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Circular RNA hsa_circ_0006168 contributes to cell proliferation, migration and invasion in esophageal cancer by regulating miR-384/RBBP7 axis *via* activation of S6K/S6 pathway

Z.-F. XIE, H.-T. LI, S.-H. XIE, M. MA

Department of Thoracic Surgery, the First Affiliated Hospital of Medical College of Shantou University, Shantou, Guangdong, China

Abstract. – OBJECTIVE: Esophageal cancer (EC) ranks as the sixth leading cause of cancer-related mortality worldwide. Circular RNAs (circRNAs) are involved in the pathogenesis of different cancers. However, the regulatory mechanism of circ_0006168 in EC progression is still unclear.

MATERIALS AND METHODS: The expression of circ_0006168, microRNA (miR)-384, and retinoblastoma binding protein 7 (RBBP7) in tumors and cells was measured by quantitative Real Time-Polymerase Chain Reaction (qRT-PCR). The stability of circ_0006168 was analyzed after RNase R treatment. 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide (MTT) assay was conducted to evaluate cell viability. Transwell assay was applied to determine cell migration and invasion. Glucose consumption and lactate production were detected using glucose detection and lactic acid detection kits. The interaction between miR-384 and circ_0006168 or RBBP7 was certified by Dual-Luciferase reporter system. Protein expression of pyruvate kinase (PK), RBBP7, S6 ribosomal protein kinase (S6K), phosphorylated S6K (p-S6K), S6, phosphorylated S6 (p-S6) was analyzed by Western blot.

RESULTS: Circ_0006168 and RBBP7 were over-expressed while miR-384 was low-expressed in EC tumors and cells. The repression of circ_0006168 attenuated cell proliferation, migration, invasion, and glycolysis in EC. Of note, circ_0006168 functioned as a sponge while RBBP7 acted as a target of miR-384 in EC. Rescue experiment revealed that miR-384 inhibitor abrogated circ_0006168 silencing-induced repression on cell proliferation, migration, and invasion in EC. Meanwhile, upregulation of RBBP7 restored the inhibition of miR-384 on EC cell progression. Moreover, circ_0006168 was able to improve RBBP7 level by interacting with miR-384. Also, circ_0006168 could activate S6K/S6 pathway by regulating RBBP7 expression.

CONCLUSIONS: Abundance of circ_0006168 contributes to cell proliferation, migration, invasion, and glycolysis in EC by competitively sponging miR-384 to facilitate RBBP7 expression, representing prospective targets for EC therapy.

Key Words:
Circ_0006168, MiR-384, RBBP7, S6K/S6 pathway, EC.

Introduction

Esophageal cancer (EC), a cancer affecting the digestive system, is a serious health burden globally[1,2]. Generally, EC patients used to be frequently diagnosed at advanced stage due to the lack of noticeable early symptoms and effective screening methods[3]. Hence, the prognosis of EC remains undesirable despite advanced treatment options[4,5]. In recent years, gene regulation was extensively studied to expound the causes of tumorigenesis and development.

Circular RNAs (circRNAs) are critical regulators in various cancers. CircRNAs participate in many pathological processes by regulating cell survival, metabolism, migration, differentiation, and autophagy. CircRNA_100290 acted as a competing endogenous RNA (ceRNA) to facilitate cell survival, glycolysis, and stimulate cell apoptosis in oral squamous cell carcinoma by sponging miR-378a to improve GLUT1 expression[6]. Consistently, circRNA_102171 acted as an oncogene to accelerate cell proliferation and colony formation in papillary thyroid cancer by interacting with CTNNBIP1 and activating β-catenin pathway[7]. On the contrary, circ-ZKSCAN1 acted

Corresponding Author: Zefeng Xie, MD; e-mail: ianyuzhilipsvxb@163.com

as a tumor suppressor to attenuate cell progression and colony formation by regulating p21 level *via* sponging miR-1178-3p in bladder cancer[8]. Hsa_circ_0007059 inhibited cell survival and epithelial-mesenchymal transition (EMT) by regulating miR-378 through Wnt/β-catenin/ERK1/2 pathway in lung cancer[9]. Whether circ_0006168 functions as oncogene or tumor suppressor in EC is still largely unknown.

MicroRNAs (miRNAs) refer to endogenous non-coding RNAs with 15-28 nucleotides in length[10]. Despite without protein-encoding ability, miRNAs could negatively regulate gene expression and further alter cell cycle, metabolism, survival, inflammation, and apoptosis[11-13]. MiR-384 was recognized as a tumor suppressor in different cancers. MiR-384 was low-expressed in colorectal cancer and was reported to restrain tumor growth by targeting AKT3[14]. Overexpression of miR-384 alleviated the malignancy of gastric cancer by reducing cell survival and metastasis by regulating metadherin gene[15]. Similarly, miR-384 was reported to decrease cell proliferation in hepatocellular carcinoma or prostate cancer by targeting IRS1 or HOXB7[16,17]. Therefore, we assumed that miR-384 might function as tumor suppressor to restrict cell progression in EC.

We aimed to reveal the molecular mechanism of EC cell progression. The role of circ_0006168, miR-384 and retinoblastoma binding protein 7 (RBBP7) were evaluated. The regulatory effects of circ_0006168/miR-384/RBBP7 axis, were assessed by loss-of-function and rescue experiments. Moreover, the underlying molecular mechanism of circ_0006168 in EC cell growth was analyzed by Western blot.

Materials and Methods

Tissue Samples

EC patients at phase I+II (n=17) and phase III+IV (n=20) were recruited from the First Affiliated Hospital of Medical College of Shantou University. Fresh EC tumors and the adjacent normal tissues were collected from the participants by surgery. All the patients signed the informed consent and the protocols have been approved by the Ethics Committee of First Affiliated Hospital of Medical College of Shantou University.

Cell Culture and RNase R Treatment

ECA-109, KYSE-510 cells, and human normal esophageal epithelial cells HET-1A were purchased from EK-Bioscience (Shanghai, China) and incubated in complete Dulbecco's Modified Eagle's Medium (DMEM, Gibco, Rockville, MD, USA). The stability of circ_0006168 was evaluated using RNase R (Geneseed Biotechnology, Guangzhou, China). In brief, ECA-109 cells were plated on 24-well plates overnight and then treated with RNase R (100 μg/mL).

Cell Transfection

Small interfering RNA targeting circ_0006168 (si-circ_0006168), negative control (si-NC), pcDNA3.1 and pcDNA3.1-RBBP7 were synthesized by GenePharma (Shanghai, China). MiR-384, miR-384 inhibitor (anti-miR-384), negative control (miR-NC) and control inhibitor (anti-miR-NC) were purchased from RioBio (Guangzhou, China). Cell transfection was carried out using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA).

