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RESEARCH PAPER

Down-regulated Circ_0000190 promotes cervical cancer by facilitating the activity of proto-oncogene protein EIF4E

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ABSTRACT

Circular RNAs (circRNAs), a new class of non-coding RNAs, have been recently confirmed to regulate cell development, functions and certain types of pathological responses. In addition, it has been proved that circ_0000190 can serve as a tumor suppressor in several cancers. However, the underlying mechanism and biological functions of it in cervical cancer (CC) remain to be revealed. In our study, relative expression of indicated molecules was detected by RT-qPCR analysis. Loss-of-function and gain-of-function experiments were conducted to detect cell functions. Mechanism experiments including RIP assay, luciferase reporter assay and pull down assay were applied to verify the interaction among the indicated molecules. Overexpressed circ_0000190 attenuated CC progression *in vitro* and *in vivo*. Circ_0000190 functioned through the modulation of miR-1252-5p/EIF4EBP2 axis. Rescue experiments found that miR-1252-5p overexpression or EIF4EBP2 knockdown could reverse the influence on CC cells caused by circ_0000190 overexpression. Interestingly, it was found that EIF4EBP2 could bind to proto-oncogene eIF4E and prevent eIF4E from forming into complex and functioning. Circ_0000190 served as a tumor suppressor in CC and down-regulated circ_0000190 expression could weaken the binding ability of EIF4EBP2 to eIF4E thus leading to CC tumorigenesis. In our investigation, a novel tumor suppressive gene circ_0000190 was recognized, which could be treated as a promising biomarker for the diagnosis of CC.

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KEYWORDS
Circ_0000190; cervical cancer; ceRNA; EIF4EBP2

Introduction

CC is the most common cancer leading to death among women in developing countries [1]. With over 500,000 cases and 275,000 deaths reported annually, CC continues to be a global threat to females. As a matter of fact, most cervical cancers can be diagnosed at an early stage and cured by surgery [2,3]. However, in resource-limited countries, due to the lack of routine population-based screening, women patients are always presented with locally advanced disease when diagnosed [4]. Therefore, identifying CC biomarkers to improve the result of both early diagnosis and prognosis is urgent.

During the past decades, new cellular roles of RNA molecules have been continuously revealed, which has contributed to our current understanding that gene expression dynamics are extremely complex in cancer pathology [5]. CircRNA serves as a highly abundant type of RNA displaying widespread expression in the tree of life [6–8]. As a novel class of competing endogenous RNAs (ceRNA), circRNAs can regulate cancer progression by modulating messenger RNA (mRNA) expression as long non-coding RNAs [9–11]. So far, several circRNAs have been reported as promising biomarkers for CC diagnosis and prognosis. For example, circRNA hsa_circRNA_101996 promotes CC progression via regulating miR-8075/TPX2 axis [12]. In addition, circ-ATP8A2 overexpression can lead to the development of CC by acting as a ceRNA [13]. Moreover, circEIF4G2 enhances the malignant phenotype of CC via activating miR-218/HOXA1 pathway [14]. Circ_0000190 has been reported as a tumor suppressive gene in gastric cancer and multiple myeloma [15,16]. During our investigation, circ_0000190 was detected to be significantly down-regulated in CC cells. Also, we observed that

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overexpressing circ_0000190 inhibited CC progression *in vitro* and *in vivo*. Here, we concluded that circ_0000190 inhibited CC progression by regulating miR-1252-5p/EIF4EBP2 axis and the down-regulated circ_0000190 stimulated EIF4EBP2 activity thus leading to CC tumorigenesis.

Materials and methods

Cell lines

CC cell lines (HeLa, SiHa, CaSki, C33A) and normal cell line (Ect1-E6E7) were acquired commercially from Shanghai Institute of Cell Biology (Shanghai, China) and kept in RPMI-1640 DMEM at 37°C in 5% CO_2 incubator. After that, 10% fetal bovine serum (FBS; Gibco, Grand Island, NY) was applied to supplement culture medium.

Quantitative real-time RT-PCR (RT-qPCR) analysis

Total RNA sample of HeLa and SiHa cells was reversely transcribed into cDNA utilizing TaKaRa Reverse Transcription Kit (TaKaRa, Dalian, China) for qPCR. BioRad SYBR Green Super Mix (Bio-Rad, Hercules, CA) was used on the Step-One Plus System (Applied Biosystems, Foster City, CA). Quantification was normalized to GAPDH or U6 using comparative $2^{-\Delta\Delta Ct}$ method.

Cell transfection

For transfection, overexpression plasmids including OE/circ_0000190 and NC, miR-1252-5p mimics and miR-NC, OE/EIF4EBP2 and NC, as well as silencing plasmids including sh-EIF4EBP2 and sh-NC, miR-1252-5p inhibitor and miR-NC, these were all designed by RiboBio (Guangzhou, China). Lipofectamine 2000 (Invitrogen, Carlsbad, CA) was used for 48 h of transfection in HeLa and SiHa cells.

Cell counting kit 8 (CCK-8) assay

Cell viability was detected by conduting Cell Counting Kit-8 (CCK-8) assay. CC cells in 96-well plates were subjected to 2 h of treatment with 10 μl of CCK-8 solution. Absorbance was monitored at the optical density of 450 nm using a microplate reader (Bio-Rad).

Colony formation assay

Transfected CC cells were planted in 6-well plates for 2 weeks. The culture medium was discarded and the cells were washed with PBS for two times. After that, cells were then fixed and stained by using 1% crystal violet. Colonies were manually counted. All the cells used for colony formation assay were transfected in a stable manner.

