Utility of Claudin-3 in extracellular vesicles from human bile as biomarkers of cholangiocarcinoma

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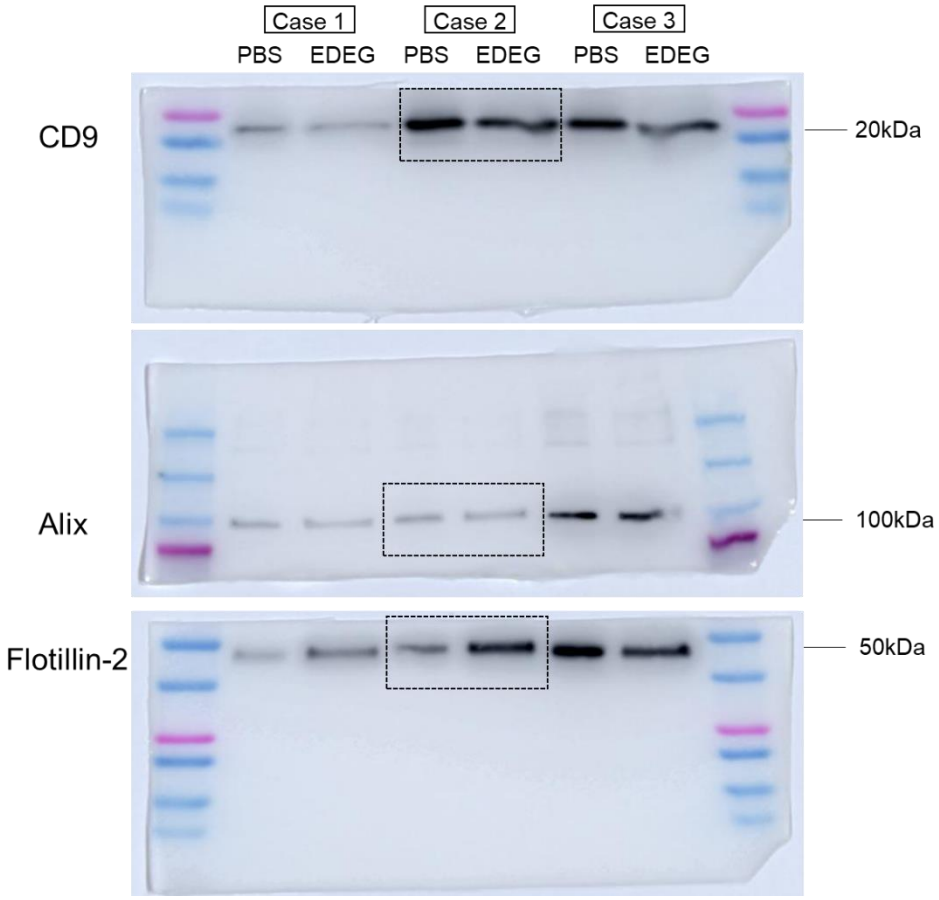
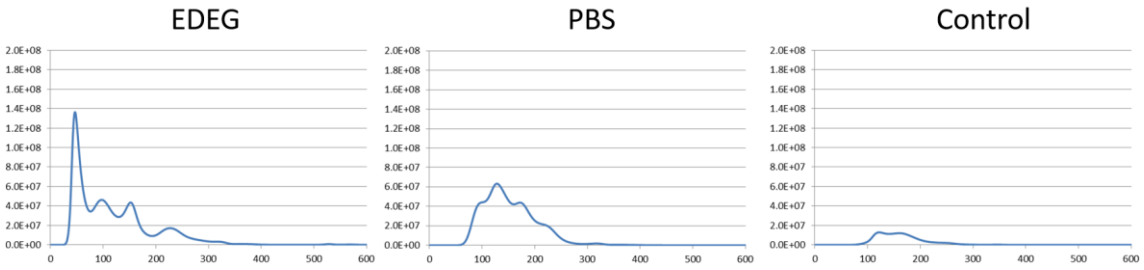
Supplementary figure S1. Full-length blots of exosome marker proteins

Western blotting showed that EVs could be isolated in both PBS and EDEG groups. For clarity, the blot for each marker in Case 2 was cut and shown in Figure 1B.



