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ORIGINAL RESEARCH

Long Non-Coding RNA PART1 Promotes Proliferation, Migration and Invasion of Hepatocellular Carcinoma Cells via miR-149-5p/MAP2K1 Axis

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Background: Hepatocellular carcinoma (HCC) is the most common primary hepatic malignancy worldwide. Long non-coding RNAs (lncRNAs) and microRNAs (miRNAs) have been identified as effective markers for the detection of multiple cancers. This study aimed to illuminate the mechanism of prostate androgen regulated transcript 1 (PART1) in HCC.

Materials and Methods: The levels of PART1, miR-149-5p and mitogen-activated protein kinase 1 (MAP2K1) mRNA were detected by quantitative real-time polymerase chain reaction (qRT-PCR) assay. Cell proliferation was assessed by Cell Counting Kit-8 (CCK-8) assay, and cell migration and invasion were evaluated by transwell assay. Dual-luciferase reporter assay was carried out to examine the relationship among PART1, miR-149-5p and MAP2K1. Western blot assay was conducted to measure the protein expression of MAP2K1.

Results: PART1 and MAP2K1 expression were greatly increased and miR-149-5p level was decreased in HCC tissues. Functional analysis revealed that the si-PART1 inhibited proliferation, migration and invasion of HCC cells. PART1 directly bound to miR-149-5p and miR-149-5p level was down-regulated by PART1. Moreover, restoration experiment demonstrated that the effect of PART1 knockdown on HCC cell progression could be partially rescued by miR-149-5p depletion. MiR-149-5p was predicted to target MAP2K1 and MAP2K1 expression was negatively modulated by miR-149-5p. Also, MAP2K1 rescued the inhibitory effects of miR-149-5p overexpression on proliferation, migration and invasion in HCC cells. Besides, the inhibition of miR-149-5p weakened the impact on MAP2K1 expression mediated by PART1 repression.

Conclusion: PART1 promoted proliferation, migration and invasion of HCC cells by regulating miR-149-5p/MAP2K1 axis.

Keywords: hepatocellular carcinoma, PART1, miR-149-5p, MAP2K1

Introduction

Hepatocellular carcinoma (HCC) is the most common primary hepatic malignancy.[1,2] It is the third leading cause of cancer-related deaths and approximately 700,000 people died of HCC each year worldwide.[3] The progression of HCC, including cell proliferation, migration and invasion, is a complicate process that involves a number of molecular mechanisms for the alteration and modulation in the extracellular matrix. Despite significant improvements in diagnostic and therapeutic techniques, the recurrence-free survival (RFS) and overall survival

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(OS) rates of HCC patients were still comparatively low.[4,5] Therefore, a better understanding of the molecular basis of HCC is urgently necessary for treatment of HCC.

Long non-coding RNAs (lncRNAs) are a class of long non-coding transcripts that contain more than 200 nucleotides.[6] Mounting evidence suggested that dysregulated lncRNA was involved in tumorigenesis and metastasis of multiple diseases, including cancer.[6–8] For instance, plenty of lncRNAs, such as MALT1,[9] XIST,[10] and HOTAIR,[11] acted as oncogenes to promote HCC tumor growth and metastasis by regulating miRNA or proteins. Recent study demonstrated that lncRNA prostate androgen regulated transcript 1 (PART1) was highly expressed and promoted cell proliferation via the inhibition of Toll-like receptor (TLR) pathway in prostate cancer.[12] Moreover, PART1 has been proved to be a promising biomarker for prognosis prediction of non-small cell lung cancer and promote gefitinib resistance in esophageal squamous cell carcinoma.[13,14] Previous studies indicated that PART1 was expressed in HCC cells and PART1 expression profile can effectively predict early recurrence after surgical resection for HCC.[15,16] However, research on the clinical utility and accuracy of PART1 in HCC remain limited.

MicroRNAs (miRNAs), a class of endogenous RNAs with approximately 22 nucleotides in length, played pivotal roles in tumorigenesis and progression.[17,18] MiRNAs have been regarded as post-transcriptional regulators that induced mRNA degradation of target genes by binding to 3′-untranslated region (3′-UTR) of mRNAs.[19] A substantial amount of evidence has proposed that miRNAs played an important role in the regulation of gene expression[20,21] and might be dysregulated in many diseases, including metabolic diseases, infectious diseases and cancers.[22,23] For example, miR-122 and miR-223 were identified as tumor suppressors in the development of HCC, while miR-21 and miR-130b were reported to promote tumor growth in HCC.[24–27] MiR-149-5p has been demonstrated to be associated with some types of cancers, including colorectal cancer, nasopharyngeal carcinoma, lung cancer and hepatocellular carcinoma.[28–31] For instance, Dong et al showed that lncRNA SNHG8 promoted the tumorigenesis and metastasis by sponging miR-149-5p in HCC.[31] However, the regulatory mechanism of miR-149-5p in HCC needs to be further explored in the future.

Mitogen-activated protein kinase (MAPK) are major components of pathways controlling embryogenesis, cell differentiation, cell proliferation, and cell death.[32] Zhou et al demonstrated that MAP2K1 exerted potent pharmacological functions of plumbagin against HCC.[33] Cui et al showed that miR-539 may act as a tumor suppressor in HCC by targeting and down-regulating apoptosis mediator MAP2K1.[34] However, there is no relevant study on the interaction mechanism between MAP2K1 or PART1 and miR-149-5p in HCC.

In the present study, we demonstrated the interaction between lncRNA PART1, miR-149-5p and MAP2K1, and their mechanism in regulating cell proliferation, migration and invasion in HCC.

Materials and Methods

Acquisition of Tissue Samples and Cell Culture

Samples of HCC tissues and adjacent normal tissues were collected from 48 patients undergoing surgical resection at the Sichuan Academy of Medical Sciences & Sichuan Provincial People's Hospital. This study was approved by the Ethical Committee of Sichuan Academy of Medical Sciences & Sichuan Provincial People's Hospital, and written informed consent was provided by all patients prior to surgery.

SK-HEP-1 cells were purchased from Korea Cell Line Bank (KCLB, Seoul, Korea) and Huh-7 cells were provided by the Japanese Collection of Research Bioresources (JCRB, Osaka, Japan). Huh-1 cells were got from Health Science Research Resources Bank (HSRRB, Japan). THLE-2 and Hep3B cells were obtained from American Type Culture Collection (ATCC, Manassas, VA, USA). All cell lines were cultured in the Dulbecco's Modified Eagle Medium (DMEM; Thermo Fisher Scientific, Rockford, IL, USA) supplemented with 10% fetal bovine serum (FBS, Thermo Fisher Scientific) and 1% penicillin/streptomycin (Invitrogen, Carlsbad, CA, USA) at 37°C in 5% CO2 atmosphere.