Flow cytometry analysis of apoptosis

CC cells were reaped after transfection and rinsed in PBS, followed by the double-staining with Annexin V fluorescein isothiocyanate (FITC)/propidium iodide (PI) detection kit (Invitrogen) for 15 min. Flow cytometer (Beckman Coulter, Brea, CA) was used for detecting cell apoptosis.

Western blot assay

The extracted protein samples were separated via electrophoresis on SDS-PAGE (12%), then loaded to PVDF membranes and treated with 5% nonfat milk. Primary antibodies against Bcl-2, Bcl-xl, Bax, cleaved caspase-3, total caspase-3 and GAPDH, along with the corresponding secondary antibodies, were acquired from Abcam (Cambridge, MA). All antibodies were diluted prior to use. After being rinsed in TBST, protein bands were analyzed through enhanced chemiluminescence (ECL) detection system (Bio-Rad).

Transwell assays

To evaluate the migratory and invasive ability of CC cells, cells were planted on the top of 24-well Transwell chambers (BD Biosciences, Franklin Lakes, NJ) coating Matrigel for invasion assay or without Matrigel for migration assay at a density of 1×10^4 cells per well. The lower chambers were added into FBS. Twenty-four hours later, cells in the upper layer were removed with caution by a cotton swab and then fixed in methanol solution for 15 min. Crystal violet was adopted to stain the membranes for 10 min, and the invaded or migrated cells were observed and counted under a microscope (10 × 10)

Animal study

The male nude mice, from the National Laboratory Animal Center (Beijing, China), were used with the approval of the Animal Research Ethics Committee of Hospital of Chengdu University of Traditional Chinese Medicine. After the subcutaneous injection of transfected CC cells for 28 days, mice were sacrificed. Finally, tumor samples were dissected and weighed.

Subcellular fractionation assay

Subcellular fraction assay was performed in CC cells using PARIS™ Kit (Invitrogen) for localization in line with the user guide. The extracted RNAs were measured by RT-qPCR analysis.

RNA binding protein immunoprecipitation (RIP) assay

With the Magna RIP Kit (EMD Millipore, Billerica, MA), RIP assay in CC cell lines was achieved with the specific antibodies and normal control anti-IgG antibody. Lysates were obtained from CC cell lines using RIP lysis buffer. The lysis was incubated with the magnetic beads conjugated to human antibodies against Ago2 or IgG as control. The precipitated RNAs were then analyzed by RT-qPCR analysis.

Luciferase reporter assays

The pmirGLO/circ_0000190 Wt/Mut and pmirGLO/EIF4EBP2 Wt/Mut reporter vectors were severally constructed and co-transfected with the indicated transfection plasmids for 48 h, finally analyzed using Dual-Luciferase Reporter Assay System (Promega, Madison, WI).

RNA pull down assay

The protein samples of CC cells were acquired from RIPA lysis buffer, cultured with beads and Bio-miR-1252-5p or Bio-NC or control. After that, the samples were rotated and incubated overnight at 4°C. Also, centrifugation was carried out after incubation, extracted by Trizol method and then sent for sequencing. After that, the pulled-down mixture was detected by RT-qPCR analysis.

Database analyses

StarBase (http://starbase.sysu.edu.cn/) was used to determine microRNAs which could bind to circ_0000190 and the binding sites between circ_0000190 and miR-1252-5p were predicted by the database. In addition, bioinformatics analysis from starBase was utilized and the binding sites between EIF4EBP2 and miR-1252-5p were predicted by the database.

Statistical analyses

All data from experiments including at least 3 biological replications were exhibited as the mean ± standard deviation (SD). Student's *t*-test and ANOVA (one-way or two-way) were applied for statistical analyses by employing the PRISM 6 (GraphPad, San Diego, CA). Statistics with a p value below 0.05 were considered to be statistically significant.

Results

Overexpression of circ_0000190 inhibits cervical cancer progression *in vitro* and *in vivo*

It has been reported that circ_0000190 is down-regulated in gastric cancer and multiple myeloma and such circRNA can inhibit the progression of those two cancers [15,16]. In our investigation, the expression of circ_0000190 was also found to be down-regulated in CC cells by RT-qPCR analysis (Figure 1(a)). HeLa and SiHa cell lines were selected for the follow-up assays. Firstly, we detected the overexpression efficiency of circ_0000190 in HeLa and SiHa cells transfected with pcDNA3.1/circ_0000190 (OE/circ_0000190) and a satisfactory result was observed (Figure 1(b)). Next, CCK-8 and colony formation assays were conducted and the results demonstrated that the overexpression of circ_0000190 inhibited the cell viability and proliferation ability (Figure 1(c,d)). According to the result of flow cytometry assay, it was presented that the apoptosis ratio was significantly increased after

