

Figure S1. Confirmation of the specificity of the core 3 synthase antibody employed in the present study. Core 3 synthase was mainly expressed in the intraepithelial non-invasive cancer cells (upper left). The specificity of the G8-144 antibody was assessed via western blotting using HAL8 and 293T cells transfected with *B3GNT6* cDNA (293T + B3GNT6). Its product, core 3 synthase, was detected at ~50 kDa, which was not observed in 293T cells (right lane). Proteins extracted from CCA tissues were also recognized by the G8-144 antibody (left lane). In addition, there were no G8-144-positive cells in the non-cancerous bile duct (lower left). Normal epithelial cells in the adnexal gland in the bile duct expressed MECA-79 (lower right). Scale bar, 100 μ m. B3GNT6, β 1,3-*N*-acetylglucosaminyltransferase 6; CCA, cholangiocarcinoma.

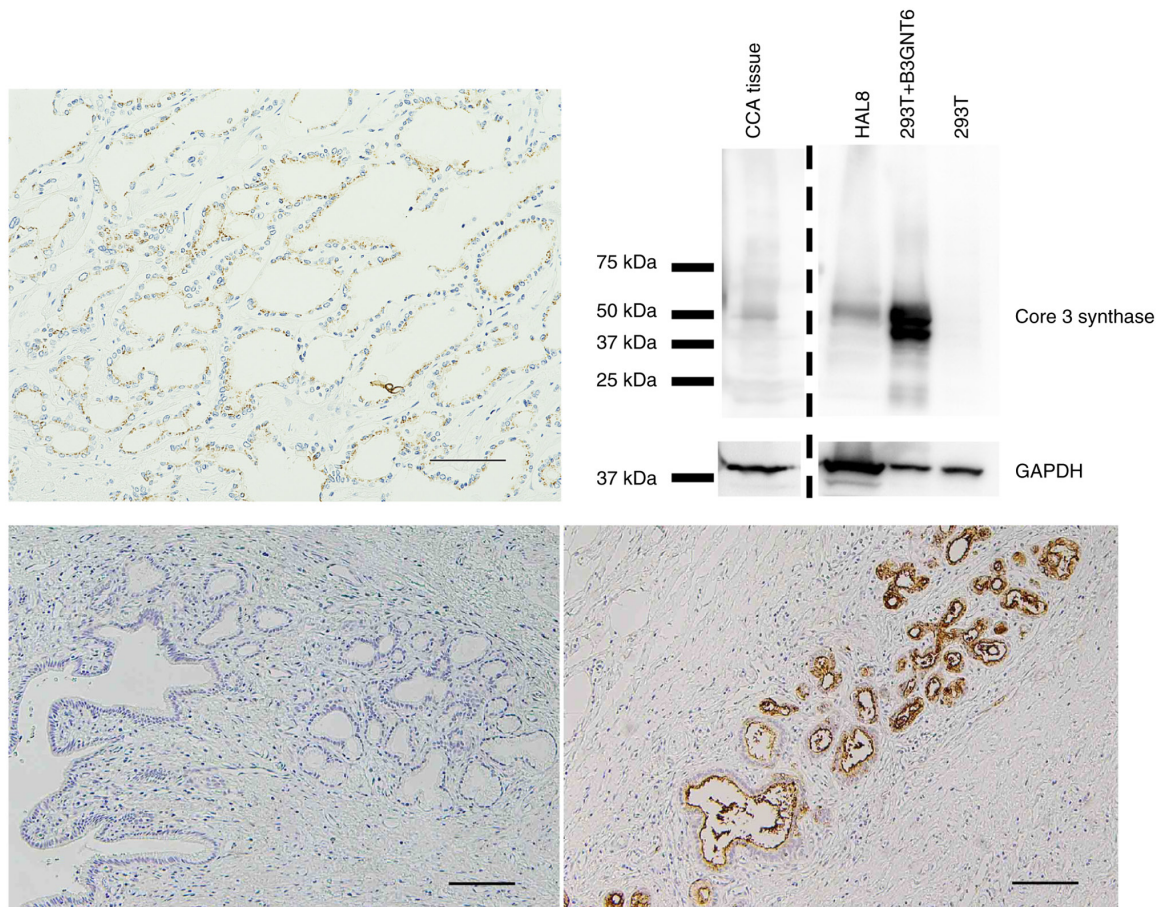


Figure S2. Survival rates of patients with extrahepatic pCCA. Kaplan-Meier survival curves were plotted for patients with G8-144(+) (red) or G8-144(-) (blue) pCCA. Postoperative OS rates are presented in the upper panels and DFS rates are presented in the lower panels. Total pCCA (left panels), non-invasive area (center panels) and invasive area (right panels). In contrast to distal CCA in Fig. 4, no association was found between the expression of core 3 synthase and the survival rate. CCA, cholangiocarcinoma; pCCA, perihilar cholangiocarcinoma; OS, overall survival; DFS, disease-free survival; (+), positive; (-), negative.