Cell Transfection

Small interference RNA (siRNA) targeting PART1 (si-PART1#1, si-PART1#2 and si-PART1#3) and their negative control (si-NC), miR-149-5p mimics, miR-149-5p inhibitor and their corresponding negative controls (miR-NC, anti-miR-NC), PART1 or MAP2K1 and their control (Vector) were synthesized by GenePharma Co., ltd (GenePharma, Shanghai, China). HCC cells were cultured in 6-well plates, and all the oligonucleotides and control vectors were transfected into HCC cells by using

Lipofectamine 2000 (Invitrogen) according to the instructions of manufacturer.

RNA Isolation and Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR) Assay

Total RNA was isolated from HCC tumor tissues and cells using TRIzol reagent (Thermo Fisher Scientific) according to the manufacturer's protocol. GoScript Reverse Transcription System (Promega, Madison, WI, USA) was used to synthesize the first strand cDNA of lncRNA PART1 and MAP2K1 mRNA, while for miRNA, cDNA was reverse transcribed by TransScript miRNA First-Strand cDNA Synthesis SuperMix (Transgen, Beijing, China). QRT-PCR was performed using iQ™ SYBR® Green Supermix (Bio-Rad Laboratories, Philadelphia, PA, USA). The qRT-PCR data was quantified using the 2-ΔΔCt method with normalization to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and U6 snRNA, respectively. The primers for qRT-PCR were purchased from Sangon Biotech (Sangon, Shanghai, China). The primers sequences of PART1, miR-149-5p, MAP2K1 and GAPDH were listed as follows: PART1 (Forward, 5′-AAGGCC GTGTCAGAACTCAA-3′; Reverse, 5′-GTTTTCCATCTC AGCCTGGA-3′), miR-149-5p (Forward, 5′-CAGGAGTTGT AAATCCGAGCCG-3′; Reverse, 5′-TTCATAGGTCAGAG CCCTGTGCA-3′), MAP2K1 (Forward, 5′-ATCTTCGGG AGAAGCACAAG-3′; Reverse, 5′-CGAAGGAGTTGGCC ATAGAG-3′), GAPDH (Forward, 5′-TATGATGATATCAA GAGGGTAGT-3′; Reverse, 5′-TGTATCCAAACTCATTGT CATAC-3′), U6: (Forward, 5′-CTCGCTTCGGCAGCACA -3′; Reverse, 5′-AACGCTTCACGAATTTGCGT-3′).

Cell Proliferation Assay

Cell proliferation was evaluated by using the Cell Counting Kit-8 (CCK-8) assay. HCC cells were seeded into 96-well plates and each well contained 10,000 cells. After being cultivated in incubator for 48 h, cells were collected and incubated with 10 μL reagent of CCK-8 (Beyotime, Shanghai, China) for 2 h at 37°C. At last, the absorbance of cells were measured at the indicated time by using a microplate reader (Bio-Rad Laboratories) at 450 nm to evaluate cell viability.

Cell Migration and Invasion Assays

Cell migration and invasion were all detected by transwell chambers (Corning Inc., Corning, NY, USA) with 8 μm-pore size membranes. Serum-free medium was added into the upper chamber, while 500 μL medium added with 10% FBS was filled into the lower chamber. First, the HCC cells suspended in serum-free medium were seeded in the upper chambers and cultivated in an incubator for 24 h at 37°C. Cell invasion were evaluated by transwell upper chambers precoated with Matrigel (Becton Dickinson, Franklin Lakes, NJ, USA), while cell migration was assessed by using the empty upper chambers. HCC cells through the membranes were collected and fixed with methanol. Then, cells were stained with 0.5% crystal violet (Sigma, St. Louis., MO, USA). Finally, migration and invasion cells incubated for 24 h were counted by a Countess automatic cell counter (Invitrogen) with at least three random views.

Dual-Luciferase Reporter Assay

LncBase Predicted v.2 prediction tool was used to determine the potential binding sites between lncRNA PART1 and miR-149-5p and Targetscan website tool was utilized to indicate the target of miR-149-5p. The partial fragments of lncRNA PART1 and MAP2K1-WT 3′-UTR containing predicted wild-type (WT) or mutant (MUT) binding sites of miR-149-5p were amplified and subcloned into psiCHECK-2 luciferase vector (Promega) to generate luciferase vectors, named as PART1-WT, PART1-MUT, MAP2K1-WT, MAP2K1-MUT. Also, the mutant sequences of PART1 and MAP2K1 3′-UTR were synthesized from Sangon Biotech. Next, SK-HEP-1 and Hep3B cells were seeded in 24-well plates, and these above constructed luciferase reporters (100 ng) and miR-149-5p mimics or miR-NC were transfected into cells using Lipofectamine 2000 transfection reagent (Invitrogen), respectively. After 48 h cultivation, the firefly luciferase activities were examined through the Dual Luciferase Reporter Assay Kit (Promega) and normalized by Ranilla luciferase activity.

Western Blot Assay

Protein samples were extracted from tumor tissues or cells by RIPA Lysis Buffer (Beyotime) and then 25 μg extracted proteins were separated using 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gels. After 2 h electrophoresis, the proteins in the gels were transferred onto polyvinylidene fluoride (PVDF) membranes (Millipore, Bedford, MA, USA). Next, the membranes were blocked in 5% non-fat milk for 2 h and subsequently incubated overnight at 4°C with the primary antibodies of MAP2K1 (ab96653; Abcam, Cambridge, MA, USA) or GAPDH (ab37168, Abcam). GAPDH acted as the internal reference in this study. The next day, the membranes were probed with horseradish peroxidase (HRP)-conjugated second antibody

(ab6721, Abcam). Finally, proteins bands were visualized using Pierce™ Enhanced chemiluminescent (ECL) Western Blotting Substrate (Thermo Fisher Scientific) with the intensity analysis via Quantity One software (Bio-Rad Laboratories).

Statistical Analysis

All data were obtained from at least three independent experiments with the quantitative data expressing as mean ± standard deviation. Difference analysis was performed by Student's *t*-test (comparison for 2 groups) or one-way analysis of variance (ANOVA; comparison for three or more groups) followed by Tukey post hoc test using GraphPad Prism 7 software (GraphPad Inc., La Jolla, CA, USA). $P<0.05$ was considered to be statistically significant. Furthermore, the correlation analysis among PART1, miR-149-5p and MAP2K1 was analyzed by Pearson's correlation coefficient.