Supplementary figure S2. Comparison of EV isolation methods by NTA

The NTA showed that the isolation method in this study using EDGE had the largest number of particles compared to PBS and previously reported methods.



Supplementary Table S1. Characteristics of CCA and stone cases

		CCA (n = 10)		Stone (n = 10)		P-value	
Age		78	(66–95)	79	(41–88)	N.S.	
Sex (male/female)			4/6		7/3		
Height	(cm)	143.0	(138.5–169.9)	150.8	(143.8–178.3)	N.S.	
Weight	(kg)	54.2	(38.7–68.1)	59.1	(40.4–90.3)	N.S.	
BMI	(kg/m^2)	23.0	(16.2–29.0)	22.9	(16.6–29.3)	N.S.	
T-bil	(mg/dL)	9.15	(0.9–22.6)	2.35	(0.5–5.7)	N.S.	
AST	(U/L)	160	(59–653)	138	(17–1536)	N.S.	
ALT	(U/L)	191.0	(32–912)	185.5	(8–1216)	N.S.	
ALP	(U/L)	1274	(208–2997)	561	(136–1086)	0.0147	*
LDH	(U/L)	285	(199–363)	213	(115–1469)	N.S.	
γ-GTP	(U/L)	641	(150–2900)	529	(24–885)	N.S.	
WBC	(/μL)	6160	(3700–7940)	6435	(3050–16900)	N.S.	
CRP	(mg/dL)	0.57	(0–3.14)	0.51	(0–22.50)	N.S.	
CEA	(ng/mL)	2.17	(1.13–6.23)	1.45	(1.04–4.40)	N.S.	
CA19-9	(U/mL)	450.1	(6.9–12000.0)	26.2	(0–1829.0)	N.S.	
Tumor location							
Hilar/distal		4/6					
TNM stage							
1/2/3/4		3/3/3/1					

Median (range)

N.S., not significant

Supplementary Table S2. Characteristics of CCA and stone cases in the validation cohort

		CCA (n＝8)		Stone (n＝8)		P-value	
Age		76.5	(64–93)	76.5	(60–89)	N.S.	
Sex (male/female)			7 / 1		3 / 5	N.S.	
Height	(cm)	162.0	(148.9–182.8)	152.2	(141.8–161.0)	0.009	*
Weight	(kg)	57.6	(49.9–74.5)	50.2	(42.7–55.9)	0.006	*
BMI	(kg/m2)	22.5	(19.7–25.8)	21.3	(20.4–25.8)	N.S.	
T-bil	(mg/dL)	3.3	(0.5–19.6)	0.8	(0.4–3.6)	0.040	*
AST	(U/L)	102	(8–994)	32	(15–436)	N.S.	
ALT	(U/L)	154	(6–1342)	36	(9–210)	N.S.	
ALP	(U/L)	1907	(200–4541)	371	(139–774)	0.010	*
LDH	(U/L)	192	(117–673)	198	(156–473)	N.S.	
γ-GTP	(U/L)	1440	(29–1846)	88	(13–584)	0.010	*
WBC	(/μL)	6530	(3810–14600)	4855	(3970–14740)	N.S.	
CRP	(mg/dL)	0.68	(0.21–6.28)	0.19	(0–5.04)	N.S.	
CEA	(ng/mL)	2.87	(1.51–5.51)	1.80	(1.11–2.82)	0.024	*
CA19-9	(U/mL)	65.7	(4.8–3746.1)	7.6	(5.1–17.1)	0.010	*
Tumor location							
Hilar/distal		4/4					
TNM stage							
1/2/3/4		2/4/2/1					

Median (range)

