

Supplementary Materials: Translating Biomarkers of Cholangiocarcinoma for Theranosis: A Systematic Review

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Table 1. Data extraction table. A summary of the 166 candidate biomarkers that met the inclusion/exclusion criteria. List of acronyms and abbreviations used: BD = bile duct, CCA = cholangiocarcinoma, i/e/pCCA = intrahepatic/extrahepatic/perihilar CCA, CCC = cholangiocellular carcinoma, NR = Not Reported, occ = occasionally, % + = percentage positive (+ve), PSC = primary sclerosing cholangitis, TMA = tissue microarray, unspec = unspecified.

AA Biomarker (Gene)	Year	First Author PMID	Eastern Study?	Site of Tumor	No. of Patients	% up-Regulation	% +	Location of Marker	Slide/TMA	Control Group	Expression in Normal Tissues	Risk of Bias
A1AT <i>SERPINA1</i>	2013	Jamnongkan, W. 23188705	Thailand	CCA	42	57.1%	-	Cytoplasm	S	Inflammatory tissues	Weak +ve normal BD	NR
A1AT <i>SERPINA1</i>	2017	Laohaviroj, M. (2017) 28618946	Thailand	CCA unspec	354	NR	50%	Cytoplasm	TMA	<i>n</i> = 2 normal tissues	NR	2 blinded independent
A1AT <i>SERPINA1</i>	2014	Khenjanta, C. 25556446	Thailand	iCCA	30	23% (7/30)	NR	Tumour cells	S	<i>n</i> = 30, adjacent normal tissue	Weak staining	2 blinded independent observers
AFP <i>AFP</i>	2017	Liu, S. 28915621	China	iCCA	60	-	53.3%	NR	S	<i>n</i> = 60 paired normal tissue	NR	NR
Akirin2 <i>AKIRIN2</i>	2019	Leng, K. 30886152	China	CCA	51	30/51	NR	Nucleus	S	Paired non-tumour <i>n</i> = 51	Normal tissue low	NR
Ang1 <i>ANGPT1</i>	2008	Mihara, Y. -	Japan	eCCA	119	-	43.7%	Cytoplasm	S	Normal mucosa <i>n</i> = 38	15.8%	2 independent investigators
Ang1 <i>ANGPT1</i>	2006	Tang, D. 16465407	Japan	CCA (CCC)	33	15.2%	42.4%	Cytoplasm	S	NR	Hepatocytes of adjacent liver tissue	NR
Ang2 <i>ANGPT2</i>	2008	Mihara, Y. -	Japan	eCCA	119	-	42.0%	Cytoplasm	S	Normal mucosa <i>n</i> = 38	26.3%	2 independent

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Ang2 <i>ANGPT2</i>	2006	Tang, D. 16465407	Japan	CCA (CCC)	33	15.2%	57.6 %	Cytoplasm	S	NR	Hepatocytes of adjacent liver tissue	NR	
Ang2 <i>ANGPT2</i>	2014	Voigtlander 24823366	No, Germany	eCCA iCCA	37 8	Strong induction in tumour vasculature	NR	Tumour vascular endothelium	S	PSC	PSC negative.	NR	
Aromatase <i>CYP19A1</i>	2018	Kaewlert, W. 30284180	Thailand	CCA	74	61%	-	Cytoplasm	S	Normal bile duct cells	Lower than CCA	NR	
CA9 CA9	2019	Bi, C. 30849962	China	iCCA	113	85.0%	-	NR	S	NR	NR	NR	2 independent pathologists
CA9 CA9	2014	Gu, M. 25755787	Korea	iCCA	85	NR	44.7 %	NR	TMA	<i>n</i> = 4 normal liver tissue	NR	NR	
Cadherin 17 <i>CDH17</i>	2015	Maeda, S. 25917007	Japan	eCCA	165	-	41%	NR	S	NR	NR	NR	3 observers
CD24 <i>CD24</i>	2006	Su, M.-C. 16125303	Taiwan	iCCA	70	NR	51%	Membrane Cytoplasm	S	Normal liver tissue	-ve	NR	
CD24 <i>CD24</i>	2013	Kim, K. 23539485	Korea	eCCA	84	NR	42.9 %	Tumour cells	TMA	None	NR	NR	
CD24 <i>CD24</i>	2010	Riener, M.- O. 20621328	Switzerland	iCCA eCCA	19 59	NR	21% 58%	Cytoplasm	TMA	<i>n</i> = 30 normal biliary epithelium of extrahepatic BD	Negative for CD24 expression	NR	
CDX2 <i>CDX2</i>	2018	Tang, H. 29862152	China	iCCA	93	-	29.0 %	Nucleus	S	NR	NR	NR	
CDX2 <i>CDX2</i>	2018	Wang, Z. No PubMed ID	Japan	iCCA	127	-	26.0 %	Nucleus	TMA	NR	NR	NR	2 blinded observers

