Down-regulated CMTM7 promotes metastasis of hepatocellular carcinoma via its family member CMTM3

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Abstract

Background

Human chemokine-like factor (CKLF)-like MARVEL transmembrane domain-containing family (CMTM) members are found deregulated in various cancers and play a role in cancer development. Recent studies showed that CMTM7 was down-regulated in liver cancer and functioned as a tumor suppressor.

Methods

The expression of CMTM7 in hepatocellular carcinoma (HCC) tissues was measured by bioinformatics analysis, Western blot, and quantitative reverse-transcription polymerase chain reaction (qRT-PCR). CCK8 and cell colony formation assays were conducted to detect the cell proliferation. Cell invasion and migration ability was assessed by wound healing and Transwell assays.

Results

We identified a decreased expression of CMTM7 in HCC tissues was closely related to the metastasis and prognosis of HCC patients. To explore the effect of CMTM7 on the biological function of HCC cells, we over-expressed CMTM7 in two HCC cell lines, SK-Hep-1 and Bel-7402 cells. Cell proliferation was reduced significantly after exogenous expression of CMTM7, detected by both CCK8 and colony formation assay. Moreover, the invasion and migration ability of HCC cells were also inhibited by exogenous expression of CMTM7 in wound healing and Transwell assay. Furthermore, there was a significantly positive correlation between CMTM7 and CMTM3 both in HCC cells and tumor tissues.

Conclusions

Our results suggest that down-regulated CMTM7 promotes metastasis of HCC through inducing the expression of its family member CMTM3.

Background

Hepatocellular carcinoma (HCC) is a complicated pathological process involved various factors and genes. That’s why the etiology of HCC has not been well clarified at present. Though surgery is still the major treatment of HCC, the complicated etiology and occult onset cause the failure of diagnosis and treatment in most HCC patients. Moreover, the recurrence rate of HCC patients undergoing radical surgery within 5 years is still as high as 60%-70% [1]. Therefore, how to find out the early biological behaviors related with HCC metastasis and recurrence becomes a critical event to enhance the treatment and prognosis of HCC patients.

Many new molecular markers have been found and applied for early diagnosis and treatment of tumors, such as human chemokine-like factor (CKLF)-like MARVEL transmembrane domain-containing family (CMTM) members, which were first proposed in 2001 and characterized by the MARVEL domain [2, 3]. Accumulated evidences have shown that CMTM members play a critical role in cancer development and progression [4–6]. Our previous studies also have found that CMTM4, CMTM6, and CMTM7 were down-regulated in HCC tissues by immunohistochemistry, and had a relationship with the poor prognosis of HCC patients after survival analysis, suggesting a tumor suppressor role of CMTM members in HCC carcinogenesis and progression [7–9].

As a CMTM family member, the CMTM7 gene locates at chromosome 3p22.3, which is a region rich of tumor suppressor genes. CMTM7 expressed widely in normal tissues of human, especially in testis, white blood cells, and spleen. It has been reported that CMTM7 is down-regulated in some tumors, such as gastric cancer, esophageal cancer and non-small cell lung cancer (NSCLC) [10–12]. CMTM7 gene can be methylated at the promoter region that results in down-regulation of CMTM7 in some tumor cells [10]. A recent study showed that the expression of CMTM7 in gastric cancer was regulated by a transcriptional regulator, SOX10 [13]. Furthermore, stable knockdown of CMTM7 was found to improve the malignant ability of NSCLC cells, decrease EGFR internalization and degradation to enhance EGFR-AKT signaling [11]. Though the exact role of CMTM7 in HCC has not been well understood, these studies suggest that CMTM7 has potential value in early diagnosis and treatment of HCC.

Based on previous studies, we performed cell proliferation, wound healing, invasion, and migration assays to explore the biological function of CMTM7 in HCC cells by exogenous expression of CMTM7. We aim to clarify the potential role of CMTM7 in the metastasis and progression of HCC and provide a novel biomarker for early diagnosis and treatment of HCC.

Materials And Methods

Bioinformatics analysis

According to the previous method [14], the expression data of CMTM7 in HCC tissues was searched from the National Center for Biotechnology Information Gene Expression Omnibus (NCBI GEO) and the Cancer Genome Atlas (TCGA) database via the cBioPortal platform (http://www.cbioportal.org/).

Tissue samples

Paired HCC and adjacent non-tumor tissues for Western blot and quantitative reverse-transcription polymerase chain reaction (qRT-PCR) test were collected from the Affiliated Hospital of Guilin Medical University between 2015 and 2016. All tissue samples were obtained from HCC patients, underwent surgery without any radiotherapy and chemotherapy, and preserved in liquid nitrogen immediately after surgery. The clinicopathological features of these patients were shown in Table 1. This study was conducted under the informed consent from all patients and approval from the Ethics Committee of the hospital (GLMC2014003).

Cell lines and plasmids

Human hepatocytes (L02) were purchased from the Cell Bank (Chinese Academy of Sciences, Shanghai, China), and hepatic tumor cell lines (SMMC-7721, HCC-97H, HCC-LM3, SK-Hep-1, Huh-7, Bel-7402and HepG2) were purchased from ATCC (Manassas, VA, USA). The cell lines were all grown in a cell incubator at 37℃ with a 5% CO_2/95% air atmosphere.

A lentiviral wild-type CMTM7 plasmid was synthesized from a GV367-puro vector by Genechem (Shanghai, China). According to the manufacturers' instructions, Bel-7402 and SK-Hep-1 cells with over-expressed CMTM7 were constructed by lentiviral infection and puromycin screening.

