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LncRNA HCG11 accelerates the progression of hepatocellular carcinoma *via* miR-26a-5p/ATG12 axis

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Abstract. – OBJECTIVE: Hepatocellular carcinoma (HCC) is one of the most commonly diagnosed cancers globally. LncRNA HLA complex group 11 (HCG11) has been reported to play an oncogenic role in multiple cancers. Nevertheless, the role and regulatory mechanism of HCG11 in HCC are not fully addressed.

PATIENTS AND METHODS: The abundance of HCG11 and miR-26a-5p was measured by quantitative Real Time-Polymerase Chain Reaction (qRT-PCR) in HCC tissues and cells. Cell proliferation, apoptosis, metastasis, and autophagy were detected by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), flow cytometry, transwell migration, invasion assays, and Western blot assay, respectively. The binding sites between miR-26a-5p and HCG11 or autophagy-related 12 (ATG12) were predicted by starBase bioinformatic software, and the combination was confirmed by Dual-Luciferase reporter assay. The abundance of ATG12 was examined by Western blot assay. Murine xenograft model was established to validate the function of HCG11 *in vivo*.

RESULTS: The enrichment of HCG11 was enhanced in HCC tissues and cells and was negatively related to the prognosis of HCC patients. The abundance of miR-26a-5p was inversely correlated with the level of HCG11 in HCC tissues. HCG11 interference suppressed the proliferation, metastasis, and autophagy while promoted the apoptosis of HCC cells. MiR-26a-5p bound to lncRNA HCG11 and ATG12. The depletion of miR-26a-5p or the accumulation of ATG12 could alleviate the suppressive effects induced by HCG11 intervention on the proliferation, metastasis, autophagy, and the promoting impact on the apoptosis of HCC cells. HCG11 promoted the growth of murine xenograft tumor and autophagy through miR-26a-5p/ATG12 axis *in vivo*.

CONCLUSIONS: LncRNA HCG11 accelerated the proliferation, metastasis, and autophagy while impeded the apoptosis of HCC cells via HCG11/miR-26a-5p/ATG12 axis. HCG11 might be a potential therapeutic target for the treatment of HCC.

Key Words:
HCC, LncRNA HCG11, MiR-26a-5p, ATG12, Proliferation, Apoptosis, Metastasis, Autophagy.

Introduction

It was estimated that 781,631 deaths of liver cancer occurred worldwide in 2018, accounting for 8.2% of all cancers[1]. Hepatocellular carcinoma (HCC) is the most common form of liver cancer. Although the therapeutic method has been improved, the survival rate of advanced-stage HCC patients remains poor due to tumor recurrence and metastasis[2,3]. Hence, finding novel markers for early-stage determination and uncovering the potential mechanism are pivotal for HCC treatment.

Long noncoding RNAs (lncRNAs) are involved in many physiological and pathological processes of cells in epigenetic and posttranscriptional levels[4-6]. Researchers[7-9] reported that lncRNAs could serve as competing endogenous RNAs (ceRNAs) to repress the biological function of microRNAs (miRNAs). LncRNA HLA complex group 11 (HCG11) has been reported to be upregulated in HCC and breast cancer (BC). Xu et al[10] claimed that the abundance of HCG11 was abnormally elevated in HCC, and HCG11 promoted the progression of HCC *via* insulin-like growth factor 2 mRNA binding protein 1 (IGF2BP1). Liu et al[11] found that the level of HCG11 was negatively related to the prognosis of BC patients. Nevertheless, the specific mechanism by which HCG11 accelerates the carcinogenesis of HCC is largely unknown.

MiRNAs could reduce the abundance of target messenger RNA (mRNA) or restrain their translation by directly binding to their 3' untranslated region (UTR)[12,13]. MiRNAs were involved in

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various cellular processes, including cell proliferation, metastasis, and apoptosis[14,15]. MiR-26a-5p was downregulated in multiple cancers, including HCC, prostate cancer (PC), and osteosarcoma. For instance, Chang et al[16] demonstrated that miR-26a-5p was decreased in HCC tumor tissues, and it suppressed the metastasis of HCC cells by modulating epithelial-mesenchymal transition (EMT). Guo et al[17] proved that miR-26a-5p inhibited the proliferation and metastasis of PC cells by inversely modulating SERBP1. Wang et al[18] reported that MALAT1 accelerated the proliferation and migration of osteosarcoma cells by sponging miR-26a-5p. However, the biological role of miR-26a-5p and the potential signal regulatory network in HCC remain poorly understood.

Autophagy is a conserved process. During the process of autophagy, impaired or superfluous organelles and proteins are degraded by lysosome. Autophagy has been reported[19-21] to promote chemoresistance and the viability of cancer cells under diverse stresses. Autophagy-related 12 (ATG12) is a crucial component in the elongation of autophagosomes[22]. Hu et al[23] claimed that ATG12-mediated autophagy regulated the radiosensitivity in colorectal cancer (CRC). An et al[24] proved that ATG12-mediated autophagy modulated chemoresistance in gastric cancer (GC). However, the role and molecular mechanism of ATG12 in HCC are not fully addressed.

In this study, we analyzed the abundance of HCG11 in HCC tissues and cells, as well as its relationship with tumor staging and survival rate of HCC patients. Loss-of-function experiments were conducted to assess the effects of HCG11 interference on the proliferation, metastasis, apoptosis, and autophagy of HCC cells. Besides, we explored whether HCG11 could sponge miR-26a-5p to modulate its level in HCC cells, and investigated the modulatory network between miR-26a-5p and ATG12.

Patients and Methods

Tissue Specimens

Sixty-five HCC tissues and their matching non-tumor tissues were collected from patients who had undergone resection in the First Affiliated Hospital of Xi'An Jiaotong University. The tissues were stored at -80°C after surgical resection for the detection of the abundance of HCG11 and miR-26a-5p. This experiment was carried out with the permission of the Ethic Committee of the First Affiliated Hospital of Xi'An Jiaotong University, and all patients signed a written informed consent.

Cell Culture

HCC cell lines MHCC97-H, Hep3B, and normal liver cell line THLE-2 were obtained from BeNa Culture Collection (Beijing, China). All cells were cultivated in Roswell Park Memorial Institute-1640 (RPMI-1640) medium (Gibco, Carlsbad, CA, USA) added with 10% fetal bovine serum (FBS; Gibco, Carlsbad, CA, USA) and 10% penicillin (100 U/mL)/streptomycin (100 μg/mL) mixed solution at 37°C with 5% CO_2.

Cell Transfection

Transfection was carried out with Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA). Small interference RNA negative control (si-NC), small interference RNA against HCG11 (si-HCG11#1, si-HCG11#2, si-HCG11#3), empty vector, HCG11 overexpression plasmid (HCG11), and ATG12 overexpression plasmid (ATG12) were obtained from GenePharma (Shanghai, China). MiR-NC, miR-26a-5p, anti-NC, and anti-miR-26a-5p were obtained from Ribobio (Guangzhou, China).

