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microRNA-489 Plays an Anti-Metastatic Role in Human Hepatocellular Carcinoma by Targeting Matrix Metalloproteinase-7

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Abstract

Dysregulation of microRNAs (miRNAs) is actively involved in the pathogenesis and tumorigenicity of hepatocellular carcinoma (HCC). miR-489 was found to play either oncogenic or tumor suppressive roles in human cancers. Recent study reported that the levels of miR-489 in late recurrent HCC patients were evidently higher than that in early recurrent cases, suggesting that miR-489 may function as a tumor suppressive miRNA in HCC. Yet, the clinical value and biological function of miR-489 remain rarely known in HCC. Here, we presented that miR-489 level in HCC tissues was notably reduced compared to matched non-cancerous specimens. Its decreased level was evidently correlated with adverse clinical parameters and poor prognosis of HCC patients. Accordingly, the levels of miR-489 were obviously down-regulated in HCC cells. Ectopic expression of miR-489 in HCCLM3 and MHCC97H cells prominently inhibits the migration and invasion of tumor cells and reduced lung metastases *in vivo*, while miR-489 knockdown increased these behaviors of HepG2 and MHCC97L cells. Mechanically, miR-489 negatively regulated matrix metalloproteinase-7 (MMP7) abundance in HCC cells. Herein, MMP7 was found to be a downstream molecule of miR-489 in HCC. An inversely correlation between miR-489 and MMP7 was confirmed in HCC specimens. MMP7 knockdown prohibited cell migration and invasion while MMP7 overexpression showed opposite effects on HCC cells. Furthermore, restoration of MMP7 expression could abrogate the anti-metastatic effects of miR-489 on HCCLM3 cells with enhanced cell migration and invasion. Altogether, miR-489 potentially acts as a prognostic predictor and a drug-target for HCC patients.

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Introduction

microRNAs (miRNAs) inhibit the expression of target genes by contributing to the degradation or translational inhibition of target mRNAs [1]. They have been found to be actively involved in different cellular processes [2,3] including proliferation, apoptosis, differentiation and movement. Emerging studies showed that abnormal expression and function of miRNAs play important roles in the pathogenesis and tumorigenicity of human malignancies [4–6]. Otherwise, miRNAs have been demonstrated to be hopeful diagnostic biomarkers and drug-targets of hepatocellular carcinoma (HCC) [7–9]. Investigating the expression and biological function of miRNAs in HCC will contribute to the discovery of new biomarkers and drug-targets for HCC patients.

miR-489 is found to be underexpressed in 4-hydroxytamoxifen-resistant and adriamycin-resistant human breast cancer cells compared to Tamoxifen-sensitive MCF-7 cells [10–12]. miR-489 overexpression reverses proliferation, migration and invasion of breast cancer cells by

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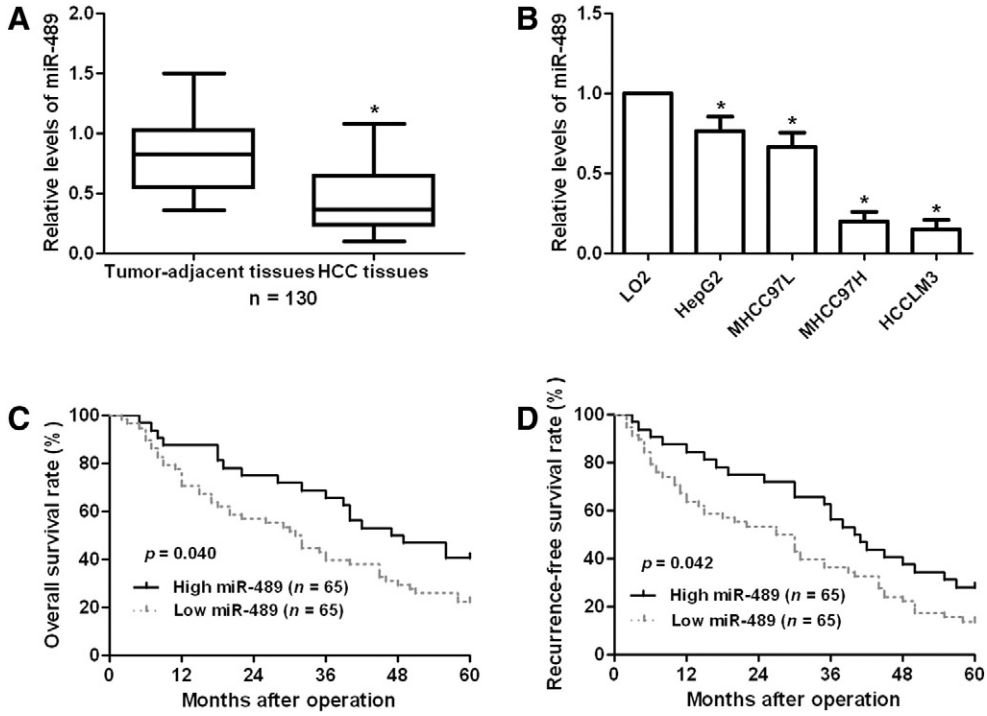

Figure 1. The status and prognostic value of miR-489 expression in HCC. (A) The alterative expression of miR-489 between HCC tissues and normal tumor-adjacent tissues. n = 130, **P* < .05 by *t* test. (B) The differences expression of 4 different HCC cells lines (HepG2, MHCC97L, MHCC97H and HCCLM3) and human immortalized hepatocyte LO2. n = 3 repeats with similar results, **P* < .05 by ANOVA. (C) and (D) The "low" or "high" of miR-489 level was defined according to the cut-off value, which was defined as the median value of the cohort of patients tested (0.83). Compared with those of high miR-489 level (n = 65), miR-489 low-expressing patients (n =65) had significantly reduced overall survival and recurrence-free survival rates. *P* < .05 by log-rank test.