Western blot

Protein was extracted from cells or powdered tissues using RIPA buffer from Beyotime (Shanghai, China) and quantified. And then the soluble protein was resolved by SDS-PAGE and transferred to PVDF membranes for Western blot using ECL detection reagents. CMTM7 antibody (PA5-103744) was obtained from Invitrogen (Carlsbad, CA, USA).

Quantitative reverse-transcription polymerase chain reaction (qRT-PCR)

Trizol was used to extract total RNA and reverse transcription reagents were used to reverse RNA to cDNA. Then, PCR reaction was started after SYBR Green Mixture (Tiangen, Beijing, China) was added according to the manufacture's procedure. QRT-PCR primers were showed in Supplementary Table 1.

CCK8 assay

CCK8 assay was conducted to detect cell proliferation using a Kit from Beyotime. 5×10^3 cells/well were seeded in triplicate in 96-well plates. After 12 h, 24 h, 48 h, 72 h, and 96 h of culture, cells were treated with CCK8 and detected for OD values at 450 nm .

Formation of cell colony

800 cells/well were grown in a 6-well plate in triplicate and cultured for 2 weeks. Then the plate was fixed for 20 min in 4% poly-formaldehyde and stained overnight with crystal violet. After washed, the cell colony was photographed and counted under a microscope.

Cell scratch detection

When cells in 6-well plates grown at a monolayer, cells were scratched by 10 μl micropipette tips and cultured with fresh serum-free medium. After 0 h, 24 h, and 48 h from scratch, cells were photographed and measured for the migration length.

Transwell assay

Cell invasion and migration ability was detected by Transwell assay. The chamber with 8 μm aperture was put in a 24-well plate with medium and activated in an incubator for 30 min. 100 μl serum-free medium of 2×10^4 cells and 600 μl medium with 10% FBS were then added to the upper chamber and the lower chamber, respectively. The chamber was incubated for 30 h, and then the cells were fixed and stained. After cleaned by PBS, cells were photographed and counted under a microscope.

Statistical analysis

All the data was analyzed on SPSS version 19.0. Chi-square and *t* tests were applied for the comparison of qualitative data and quantitative data between two groups, respectively. $P<0.05$ was defined as statistical significant for all tests.

Results

CMTM7 is down-regulated in HCC tissues

We previously measured CMTM7 expression in 75 paired HCC and adjacent non-tumor tissues by immunohistochemistry and found that the positive expression of CMTM7 was significantly lower in HCC tissues than that of CMTM7 in adjacent non-tumor tissues [8]. We also conducted bioinformatics analysis to check CMTM7 expression in HCC tissues from GEO and TCGA databases. As shown in Figure 1A, we found a lower expression of CMTM7 in HCC tissues than normal liver tissues in GSE3500 ($P<0.001$), but CMTM7 expression had no difference between normal liver tissues and HCC tissues in TCGA database.

To further confirm the expression of CMTM7 in HCC tissues, we chose another 20 paired HCC and adjacent non-tumor tissues to perform Western blot. As Figure 1B shown, down-regulated CMTM7 was determined in 80% (16/20) of HCC tissues. We also detected significantly decreased mRNA expression of CMTM7 in 75.7% (53/70) of HCC tissues by qRT-PCR (Figure 1C). In consistent with the previous studies, we detect a down-regulated expression of CMTM7 in HCC tissues.

CMTM7 expression is correlated with the clinical characteristics of HCC patients

We first explored the relationship between CMTM7 expression and the clinical characteristics of HCC patients from online databases. It was found that the expression of CMTM7 was highly expressed in cirrhosis liver tissues than normal liver tissues in GSE6764 ($P<0.001$, Figure 2A). However, we found no difference between the copy number of CMTM7 in HCC tissues and normal liver tissues in GSE32649 (Figure 2B). The relationship between CMTM7 expression and the prognosis of HCC patients was also analyzed from the TCGA database by Kaplan-Meier method. As Figure 2C and 2D shown, the overall survival time of HCC patients in CMTM7 low expression group was significant shorter than that in CMTM7 high expression group both in five-year and ten-year of survival analysis ($P<0.05$).

We also collected clinical information from the 70 HCC patients for qRT-PCR detection to analyze the correlation between CMTM7 expression and the clinical characteristics of HCC patients. The 70 HCC patients were divided into CMTM7 high and low expression groups according to the qRT-PCR results (LgT/N≥0 means high expression and LgT/N<0 means low expression). The result showed that CMTM7 expression was significantly correlated with HCC metastasis (P<0.05), while had no relationship with other clinicopathological features of HCC patients (Table 1). These results suggest that CMTM7 expression had a relationship with HCC metastasis and prognosis.

Over-expression of CMTM7 inhibits HCC cell proliferation

In order to explore the biological functions of CMTM7 in HCC cells, we detected CMTM7 expression in seven cell lines, SMMC-7721, HCC-97H, HCC-LM3, SK-Hep-1, Bel-7402, Huh-7 and HepG2 by Western blot. Compared with the hepatocytes L02, the expression of CMTM7 was decreased significantly in SK-Hep-1, Bel-7402, Huh-7, and HepG2 cells (Figure 3A). There were only 20% of CMTM7 expression in SK-Hep-1 and 10% in Bel-7402 cells as compared with L02 cells. SK-Hep-1 and Bel-7402 cell lines were then selected for subsequent cell function test.