CDX2 <i>CDX2</i>	2019	Ishida, K. 31401234	Japan	eCCA	84	NR	NR	Nucleus	S	NR	NR	3 blinded independent observers
CDX2 <i>CDX2</i>	2007	Chang, Y. 17295772	Thailand	eCCA	49	-	22.5 %	Nucleus	S	NR	NR	Single independent pathologist
CDX2 <i>CDX2</i>	2006	Jinawath, A. 16794828	Thailand	iCCA eCCA	36 23	-	37.4 %	NR	S	NR	NR	NR
CDX2 <i>CDX2</i>	2008	Chiu, C.-T. 18329969	Taiwan	iCCA	30	NR	60%	Nucleus	S	None	NR	NR
CDX2 <i>CDX2</i>	2017	Hass, H.G.	Germany	iCCA	36	NR	25%	Nucleus	S	NR	NR	NR
Claudin 18 <i>CLDN18</i>	2015	Keira, Y. 25503275	Japan	iCCA eCCA	27 32	NR	NR	Basolateral membrane	S	NR	non-neoplastic cells -ve -ve.	NR
Claudin 18 <i>CLDN18</i>	2011	Shinozaki, A. 21607649	Japan	eCCA iCCA	99 83	NR	90% 43%	Membrane	S	Normal intrahepatic and extrahepatic biliary epithelial	Inflammatory mucosa basolateral membrane occ +ve	NR
CTLA4 <i>CTLA4</i>	2017	Lim, Y.J. 28084572	Korea	eCCA	77	79.2% (61/77)	NR	Tumour cells and infiltrating lymphocytes	TMA	None	NR	2 blinded independent observers confirmed the results
CUL4A <i>CUL4A</i>	2017	Huang, G.- K.	Taiwan	iCCA	105	NR	32.4 %	Nucleus	TMA	Normal BD	NR	Two blinded

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CUL4A <i>CUL4A</i>	2017	Zhang, T.-J. 28428711	China	pCCA	72	NR	NR	Nucleus	S	<i>n</i> = 12 adjacent normal BD from the 72 patients	Expressed in normal BD	
CXCL12 <i>CXCL12</i>	2019	Miyata, T. 31361379	Japan	iCCA	127	52% (66/127)	NR	Nucleus and cytoplasm	S	NR	NR	
CXCR2 <i>CXCR2</i>	2014	Sueoka, H. 24582495	Japan	iCCA	34	64.7% (22/34)	NR	Tumour cells	S	<i>n</i> = 30 adjacent normal liver tissue	Weak expression	
CYP39A1 <i>CYP39A1</i>	2014	Khenjanta, C. 25556446	Thailand	CCA unspec	30	30% (9/30)	NR	Tumour cells and hepatocytes	S	<i>n</i> = 30, adjacent normal tissue	Weak staining	
DKK1 <i>DKK1</i>	2013	Shi, R.Y. 23132676	China	iCCA	138	-	38.4 %	Cytoplasm	TMA	NR	NR	
DKK1 <i>DKK1</i>	2016	Shi, X.-D. 27608843	China	Hilar CCA	37	64.9%	NR	Cytoplasm	S	Peritumoral tissue	Very low expression	

