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MIR-147B REGULATED PROLIFERATION AND APOPTOSIS OF GASTRIC CANCER CELLS BY TARGETING *CPEB2* VIA THE PTEN PATHWAY

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ABSTRACT

The present study has been performed to illustrate the role and mechanism of microRNA-147b (*miR-147b*) in the cellular viability and apoptosis of gastric cancer (GC) cells. The GC tissues of 50 patients with complete data and the adjacent tissues were selected from Shanxi Cancer Hospital, and 3 pairs of tissues were randomly selected for microarray detection of high-expressing microRNAs. The expressions of *miR-147b* were quantified in numerous GC cell lines, i.e., BGC-823, SGC-7901, AGS, MGC-803 and MKN-45, normal tissue cell lines and 50 pairs of gastric cancer tissues. Moreover, two cell lines of *miR-147b* high-expressing used PCR quantitative analysis were selected for transfection experiments. The differentially expressed *miR-147b* was screened from 3 pairs of samples by miRNA chip. The expression of*miR-147b* was found highly expressed in gastric cancer tissues of 50 pairs of gastric cancer and adjacent tissues. The *miR-147b* found in diverse range in each of GC cell line. Therefore, two cell lines, BGC-823 and MGC-803, with relatively high expression levels of *miR-147b* were selected for further analysis and research. Scratch analysis results showed that compared with *miR-147b* NC, the *miR-147b* inhibitor group inhibited GC cell growth and reduced cell migration. The early apoptosis of MGC-803, and BGC-823 cells was enhanced by *miR-147b* inhibitor. *miR-147b* inhibitor significantly repressed the proliferation of BGC-823 and MGC-803 cells. Our study showed that the high expression of *miR-147b* is positively correlated with the occurrence and development of gastric cancer.

Keywords: Gastric cancer; microRNA-147b, apoptosis, cell viability.

INTRODUCTION

Over the last four decades, the world has experienced rapid demographic and epidemiological transitions. Rapid industrialization, urbanization, aging, and changes in lifestyle have led to a shift in the burden of the disease spectrum from infectious diseases to non-communicable diseases. China has been an important contributor to the global cancer burden because of its large population. Among the cancer related deaths, Gastric cancer (GC) ranked fifth and was responsible for a significant mortality rates across the globe[1]. Particularly in China, the survivability of patients in after 5years is only 30 % after radical resection[2, 3]. Therefore, it is urgent to study the mechanism of GC occurrence and development[4].

MicroRNAs (miRNAs) are a family of small non-coding RNAs of 20–22 nucleotides that regulate a wide array of biological processes including carcinogenesis. Under normal physiological conditions, miRNAs function in feedback mechanisms by safeguarding key biological processes including cell proliferation, differentiation and apoptosis[5]. However, in cancer cells, miRNAs have been found to be heavily dysregulated and are considered to play a pivotal role in the progression of a variety of tumors. It can reverse mediate about 30-40% of human genes by binding to the complementary 3 '- non expression region (3' - UTR)[6,7]. Recent studies have shown that *miR-147b* is related to some cancers [8], which may be the tumor suppressor gene or oncogene. Over-expression of the *miR-147b* inhibitor or *SOX15* can be seen through the

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Wnt/β-Catenin signal transduction pathway, delaying the growth of cancer cells in vivo. The role of *miR-147b* in GC is not clear; therefore, we have conducted the present study to elucidate its probable mechanism in GC.

MATERIALS AND METHODS

Materials

The five Gastric Cancer cell lines, i.e., SGC-7901, MGC-803, BGC-823, AGS and MKN-45 and normal gastric tissue cell lines were obtained from the sample bank at Shanxi Tumor Hospital. The specific information of 50 pairs of gastric cancer specimens in the biological sample bank is related to gastric body cancer patients who underwent radical gastrectomy at the Shanxi Tumor hospital from January 2014 to January 2015. Of these patients, 29 were male and 21 female. The age ranged from 43 to 70 years. The TNM system was used for clinical staging. There were 20 cases of stage I+II and 30 cases of stage III.

