade		5 g
Distilled water	to	50 ml

Note: Filter solution through a 0.45-μm filter. Store at RT.

III. **Reagents for Western blot**

1. **5X Stock Transfer buffer**

Tris base		15.1425 g
Glycine		72.0672 g
Distilled water	to	1 liter

pH is adjusted at 8.4

2. **1X Working Transfer buffer**

5X Stock Transfer buffer		600 ml
Methanol		600 ml
Distilled water	to	3 liters

3. **1X phosphate-buffered saline with 0.05% Tween-20 (PBST)**

10 X PBS		100 ml
Tween-20		500 μl
Distilled water	to	1 liter

4. **5% skimmed milk in PBST**

Instant nonfat dry milk	5 g
1X PBST	100 ml

5. **2% skimmed milk in PBST**

Instant nonfat dry milk	2 g
1X PBST	100 ml

IV. **Reagents for immunohistochemistry**

1. **0.01M Citrate buffer pH 6.0**

Citric acid		4.2 g

Dissolve and adjust to pH 6.0 with NaOH

Distilled water	to	2 liter

2. **0.05M Tris buffer pH 7.6 for DAB**

Tris base		6.1 g
Distilled water	to	800 ml
Dissolve and adjust to pH 7.6 with NaOH		
Distilled water	to	1 liter

3. **PBS for diluting with 0.05% NaN$_3$**

NaN$_3$		0.05 g
Distilled water	to	100 ml

4. **Stock DAB (25 mg/ml)**

DAB		1 g
Distilled water	to	40 ml
Aliquot 1 ml to each microtubes.		

5. **10X phosphate buffered saline (PBS)**

NaCl		40 g
KCl		1 g
Na$_2$HPO$_4$		7.2 g
KH$_2$PO$_4$		1.2 g
Distilled water	to	500 ml
pH is adjusted at 7.4		

6. **1X PBS**

10X PBS		100 ml
Distilled water	to	1 liter

Table A.1 *Opisthorchis viverrini*-induced cholangiocarcinoma cell lines and normal biliary cell line.

Cell lines	Description
OCA17	Well differentiated adenocarcinoma CCA cell line
M156	Moderately differentiated adenocarcinoma CCA cell line
K100	Poorly differentiated adenocarcinoma CCA cell line
M139	Squamous cell carcinoma CCA cell line
M213	Adenosquamous cell carcinoma CCA cell line
H69	Immortalized normal cholangiocyte cell line

Note: CCA cell lines were established at Division of Experimental Pathology, Department of Pathology, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand. Immortalized cholangiocyte cell line H69 was kindly provided by Dr. Gregory J. Gores, Mayo clinic, Rochester, MN, USA.

Table A.2 Immunohistochemical grading of ANXA2, PRX1, EBP50 and ENO1 proteins in human CCAs.

No.	Code	Immunohistochemical Grading			
		ANXA2[a]	PRX1[b]	EBP50[b]	ENO1[b]
1	40-059	1	0	1	1
2	40-108	1	0	1	1
3	40-116	1	1	1	1
4	41-122	1	0	0	1
5	41-123	1	0	1	1
6	M005	0	1	0	1
7	M009	1	1	1	0
8	M030	1	1	0	0
9	M040	0	1	1	0
10	M037	1	0	1	1
11	M043	1	1	0	0
12	M055	1	0	0	1
13	M068	1	1	1	0
14	M071	1	0	0	1
15	M079	1	0	1	1
16	M081	1	0	1	1
17	M082	0	1	1	0
18	M090	1	0	1	1
19	M101	0	1	1	0
20	M102	1	0	1	1
21	M111	1	1	0	1
22	M119	0	1	1	0
23	M132	1	0	1	1
24	M136	0	1	1	0
25	M138	0	1	0	0
26	M140	0	0	1	1
27	M148	0	0	1	1
28	M152	0	1	1	0
29	M155	0	1	1	0
30	M156	0	1	1	0
31	M157	0	0	1	1
32	M171	1	0	1	1
33	M187	1	0	1	1
34	M209	0	1	1	0
35	M213	1	0	0	1
36	M214	1	0	1	1
37	M229	1	1	1	1
38	M240	0	1	1	0
39	M249	1	0	1	1
40	M250	0	1	0	1
41	M259	1	0	0	1
42	M265	1	0	1	1
43	M270	1	1	1	1
44	M274	1	1	0	0
45	M276	0	1	1	0
46	M279	0	0	1	1
47	M283	1	0	0	1
48	M286	1	0	0	1
49	M287	1	0	1	1