targeting Gse1 coiled-coil protein (GSE1) [13]. Restoration of miR-489 suppresses HER2-positive breast cancer cell growth and inhibits tumorigenesis and tumor growth *in vivo* [14]. Otherwise, miR-489 exerts a tumor suppressive role by inhibiting cell growth *via* inhibition of protein tyrosine phosphatase, non-receptor type 11 (PTPN11) [15] in hypopharyngeal squamous cell carcinoma. The level of miR-489 is reduced in cisplatin-resistant ovarian cancer (OC) cells and miR-489 overexpression inhibits OC cell survival, cell growth and induces apoptosis by targeting Akt3 [16]. In non-small cell lung cancer, knockdown of miR-489 facilitates cell invasion and epithelial mesenchymal transition (EMT) [17]. However, miR-489 is significantly overexpressed in oral squamous cell carcinoma [18], clear cell renal cell carcinoma [19,20]. Recently, Yang Z et al. found that the levels of miR-489 in late recurrent HCC patients were notably lower

Table 1. Correlation between the Clinicopathologic Features and miR-489 Expression in HCC

Characteristics		Total No. of Patients, n = 130	miR-489 Expression Status		*P*
			Low	High	
Age (y)	<50	54	26	28	.722
	≥50	76	39	37	
Sex	Male	98	50	48	.684
	Female	32	15	17	
HBV	Absent	42	17	25	.134
	Present	88	48	40	
Serum AFP level (ng/mL)	<400	48	23	25	.716
	≥400	82	42	40	
Tumor size (cm)	<5	49	23	26	.587
	≥5	81	42	39	
No. of tumor nodules	1	102	45	57	.010 *
	≥2	28	20	8	
Cirrhosis	Absent	54	28	26	.722
	Present	76	37	39	
Venous infiltration	Absent	96	43	53	.046 *
	Present	34	22	12	
Edmondson-Steiner grading	I + II	98	45	53	.103
	III + IV	32	20	12	
TNM tumor stage	I + II	97	42	55	.009 *
	III + IV	33	23	10	

HCC, hepatocellular carcinoma; HBV, hepatitis B virus; AFP, alpha-fetoprotein; TNM, tumor-node-metastasis.
* Statistically significant.

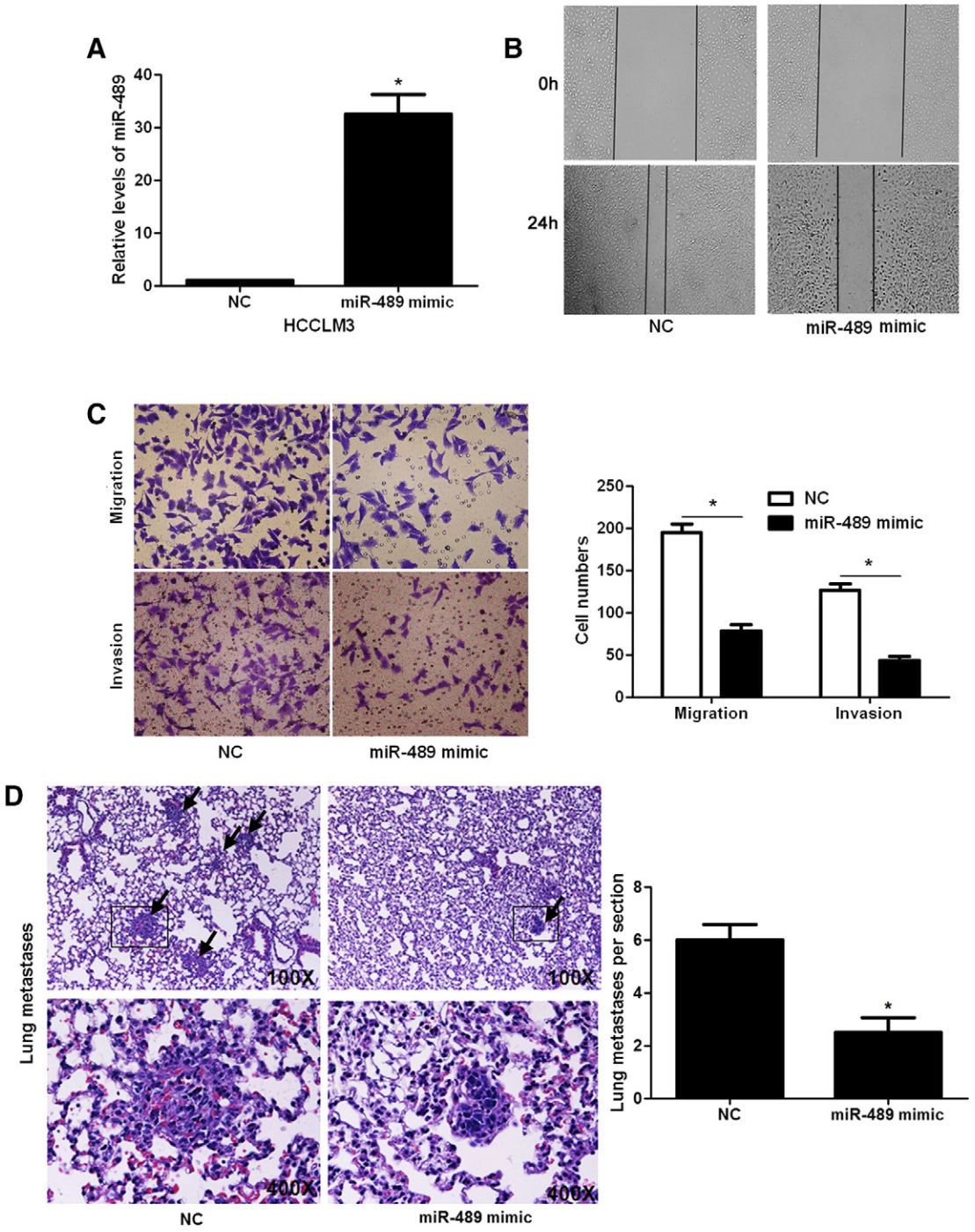

Figure 2. miR-489 overexpression inhibits the mobility of HCCLM3 cells. (A) HCCLM3 cells that were transduced with miRNA scrambled control clones (NC) or miR-489 mimics were confirmed by qRT-PCR. n = 3 repeats with similar results, *$P < .05$ by *t* test. (B) Wound healing assays indicated that miR-489 overexpression inhibited the migration of HCCLM3 cells. (C) Transwell assays confirmed that miR-489 overexpression restrained HCCLM3 cell migration and invasion. n = 3 repeats with similar results, *$P < .05$ by *t* test. (D) HCCLM3 cells that were transfected miR-489 mimic and miRNA scrambled control clones (NC) were intravenously injected into nude mice (n = 8). HE staining revealed that miR-489 overexpression significantly reduced lung metastases of HCCLM3 cells *in vivo*. *$P < .05$ by *t* test.

than those in early recurrent cases [21]. Yet, the clinical value and biological role of miR-489 in HCC remain largely unknown.