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EGFR ERBB1	2007	Schmitz 18161916	No, Germany	iCCA	61	21.3% 13/61	68.9 %	Tumour cell membrane	S	None	NR	
EGFR ERBB1	2014	Simbolo 24867389	No, Italy	iCCA eCCA	57 40	NR	49% 18%	Membranous	TMA	None	NR	NR
EGFR ERBB1	2017	Padthaisong, S. 28668850	Thailand	CCA	94	55%	-	NR	S	NR	NR	NR
EGFR ERBB1	2019	Zhao, L. 31307551	China	iCCA	47	-	76.6 %	NR	S	NR	NR	1 blinded patholog ist 3
EGFR ERBB1	2011	Miyamoto	Japan	iCCA	111	-	26.1 %	NR	TMA	NR	NR	blinded observer s
EGFR ERBB1	2010	Pignochino, Y. 21087480	No, Italy	iCCA eCCA	17 19	76.5% 26.3%	100 % 52.6 %	Membrane	S	Normal cholangiocyte and hepatocyte membranes	All positive	NR
EGFR ERBB1	2006	Ogo, Y. 16739351	Japan	CCA unspec	44	43%	-	Membrane	S	+ control Lung Ca, no normal tissues	+ lung Ca	NR
EGFR ERBB1	2018	Xu, Y.F. 30177842	China	pCCA	121	45.5%	-	NR	S	NR	NR	NR
EGFR ERBB1	2018	Gomes, R.V. 29995151	No, Brazil	CCA (inc pCCA)	35	-	28.6 %	Cell membrane	S	NR	NR	1 blinded experien ced patholog ist
EGFR ERBB1	2010	Shafizadeh, N. 20040392	No, USA	iCCA eCCA	26 19	-	81% 79%	Membrane	S	NR	NR	NR

EGFR <i>ERBB1</i>	2014	Yang, X. 24927194	China	iCCA eCCA	65 110	30.8% (20/65) 20.9% (23/110)	NR	Membrane	TMA	NR	NR	2 blinded independ ent observer s
EGFR <i>ERBB1</i>	2008	Yoshikawa, D. 18087285	Japan	iCCA eCCA	106 130	NR	27.4 % 19.2 %	Membrane	S	None	NR	3 Blinded authors
EGFR <i>ERBB1</i>	2012	Gu, M.J. 22281980	Korea	iCCA	83	NR	55.4 %	Membrane	S	None	NR	NR (althoug h no access to full article)
EGFR <i>ERBB1</i>	2016	Moon, A. 27020207	Korea	eCCA	84	NR	38.1 %	Cytoplasm Membrane	TMA	NR	NR	Two authors
EGFR <i>ERBB1</i>	2009	Iguchi, T. 19262069	Japan	iCCA	61	NR	67.2 %	Cytoplasm, membranous	S	None	NR	NR
eNOS <i>NOS3</i>	2017	Suksawat, M. 27143607	Thailand	CCA	171	100%	50.9 %	Epithelial cells	S	Normal BD	NR	NR
ER α <i>ESR1</i>	2018	Kaewlert, W. 30284180	Thailand	CCA	74	78%	-	Cytoplasm and Nucleus	S	Adjacent normal tissue 1. BD epithelial cells 2. Hepatocytes	1. Sig lower expression in cytoplasm	NR
ER β <i>ESR2</i>	2018	Kaewlert, W. 30284180	Thailand	CCA	74	50%	-	Cytoplasm and Nucleus	S	Adjacent normal tissue 1. BD epithelial cells 2. Hepatocytes	1. Sig lower expression in cytoplasm	NR
Etk/Bmx <i>BMX</i>	2008	Guo, L. 18270973	China	iCCA	57	-	33.3 %	Cytoplasm and nucleus	S	Background liver tissue (normal hepatocytes, BD)	Negative	NR
FOXC2 <i>FOXC2</i>	2013	Watanabe, A.	Thailand	eCCA	77	-	23.3 %	Cytoplasm	S	NR	NR	2 independ

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GLUT-1 SLC2A1	2018	Ikeno, Y. 29642912	Japan	iCCA	50	52%	-	Tumour cell memb	S	NR	NR	NR	
GLUT-1 SLC2A1	2014	Kubo, Y. 24824030	Japan	iCCA	149	NR	46.3 %	Membrane	S	NR	NR	NR	
HES1 HES1	2016	Aoki, S. 27821106	Japan	eCCA	132	Unknown	81.8 %	Nucleus	TMA	N = 8 normal BD	Normal BD negative	NR	
Hes1 Hes1	2013	Gandou, C. 23134772	Japan	pCCA iCCA	25 21	-	84% 62%	Nucleus, cytoplasm	S	Intra-hepatic and extra hepatic BD	Infrequently positive 57% (16/28) Hes1 expression in the Nucleus of lining epithelial	NR	
Hes1 HES1	2012	Igarashi, S. 22594685	Japan	pCCA	43	NR	35%	NR	S	n = 28 normal foetal and adult BD		NR	
ID2 ID2	2013	Harder, J. 24409060	No, Germany	iCCA eCCA	50 45	-	29% 28%	Mainly cytoplasm	S	Normal BD	Low or negative		2 independ ent blinded observer s
IGF-1 IGF1	2012	Ohashi, H. 22044563	Japan	eCCA	30	-	60%	NR	S	NR	NR	NR	
IGF-2 IGF2	2012	Ohashi, H. 22044563	Japan	eCCA	30	-	53%	NR	S	NR	NR	NR	
IGFBP3 IGFBP3	2006	Jinawath, N. 17006947	Thailand	iCCA	273	NR	55.3 4%	Cytoplasm	TMA	Noncancerous liver tissue from n = 10 patients with liver metastasis from CRC	Negative expression		Three independ ent investiga tors