Results

PART1 Was Highly Expressed in HCC Tissues and Cell Lines

To begin with, qRT-PCR was conducted to detect the expression of PART1 in HCC tumor tissues and cells. Compared with the adjacent normal tissues, PART1 expression was noticeably increased in HCC tissues (n = 48) (Figure 1A). In addition, PART1 level was significantly elevated in SK-HEP-1, Huh-1, Hep3B and Huh-7 cells versus human normal liver cells THLE-2 (Figure 1B). These results indicated that PART1 might play a role in the development of HCC.

PART1 Knockdown Suppressed Proliferation, Migration and Invasion in HCC Cells

Due to the higher expression of PART1 in SK-HEP-1 and Hep3B cells than other two cell lines, these two cells were selected for further functional analysis. To elucidate the function of lncRNA PART1 in HCC, SK-HEP-1 and Hep3B cells were transfected with si-PART1#1, si-PART1#2 or si-PART1#3. QRT-PCR assay showed that the expression of PART1 was sharply inhibited by the interference of PART1 and the lowest expression was detected in the si-PART1#1 group (Figure 2A). Next, CCK-8 assay demonstrated that the silence of PART1 notably suppressed viability of SK-HEP-1 and Hep3B cells (Figure 2B and C). Besides, the migration and invasion cells were evaluated by transwell assay. The results demonstrated that migration and invasion of SK-HEP-1 and Hep3B cells transfected with si-PART1#1 were both significantly reduced compared with the negative control (Figure 2D and E). These data revealed that PART1 knockdown repressed proliferation, migration and invasion of HCC cells in vitro.

PART1 Directly Interacted with miR-149-5p

To explore the regulatory network of PART1 in HCC, LncBase Predicted v.2 was used to predict the potential target of PART1. The putative binding sites between PART1 and miR-149-5p and the mutant sequences of PART1 were shown in Figure 3A. To confirm this prediction, luciferase reporter vectors were co-transfected into SK-HEP-1 and Hep3B cells with miR-149-5p or miR-NC. Luciferase activity of SK-HEP-1 and Hep3B cells in

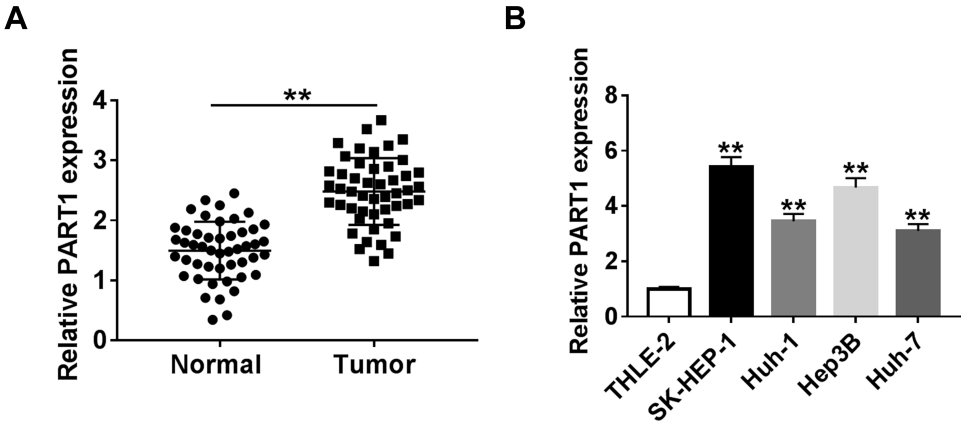

Figure 1 PART1 was highly expressed in HCC tissues and cell lines. (**A**–**B**) The expression of PART1 was measured in 46 pairs of HCC tissues and adjacent normal tissues, as well as human normal liver cell line (THLE-2) and HCC cell lines (SK-HEP-1, Huh-1, Hep3B and Huh-7) by qRT-PCR assay. **P < 0.01.