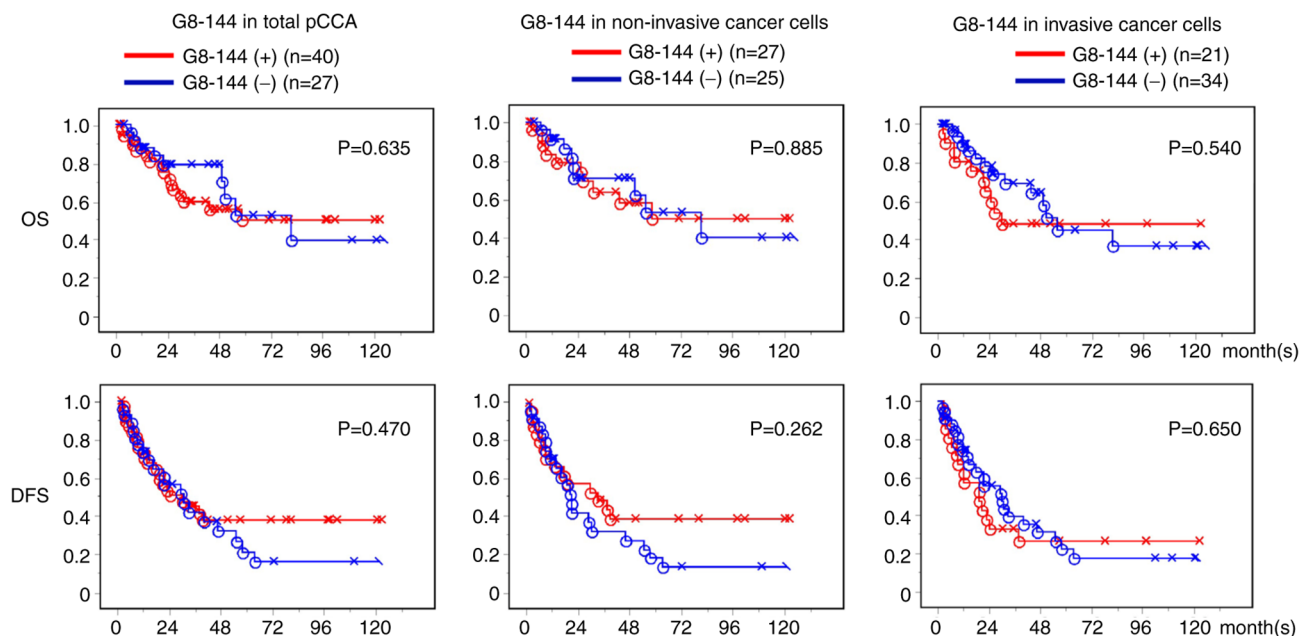


Figure S3. Survival rates of patients with extrahepatic pCCA. Kaplan-Meier survival curves were plotted for patients with MECA-79(+) (red) or MECA-79(-) (blue) pCCA. Postoperative OS rates are presented in the upper panels and DFS rates are presented in the lower panels. Total pCCA (left panels), non-invasive area (center panels) and invasive area (right panels). In contrast to distal CCA in Fig. 5, no association was found between the expression of MECA-79 and the survival rate. CCA, cholangiocarcinoma; pCCA, perihilar cholangiocarcinoma; OS, overall survival; DFS, disease-free survival; (+), positive; (-), negative.

