Circular RNA BMI1 Serves as a Potential Target for Diagnosis and Treatment in Esophageal Cancer

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Abstract

Aims: Previous studies have confirmed that BMI1 is elevated in esophageal cancer, which is a potential therapeutic target for esophageal cancer. However, the clinical significance of circular RNA BMI1 (circ-BMI1) in esophageal cancer is not yet clear. Herein, we revealed the clinical implication of circ-BMI1 in esophageal cancer, and provided a theoretical basis for molecular diagnosis and potential targeted therapy of esophageal cancer. **Methods:** Firstly, 10 fresh paired esophageal cancer tissues and paracancer tissues, 49 esophageal cancer serum samples and 28 healthy control serum samples were involved in our study. Differential expression and clinical significance of circ-BMI1 in esophageal cancer patients and healthy controls were evaluated by quantitative Real-time RT-PCR (RT-qPCR). Secondly, effects of circ-BMI1 differential expression on biological function of esophageal cancer cell line Eca109 were analyzed. Effects of circ-BMI1 on cell proliferation, migration and colony forming ability were evaluated by CCK-8, wound healing, and colony-forming assay. Cell apoptosis, drug sensitivity tests were also be conducted. Finally, influence of Eca109 cells differentially expressed by circ-BMI1 on tumorigenicity in nude mice was studied. **Results:** Expression of circ-BMI1 in serum and tissues of esophageal cancer patients was significantly decreased compared to controls ($P < 0.001$ and $P = 0.003$, respectively). Area under the receiver operating characteristic curve (ROC) was 0.726. Cell proliferation, migration and colony forming ability of circBMI1-Eca109 cells were obviously decreased than that of NC-Eca109 cells ($P < 0.05$). circBMI1-Eca109 cells were more sensitive to 5-fluorouracil and cisplatin, and tumor volume of nude mice in circBMI1-Eca109 group was smaller ($P < 0.05$). **Conclusions:** The study indicated that expression of circ-BMI1 was significantly down-regulated in esophageal cancer. Overexpression of circ-BMI1 inhibited proliferation, migration, colony formation of Eca109 cells, and tumor growth of Eca109 cells in nude mice. circ-BMI1 may be a potential target for diagnosis and treatment in esophageal cancer.

Introduction

Esophageal cancer is the eighth most common cancer globally and the sixth leading cause of cancer-related deaths worldwide.[1,2] Although surgery, chemotherapy, radiotherapy and biological targeted therapy have been used to treat esophageal cancer, the overall prognosis of patients is still inferior, with a global 5-year survival rate of less than 20%.[3] With the improvement of clinical stage and pathological grade, the prognosis of esophageal cancer has deteriorated significantly. Early detection and treatment can improve the prognosis of esophageal cancer.[4] At present, there is an urgent demand to find suitable molecular biomarkers and therapeutic targets for early diagnosis, improving the 5-year survival rate of patients with esophageal cancer.

Circular RNA (circRNA) is a type of non-coding RNA formed by reverse splicing, without 5′-3′ polarity and polyadenine tail. In recent years, it has been proven to have critical regulatory role in cancer biology,[5] such as acting as a

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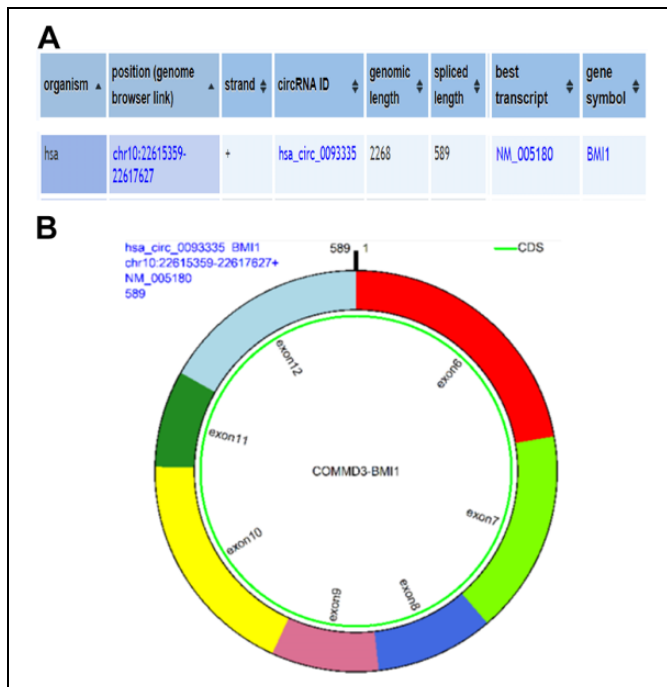Email: zqdeng2002@163.com

Figure 1. Basic information and structure diagram of circ-BMI1. A, Search the circular RNA of BMI1 through the CircBase database. B, Structure diagram of circ-BMI1.

microRNA sponge or combining with RNA-related proteins to form RNA-protein complexes that regulate gene transcription and encode regulatory peptides.[6,7] B-Cell-specific Moloney murine leukemia virus integration site-1 (BMI1) is a stem cell-related gene, which plays an essential role in maintaining normal stem cell self-renewal and multi-directional differentiation.[8,9] Studies showed that BMI1 was overexpressed in esophageal cancer and was related to the disease's aggressive clinicopathological characteristics.[10,11] In addition, BMI1 downregulation in esophageal carcinoma cells inhibited cell proliferation, cell cycle, and cell migration, while increased cell apoptosis, suggesting that BMI1 was a potential molecular target of treating esophageal cancer.[12] In 2018, a meta-analysis showed that BMI1 was involved in the progression and invasion of esophageal cancer, and could be used as a target for the prognosis and treatment of esophageal cancer.[13] In the same year, Wang *et al* reported that targeting BMI1 and Mel18 could make esophageal squamous cell carcinoma sensitive to chemotherapy.[14] However, the clinical significance of circ-BMI1 in esophageal cancer was not yet clear.

circ-BMI1 has not been reported yet. According to the CircBase database, circ-BMI1, namely has_circ_0093335, has been found (Figure 1A). circ-BMI1 is formed by circularization of exons 6-12 of BMI1, with a total of 589 bp, which is a non-coding RNA (Figure 1B).