Here, we confirmed that miR-489 was underexpressed in HCC specimens and cells. The low level of miR-489 associated with malignant clinical features of HCC patients and decreased survival rates. Our results showed that miR-489 inhibited the invasive ability of cancer cells in HCC. Moreover, matrix metalloproteinase-7 (MMP7) was recognized as a downstream molecule of miR-489 and possibly mediated the biological functions of miR-489 in HCC.

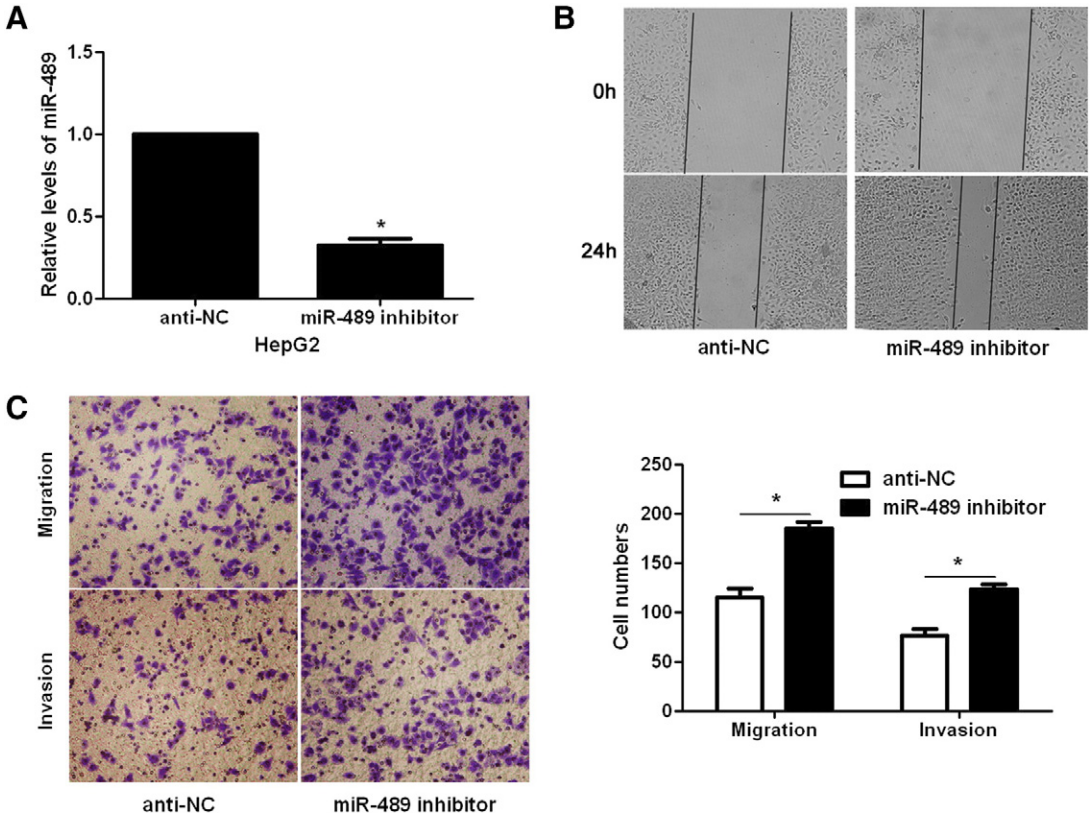

Figure 3. miR-489 knockdown facilitates the metastasis of HepG2 cells. (A) HepG2 cells that were transduced with miRNA inhibitor control clones (anti-NC) or miR-489 inhibitors were confirmed by qRT-PCR. n = 3 repeats with similar results, $*P < .05$ by *t* test. (B) miR-489 knockdown notably facilitated the migration of HepG2 cells. (C) miR-489 knockdown prominently increased HepG2 cell migration and invasion. n = 3 repeats with similar results, $*P < .05$ by *t* test.

Material and Methods

Clinical Samples

Clinical specimens were obtained from 130 patients histologically diagnosed as HCC in the Department of Hepatobiliary Surgery at the Nanfang Hospital of Southern Medical University. Patients who received immunotherapy, chemotherapy or radiotherapy before surgical treatment were excluded. Informed consent was signed by each patient before clinical specimens collected and used. All specimens were stored in liquid nitrogen or fixed with formalin for further investigation. The protocol involved clinical specimens in this study was permitted by the Research Ethics Committee of Southern Medical University.

Cell Culture and Transfection

Human HCC cell lines including HepG2, MHCC97L, MHCC97H and HCCLM3, a human immortalized normal hepatocyte cell line (LO2) and a human embryonic kidney (HEK293T) cell line were obtained from Chinese Academy of Sciences (Shanghai, China). All cells were cultured in DMEM (HyClone, Logan, UT, USA) along with fetal bovine serum (10%) (FBS; HyClone) and anti-biotics (Sigma-Aldrich, St. Louis, MO, USA). Cell cultures were kept in an incubator containing of 5% CO2 and humidified atmosphere at 37 °C.

miR-489 mimic (HmiR0107-MR04), miR-489 inhibitor (HmiR-AN0528-AM04) and the corresponding control vectors (CmiR0001-MR04; CmiR-AN0001-AM04) were bought from Genecopoeia (Guangzhou, China). MMP7 siRNA and scrambled siRNA were designed and synthesized by GenePharma (Shanghai, China). The above vectors were then tranduced into HCC cells with lippofectamine 2000 following manufactures' protocol. Retroviral vectors pMMP-MMP7 were constructed by inserting the corresponding cDNA into pMMP. The retroviruses were packaged and tranfected into HCC cells as previously described [22].

Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR)

Total RNA from HCC cells was isolated by miRNeasy Mini Kit (Qiagen, Hilden, Germany) and total RNA from HCC tissues were extracted with Trizon reagent (Invitrogen, Carlsbad, CA, USA). cDNAs were synthesized from 2 μg total RNA using TIANScript RT Kit (Tiangen biotech, Beijing, China). miR-489 levels in these samples were detected using TaqMan™ MicroRNA Assays based on the manufacturer's instructions (Applied Biosystems™, Carlsbad, CA, USA). Quantitative real-time PCR was performed using UltraSYBR Mixture (CW0957, CWBIO, Beijing, China) in a final volume of 10 μL in LC 480 PCR System (Roche, Indianapolis, IN, USA). The primers for miR-489 and U6, MMP7 and GAPDH were bought from Genecopoeia (Guangzhou, China). U6 was used as the control gene for the relative level of miR-489 while GAPDH served as internal control for MMP7. Data were presented as relative quantification based on the calculation of $2^{-\Delta Ct}$. ΔCt was derived from subtracting the Ct value of reference cDNA from the Ct value of the cDNA of interest. All primers for RT and PCR are shown as follows: miR-489 RT primer: 5′-CTC AAC TGG TGT CGT GGA

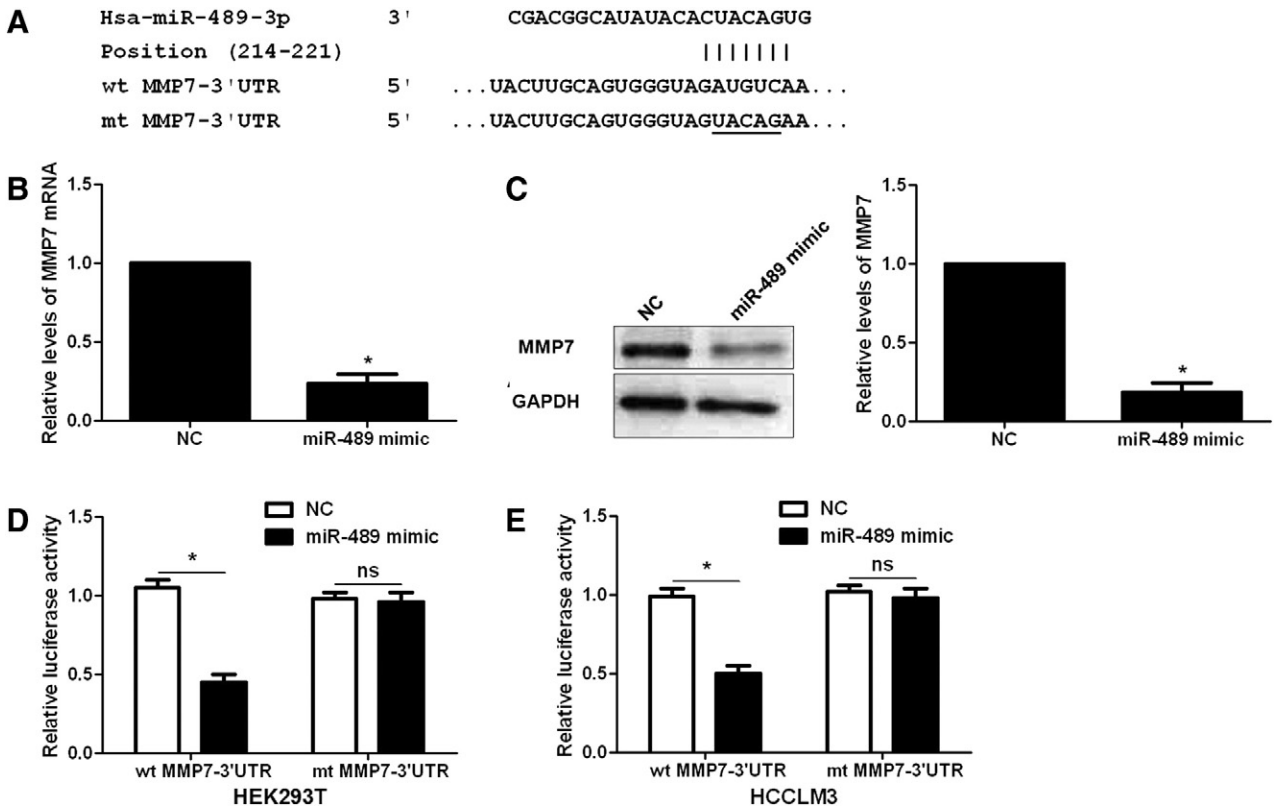

Figure 4. MMP7 is a downstream molecule of miR-489. (A) The potential miR-489 binding site in wild type (wt) 3′-UTR sequence of MMP7. The underlined part is the mutant site designed for mutant (mt) 3′-UTR sequence of MMP7. (B) and (C) HCCLM3 cells that were transduced with NC or miR-489 mimics were confirmed by qRT-PCR and immunoblotting for MMP7 mRNA and protein expression. n = 3 repeats with similar results, *$P < .05$ by *t* test, respectively. (D) and (E) Overexpression of miR-489 decreased the luciferase activity of wild type (wt) MMP7 3′-UTR in both HEK293T and HCCLM3 cells. While alteration of miR-489 showed non-effect on the luciferase activity of mutant (mt) MMP7 3′-UTR. n = 3 repeats with similar results, *$P < .05$ by *t* test, respectively.

GTC GGC AAT TCA GTT GAG AGC TGC CGT-3′; miR-489 forward: 5′-ACA CTC CAG CTG GGG TGA CAT CAC ATA-3′; miR-489 reverse primer: 5′-TGG TGT CGT GGA GTC G-3′; MMP-7 primers were: 5′-GGT CAC CTA CAG GAT CGT ATC ATA T-3′(forward) and 5′-CAT CAC TGC ATT AGG ATC AGA GGA A-3′ (reverse); GAPDH primers were: 5′-CAA GCT CAT TTC CTG GTA TGA C-3′(forward) and 5′-CAG TGA GGG TCT CTC TCT TCC T-3′(reverse).

Luciferase Reporter Assay

To investigate whether miR-489 could interact with the 3′-UTRs of MMP7, wild type (wt) 3′-UTR of MMP7 predicted to interact with miR-489 or the mutant (mt) MMP7 3′-UTR was amplified. Then, the wt 3′-UTR of MMP7 or mt 3′-UTR of MMP7, and miR-489 mimic or miRNA scrambled control clones (NC) were co-transduced into HCC and HEK293T cells, respectively by lippofectamine 2000. 48 hours after co-transduction, the cells were lysed and detected using a Dual-Luciferase® Reporter Assay Kit (Promega, Madison, WI, USA) based on the manufacturer's protocols.

