Figure S1. Wnt5a expression in liver cancer cell lines. (A) Expression levels of Wnt5a in liver cancer cell lines (HLE, HLF, HepG2 and Huh7) were examined by western blotting. (B) Expression levels of Wnt5a in relation to GAPDH expression as a control. The intensity ratio of expression to GAPDH was 20.2% in HLE, 23.5% in HLF, 40.2% in HepG2 and 85.5% in Huh7 cells. Wnt5a expression was lower in poorly-differentiated HLE and HLF cells than in well-differentiated HepG2 and Huh7 cells.

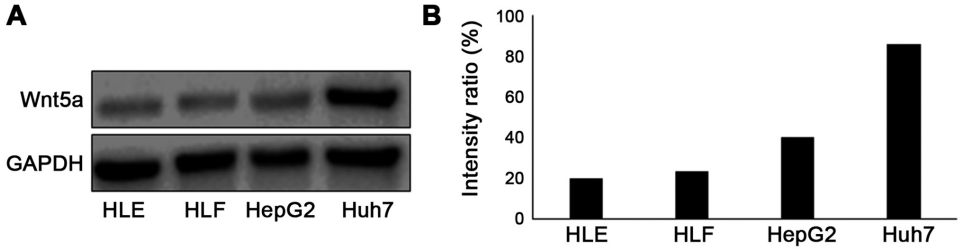

Figure S2. Relative changes of SNAIL, ZEB and TWIST families following overexpression of Wnt5a in HLF cells were examined using a DNA microarray. The expression levels of SNAI2 and SNAI3 were decreased following overexpression of Wnt5a. SNAI, snail family transcriptional repressor; TWIST, twist family bHLH transcription factor; ZEB, zinc finger E-box binding homeobox.

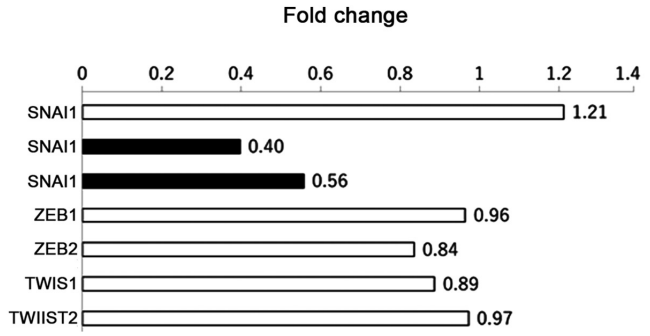

Figure S3. Relative changes of the MMP family following overexpression of Wnt5a in HLF cells were examined using a DNA microarray. The expression levels of MMP2, MMP7, MMP9, MMP10 and MMP23B were decreased following overexpression of Wnt5a. MMP, matrix metalloproteinase.

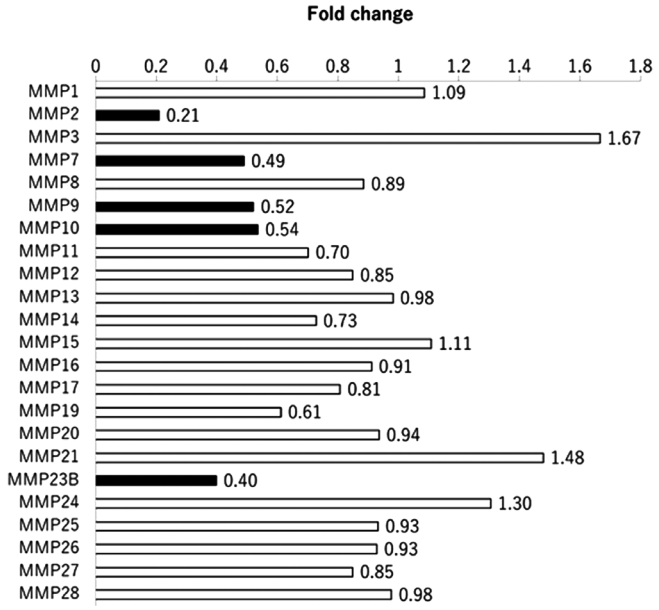

Figure S4. Survival analysis by cytoplasmic Wnt5a expression. (A) OS and (B) RFS curves were generated using the Kaplan-Meier method. OS in the cytoplasmic Wnt5a-negative group was significantly poorer compared with that in the cytoplasmic Wnt5a-positive group (P=0.018). There was no significant difference between the groups for RFS (P=0.661). (C) Survival analysis using the combination of cytoplasmic and membrane staining patterns. OS in the cytoplasmic and membrane-negative group was significantly poorer compared with that in the positive group (P=0.005). There was no significant difference between the other groups. OS, overall survival; RFS, relapse-free survival.