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

The reverse transcription of HCG11 was performed using M-MLV reverse transcriptase kit (Invitrogen, Carlsbad, CA, USA). The reverse transcription of miR-26a-5p was conducted by using All-in-One™ miRNA First stand cDNA Synthesis Kit (GeneCopoeia, Rockville, MD, USA). U6 small RNA or glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was regarded as the internal reference. The $2^{-\Delta\Delta Ct}$ method was used for the detection of the abundance of HCG11 and miR-26a-5p[25]. The primer sequences were shown as below: HCG11 (Forward, 5'-GCTCTATG CCATCCTGCTT-3'; Reverse, 5'-TCCCATCTC-CATCAACCC-3'), miR-26a-5p (Forward, 5'-CCGCCGTTCAAGTAA TCCAG-3'; Reverse, 5'-AGTGCAGGGTCCGAGGTATT-3'), U6 (Forward, 5'-CCTGCGCAAGGATGAC-3'; Reverse, 5'-GTGCAGGGTCCGAGGT-3'), GAPDH (Forward, 5'-CTGGGCTACACTGAGCACC-3'; Reverse, 5'-AAGTGG TCGTTGAGGGCAATG-3').

3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) Assay

10 μL MTT (Invitrogen, Carlsbad, CA, USA) was added to the wells of 96-well plates after transfection for 0 h, 24 h, 48 h, and 72 h for additional 4 h. After removing the cell supernatant, the dimethyl sulfoxide (DMSO; Sigma-Aldrich,

St. Louis, MO, USA) was added to the wells. The optical density was detected by a microplate reader at 490 nm.

Cell Apoptosis Analysis

Flow cytometry was carried out to measure the apoptosis of HCC cells. HCC cells were seeded into 6-well plate and cultured at 37°C. After transfection with si-NC or si-HCG11, HCC cells were harvested using cold phosphate-buffered saline (PBS) buffer for twice. Then, the cells were stained with 5 μL Annexin V combined fluorescein isothiocyanate (FITC) and propidium iodide (PI; Solarbio, Beijing, China) for 10 min at room temperature. Then, the apoptotic cells (FITC+, PI+/-) were identified by the flow cytometer (BD Biosciences, Franklin Lakes, NJ, USA).

Transwell Migration and Invasion Assays

To assess the migration capacity of MHCC97-H and Hep3B cells, MHCC97-H and Hep3B cells in medium without serum were seeded into the upper chambers. The lower chambers were filled with medium, were added with 10% FBS. The migrated cells were dyed and counted after 48-h incubation.

To evaluate the invasion ability of MHCC97-H and Hep3B cells, the polycarbonate membrane was pre-coated with BD Matrigel matrix (BD Biosciences, Franklin Lakes, NJ, USA); MHCC97-H and Hep3B cells were then seeded into the pre-coated upper chambers. The other steps were the same as above.

Western Blot Assay

MHCC97-H and Hep3B cells were harvested using cold PBS buffer and lysed using RIPA lysis solution (Beyotime, Shanghai, China). The quantified proteins were loaded onto sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA, USA). After the nonspecific binding sites were blocked for 1 h, the membranes were incubated with antibody against LC3 (ab51520, Abcam, Cambridge, MA, USA), p62 (ab109012, Abcam, Cambridge, MA, USA), ATG12 (ab155589, Abcam, Cambridge, MA, USA) or β-actin (ab8226, Abcam, Cambridge, MA, USA) at 4°C for 12 h. The PVDF membranes were then incubated with secondary antibody (ab205718, Abcam, Cambridge, MA, USA). The protein signal was measured *via* enhanced chemiluminescence (ECL) system (Beyotime, Shanghai, China).

Dual-Luciferase Reporter Assay

The putative combination sequences were predicted by starBase bioinformatic software. For the confirmation of the combination between miR-26a-5p and lncRNA HCG11, the sequences of lncRNA HCG11 containing wild-type or mutant type binding sites were amplified and inserted to the Luciferase reporter vector, namely HCG11 WT or HCG11 MUT. The above Luciferase reporter vectors were co-transfected with miR-26a-5p or miR-NC in MHCC97-H and Hep3B HCC cells. After transfection for 48 h, the cells were collected and lysed, and Luciferase activity was examined.

The combination between ATG12 and miR-26a-5p was also validated by Dual-Luciferase reporter assay. The 3' UTR of ATG12, including wild-type or mutant type binding sites, was amplified and inserted to the Luciferase reporter vector, generating ATG12 WT or ATG12 MUT. The other steps were the same as above.

Murine Xenograft Assay

Nude mice xenograft experiments were approved by the Animal Research Committee of the First Affiliated Hospital of the Xi'An Jiaotong University. Tumor xenograft model was built using MHCC97-H cells stably transfected with sh-HCG11 or sh-NC. The mice were subcutaneously injected with the above MHCC97-H cells, and the tumor volume was measured per week. The mice were sacrificed after five-week following the inoculation, and the weight of tumors was recorded. Tumor tissues were used to detect the levels of HCG11, miR-26a-5p, ATG12, and autophagy-related proteins (LC3 I, LC3 II, and p62).

Statistical Analysis

All data from three independent experiments were analyzed using GraphPad Prism 7 software (La Jolla, CA, USA) and were displayed as mean±standard deviation (SD). Student's *t*-test and One-way analysis of variance (ANOVA) followed by Tukey's test were used for the analysis of differences between the two groups or among multiple groups. Survival curve of patients was generated by Kaplan-Meier plot and analyzed by log-rank test. The linear relationship between the level of miR-26a-5p and the expression of HCG11 in HCC tissues was assessed by Spearman's correlation coefficient. $p<0.05$ was considered to be statistically significant.

Results

The Enrichment of LncRNA HCG11 is Elevated in HCC Tissues and Cells, and is Positively Related to Tumor Staging of HCC Patients

To investigate the potential role of HCG11 in HCC, we first examined the expression of HCG11 in HCC tissues and adjacent normal tissues. The abundance of HCG11 was elevated in HCC tissues compared with that in the corresponding normal tissues (Figure 1A). The HCC patients were divided into two groups in terms of the TNM staging, and the statistics data showed that the expression of HCG11 was positively related to TNM staging (Figure 1B). The survival curve was analyzed in HCG11 low and high expression groups, and the five-year survival rate of HCC patients was prominently decreased in HCG11 high expression group, compared with that in the HCG11 low expression group (Figure 1C). These findings suggested that HCG11 was abnormally upregulated in HCC tissues, and the high expression of HCG11 was positively related to the TNM staging and was inversely related to the prognosis of HCC patients. The enrichment of HCG11 was higher in HCC cells than that in normal liver cells THLE-2 (Figure 1D). MiR-26a-5p was downregulated in HCC tissues and cells (Figure 1E and 1F), and the abundance of miR-26a-5p was negatively related to the enrichment of HCG11 in HCC tissues (Figure 1G).