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

Total RNA was extracted from EC tissues and normal tissues using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). Nuclear and Cytoplasmic Extraction Kit (Yasen, Shanghai, China) was used to extract RNA from the nuclear and cytoplasm of EC cells. The cDNA for circ_0006168, miR-384, and RBBP7 was synthesized by All-in-One™ Synthesis Kit (FulenGen, Guangzhou, China). Subsequently, qRT-PCR was performed using SYBR Green (Applied Biosystems, Foster City, CA, USA). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and U6 were exploited as internal references. The primers for circ_0006168, miR-384, and RBBP7 are listed as follows: circ_0006168, (Forward, 5'-ACCAGCAGAACTAGGAAACA-3'; Reverse, 5'-TGGCATCCCTATTAGTCTTTC-3'); miR-384, (Forward, 5'-TGTTAAATCAGGAATTTTAA-3'; Reverse, 5'-TGTTACAGGCATTATGAA-3'); RBBP7 (Forward, 5'-CTTCTAAGCCGAGCCATTT-3'; Reverse, 5'-GATTACGCAGGTCCCATA-3'); GAPDH, (Forward, 5'-AGGTCGGTGTGAACGGATTTG-3'; Reverse, 5'-GGGGTCGTTGATGGCAACA-3'); U6, (Forward, 5'-ACCCTGAGAAATACCCTCACAT-3'; Reverse, 5'-GACGACTGAGCCCCTGATG-3').

3-(4,5-Dimethyl-2-Thiazolyl)-2,5-Diphenyl-2-H-Tetrazolium Bromide (MTT) Assay

ECA-109 and KYSE-510 cells were inoculated in 96-well plates and transfected with the vectors for 24 h, 48 h, and 72 h. Next, the cells were added with 10 μL MTT (Beyotime, Shanghai, China) for 4 h followed by 100 μL dimethyl sulfoxide (DMSO, Sigma-Aldrich, St. Louis, MO, USA) for 2 h. The optical density (OD) value at 490 nm was measured by a spectrophotometer.

Transwell Assay

For cell invasion detection, transfected ECA-109 and KYSE-510 cells were placed on the upper chamber of the transwell (Corning, Corning, NY, USA) pre-coated with Matrigel (Sigma-Aldrich, St. Louis, MO, USA). As for cell migration evaluation, the cells were placed on the upper chamber of the transwell. After migration or invasion for 48 h, the cells at lower chamber were stained with 0.1% crystal violet (Sigma-Aldrich, St. Louis, MO, USA). Then, the cells were captured by a microscope.

Evaluation of Glycolysis with Glucose, Lactate and Pyruvate Kinase (PK)

Glycolysis was evaluated by detecting glucose, lactate and PK level. Glucose consumption and lactate production were determined using glucose detection and lactic acid detection kits (Sigma-Aldrich, St. Louis, MO, USA) following the manufacturer's instruction. Protein expression of PK was analyzed by Western blot.

Western Blot

Total protein was isolated from ECA-109 and KYSE-510 cells, separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA, USA). After blocking by 5% nonfat milk, the membranes were incubated with primary antibodies against PK, RBBP7, S6 ribosomal protein kinase (S6K), phosphorylated S6K (p-S6K), S6, phosphorylated S6 (p-S6) and GAPDH and horseradish peroxidase (HRP)-conjugated secondary antibody (Sangon, Shanghai, China).

Dual-Luciferase Reporter Assay

The interaction between miR-384 and circ_0006168 or RBBP7 was validated by Dual-Luciferase reporter assay. In brief, wild type circ_0006168 (circ_0006168-WT), RBBP7 (RBBP7-WT), mutant type circ-0001801 (circ_0006168-MUT), and RBBP7 (RBBP7-MUT) Luciferase vectors were constructed. Next, those vectors were co-transfected into ECA-109 and KYSE-510 cells with miR-384 or miR-NC. Luciferase activities were determined using a luminometer.

Statistical Analysis

The data were presented as means ± standard deviation (SD). Statistical analysis was performed by GraphPad Prism 7 (San Diego, CA, USA). The two-tailed Student's *t*-test was used to analyze the differences between the groups. *p*-value less than 0.05 ($p<0.05$) was considered statistically significant.

Results

Overexpression of Circ_0006168 in EC

The underlying role of circ_0006168 in EC cell progression was evaluated by measuring the expression of circ_0006168 in EC. As illustrated in Figure 1A, the level of circ_0006168 was upregulated in EC tumors compared with the adjacent tissues. More specifically, circ_0006168 expression was relatively higher in tumors from phase III+IV patients (n=20) than that of phase I+II patients (n=17) (Figure 1B). Meanwhile, circ_0006168 also over-expressed in EC cells (ECA-109, KYSE-510) compared with human normal esophageal epithelial cells HET-1A (Figure 1C). Then, ECA-109 cells were reversely transcribed with random hexamer or oligo $(dT)_{18}$ primers to exhibit the characterization of circ_0006168. The downregulation of circ_0006168 in oligo $(dT)_{18}$ primers group revealed that circ_0006168 had no poly-A tail (Figure 1D). Furthermore, the stability of circ_0006168 was proved since circ_0006168 was resistant to RNase R (Figure 1E). We also detected the aggregation of circ_0006168 at the subcellular level and found that circ_0006168 was mainly expressed in cytoplasm rather than nuclear (Figure 1F). Taken together, circ_0006168 played an essential role in EC progression.

Elimination of Circ_0006168 Inhibited Cell Proliferation, Migration, and Invasion in EC

Loss-of-function experiment was performed to explore the function of circ_0006168 in EC. Circ_0006168 expression in ECA-109 and KYSE-510 cells was distinctly reduced by circ_0006168 knockdown (Figure 2A-B). MTT results indicated that circ_0006168 knockdown had a negative effect on cell proliferation (Figure 2C-D). Consistently, cell migration and invasion were restricted by the deficiency of circ_0006168 (Figure 2E-F). What's more, depletion of circ_0006168 inhibited glucose consumption, as well as lactate production, clarifying that