To identify the role of circ-BMI1 in esophageal cancer, we detected the expression level of circ-BMI1 in newly diagnosed patients with esophageal cancer, and analyzed the relationship between circ-BMI1 expression and clinical parameters. We found that the expression of circ-BMI1 in esophageal cancer was significantly down-regulated, and the expression level of circ-BMI1 could distinguish patients with esophageal cancer from healthy controls. Given the critical role of circ-BMI1 in the occurrence and development of esophageal cancer, we hypothesized that the overexpression of circ-BMI1 might promote the occurrence and progression of esophageal cancer. In our study, we validated this hypothesis with esophageal cancer cell line *in vitro* and *in vivo*.

Material and Methods

Patients and Ethics

Collected esophageal cancer tissues and matched paracancer tissues (3 to 5 cm from the tumor edge) of 10 patients and serum samples of 49 patients. These patients were diagnosed with esophageal cancer at the Affiliated People's Hospital of Jiangsu University (Zhenjiang, China) from April 2018 to April 2019. During the same period, 28 healthy controls were randomly selected from the same hospital. The detailed clinical characteristics of serum samples from 49 patients (such as age, gender, smoking, drinking, TNM stage, histological type, lymph node metastasis and differentiation status) were listed in Table 1. Patients did not receive chemotherapy, radiotherapy or other anti-cancer treatments before surgery. The whole participants signed an informed consent form, and this study was approved by the Ethical Review Committee of the Affiliated People's Hospital of Jiangsu University (approval number: K-20200142-W).

RNA Isolation, Reverse Transcription, and Quantitative Real-time PCR (qPCR)

According to the manufacturer's protocol, trizol reagent (Invitrogen, USA) was used to isolate RNA from esophageal cancer tissues samples, and RNA was extracted from serum samples of esophageal cancer using RNeasy Serum/Plasma Kit (Qiagen, Germany). The concentration and purity of all RNA samples were detected by a NanoDrop One spectrophotometer (Thermo, USA). Then, reverse transcription of each RNA sample of 2 μg was completed synthesizing cDNA using the cDNA synthesis kit (TaKaRa, Japan) following the manufacturer's protocol (step 1: 65°C for 5 min; step 2: 30°C for 10 min, 45°C for 45 min, 95°C for 5 min).

Real-time PCR was performed to detect the expression of circ-BMI1 with TB Green Premix Ex Taq II (TaKaRa, Japan), and 2 × SYBR Green PCR Mix (Multisciences, China) for housekeeping gene ABL expression detection, according to the manufacturer's protocol. The primers used for circ-BMI1 and ABL expression were illustrated in Table 2. CircPrimer1.2 software was used to design primers based on gene sequences in Circbase, and the primers were synthesized by Beijing Liuhe Huada Gene Technology Co., Ltd. (Beijing, China). The qPCR reaction conditions: predenaturation of 1 cycle (95°C for 30 sec), amplification of 40 cycles (95°C for 5 sec, 66.7°C for

Table 1. Comparison of Serum Circ-BMI Expression and Clinical Parameters in Patients With Esophageal Cancer.

Patient's parameters	Low (n = 23)	High (n = 26)	P value
Mean age, years (range)	63.70 (49-81)	67.85 (52-85)	0.089
Sex, n (%)			1.000
Male	14 (60.9%)	15 (57.7%)	
Female	9 (39.1%)	11 (42.3%)	
Smoking, n (%)			0.761
No	17 (73.9%)	18 (69.2%)	
Yes	6 (26.1%)	8 (30.8%)	
Drinking, n (%)			1.000
No	17 (73.9%)	19 (73.1%)	
Yes	6 (26.1%)	7 (26.9%)	
Histological type, n (%)			
Squamous cell carcinoma	19 (82.6%)	21 (80.8%)	0.943
Adenocarcinoma	2 (8.7%)	2 (7.7%)	
Other types	2 (8.7%)	3 (11.5%)	
Differentiation status, n (%)			
Moderate differentiation	11 (47.8%)	21 (80.8%)	0.028
Poor differentiation	7 (30.4%)	2 (7.7%)	
No data	5 (21.8%)	3 (11.5%)	
Lymph node metastasis, n (%)			0.042
No	6 (26.1%)	15 (57.7%)	
Yes	17 (73.9%)	11 (42.3%)	
TNM stage, n (%)			1.000
0	1 (4.3%)	0 (0.0%)	
I	1 (4.3%)	3 (11.5%)	
II	3 (13.0%)	6 (23.1%)	
III	5 (21.7%)	4 (15.4%)	
IV	7 (30.4%)	7 (26.9%)	
No data	6 (26.1%)	6 (23.1%)	

Table 2. Primers Sequencing for qPCR.