IL-17 IL17	2015	Asukai, K. 26228109	Japan	iCCA	72	38.9%	-	Cytoplasm membrane	S	Pancreas exocrine tissue +ve control. Peritumoral normal liver	Pancreas +ve, normal liver	NR
IL-6 IL6	2015	Asukai, K. 26228109	Japan	iCCA	72	47%	-	Cytoplasm membrane	S	Appendix +ve control	Appendix +ve	NR
IMP3 IMP3	2014	Gao, Y. 24745619	China	iCCA	72	-	82%	Tumour nests	S	Adjacent normal tissue	No expression	NR
IMP3 IMP3	2009	Riener, M.- O. 19467694	No, Switzerlan d	iCCA eCCA	19 58	-	36.8 % 50.0 %	Cytoplasm	TMA	Normal BD ($n = 36$), acute inflammation ($n = 36$), dysplasia (low grade $n = 9$, high grade $n = 11$)	Normal BD, inflammation, low grade dysplasia negative/weak , high grade all +	3 patholog ists
IMP3 IMP3	2014	Lok, T. 24439226	China	iCCA	41	68%	90%	Cytoplasm	S	NR	NR	3 investiga tors
IMP3 IMP3	2012	Chen, Y.-L. 23246869	Taiwan	iCCA	61	-	41.0 %	Tumour cells	S	Adjacent normal/ non tumorous liver tissue	Negative	2 blinded independ ent patholog ists
IMP3 IMP3	2013	Kawashima, H. 23269460	Japan	eCCA	68	NR	74.9 %	Cytoplasm	S	$n = 12$ benign	Detected in 1/12 samples	Two independ ent observes
Laminin 5 LAMA5	2018	Soejima, Y. 29584696	Japan	iCCA	48	54.2% (26/48)	NR	Cytoplasm of tumour cells and intratumoral stroma	S	None	NR	NR
LEF1 LEF1	2012	Coulouarn, C. 22696594	France	CCA	54	NR	32%	Nucleus	TMA	Normal biliary cells	Weak expression?	NR

LI-cadherin <i>CDH17</i>	2009	Takamura, M. 23852458	Japan	iCCA	34	NR	52.9 %	NR	S	Non-neoplastic biliary epithelium negative	Non- neoplastic biliary epithelium negative	NR
Lipocalin 1 <i>LCN1</i>	2018	Tian, Y. AN: 624752303	China	iCCA eCCA	100 27	44.1%	-	NR	TMA	Adjacent non- tumour liver tissue (<i>n</i> = unknown)	CC > than adjacent normal tissues	2 independ ent blinded patholog ists
MAGE-A1 <i>MAGEA1</i>	2011	Zhou, J.X. 21211023	China	iCCA	89	-	29.2 %	Cytoplasm	S	NR	NR	NR
MAGE-A3/4 <i>MAGEA3</i> <i>MAGEA4</i>	2011	Zhou, J.X. 21211023	China	iCCA	89	-	27.1 %	Cytoplasm	S	NR	NR	NR
Matripase <i>ST14</i>	2019	Komatsubar, A.T. 30729623	Japan	eCCA	256	Cells 48% Stroma 40%	-	Mainly cytoplasm	TMA	Non-malignant epithelial cells	Matriptase + in some tumors	2 blinded indep patholog ists
M-CSF <i>CSF1</i>	2015	Oishi 25052379	Japan	iCCA	39	NR	35.9 %	Cytoplasm	S	Adjacent hepatic paranchyma	None	Single blinded patholog ist
Mesothilin <i>MSLN</i>	2012	Kawamata, F. 23064529	Japan	eCCA	61	47.5%	72%	luminal membrane/ cytoplasm	S	NR	NR	3 independ ent blinded patholog ists
MMP7 <i>MMP7</i>	2009	Oka, T. 19701966	Japan	CCA	30	NR	80%	Cytoplasm	S	None	NR	NR
MMP7 <i>MMP7</i>	2011	Ohashi, H. 22044563	Japan	eCCA	30	-	80%	NR	S	NR	NR	NR