To next study the role of CMTM7 in cell proliferation, CMTM7 was over-expressed in SK-Hep-1 and Bel-7402 cell lines by exogenous introduction with a lentiviral wild-type CMTM7 plasmid (named as SK-Hep-1-CMTM7 and Bel-7402-CMTM7 cells). As shown in Figure 3B, CMTM7 was increased by 1.5 fold in Bel-7402 cells and 1 fold in SK-Hep-1 cells, compared with the negative control group (named as SK-Hep-1-Vector and Bel-7402-Vector cells). After CMTM7 was over-expressed in SK-Hep-1 and Bel-7402 cell lines, we detected the proliferation ability of these cells by conducting CCK8 and colony formation assay. We found that the proliferation ability of SK-Hep-1-CMTM7 and Bel-7402-CMTM7 cells was decreased significantly than SK-Hep-1-Vector and Bel-7402-Vector cells, respectively (Figure 3C and 3D, P<0.05). In addition, the colony formation test showed the result as same as the CCK8 assay (Figure 3E, P<0.01). These results suggest that over-expressed CMTM7 inhibits the proliferation of HCC cells.

The function of CMTM7 in HCC cell metastasis

To find out whether CMTM7 plays a role in HCC cell metastasis, we performed a wound healing assay to measure the metastatic ability of SK-Hep-1-CMTM7 and Bel-7402-CMTM7 cells (Figure 4A and 4B). As compared with SK-Hep-1-Vector and Bel-7402-Vector cells, the cell migration distance was wider in SK-Hep-1-CMTM7 and Bel-7402-CMTM7 cells after 48 h of wound (P<0.01).

Cell invasion and migration were the major features of most malignant tumors. Transwell assay was performed to detect the invasion and migration ability of HCC cells after exogenous expression of CMTM7. As shown in Figure 4C and 4D, CMTM7 over-expression caused an obvious reduced invasion and migration of Bel-7402 cells and SK-Hep-1 cells (P<0.01). These results show the exogenous expression of CMTM7 disturbs the metastasis of HCC cells, further support that CMTM7 expression is correlated with tumor metastasis of HCC patients.

Over-expression of CMTM7 inhibits HCC metastasis via up-regulating CMTM3

Epithelial mesenchymal transition (EMT) is a critical process in HCC metastasis [15]. To determine the role of CMTM7 in HCC metastasis, the expression of EMT factors were measured in CMTM7 over-expressed SK-Hep-1 cells. As compared to SK-Hep-1-Vector cells, the epithelial markers E-cadherin and β-catenin in SK-Hep-1-CMTM7 cells were induced by 100%±32% and 281%±72%, while the mesenchymal markers N-cadherin and Vimentin in SK-Hep-1-CMTM7 cells were redued by 47%±11% and 40%±12%, respectively (Figure 5A). These results show an inhibitory role of over-expressed CMTM7 in the EMT process.

To further explore the mechanism of CMTM7 in HCC metastasis, we next searched CMTM7 co-expressed genes in TCGA liver cancer data. As shown in Figure 5B, of the topmost 10 positively and 10 negatively CMTM7-correlated genes, a CMTM family member, CMTM3 attracted our interest. We detected the expression of CMTM3 in CMTM7 over-expressed SK-Hep-1 cells and found that CMTM3 was increased by 35%±15% than SK-Hep-1-Vector cells (Figure 5A). Moreover, in the same 30 paired HCC and adjacent non-tumor tissues as CMTM7, the mRNA expression of CMTM3 was down-regulated in 73.3% (22/30) of HCC tissues by qRT-PCR (Figure 5C). In addition, CMTM3 also had a relationship with metastasis of HCC patients (P<0.05, Table 2). In consistent with the results of HCC cells, we found a significantly positive correlation between CMTM7 and CMTM3 in HCC tissues (r=0.489, P<0.01, Table 3). These results show that down-regulated expression of CMTM7 might promote HCC metastasis via its family member CMTM3.

Discussion

In this study, after confirmation of down-regulated CMTM7 in HCC tissues by bioinformatics analysis, Western blot, and qRT-PCR, we used exogenous introduction to increase the expression of CMTM7 in HCC cells. As a result, the up-regulation of CMTM7 inhibited HCC cell proliferation, invasion, and migration, suggesting a suppressor role of CMTM7 in HCC pathogenesis and progression. Moreover, we found a significantly positive correlation between CMTM7 and CMTM3 both in HCC cells and tumor tissues, further supporting that there was a combining effect between CMTM family members in HCC metastasis.

In recent years, the CMTM family members were found deregulated in tumors and acted as valuable tumor suppressor genes with critical roles in the immune, male reproduction, and hematopoietic systems. Studies have shown a close relationship between CMTM family members and tumor carcinogenesis [5, 16, 17]. Importantly, in a genome-wide CRISPR–Cas9 screening, CMTM6 was identified as a key regulator of PD-L1 in many kinds of cancer cells. And CMTM6 can protect PD-L1 from being targeted for lysosomal degradation by association with PD-L1 at the plasma membrane and in recycling endosomes [5]. Moreover, the expression interference of CMTM6 can result in impaired PD-L1 protein expression in all tested human tumor cell types, and this function was shared by CMTM4 [6]. This is the first study showed that CMTM family members function together in cancer immunity and are proposed to have a significant clinical value in the diagnosis and treatment of tumors.

As a CMTM family member, CMTM7 is a newly discovered tumor suppressor gene, which is highly conserved and widely expressed in human normal tissues. However, the expression of CMTM7 was down-regulated or absent in most tumor tissues and cell lines [11]. It has been studied that down-regulated or absent CMTM7 was found in esophageal carcinoma because of promoter CpG methylation and loss of heterozygosity at the 3p22.3 locus [10]. Our previous study also found CMTM7 was down-regulated in HCC tissues by immunohistochemistry and had a correlation with the metastasis and prognosis of HCC patients [8]. A study showed that the decrease or absence of CMTM7 expression in lung cancer cell line A549 could inhibit the activation of Rab5 and the internalization of EGFR to enhance tumor cell proliferation, invasion and migration through activating the downstream PI3K/AKT signaling pathway [11]. In addition, another study has reported CMTM7 implicated in BCR expression and survival in B-1a cells [18]. Consistent with these studies, especially a recent study by Huang ZM et al., [19] CMTM7 exogenous expression in HCC cells significantly decreased cell proliferation and invasion, further confirmed the relationship between CMTM7 and tumor metastasis in HCC tissues.