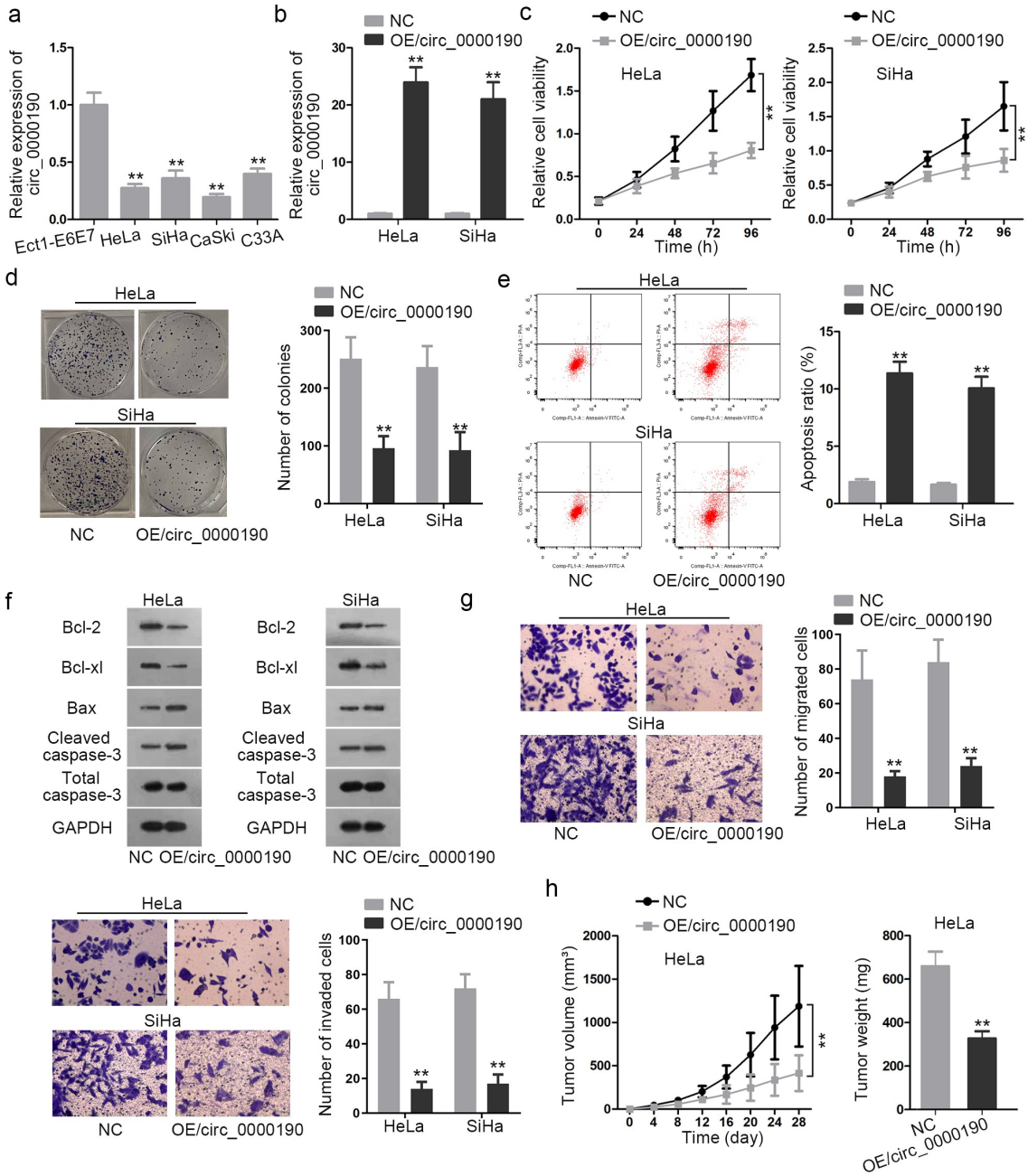

Figure 1. Overexpression of circ_0000190 inhibits cervical cancer progression *in vitro* and *in vivo*.

a. Relative expression of circ_0000190 in CC cells was detected by RT-qPCR analysis. b. Circ_0000190 overexpression efficiency was measured. c-d. CCK-8 and colony formation assays were carried out to detect the cell proliferation upon circ_0000190 overexpression. e-f. Flow cytometry and Western blot assays were utilized to detect cell apoptosis. g. Transwell assays were carried out to detect cell migration and invasion. h. Tumor volume and tumor weight were measured when cells transfected with OE/circ_0000190 were injected in to mice. **p < 0.01.

circ_0000190 overexpression (Figure 1(e)). Also, Western blot assay was utilized to measure the level of apoptosis-associated proteins. It was verified that the protein level of anti-apoptotic proteins (Bcl-2 and Bcl-xl) was sharply decreased while that of pro-apoptotic proteins (Bax and cleaved caspase-3) was prominently increased after the transfection with OE/circ_0000190 (Figure 1(f)). Moreover, by conducting Transwell assays, we observed that the numbers of migrated and invaded cells were both significantly decreased after the CC cells were transfected with OE/circ_0000190 (Figure 1(g)). Finally, *in vivo* experiments were carried out to further confirm our

conclusion. According to the results, the tumor volume and tumor weight were greatly reduced when cells transfected with OE/circ_0000190 were injected into the mice, which demonstrated that circ_0000190 could inhibit CC tumor growth (Figure 1(h)). Collectively, circ_0000190 functioned as a tumor suppressor in CC cells.