Primers	Sequence (5′-3′)
circ-BMI1-Forward	CTTCTGCTGATGCTGCCAATG
circ-BMI1-Reverse	CTGGTCTCCAGGTAACGAACA
ABL-Forward	TCCTCCAGCTGTTATCTGGAAGA
ABL-Reverse	TCCAACGAGCGGCTTCAC

transfected into Eca109 cells, respectively. After 6 h of transfection, it was replaced with a complete medium containing 10% FBS. After Screening with puromycin (ScienCell, USA) and cell sorting by flow cytometry (BD FACSAria II, USA), RT-qPCR was used to detect circ-BMI1 expression in each transfected cells.

Cell Proliferation Assay

Took NC-Eca109 and circBMI1-Eca109 cells in the logarithmic growth phase respectively, and prepared the cell suspension with RPMI-1640 culture medium containing 10% FBS. Cells were seeded into 96-well plates at a density of 3×10^4/mL cells in 0.1 mL. Set zero adjustment groups at the same time, each group had 6 holes to repeat, and a total of 5 plates were laid. On days 0, 1, 2, 3, and 4 of culture, 10 μL of CCK-8 (Dojindo, Japan) was added to each well. After incubating for 3 h, the optical density value (OD value) of each well at 450 nm was detected by a microplate reader (BioTek, USA). Then compared the difference of OD value of 2 groups to draw a cell growth curve.

Wound Healing Array

Two groups of cells in the logarithmic growth phase were adopted, and the cells were seeded into a 6-well plate at a density of 6×10^5 /mL cells in 2 mL. Each group had 3 multiple holes. Wounds were evenly scratched with 10 μL plastic pipette tip. The cells were washed 3 times with PBS (Procell, China) and added with fresh medium. Then incubated for 0 h and 24 h, and examined the wounds under a photomicroscope (Olympus, Japan). Marked 10 identical positions of the wounds, calculated the cell migration rate with Image-Pro Plus 6.0 (Media Cybernetics, USA), and analyzed the statistical differences.

32 sec, 72°C for 30 sec, and 80°C for 30 sec to collect fluorescence), release procedure (95°C for 15 sec, 60°C for 60 sec, 95°C for 15 sec, and 60°C for 15 sec). The relative expression levels of circ-BMI1 were calculated by the $2^{-\Delta\Delta CT}$ method.[15]

Cell Culture

The human Eca109 cell line of esophageal cancer was purchased from the Cell Center of Basic Medicine, Peking Union Medical College. Eca109 cells were incubated in RPMI-1640 medium (Wisent, China) with 10% fetal bovine serum (FBS, Excell, China) and 100 μg/mL penicillin/streptomycin (Hyclone, USA). A temperature of 37°C and a carbon dioxide concentration of 5% were set as the cell culture condition.

Cell Transfection

Eca109 cells in the logarithmic growth phase were seeded with 2×10^5 cells per well in a 6-well plate, and the cells were confluent approximately 80%-90% after overnight culture. According to Lipofectamine 2000 (Invitrogen, USA) manufacturer's protocol, serum-free RPMI-1640 medium was used to prepare transfection reagent and plasmid working solution, and pcircRNA-circ-BMI1 plasmid and pcircRNA-NC were

Plate Clone Formation Assay

NC-Eca109 and circBMI1-Eca109 cells were inoculated with 400 cells per well in a 6-well plate and shaken gently to distribute the cells evenly. Observed cell growth under microscope (Olympus, Japan) every 3 days and regularly changed the medium. Two weeks later, the cells were stained with crystal violet staining solution (Beyotime, China), and the number of cell colonies were counted. Repeated the experiment 3 times, and statistically analyzed the results.

Cell Apoptosis Assay

In order to study cell apoptosis, circ-BMI1/mock transfected cells were resuspended in FBS-free RPMI-1640 medium, and 2×10^5 cells were seeded in a 6-well plate for 48 h. The day before the flow cytometry, set up an internal reference tube, took 2×10^5 cells, and fixed the cells with 2% paraformaldehyde (Beyotime, China). On the day of the flow cytometry, diluted Annexin V binding buffer (BD Biosciences, USA) 10 times, transferred the cells to flow tubes, and washed the cells twice with PBS and once with binding buffer. Then, the cells were mixed gently with 100 μL binding buffer and stained (15 min in the dark at room temperature) with an Annexin V apoptosis detection Kit (Annexin V-PE/7AAD, BD Biosciences, USA) according to the manufacturer's instructions. After the cells were washed with PBS, 400 μL of binding buffer was added and analyzed by flow cytometry (Beckman, USA).

Drug Sensitivity Test

The resuspended circ-BMI1/mock transfected cells were cultured in a 96-well plate in complete medium with a density of 5000/well cells and a volume of 50 μL. Added different concentrations of cisplatin (Meilun Biotechnology, China) and 5-fluorouracil (Meilun Biotechnology, China) prepared with complete medium to each well, totaling 50 μL. The cisplatin concentrations were 25 μmol/L, 50 μmol/L, 100 μmol/L, 150 μmol/L, 200 μmol/L, and the concentrations of 5-fluorouracil were 2.5 μmol/L, 10 μmol/L, 40 μmol/L, 80 μmol/L, 160 μmol/L, respectively. 10 μL of CCK-8 was added the next day. After 3 h of incubation, the OD value at 450 nm was measured by a microplate reader to calculate the growth inhibition rate (IR).

