Supplementary

Table S1 Sequences of miR-552-3p mimic and RGMA siRNA

Primers	Sequences (5'-3')
MiR-552-3p mimic	micrON hsa-miR-552-3p mimic (#miR1151102104642-1-5, RIBOBIO)
MiR-552-3p mimic negative control	micrON mimic NC (#miR1N0000001-1-5, RIBOBIO)
RGMA siRNA-Forward	CCGCUCAUCGACAAUAAUUTT
RGMA siRNA-Reverse	AAUUAUUGUCGAUGAGCGGTT
RGMA siRNA negative control-Forward	UUCUCCGAACGUGUCACGUTT
RGMA siRNA negative control-Reverse	ACGUGACACGUUCGGAGAATT

Table S2 Sequences for quantitative real-time PCR

Primers	Sequences (5'-3')	
has-miR-552-3P (bulge-loop RT primer, Forward primer, Reverse primer)	Bulge-loop hsa-miR-552-3p primer set (#MQPS0000002-1-100, RIBOBIO)	
U6 (bulge-loop RT primer, forward primer, reverse primer)	Bulge-loop U6 qPCR primer set (#MQPS0001858-1-100, RIBOBIO)	
RGMA-forward	AACCAGCAGATCGACTTCCAG	
RGMA-reverse	ACGGCTGTCTCGTATGGGA	
ABCG2-forward	CGCACAGAGCAAAGCCATTT	
ABCG2-reverse	GCAAGGGGCTAGAAGAAGGG	
MDR1-forward	CCTAGGAGTACTCACTTCAGGA	
MDR1-reverse	CCAATCAGCCTCACCACAGA	
CD34-forward	AGGAGAAAGGCTGGGCGAAG	
CD34-reverse	GTTGTCTTGCTGAATGGCCG	
OCT4-forward	CTTGCTGCAGAAGTGGGTGGAGGAA	
OCT4-reverse	CTGCAGTGTGGGTTTCGGGCA	
GAPDH (glyceraldehyde-3-phosphate dehydrogenase)- forward	CATGAGAAGTATGACAACAGCCT	
GAPDH-reverse	AGTCCTTCCACGATACCAAAGT	



Figure S1 The expressions of miR-552-3p in GBC spheroids. (A) The expression of miR-552-3p in GBC spheroids and attached cells was examined by qRT-PCR; (B) SGC-996 and GBC-SD cells were infected with miR-552-3p knockdown virus, and the expression of miR-552-3p was detected by qRT-PCR; (C) EdU immunofluorescence staining was used to detect the proliferation of miR-552-3p knockdown and control GBC cells (dyeing with EdU & DAPI; scale bar =50 μ m); (D) the migratory ability of miR-552-3p knockdown or overexpression in GBC cells was measured by wound-healing assay with mitomycin C (10 μ M mitomycin C, GlpBio, #GC12353, USA) for 72 h in medium containing 5% serum (scale bar =500 μ m). *, P<0.05. GBC, gallbladder cancer.



Figure S2 The relationship between miR-552-3p and RGMA. (A) The expression of RGMA in nontumor tissues and tumor tissues was detected by immunohistochemistry (scale bar =100 μ m); (B) correlation between miR-552-3p vs. RGMA in several tumors. RGMA, repulsive guidance molecule BMP co-receptor a.

Variable	Category	Association of miR-552-3p expression		
		Low (n=20)	High (n=21)	- P value
Age (years)	<60	6	9	0.393
	≥60	14	12	
Gender	Male	7	10	0.412
	Female	13	11	
Tumor size (cm)	<3	12	6	0.043*
	≥3	8	15	
Histological differentiation	Well or moderate	16	14	0.336
	Poor	4	7	
Lymph node metastasis	No	8	10	0.623
	Yes	12	11	
Tumor invasion (AJCC 8th)	Tis-T2	9	7	0.444
	T3-T4	11	14	
Liver metastasis	No	11	5	0.041*
	Yes	9	16	

Table S3 Association of miR-552-3p expression with the clinicopathological features of the GBC patients

*, P<0.05. AJCC, American Joint Committee on Cancer; GBC, gallbladder cancer.