N.S. not significant

Supplementary Table S3. All CCA-specific proteins

	Accession number	Description	Gene name	P-value	Fold change
1	O15551	Claudin-3	CLDN3	0.000232	2.58
2	Q9P2J5	Leucine--tRNA ligase, cytoplasmic	LARS	0.000233	3.29
3	Q96CS3	FAS-associated factor 2	FAF2	0.000491	6.51
4	Q9NX57	Ras-related protein Rab-20	RAB20	0.000721	21.48
5	Q9Y678	Coatomer subunit gamma-1	COPG1	0.000825	2.75
6	P04114	Apolipoprotein B-100	APOB	0.001219	2.08
7	P22694	cAMP-dependent protein kinase catalytic subunit beta	PRKACB	0.001835	2.51
8	P62913	60S ribosomal protein L11	RPL11	0.002573	8.13
9	Q9HD43	Receptor-type tyrosine-protein phosphatase H	PTPRH	0.002640	2.93
10	P02760	Protein AMBP	AMBP	0.002855	2.74
11	Q86YQ8	Copine-8	CPNE8	0.003307	3.44
12	Q6P6B1	Glutamate-rich protein 5	ERICH5	0.003354	10.02
13	Q969L2	Protein MAL2	MAL2	0.003637	2.33
14	Q14651	Plastin-1	PLS1	0.004065	5.18
15	Q96TA1	Niban-like protein 1	FAM129B	0.005052	2.66
16	Q14451	Growth factor receptor-bound protein 7	GRB7	0.005113	4.72
17	P00742	Coagulation factor X	F10	0.005602	31.39
18	Q4VNC1	Probable cation-transporting ATPase 13A4	ATP13A4	0.005734	2.89

19	P09525	Annexin A4	ANXA4	0.005813	3.09
20	P37059	Estradiol 17-beta-dehydrogenase 2	HSD17B2	0.005920	8.15
21	P60903	Protein S100-A10	S100A10	0.006000	2.11
22	P08865	40S ribosomal protein SA	RPSA	0.006072	15.09
23	Q9UHD8	Septin-9	SEPT9	0.006345	5.31
24	H3BN98	Uncharacterized protein (fragment)		0.006381	67.62
25	P46776	60S ribosomal protein L27a	RPL27A	0.006564	6.98
26	Q8WUM4	Programmed cell death 6-interacting protein	PDCD6IP	0.006578	2.83
27	Q15796	Mothers against decapentaplegic homolog 2	SMAD2	0.006663	5.91
28	Q9BW30	Tubulin polymerization-promoting protein family member 3	TPPP3	0.006769	2.66
29	P98196	Probable phospholipid-transporting ATPase IH	ATP11A	0.006838	2.33
30	Q99816	Tumor susceptibility gene 101 protein	TSG101	0.006861	3.35
31	P19823	Inter-alpha-trypsin inhibitor heavy chain H2	ITIH2	0.007085	2.32
32	Q06210	Glutamine–fructose-6-phosphate aminotransferase [isomerizing] 1	GFPT1	0.007092	5.77
33	P34096	Ribonuclease 4	RNASE4	0.007147	4.23
34	P68371	Tubulin beta-4B chain	TUBB4B	0.007213	2.74
35	Q2M3M2	Sodium/glucose cotransporter 4	SLC5A9	0.007502	13.70
36	Q9GZP0	Platelet-derived growth factor D	PDGFD	0.007548	9.26
37	P78369	Claudin-10	CLDN10	0.007737	6.28
38	P11021	Endoplasmic reticulum chaperone BiP	HSPA5	0.008142	11.57
39	Q13393	Phospholipase D1	PLD1	0.008517	2.56
40	P11586	C-1-tetrahydrofolate synthase, cytoplasmic	MTHFD1	0.008596	2.25