Inclusion criteria:

1. Gastric body cancer
2. Total gastrectomy
3. 3.ECoG score ≤ 1 point.

Exclusion criteria

1. >70 years old
2. Patients with more serious complications or chronic diseases requiring consultation and treatment
3. Other tumor history or related treatment history
4. Serious complications which occurred after operation.
5. Follow up data were missing.

The tissue samples taken in this study were obtained from the patients after their written consent was obtained. This study has been duly approved by the medical ethics committee of the Beijing Friendship Hospital, Capital Medical University.

Methods

The GC tissues of 50 patients with gastric cancer with complete data and the adjacent tissues > 5cm were divided into an experimental group and a control group in order to determine the expression level of the target gene *miR-147b* in the selected tissues. BGC-823, SGC-7901, AGS, MGC-803 and MKN-45 GC cell lines served as markers for the experimental group, whereas normal gastric tissue cell lines serve as the control group to quantify the expression of *miR-147b*. Two groups of cell lines with high expression of *miR-147b* were selected for transfection experiment: 20 ~ 50 nM *miR-147b* inhibitor was used as the experimental group and *miR-147b* NCas was used as the negative control group. We measured the cell cycle and colony formation after 24h of transfection and cell migration after 48h of transfection of the two groups of transfected cell lines. The proliferation rate of GC cells at 0, 24, 48, 60, and 72 hours after transfection was determined by the CCK8 method.

Cell resuscitation and culture

All cell lines were maintained in complete Dulbecco's Modified Eagle Medium, supplemented with 10% (vol./vol.) fetal calf serum, penicillin G (100 μg/mL) and streptomycin (100 μg/mL). All cell lines were grown at 37°C in humidified 5% CO_2 and 95% air atmosphere. All cell lines were incubated for 24h prior to experiments.

RNA isolation and quantitative qrtPCR

Three pairs of gastric cancer tissues were randomly selected for tissue microarray to detect the expression of the target and related genes in gastric cancer tissues. The expression of the target gene in 50 pairs of gastric cancer tissues was also determined. Total RNA was extracted from GC cell lines, normal tissues, and tissues using Trizol reagent (Invitrogen, USA) to determine the expression of *miR-147b* gene. The expression of *miR-147b* in the cells of each group was analyzed and compared. *miR-147b* was amplified by the upstream primer 5'-GGGGTGTGTGGAAAT-3' and reverse primer 5'- AACTGGTGTCGTGGAGTCGGC-3', and reverse transcribed into cDNA using RNA to cDNA premix. QRT-PCR was performed with SYBR Green master mix according to the protocol provided (all from vii7 Q-PCR system). The concentration of RNA was quantified by Nanodrop-2000 (Nanodrop, USA). The relative expression of *miR-147b* was calculated by $2^{-\Delta\Delta Ct10}$.

Transfection experiment

After the PCR quantitative analysis, two groups of cell lines with high expression of *miR-147b* were selected for the transfection experiment. 20 ~ 50 nM *miR-147b* inhibitor was used as the experimental group and *miR-147b* NC serves as the control group. The cells were cultured at ambient temperature and humidity at 5 % CO_2 for 36 ~ 48h. The clone with the highest expression of foreign protein in monoclonal cells was used in Western blot, and scratch test, apoptosis test, clone formation test and cell proliferation were detected.

Scratch test

Exponentially, cells that were growing were trypsinized and seeded at a density of 200,000 cells per well into 12-well plate for a 24h incubation (~90% confluence). The scratch wounds were made by a sterile 1 mL pipette tip through a pre-marked line. After removal of the resulting debris from the five lineal scratches, the cell monolayer was subsequently rinsed three times with PBS, followed by incubation with sample for 24h. The wound areas were

displayed by taking images just above the interchanges between scratched wound areas and pre-marked lines. The effect of the sample on the wound closure was determined microscopically (Olympus IX - 7) after 0, 24, and 48h,of incubation. The effect of the sample on the wound closure was captured using MRI imaging software.