As the same as CMTM7, CMTM3 is also found as tumor suppressor in gastric cancer, prostate cancer, and clear cell renal cell carcinoma [20–22]. CMTM3 locates at 16q22.1, and can be significantly methylated at the promoter detected in colorectal cancer and laryngeal squamous cell carcinoma [23, 24]. Over-expression of CMTM3 was reported to inhibit the growth and migration of oral squamous cell carcinoma cells, and HCC cells [25, 26]. While knockdown of CMTM3 was found to enhance migration and invasion of PC3 cells and LNCaP cells [21, 27]. In addition, CMTM3 can suppress epidermal growth factor (EGF)-mediated migration and decrease EGFR expression in gastric cancer [28]. Though locates at different chromosomes, CMTM3 shares so many roles the same as CMTM7 in cancer development and progression. Our study also explored a positive correlation between CMTM7 and CMTM3 in HCC tissues and cells, which might provide some clues for HCC treatment by targeting CMTM family members.

Conclusions

In conclusion, we report for the first time that down-regulated CMTM7 promotes the metastasis of HCC via CMTM3. Further study is needed to clarify the exact regulation between CMTM7 and CMTM3 in HCC progression to support the application of CMTM7 as a new biomarker in the diagnosis and treatment of HCC.

Declarations

ethics approval and consent to participate

This study was approved by the Institutional Research Ethics Committee of Guilin Medical University. Informed consents were obtained from all patients.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analyzed during this study are included in this published article and its supplementary information files.

Competing interests

The authors declare no conflict of interest.

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Authors' contributions

XNZ, CHB and SKT made contributions to the conception and design of the study. DL, HXZ and WZ analyzed the data and wrote the first draft of the article. All the authors read and approved the final manuscript.

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Tables

Table 1. Correlation between CMTM7 expression and clinic-pathological characteristics of HCC patients

Variables	Total	CMTM7 expression		χ^2 value	*P* value
		Low	High		
Gender					
Male	47	36	11	0.060	0.806
Female	23	17	6		
Age-yr					
≥50	32	24	8	0.016	0.898
50	38	29	9		
Alcohol intake					
No	39	28	11	0.736	0.391
Yes	31	25	6		
HCC family history					
No	62	48	14	0.238	0.625
Yes	8	5	3		
HBV infection					
No	25	21	4	1.452	0.228
Yes	35	32	13		
Liver cirrhosis					
No	20	14	6	0.157	0.692
Yes	50	39	11		
AFP (ng/ml)					
≥20	39	28	11	0.736	0.391
20	31	25	6		
Tumor diameter (cm)					
≥5	37	27	10	0.321	0.571
5	33	26	7		
Tumor number					
1	42	31	11	0.207	0.649
1	28	22	6		
Tumor grade					
+	33	27	6	1.265	0.261
	37	26	11		
Tumor stage					
+	43	33	10	0.064	0.800
+	27	20	7		
Vascular invasion					
No	38	29	9	0.016	0.898
Yes	32	24	8		
Metastasis					
No	49	41	8	5.627	**0.018**

Yes	21	12	9

Notes: Bold values indicate significance. *P* value is based on the χ^2 test.

Abbreviations: AFP, α-fetoprotein.

Table 2. Correlation between CMTM3 expression and clinic-pathological characteristics of HCC patients

Variables	Total	CMTM3 expression		*P* value
		Low	High	
Gender				
Male	28	21	7	0.469
Female	2	1	1	
Age-yr				
≥50	18	13	5	1.000
50	12	9	3	
Alcohol intake				
No	20	15	5	1.000
Yes	10	7	3	
HCC family history				
No	27	19	8	0.545
Yes	3	3	0	
HBV infection				
No	5	4	1	1.000
Yes	25	18	7	
Liver cirrhosis				
No	12	9	3	1.000
Yes	18	13	5	
AFP (ng/ml)				
≥20	20	15	5	1.000
20	10	7	3	
Tumor diameter (cm)				
≥5	17	11	6	0.407
5	13	11	2	
Tumor number				
1	24	16	8	0.155
1	6	6	0	
Tumor grade				
+	14	11	3	0.689
	16	11	5	
Tumor stage				
+	22	17	5	0.643
+	8	5	3	
Vascular invasion				
No	19	16	3	0.068
Yes	9	4	5	
Metastasis				
No	26	21	5	**0.048**

Yes	4	3	1	

Notes: Bold values indicate significance. *P* value is based on Fisher's exact test.

Abbreviations: AFP, α-fetoprotein.

Table 3. The correlation between CMTM7 and CMTM3 in HCC tissues

Correlation		CMTM7		χ^2 value	r value	*P* value
		High	Low			
CMTM3	High	5	3	7.163	0.489	**0.007**
	Low	3	19			

Notes: Bold values indicate significance. *P* value is based on Spearman correlation test.