41	Q717R9	Cystin-1	CYS1	0.008785	6.41
42	Q9UK41	Vacuolar protein sorting-associated protein 28 homolog	VPS28	0.008799	3.25
43	Q9HD42	Charged multivesicular body protein 1a	CHMP1A	0.008976	7.50
44	P05787	Keratin, type II cytoskeletal 8	KRT8	0.009257	3.58
45	Q9NZZ3	Charged multivesicular body protein 5	CHMP5	0.009466	2.91
46	P98160	Basement membrane-specific heparan sulfate proteoglycan core protein	HSPG2	0.009573	3.05
47	P13611	Versican core protein	VCAN	0.009820	6.69
48	Q14204	Cytoplasmic dynein 1 heavy chain 1	DYNC1H1	0.010018	2.84
49	Q9H0E2	Toll-interacting protein	TOLLIP	0.010042	7.44
50	Q9BQE3	Tubulin alpha-1C chain	TUBA1C	0.010217	2.17
51	P39060	Collagen alpha-1(XVIII) chain	COL18A1	0.010323	7.26
52	Q08257	Quinone oxidoreductase	CRYZ	0.010877	3.32
53	Q9NP79	Vacuolar protein sorting-associated protein VTA1 homolog	VTA1	0.010888	8.96
54	Q9BVA1	Tubulin beta-2B chain	TUBB2B	0.010895	15.02
55	P07437	Tubulin beta chain	TUBB	0.011092	3.02
56	P05023	Sodium/potassium-transporting ATPase subunit alpha-1	ATP1A1	0.011095	4.42
57	P50914	60S ribosomal protein L14	RPL14	0.011483	4.20
58	O75608	Acyl-protein thioesterase 1	LYPLA1	0.011817	5.17
59	Q14108	Lysosome membrane protein 2	SCARB2	0.011822	2.36
60	P62847	40S ribosomal protein S24	RPS24	0.011882	4.26
61	P10909	Clusterin	CLU	0.012077	3.98
62	O14979	Heterogeneous nuclear ribonucleoprotein D-like	HNRNPDL	0.012158	4.13

63	Q86XT2	Vacuolar protein sorting-associated protein 37D	VPS37D	0.013068	7.71
64	Q9H4G0	Band 4.1-like protein 1	EPB41L1	0.013423	7.78
65	Q9GZM7	Tubulointerstitial nephritis antigen-like	TINAGL1	0.015175	6.29
66	Q01813	ATP-dependent 6-phosphofructokinase, platelet type	PFKP	0.015896	4.36
67	P62879	Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-2	GNB2	0.015909	2.10
68	Q9Y6W3	Calpain-7	CAPN7	0.015976	3.18
69	Q3B8N2	Galectin-9B	LGALS9B	0.016036	4.95
70	Q9NYS7	WD repeat and SOCS box-containing protein 2	WSB2	0.016491	2.20
71	P36955	Pigment epithelium-derived factor	SERPINF1	0.017125	3.39
72	Q14435	Polypeptide N-acetylgalactosaminyltransferase 3	GALNT3	0.017145	16.51
73	P12236	ADP/ATP translocase 3	SLC25A6	0.017950	4.47
74	Q9Y2T3	Guanine deaminase	GDA	0.018061	12.02
75	P02747	Complement C1q subcomponent subunit C	C1QC	0.018073	2.62
76	O43520	Phospholipid-transporting ATPase IC	ATP8B1	0.018216	3.10
77	O60658	High-affinity cAMP-specific and IBMX-insensitive 3',5'-cyclic phosphodiesterase 8A	PDE8A	0.018597	3.17
78	P26572	Alpha-1,3-mannosyl-glycoprotein 2-beta-N-acetylglucosaminyltransferase	MGAT1	0.019067	6.67
79	Q99747	Gamma-soluble NSF attachment protein	NAPG	0.019490	9.35
80	O75351	Vacuolar protein sorting-associated protein 4B	VPS4B	0.019602	3.14
81	P62829	60S ribosomal protein L23	RPL23	0.019631	3.37
82	P51178	1-PhosphatidylInositol 4,5-bisphosphate phosphodiesterase delta-1	PLCD1	0.019640	4.63
83	Q5VW32	BRO1 domain-containing protein BROX	BROX	0.019844	2.56
84	O15438	Canalicular multispecific organic anion transporter 2	ABCC3	0.020068	4.30