HCG11 Promotes the Proliferation, Metastasis, and Autophagy while Restrains the Apoptosis of HCC Cells

We further explored the biological significance of HCG11 in HCC cells by loss-of-function experiments. The knockdown efficiency of small interference RNA against HCG11 (si-HCG11#1, si-HCG11#2, and si-HCG11#3) was assessed in MHCC97-H and Hep3B cells. As mentioned in Figures 2A and 2B, the expression of HCG11 was notably declined by the transfection of si-HCG11#1, si-HCG11#2, and si-HCG11#3. Due to the highest knockdown efficiency of HCG11, si-HCG11#1 was chosen for the following experiments. Cell proliferation was prominently restrained after si-HCG11#1 transfection (Figures 2C and 2D). Flow cytometry was conducted to evaluate the apoptosis rate of HCC cells in si-HCG11#1 group and control group, and the intervention of HCG11 facilitated the apoptosis of HCC cells (Figure 2E). Meanwhile, the migration and invasion of MHCC97-H and Hep3B cells were inhibited by the transfection of si-HCG11#1 with the detection by transwell migration and invasion assays (Figures 2F and 2G). Besides, the Western blot assay showed that the ratio of LC3 II/ LC3 I was decreased, and the expression of p62 was enhanced with HCG11 the knockdown in MHCC97-H and Hep3B cells (Figure 2H). These findings indicated that HCG11 served as an oncogenic RNA to accelerate the proliferation, metastasis, and autophagy and impede the apoptosis of HCC cells.

MiR-26a-5p Plays a Suppressive Role in the Progression of HCC

MiR-26a-5p has been reported to be downregulated in multiple cancers[16-18], implying that it might serve as a tumor suppressor to exert its function. We first investigated the effects of miR-26a-5p overexpression on the proliferation, apoptosis, metastasis, and autophagy of HCC cells by performing MTT assay, flow cytometry, transwell migration, invasion assays, and Western blot. The abundance of miR-26a-5p was enhanced by the transfection of miR-26a-5p mimics, and it was reduced with the addition of anti-miR-26a-5p in HCC cells (Figure 3A). Subsequently, we found that the overexpression of miR-26a-5p suppressed the proliferation, as well as promoted the apoptosis of HCC cells (Figure 3B-3D). Furthermore, the migration and invasion of HCC cells were inhibited by the accumulation of miR-26a-5p (Figure 3E and 3F). The abundance of LC3 II/ LC3 I was decreased, and the enrichment of p62 was elevated by the transfection of miR-26a-5p, indicating that the addition of miR-26a-5p repressed the autophagy of HCC cells (Figure 3G). These results revealed that miR-26a-5p played a tumor suppressor role in HCC.

MiR-26a-5p is a Direct Target of HCG11

LncRNA could serve as a ceRNA of miRNA to exert its function. StarBase online software was used to screen the targets of HCG11. Although many genes were predicted to bind to HCG11, we focused on miR-26a-5p because it was negatively regulated by HCG11. As showed in Figure 4A, the binding sites between HCG11 and miR-26a-5p were predicted by starBase. The Luciferase reporter vector containing the wild-type or mutant type binding sites of HCG11, named as HCG11 WT or HCG11 MUT, was constructed to confirm the combination between HCG11 and miR-26a-5p.

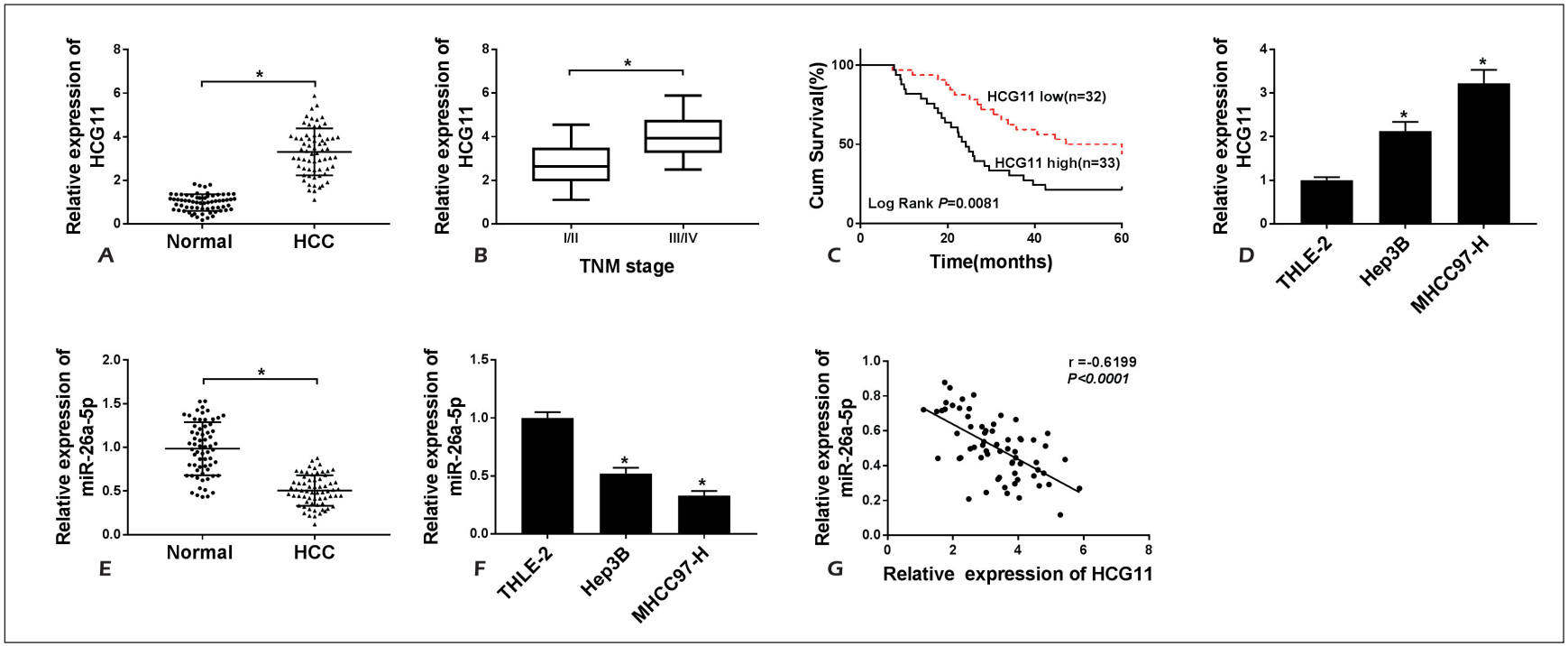

Figure 1. The enrichment of lncRNA HCG11 is elevated in HCC tissues and cells and it is positively related to tumor staging of HCC patients. **A**, The expression of HCG11 was detected in HCC tissues (n=65) and adjacent normal tissues (n=65) by qRT-PCR. **B**, The abundance of HCG11 was measured in terms of TNM staging by qRT-PCR. **C**, The survival rate of HCC patients was analyzed in HCG11 high expression and low expression groups using Kaplan-Meier analysis and log-rank test. **D**, The relative expression of HCG11 was determined in normal liver cells THLE-2 and HCC cells, including Hep3B and MHCC97-H by qRT-PCR. **E**, The level of miR-26a-5p was examined in HCC tissues (n=65) and corresponding non-tumor tissues (n=65) by qRT-PCR. **F**, QRT-PCR was conducted to detect the level of miR-26a-5p in normal liver cells and HCC cells. **G**, Spearman's correlation analysis was conducted to analyze the correlation between the expression of miR-26a-5p and the abundance of HCG11. *$p<0.05$.