Figures

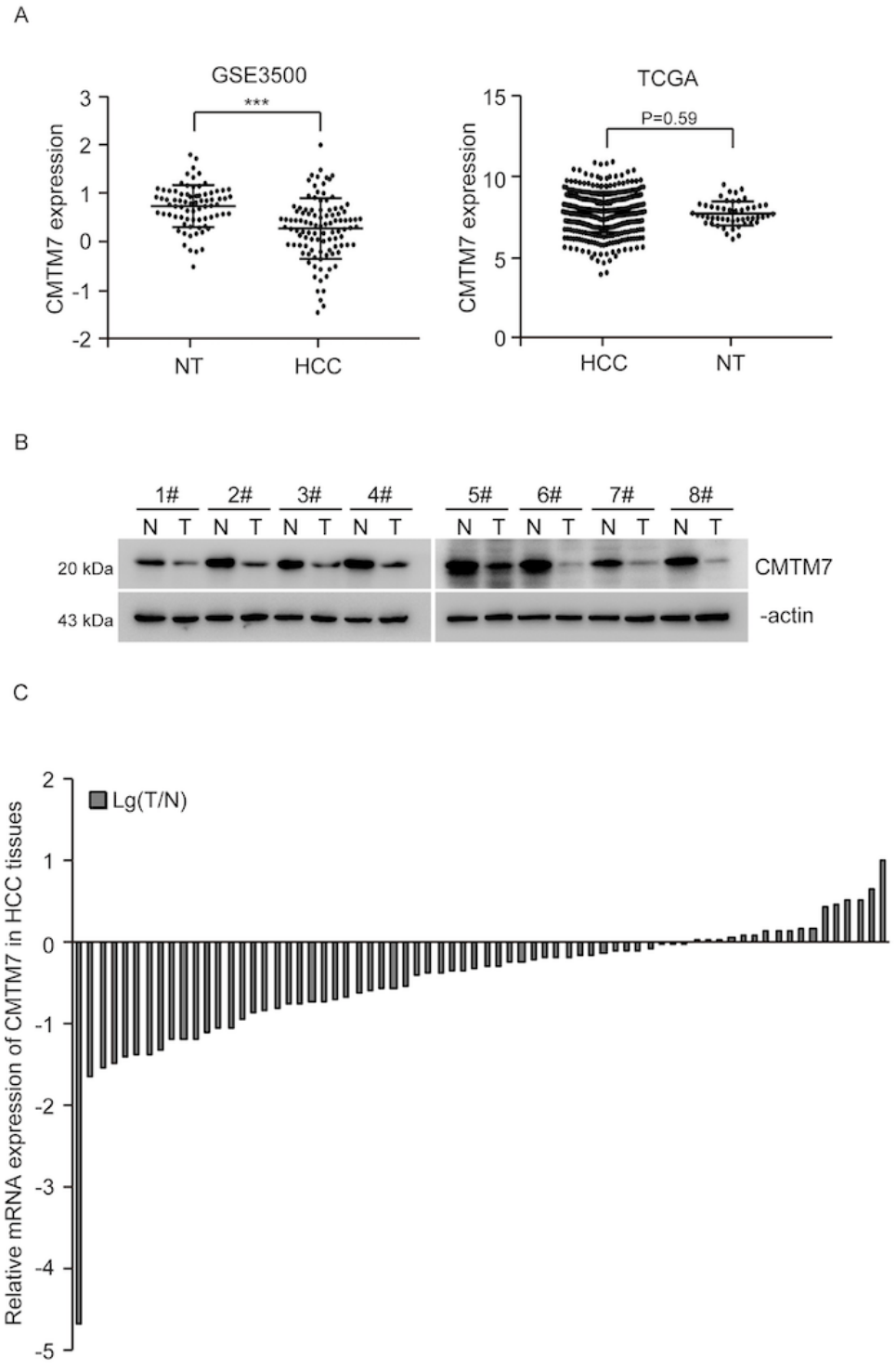

Figure 1

CMTM7 is down-regulated in HCC tissues. (A) Relative expression of CMTM7 in HCC and normal liver tissues (NT) from GSE3500 and TCGA database. (B) CMTM7 protein expression was analyzed in paired-HCC (T) and adjacent non-tumor tissues (N) by Western blot. (C) Relative mRNA expression of CMTM7 in 70 HCC tissues. Lg(T/N) is logarithmic transformation of the CMTM7 expression in paired-HCC (T) and adjacent non-tumor tissues (N) by qRT-PCR. ***, P<0.001 compared to normal liver tissues.