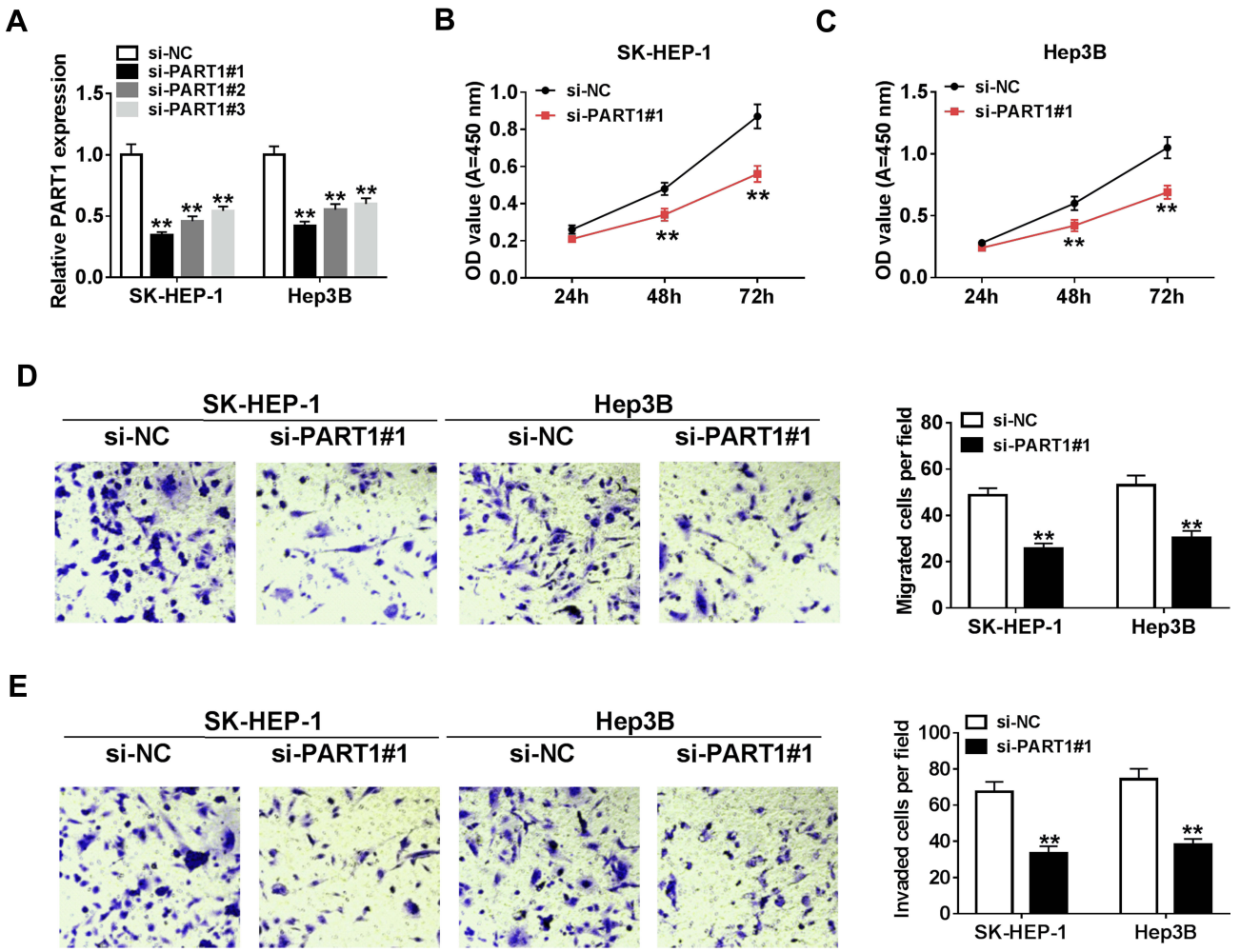

Figure 2 PART1 knockdown suppressed proliferation, migration and invasion in HCC cells. (**A**) The transfection efficacy of si-PART1#1/#2/#3 was measured by qRT-PCR assay. (**B**–**E**) SK-HEP-1 and Hep3B cells were transfected with si-PART1#1 or si-NC. (**B**–**C**) Cell viability was evaluated by CCK-8 assay. (**D, E**) The migration and invasion of transfected HCC cells were detected by transwell assay. **P < 0.01.

PART1-WT group was significantly decreased by the introduction of miR-149-5p, whereas no changes of activities were observed in PART1-MUT group (Figure 3B and C). Moreover, the expression of miR-149-5p was largely promoted by PART1 silencing in SK-HEP-1 and Hep3B cells, which was hindered following transfection with PART1 (Figure 3D and E). Similarly, PART1 level was prominently blocked in SK-HEP-1 and Hep3B cells by miR-149-5p up-regulation, while PART1 expression was increased by transfection with miR-149-5p inhibitor (Figure 3F and G). Furthermore, miR-149-5p expression was drastically decreased in HCC tumor tissues and was negatively correlated with PART1 expression (R2=0.561, P<0.0001) (Figure 3H and I). All above data implied that miR-149-5p was targeted by PART1 and miR-149-p expression was down-regulated by PART1.

LncRNA PART1 Regulated Proliferation, Migration and Invasion of HCC Cells via miR-149-5p

To explore the interaction between PART1 and miR-149-5p in HCC, SK-HEP-1 and Hep3B cells were transfected with si-PART1#1 or si-PART1#1+miR-149-p inhibitor. QRT-PCR assay determined that the expression of miR-149-5p was up-regulated by PART1 silencing in SK-HEP -1 and Hep3B cells, which was abated by co-transfection with miR-149-5p inhibitor (Figure 4A). Also, CCK-8 assay indicated that proliferation of SK-HEP-1 and Hep3B cells transfected with PART1 knockdown was significantly inhibited, whereas the effect was attenuated by the interference of miR-149-5p (Figure 4B and C). Meanwhile, transwell assay implicated that miR-149-5p