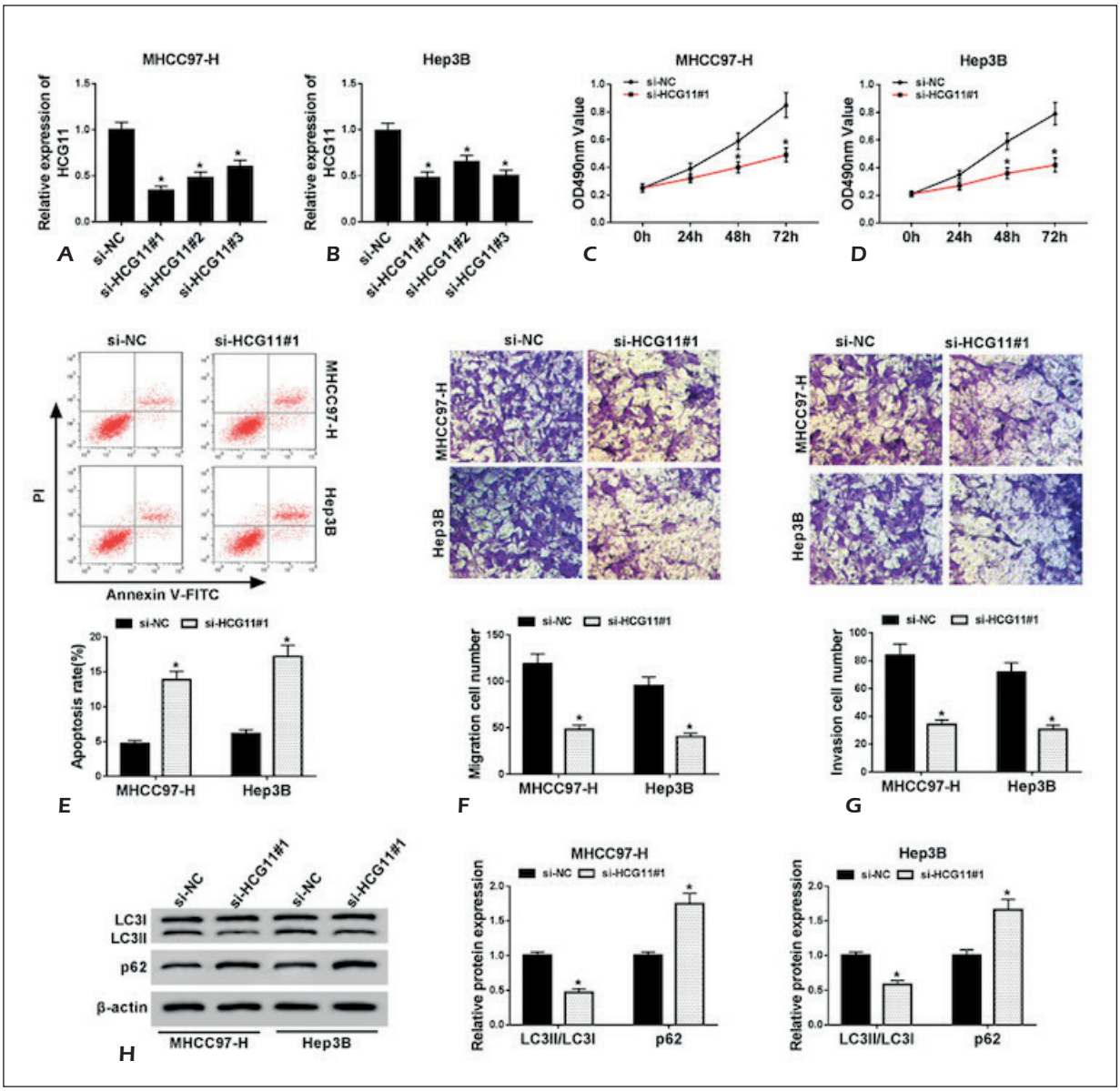

Figure 2. HCG11 promotes the proliferation, metastasis and autophagy while restrains the apoptosis of HCC cells. **A**-**B**, The knockdown efficiency was analyzed in MHCC97-H and Hep3B cells transfected with si-HCG11#1, si-HCG11#2, si-HCG11#3 or si-NC by qRT-PCR. **C**-**D**, MTT assay was performed to detect the proliferation of HCC cells transfected with si-HCG11#1 or si-NC. **E**, The apoptosis of HCC cells with the depletion of HCG11 was examined by flow cytometry. **F**-**G**, (x 300), The migration and invasion of HCC cells transfected with si-HCG11#1 or si-NC were determined by transwell migration and invasion assays. H, Western blot was carried out to detect the abundance of autophagy markers (LC3 I, LC3 II, and p62) in the HCC cells of different groups. $^{**}p<0.05$.

As indicated in Figures 4B and 4C, the Luciferase activity was dramatically declined with the accumulation of miR-26a-5p in HCG11 WT group compared with that in the HCG11 MUT group, suggesting that miR-26a-5p was a direct target of HCG11 in HCC cells. Subsequently, we found an inverse relationship between the expression of miR-26a-5p and the enrichment of HCG11 in MHCC97-H and Hep3B cells. The abundance of miR-26a-5p was increased with the depletion of HCG11, which was notably reduced with the accumulation of HCG11 (Figures 4D and 4E).