MMP7 <i>MMP7</i>	2016	Shi, X.-D. 27608843	China	pCCA	37	62.2%	NR	Cytoplasm	S	Peritumoral tissue	Very low expression	Two blinded independent observers	
MMP9 <i>MMP9</i>	2018	Park, Y. 29422250	Korea	eCCA	66	50%	-	NR	S	NR	NR	NR	
MMP9 <i>MMP9</i>	2014	Sun, Q. 25337264	China	pCCA	58	46.6%	67.2 %	Cytoplasm	S	NR	NR	2 independent blinded observers	
MMP9 <i>MMP9</i>	2009	Itatsu, K. 19218340	Japan	eCCA iCCA	75 35	NR	58%	Cytoplasm	S	Adjacent normal non-neoplastic BD	Faint or absent expression	NR	
MMP9 <i>MMP9</i>	2015	Tian, X. 24985031	China	iCCA	65	-	53.8 %	Cytoplasm	S	NR	NR	2 blinded independent observers	
MMP9 <i>MMP9</i>	2010	Subimerb	Thailand	iCCA eCCA	32 18	Overall 6% CCA cells, 44% of TAMs in invasive margin		- -	CCA cells Tumour associated macrophages	S	No control	NR	NR
MMP9 <i>MMP9</i>	2015	Sun, Q. 26339407	China	pCCA	62	32.2%	67.7 %	Cytoplasm	S	NR	NR	2 independent blinded observers	
MMP9 <i>MMP9</i>	2013	Shi, R.Y. 23132676	China	iCCA	138	-	45.6 %	Cytoplasm	TMA	NR	NR	2 observer	

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MMP9 MMP9	2009	Onodera, M. 19721413	Japan	eCCA iCCA	55 32	-	47.3 % 62.5 %	Cytoplasm	S	Non-neoplastic biliary epithelium (<i>n</i> = 13)	Negative	
Nanog NANOG	2019	Zhang, M.-X. 30854141	China	iCCA	116	61.2%	-	Cytoplasm	TMA	Para-carcinoma tissues	Weak/ negative	
NGF NGF	2014	Yang, X.-Q. 24744599	China	iCCA	83	27.7% (23/83)	NR	Cytoplasm	S	None	NR	NR
Notch4 Notch4	2014	Wu, W.-R. 25031748	China	iCCA	41	NR	34.1 %	Nucleus and cytoplasm	S	Adjacent non- tumour liver	Expressed in normal liver tissue	NR
NY-ESO-1 CTAG1B	2011	Zhou, J.X. 21211023	China	iCCA	89	-	21.3 %	Cytoplasm	S	NR	NR	NR
OPCML OPCML	2011	Sriraksa, R. 21448164	Thailand	CCA	92	NR	26%	NR	S	<i>n</i> = 29 tumour adjacent normal samples	NR	Two independ ent observer s
p53 TP53	2006	Hughes, N.R. 16679351	Australia & Thailand	iCCA eCCA iCCA eCCA	27 38 13 34	44% 47% 15% 26%	+ve only if stro ng stain ing of mos t cells	Nucleus	S	Normal donor livers <i>n</i> = 6, non- neoplastic biliary epithelium	Normal bile ducts and liver negative	NR