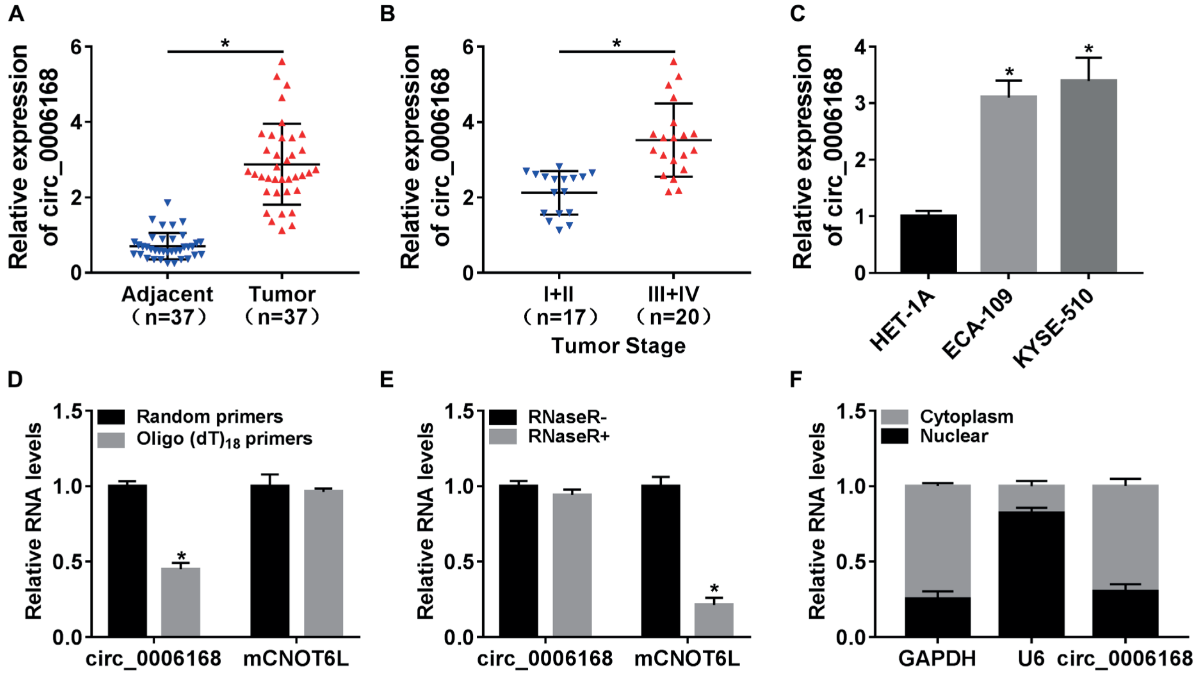

Figure 1. Circ_0006168 was upregulated in EC. **A,** The expression of circ_0006168 in EC tumors and the adjacent normal tissues was measured by qRT-PCR. **B,** The expression of circ_0006168 in EC tumors collected from phase I+II patients (n=17) compared with phase III+IV patients (n=20). **C,** The expression of circ_0006168 in EC cells (ECA-109, KYSE-510) compared with human normal esophageal epithelial cells HET-1A. **D,** The expression of circ_0006168 and mCNOT6L in ECA-109 cells reversely transcribed with random hexamer or oligo (dT)$_{18}$ primers, was evaluated by qRT-PCR. **E,** The expression of circ_0006168 and mCNOT6L in ECA-109 cells treated with RNase R was examined by qRT-PCR. **F,** The expression of circ_0006168, GAPDH and U6 in the cytoplasm and nuclear of ECA-109 cells. *p<0.05.

circ_0006168 knockdown weakened glycolysis in EC cells (Figure 2G-H). As expected, protein PK expression was reduced by circ_0006168 silencing (Figure 2I). Collectively, deficiency of circ_0006168 suppressed cell proliferation, migration, invasion, and glycolysis in EC.

Circ_0006168 Was a Sponge of MiR-384

Then, the molecular mechanism of circ_0006168 on EC development was studied. According to bioinformatics prediction by starBase, there were potential binding sites between circ_0006168 and miR-384 (Figure 3A). Luciferase activity of circ_0006168-WT was decreased by miR-384 in ECA-109 and KYSE-510 cells, confirming the interaction between circ_0006168 and miR-384 (Figure 3B-C). Of note, miR-384 expression was downregulated in EC tumors and cells compared with the normal counterparts, suggesting the inhibitive role of miR-384 in EC (Figure 3D-E). Furthermore, miR-384 expression was enhanced by si-circ_0006168 in comparison with si-NC group (Figure 3F-G). These findings revealed that circ_0006168 could regulate miR-384 expression in EC.

Circ_0006168 Regulated EC Cell Proliferation, Migration, and Invasion by Sponging MiR-384

Rescue experiment was conducted to evaluate the regulatory effects of circ_0006168/miR-384 axis on EC cell progression. Clearly, miR-384 expression was inhibited by miR-384 inhibitor, indicating a high transfection efficiency (Figure 4A-B). What's more, miR-384 inhibitor rescued the inhibition of circ_0006168 silencing on cell viability in EC (Figure 4C-D). Consistently, the repression of circ_0006168 knockdown on EC cell migration and invasion was reversed by miR-384 inhibitor (Figure 4E-H). Therefore, circ_0006168 could sponge miR-384 and further regulate cell progression in EC.

RBBP7 Directly Interacted with MiR-384

By searching from starBase, we discovered that miR-384 contained the binding sites of RBBP7