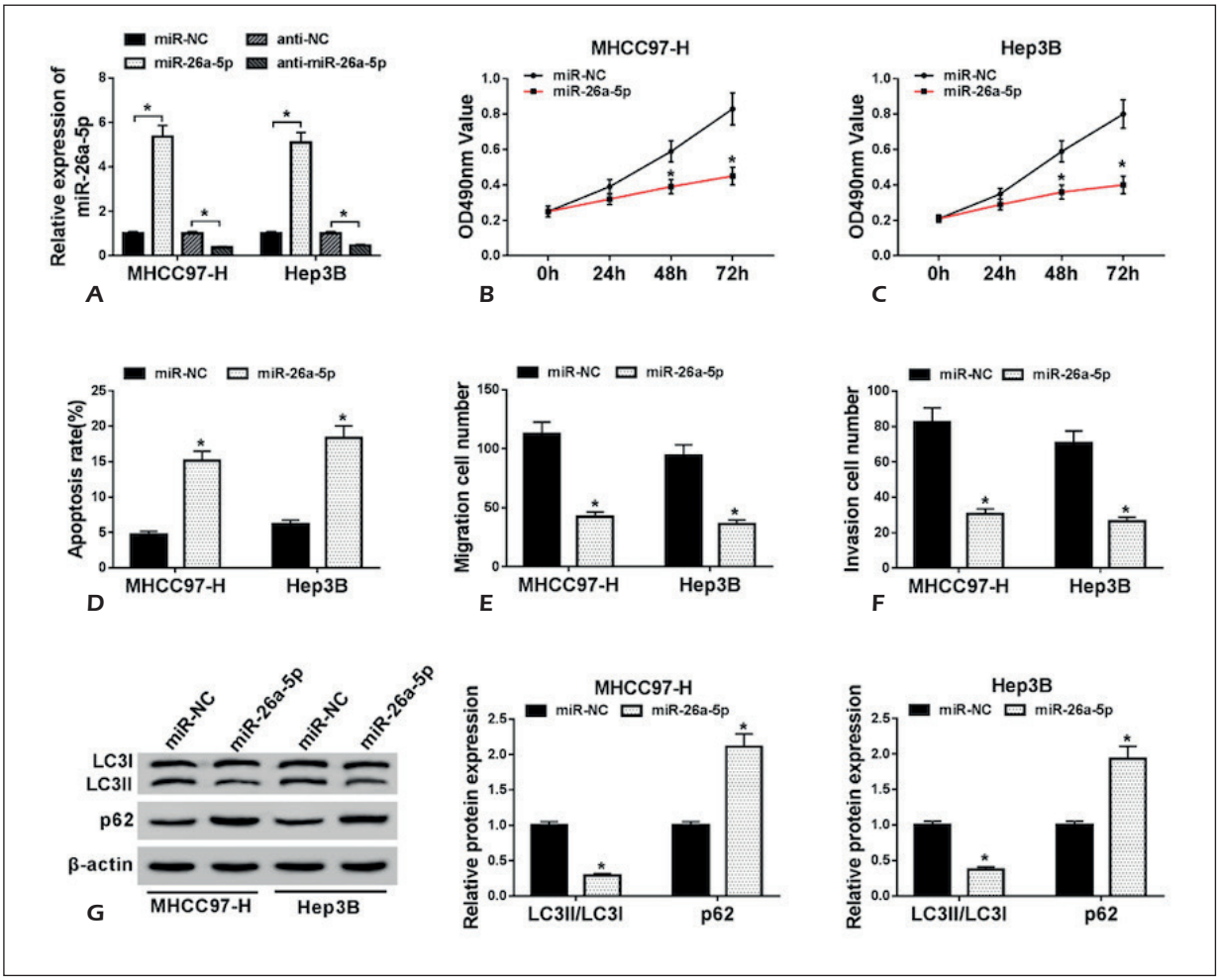

Figure 3. MiR-26a-5p plays a suppressive role in the progression of HCC. Hep3B and MHCC97-H cells transfected with miR-NC, miR-26a-5p, anti-NC or anti-miR-26a-5p were used for the following experiments. **A**, The abundance of miR-26a-5p was examined in HCC cells by qRT-PCR. **B-C**, The proliferation of HCC cells was measured by MTT assay. **D**, Flow cytometry was applied to detect the apoptosis of HCC cells. **E-F**, Transwell migration and invasion assays were carried out to detect the motility of HCC cells. **G**, Western blot assay was performed to detect the enrichment of LC3 I, LC3 II, and p62 in HCC cells, and β-actin was regarded as the internal reference. *$p<0.05$.

ATG12 is a Target of MiR-26a-5p

To illustrate the underlying mechanism by which miR-26a-5p restrains the development of HCC, we aimed to find the target of miR-26a-5p. As mentioned in Figure 5A, starBase bioinformatic software predicted that miR-26a-5p bound to ATG12. The 3' UTR of ATG12 containing wild-type or mutant type binding sites was amplified and inserted to the Luciferase reporter vector and co-transfected with miR-NC or miR-26a-5p into MHCC97-H and Hep3B cells. The transfection of miR-26a-5p markedly decreased the Luciferase activity in ATG12 WT group, whereas it had a little effect on ATG12 MUT group, demonstrating that ATG12 bound to miR-26a-5p in HCC cells (Figures 5B and 5C). To assess the regulatory relationship between miR-26a-5p and ATG12, miR-NC, miR-26a-5p, anti-NC or anti-miR-26a-5p were transfected into MHCC97-H or Hep3B cells. As indicated in Figures 5D and 5E, the abundance of ATG12 was decreased by the overexpression of miR-26a-5p, while it was elevated with miR-26a-5p inhibition in MHCC97-H and Hep3B cells. To evaluate the modulation among HCG11, miR-26a-5p and ATG12, MHCC97-H and Hep3B cells were transfected with si-NC, si-HCG11#1, si-HCG11#1 + anti-NC or si-HCG11#1 + anti-miR-26a-5p, respectively. The expression of ATG12 was declined with the interference of HCG11, and the inhibition of miR-26a-5p reversed the suppressive effect of HCG11 depletion on the level of ATG12 in