Nude Mice Tumor Formation Experiments

10 BALB/C nude mice (female, 5 weeks old, weighing 20 ± 2 g) were purchased from Changzhou Cavens Laboratory Animal Co., Ltd. (Changzhou, China). The mice were randomly divided into 2 groups and housed in 2 animal cages (n = 5): circBMI1-Eca109, NC-Eca109. Each mouse was marked with an ear tag to prevent confusion in the experimental process. circBMI1-Eca109 or NC-Eca109 cells at logarithmic growth phase (1.0×10^7 cells/100 μL for each nude mouse) were injected subcutaneously into flanks of mice. After injection, the volume of transplanted tumor was measured regularly with a vernier caliper every week. Tumor volume was calculated with the following formula: volume = (length × width × width)/2.[16] 25 days after the cells injection, the mice were anesthetized by intraperitoneal injection of 10% chloral hydrate (Ameko, China) and sacrificed by cervical dislocation. The tumor was separated from mice and tumor specimens were collected to detect the expression level of circ-BMI1. According to the Guide for the Care and Use of Laboratory Animals, all mice were raised in an environment free of specific pathogens.[17] All animal studies were approved by the Animal

Experiment Ethics Committee of Jiangsu University (approval number: UJS-IACUC-2020111802).

Statistical Analysis

All statistical analyses were conducted by IBM SPSS software package version 20.0 (SPSS Inc., USA) and GraphPad Prism 7.0 (GraphPad Inc., USA). Measurement data were expressed as mean $\pm$ standard error, and comparisons between different groups were performed by Student's *t*-test. Pearson Chi-square analysis or Fisher exact test was utilized to compare the differences between categorical variables. ROC curve and area under ROC curve (AUC) analysis were applied to evaluate the diagnostic value of circ-BMI1 expression for esophageal cancer. A 2-sided *P* value of 0.05 or less was considered statistically significant.

Results

Expression of circ-BMI1 in Tissue Samples

The relative expression level of circ-BMI1 corresponding adjacent normal esophagus tissues ranged 0.0037-1.0000 with median level 0.1789. circ-BMI1 expression in esophageal cancer tissues ranged 0.0008-0.5178 with median level 0.0038 was significantly down-regulated compared with the adjacent tissues (*P* = 0.003, Figure 2A).

Expression of circ-BMI1 in Serum Samples

Relative expression of circ-BMI1 in serum of patients with esophageal cancer (range 0.0026-11.8126, median 0.0878) was significantly lower than that of control group (range 0.0425-14.5797, median 0.2196) (*P* < 0.001, Figure 2B). Based on ROC analysis, the level of serum circ-BMI1 could be used as a potential biomarker to distinguish esophageal cancer patients from healthy controls. Area under ROC curve was 0.726, and 95% confidence interval was 0.615-0.836 (*P* = 0.001, Figure 2C). The value of cut-off was 0.433, with sensitivity was 0.964 and specificity was 0.469. When esophageal cancer patients were grouped according to age and clinicopathological characteristics, expression of circ-BMI1 was statistically significant. The results revealed that the expression level of circ-BMI1 in the elderly was significantly higher than that in younger ones (*P* = 0.036, Figure 2D). Meanwhile, circ-BMI1 expression in a poorly differentiated group was decreased than in the moderately differentiated group (*P* = 0.001, Figure 2E), and in the lymph node metastasis group, it was significantly decreased compared with non-lymph node metastasis group (*P* = 0.003, Figure 2F).

Comparison of Serum circ-BMI1 Expression and Clinical Characteristics in Esophageal Cancer Patients

To further analyze the clinical impact of circ-BMI1 expression, we divided whole esophageal cancer patients into high expression group and low expression group according to its cut-off value. Combined with patients with esophageal cancer's clinical
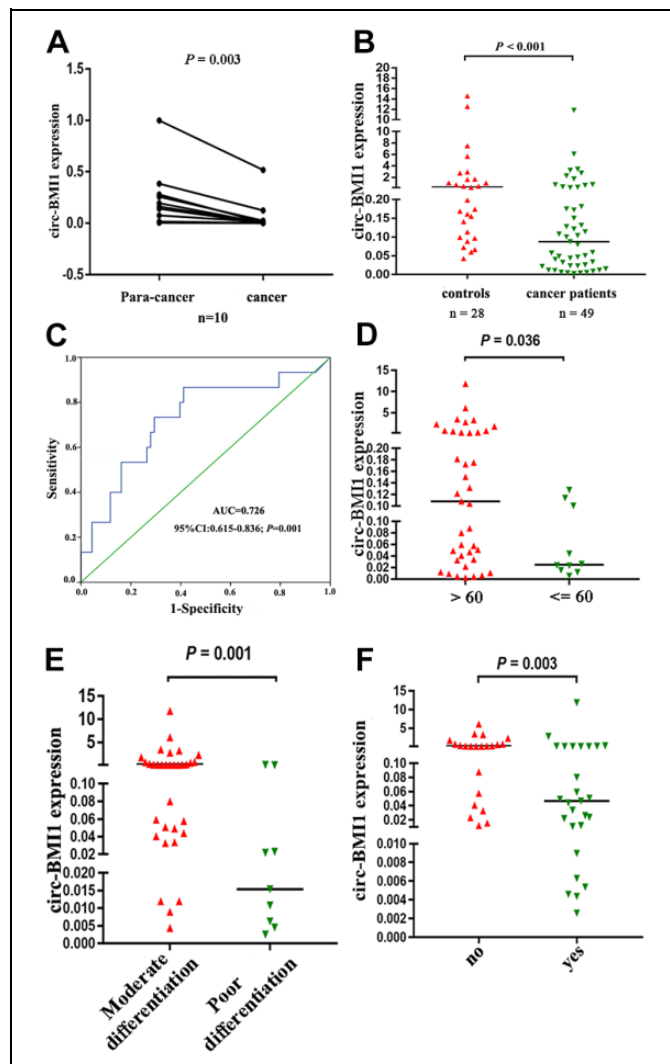
Figure 2. Expression of circ-BMI1 in cancer tissues and serum samples of patients with esophageal cancer. A, Expression of circ-BMI1 in cancer tissues of esophageal cancer patients was down-regulated compared with the paired paracancer tissues. B, circ-BMI1 expression in serum of esophageal cancer patients and healthy controls. C, ROC analysis for circ-BMI1 in distinguishing esophageal cancer patients from controls. D, circ-BMI1 expression in serum was related to age group. E, circ-BMI1 expression was correlated with the level of differentiation. F, circ-BMI1 expression was connected with the presence or absence of lymph node metastasis.