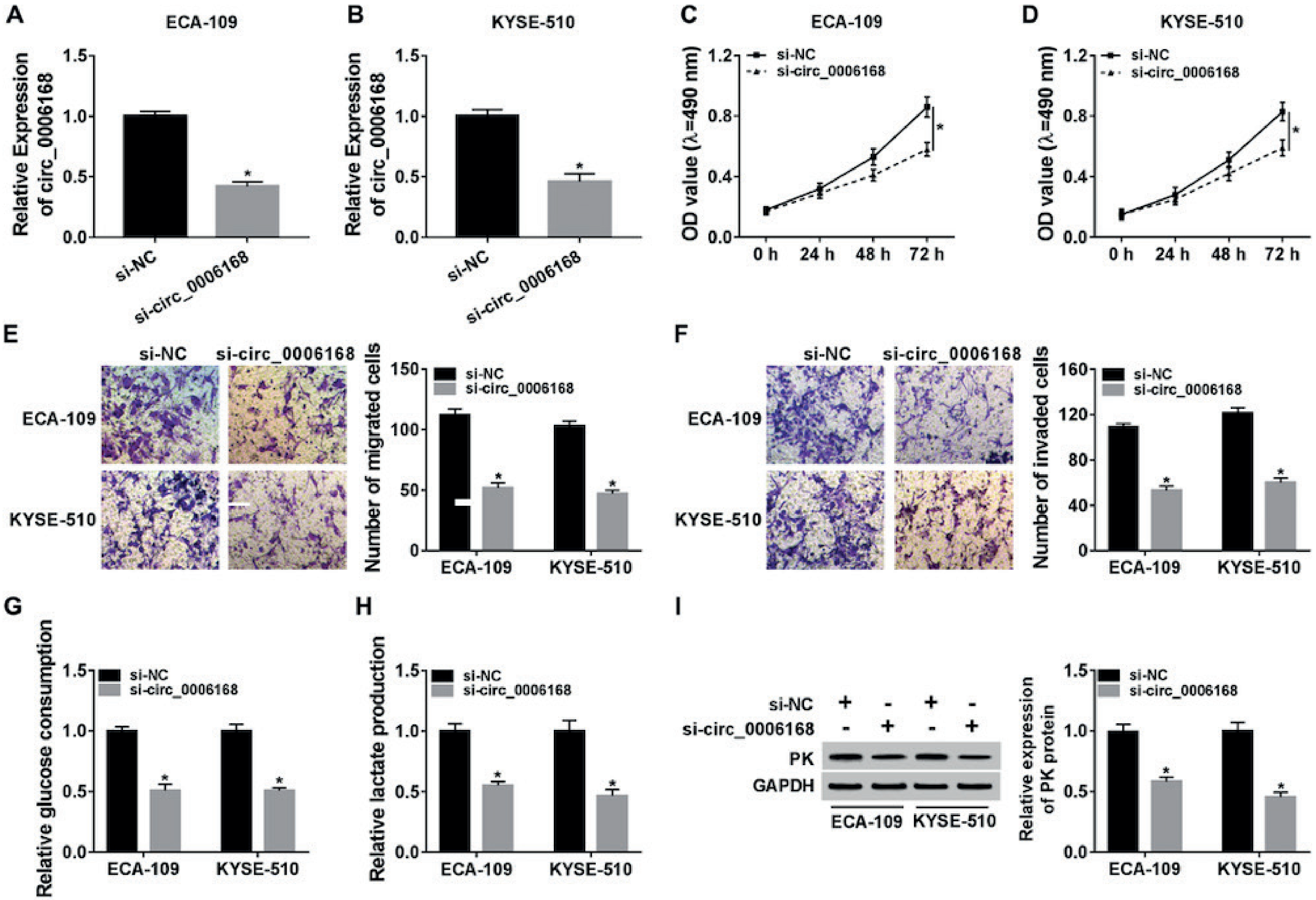

Figure 2. Circ_0006168 knockdown repressed cell proliferation, migration and invasion in EC. ECA-109 and KYSE-510 cells were transfected with si-NC and si-circ_0006168. **A-B,** Analysis of circ_0006168 expression in transfected cells by qRT-PCR. **C-D,** Cell viability was detected by MTT assay. **E-F,** Cell migration and invasion were determined by transwell assay (100×). **G,** The consumption of glucose was evaluated by glucose detection kits. **H,** The production of lactate was assessed by lactic acid detection kits. **I,** Protein expression of PK was analyzed by Western blot. $^{*}p<0.05$.

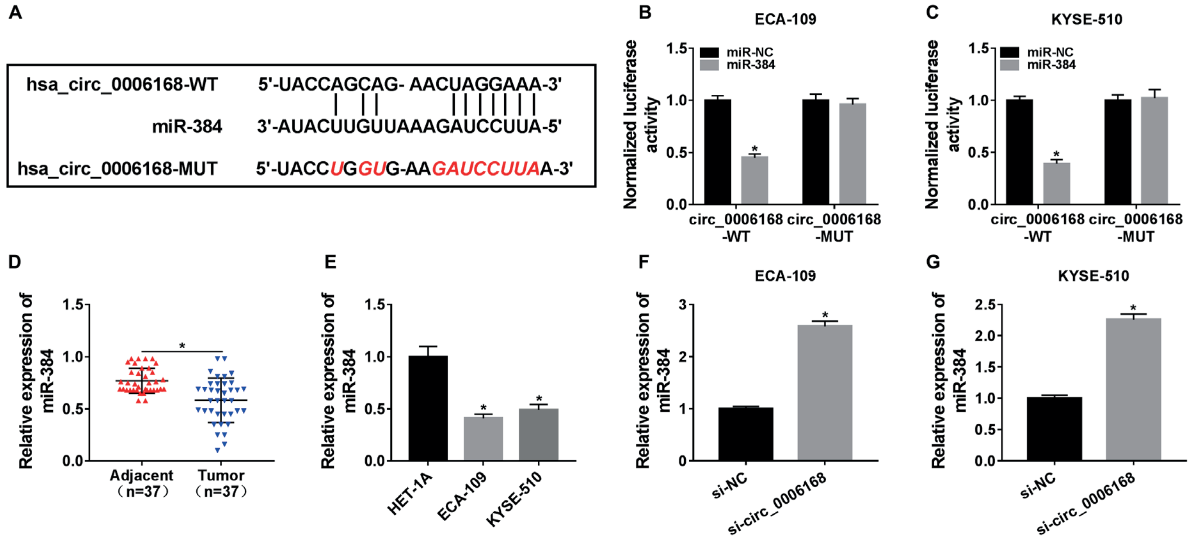

Figure 3. Circ_0006168 directly interacted with miR-384. **A,** The potential binding sites between circ_0006168 and miR-384 were predicted by starBase. **B-C,** Luciferase activity of ECA-109 and KYSE-510 cells co-transfected with circ_0006168-WT or circ_0006168-MUT and miR-384 or miR-NC was determined by Dual-Luciferase reporter assay. **D-E,** The expression of miR-384 in EC tumors and cells compared with the normal tissues and cells. **F-G,** QRT-PCR was used to detect the expression of miR-384 in ECA-109 and KYSE-510 cells transfected with si-NC and si-circ_0006168. *$p<0.05$.

(Figure 5A). As displayed in Figure 5B-C, Luciferase activity was reduced in ECA-109 and KYSE-510 cells co-transfected with RBBP7-WT and miR-384, validating the interaction between RBBP7 and miR-384. In addition, RBBP7 mRNA and protein expression were evidently higher in EC tumors and cells than that of the normal ones, implicating the oncogenic role of RBBP7 in EC (Figure 5D-G). We also observed that RBBP7 mRNA and protein expression were strengthened by miR-384 inhibitor (Figure 5H-K). Altogether, RBBP7 acted as a target of miR-384 in EC.

Restoration of RBBP7 Counteracted MiR-384-Induced Inhibition of EC Cell Proliferation, Migration and Invasion

The interrelation of miR-384 and RBBP7 on EC cell growth was further investigated by transfecting miR-NC, miR-384, miR-384+pcDNA3.1, and miR-384+pcDNA3.1-RBBP7 in ECA-109 and KYSE-510 cells. Enhanced expression of miR-384 in EC cells transfected with miR-384 indicated a high transfection efficiency (Figure 6A-B). Similarly, RBBP7 mRNA and protein expression were increased in EC cells transfected with pcDNA3.1-RBBP7 (Figure 6C-F). Essentially, the overexpression of RBBP7 neutralized the repression of miR-384 on EC cell growth (Figure 6G-H). Meanwhile, RBBP7 alleviated miR-384-mediated inhibition of cell migration and invasion in EC (Figure 6I-L). In addition, glucose consumption and lactate production were inhibited by miR-384 and the inhibitive effects were inversed by pcDNA3.1-RBBP7 (Figure 6M-N). Also, the protein expression of PK was blocked by miR-384 and boosted by pcDNA3.1-RBBP7 (Figure 6O). Hence, miR-384 could regulate cell proliferation, migration, invasion, and glycolysis by targeting RBBP7 in EC.