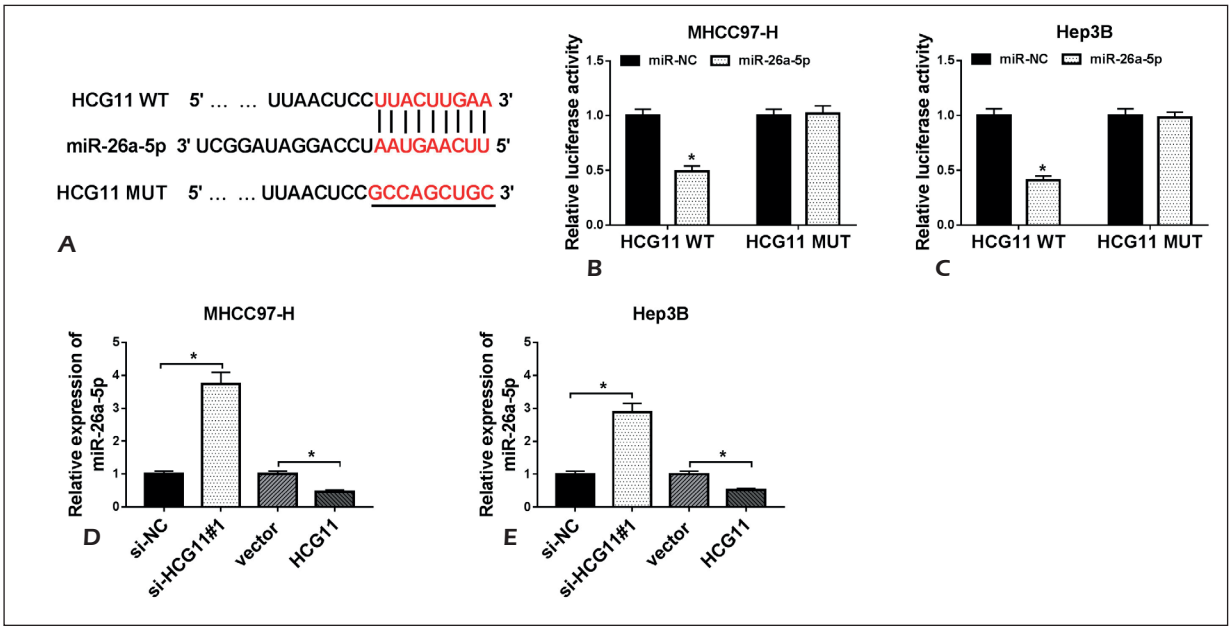

Figure 4. MiR-26a-5p is a direct target of HCG11. **A**, MiR-26a-5p was predicted to be a target of HCG11 based on the information of starBase. **B**-**C**, Dual-Luciferase reporter assay was conducted to confirm the combination between miR-26a-5p and HCG11 in MHCC97-H and Hep3B cells. **D**-**E**, The relative expression of miR-26a-5p was determined in MHCC97-H and Hep3B cells transfected with si-NC, si-HCG11#1, vector or HCG11 overexpression plasmid by qRT-PCR. *p<0.05.

MHCC97-H and Hep3B cells (Figures 5F and 5G). These findings suggested that ATG12 was a functional target of miR-26a-5p in HCC cells. The abundance of ATG12 was positively regulated by HCG11 and was inversely modulated by miR-26a-5p.

The Inhibition of MiR-26a-5p or the Accumulation of ATG12 Could Reverse the Suppressive Effects of HCG11 Interference on the Proliferation, Metastasis, and Autophagy of HCC Cells

The abundance of ATG12 was notably enhanced by the transfection of ATG12 overexpression plasmid in MHCC97-H and Hep3B cells (Figure 6A). To illustrate whether miR-26a-5p or ATG12 was involved in HCG11-mediated proliferation, apoptosis, metastasis, and autophagy in HCC cells, MHCC97-H and Hep3B cells were transfected with si-NC, si-HCG11#1, si-HCG11#1 + anti-NC, si-HCG11#1 + anti-miR-26a-5p, si-HCG11#1 + vector or si-HCG11#1 + ATG12. As mentioned in Figures 6B-6D, the inhibition of miR-26a-5p or the accumulation of ATG12 alleviated the inhibitory effect of HCG11 intervention on the proliferation and the promoting impact on the apoptosis in HCC cells. The depletion of miR-26a-5p or the overexpression of ATG12 also abolished the suppressive effects of HCG11 depletion on the migration and invasion of HCC cells (Figures 6E and 6F). Western blot assay was conducted to detect the changes in the abundance of LC3 I, LC3 II, and p62 in different groups. The inhibition of miR-26a-5p or the overexpression of ATG12 counteracted the suppressive effect of HCG11 interference on autophagy of HCC cells (Figure 6G). Collectively, HCG11 promoted the proliferation, metastasis, and autophagy while impeded the apoptosis of HCC cells by elevating the enrichment of ATG12 by sponging miR-26a-5p.

HCG11 Accelerates the Progression of HCC Through Enhancing the Enrichment of ATG12 by Sponging MiR-26a-5p in vivo

To confirm the biological role of HCG11 *in vivo*, we established the murine xenograft model using MHCC97-H cells stably transfected with sh-NC or sh-HCG11. The tumor volume was recorded once a week. Tumor was resected after five-week inoculation and was weighed. As showed in Figures 7A and 7B, the tumor volume and weight were less in the sh-HCG11 group compared with that in the sh-NC group. The abundance of HCG11, miR-26a-5p, ATG12, and autophagy markers (LC3 I, LC3 II, and p62) was measured in resected tumor tissues. The abundance of HCG11 was significantly reduced in the sh-HCG11 group (Figure 7C).

