OMTN, Volume 18

Supplemental Information

IncRNA MAGI2-AS3 Prevents the Development

of HCC via Recruiting KDM1A and Promoting

H3K4me2 Demethylation of the RACGAP1 Promoter

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Inhibition of MAGI2-AS3 induces proliferation, migration and invasion and inhibits apoptosis of HCCLM3 cells

RT-qPCR determination presented that the expression of MAGI2-AS3 in HCCLM3 cells decreased after infection with lentivirus expressing sh-MAGI2-AS3 (Supplementary Figure 1A, p < 0.05). The results of EdU assay showed that the number of EdU positive cells increased remarkably after MAGI2-AS3 silencing (Supplementary Figure 1B, p < 0.05). TUNEL staining showed that the number of apoptotic cells was reduced dramatically after MAGI2-AS3 silencing (Supplementary Figure 1C, p < 0.05). Transwell migration and invasion assays showed that the numbers of migrated and invaded cells were elevated after MAGI2-AS3 silencing (Supplementary Figure 1D and 1E, p < 0.05). These results suggest that knockdown of MAGI2-AS3 could accelerate the proliferating, migrating and invading capabilities but inhibit apoptosis of HCCLM3 cells.



Supplementary Figure 1. Suppression of MAGI2-AS3 enhances the proliferative, migrating and invading HCCLM3 cells but reduce apoptotic HCCLM3 cells. HCCLM3 cells were infected with lentivirus harboring sh-MAGI2-AS3 or sh-NC. (A) The expression of MAGI2-AS3 was determined by RT-qPCR following infection. (B) The proliferation of HCCLM3 cells was evaluated by EdU staining following infection (× 200). (C) TUNEL staining assessed the apoptosis of HCCLM3 cells following infection (× 200). (D, E) Cell migration and invasion assessed by Transwell assay (× 200). The experiments were repeated 3 times independently; * p < 0.05, vs. the sh-NC group, data were analyzed using unpaired *t*-test.

<u>patients</u>			
Characteristics	Patients $(n = 70)$	MAGI2-AS3	р
Age (years)			0.212
\leq 50	31	0.326 ± 0.037	
> 50	27	0.311 ± 0.053	
Gender			0.716
Male	49	0.318 ± 0.043	
Female	9	0.324 ± 0.057	
Tumor size (cm)			0.023
\geq 5	12	0.293 ± 0.058	
< 5	46	0.326 ± 0.039	
TNM stage			0.048
Ι	16	0.341 ± 0.034	
II	24	0.316 ± 0.045	
III	18	0.304 ± 0.048	
Cirrhosis			0.764
Yes	16	0.322 ± 0.055	
No	42	0.318 ± 0.041	
Hepatitis (HBV or HCV)			0.246
Negative	32	0.325 ± 0.049	
Positive	26	0.311 ± 0.040	
Lymph node metastasis			< 0.001
Yes	19	0.270 ± 0.032	
No	39	0.343 ± 0.028	

Supplementary Table 1 Associations between MAGI2-AS3 expression and characteristics of HCC patients

Notes: HCC, hepatocellular carcinoma; TNM, tumor lymph node metastasis; HBV, hepatitis B virus; HCV, hepatitis C virus.

Supplementary Table 2 Primer sequences for RT-qPCR		
Gene	Sequence	
MAGI2-AS3	F: 5'-CACCTTGCTTGACAACTTGA-3'	
	R: 5'-CATTACAGCTCGGCTACTGC-3'	
β-actin	F: 5'-GGGACCCTTCAGAACCAA-3'	
	R: 5'-TAGCCTGATGCTCGAATCCT-3'	
RACGAP1	F: 5'-TGATGGTGGAGCAAGAG-3'	
	R: 5'-GGGAAGTAACAGGCAGAT-3'	

Notes: RT-qPCR, reverse transcription-quantitative polymerase chain reaction; MAGI2-AS3, MAGI2 antisense RNA 3; RACGAP1, Rac GTPase-activating protein 1; F, forward; R, reverse.