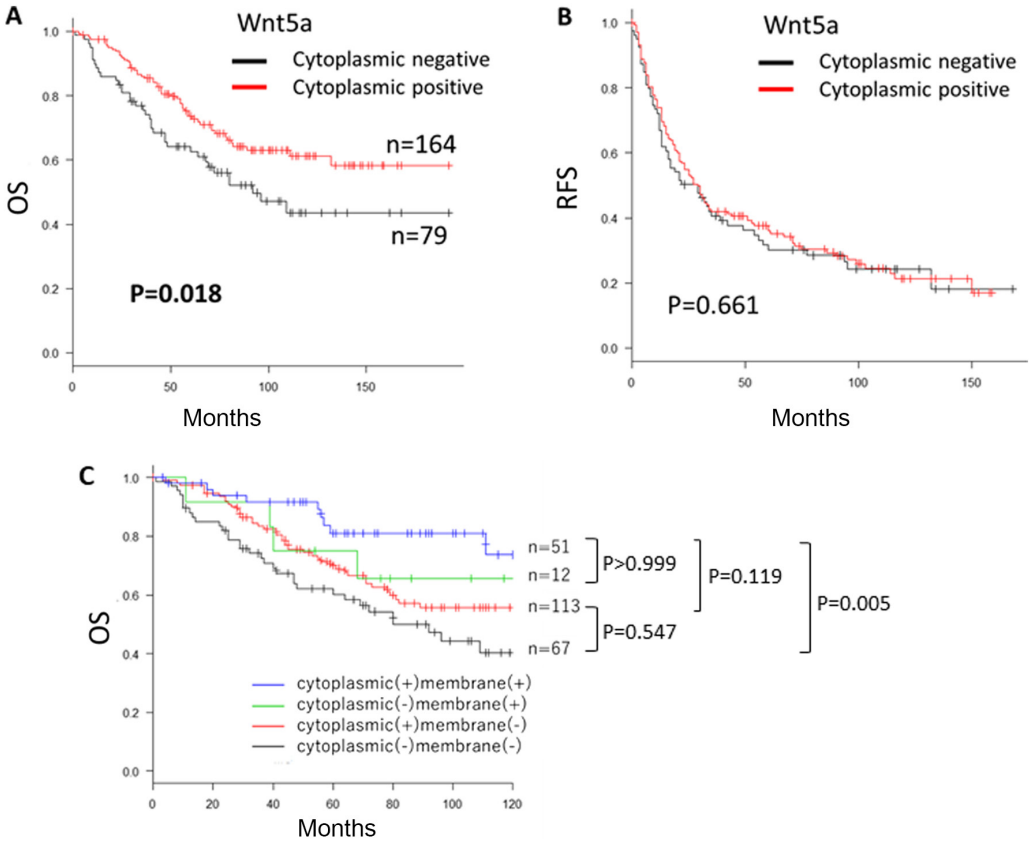

Figure S5. Relative Wnt5a mRNA expression in HLE, HLF, HepG2 and Huh7 cells was examined by reverse transcription-quantitative PCR. The expression levels were compared by the comparative threshold cycle method using GAPDH as an internal control. There was no association between mRNA and protein expression of Wnt5a.

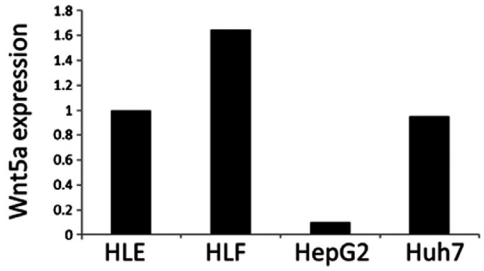

Table SI. Recurrence patterns and its management in Wnt5a-negative (n=133) and Wnt5a-positive (n=38) patients.