Wound Healing Assay

HCC cells transduced with corresponding vectors were subsequently seeded in 6-well plates to form the single confluent cell layer. The wound were made with 100ul tips in the confluent cell layer.0 and 24 hours after would scratching, the width of wound was photographed with phase-contrast microscope.

Transwell Migration and Invasion Assay

The migration and invasion ability of HCC cells were detected with Transwell chambers (BD Biosciences, Franklin Lakes, NJ, USA). 5–10 × 10^4 HCC cells suspended in 100ul medium without serum were seeded into the upper chamber, and lower chamber was full of 20% FBS to induce HCC cells migrating or invading through the membrane. Matrigel (1:6 dilution) was added on the upper chamber for invasion assay. Twenty-four hours later, cells with crystal violet staining that migrated or invaded across the Transwell membrane were numbered under optical microscope.

Western Blotting

Two days post transfection, total protein was extracted, following by quantification with BCA protein assay kit (Pierce, Bonn, Germany). Protein were separated by 10% SDS-polyacrylamide gels and subsequently transferred to PVDF membranes. Then, 5% milk blocked membranes were incubated with MMP7 (10374–2-AP, Proteintech, Rosemont, IL, USA) antibody. The secondary antibodies were obtained from Cell signaling (Danvers, MA, USA). GAPDH (G8140, US Biological, Swampscott, MA, USA) was used as a loading control.

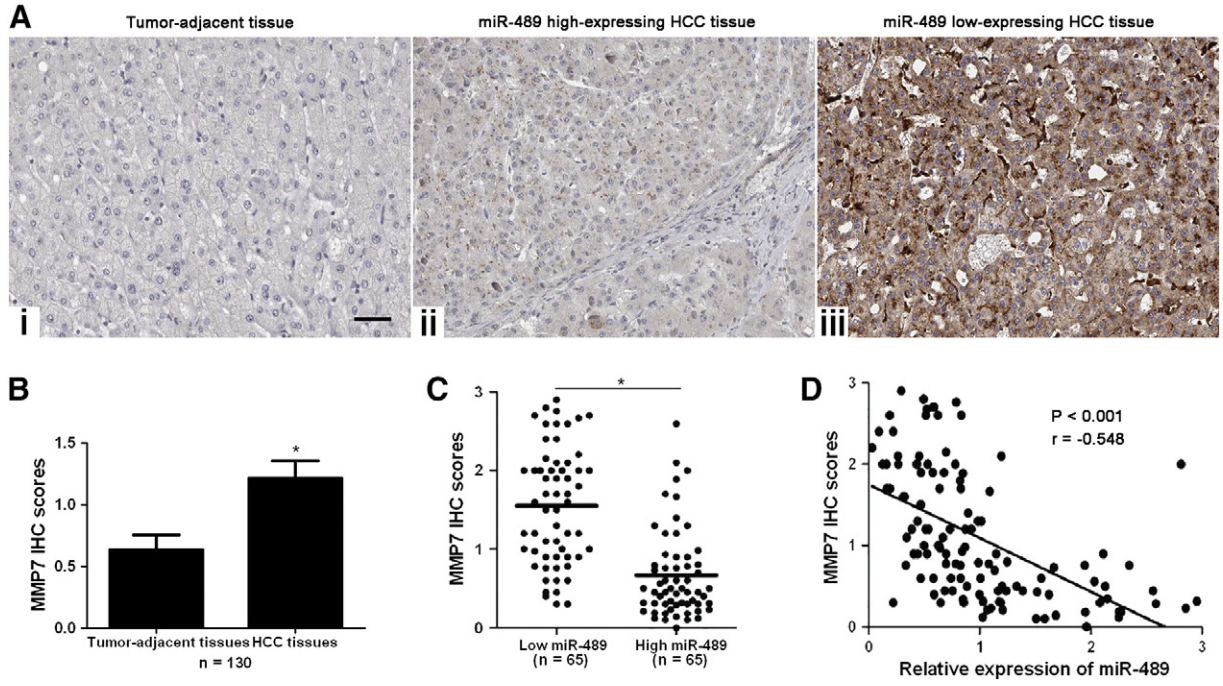

Figure 5. The difference of MMP7 expression between HCC and non-tumor tissues. (A) Representative IHC staining of MMP7 in HCC and tumor-adjacent tissues. miR-489 high expressing tumors showed weak staining of MMP7 (ii), while miR-489 low expressing tumors showed strong staining of MMP7 (iii). (B) The expression difference of MMP7 between HCC and adjacent non-tumor tissues. n = 130, *$P < .05$ by *t* test. (C) The expression difference of MMP7 between miR-489 low and high expressing HCC specimens. n = 65, *$P < .05$ by *t* test. (D) An negative correlation between miR-489 and MMP7 was confirmed in HCC tissues. n = 130, $P < .05$ by Spearman's rank correlation analysis.

Immunohistochemistry (IHC)

The tumor tissues that were previously formalin-fixed and paraffin-embedded were sliced into 4 μm sections, and underwent deparaffination and then rehydration. Antigen retrieval, suppression of endogenous peroxidase activity and 10% skim milk blocking were performed before primary antibody incubation. MMP7 (Proteintech) antibody was used as primary antibody overnight at 4 °C. The following day, the slides were incubated with peroxidase conjugated secondary antibody (ZSGB BIO, Beijing China) for 90 min, and a peroxidase-labeled polymer, DAB solution was used for signal development for 5 min. The sections were counterstained with hematoxylin followed by dehydrating and mounting.

In Vivo Pulmonary Metastasis Assay

6×10^6 HCCLM3 cells that were transfected with miR-489 mimic or control vector were intravenously injected into nude mice. Five weeks after injection, all mice were euthanized, and the lungs were sectioned and stained by H&E to check if pulmonary metastatic foci formed. The protocol for these animal experiments were approved by the Ethics Review Committee of Southern Medical University.

Statistical Analysis

Data were presented as Mean ± SEM and analyzed by GraphPad Prism 5 software (GraphPad Software, Inc., San Diego, *CA*, USA). Chi-squared test was employed to explore the association between two variables. The Student's *t* test and ANOVA were carried out to analyze continuous variable. Survival curves were constructed and differences among groups were calculated using the Kaplan–Meier method and Log-rank test. Spearman's rank correlation analysis was performed to reveal the correlation between two factors. The value of *P* less than .05 was considered to have statistical significance.