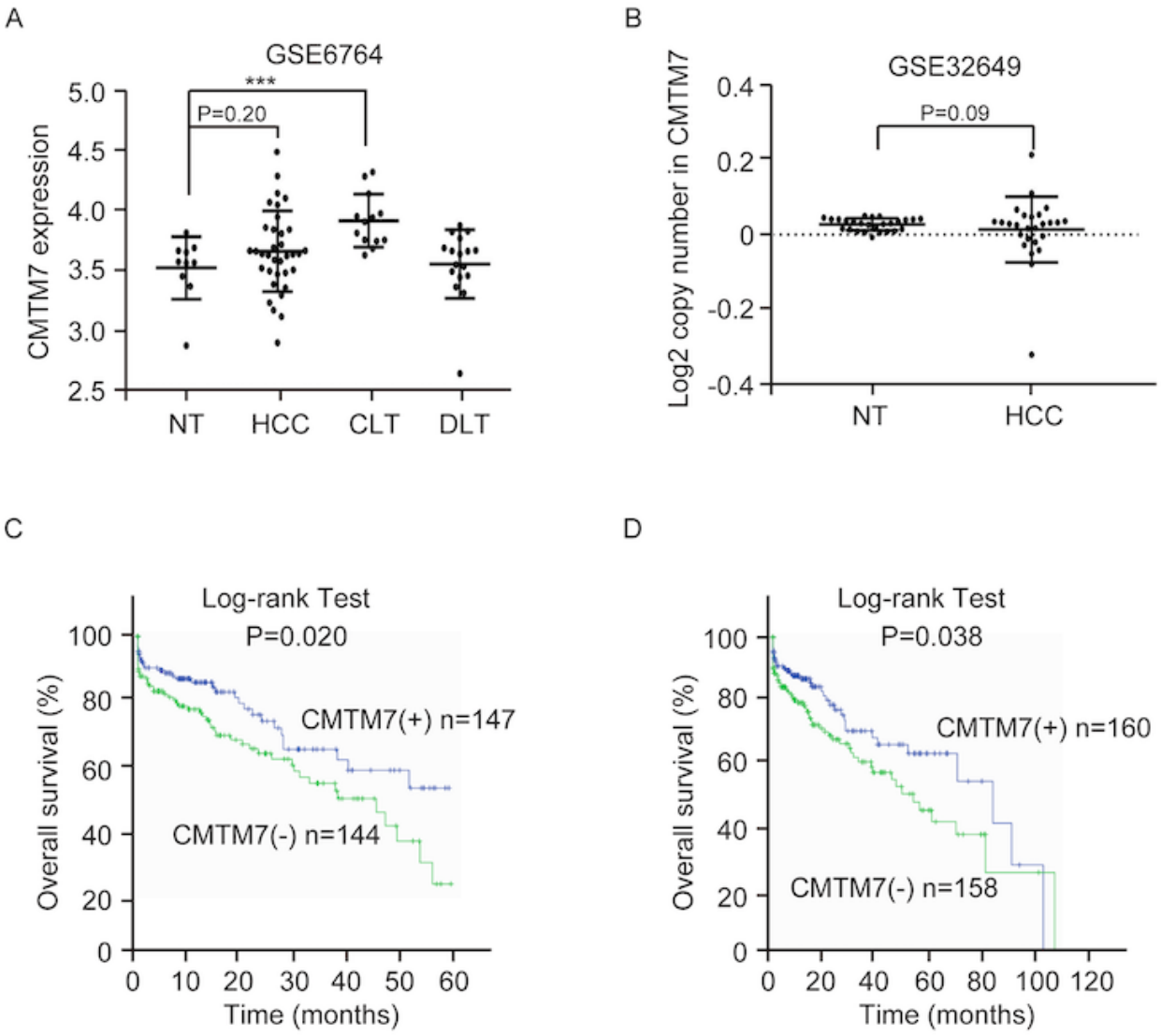

Figure 2

Correlation between CMTM7 expression and clinical characteristics of HCC patients. (A) Relative expression of CMTM7 in normal liver tissues (NT), HCC tissues, cirrhosis liver tissues (CLT) and dysplastic liver tissues (DLT) from GSE6764. (B) The copy number of CMTM7 in HCC tissues and normal liver tissues (NT). (C) Five-year and (D) ten-year of overall survival analysis for HCC patients between CMTM7 high and low expression groups. ***, P<0.001 compared to normal liver tissues.