	Wnt5a expression		
Variable	Negative	Positive	P-value
Location, n (%)			
Intrahepatic	107 (80.5)	32 (84.2)	0.813
Extrahepatic	16 (12.0)	4 (10.5)	0.518
Both	9 (6.8)	2 (5.3)	>0.999
Intrahepatic recurrence			
Tumor number, n	1.97±2.19	1.68±1.19	0.481
Tumor diameter, cm	1.75±0.99	1.67±0.70	0.668
Management, n (%)			
TACE	46 (35.9)	16 (44.4)	0.437
RFA (MCT, PEIT)	38 (29.7)	9 (25.0)	0.679
Resection	19 (14.8)	6 (16.7)	0.795
Metastatic resection	13 (10.2)	3 (8.3)	>0.999
Chemotherapy	8 (6.3)	1 (2.8)	0.685
BSC	4 (3.1)	0 (0.0)	0.577
Radiotherapy	0 (0.0)	1 (2.8)	0.220

TACE, transcatheter arterial chemoembolization; RFA, radiofrequency ablation; MCT, microwave coagulation therapy; PEIT, percutaneous ethanol injection therapy; BSC, best supportive care.

Table SII. Association between cytoplasmic expression levels of Wnt5a and clinicopathological characteristics.

Characteristics	Wnt5a expression		P-value
	Negative, n (n=79)	Positive, n (n=164)	
Sex			
Male	62	138	0.286
Female	17	26	
Age, years			
≤60	35	67	0.678
>60	44	97	
HBV			
Negative	45	100	0.576
Positive	34	64	
HCV			
Negative	45	98	0.679
Positive	34	66	
Albumin, g/dl			
<4	24	72	0.050
≥4	55	92	
AFP, ng/ml			
≤10	31	96	0.009[a]
>10	46	68	
PIVKAII, mAU/ml			
≤40	36	65	0.402
>40	41	95	
Differentiation			
Well-moderate	52	142	<0.001[a]
Poor	27	22	
Tumor number			
Solitary	68	119	0.023[a]
Multiple	11	45	
Tumor size, cm			
≤5	51	114	0.464
>5	28	50	
Vascular invasion			
Negative	60	144	0.025[a]
Positive	19	20	
Non-cancerous liver			
Non-cirrhosis	52	105	0.886
Cirrhosis	27	59	

[a]P<0.05. HBV, hepatitis B virus; HCV, hepatitis C virus; NBNC, non-hepatitis B virus and non-hepatitis C virus; AFP, α-fetoprotein; PIVKAII, protein induced by vitamin K absence or antagonist-II.

Table SIII. Univariate and multivariate analysis of prognostic factors for overall survival.

Characteristics	Univariate analysis			Multivariate analysis		
	HR	95% CI	P-value	HR	95% CI	P-value
Sex (male vs. female)	1.030	0.581-1.823	0.921			
Age (>60 vs. ≤60 years)	1.426	0.926-2.196	0.107			
HBV (positive vs. negative)	0.873	0.569-1.339	0.533			
HCV (positive vs. negative)	1.132	0.743-1.724	0.565			
AFP (>10 vs. ≤10 ng/ml)	1.668	1.090-2.553	0.018[a]	1.447	0.923-2.270	0.108
PIVKAII (>40 vs. ≤40 mAU/ml)	1.570	1.002-2.459	0.049[a]	1.546	0.931-2.566	0.092
Albumin (<4 vs. ≤4 g/dl)	1.826	1.204-2.770	0.005[a]	1.554	0.991-2.437	0.055
Tumor number (multiple vs. solitary)	1.824	1.134-2.932	0.013[a]	2.040	1.236-3.367	0.005[a]
Tumor size (>5 vs. ≤5 cm)	2.110	1.384-3.218	0.001[a]	1.673	1.016-2.753	0.043[a]
Vascular invasion (positive vs. negative)	2.630	1.645-4.205	<0.001[a]	2.351	1.375-4.020	0.002[a]
Differentiation (poor vs. well + moderate)	1.361	0.826-2.241	0.226			
Non-cancerous liver (cirrhosis vs. non-cirrhosis)	1.721	1.130-2.623	0.012[a]	1.566	1.005-2.440	0.047[a]
Wnt5a (cytoplasmic negative vs. cytoplasmic positive)	1.656	1.086-2.525	0.019[a]	1.520	0.953-2.422	0.078

[a]P<0.05. HBV, hepatitis B virus; HCV, hepatitis C virus; NBNC, non-hepatitis B virus and non-hepatitis C virus; AFP, α-fetoprotein; PIVKAII, protein induced by vitamin K absence or antagonist-II; HR, hazard ratio.