Circ_0006168 Targeted MiR-384 to Improve RBBP7 Expression in EC

Then, qRT-PCR and Western blot were performed to explore the interrelation among circ_0006168, miR-384, and RBBP7. As exhibited in Figure 7A-D, miR-384 inhibitor abolished circ_0006168 knockdown-induced suppressive effects on the expression of RBBP7 mRNA and protein. These results clarified that circ_0006168 could regulate RBBP7 expression by absorbing miR-384 in EC.

Circ_0006168 Stimulated the Activation of S6K/S6 Pathway by Regulating RBBP7 Expression

To further illuminate the molecular mechanism of circ_0006168 in EC cell regulation, ECA-109 and KYSE-510 cells were transfected with si-NC, si-circ_0006168, si-circ_0006168+pcDNA3.1, and si-circ_0006168+pcDNA3.1-RBBP7.

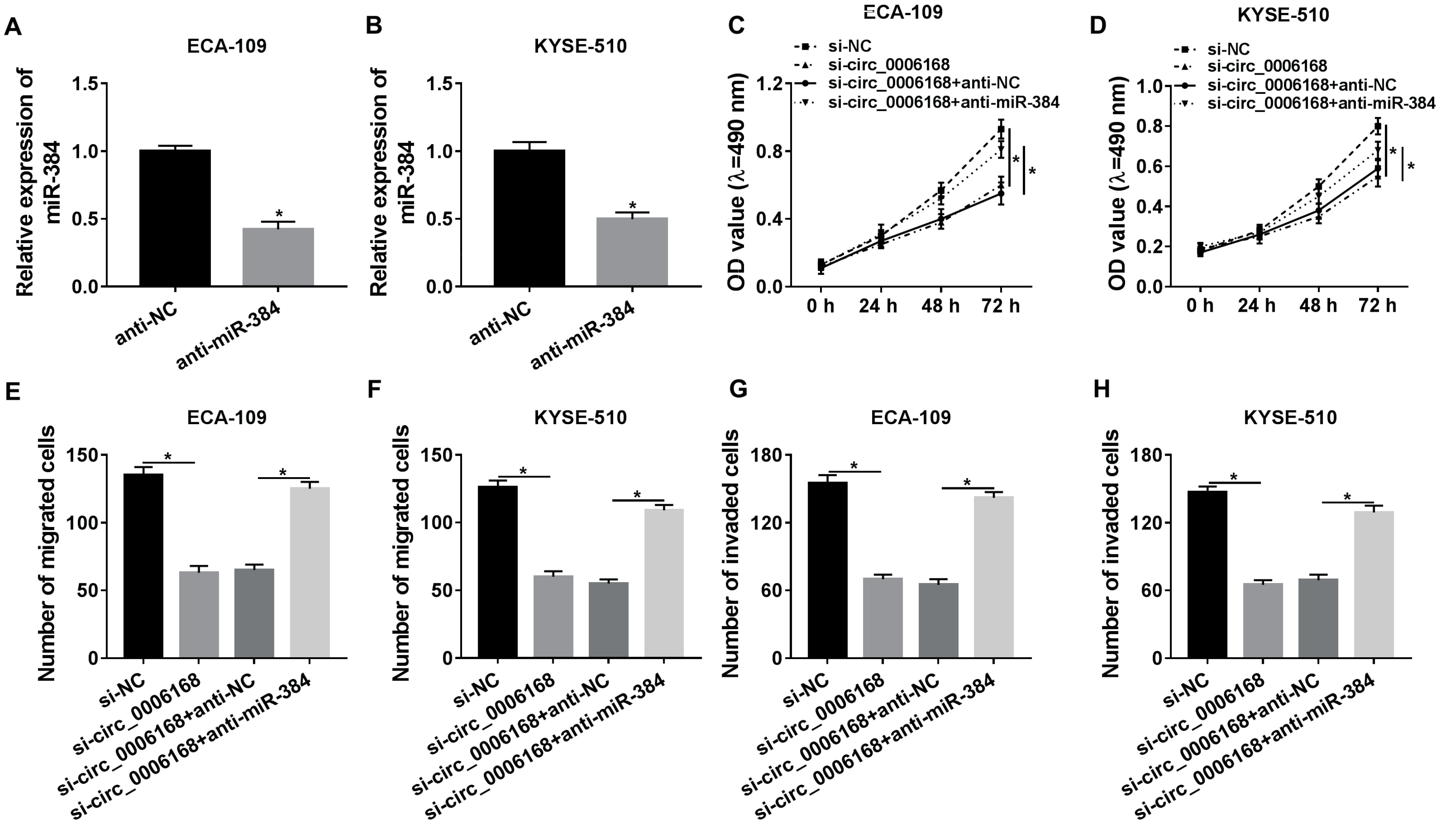

Figure 4. MiR-384 inhibitor attenuated circ_0006168 silencing-induced inhibition on cell proliferation, migration and invasion in EC. ECA-109 and KYSE-510 cells were transfected with si-NC, si-circ_0006168, si-circ_0006168+anti-NC and si-circ_0006168+anti-miR-384. **A-B,** The expression of miR-384 in transfected cells was detected by qRT-PCR. **C-D,** Cell viability was evaluated by MTT assay. **E-H,** Cell migration and invasion were examined by transwell assay. *$p<0.05$.