Circ_0000190 serves as a sponge for miR-1252-5p

To further study the underlying mechanism of circ_0000190 in CC cells, we firstly analyzed circ_0000190 location in the nucleus and cytoplasm fraction of CC cells to verify our ceRNA hypothesis. The results demonstrated that circ_0000190 was predominantly distributed in the cytoplasm of CC cells, suggesting the possibility of our assumption (Figure 2(a)). Next, according to the data from starBase (http://starbase.sysu.edu.cn/), five microRNAs (miRNAs) (miR-1252-5p, miR-22-3p, miR-3164, miR-6820-3p, miR-873-5p) sharing binding sites with circ_0000190 were sifted out under certain screening conditions (Clip Data: medium, Degradome Data: low) (Figure 2(b)). After that, RT-qPCR analysis was utilized to measure their relative expression in CC cells and only the expression of miR-1252-5p was significantly elevated in CC cells compared to that in normal cell line Ect1-E6E7 (Figure 2(c)). Also, we examined miR-1252-5p expression in CC cells after overexpressing circ_0000190 and detected a prominent down-regulation of miR-1252-5p. As shown by the result, it was indicated that miR-1252-5p could be modulated by circ_0000190 (Figure 2(d)). MiR-1252-5p loss-of-function experiments were performed next. Firstly, we detected the interference efficiency of miR-1252-5p inhibitor and obtained a desirable result (Figure S1A). After that, by conducting functions assay including colony formation assay, CCK-8 assay and flow cytometry assay, it was confirmed that the knock-down of miR-1252-5p could inhibit cell proliferation while enhancing the apoptosis of CC cells (Figure S1B-D). Also, the protein level of apoptosis-associated proteins was detected (Figure S1E). As shown by the result, it was verified that the protein level of anti-apoptotic proteins (Bcl-2 and Bcl-xl) was significantly decreased while that of pro-apoptotic proteins (Bax and cleaved caspase-3) was prominently increased after the transfection with miR-1252-5p inhibitor. Moreover, cell migration and invasion change was detected by Transwell assays (Figure S1F). As shown by the results, both the migration and invasion ability of CC cells were significantly decreased after the silencing of miR-1252-5p. Moreover, *in vivo* assay was conducted to detect the effect of miR-1252-5p silencing on tumor volume and weight (Figure S2A). As shown by the result, the tumor volume and tumor weight were greatly reduced when cells transfected with miR-1252-5p inhibitor were injected into the mice. Therefore, miR-1252-5p could act as a tumor suppressive role in CC cells. After that, RIP assay was conducted to verify the coexistence of miR-1252-5p and circ_0000190 in RISC and the interaction between them was further verified by luciferase reporter assay after their binding sites were predicted (Figure 2(e,f)). As the luciferase activity of cells transfected with circ_0000190 Wt and miR-1252-5p mimics was significantly decreased, it was validated that miR-1252-5p could bind to circ_0000190 in CC cells. Furthermore, rescue experiments with miR-1252-5p mimics were utilized to further verify the effect of miR-1252-5p on CC cells. According to the results of CCK-8 and colony formation assays, cell viability and proliferation ability reduced by OE/circ_0000190 were partially rescued by the co-transfection of OE/circ_0000190+ miR-1252-5p mimics (rescue group) (Figure 2(g,h)). Also, flow cytometry and Western blot assays were utilized to evaluate cell apoptosis (Figure 2(i,j)). As shown by the results, the enhanced cell apoptosis caused by circ_0000190 overexpression was greatly reversed by the co-transfection of OE/circ_0000190+ miR-1252-5p mimics. Moreover, cell migration and invasion change was detected by Transwell assays (Figure 2(k)). According to the results, both the migration and invasion ability of CC cells significantly decreased after the overexpression of circ_0000190 were partially restored by the co-transfection of OE/circ_0000190+ miR-1252-5p mimics. In addition, *in vivo* assay was conducted to detect the effect of OE/circ_0000190+ miR-1252-5p mimics on tumor volume and weight (Figure S2B). As shown by the result, the tumor volume and weight decreased by the injection of cells transfected with OE/circ_0000190 could be greatly recovered after the co-transfection of miR-1252-5p inhibitor. Taken that, miR-1252-5p