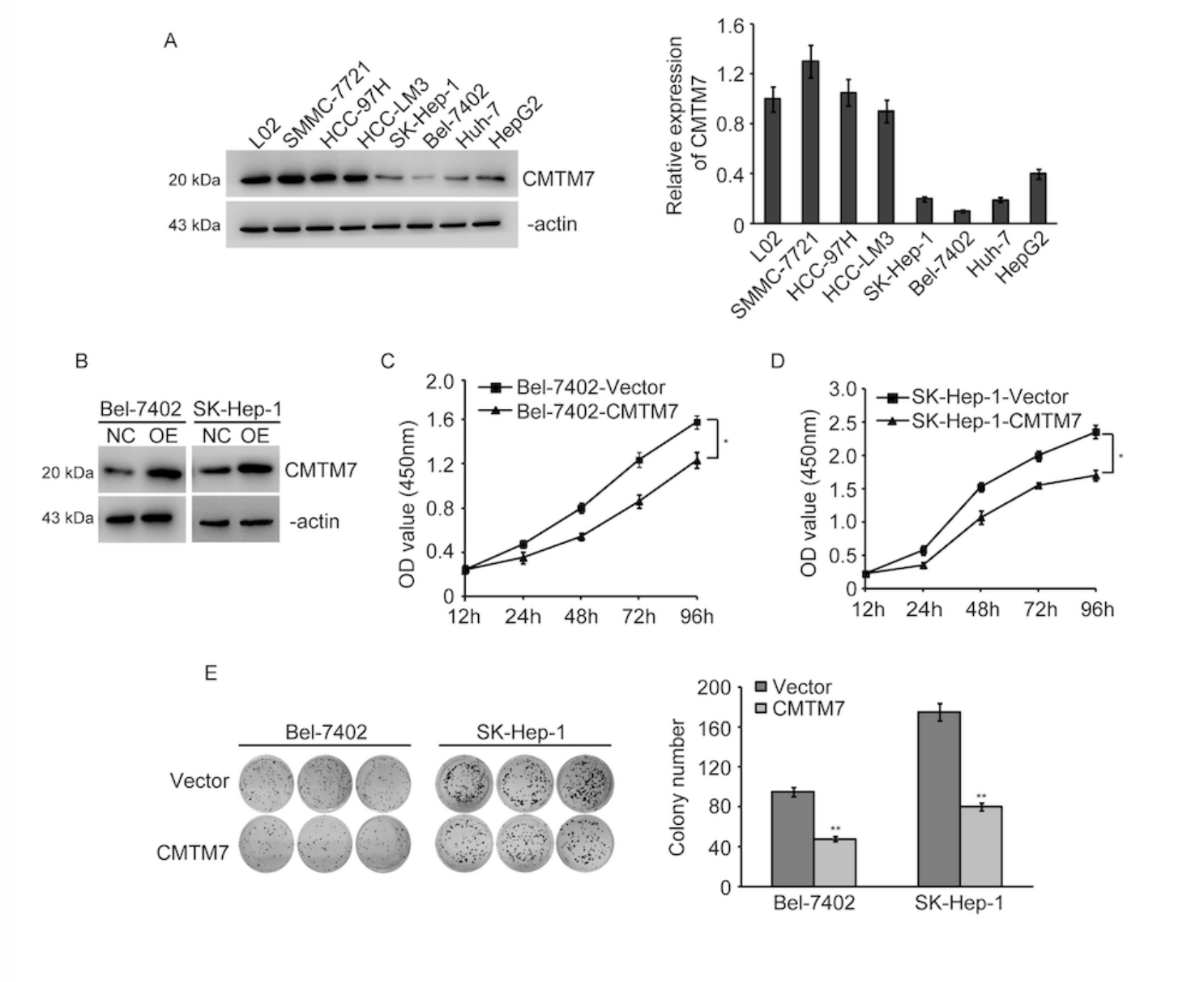

Figure 3

The role of CMTM7 in HCC cell proliferation. (A) CMTM7 protein expression in L02 cell line and HCC cell lines as indicated was detected by Western blot. The right column shows the relative expression of CMTM7 in HCC cells from gray scan results normalized to β-actin. (B) CMTM7 protein expression in Bel-7402 and SK-Hep-1 cells after exogenous expression of CMTM7. NC means vector and OE means CMTM7. (C) and (D) Cell proliferation was detected by CCK-8 in Bel-7402 and SK-Hep-1 cells after exogenous expression of CMTM7. *, P<0.05 is based on the Student t test compared to the negative control cells. (E) Cell proliferation was detected by colony formation assay in Bel-7402 and SK-Hep-1 cells after exogenous expression of CMTM7. **, P<0.01 is based on the Student t test compared to the negative control cells.The role of CMTM7 in HCC cell proliferation. (A) CMTM7 protein expression in L02 cell line and HCC cell lines as indicated was detected by Western blot. The right column shows the relative expression of CMTM7 in HCC cells from gray scan results normalized to β-actin. (B) CMTM7 protein expression in Bel-7402 and SK-Hep-1 cells after exogenous expression of CMTM7. NC means

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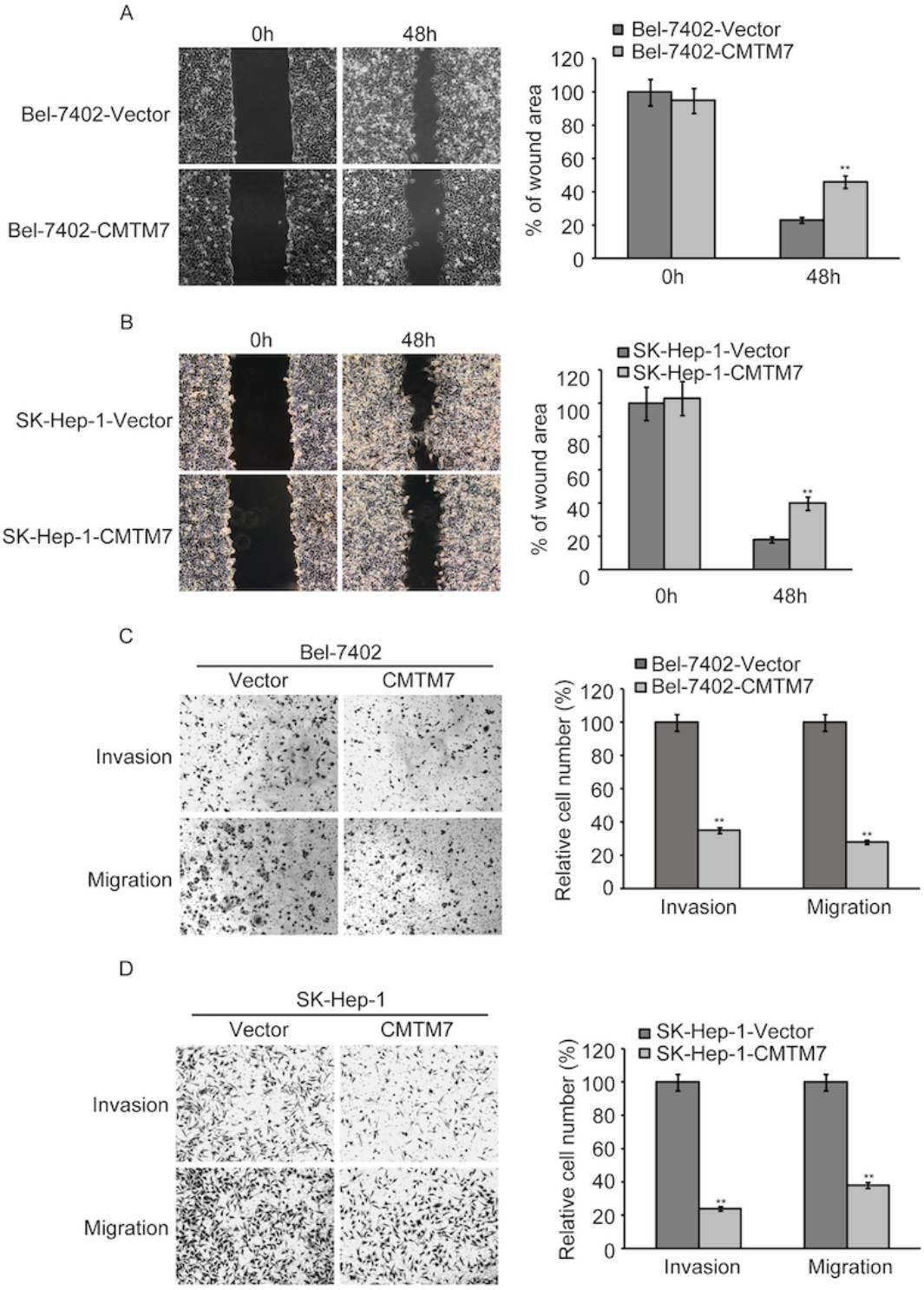