Table A.2 Immunohistochemical grading of ANXA2, PRX1, EBP50 and ENO1 proteins in human CCAs (Cont.)

No.	Code	Immunohistochemical Grading			
		ANXA2[a]	PRX1[b]	EBP50[b]	ENO1[b]
50	M294	0	1	1	0
51	M298	1	0	1	1
52	M303	1	0	1	1
53	M311	0	1	1	0
54	N013	1	0	0	1
55	N017	0	0	1	1
56	N019	0	1	1	0
57	N021	1	1	0	0
58	N022	1	0	1	1
59	N023	1	1	0	0
60	N024	0	1	0	0
61	N029	1	0	0	1
62	N044	1	0	1	1
63	N046	1	1	0	0
64	N048	1	0	1	1
65	N052	1	0	0	1
66	N069	1	0	1	1
67	N059	1	1	1	1
68	N082	1	0	1	1
69	N088	1	0	1	1
70	N091	1	0	0	1
71	N104	1	0	1	1
72	P066	0	1	1	0
73	N105	1	0	0	1
74	N108	1	0	0	1
75	N110	1	1	1	0
76	N112	1	0	0	1
77	N114	1	0	1	1
78	N127	0	1	0	0
79	N128	1	1	0	1
80	N136	1	0	1	1
81	N140	1	1	1	1
82	N146	1	1	1	1
83	N154	0	1	1	0
84	N157	1	0	1	1
85	N158	1	0	1	1
86	P006	0	1	0	0
87	P011	1	0	1	1
88	P020	1	0	1	1
89	P029	1	0	0	1
90	P031	1	0	1	1
91	P040	1	0	1	1
92	P048	1	0	1	1
93	P055	1	0	1	1
94	P056	0	1	1	0
95	P059	1	0	1	1
96	P060	1	0	0	1
97	P068	1	0	0	1
98	P071	0	1	0	0
99	P077	1	0	1	1
100	P086	1	0	1	1

Table A.2 Immunohistochemical grading of ANXA2, PRX1, EBP50 and ENO1 proteins in human CCAs (Cont.)

No.	Code	Immunohistochemical Grading			
		ANXA2[a]	PRX1[b]	EBP50[b]	ENO1[b]
101	P090	1	0	0	1
102	P091	0	1	1	0
103	P092	1	0	1	1
104	P093	1	0	1	1
105	P094	1	0	1	1
106	P095	0	1	1	0
107	P112	1	1	0	1
108	P126	0	1	0	0
109	P120	1	0	1	1
110	P127	1	0	1	1
111	N135	1	0	0	1
112	P145	1	0	0	1
113	P147	1	1	1	1
114	P149	1	0	0	1
115	P152	1	0	1	1
116	Q006	1	0	1	1
117	Q007	1	0	1	1
118	Q008	1	1	0	1
119	Q013	1	1	0	1
120	Q015	1	0	1	1
121	Q021	1	0	1	1
122	Q023	1	0	1	1
123	Q025	1	0	0	1
124	Q036	1	0	0	1
125	Q037	0	1	0	0
126	Q041	1	0	0	1
127	Q043	1	0	1	1
128	Q045	0	1	0	0
129	Q048	1	0	0	1
130	Q053	1	0	1	1
131	Q058	1	0	0	1
132	Q062	1	0	1	1
133	Q063	0	1	0	0
134	Q066	1	1	1	1
135	Q071	1	0	1	1
136	Q074	1	1	1	1
137	Q089	0	1	1	0
138	Q093	1	0	1	1
139	Q095	1	0	1	1
140	Q099	1	1	1	1
141	Q101	1	0	0	1
142	Q102	1	0	1	1
143	Q103	1	0	1	1
144	Q106	1	0	1	1
145	Q111	1	0	1	1
146	Q114	1	0	1	1
147	Q123	1	0	0	1
148	Q126	1	0	1	1
149	R001	0	1	0	0

Table A.2 Immunohistochemical grading of ANXA2, PRX1, EBP50 and ENO1 proteins in human CCAs (Cont.)