Apoptosis test

The cells were seeded in 12-well plates (2×10^5 cells/well). The cells were cultured and incubated (with 5% CO_2 and 95% air) at 37°C. Various concentrations of test drugs were dissolved in DMSO and incubated with the cells for 48h. DMSO in the culture medium never exceeded 0.1% (v/v), the concentration known not to affect cell proliferation. The Annexin V-FITC/PI apoptosis kit (Keygen Biotech, China) was used. For this purpose, the cells were incubated with 5 µl Annexin V-FITC and 5 µl PI for 5 min in the dark. Data were collected with moflow (Beckman Coulter, USA).

Clone formation assay

One thousand cells were plated in a 35 mm Petri dish after a different treatment was applied for 24h and allowed to grow for two weeks. The medium was changed twice per week. Then, the colonies were fixed with 4% paraformaldehyde, stained with methyl violet. The colonies were observed under a microscope. Colony inhibition rate = [(1-number of colonies in experimental group / control group) ×100%; colony formation efficiency = 1-colony inhibition rate.

Cell proliferation test

Cell suspensions of the two cell lines were inoculated into 96 well plates, incubated at ambient temperature and humidity at 5 % CO_2, and further cultured after adding 10µL CCK-8 reagent (Dojindo, Japanese) to each well. CCK-8, being nonradioactive, allows sensitive colorimetric assays for the determination of the number of viable cells in cell proliferation and cytotoxicity assays. It works on the principle of highly water-soluble tetrazolium salt. WST-8 [2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4- disulfophenyl)-2H-tetrazolium, monosodium salt] produces a water-soluble formazan dye upon reduction in the presence of an electron mediator. WST8 is reduced by dehydrogenases in cells to give an orange colored product (formazan), which is soluble in the tissue culture medium. The amount of the formazan dye generated by dehydrogenases in cells is directly proportional to the number of living cells. The absorbance values of each well were measured at 450 nm and 630 nm at 0, 24, 48, 60 and 72 hours after transfection. The experiments were conducted three times. Cell viability has been calculated using the formula * (%) = [a (dosing) - A (blank)] / [A (0 dosing) - A (blank)] × 100.

Vector construction and luciferase analysis

The target scan database was selected to search for the target gene that can be combined with *miR-147b* and map the base pairs that cross the 3 'untranslated region of *miR-147b* and the predicted target gene. *CPEB2* plasmid was constructed by the pGL3 promoter vector: wt*CPEB2* (5′-GUUUCUGUAUAGAAUCGCACAAG-3′) and mutant mut*CPEB2* (5′-GUUUCUGUAUAGAAUTTCTC-GAG3′). MGC-803 cells were cultured until the cell density grew to 80% - 90%. The cells were divided into an experimental group and a control group. Lipofectamine™ 2000 transfection reagent was used for co-transfection of the MGC-803 cells with the wt-*CPEB2* plasmid and scramble or *miR-147b* inhibitor. The second group was divided into an experimental group and a control group. Lipofectamine™ 2000 transfection reagent was used for co-transfection of MGC-803 cells with mut-*CPEB2* plasmid and scramble or *miR-147b* inhibitor were co transfected into MGC-803 cells by using. Double glo stop and glo reagent were added into 24 well plate cells at 48h. After full reaction at room temperature, the fluorescence intensity was detected according to the instructions of double luciferase reporting system (Promega, USA) and the luciferase activity in MGC-803 cells was analyzed.

Western blotting

MGC-803 cells were co-transfected with *miR-147b* NC, *miR-147b*inhibitor and *miR-147b* NC+*CPEB2* and were further categorized into two groups to identify the expression of *CPEB2* and *GAPDH* (control group).MGC-803 and BGC-823 cells were separated into two groups after the culture. One group was added with an anti-*CPEB2* antibody (Abcam, ab222070) to establish the si-*CPEB2* and si-control experimental group and control group. After loading (25 µg/lane), the protein concentration was estimated using the BCA Kit and protein was separated using SDS-PAGE. Thereafter, the western transfer was performed with PVDF membranes (Millipore, Bedford, MA, USA) using the primary and secondary antibodies as per the earlier reported procedure. [9]

H&E staining and immunohistochemistry

Routine H&E staining and immunohistochemical analysis was conducted to determine the expression of *CPEB2* in the gastric cancer tissues and was observed using a Zeiss UV LSM confocal microscope.