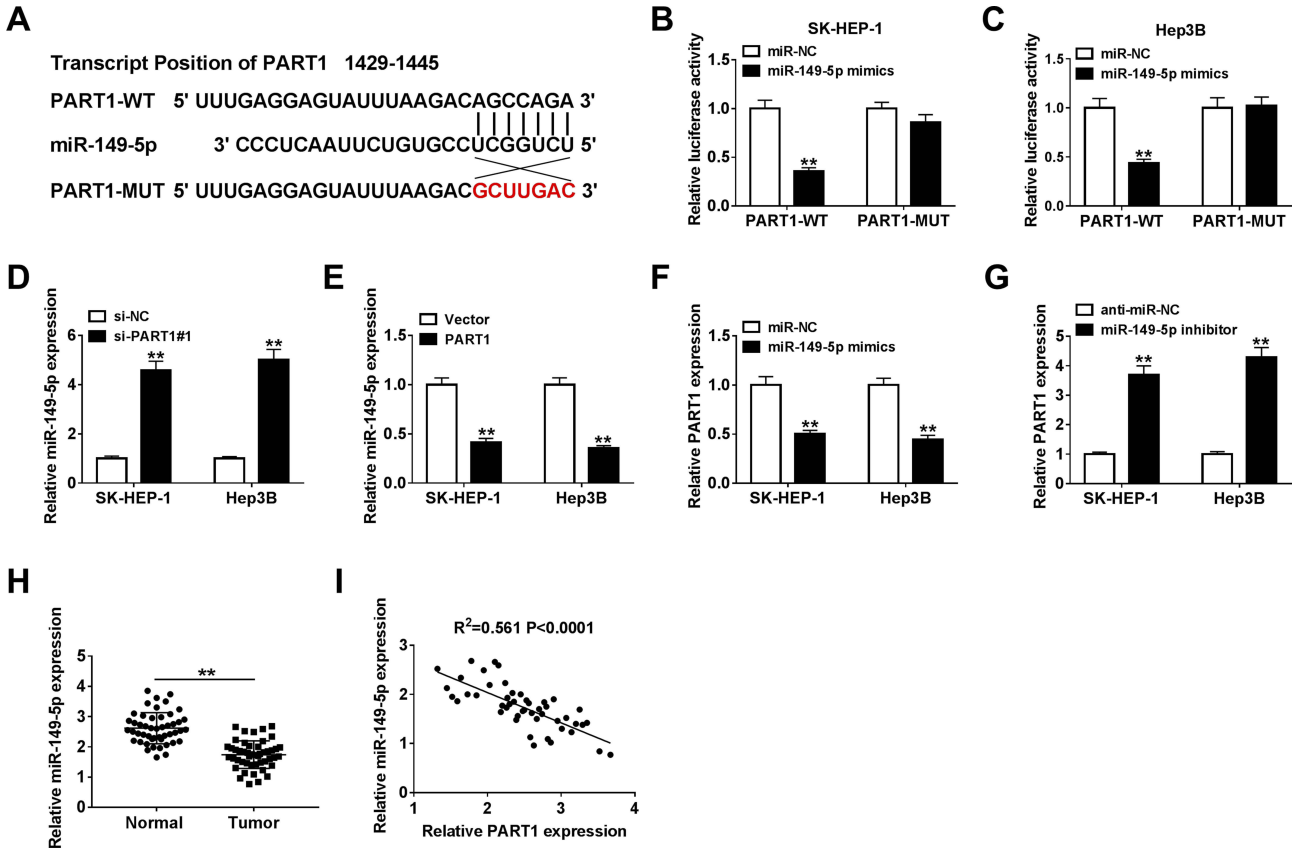

Figure 3 PART1 could bind miR-149-5p specifically and suppressed the miR-149-5p expression. (**A**) The putative binding sites between PART1 and miR-149-5p and the mutant sequences of PART1 were shown. (**B, C**) Luciferase activity was detected in SK-HEP-1 and Hep3B cells co-transfected with PART1-WT or PART1-MUT and miR-149-5p mimics or miR-NC. (**D, E**) The expression of miR-149-5p was examined by qRT-PCR in SK-HEP-1 and Hep3B cells transfected with si-NC, si-PART1#1, Vector or PART1. (**F, G**) The expression level of PART1 was detected in SK-HEP-1 and Hep3B cells transfected with miR-NC, miR-149-5p mimics, anti-miR-NC or miR-149-5p inhibitor. (**H**) The miR-149-5p expression was measured in HCC tissues and adjacent normal tissues. (**I**) Correlation analysis between PART1 and miR-149-5p expression was conducted by Pearson analysis. **P<0.01.

depletion weakened PART1 silencing-mediated anti-migration and anti-invasion of SK-HEP-1 and Hep3B cells (Figure 4D and E). In total, PART1 modulated HCC cells progression by suppressing miR-149-3p expression.

MAP2K1 Was a Target of miR-149-5p

To illuminate the underlying network of miR-149-5p in HCC, bioinformatic analysis was performed to predict the potential target. The binding sites between MAP2K1 and miR-149-5p were described in Figure 5A. Then, luciferase plasmids containing 3′-UTR of wild type MAP2K1 (MAP2K1-WT) and mutant type MAP2K1 (MAP2K1-MUT) were constructed. After transfection for 48 h, luciferase activities of both SK-HEP-1 and Hep3B cells transfected with miR-149-5p were markedly decreased in MAP2K1-WT group versus the negative control, while there were no obvious changes of activities in MAP2K1-MUT group (Figure 5B and C). Then, the mRNA and protein levels of MAP2K1 were detected by qRT-PCR or Western blot assay, respectively. The data showed that both mRNA and protein levels of MAP2K1 were significantly reduced in SK-HEP-1 and Hep3B cells when transfected with miR-149-5p and were greatly promoted by the knockdown of miR-149-5p (Figure 5D–G). Also, qRT-PCR assay indicated that MAP2K1 expression was noticeably increased in HCC tissues compared with the adjacent normal tissues (Figure 5H). The correlation analysis disclosed that there was an opposite trend between MAP2K1 and miR-149-5p expression (R2=0.517, P<0.0001) (Figure 5I). All data suggested that miR-149-5p targetedly suppressed MAP2K1 expression.

MiR-149-5p Suppressed Proliferation, Migration and Invasion of HCC Cells via MAP2K1

To further investigate the function of miR-149-5p, SK-HEP-1 and Hep3B cells were transfected with miR-149-5p mimics or miR-149-5p mimics + MAP2K1. QRT-PCR and Western blot

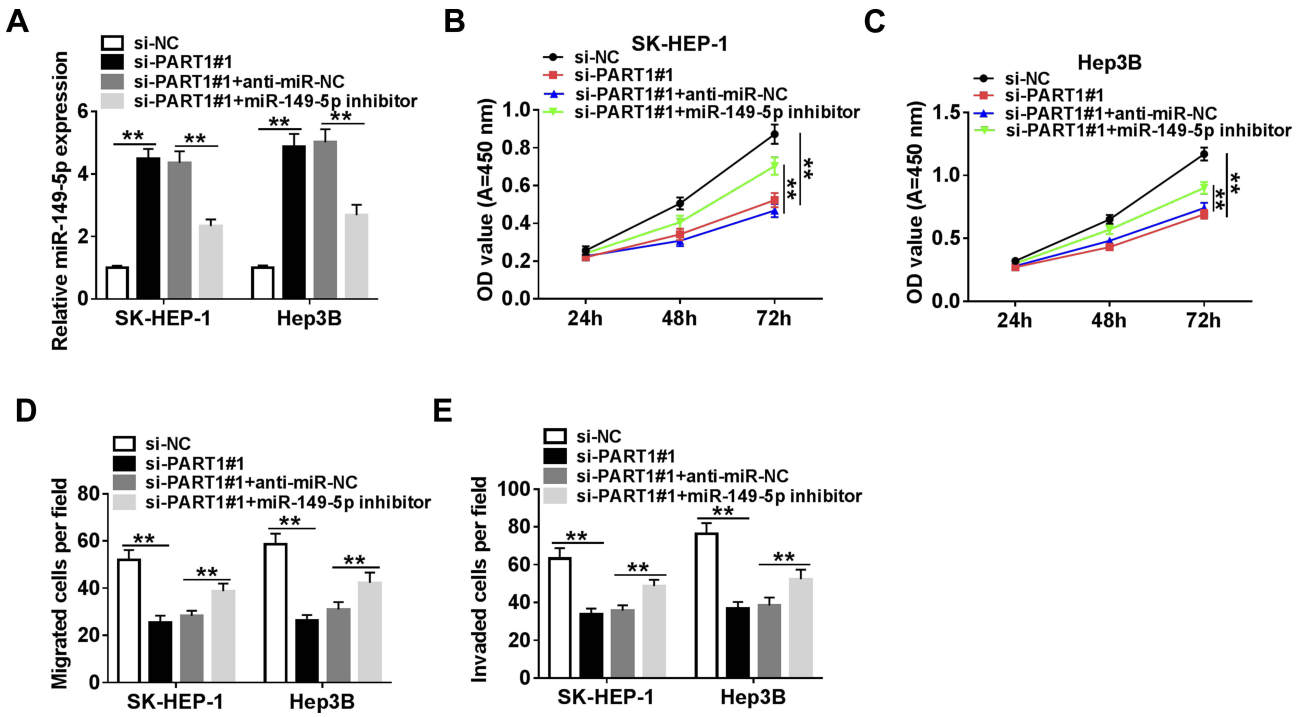

Figure 4 PART1 regulated proliferation, migration and invasion of HCC cells via miR-149-5p. SK-HEP-1 and Hep3B cells were transfected with si-NC, si-PART1#1, si-PART1#1+anti-miR-NC, or si-PART1#1+miR-149-5p inhibitor. (**A**) QRT-PCR was carried out to detect the expression of miR-149-5p in transfected HCC cells. (**B, C**) CCK-8 was used to assess the proliferation. (**D, E**) The migration and invasion were detected by transwell assay. **P<0.01.

assays determined that the expression of MAP2K1 was remarkably hindered in SK-HEP-1 and Hep3B cells transfected with miR-149-5p, which was abrogated by co-transfection with MAP2K1 (Figure 6A–C). Furthermore, the overexpression of MAP2K1 rescued miR-149-5p up-regulation-mediated anti-proliferation, anti-migration and anti-invasion effects of SK-HEP-1 and Hep3B cells (Figure 6D–G). These data implied that miR-149-5p regulated proliferation, migration and invasion of HCC cells through targeting MAP2K1.