85	Q16629	Serine/arginine-rich splicing factor 7	SRSF7	0.020626	14.28
86	Q16555	Dihydropyrimidinase-related protein 2	DPYSL2	0.020749	9.03
87	P55056	Apolipoprotein C-IV	APOC4	0.021059	8.87
88	P62241	40S ribosomal protein S8	RPS8	0.021239	2.55
89	Q13557	Calcium/calmodulin-dependent protein kinase type II subunit delta	CAMK2D	0.021345	5.89
90	Q13418	Integrin-linked protein kinase	ILK	0.021600	3.86
91	P84077	ADP-ribosylation factor 1	ARF1	0.021632	5.40
92	Q9Y2A9	N-Acetyllactosaminide beta-1,3-N-acetylglucosaminyltransferase 3	B3GNT3	0.021897	9.32
93	P13866	Sodium/glucose cotransporter 1	SLC5A1	0.022042	2.09
94	Q00610	Clathrin heavy chain 1	CLTC	0.022530	2.44
95	P27216	Annexin A13	ANXA13	0.022902	3.12
96	P78417	Glutathione S-transferase omega-1	GSTO1	0.022956	9.36
97	P16422	Epithelial cell adhesion molecule	EPCAM	0.023022	13.02
98	P36405	ADP-ribosylation factor-like protein 3	ARL3	0.023568	5.58
99	P42330	Aldo-keto reductase family 1 member C3	AKR1C3	0.023765	2.53
100	Q14520	Hyaluronan-binding protein 2	HABP2	0.024753	2.79
101	Q14376	UDP-glucose 4-epimerase	GALE	0.025093	4.25
102	P20337	Ras-related protein Rab-3B	RAB3B	0.025594	2.90
103	O14672	Disintegrin and metalloproteinase domain-containing protein 10	ADAM10	0.026215	5.93
104	P05026	Sodium/potassium-transporting ATPase subunit beta-1	ATP1B1	0.026245	2.53
105	O14950	Myosin regulatory light chain 12B	MYL12B	0.026341	5.31
106	Q14116	Interleukin-18	IL18	0.026552	2.81

107	Q9H9H4	Vacuolar protein sorting-associated protein 37B	VPS37B	0.026652	2.95
108	P00352	Retinal dehydrogenase 1	ALDH1A1	0.027009	2.01
109	O43633	Charged multivesicular body protein 2a	CHMP2A	0.027210	3.21
110	Q8TE68	Epidermal growth factor receptor kinase substrate 8-like protein 1	EPS8L1	0.028753	2.10
111	P15291	Beta-1,4-galactosyltransferase 1	B4GALT1	0.029258	3.08
112	P30085	UMP-CMP kinase	CMPK1	0.029344	2.05
113	Q15366	Poly(rC)-binding protein 2	PCBP2	0.029939	2.25
114	P20339	Ras-related protein Rab-5A	RAB5A	0.030110	4.17
115	Q15181	Inorganic pyrophosphatase	PPA1	0.030279	2.20
116	Q9Y224	RNA transcription, translation and transport factor protein	RTRAF	0.031267	3.41
117	P08754	Guanine nucleotide-binding protein G(k) subunit alpha	GNAI3	0.031363	2.42
118	Q6PCB0	von Willebrand factor A domain-containing protein 1	VWA1	0.031511	3.29
119	Q12797	Aspartyl/asparaginyl beta-hydroxylase	ASPH	0.031742	3.48
120	Q9P0V3	SH3 domain-binding protein 4	SH3BP4	0.032538	3.85
121	Q5XXA6	Anoctamin-1	ANO1	0.032636	2.13
122	P53990	IST1 homolog	IST1	0.032639	2.12
123	O95471	Claudin-7	CLDN7	0.032680	8.21
124	P14866	Heterogeneous nuclear ribonucleoprotein L	HNRNPL	0.033079	6.48
125	Q05209	Tyrosine-protein phosphatase non-receptor type 12	PTPN12	0.033402	2.25
126	Q9UBI6	Guanine nucleotide-binding protein G(I)/G(S)/G(O) subunit gamma-12	GNG12	0.033635	3.32
127	Q8ND76	Cyclin-Y	CCNY	0.034088	2.57
128	P08183	Multidrug resistance protein 1	ABCB1	0.034098	3.43