p53 TP53	2018	Kim, S.J. 29309301	Korea	iCCA	213	NR	43.7 %	Nucleus	TMA	None	NR	NR
p53 TP53	2009	Shen, Y.C. 19190145	Taiwan	iCCA eCCA	74 49	NR	27% 36%	Nucleus	S	NR	NR	NR
p53 TP53	2019	Zhao, L. 31307551	China	iCCA	47	-	31.9 %	NR	S	NR	NR	NR
p53 TP53	2017	Okada, R. 28508195	Japan	eCCA	61	34.4%	-	Nucleus	S	NR	NR	2 observers 2 blinded independent pathologists
p53 TP53	2012	Tan, X.-P. 22783387	China	CCA	69	-	84%	Nucleus	S	No control	NR	NR
p53 TP53	2017	Liu, S. 28915621	China	iCCA	60	-	46.7 %	NR	S	<i>n</i> = 60 paired normal tissue	NR	NR
p53 TP53	2011	Ohashi, H. 22044563	Japan	eCCA	30	-	46.7 %	NR	S	NR	NR	NR
p53 TP53	2010	Riener, M.- O. 20621328	Switzerland	iCCA eCCA	19 59	NR	37% 46%	Nucleus	TMA	<i>n</i> = 30 normal biliary epithelium of extrahepatic BD	Negative for p53 expression	NR
p53 TP53	2008	Karamitopoulou, E. 18854271	Switzerland	eCCA	62	NR	32%	Nucleus staining	TMA	<i>n</i> = 24 normal BD	Not expressed in normal tissue	NR
p53 TP53	2013	Kaira, K. 24131658	Japan	eCCA	89	49.4% (44/89)	NR	Nucleus	S	<i>n</i> = 16 benign biliary tract lesions	Not expressed in control tissue 0%	2 blinded authors Two blinded independent observers
p53 TP53	2010	Won, H.S. 20955617	Korea	eCCA inc. pCCA	75	NR	53.3 %	Nucleus	TMA	None	NR	NR
p53 TP53	2006	Liu, X.-F. 16937443	China	CCA	36	NR	52.8 %	Nucleus	S	None	NR	NR

p53 TP53	2013	Hirashima, K. 22927259	Japan	eCCA	71	NR	39.4 %	Nucleus	S	Normal BD epithelium of the samples	NR	Two blinded authors
p53 TP53	2015	Vasuri, F. 25367684	Italy	iCCA	60	NR	38.3 %	Nucleus	TMA	<i>n</i> = 10 normal liver tissue	NR	NR
p53 TP53	2009	Iguchi, T. 19262069	Japan	iCCA	61	NR	36.1 %	Nucleus	S	None	NR	NR
p53 TP53	2015	Keira, Y. 25503275	Japan	iCCA eCCA	27 32	NR	NR	Nucleus of adenocarcino mas	S	NR	NR	NR
p53 TP53	2016	Moro, C.F. 27829056	No, Sweeden	iCCA	97	-	30%	Nucleus	S	NR	NR	2 patholog ists
p53 TP53	2017	Bhalla, A. 28823571	No, USA	iCCA	56	-	55.4 %	Nucleus	S	Liver with Von Meyenburg complex without dysplasia and with low grade dysplasia	No p53 expression at 10% cut off	NR
p53 TP53	2014	Lee, C.T. 25245603	China	iCCA	54	-	59%	Nucleus	S	NR	NR	2 patholog ists
p53 TP53	2013	Park, K.W. 23613672	Korea	eCCA pCCA iCCA	46 41 17	-	52.8 % 47.4 % 46.2 %	Nucleus	TMA	NR	NR	2 blinded independ ent patholog ists
PAI-2 SERPINB2	2018	Utaijaratras mi, P. 29347950	Thailand	iCCA	72	63%	-	Tumour cell	S	NR	NR	2 blinded independ ent observer s

PBK/TOPK <i>PBK</i>	2010	He, F. 19954816	China	iCCA pCCA	51 23	-	91.9 %	Cytoplasm, occ membranous	S	N = 10 normal donor livers	Expressed in N BD but -ve in hepatocytes	NR
PDGF-D <i>PDGFD</i>	2019	Komatsubar a, T. 30729623	Japan	eCCA	256	Cells 32% Stroma 26%	-	Mainly cytoplasm	TMA	Adjacent non- malignant epithelial cells	weak or no staining of PDGF-D	2 blinded indep patholog ists Three independ ent investiga tors
PGF <i>PGF</i>	2006	Jinawath, N. 17006947	Thailand	iCCA	273	NR	70.8 3%	Cytoplasm	TMA	Noncancerous liver tissue from <i>n</i> = 10	NR	NR
PIWIL2 <i>PIWIL2</i>	2015	Chen, Y.J. 26125915	China	pCCA	41	80.5%	-	Cytoplasm	S	Control tissues <i>n</i> = 10	90% low or negative	NR
PRL-2 <i>PTP4A3</i>	2010	Xu, Y. 19757198	China	iCCA	102	47.1%	-	Cancer cells	S	Adjcent non- cancerous liver <i>n</i> = 102	Negative or low	All sections scored twice 2 blinded independ ent patholog ists
ROS1 <i>ROS1</i>	2016	27136744	Taiwan	iCCA	85	-	73%	Cytoplasm	S	NR	NR	NR
ROS1 <i>ROS1</i>	2015	Lee, K.-H. 26475437	Korea	iCCA	194	NR	37.1 %	Cytoplasm	TMA	Each TMA had 4 cores of normal tissue	NR	NR
SALL4 <i>SALL4</i>	2015	Deng, G. 26317546	China	iCCA	175	-	58%	Nucleus	S	Adjacent non- tumour tissue	N = 28 negative	3 experien ced patholog ists
SFRP1 <i>SFRP1</i>	2016	Davaadorj 28062160	Japan	iCCA	50	NR	60%	NR	S	None	NR	Single blinded