Statistical analysis

Statistical analysis was evaluated using a two tailed t-test and one-way ANOVA with SPSS packaging program

(SPSS17.0, USA). The Pearsonχ^2 test was used to compare the differences between groups. If the conditions were not met, continuity correction χ^2 analysis was further used. Statistical significance was set as $P < 0.05$.

RESULTS

***miR-147b* is up-regulated in GC cells and tissues**

The differentially expressed *miR-147b* was screened out in 3 pairs of samples by the miRNA chip (Fig. 1A), which was found three times higher than that of adjacent tissues in three random pairs of gastric cancer samples (Fig. 1B). Q-PCR further detected the expression of *miR-147b* in 50 pairs of gastric cancer and adjacent tissues, and *miR-147b*was-found to be highly expressed in the gastric cancer tissues (0.53±0.02 vs 1.35±0.03, P<0.05) (Fig. 1C). The expression of *miR-147b* in each GC cell line was recorded as Normal cell0.99±0.01, SGC-7901: 1.55±0.03, BGC-8231.74±0.12, AGS: 1.47±0.02, MGC-803, 1.8±0.04, MKN-45, 1.44±0.03, P<0.05) (Fig. 1D, table 1). BGC-823 and MGC-803 cell lines with increased expression level of *miR-147b* were selected for further analysis. After transfection, the relative gene expression of *miR-147b* nc in the control group and *miR-147b* inhibitor group were found to be 1.00±0.09, and 0.23±0.07 respectively, with a significant difference (t = 3.07, P < 0.05), indicating successful transfection (Fig. 1D).

Table 1. Expression of transfected *miRNA-147b* in normal cells and different gastric cancer cells detected by RT-PCR ($\bar{x}$±s)

Cell grouping	Number of samples	*miR-147b*	T value	Pvalue
Normal cell	3	0.99±0.01		
SGC-7901	3	1.55±0.03	2.91	<0.05
BGC-823	3	1.74±0.12	2.91	<0.05
AGS	3	1.47±0.02	2.35	<0.05
MKN-45	3	1.44±0.03	2.91	<0.05
MGC-803	3	1.8±0.04	2.91	<0.05

Note: *miR-147b*nc is an empty carrier

***miR-147b* promotes tumor growth in human cell lines**

Scratch analysis showed that the *miR-147b* inhibitor group inhibited GC cell growth and reduced cell migration compared with *miR-147b* NC (Fig. 1E). In addition, by flow cytometry, we have also analyzed the apoptotic effect of *miR-147b*. As compared with *miR-147b* NC, *miR-147b* inhibitor increased the early apoptosis of MGC-803 cells [(61.8±0.2)% vs (55.4±0.1)%]. It increased the early apopto-