PART1 Silencing Constrained MAP2K1 Expression by Restoring miR-149-5p Interference-Mediated Inhibitory Effect on MAP2K1 Expression in HCC Cells

To make clear the interaction among PART1/miR-149-5p/MAP2K1 axis, we measured the expression of MAP2K1 in SK-HEP-1 and Hep3B cells transfected with si-PART1 or si-PART1 + miR-149-5p inhibitor. As described above, PART1 and MAP2K1 levels were evaluated by qRT-PCR in HCC tumor tissues and there was a positive trend between the expression of them (Figure 7A). Furthermore, the mRNA and protein expression of MAP2K1 was measured by qRT-PCR or Western blot assay, respectively. Compared with the negative control, the level of MAP2K1 was conspicuously reduced by the knockdown of PART1 in SK-HEP-1 and Hep3B cells, however, MAP2K1 expression was apparently promoted when HCC cells were co-transfected with miR-149-5p inhibition (Figure 7B–D). Collectively, the effect on MAP2K1 expression caused by PART1 down-regulation could be weakened by miR-149-5p inhibition in SK-HEP-1 and Hep3B cells.

Discussion

HCC has a high morbidity and mortality.1 With the development of noninvasive radiological techniques and HCC management, HCC patients are increasingly being diagnosed early. Unfortunately, there is no effective neoadjuvant or adjuvant treatment to reduce the risk of recurrence,[35] 70% of HCC cases will encounter with recurrence in 5 years.[36] Therefore, it is urgently necessary to understand the molecular basis of HCC and develop a novel strategy for early diagnosis and treatment of HCC.

LncRNA PART1 has been reported to be an oncogenic factor in HCC.[15,16] For instance, Ye et al showed that the higher expression of PART1 was associated with a higher risk of recurrence in HCC.[15] Lv et al demonstrated that lncRNA PART1 expression profile can effectively predict

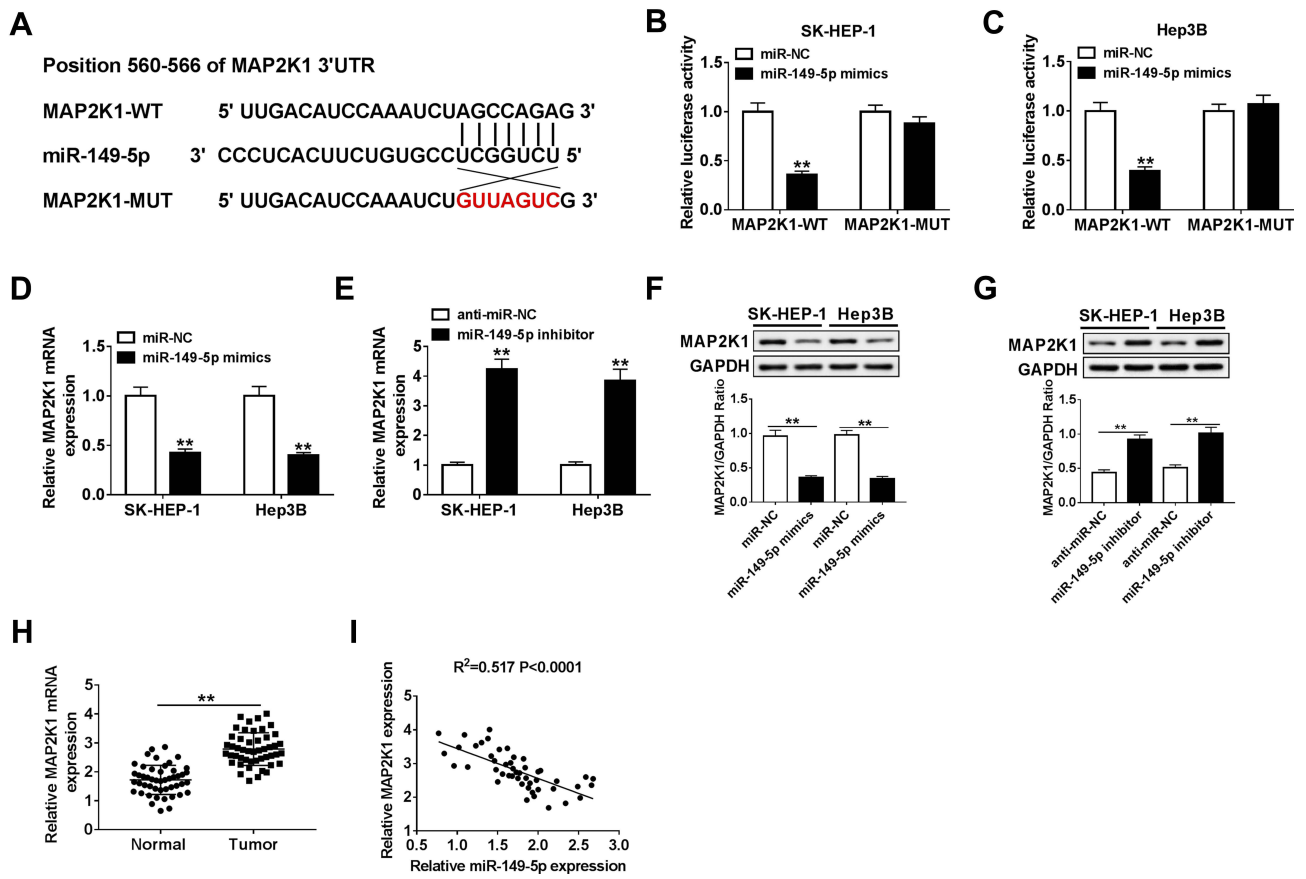

Figure 5 MAP2K1 was targeted by miR-149-5p and MAP2K1 expression was down-regulated by miR-149-5p. (**A**) The putative binding sites between MAP2K1 and miR-149-5p and the mutant sites of MAP2K1 were exhibited. (**B, C**) Luciferase activity was detected in SK-HEP-1 and Hep3B cells co-transfected with MAP2K1-WT or MAP2K1-MUT and miR-149-5p mimics or miR-NC. (**D**–**G**) The MAP2K1 mRNA and protein levels were measured in SK-HEP-1 and Hep3B cells transfected with miR-NC, miR-149-5p mimics, anti-miR-NC or miR-149-5p inhibitor. (**H**) The MAP2K1 expression was evaluated in HCC tissues and adjacent normal tissues. (**I**) Correlation analysis between MAP2K1 and miR-149-5p expression in HCC tissues was performed by Pearson analysis. **P<0.01.