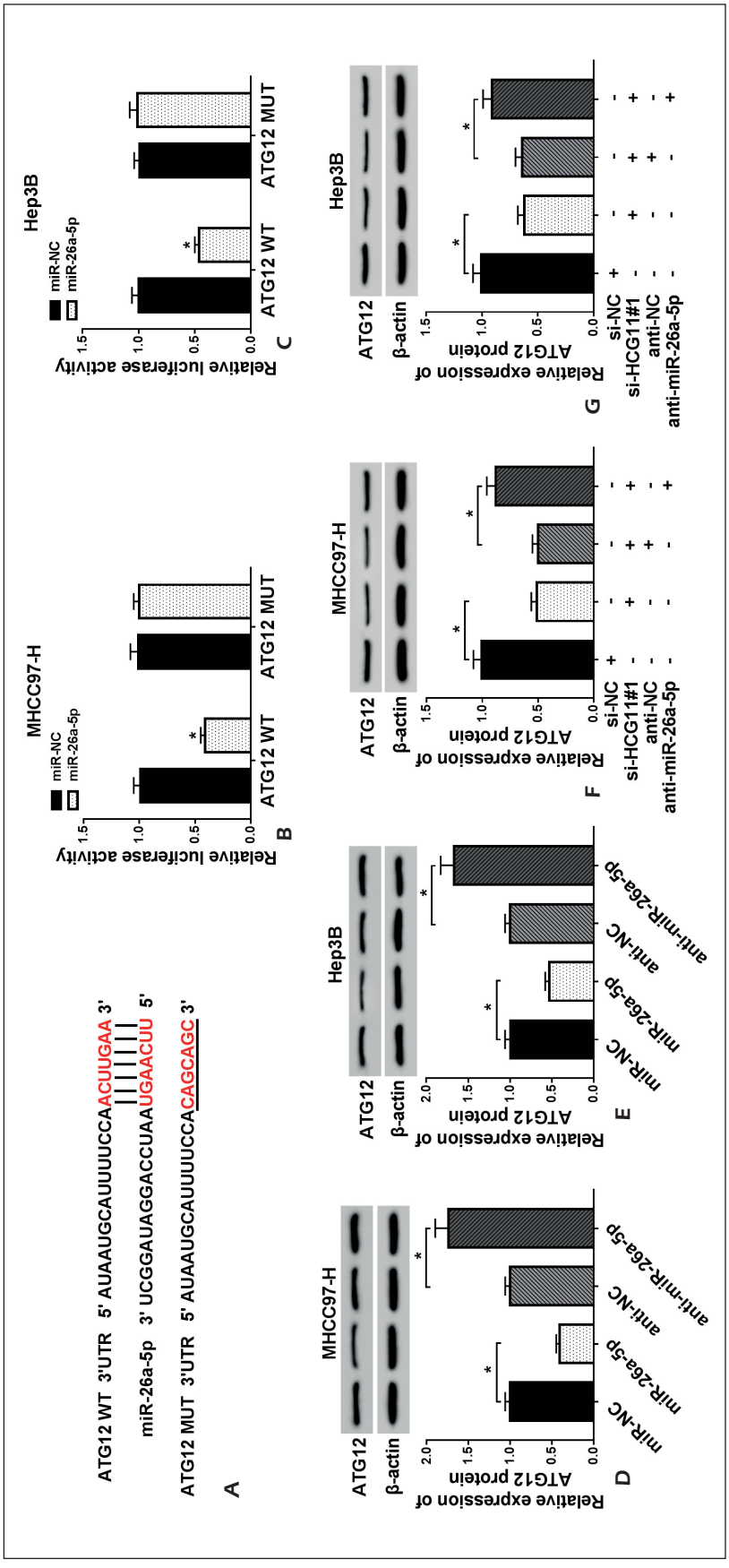

Figure 5. ATG12 is a target of miR-26a-5p. **A**, The binding sites between ATG12 and miR-26a-5p were predicted by starBase online software. **B-C**, Luciferase activity was detected in MHCC97-H and Hep3B cells co-transfected with miR-NC or miR-26a-5p and ATG12 WT or ATG12 MUT. **D-E**, The protein level of ATG12 was determined in MHCC97-H and Hep3B cells transfected with miR-NC, miR-26a-5p, anti-NC or anti-miR-26a-5p by Western blot assay. **F-G**, The abundance of ATG12 was examined in MHCC97-H and Hep3B cells transfected with si-NC, si-HCG11#1, si-HCG11#1 + anti-NC or si-HCG11#1 + anti-miR-26a-5p by Western blot. *$p<0.05$.

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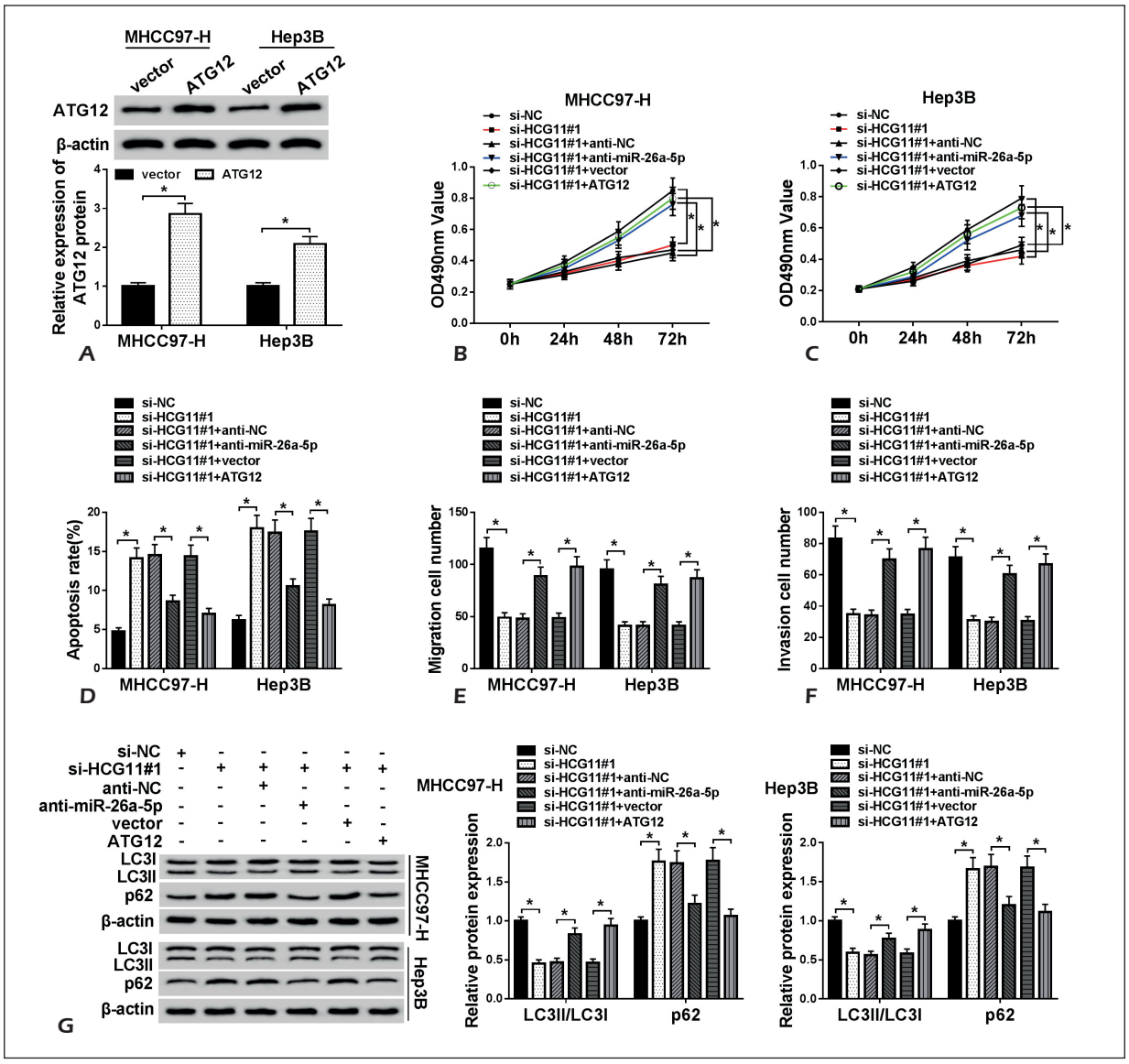

Figure 6. The inhibition of miR-26a-5p or the accumulation of ATG12 could reverse the suppressive effects of HCG11 interference on the proliferation, metastasis, and autophagy of HCC cells. **A**, The abundance of ATG12 was measured in MHCC97-H and Hep3B cells transfected with empty vector or ATG12 overexpression vector by Western blot assay. MHCC97-H and Hep3B cells transfected with si-NC, si-HCG11#1, si-HCG11#1 + anti-NC, si-HCG11#1 + anti-miR-26a-5p, si-HCG11#1 + vector or si-HCG11#1 + ATG12 were used for the detection of cell proliferation, apoptosis, metastasis, and autophagy. **B-C**, MTT assay was carried out to detect the proliferation of HCC cells. **D**, Flow cytometry was performed to determine the apoptosis of HCC cells. **E-F**, Transwell migration and invasion assays were conducted in HCC cells, and the number of migration and invasion cells was counted. **G**, The protein abundance of LC3 I, LC3 II, and p62 was examined in HCC cells by Western blot assay. *$p<0.05$.