Figure 3. Overexpression of circ-BMI1 affected migration, colony formation and apoptosis of Eca109 cells. A, Fluorescence microscopic observation of circBMI1-Eca109 and NC-Eca109 cells. B, Relative expression of circ-BMI1 in circBMI1-Eca109 and NC-Eca109 cells. C, Migration of circBMI1-Eca109 and NC-Eca109 cells at different time. D, The relative migration rate of circBMI1-Eca109 was significantly decreased than the control, circ-BMI1 inhibited the migration ability of Eca109 cells. E, Representative micrographs of crystal violet-stained cell colonies. F, Number of crystal violet-stained cell colonies, circ-BMI1 inhibited the colon ability of Eca109 cells. G, NC-Eca109 and circBMI1-Eca109 cells were analyzed by flow cytometry. H, There was no difference in apoptosis rate between circBMI1-Eca109 and control cells.

data, the low-expression group was significantly different from the circ-BMI1 high-expression group in the differentiation status and lymph node metastasis ($P = 0.028$, $P = 0.042$, respectively, Table 1). However, no significant differences were observed in age, gender, smoking, drinking, histological type and TNM stage between the 2 groups ($P > 0.05$, Table 1).

Overexpression of circ-BMI1 Inhibited Eca109 Cell Proliferation, Migration and Colony Formation

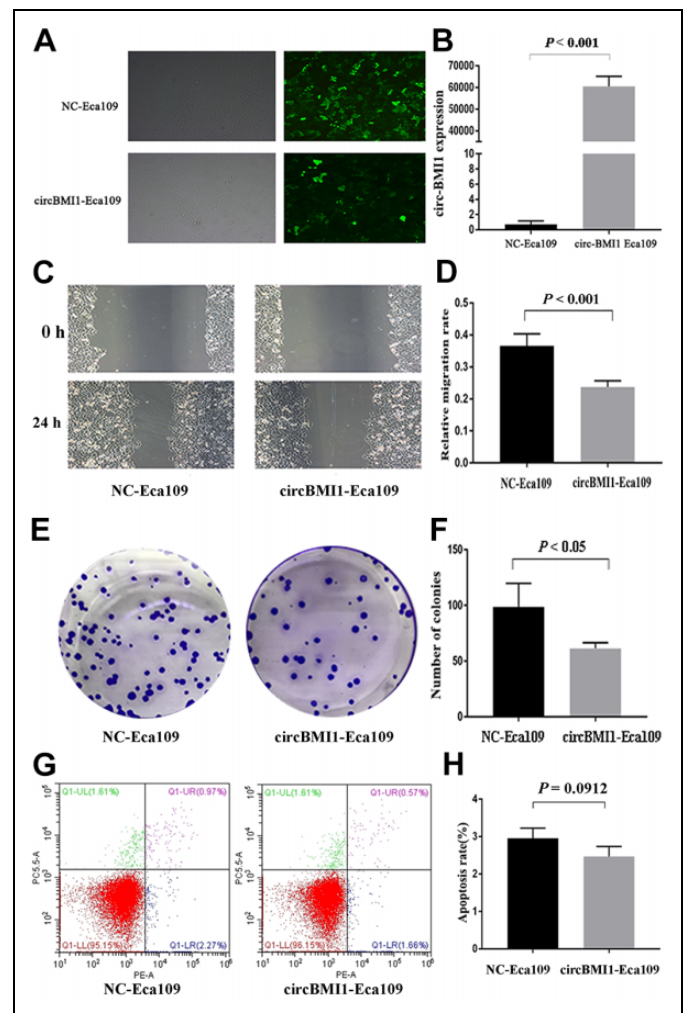The expression of circ-BMI1 in esophageal cancer decreased remarkably, prompting us to reveal circ-BMI1's biological function in esophageal cancer. After successfully transfecting Eca109 cells with circ-BMI1 or mock vector, cells were selected with puromycin and cell sorting. Transfection effect was measured by fluorescence microscope (Olympus, Japan), and circ-BMI1 expression was detected by RT-qPCR. The expression of circ-BMI1 increased obviously in Eca109 transfection group ($P < 0.001$, Figure 3A and B).