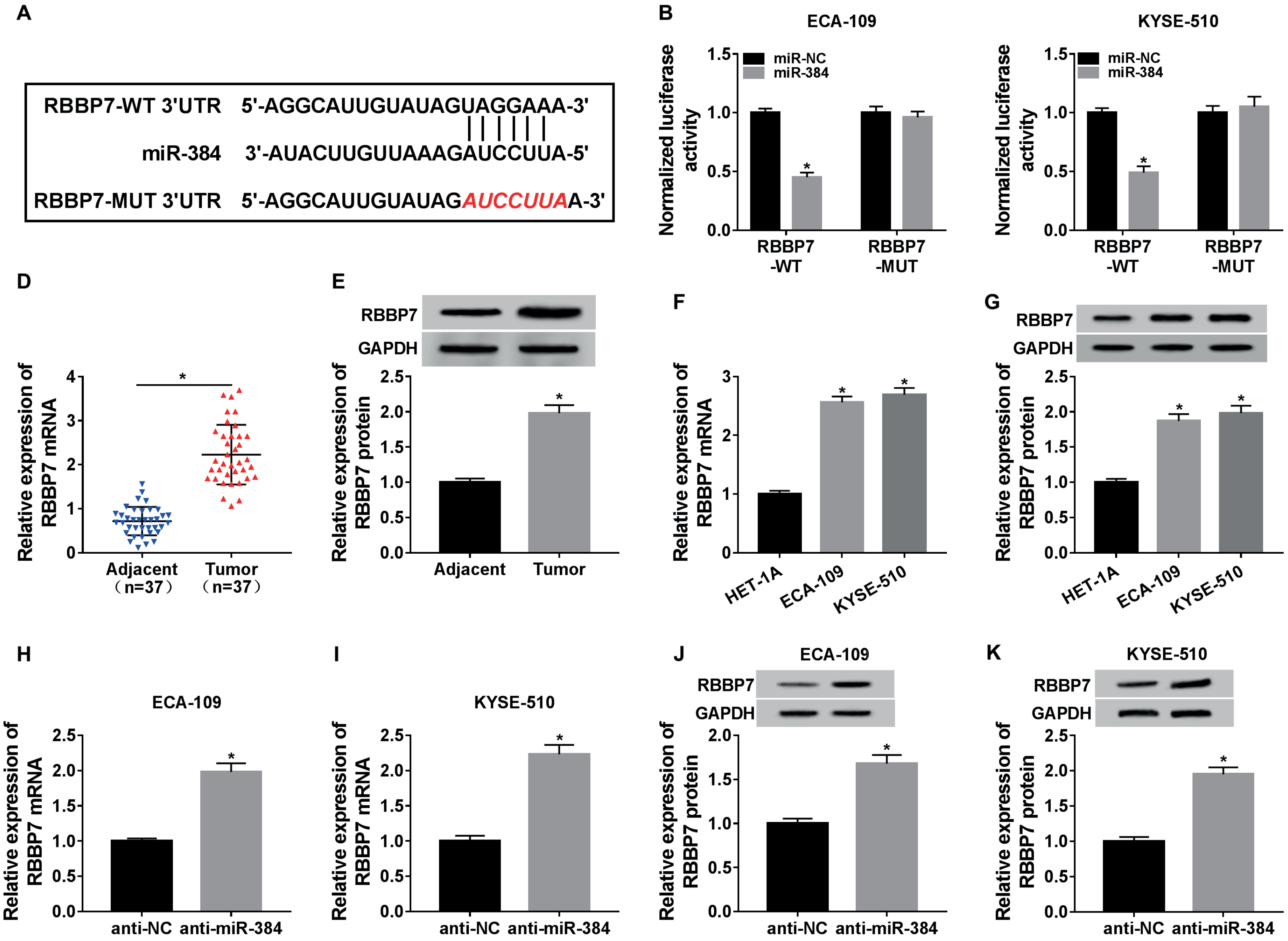

Figure 5. RBBP7 acted as a target of miR-384. **A,** The potential binding sites between RBBP7 and miR-384 were predicted by starBase. **B-C,** Dual-Luciferase reporter assay was used to analyze Luciferase activity of ECA-109 and KYSE-510 cells co-transfected with RBBP7-WT or RBBP7-MUT and miR-384 or miR-NC. **D-G,** The expression of RBBP7 mRNA and protein in EC tumors and cells compared with the normal tissues and cells. **H-K,** The expression of RBBP7 mRNA and protein in ECA-109 and KYSE-510 cells transfected with anti-NC and anti-miR-384 was detected by qRT-PCR and Western blot, respectively. *$p<0.05$.