early recurrence after surgical resection for HCC.[16] However, more research is needed on the regulatory mechanism of lncRNA PART1 in HCC. Consistent with above studies, we discovered that PART1 expression was noticeably increased in HCC tissues and cell lines, and PART1 knockdown suppressed proliferation, migration and invasion in HCC cells. Therefore, PART1 might be involved in the pathogenesis of HCC.

Recently, emerging evidence suggests that lncRNAs can act as competing endogenous RNAs (ceRNAs) of specific miRNAs to prevent miRNAs from interacting with target mRNAs and regulate downstream target mRNAs expression in HCC.[15] However, whether PART1 could serve as a ceRNA of miR-149-5p in HCC progression remained undefined and the lncRNA-miRNA-mRNA ceRNA regulatory network about PART1 needed to be elucidated. Previously, miR-149 has been demonstrated to function as a tumor suppressor in multiple malignant diseases.[37–40] MiR-149-5p suppressed cell proliferation and invasion by targeting FOXM1 in CRC,[38] GIT1 in breast cancer,[39] and ZBTB2 in GC.[40] In the present study, we found that miR-149-5p was downregulated in HCC tissues, which was in line with previous reports.[37–40] More importantly, we discovered that miR-149-5p was targeted by PART1 and miR-149-5p expression was down-regulated by PART1 in HCC cells. Rescues assay showed the effect of PART1 knockdown on cell proliferation, migration and invasion could be partially rescued by miR-149-5p interference. All above data implicated that lncRNA PART1 regulated the progression of HCC cells via targeting miR-149-5p.

It has been shown that mutations and deletions in the activation region of MAP2K1 constitutively activated proteins.[34,41] Hirata et al showed that knockdown of MAP2K1 reproduced the tumor-suppressive effect of miR-1826.[41] Cui et al demonstrated that overexpression of miR-539 inhibited cell proliferation, migration, invasion and increased apoptosis rate, and miR-539 suppressed the

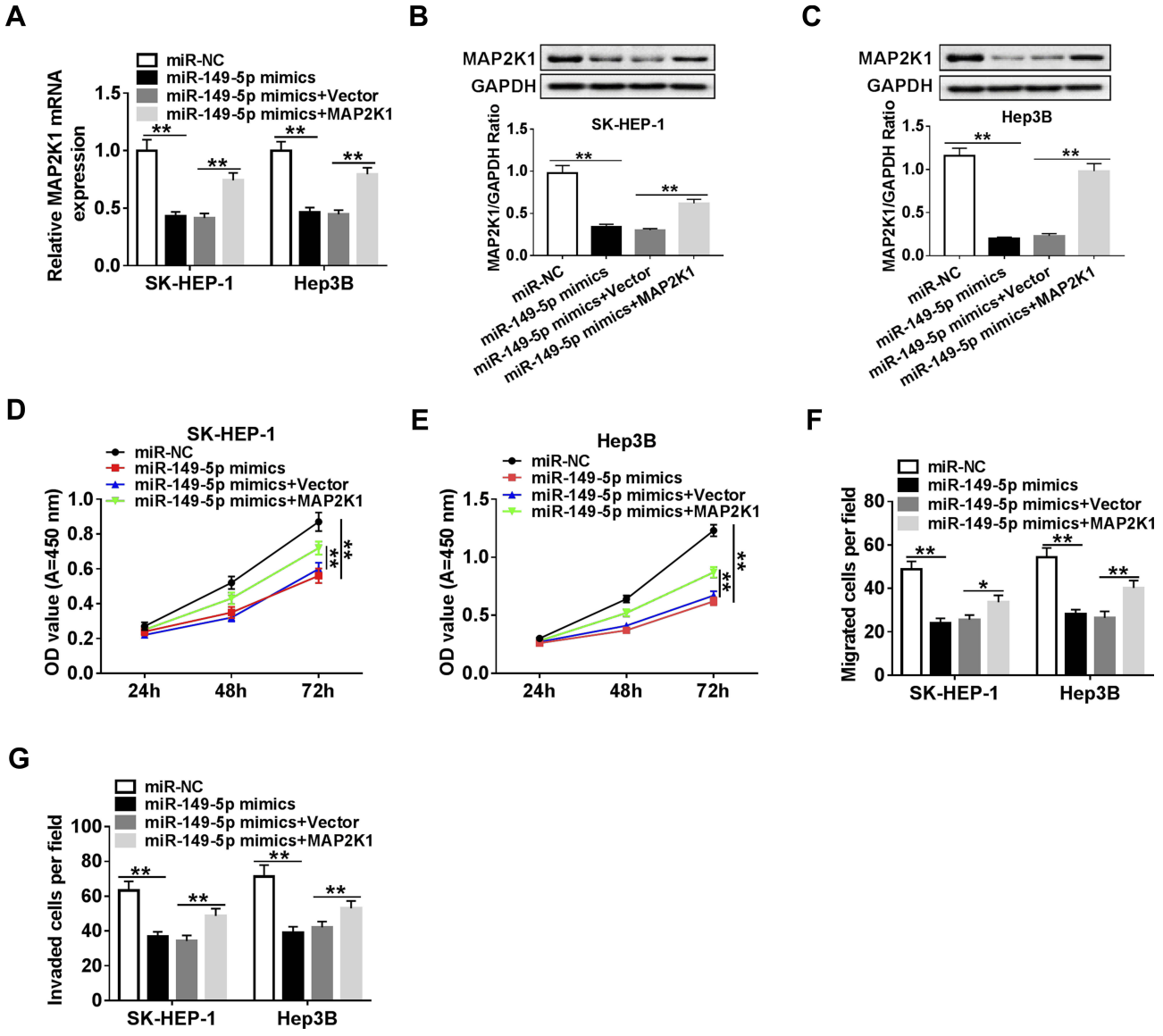

Figure 6 miR-149-5p inhibited MAP2K1 expression and regulated proliferation, migration and invasion of HCC cells via MAP2K1. SK-HEP-1 and Hep3B cells were transfected with miR-NC, miR-149-5p mimics, miR-149-5p mimics + Vector, or miR-149-5p mimics+MAP2K1. (**A**) The expression level of MAP2K1 mRNA was measured by qRT-PCR assay. (**B, C**) The MAP2K1 protein level was evaluated by Western blot in transfected SK-HEP-1 and Hep3B cells. (**D, E**) Cell proliferative ability of transfected SK-HEP-1 and Hep3B cells was assessed by CCK-8 assay. (**F, G**) Cell migration and invasion of transfected SK-HEP-1 and Hep3B cells were detected by transwell assay. *P < 0.05, **P<0.01.