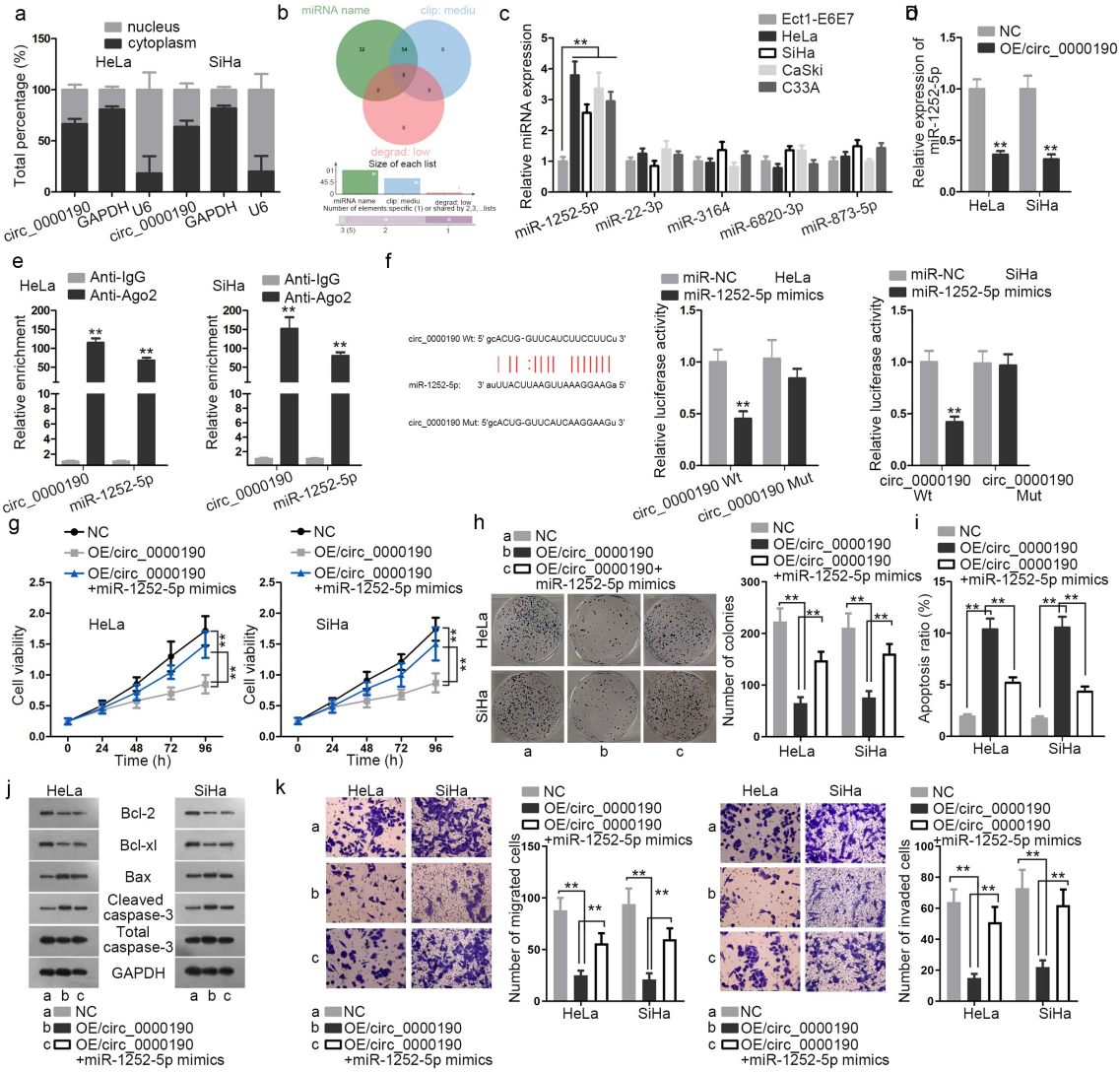

Figure 2. Circ_0000190 serves as a sponge for miR-1252-5p.

a. Subcellular fractionation analysis was conducted to verify the location of circ_0000190 in CC cells. b. Bioinformatics analysis was used to sift out miRNA candidates. **C**. The expression of five potential miRNAs was detected. d. MiR-1252-5p expression in response to circ_0000190 overexpression was verified. e. RIP assay was conducted to examine the coexistence of the indicated molecules. f. Luciferase reporter assay was utilized to verify the interaction between the indicated molecules. g-h. The proliferation of cells transfected with OE/circ_0000190+ miR-1252-5p mimics was detected. i-j. Flow cytometry and Western blot assays were utilized to detect the apoptosis of cells transfected with OE/circ_0000190+ miR-1252-5p mimics. k. Transwell assays were carried out to detect cell migration and invasion of cells transfected with OE/circ_0000190+ miR-1252-5p mimics. **p < 0.01.

could bind to circ_0000190 and partially rescue the influence caused by circ_0000190 overexpression on CC cells.

EIF4EBP2 functions as an endogenous competing RNA for miR-1252-5p

After that, bioinformatics analysis from starBase was utilized to determine the downstream target genes (PPT1, EIF4EBP2, HNRNPUL2-BSCL2, UCP2, SOX4) of miR-1252-5p under certain screening conditions (Clip Data: strict, Degradome Data: high, Pan-cancer Number: 2, Program Number: 3) (Figure 3(a)). After that, RT-qPCR analysis was conducted and it was demonstrated that only EIF4EBP2 expression was down-regulated significantly in CC cells (Figure 3(b)). Then, the fact that EIF4EBP2 expression was found to be up-regulated in response to circ_0000190 overexpression further elucidated

that EIF4EBP2 could be modulated by circ_0000190 (Figure 3(c)). Subsequently, we conducted EIF4EBP2 gain-of-function experiments. Firstly, EIF4EBP2 overexpression efficiency was detected by RT-qPCR assay and a satisfactory result was observed (Figure 3(d)). In addition, CCK-8 and colony formation assays were conducted and it was demonstrated that the cell viability and proliferation ability were decreased after EIF4EBP2 overexpression (Figure 3(e,f)). After that, flow cytometry assay and Western blot assay demonstrated that EIF4EBP2 overexpression could promote cell apoptosis (Figure 3(g,h)). Transwell assays were then carried out and it was