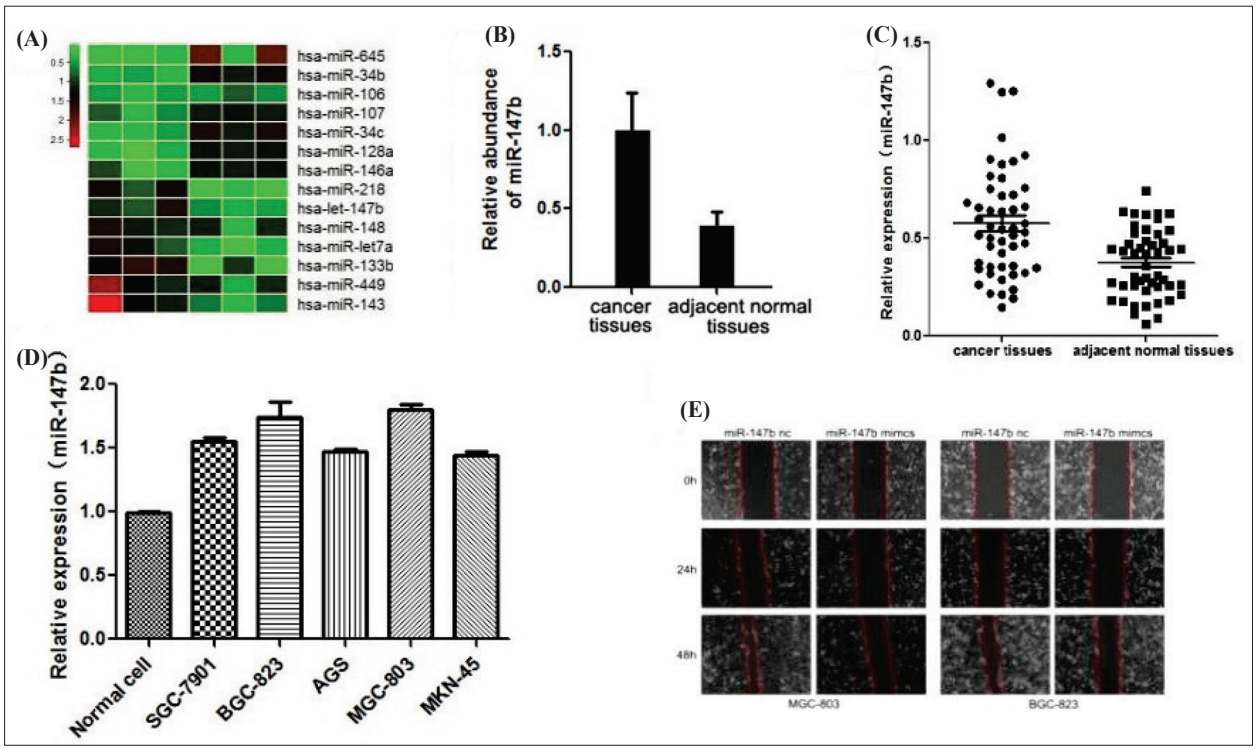

Figure 1. (A) MiRNA expression in 3 GC tissues and adjacent normal tissues was analyzed by microarray analysis, (B) Quantification of microarray data showed that *miR-147b* was up-regulated in GC tissues. The data are expressed as mean multiple changes in *miR-147b* levels in GC tissues normalized to their corresponding controls.* P < 0.05, t test; (C) QPCR analysis showed that *miR-147b* was up-regulated in most GCs. The data shown are multiples of *miR-147b* expressions in GC cases normalized to paired adjacent normal tissues; (D) The expression of *miR-147b* in cultured GC cell lines and normal cells was analyzed by qPCR; (E) Wound healing analysis of BGC-823 and MGC-803 cells treated with *miR-147b* inhibitor or *miR-147b* NC.

sis of BGC-823 cells [(35.5 ± 0.2)% vs. (23.1 ± 0.2)%] (Fig. 2A). On the other hand, clone formation experiments showed that the*miR-147b* inhibitor drastically restricts the proliferation of BGC-823 and MGC-803 cells (Fig. 2B, table 2). CCK-8 proliferation experiment showed that the*miR-147b* inhibitor significantly inhibited the growth of BGC-823 and MGC-803 cells, and inhibition rate was more prominent as the time increases (t = 2.78, P < 0.05) (Fig. 2C and D, table 3).

Table 2. Effect of transfection of the *miR-147b* inhibitor on the number of clones of gastric cancer MGC-803 and BGC-823 cells

Group	MGC-803	BGC-823
***miR-147b* nc**	638±15	632±17
***miR-147b* inhibitor group**	464±11	448±15
T value	2.35	2.35
P value	< 0.05	< 0.05

Note: *miR-147b* nc is an empty carrier

Table 3. Effect of transfection of the *miR-147b* inhibitor on the proliferation inhibition rate of gastric cancer cells by CCK-8 method ($\bar{x}$±s)