Results

miR-489 Expression is Decreased in HCC

To examine the status of miR-489 in HCC, qRT-PCR was performed for 130 HCC cases. Our data disclosed that HCC tissues had significant decreased expression levels of miR-489 ($P < .05$, Figure 1*A*). Next, we compared the expression level of miR-489 between HCC cells lines and LO2 cells. Compared with LO2 cells, the levels of miR-489 in all HCC cells (HepG2, MHCC97L, MHCC97H, and HCCLM3) were significantly reduced, especially in high-metastatic cells (MHCC97H and HCCLM3) ($P < .05$, Figure 1*B*). These data indicate miR-489 probably plays a suppressive role in HCC.

Underexpression of miR-489 Correlates with Adverse Clinical Parameters and Prognosis of HCC Patients

To clarify the clinical value of miR-489 in HCC, all patients were grouped into miR-489 low group and miR-489 high group according to the cut-off value, which was defined as the median value of the cohort of patients tested (0.83). As shown in Table 1, HCC patients with low expression of miR-489 had more tumor nodes ($P = .010$), venous infiltration ($P = .046$) and advanced tumor-node-metastasis (TNM) stage ($P = .009$). Furthermore, survival analyses indicated that patients with low expression showed significantly reduced 5-year overall and recurrence-free survival ($P = .040$ and $P = .042$, respectively, Figure 1*C* and *D*). We suggest that miR-489 is a possible prognostic biomarker for HCC patients.

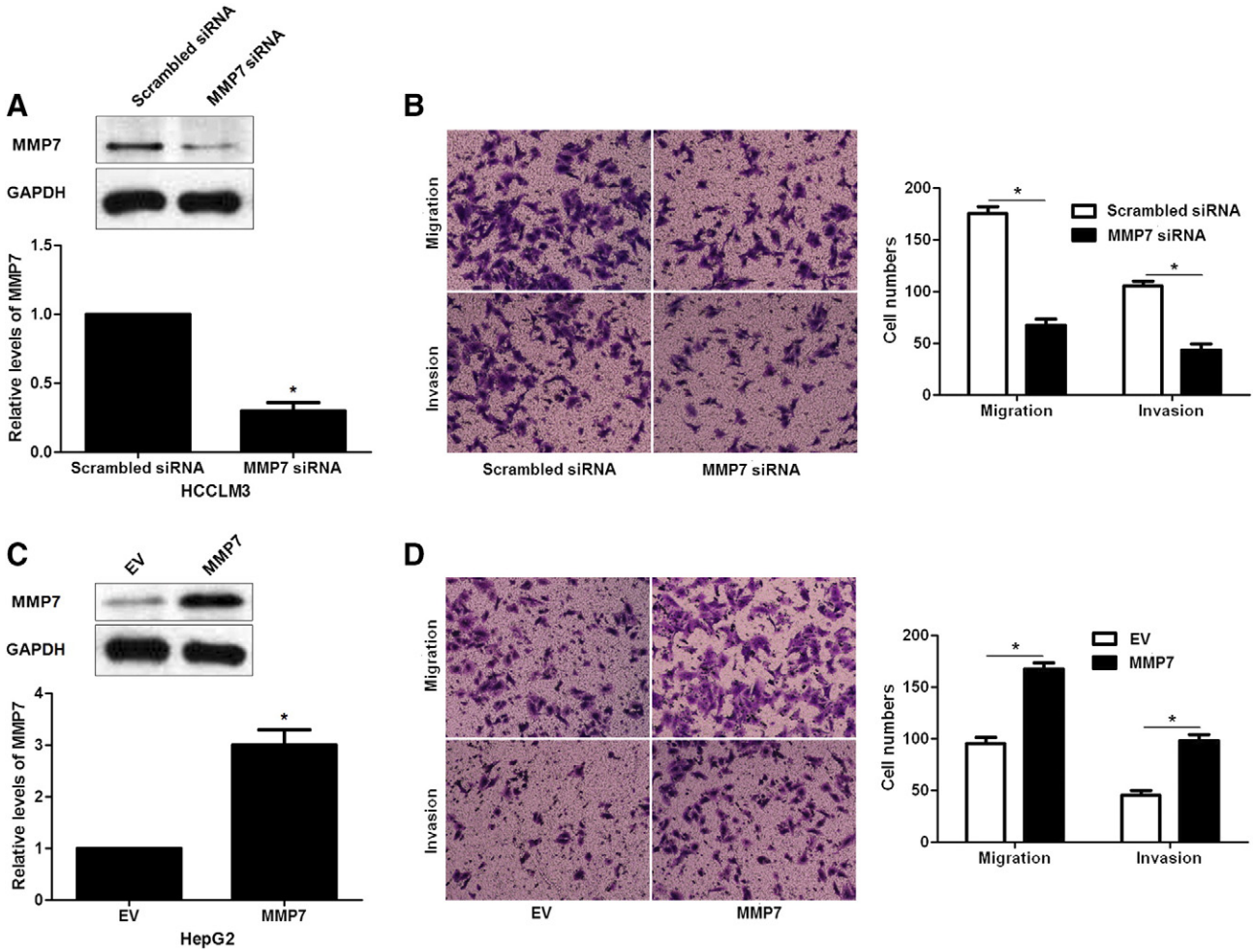

Figure 6. MMP7 regulates the metastatic behaviors of HCC cells. (A) HCCLM3 cells that were transduced with MMP7 siRNA or scrambled siRNA were subjected to western blotting for MMP7 expression. n = 3 repeats with similar results, *$P < .05$ by *t* test. (B) Quantitative data indicated that MMP7 knockdown inhibited the migration and invasion of HCCLM3 cells. n = 3 repeats with similar results, *$P < .05$ by *t* test. (C) HepG2 cells that were transduced with empty vector (EV) or MMP7 retroviruses were subjected to immunoblotting for MMP7 expression. n = 3 repeats with similar results, *$P < .05$ by *t* test. (D) Transwell assays demonstrated that MMP7 overexpression facilitated the metastatic behaviors of HepG2 cells with increased cell migration and invasion. n = 3 repeats with similar results, *$P < .05$ by *t* test.