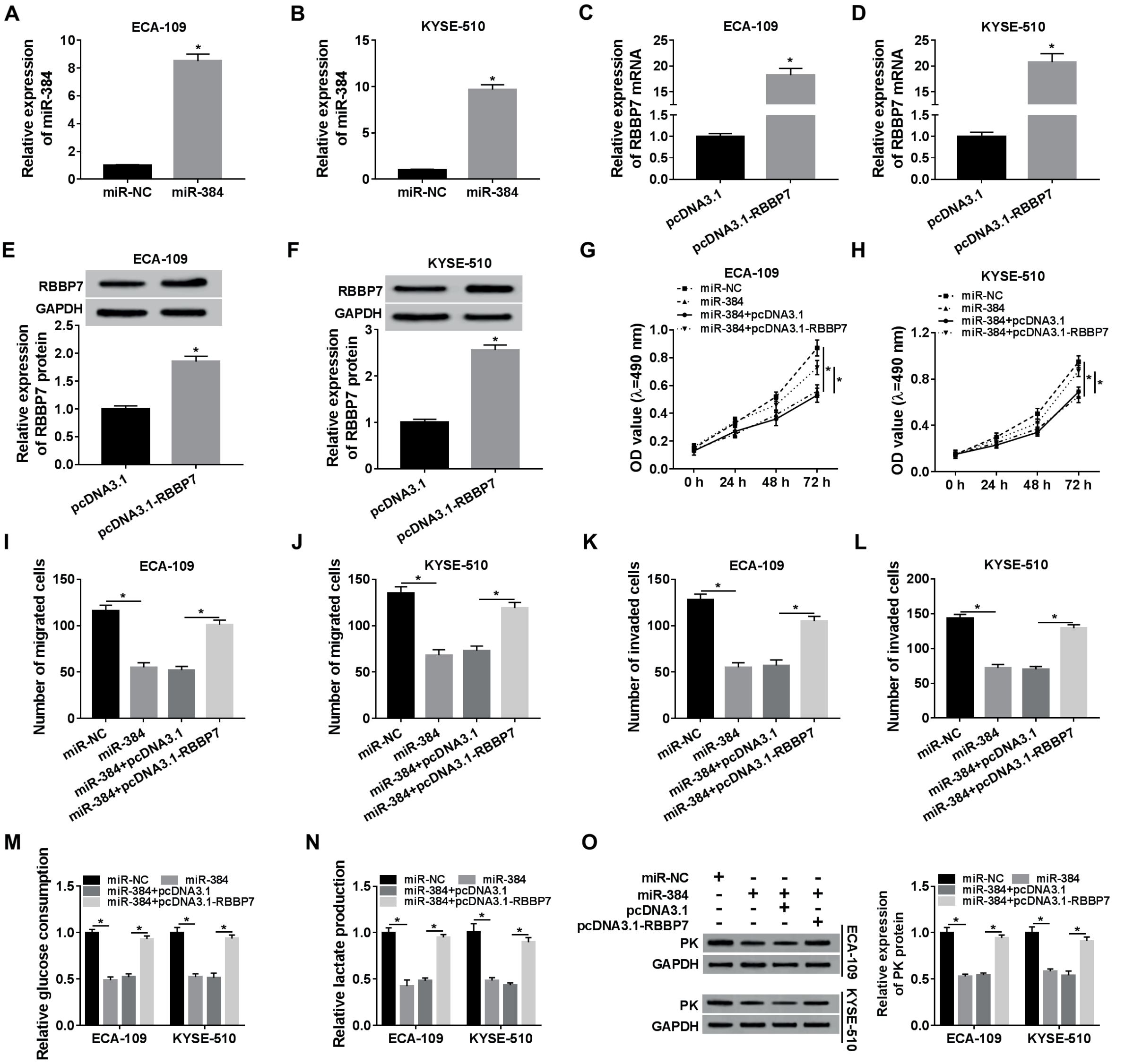

Figure 6. RBBP7 restored the suppression of miR-384 on cell proliferation, migration and invasion in EC. ECA-109 and KYSE-510 cells were transfected with miR-NC, miR-384, miR-384+pcDNA3.1 and miR-384+pcDNA3.1-RBBP7. **A-B,** The expression of miR-384 in cells transfected with miR-NC and miR-384 was detected by qRT-PCR. **C-F,** The expression of RBBP7 mRNA and protein in cells transfected with pcDNA3.1 and pcDNA3.1-RBBP7 was measured by qRT-PCR and Western blot. **G-H,** Cell viability was examined by MTT assay. **I-L,** Cell migration and invasion were assessed by transwell assay. **M-N,** Relative glucose consumption and lactate production were detected by glucose and lactic acid detection kits. **O,** Protein expression of PK was determined by Western blot. $^*p<0.05$.

As illustrated in Figure 8A-B, the relative expression of p-S6K and p-S6 was inhibited by circ_0006168 knockdown and the inhibition was reversed by RBBP7. The data demonstrated that circ_0006168 modulated EC cell progression by regulating RBBP7 expression and activating S6K/S6 pathway.

Discussion

Increasing studies[18-20] indicated that the dysregulation of circRNAs was associated with the prognosis of a variety of cancers, such as multiple myeloma, pancreatic, and colorectal cancer. The overexpression of cir-

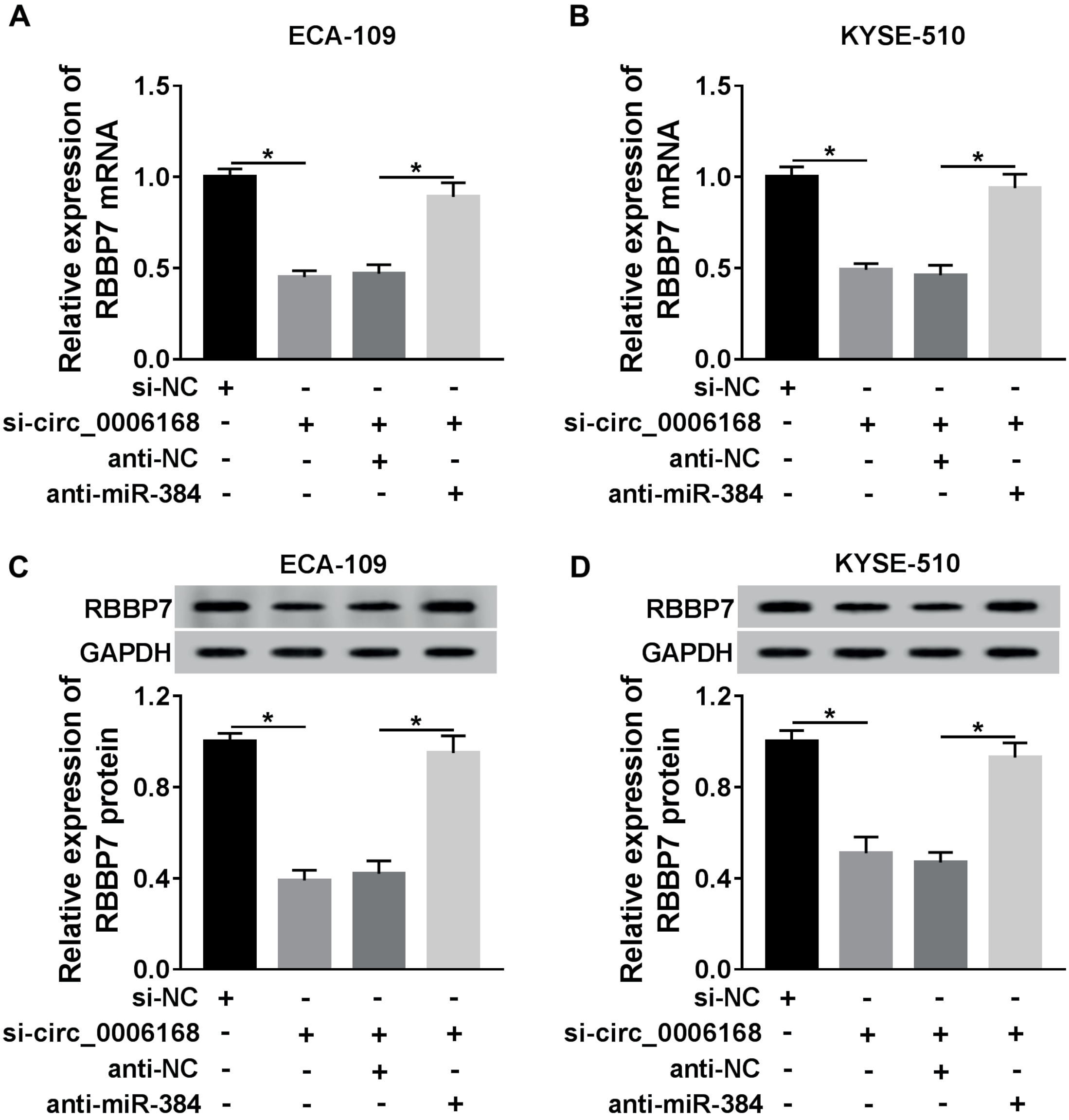

Figure 7. Circ_0006168 promoted RBBP7 expression by sponging miR-384 in EC. ECA-109 and KYSE-510 cells were transfected with si-NC, si-circ_0006168, si-circ_0006168+anti-NC and si-circ_0006168+anti-miR-384. **A-B,** RBBP7 mRNA expression was measured by qRT-PCR. **C-D,** RBBP7 protein expression was examined by Western blot. *$p<0.05$.

cRNA-100338 indicated poor prognosis of hepatocellular carcinoma and circRNA-100338 expedited the malignancy by binding to miR-141-3p to enhance RHEB expression *via* mTOR pathway[21]. The abundance of hsa_circRNA_102958 expedited the deterioration of colorectal cancer by accelerating tumor growth by binding to miR-585 and increasing CDC25B expression[22]. Consistently, the upregulation of hsa_circ_0067997 contributed to gastric cancer cell proliferation, colony formation, and invasion by repressing miR-515-5p and enhancing XIAP[23]. Enhanced expression of hsa_circRNA_101996 was reported to expedite cell progression by interacting with miR-8075 to alter TPX2 level[24]. By contrast, hsa_circ_0042666 was low-expressed in laryngeal squamous cell carcinoma and hsa_circ_0042666 could repress cell progression by upregulating TGFBR3 level *via* interacting with miR-223[25]. Hence, it is imperative to illuminate the function of circ_0006168 in EC.