No.	Code	Immunohistochemical Grading			
		ANXA2[a]	PRX1[b]	EBP50[b]	ENO1[b]
150	R003	0	1	0	0
151	R004	1	0	0	1
152	R009	1	0	1	1
153	R012	1	0	0	1
154	R023	1	0	0	1
155	R025	0	1	0	0
156	R026	1	0	0	1
157	R031	0	1	0	0
158	R038	1	0	1	1
159	R043	1	0	1	1
160	R049	1	0	1	1
161	R50	1	0	0	1
162	R052	0	1	0	0
163	R053	1	0	0	1
164	R054	1	0	0	1
165	R061	1	0	0	1
166	R062	1	0	1	1
167	R066	1	0	0	1
168	R074	0	1	0	0
169	R077	1	0	0	1
170	R078	0	1	1	0
171	R081	1	0	0	1
172	R085	0	1	1	0
173	R086	1	0	0	1
174	R087	1	0	1	1
175	R092	1	0	0	1
176	R093	1	0	1	1
177	R096	0	1	0	0
178	R100	1	0	1	1
179	R101	1	0	0	1
180	R103	1	1	0	1
181	R104	1	0	1	1
182	R105	1	0	0	1
183	R106	0	1	1	0
184	R118	1	0	1	1
185	R121	1	0	1	1
186	R123	0	1	1	0
187	R128	1	0	0	1
188	R133	1	1	1	1
189	R134	1	0	0	1
190	R137	1	0	0	1
191	R138	1	0	0	1
192	R141	0	1	1	0
193	R144	1	0	1	1
194	R145	0	1	0	0
195	R149	1	0	1	1
196	R155	1	0	1	1
197	R156	1	0	1	1
198	T001	0	1	0	0
199	T003	0	1	0	0
200	T005	1	0	1	1

Table A.2 Immunohistochemical grading of ANXA2, PRX1, EBP50 and ENO1
proteins in human CCAs (Cont.)

No.	Code	Immunohistochemical Grading			
		ANXA2[a]	PRX1[b]	EBP50[b]	ENO1[b]
201	T006	0	1	1	0
202	T010	0	1	1	0
203	T015	1	0	0	1
204	T016	1	0	0	1
205	T017	0	1	1	0
206	T026	1	0	1	1
207	T036	1	0	0	1
208	T041	0	1	0	0
209	T042	1	0	0	1
210	T043	1	0	1	1
211	T046	1	0	1	1
212	T049	0	1	0	0
213	T051	1	0	1	1
214	T058	0	1	1	0
215	T068	1	0	1	1
216	T071	1	0	0	1
217	T077	0	1	0	0
218	T079	1	0	0	1
219	T090	1	0	1	1
220	T092	1	0	1	1
221	T102	1	0	0	1
222	T117	1	0	0	1
223	T124	1	0	0	1
224	T151	1	0	1	1
225	T160	0	1	1	0
226	T162	1	0	0	1
227	T165	1	0	1	1
228	T168	1	0	0	1
229	T174	1	0	1	1
230	U004	1	0	1	1
231	U005	1	0	0	1
232	U010	0	1	0	0
233	U018	1	1	1	1
234	U019	1	0	0	1
235	U027	1	0	1	1
236	U028	0	1	1	0
237	U030	1	0	0	1
238	U034	1	0	0	1
239	U041	1	0	1	1
240	U044	1	1	0	1
241	U051	0	1	0	0
242	U052	1	0	0	1
243	U054	0	1	1	0
244	U059	1	0	1	1
245	U090	1	0	1	1
246	U107	0	1	0	0
247	U109	1	0	1	1
248	U110	1	0	0	1
249	U120	1	0	1	1
250	U122	1	0	1	1
251	U126	1	1	1	1

Table A.2 Immunohistochemical grading of ANXA2, PRX1, EBP50 and ENO1 proteins in human CCAs (Cont.)