miR-489 Inhibits the Mobility of HCC Cells

Since increased cancer cell mobility is an important reason for the metastasis and recurrence of human cancer [23], we explored whether miR-489 could modulate the migration and invasion of HCC cells. Transfection of miR-489 mimic obviously up-regulated the level of miR-489 in HCCLM3 cells ($P < .05$, Figure 2*A*). The wound healing assays showed that miR-489 overexpression notably reduced cell migration in HCCLM3 cells ($P < .05$, Figure 2*B*). And Transwell assays explored that ectopic expression of miR-489 significantly reduced the numbers of migrated and invaded HCCLM3 cells ($P < .05$, Figure 2*C*). In a pulmonary metastasis model, miR-489 overexpression remarkably reduced the number of lung metastases in nude mice ($P < .05$, Figure 2*D*). In turn, miR-489 inhibitor significantly decreased the level of miR-489 in HepG2 cells ($P < .05$, Figure 3*A*). Subsequently, miR-489 silencing notably facilitated HepG2 cell migration and invasion ($P < .05$, respectively, Figure 3, *B* and *C*). Furthermore, overexpressing and underexpressing miR-489 were performed in MHCC97H and MHCC97L cells, respectively. miR-489 showed similar effects on migration and invasion of MHCC97H and MHCC97L cells ($P < .05$, respectively, Supplementary Figure 1). Notably, CCK-8 assays indicated that miR-489 alteration did not significantly influenced HCC cell growth (Supplementary Figure 2). Thus, miR-489 exerts a anti-metastatic role in HCC cells.

miR-489 Post-Transcriptionally Regulates MMP7 Expression

To disclose the potential molecular mechanisms involved in the role of miR-489 in HCC cells, we searched for candidate target genes of miR-489 using publicly available databases, including TargetScan, miRanda and PicTar. MMP7, a pro-metastatic molecule in HCC [24], was considered as one of the candidates with which miR-489 could bind directly (Figure 4*A*). Further experiments were performed to confirm the above hypothesis. Interestingly, miR-489 overexpression reduced the expressions of MMP7 mRNA and protein ($P < .05$, respectively, Figure 4, *B* and *C*). We further explored whether MMP7 was a downstream target molecule of miR-489. Then, our data indicated that miR-489 overexpression decreased the luciferase activity of wt MMP7 3′-UTR ($P < .05$, respectively, Figure 4, *D* and

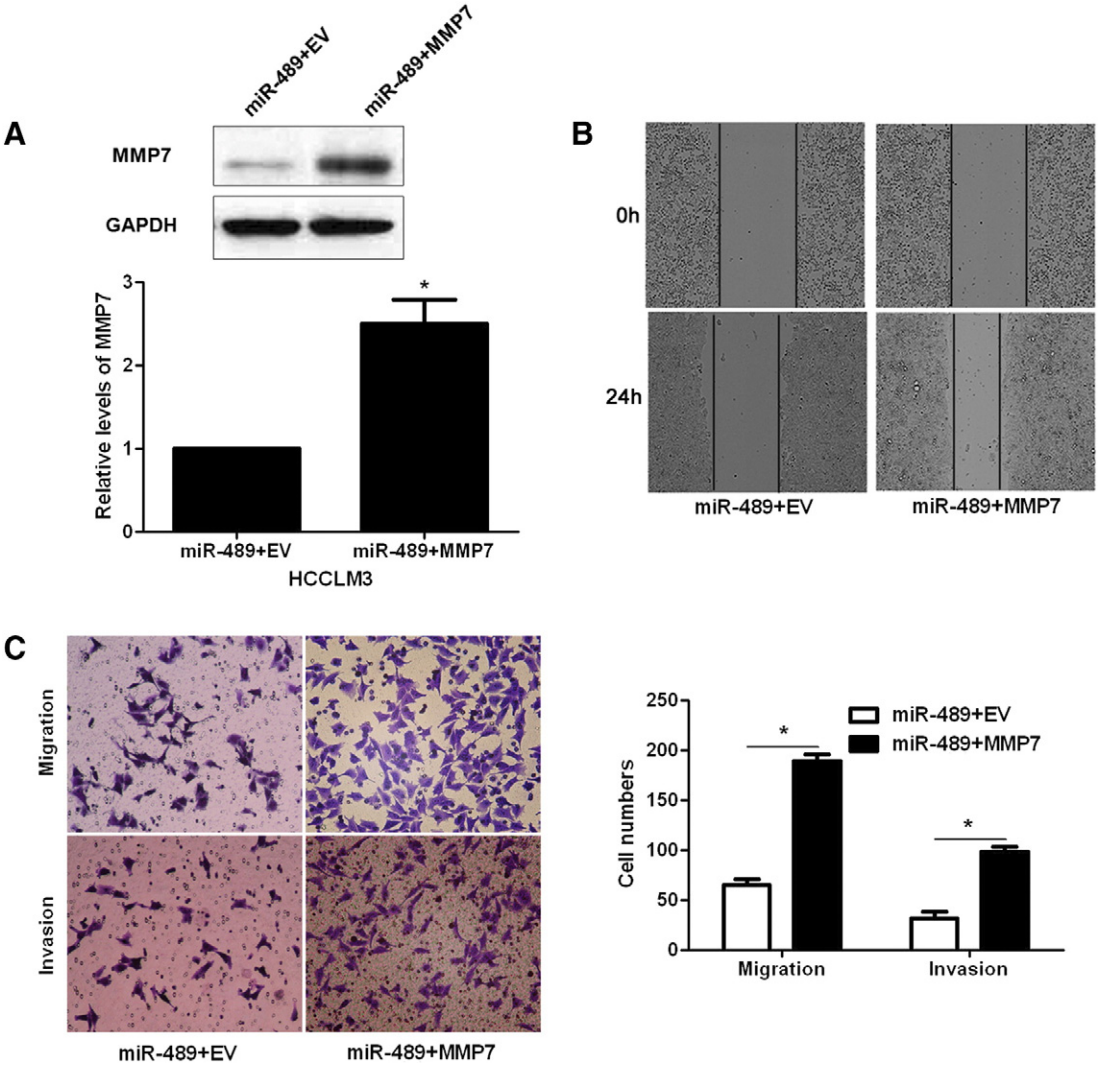

Figure 7. MMP7 restoration reverses the effects of miR-489. (A) miR-489 overexpressing HCCLM3 cells that were infected with empty vector (EV) or MMP7 retroviruses were confirmed by western blotting for MMP7 expression. n = 3 repeats with similar results, *$P < .05$ by *t* test. (B) MMP7 restoration significantly promoted the migration of miR-489 overexpressing HCCLM3 cells. (C) MMP7 restoration evidently facilitated cell migration and invasion in miR-489 overexpressing HCCLM3 cells. n = 3 repeats with similar results, *$P < .05$ by *t* test.