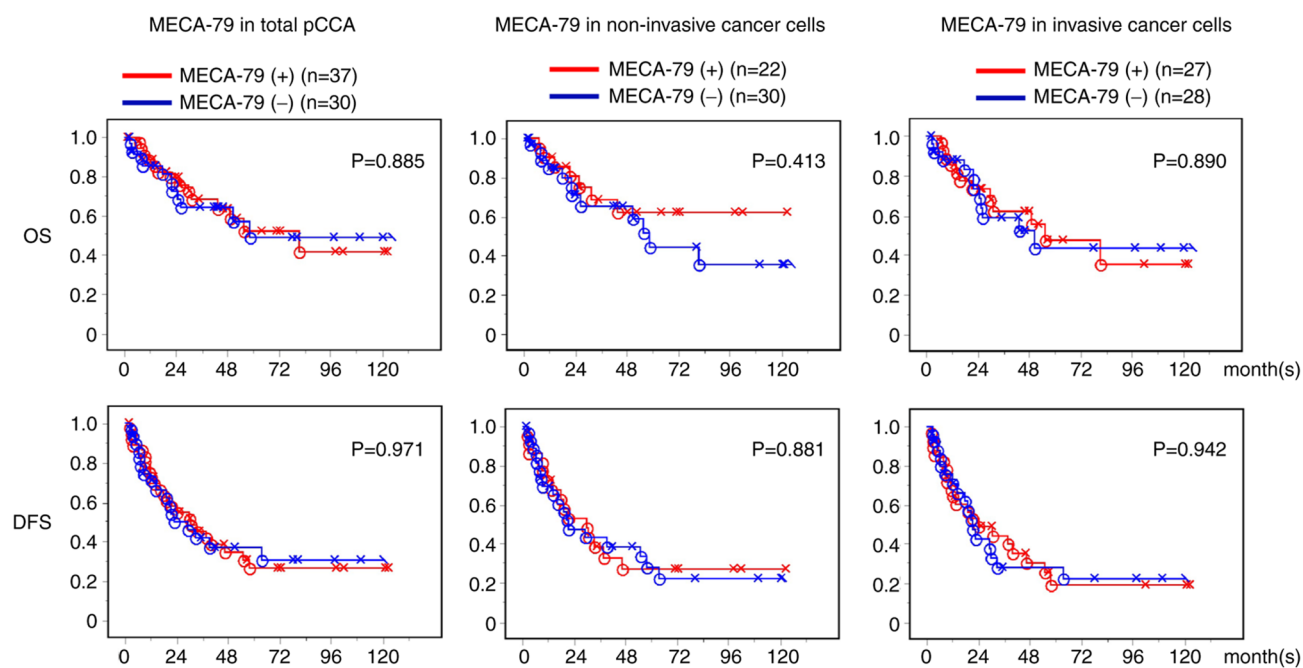


Table SI. Relationship between clinicopathological variables and expression of core 3 synthase/B3GNT6 or MECA-79 antigen in cancer cells of dCCA.

A, Core 3 synthase/B3GNT expression

Variables	Cases, n	Core 3 synthase/B3GNT expression status in cancer cells of dCCA cases								
		Total (n=118)			Non-invasive (n=112)			Invasive (n=110)		
		Positive, n	Negative, n	P-value	Positive, n	Negative, n	P-value	Positive, n	Negative, n	P-value
Age, years				0.855			0.705			0.158
≤72	61	33	28		28	28		23	34	
>72	57	32	25		31	25		14	39	
Sex				0.631			0.470			0.800
Male	98	55	43		50	42		31	59	
Female	20	10	10		9	11		6	14	
Pathological tumor status				0.382			0.294			N.A.
Tis	3	3	0		3	0		0	0	
T1	38	20	18		18	19		14	22	
T2	61	32	29		28	28		20	39	
T3	16	10	6		10	6		3	12	
T4	0	0	0		0	0		0	0	
Pathological node status				0.725			0.523			0.514
N0	61	35	26		34	25		16	39	
N1	40	20	20		17	20		14	25	
N2	17	10	7		8	8		7	9	
Pathological metastasis status				0.465			0.473			0.715
M0	110	63	47		56	48		35	67	
M1	8	2	6		3	5		2	6	
Stage				0.616			0.588			N.A.
0	3	3	0		3	0		0	0	
I	22	12	10		11	10		8	12	
IIA	41	22	19		21	19		12	28	
IIB	32	18	14		16	13		10	21	
IIIA	12	7	5		5	6		5	6	
IIIB	0	0	0		0	0		0	0	
IV	8	3	5		3	5		2	6	
Macroscopic type				0.600			0.688			0.758
Nodular	78	41	37		36	36		27	50	
Flat	15	8	7		8	7		5	14	
Papillary	25	16	9		15	10		5	9	
Histological type				0.845			0.691			0.144
Pap	17	10	7		10	7		2	10	
Tub	79	44	35		39	39		30	46	
Por	22	11	11		10	7		5	17	
Tumor histological grade				0.665			0.782			0.475
G1	34	18	16		17	17		8	19	
G2	62	36	26		32	29		24	37	
G3	21	10	11		9	7		5	16	
G4	1	1	0		1	0		0	1	

Table SI. Continued.