No.	Code	Immunohistochemical Grading			
		ANXA2[a]	PRX1[b]	EBP50[b]	ENO1[b]
252	U137	1	0	1	1
253	W002	0	1	1	0
254	W004	1	1	1	1
255	W005	1	0	1	1
256	W008	0	1	1	0
257	W011	0	1	0	0
258	W012	1	0	0	1
259	W016	1	1	0	1
260	W017	1	0	0	1
261	W022	1	0	1	1
262	W025	1	0	1	1
263	W027	1	1	1	1
264	W035	1	0	1	1
265	W039	1	1	1	1
266	W040	1	0	0	1
267	W048	1	0	1	1
268	W049	1	0	1	1
269	W050	1	1	0	1
270	W056	1	0	1	1
271	W060	1	0	1	1
272	W061	1	0	1	1
273	W063	0	1	0	0
274	W068	0	1	0	0
275	W069	1	0	1	1
276	W077	1	0	0	1
277	W080	0	1	1	0
278	W084	1	0	0	1
279	W085	1	0	0	1
280	W087	1	0	0	1
281	W090	1	0	1	1
282	W092	1	0	1	1
283	W093	1	0	1	1
284	W100	1	0	1	1
285	W128	0	1	1	0
286	W130	1	0	1	1
287	W132	1	0	0	1
288	W147	0	1	1	0
289	W149	1	0	1	1
290	W151	1	0	1	1
291	W153	1	0	1	1
292	W164	1	0	0	1
293	W169	1	0	1	1
294	W171	1	1	1	1
295	W176	1	0	1	1
296	W181	1	0	0	1
297	W187	1	0	1	1
298	W195	1	0	1	1
299	W196	1	0	1	1
300	OCA17	0	1	0	0

Note: '0'= low expression group (positive staining cells ≤ 10%); '1'= high expression group (positive staining cells >10%). [a]Membrane protein, [b]Cytosolic proteins.

RESEARCH PUBLICATIONS

1. **Yonglitthipagon P**, Pairojkul C, Chamgramol Y, Mulvenna J, Sripa B. Up-regulation of annexin A2 in cholangiocarcinoma caused by *Opisthorchis viverrini* and its implication as a prognostic marker. **Int J Parasitol** 2010; 40: 1203-1212.

2. **Yonglitthipagon P**, Pairojkul C, Chamgramol Y, Mulvenna J, Loukas A, Bethony J, Sripa B. Prognostic significance of peroxiredoxin 1 and ezrin-radixin-moesin-binding phosphoprotein 50 in cholangiocarcinoma. *(submitted to Human Pathol)*.

3. **Yonglitthipagon P**, Pairojkul C, Chamgramol Y, Mulvenna J, Loukas A, Sripa B. Proteomics-based identification of enolase 1 as a potential prognostic marker in cholangiocarcinoma. *(submitted to Clin Biochem)*.

CURRICULUM VITAE

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2002-2005   Bachelor of Science (Physiotherapy), Faculty of Associated Medical Science. Khon Kaen University, Khon Kaen, Thailand.

2006-2011   Doctor of Philosophy in Pathobiology, Department of Pathology, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand.

Training and visiting scholar:

2009   Application of laser capture microdissection and proteomics in cholangiocarcinoma, Queensland Institute of Medical Research (Brisbane), Queensland, Australia.

2010   Quantitative proteomics analysis of cholangiocarcinoma cell lines using OFFGEL, iTRAQ labeling and mass spectrometry (LC-MS/MS), Queensland Tropical Health Alliance, James Cook University (Cairns), Australia.

Honors:

2005   First class honor (Physiotherapy), Khon Kaen University, Khon Kaen, Thailand.

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Outstanding poster presentation awards:

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