E), while alteration of miR-489 did not have any influence on the luciferase activity of mt MMP7 3′-UTR in HEK293T and HCCLM3 cells (Figure 4, *D* and *E*). Therefore, these data indicate miR-489 can regulate the expression of MMP7 by directly interacting with its 3′-UTR in HCC.

miR-489 Inversely Correlates with MMP7 in HCC Specimens

Next, IHC was performed to detect MMP7 in HCC and noncancerous tissues. IHC scores evaluation indicated that MMP7 was overexpressed in HCC tissues ($P < .05$, Figure 5, *A* and *B*). Notably, miR-489 high expressing tumors showed weak staining of MMP7 (Figure 5*A*–ii), while miR-489 low expressing tumors showed strong staining of MMP7 (Figure 5*A*–iii). Quantification data suggested that the expressions of MMP7 in miR-489 low expressing tumors were notably higher than those in miR-489 high expressing cases ($P < .05$, Figure 5*C*). Spearman's correlation analysis disclosed that miR-489 was negatively correlated with MMP7 in HCC specimens ($r = -0.548$, $P < .001$, Figure 5*D*). These data showed a negative correlation between miR-489 and MMP7 in HCC.

Restoration of MMP7 Abrogates the Anti-Metastatic Role of miR-489

The role of MMP7 was further confirmed in our study. MMP7 knockdown by a specific siRNA showed a significant reduction of migrated and invaded cells in HCCLM3 cells ($P < .05$, respectively, Figure 6, *A* and *B*). In contrast, MMP7 overexpression that was assessed by immunoblotting promoted cell migration and invasion in HepG2 cells ($P < .05$, respectively, Figure 6, *C* and *D*). Since we confirmed MMP7 was a target molecule of miR-489, MMP7 retroviruses were employed to disclose whether MMP7 restoration could abolish the anti-metastatic role of miR-489 in HCC cells. As shown in Figure 7*A*, MMP7 retroviruses infection significantly increased the level of MMP7 in miR-489 overexpressing HCCLM3 cells ($P < .05$). Consequently, restoration of MMP7 promoted the metastatic behavior of miR-489 overexpressing HCCLM3 cells with enhanced cell migration and invasion ($P < .05$, respectively, Figure 7, *B* and *C*). These rescue experiments suggest that miR-489 inhibits the migration and invasion of HCC cells possibly by targeting MMP7.

Discussion

Emerging evidences have confirmed that miRNAs are actively involved in the pathogenic process of HCC [25]. And miRNAs have been reported to be important mediator of the metastasis and epithelial mesenchymal transition of HCC cells [26]. According to the important function of miRNAs in HCC, miRNAs have been considered as potential diagnostic biomarkers and drug-targets of HCC [27]. In this study, miR-489 was found to be significantly under-expressed in HCC. HepG2 and MHCC97L were low invasive cell lines, while MHCC97H and HCCLM3 were high metastatic cell lines. Thus, the relative levels of miR-489 in MHCC97H and HCCLM3 cells were lower than those in HepG2 and MHCC97L cells. And low expression of miR-489 conferred adverse clinical parameters of HCC patients including more tumor nodes, venous invasion and advanced clinical stage. More importantly, decreased expression of miR-489 correlated with shortened 5-year overall and recurrence-free survival. Therefore, miR-489 played tumor suppressive role in HCC and could potentially serve as a promising biological tag for the prognosis of patients.

Systemic metastasis is the important reason for the unsatisfactory prognosis of HCC patients [28]. Increased migratory and invasive ability of cancer cells underlies the systemic metastasis of HCC. Thus, it is fundamental to disclose the underlying mechanisms for the metastasis of HCC cells. Here, we explored that miR-489 inhibited the migration and invasion of HCC cells *in vitro* and *in vivo*. These data confirmed that miR-489 exerted tumor suppressive role in HCC by inhibiting metastatic behaviors of HCC cells. MMP7, a secreted protein, exerts a role in the destruction of extracellular matrix substrates in human cancers [24]. Otherwise, MMP7 was found to function as a pro-metastatic factor by promoting the migratory and invasive ability of cancer cells [29]. Recent studies reported that overexpression of MMP7 was found in HCC specimens and cells [30,31]. Moreover, MMP7 was considered as an crucial target molecule of dickkopf1 (DKK1) and mediated DKK1-induced HCC cell migration and invasion [24]. Here, we also revealed that MMP7 promoted HCC cell migration and invasion. Notably, we disclosed that miR-489 suppressed the expression of MMP7 in HCC cells. And the levels of MMP7 in HCC tissues were negatively correlated with the expressions of miR-489. Moreover, we found that miR-489 could directly interact with the 3′-UTR of MMP7. These experiments suggest that MMP7 is a downstream molecule of miR-489. Furthermore, we found that restoration of MMP7 could abrogate the anti-metastatic effects of miR-489 on HCC cells migration and invasion. These suggest that miR-489 inhibits the migration and invasion of HCC cells possibly by targeting MMP7.

Altogether, our study demonstrates that miR-489 expression is significantly decreased in HCC. The low level of miR-489 correlates with adverse clinical parameters of HCC patients and shortened survival. And miR-489 inhibits the metastasis of HCC cells. Furthermore, MMP7 is a direct target of miR-489 in HCC. Altogether, miR-489 exerts its inhibitory effects on HCC metastasis, at least in part, by targeting MMP7.

Conclusions

To conclude, we recognize miR-489 down-regulation as a biomarker for poor prognosis prediction. The underexpression of miR-489 creates a milieu of metastasis facilitation that plays a role in HCC progression. A mechanism by which underexpressed miR-489 promotes the metastasis by targeting MMP7 plays an important role in this process. This finding will improve understanding of cancer progression mechanism and provide novel targets for the molecular treatment of HCC.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.tranon.2017.01.010.

Conflicts of Interest

The authors declare that they have no competing interests.

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