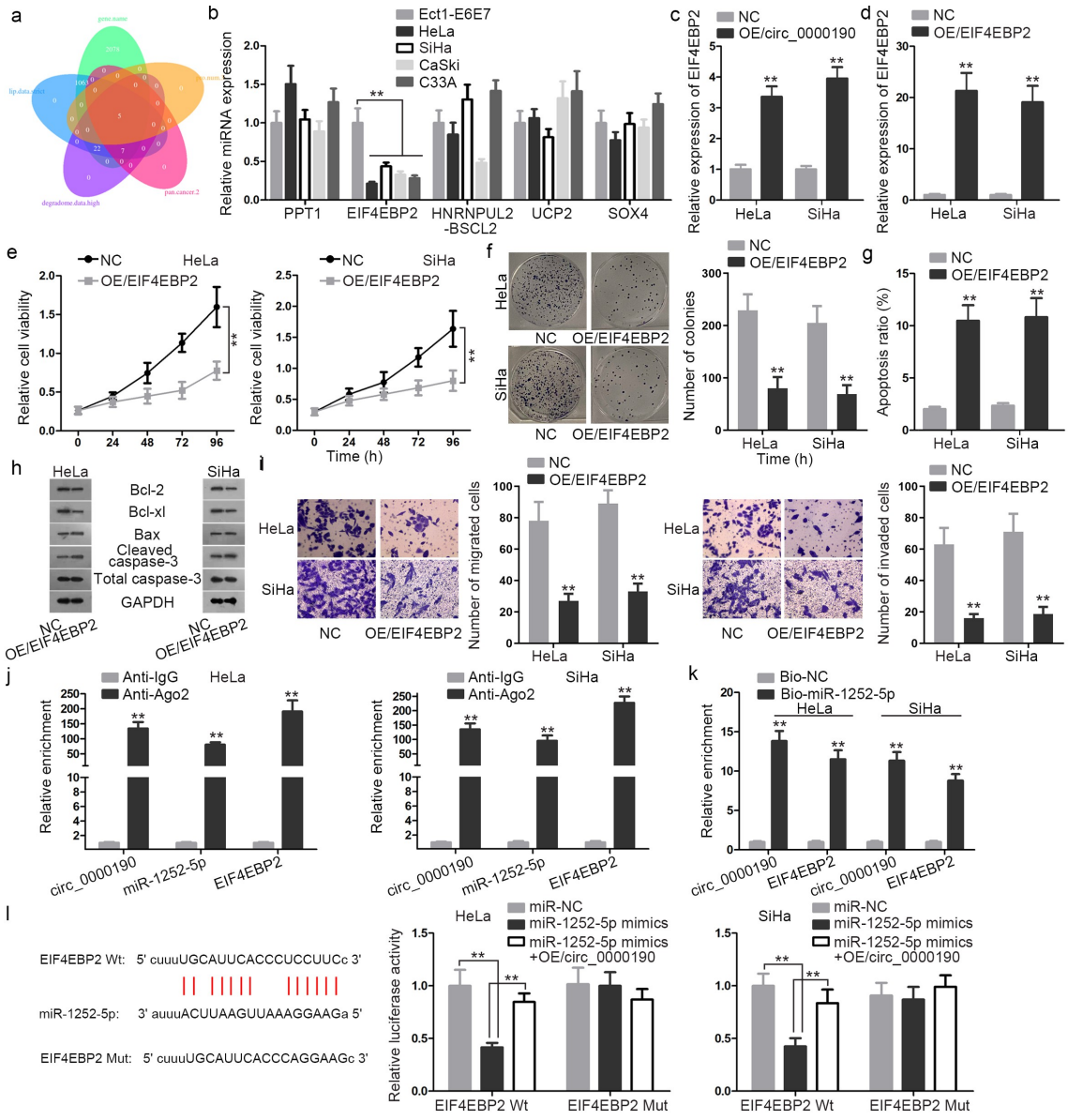

Figure 3. EIF4EBP2 functions as an endogenous competing RNA for miR-1252-5p.

a. Bioinformatics analysis of potential target genes of miR-1252-5p was presented. b. Relative expression of selected mRNAs in CC cells was detected. c. EIF4EBP2 expression in response to circ_0000190 overexpression was detected. d. EIF4EBP2 overexpression efficiency was detected. e-f. CCK-8 and colony formation assays were carried out to detect the proliferation of cells transfected with OE/EIF4EBP2. g-h. Flow cytometry and Western blot assays were utilized to detect the apoptosis of cells transfected with OE/EIF4EBP2. i. Transwell assays were carried out to detect the migration and invasion of cells transfected with OE/EIF4EBP2. j. RIP assay was conducted to examine the coexistence of the indicated molecules. k. RNA pull down assay was carried out to further verify the binding relationship among miR-1252-5p, EIF4EBP2 and circ_0000190. l. Luciferase reporter assay was utilized to confirm the interaction among the indicated molecules. **p < 0.01.

shown that both the cell migration and invasion were attenuated by EIF4EBP2 overexpression (Figure 3(i)). After that, *in vivo* assay was conducted to detect the influence of OE/EIF4EBP2 on tumor volume and weight (Figure S2C). According to the result, the tumor volume and weight were obviously reduced when cells transfected with OE/EIF4EBP2 were injected into the mice. Collectively, EIF4EBP2 was up-regulated in CC cells and the overexpression of EIF4EBP2 inhibited CC progression. To further verify the mechanism among circ_0000190, miR-1252-5p and EIF4EBP2, mechanism experiments including RIP, RNA pull down and luciferase reporter assays were conducted next. From RIP assay, we could observe that the three indicated molecules circ_0000190, miR-1252-5p and EIF4EBP2 could coexist in RISC (Figure 3(j)). RNA pull down assay further verified that both circ_0000190 and EIF4EBP2 could be pulled down by Bio-miR-1252-5p, suggesting that miR-1252-5p could bind to those two molecules (Figure 3(k)). At last, after the binding sites between EIF4EBP2 and miR-1252-5p were predicted, we applied luciferase reporter assay to further verify whether there existed interaction among them (Figure 3(l)). The results showed that relative luciferase activity of cells transfected with EIF4EBP2 Wt was decreased after the transfection with miR-1252-5p mimics but recovered after the co-transfection with miR-1252-5p mimics+OE/circ_0000190, indicating miR-1252-5p could bind to circ_0000190 and EIF4EBP2 respectively.