Logarithmic growth phase circBMI1-Eca109 and NC-Eca109 cells were subjected to study the role of circ-BMI1 in adjusting cell proliferation, migration and colony formation. After 1, 2, 3 and 4 days of culture, cell proliferation
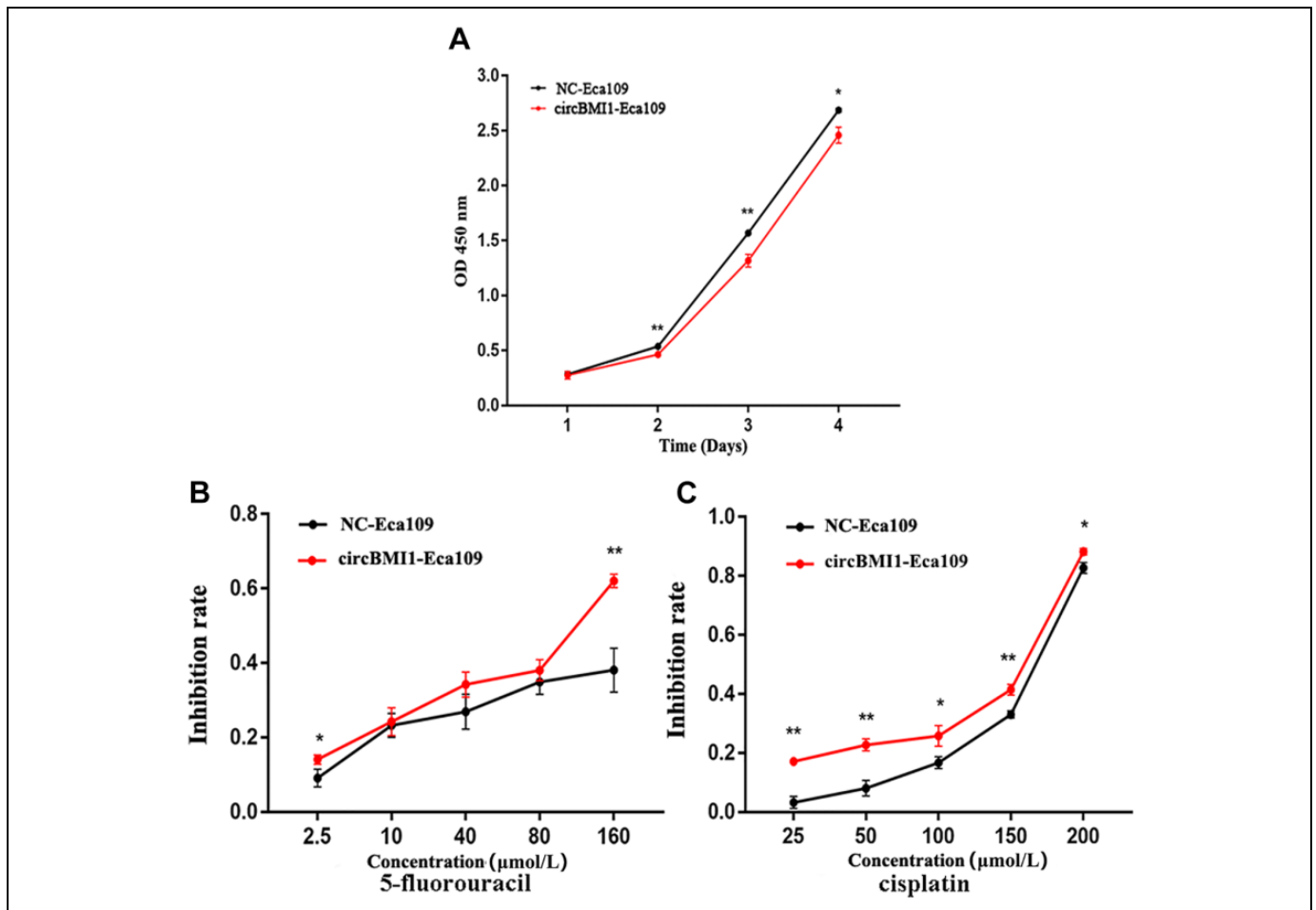
Figure 4. Overexpression of circ-BMI1 affected cell proliferation and chemotherapeutic drug sensitivity of Eca109 cells. A, circ-BMI1 inhibited the growth of Eca109 cells (**, $P < 0.01$; *, $P < 0.05$). B, circ-BMI1 enhanced sensitivity of Eca109 cells to 5-fluorouracil (**, $P < 0.01$; *, $P < 0.05$). C, circ-BMI1 enhanced sensitivity of Eca109 cells to cisplatin (**, $P < 0.01$; *, $P < 0.05$).

was measured by CCK8. The results showed that *in vitro* NC-Eca109 cells proliferated faster than circBMI1-Eca109 cells ($P < 0.05$, Figure 4A). Wound healing array was scratched with a pipette tip and then observed with a photomicroscope. The outcomes illustrated that the number of migrated cells in circBMI1-Eca109 group was significantly decreased than that in NC-Eca109 group ($P < 0.001$, Figure 3C and D). Plate clone formation assay was detected by crystal violet Staining Solution. The results demonstrated that the number of cell colonies of circBMI1-Eca109 cells was significantly decreased than that of control group ($P < 0.05$, Figure 3E and F). These outcomes revealed expression of circ-BMI1 inhibited Eca109 cells proliferation, migration and colony formation.

circ-BMI1 Had No Effect on Cell Apoptosis

To analyze the functions of circ-BMI1 in cell apoptosis, circBMI1-Eca109 and NC-Eca109 cells with the same cell density were collected in 0% FBS 1640 medium and cultured for 48 h. After staining with Annexin V-PE/7AAD, cells were detected by flow cytometry. There was no significant

difference in apoptosis between circBMI1-Eca109 and control cells ($P = 0.0912$, Figure 3G and H).