Based on bioinformatics analysis using starBase, miR-384 comprised the binding sites of circ_0006168. Guo et al[26] and Yu et al[27] showed that miR-384 was a critical anti-tumor molecular, as well as prognostic biomarker in many cancers' types. For instance, the abundance of miR-384 weakened cell survival and invasion by regulating AEG-1/Wnt pathway in non-small-cell lung cancer[28]. Increased expression of miR-384 was able to interact with pleiotrophin and further inhibit tumor development in HBV-induced hepatocellular carcinoma through N-syndecan/PI3K/Akt/mTORC1 pathway[29]. Similarly, miR-384 was reported to restrain cell proliferation and induce cell apoptosis in laryngeal cancer by regulating WISP1 expression[30]. Excess of miR-384 attenuated cell viability, migration, and invasion in renal cell carcinoma or glioma by regulating RAB23 or CDC42 expression[31,32]. Therefore, we speculated that circ_0006168 could bind to miR-384 and regulate tumorigenesis and progression in EC.

We assumed that circ_0006168 exerted its oncogenic function by interacting with miR-384 to enhance RBBP7 expression in EC. Solid tumor proliferation requires nutrients, as well as energy supply, under hypoxia microenvironment. Aerobic glycolysis is a well-known metabolic pattern under hypoxia to support cancer cell growth by converting glucose to lactate[33]. Therefore, we investigated the influence of circ_0006168 on glycolysis by detecting glucose, lactate, and PK levels. The overexpression of circ_0006168 and low-expression of miR-384 implicated the oncogenic role of circ_0006168 and the suppressive role of miR-384. Loss-of-function experiment revealed that circ_0006168 knockdown had negative impacts on EC cell progression and glycolysis. Essentially, we discovered that circ_0006168 was a sponge of miR-384 and RBBP7 was a target of miR-384. As expected, circ_0006168 could enhance the expression of RBBP7 mRNA and protein by interacting with miR-384 in EC. In addition, inhibition of miR-384 counteracted

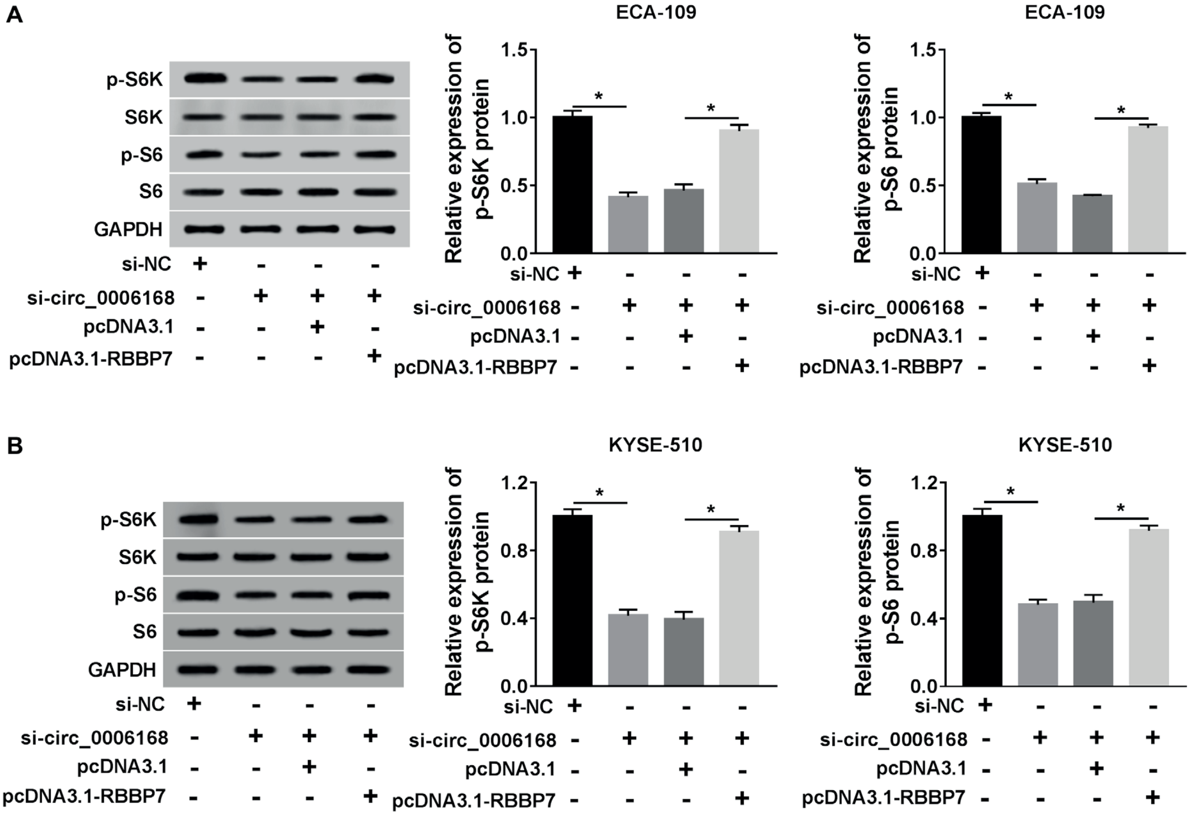

Figure 8. Circ_0006168 accelerated EC cell progression by activation of S6K/S6 pathway through regulating RBBP7 expression. ECA-109 and KYSE-510 cells were transfected with si-NC, si-circ_0006168, si-circ_0006168+pcDNA3.1 and si-circ_0006168+pcDNA3.1-RBBP7. **A-B,** Protein expression of p-S6K, S6K, p-S6 and S6 was analyzed by Western blot. *$p<0.05$.

circ_0006168 silencing-mediated regulatory effects on EC cell development. Restoration of RBBP7 alleviated the repression of miR-384 on cell progression in EC. Furthermore, we discovered that circ_0006168 could activate S6K/S6 pathway by measuring protein expression of p-S6K and p-S6.

Conclusions

Our study demonstrated that circ_0006168 facilitated cell growth, migration, invasion, and glycolysis in EC by regulating miR-384/RBBP7 axis through activation of the S6K/S6 pathway, providing alternative treatment strategies for EC.

Conflict of Interests

The Authors declare that they have no conflict of interests.

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