Variables	Cases, n	Core 3 synthase/B3GNT expression status in cancer cells of dCCA cases								
		Total (n=118)			Non-invasive (n=112)			Invasive (n=110)		
		Positive, n	Negative, n	P-value	Positive, n	Negative, n	P-value	Positive, n	Negative, n	P-value
Tumor margin				0.707			0.702			0.310
Negative	48	25	23		23	23		13	34	
Positive	70	40	30		36	30		24	39	
Lymphatic invasion				0.853			1.000			0.423
Low, ly0 and ly1	13	7	5		29	26		15	36	
High, ly2 and ly3	45	23	22		30	27		22	37	
Venous invasion				1.000			0.444			0.155
Low, v0 and v1	16	8	8		37	29		17	45	
High, v2 and v3	53	30	23		22	24		20	28	
Perineural invasion				0.665			0.372			0.042 ^a
Low, pn0 and pn1	15	9	6		16	10		3	19	
High, pn2 and pn3	12	7	5		43	43		34	54	
Total	N.A.	65	53	N.A.	59	53	N.A.	37	73	N.A.

B, MECA-79 expression

Variables	Cases, n	MECA-79 antigen expression status in cancer cells of dCCA cases								
		Total (n=118)			Non-invasive (n=112)			Invasive (n=110)		
		Positive, n	Negative, n	P-value	Positive, n	Negative, n	P-value	Positive, n	Negative, n	P-value
Age, years				0.690			0.130			0.556
≤72	61	41	20		23	33		34	23	
>72	57	41	16		32	24		35	18	
Sex				0.790			0.806			0.307
Male	98	67	31		46	46		54	36	
Female	20	15	5		9	11		15	5	
Pathological tumor status				0.763			0.586			N.A.
Tis	3	2	1		2	1		0	0	
T1	38	24	14		20	17		18	18	
T2	61	44	17		24	32		40	19	
T3	16	12	4		9	7		11	4	
T4	0	0	0		0	0		0	0	
Pathological node status				0.028 ^a			0.070			0.017 ^a
N0	61	36	25		26	33		28	27	
N1	40	31	9		18	19		27	12	
N2	17	15	2		11	5		14	2	
Pathological metastasis status				1.000			0.158			0.708
M0	110	76	34		49	55		63	39	
M1	8	6	2		6	2		6	2	

Table SI. Continued.

Variables	MECA-79 antigen expression status in cancer cells of dCCA cases								
	Cases, n	Total (n=118)			Non-invasive (n=112)			Invasive (n=110)	
		Positive, n	Negative, n	P-value	Positive, n	Negative, n	P-value	Positive, n	Negative, n
Stage				0.547			0.594		N.A.
0	3	2	1		2	1		0	0
I	22	12	10		11	10		8	12
IIA	41	28	13		18	22		24	16
IIB	32	24	8		12	17		22	9
IIIA	12	10	2		6	5		9	2
IIIB	0	0	0		0	0		0	0
IV	8	6	2		6	2		6	2
Macroscopic type				0.081			0.026 ^a		0.248
Nodular	78	55	23		36	36		50	27
Flat	15	7	8		3	12		6	8
Papillary	25	20	5		16	9		13	6
Histological type				0.803			0.685		0.353
Pap	17	12	5		10	7		6	6
Tub	79	56	23		37	41		51	25
Por	22	14	8		8	9		12	10
Tumor histological grade				0.719			0.746		0.179
G1	34	23	11		17	17		14	13
G2	62	45	17		30	31		43	18
G3	21	13	8		7	9		12	9
G4	1	1	0		1	0		0	1
Tumor margin status				0.685			0.184		0.558
Negative	48	50	20		36	30		31	15
Positive	70	32	16		19	27		38	26
Lymphatic invasion				0.110			0.710		0.010 ^a
Low, ly0 and ly1	13	36	22		26	29		25	26
High, ly2 and ly3	45	46	14		29	28		44	15
Venous invasion				0.155			1.000		0.010 ^a
Low, v0 and v1	16	44	25		32	34		32	30
High, v2 and v3	53	38	11		23	23		37	11
Perineural invasion				1.000			0.182		0.461
Low, pn0 and pn1	15	19	8		16	10		12	10
High, pn2 and pn3	12	63	28		39	47		57	31
Total	N.A.	82	36	N.A.	55	57	N.A.	69	41

^aSignificant value. B3GNT6, β 1,3-*N*-acetylglucosaminyltransferase 6; dCCA, distal cholangiocarcinoma; N.A., not applicable.

Table SII. Associations between G8 expression and MECA-79 expression.

A, Invasive cancer cells

G8 expression	MECA-79		Total	P-value
	Positive	Negative		
Positive	27	10	37	0.146
Negative	42	32	74	
Total	69	42	111	

B, Non-invasive cancer cells

G8 expression	MECA-79		Total	P-value
	Positive	Negative		
Positive	29	30	59	1.000
Negative	29	30	59	
Total	58	60	118	

C, Non-invasive or invasive cancer cells

G8 expression	MECA-79		Total	P-value
	Positive	Negative		
Positive	46	19	65	0.841
Negative	36	17	53	
Total	82	36	118	