EIF4EBP2 inhibited carcinogenesis by modulating eIF4E activity

The above experiments have elaborated that circ_0000190 could regulate CC by binding to miR-1252-5p and modulating EIF4EBP2. In addition, rescue experiments had further verified that miR-1252-5p mimicscould partially reverse the effect on cell proliferation, apoptosis, migration and invasion caused by circ_0000190 overexpression. To further verify the rescue effects of EIF4EBP2 on circ_0000190 overexpression, we applied the rescue experiments with OE/circ_0000190+ sh-EIF4EBP2 as the rescue group. According to the results of CCK-8 and colony formation assays, reduced cell viability and proliferation ability caused by circ_0000190 overexpression were partially recovered after the co-transfection of OE/circ_0000190+ sh-EIF4EBP2 (Figure 4(a,b)). After that, flow cytometry assay and Western blot assay demonstrated that the co-transfection of OE/circ_0000190+ sh-EIF4EBP2 could significantly reverse the enhanced cell apoptosis caused by circ_0000190 overexpression (Figure 4(c,d)). Transwell assays were then carried out and it was shown that both the cell migration and invasion attenuated by circ_0000190 overexpression could be greatly rescued by the co-transfection of OE/circ_0000190+ sh-EIF4EBP2 (Figure 4(e)).

Furthermore, it has been reported that EIF4EBP2 down-regulates cap-dependent translation through the sequestration of eIF4E from the eukaryotic translation initiation factor complex [17]. Also, phosphorylated eIF4E has been reported to promote tumorigenesis [18]. To explore the effects of circ_0000190 on eIF4E, we detected the protein levels of EIF4EBP2, phosphorylated eIF4E, eIF4E and target mRNAs of eIF4E: ODC1, VEGFA, FGF2 [19–21]. As shown by the result, the overexpression of circ_0000190 could elevate the protein level of EIF4EBP2 protein level while reducing phosphorylated eIF4E, ODC1, VEGFA and FGF2 protein level. After the co-transfection of OE/circ_0000190+ sh-EIF4EBP2, such results were significantly reversed, suggesting that overexpressed circ_0000190 could elevate the expression of EIF4EBP2 and facilitating eIF4E to bind to EIF4EBP2 therefore preventing eIF4E from functioning (Figure 4(f)). Taken together, circ_0000190 overexpression resulted in the high expression of EIF4EBP2 thus inactivating eIF4E activity. Moreover, *in vivo* assay was conducted to detect the influence of OE/circ_0000190 + sh-EIF4EBP2 on tumor volume and weight (Figure S2D). As shown by the result, the tumor volume and weight decreased by the injection of cells transfected with OE/circ_0000190 could be greatly recovered after the co-transfection of sh-EIF4EBP2. To conclude, down-regulated circ_0000190 could promote cervical cancer by facilitating the activity of EIF4E.

Discussion

CircRNAs, a novel class of non-coding RNAs with a special circular covalently bonded structure, are promising molecular biomarkers in diseases