Gene	Year	Author	Country	Cancer Type	n	Sensitivity	Specificity	Location	Staining	Control	Positive %	Notes	pathologist
													2 blinded independent observers
sFRP1 <i>SFRP1</i>	2014	Kang, P. 24594839	China	iCCA eCCA	20 58	-	30% 34.5%	Cytoplasm	S	Normal BD <i>n</i> = 36	69.4%		NR
SHH <i>SHH</i>	2013	Tang, L. 23493353	China	iCCA	108	-	93.5%	Cytoplasm	TMA	Normal liver <i>n</i> = 8	Normal BD -ve/weak		NR
SHH <i>SHH</i>	2012	Kim, Y.J. 21946948	Korea	eCCA	42	38.1%	85.7%	Cytoplasm	S	Paired normal liver <i>n</i> = 42	35.7% normal liver positive		NR
TBX4 <i>TBX4</i>	2013	Zong, M. 23341740	China	iCCA	72	-	52.8%	Nucleus, occ cytoplasm	S	Paired adjacent non-cancerous tissue	2.78% positive		NR
TCF7 <i>TCF7</i>	2019	Liu, Z. 31248836	China	pCCA	103 + 57	-	43.8%	NR	TMA	No control	NR		NR
Tenascin <i>TNC</i>	2009	Iguchi, T. 19262069	Japan	iCCA	61	NR	63.9%	Intra- tumoral stroma	S	None	NR		NR
Tenascin-C <i>TNC</i>	2018	Soejima, Y. 29584696	Japan	iCCA	48	37.5% (18/48)	NR	Cytoplasm of tumour cells and intratumoral stroma	S	None	NR		NR
TFF1 <i>TFF1</i>	2007	Thuwajit, P. 17397518	Thailand	CCA	61	91.8%	98.4%	Cytoplasm, apical biliary epithelium, lumen of BD, stroma	S	Adjcent normal liver	+ large BD but negative hepatocytes and small BD		NR
TFF1 <i>TFF1</i>	2008	Chiu, C.-T. 18329969	Taiwan	iCCA	30	NR	56%	NR	S	None	NR		NR
TFF1 <i>TFF1</i>	2006	Jinawath, N. 17006947	Thailand	iCCA	273	NR	28.5 7%	Cytoplasm	TMA	Noncancerous liver tissue from <i>n</i> = 10	NR		Three independ ent

TFF1 <i>TFF1</i>	2018	Kaewlert, W. 30284180	Thailand	CCA	74	53%	-	Cytoplasm	S	Adjacent normal BD epithelial cells	Negative	investiga tors NR
TFF2 <i>TFF2</i>	2008	Thuwajit, P. 18410610	Thailand	CCA	51	31% (16/51)	NR	Cytoplasm and lumen	S	NR	NR	Two blinded observer s NR
TGF beta 1 <i>TGFB1</i>	2015	Chen, Y. 25993985	China	iCCA	78	29.5%	47.4 %	Cytoplasm	S	Normal liver tissue	Hepatocytes and BD -ve	NR
TGFβ1 <i>TGFB1</i>	2018	Soejima, Y. 29584696	Japan	iCCA	48	45.8% (22/48)	NR	Cytoplasm	S	None	NR	NR
Tie2 <i>TEK</i>	2008	Mihara, Y. -	Japan	eCCA	119	-	774. 8%	Cytoplasm	S	Normal mucosa <i>n</i> = 38	23.7%	2 indep endent investiga tors NR
TLR2 <i>TLR2</i>	2016	Liu, B. 27616304	China	iCCA	51	47%	-	NR	S	Paired normal <i>n</i> = 51	Significantly lower	NR
Transferrin <i>TF</i>	2017	Jamnongkan, W. 28671021	Thailand	CCA	49	41%	-	Cytoplasm	S	Cadaveric liver donors <i>n</i> = 3	Lower in N BD cells compared to CCA	NR
Twist <i>TWIST1</i>	2014	Nitta, T. 25077440	Japan	eCCA	117	57.3%	-	NR	TMA	NR	NR	2 blinded investiga tors Single senior patholog ist NR
Twist <i>TWIST1</i>	2016	Chu, C. 26479153	China	CCA	54	-	27.7 %	NR	S	Choledochal cyst (<i>n</i> = 16)	87.5% positive	
Twist <i>TWIST1</i>	2014	Duangkump ha, K. 24969562	Thailand	CCA	30	30% (9/30)	NR	Nucleus and cytoplasm of tumour cells	S	None	NR	NR