Expression of circ-BMI1 Enhanced Drug Sensitivity to 5-Fluorouracil and Cisplatin

We conducted an analysis of influence of circ-BMI1 on drug sensitivity of 5-fluorouracil and cisplatin, and found that as the concentration of 5-fluorouracil and cisplatin increased, the growth inhibition rate of circBMI1-Eca109 cells raised. The results revealed that overexpression of circBMI1 enhanced the sensitivity of Eca109 cells to 5-fluorouracil and cisplatin (Figure 4B and C).

circ-BMI1 Suppressed Tumor Growth of Eca109 Cells *In Vivo*

Finally, the involvement of circ-BMI1 in esophageal cancer was evaluated *in vivo* model. The results illustrated that tumor volume of nude mice in circBMI1-Eca109 group was significantly smaller than that of control group ($P < 0.05$, Figure 5A
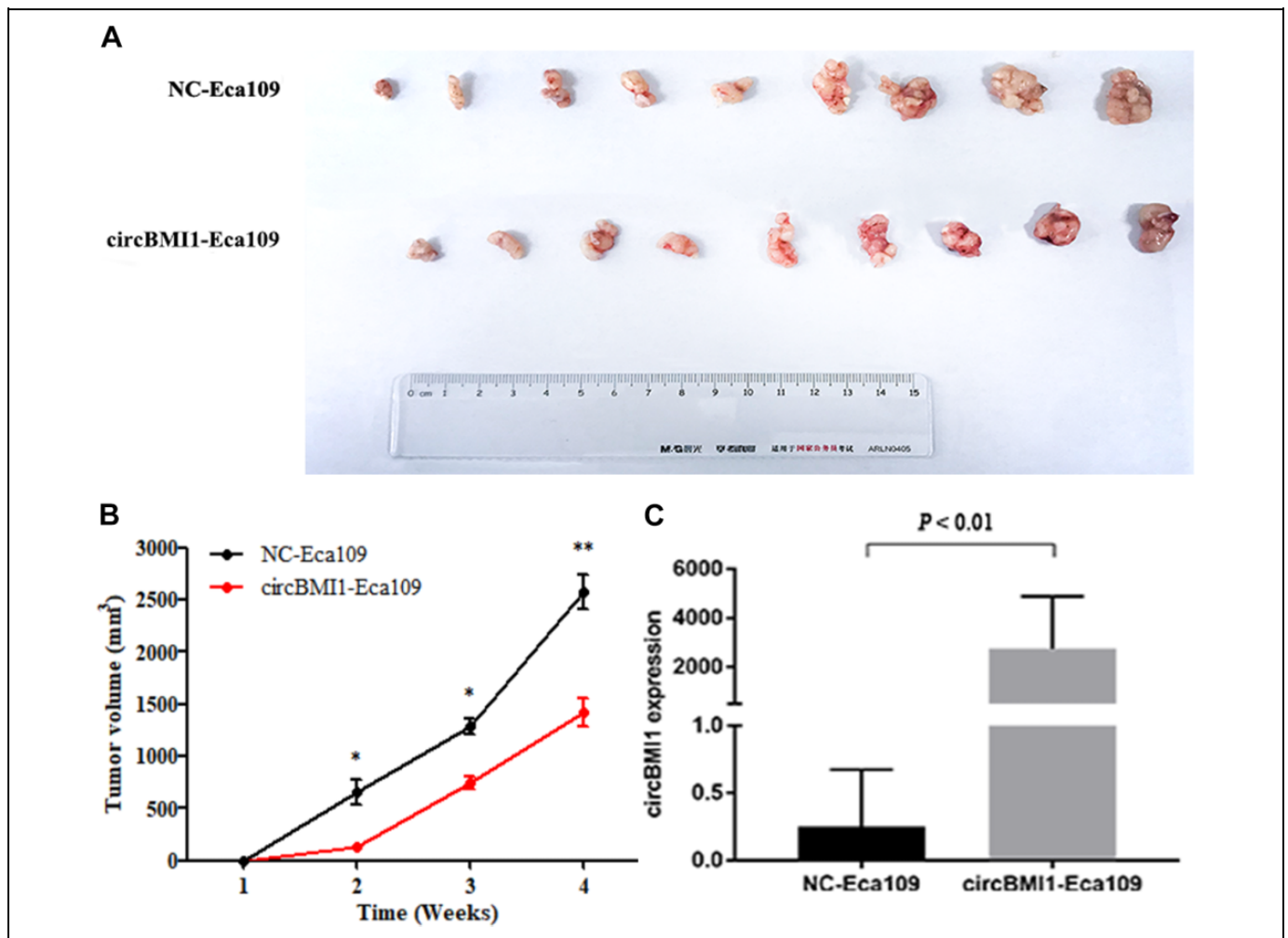
Figure 5. circBMI1 suppressed tumor growth in nude mice. A, Photograph of tumors separated from nude mice 25 days after NC-Eca109 or circBMI1-Eca109 cells injection. B, Tumor volume of nude mice in circBMI1-Eca109 group was significantly smaller than that of control group (**, $P < 0.01$; *, $P < 0.05$). C, Compared with NC-Eca109 group, the expression in circBMI1-Eca109 group was significantly higher.

and B). Moreover, expression of circ-BMI1 in the circBMI1-Eca109 group was significantly higher than that in the control group ($P < 0.01$, Figure 5C). These results implied that overexpression of circ-BMI1 suppressed the tumor growth of Eca109 cells in nude mice.