Cell grouping	Number of samples	*miR-147b*nc	*miR-147b* inhibitor	T value	P value
MGC-803					
0h	3	**0.200±0.04**	**0.20±0.03**	3.11	>0.05
24h	3	**0.395±0.05**	**0.33±0.03**	2.76	>0.05
48h	3	**1.030±0.07**	**0.67±0.05**	2.77	<0.05
60h	3	**1.720±0.09**	**0.98±0.05**	2.77	<0.05
72h	3	**2.14±0.09**	**1.23±0.07**	2.78	<0.05
BGC-823					
0 h	3	**0.20±0.04**	**0.20±0.03**	3.07	>0.05
24h	3	**0.49±0.05**	**0.33±0.03**	2.78	>0.05
48h	3	**1.13±0.07**	**0.62±0.05**	2.18	<0.05
60h	3	**1.56±0.09**	**0.85±0.05**	2.77	<0.05
72h	3	**1.84±0.1**	**1.02±0.07**	2.78	<0.05

Note: *miR-147b* nc is an empty carrier

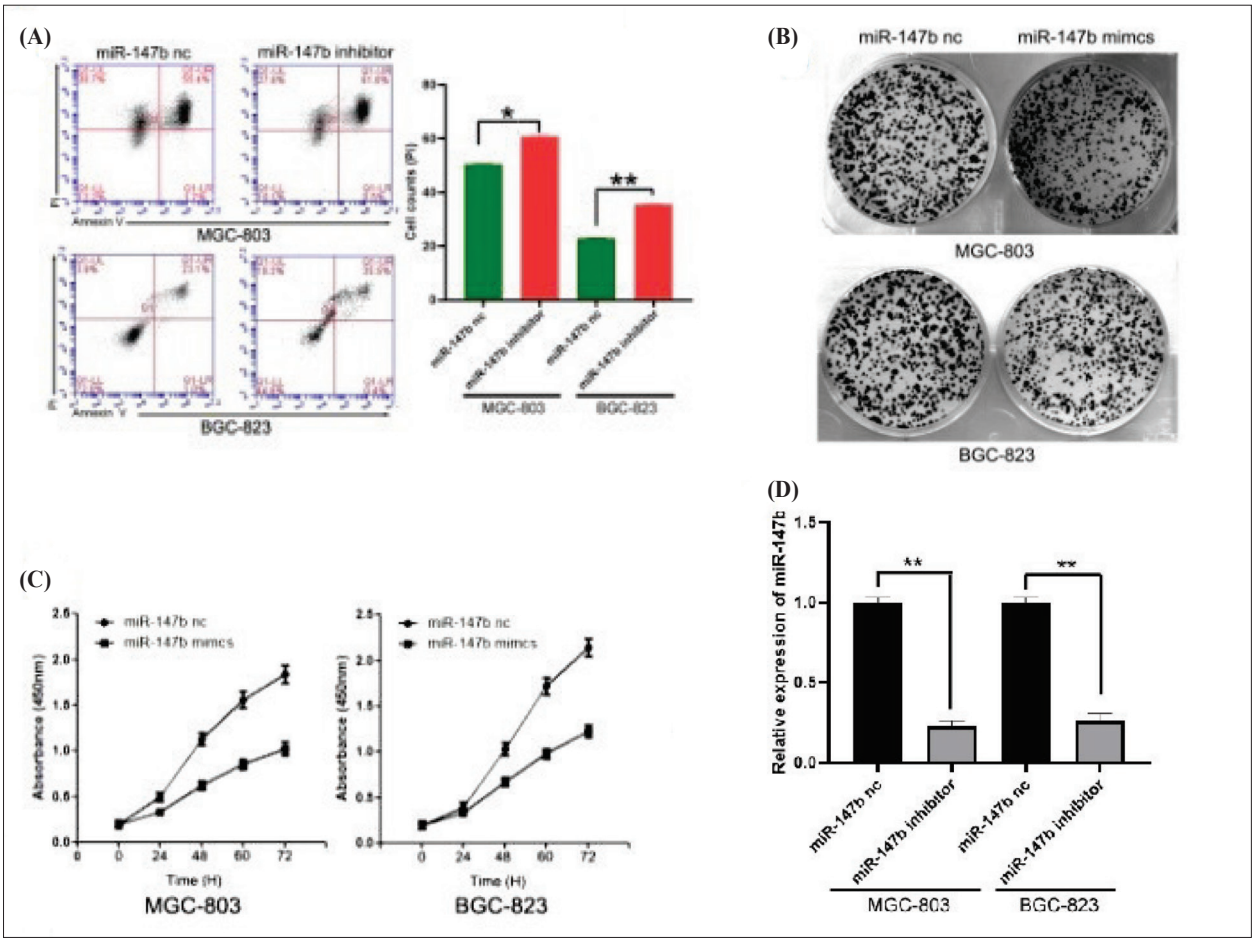

Figure 2. (A) After treatment with *miR-147b* inhibitor or *miR-147b* NC and staining with apoptosis markers (FITC annexin V and PI), the apoptosis of BGC-823 and MGC-803 cells was examined by flow cytometry. In the apoptosis diagram, FITC Annexin V + / PI + represents late apoptosis, FITC Annexin V + / pi - represents early apoptosis, and FITC Annexin V - / pi - represents normal living cells; (B) Clonogenic assay of BGC-823 and MGC-803 cells treated with *miR-147b* inhibitor or *miR-147b* NC. For all qPCR results, the data are expressed as mean ± SEM, and the error line represents the standard deviation obtained from three independent experiments; (C) Cell proliferation assay of BGC-823 and MGC-803 cells treated with *miR-147b* inhibitor of CCK-8 or *miR-147b* NC. (D) *MiR-147b* was up-regulated in GC cells.

miR-147b affects the expression of *CPEB2* in GC cells