Figure 4

The role of CMTM7 in HCC metastasis. (A) and (B) Metastatic ability of Bel-7402 and SK-Hep-1 cells were analyzed by wound healing after exogenous expression of CMTM7. (C) and (D) Invasion and migration ability of Bel-7402 and SK-Hep-1 cells were analyzed by Transwell assay after exogenous expression of CMTM7. **, P<0.01 is based on the Student t test compared to the negative control cells.

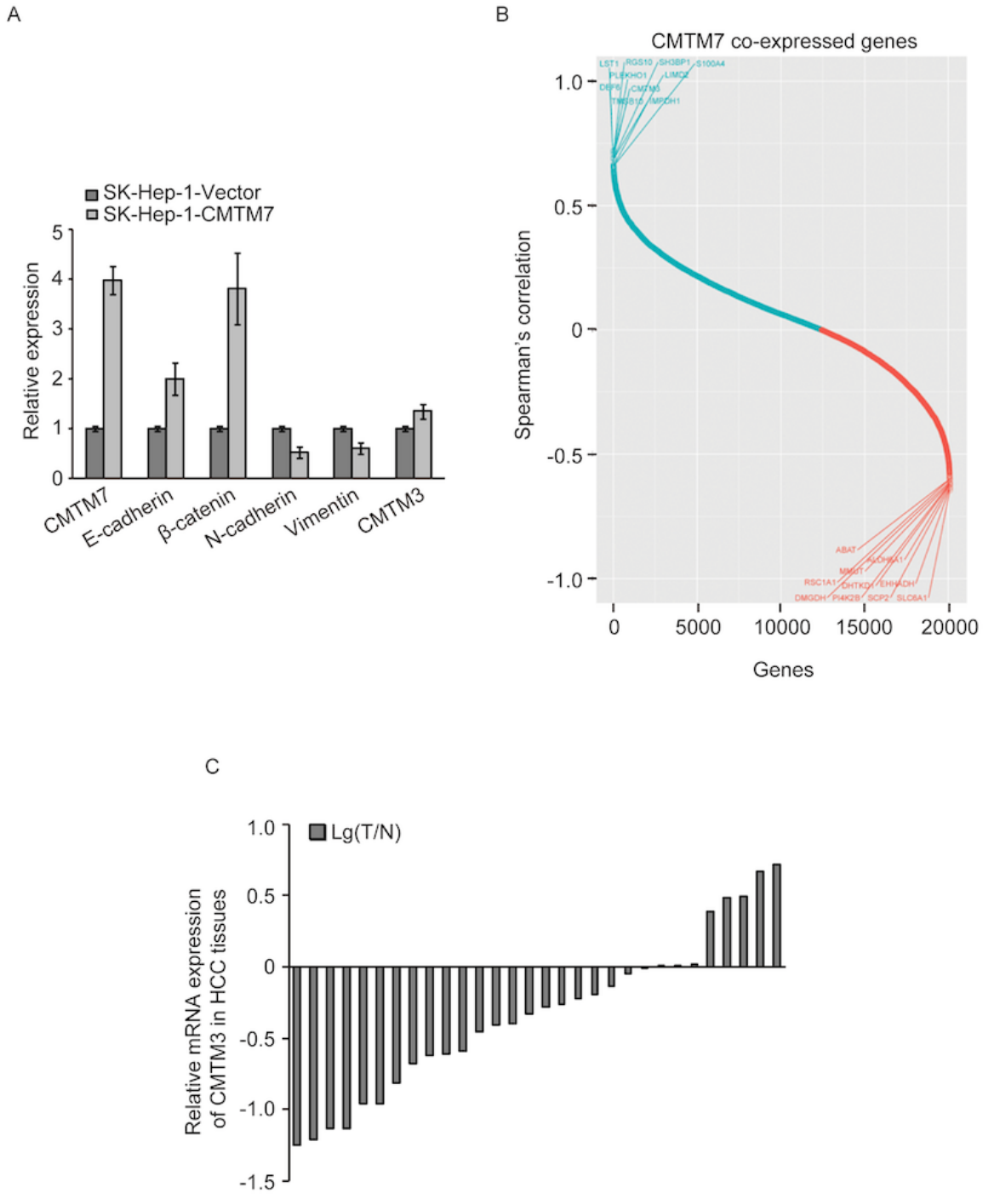

Figure 5

Over-expression of CMTM7 inhibits HCC metastasis via up-regulating CMTM3. (A) Relative gene expression after exogenous expression of CMTM7 in SK-Hep-1 cells. (B) CMTM7 co-expressed genes searched from TCGA liver cancer data. (C) Relative mRNA expression of CMTM3 in 30 HCC tissues. Lg(T/N) is logarithmic transformation of the CMTM3 expression in paired-HCC (T) and adjacent non-tumor tissues (N) by qRT-PCR.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryTable1.docx