Discussion

Diener first observed circRNA during the study of potato spindle tuber disease.[18] With the development of high-throughput sequencing technology, circRNAs were gradually entering the field of human biology.[19] In recent years, a large number of reports showed that circRNAs were expressed in human body fluids, such as plasma,[20] saliva,[21] and hematopoietic system,[22] indicating that circRNAs were potential disease biomarkers. Studies discovered that circRNAs played essential regulatory roles in the occurrence and development of diseases, especially cancer. They were expected to become new tumor biomarkers

and targets for the diagnosis of cancers such as gastric cancer,[23] colorectal cancer,[24] lung cancer,[25] and esophageal cancer.[26]

Our current study observed that the expression level of circ-BMI1 in esophageal cancer was significantly down-regulated compared with the control group. ROC curve analysis indicated that circ-BMI1 low expression might be a promising diagnostic biomarker for screening all patients with esophageal cancer from healthy controls. As far as we know, this was the first reported case of circ-BMI1 expression in cancer, especially esophageal cancer. We predicted that circ-BMI1 was expected to be used in the diagnosis and treatment of esophageal cancer patients in the near future through further research. Simultaneously, the analysis of the patients' clinical data revealed that the expression level of circ-BMI1 in a poorly differentiated group was decreased than in the moderately differentiated group, and in the lymph node metastasis group, it was significantly decreased compared with non-lymph node metastasis group. In order to clarify the potential effect of circ-BMI1 in the occurrence and development of esophageal cancer, circ-

BMI1 overexpression plasmid and mock vector were used to transfect esophageal cancer Eca109 cells, and cell function experiments were performed. The results suggested that overexpression of circ-BMI1 inhibited the cell proliferation, migration and colony formation of Eca109 cells, and circ-BMI1 suppressed tumor growth of Eca109 cells in nude mice. These outcomes indicated that circ-BMI1 might be a potential biomarker for the diagnosis, risk assessment and treatment of esophageal cancer. However, the exact mechanism of circ-BMI1's effect on esophageal cancer still need further research to explore and clarify. Studies had found that circRNA might act as a miRNA sponge or competitive endogenous RNA to regulate the expression of miRNA target genes.[27] In 2019, Shi *et al* reported circ_0006168 adjusted mTOR expression through sponge adsorption of miR-100 to promote the proliferation, migration and invasion of esophageal cancer.[28] In the same year, Huang *et al* discovered circular RNA ciRS-7 could trigger the migration and invasion of esophageal cancer through miR-7/KLF4 and NF-κB signals, and targeted inhibition of ciRS-7 might be a way to treat esophageal cancer.[29] Our experimental study showed that the expression level of circ-BMI1 in the serum of patients with esophageal cancer was significantly reduced. Overexpression of circ-BMI1 could significantly inhibit the proliferation, migration and colony forming ability of esophageal cancer cells. Therefore, circ-BMI1 may also act as a miRNA sponge, regulating the expression of miRNA target genes to inhibit the occurrence and development of esophageal cancer, which is worthy of further study in the future.

Early diagnosis of esophageal cancer improves the prognosis of patients. However, in the early stages of the disease is relatively asymptomatic, the majority of patients presenting with symptoms were diagnosed with advanced disease. Combination chemotherapy with cisplatin and 5-fluorouracil is the standard treatment for inoperable esophageal cancer, with response rates ranging from 15% to 45%.[30] Our experiments found that overexpression of circ-BMI1 enhanced the sensitivity of Eca109 cells to 5-fluorouracil and cisplatin, suggesting that circ-BMI1 may be a potential target for treatment in esophageal cancer. The association between circ-BMI1 and the chemosensitivity of 5-fluorouracil and cisplatin needs further study.

At present, due to our limited sample size, no significant differences were observed in variables such as age, gender, smoking, drinking, histological type and TNM stage between the 2 groups with high and low expression of circ-BMI1. There was no report about circ-BMI1, which limited our research on the function of circ-BMI1 in esophageal cancer. Since the return time visit was not long enough, it was impossible to analyze the prognosis of patients' survival and disease-free survival time. Moreover, the potential mechanism of circ-BMI1 was unclear. Later, we plan to further verify the value of circ-BMI1 in diagnosing esophageal cancer through multi-center and large-sample researches, track patients' condition to assess patients' prognosis, and conduct more studies to confirm the mechanism of circ-BMI1 in esophageal cancer. Shortly, prospective detection of circ-BMI1 expression and targeted intervention of circ-BMI1 may be a new approach to diagnosing and treating esophageal cancer.

Conclusions

Our research suggested that down-regulation of circ-BMI1 expression was a joint event in patients with newly diagnosed esophageal cancer, and expression level of serum circ-BMI1 could be used as a new potential biomarker for distinguishing esophageal cancer patients from healthy controls. Overexpression of circ-BMI1 inhibited proliferation, migration, colony formation of Eca109 cells, enhanced the sensitivity of Eca109 cells to 5-fluorouracil and cisplatin, and suppressed tumor growth of Eca109 cells in nude mice. Our findings were new insights into understanding the occurrence, progression and drug sensitivity of esophageal cancer. In the future, circ-BMI1 may be a new potential target for diagnosis and treatment in esophageal cancer.

Authors' Note

Qian Zhao and Xin Zhu contributed equally to this work. Zhao-qun Deng and Jiang Lin conceived and designed the study. Qian Zhao and Xin Zhu collected clinical specimens, performed the experiments and analyzed the data. Qian Zhao wrote the manuscript. Jin-ming Ke, Xiao-yu Su, Jing Yi and De-long Wu assisted with the experiments. All authors read and approved the final manuscript. The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request. The study was approved by the Ethical Review Committee of the Affiliated People's Hospital of Jiangsu University (approval number: K-20200142-W). We have obtained written informed consent from all study participants.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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