129	O94826	Mitochondrial import receptor subunit TOM70	TOMM70	0.034329	3.31
130	P31943	Heterogeneous nuclear ribonucleoprotein H	HNRNPH1	0.034648	2.89
131	Q9BQL6	Fermitin family homolog 1	FERMT1	0.034820	2.19
132	P05162	Galectin-2	LGALS2	0.035133	5.26
133	Q93052	Lipoma-preferred partner	LPP	0.035138	4.50
134	P52895	Aldo-keto reductase family 1 member C2	AKR1C2	0.035570	2.77
135	Q99805	Transmembrane 9 superfamily member 2	TM9SF2	0.035677	8.00
136	Q07960	Rho GTPase-activating protein 1	ARHGAP1	0.035950	5.26
137	O43396	Thioredoxin-like protein 1	TXNL1	0.037047	4.72
138	P49411	Elongation factor Tu, mitochondrial	TUFM	0.037328	7.82
139	Q16650	T-box brain protein 1	TBR1	0.037824	3.42
140	Q71U36	Tubulin alpha-1A chain	TUBA1A	0.038011	10.87
141	Q7KZF4	Staphylococcal nuclease domain-containing protein 1	SND1	0.038245	4.51
142	P02649	Apolipoprotein E	APOE	0.038489	3.30
143	P83731	60S ribosomal protein L24	RPL24	0.038670	13.28
144	Q8TAD7	Overexpressed in colon carcinoma 1 protein	OCC1	0.038839	47.32
145	P62424	60S ribosomal protein L7a	RPL7A	0.040084	4.40
146	P40925	Malate dehydrogenase, cytoplasmic	MDH1	0.040101	20.03
147	O00159	Unconventional myosin-Ic	MYO1C	0.040211	2.63
148	Q9UP52	Transferrin receptor protein 2	TFR2	0.040977	3.91
149	O94832	Unconventional myosin-Id	MYO1D	0.041215	2.19
150	P27635	60S ribosomal protein L10	RPL10	0.041502	3.28

151	P10646	Tissue factor pathway inhibitor	TFPI	0.041861	3.89
152	P62266	40S ribosomal protein S23	RPS23	0.042762	4.63
153	Q9C0K7	STE20-related kinase adapter protein beta	STRADB	0.043303	4.18
154	Q92599	Septin-8	8-Sep	0.043528	7.52
155	P53621	Coatomer subunit alpha	COPA	0.043862	2.48
156	Q9BUF5	Tubulin beta-6 chain	TUBB6	0.043887	2.09
157	P11172	Uridine 5'-monophosphate synthase	UMPS	0.043888	5.62
158	Q9GZU7	Carboxy-terminal domain RNA polymerase II polypeptide A small phosphatase 1	CTDSP1	0.044412	13.31
159	Q8TBF2	Prostamide/prostaglandin F synthase	PRXL2B	0.044498	2.39
160	Q9UHG0	Doublecortin domain-containing protein 2	DCDC2	0.045061	2.90
161	P11182	Lipoamide acyltransferase component of branched-chain alpha-keto acid dehydrogenase complex, mitochondrial	DBT	0.045542	2.38
162	P62851	40S ribosomal protein S25	RPS25	0.047421	10.04
163	Q9Y625	Glypican-6	GPC6	0.047553	4.88
164	P18124	60S ribosomal protein L7	RPL7	0.047780	3.11
165	P39023	60S ribosomal protein L3	RPL3	0.047995	4.56
166	O60513	Beta-1,4-galactosyltransferase 4	B4GALT4	0.048659	13.28

Supplementary Table S4. Comparison of CLDN3 by immunohistochemical staining

	Case 1	Case 2	Case 3	Case 4	Case 5	P-value	
Normal	1 (1,1)	0 (0,0)	1 (0,0)	4 (2,2)	2 (1,2)	0.0153	*
CCA	8 (2,4)	9 (3,3)	4 (2,2)	9 (3,3)	12 (3,4)		

IHC score (intensity, positive percentage)

Normal, normal bile duct epithelium

CCA, cholangiocarcinoma