progression of HCC by directly targeting and regulating MAP2K1.[34] Our research revealed that MAP2K1 was a target of miR-149-5p and miR-149-5p targetedly suppressed MAP2K1 expression in HCC. Moreover, the effect of miR-149-5p overexpression on cell proliferation, migration and invasion could be partially rescued by MAP2K1 up-regulation. These results implied that miR-149-5p regulated proliferation, migration and invasion of HCC cells through targeting MAP2K1.

Interestingly, to explore the interaction of PART1/miR-149-5p and miR-149-5p/MAP2K1 axis, we analyzed the relationship between MAP2K1 and PART1 and found that MAP2K1 expression was positively correlated with PART1 expression in HCC tissues. Furthermore, after transfection with si-PART1, the level of MAP2K1 expression was conspicuously reduced in HCC cells, whereas, miR-149-5p inhibitor enhanced MAP2K1 expression. Thus, these data suggested that the effect on MAP2K1 expression caused by PART1 down-regulation could be weakened by miR-149-5p inhibition in vitro. Therefore, it was concluded that PART1 could function as a ceRNA by competitively binding to miR-149-5p and thus improving MAP2K1 expression in HCC tumorigenesis and progression. Finally, we constructed the lncRNA PART1-associated ceRNA

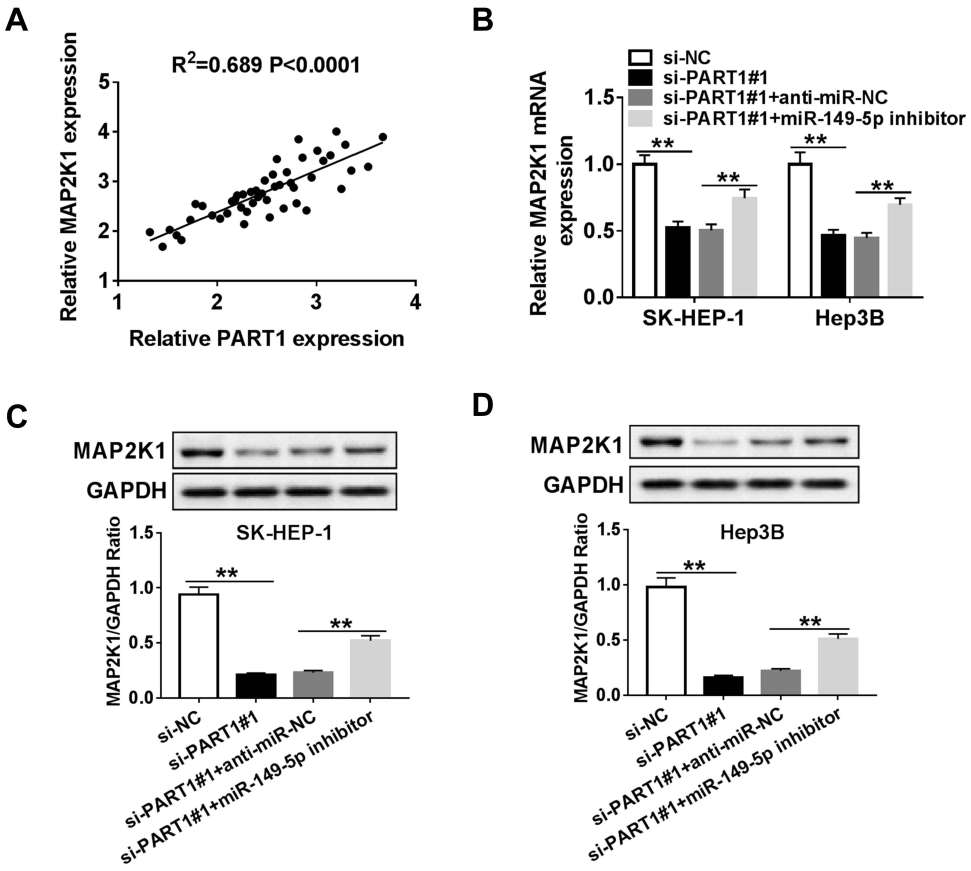

Figure 7 PART1 increased MAP2K1 level by weakening miR-149-5p-mediated inhibitory effect on MAP2K1 in SK-HEP-1 and Hep3B cells. (**A**) Correlation analysis between MAP2K1 and PART1 expression in HCC was performed by Pearson analysis. (**B**–**D**) SK-HEP-1 and Hep3B cells were transfected with si-NC, si-PART1#1, si-PART1#1+anti-miR-NC, or si-PART1#1+miR-149-5p inhibitor. (**B**) The MAP2K1 mRNA expression was assessed by qRT-PCR. (**C, D**) MAP2K1 protein level was determined by Western blot in transfected SK-HEP-1 and Hep3B cells. **P<0.01.

regulatory network of PART1/miR-149-5p/MAP2K1 axis in HCC cells.

All in all, this research revealed that lncRNA PART1 could promote cell proliferation, migration and invasion in HCC via miR-149-5p/MAP2K1 axis, providing potential therapeutic targets for HCC and deepening our understanding on the role of lncRNA-miRNA functional network in HCC. However, there still exist limitations in our article. In vivo data of PART1 in HCC and the clinical utility and accuracy for treatment of HCC remain to be further investigated.

Conclusion

PART1 expression was largely increased in HCC tissues and cells. Moreover, PART1 could bind miR-149-5p, and the knockdown of PART1 distinctly restrained proliferation, migration and invasion of HCC cells which were enhanced partially by co-transfection with miR-149-5p inhibition. Meanwhile, MAP2K1 was directly targeted by miR-149-5p. MAP2K1 overexpression abolished miR-149-5p up-regulation-mediated anti-proliferation, anti-migration and anti-invasion effects on HCC cells. Besides, MAP2K1 level was greatly decreased by PART1 depletion, which was abated by the introduction of miR-149-5p inhibitor.

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Disclosure

The authors declare that they have no financial conflicts of interest.

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