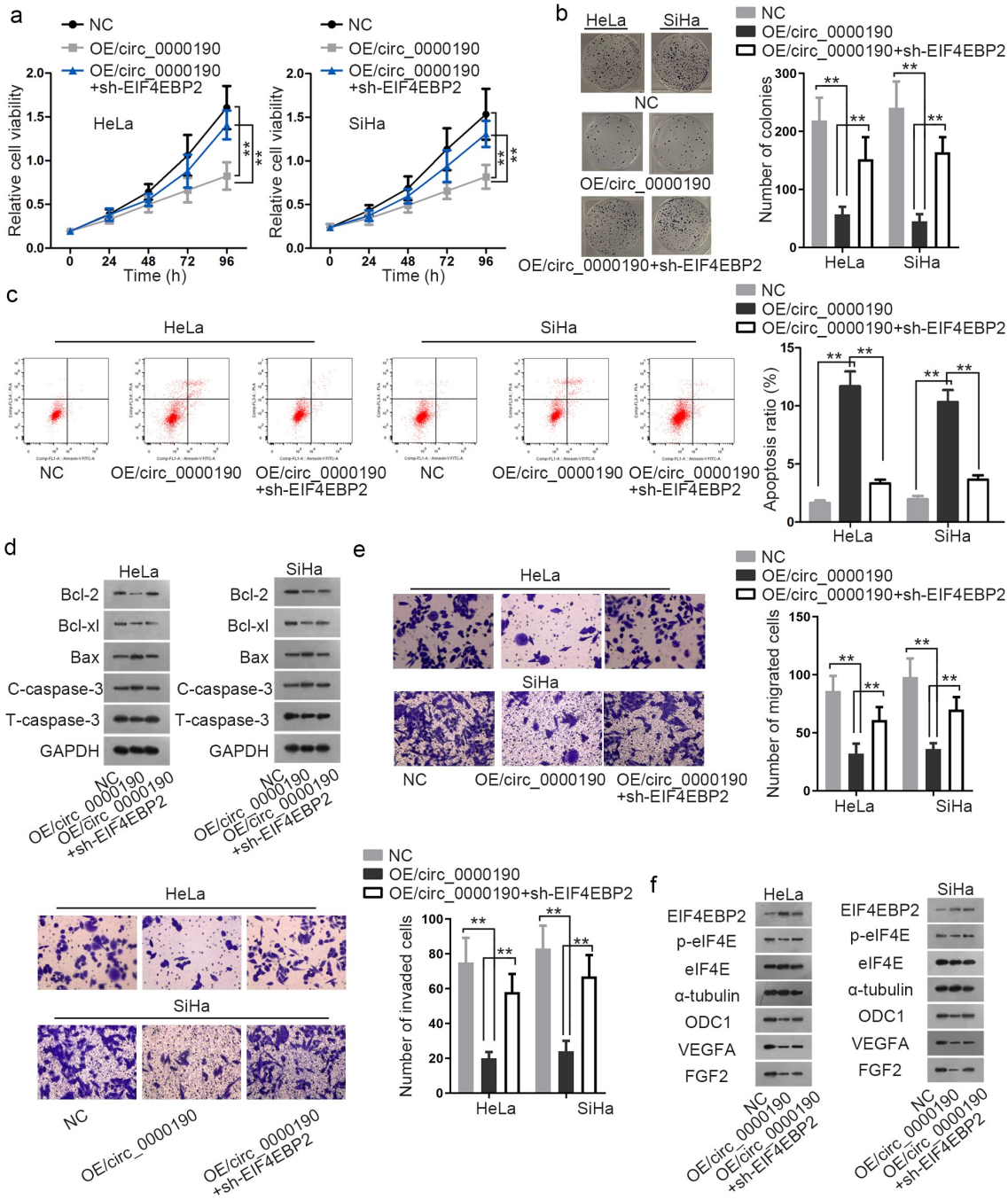

Figure 4. EIF4EBP2 inhibits carcinogenesis by modulating eIF4E activity.

a-b. CCK-8 and colony formation assays were carried out to detect the proliferation of cells co-transfected with OE/circ_0000190+ sh-EIF4EBP2. c-d. Flow cytometry and Western blot assays were utilized to detect the apoptosis of cells co-transfected with OE/circ_0000190+ sh-EIF4EBP2. i. Transwell assays were carried out to detect the migration and invasion of cells co-transfected OE/circ_0000190+ sh-EIF4EBP2. f. Western blot assay was utilized to detect the protein levels of phosphorylated eIF4E and eIF4E-associated mRNAs in cells co-transfected with OE/circ_0000190+ sh-EIF4EBP2. **p < 0.01.

including cancers as they are features with unique conservation, abundance and tissue specificity [5]. Recently, more and more circRNAs have been identified owing to the development of high-throughput sequencing technology [22]. Previous studies have revealed the tumor suppressive role of circ_0000190 in gastric cancer and multiple myeloma [15,16]. To start with our research on circ_0000190 in cervical cancer cells, we firstly detected circ_0000190 expression in CC cells. According to the result of RT-

qPCR analysis, we detected a down-regulated expression of circ_0000190 in CC cells. Moreover, the subsequent functional experiments and *in vivo* experiments further confirmed the tumor suppressive property of circ_0000190.

To verify our assumption of ceRNA mechanism of circ_0000190 in CC cells, we firstly detected circ_0000190 location by nucleus and cytoplasm fractionation analysis and found that circ_0000190 was predominantly located in the cytoplasm. Next, according to the data from starBase, the miRNA candidates sharing binding sites with circ_0000190 were determined. By detecting their relative abundance and response to circ_0000190 overexpression, we confirmed that miR-1252-5p was down-regulated in CC cells and could be modulated by circ_0000190. Interestingly, there haven't been quite many researches on miR-1252-5p. To unveil the effects of miR-1252-5p on cell functions, loss-of-functions were performed. According to the results, miR-1252-5p was revealed to promote the cell proliferation, migration and invasion of CC cells. Next, mechanism experiments further proved the interaction between circ_0000190 and miR-1252-5p. Then, several assays were carried out to find the downstream target gene of miR-1252-5p. By using bioinformatics analysis, five mRNAs having binding sites with miR-1252-5p were sifted out. According to the RT-qPCR analysis, it was demonstrated that only eukaryotic translation initiation factor 4E binding protein 2 (EIF4EBP2) expression level was down-regulated in CC cells. Also, functional experiments further confirmed the oncogenic character of EIF4EBP2. Moreover, mechanism experiments were conducted which proved the interaction among circ_0000190, miR-1252-5p and EIF4EBP2. Therefore, we could conclude that circ_0000190 regulated CC progression via sponging miR-1252-5p and regulating EIF4EBP2. During our investigation of EIF4EBP2, we studied several articles about the EIF4EBP2 mechanism which may suppress the carcinogenesis by binding to proto-oncogenic eIF4E, which greatly aroused our interest. According to the searching results in human gene database GeneCards (https://www.genecards.org/), we discovered that EIF4EBP2 could competitively bind to eIF4E and prevent eIF4E from forming into complex thus functioning as an oncogene. It has been confirmed that eIF4E can act as an oncogene and the phosphorylation of eIF4E at serine 209 can promote tumorigenesis [18,23]. Also, previous studies have revealed that altered expression of EIF4E-BPs led to the altered phosphorylation status of eIF4E. Based on the previous reports, we conjectured that EIF4EBP2 could bind to phosphorylated eIF4E thus suppresses eIF4E from exerting functions. By carrying out Western blot assay, we found the down-regulation of phosphorylated eIF4E after the overexpression of circ_0000190 and such result was reversed after cells were co-transfected with sh-EIF4EBP2. We also examined the protein levels of ODC1, VEGFA, FGF2, which were all target mRNAs translated by eIF4E. Therefore, we could conclude that it was the circ_0000190 overexpression that led to the high expression of EIF4EBP2 in CC cells and inhibited phosphorylated eIF4E activity.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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