The enrichment of miR-26a-5p was negatively related to the expression of HCG11 in resected tumor tissues (Figure 7D). Besides, the depletion of HCG11 decreased the abundance of ATG12 and restrained cell autophagy (Figure 7E). Taken together, HCG11 promoted the progression of HCC by HCG11/miR-26a-5p/ATG12 axis *in vivo*.

Discussion

LncRNAs are involved in multiple types of cellular processes, including proliferation and metastasis[26-28]. Some researchers[10,11] proved that HCG11 was abnormally upregulated in many cancers, and it might be a potential prognosis

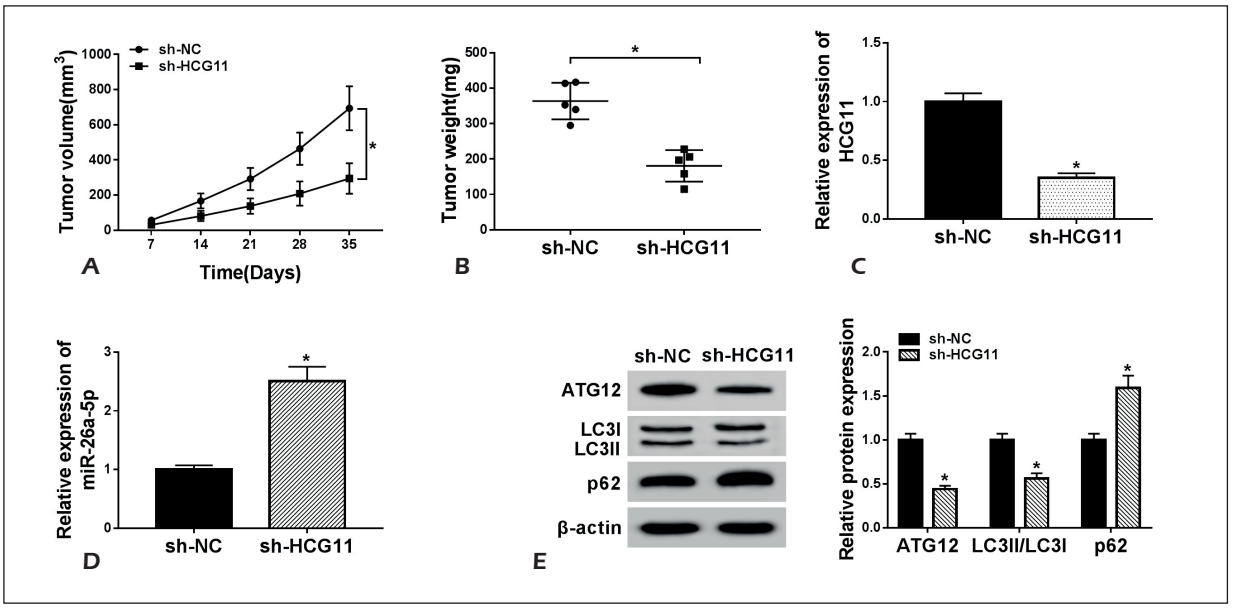

Figure 7. HCG11 accelerates the progression of HCC through enhancing the enrichment of ATG12 by sponging miR-26a-5p in vivo. **A**, Tumor volume was recorded every 7 d. **B**, Tumor was weighed after five-week inoculation. **C-D**, The expression of HCG11 and miR-26a-5p was examined in resected tumor tissues by qRT-PCR. **E**, The abundance of autophagy markers (LC3 I, LC3 II, and p62) and ATG12 was measured in sh-NC and sh-HCG11 groups by Western blot. *$p<0.05$.

marker of multiple cancers. Consistent with the above findings, we found that the abundance of HCG11 was elevated in HCC tissues compared with that in the corresponding normal tissues. The high expression of HCG11 was closely related to the poor prognosis of HCC patients. Loss-of-function experiments were performed to assess the biological role of HCG11 *in vitro*. The intervention of HCG11 not only restrained the proliferation, metastasis, and autophagy of MHCC97-H and Hep3B cells, but also led to the promotion of apoptosis.

To investigate the molecular mechanism of HCG11 in the development of HCC, starBase software was used for the screening targets of HCG11. MiR-26a-5p was predicted to be a target of HCG11 and Dual-Luciferase reporter assay confirmed the combination between miR-26a-5p and HCG11. Accruing researches claimed that miR-26a-5p served as a tumor suppressor in HCC. Li et al[29] demonstrated that miR-26a-5p suppressed the proliferation and metastasis of HCC cells through decreasing the abundance of maternally expressed gene 3 (MEG3) via DNA methyl-transferase 3b (DNMT3B). Ma et al[30] claimed that miR-26a restrained the proliferation and metastasis of HCC cells by reducing the enrichment of F-box protein 11 (FBXO11). Jin et al[31] found that miR-26 promoted the chemosensitivity and apoptosis of HCC cells by inhibiting autophagy. Consistent with the above findings, the accumulation of miR-26a-5p inhibited the proliferation, metastasis, and autophagy of HCC cells. The inhibition of miR-26a-5p alleviated the suppressive effects of HCG11 knockdown on the proliferation, metastasis, and autophagy of HCC cells and the promoting impact on the apoptosis of HCC cells.

To illustrate the mechanism by which miR-26a-5p suppresses the progression of HCC, we aimed to find the downstream component of miR-26a-5p. ATG12 was predicted to be a functional target of miR-26a-5p by bioinformatics analysis, and the Dual-Luciferase reporter assay validated the relationship between miR-26a-5p and ATG12. Kunanopparat et al[32] found that the expression of ATG12 was elevated in hepatitis B virus-associated HCC. We found that the abundance of ATG12 was negatively regulated by miR-26a-5p and was positively modulated by HCG11. The accumulation of ATG12 attenuated the inhibitory effects of HCG11 depletion on the proliferation, metastasis, and autophagy of HCC cells and the promoting impact on the apoptosis of HCC cells. Collectively, HCG11 promoted the proliferation, metastasis, and autophagy while impeded the apoptosis of HCC cells by enhancing the abundance of ATG12 by sponging miR-26a-5p.

The murine xenograft model was established using MHCC97-H cells stably transfected with sh-HCG11 or sh-NC to assess the effect of HCG11 depletion *in vivo*. The knockdown of HCG11 inhibited the growth of tumors and autophagy *via* miR-26a-5p/ATG12 axis. More efforts are needed to explore the downstream signaling pathways of ATG12 in HCC.

Conclusions

HCG11 was identified as an oncogene in HCC. HCG11 accelerated the proliferation, metastasis, and autophagy and impeded the apoptosis of HCC cells partly through the miR-26a-5p/ATG12 axis. The HCG11/miR-26a-5p/ATG12 axis might provide new insight into developing an effective strategy for the treatment of HCC.

Conflict of Interests

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

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