Type IV Collagen a6 <i>COL4A6</i>	2013	Hirashima, K. 22927259	Japan	eCCA	71	NR	38%	Membrane	S	Normal BD epithelium of the samples	Weak expression	Two blinded authors
ULBP1	2016	Tsukagoshi, M. 26608587	Japan	eCCA	82	51.2%	100 %	Tumour cells	S	NR	NR	NR
ULBP2	2016	Tsukagoshi, M. 26608587	Japan	eCCA	82	61.0%	76.8 %	Tumour cells	S	NR	NR	NR
uPA <i>PLAU</i>	2012	Thummarati, P. 22294827	Thailand	CCA	174	49.4%	75.3 %	Cytoplasm	TMA	NR	NR	NR
VEGF C <i>VEGFC</i>	2008	Aishima 18192971	Japan	iCCA	88	NR	30%	Cytoplasm	S	None	NR	Two blinded patholog ists
VEGF C	2006	Taniguchi, K. 16688800	Japan	pCCA	34	-	47%	Cancer cells	S	NR	NR	Single blinded patholog ist
VEGF C <i>VEGFC</i>	2015	Xu, Y.F. 25805932	China	iCCA	65	-	49.2 %	Cytoplasm	TMA	NR	NR	2 independ ent blinded observer s
VEGFC <i>VEGFC</i>	2017	Suksawat, M. 27143607	Thailand	CCA	171	100%	66.7 %	Cytoplasm and membrane	S	No control	NR	NR
VEGF-C <i>VEGFC</i>	2006	Park, B.K. 16601431	Korea	iCCA	36	41.7%	69.5 %	Cytoplasm	S	Normal epithelia	Negative	2 blinded observer s
VEGF-C <i>VEGFC</i>	2015	Zhao, R. 26622616	China	CCA	65	NR	75.4 %	NR	S	<i>n</i> = 5 normal cholangiolar biopsies	Moderate to low in 5% of BD	NR
VEGF-C <i>VEGFC</i>	2013	Shi, R.Y. 23132676	China	iCCA	138	-	72.5 %	Cytoplasm	TMA	NR	NR	2 observer

												s, 1 blinded
VitD R VDR	2007	Seubwai, W. 17487855	Thailand	CCA	111	40%	74%	Cytoplasm	S	Normal BD	20% low expression	NR
Wnt3 WNT3	2014	Loilome, W. 24549785	Thailand	CCA	38	Unknown	42.1 %	Tumour cells	S	Cadaveric donor liver. Adjacent non- tumour tissue	Normal BD & hepatocytes - ve.	NR
Wnt3a WNT3A	2014	Loilome, W. 24549785	Thailand	CCA	38	Unknown	92.1 %	Cytoplasm	S	Cadaveric donor liver. Adjacent non- tumour tissue	Normal BD low expression.	NR
Wnt5a WNT5A	2014	Loilome, W. 24549785	Thailand	CCA	38	Unknown	76.3 %	Tumour cells	S	Cadaveric donor liver. Adjacent non- tumour tissue	Normal BD epithelia weak cytoplasm +ve.	NR
Wnt7b WNT7B	2014	Loilome, W. 24549785	Thailand	CCA	38	34.2%	100 %	Cytoplasm	S	Cadaveric donor liver. Adjacent non- tumour tissue	Nucleus +ve hyperplastic BD. +ve	NR
ZNF423 ZNF423	2019	Chaiprasert, T. 31284679	Thailand	iCCA	75	41%	-	NR	S	Adjacent non- cancer areas	NR	NR



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