We searched the target gene *CPEB2* that can bind to *miR-147b* through the target scan database, and we mapped the base pairs that *miR-147b* crosses with the 3 'untranslated region of the predicted target gene *CPEB2* (Fig. 3A). We selected MGC-803 with relatively high endogenous *CPEB2* content to detect whether the *miR-147b* inhibitor of *miR-147b* NC affects *CPEB2* expression. The double luciferase reporter vectors of *CPEB2* wild-type and mutant were further constructed (Fig.3A). The above vectors were co-transfected with NC and *miR-147b* inhibitor into MGC-803 cells, respectively. It was found that *CPEB2* was the target gene directly affected by *miR-147b*. In the presence of *CPEB2*3 '- UTR, this activity produced about 20% (Fig. 3B), indicating that the expression of *miR-147b* inhibitor accelerated the activity of *CPEB2*3'-UTR. Western blot showed that the expression of *CPEB2* in MGC-803 cells in *miR-147b* inhibitor group and *miR-147b* NC+*CPEB2* group showed higher concentration as compared to negative control group (Fig. 3C). These results suggest that *CPEB2* is the target of *miR-147b* and is negatively regulated by it.

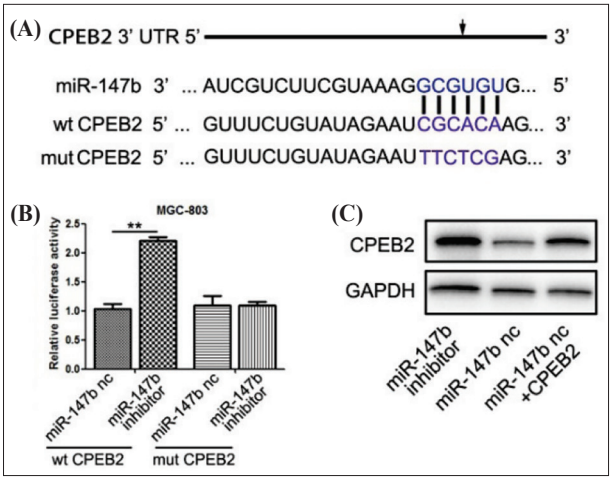

Figure 3. (A) Double luciferase reporter vector of *CPEB2* wild type and mutant, (B) Expression of *miR-147b* inhibitor accelerates *CPEB2* 3 '- UTR activity, (C) Expression of *CPEB2* in MGC-803 cells of different groups.

B2 was highly expressed in GC cells and correlates with prognosis

Quantitative PCR (qPCR) analysis of 50 pairs of clinical GC tissues and adjoining normal tissue samples showed that *CPEB2* mRNA expression in tumor tissues was down regulated in 40 patients (80%) (Fig. 4A). The non-parametric test results of the relationship between *CPEB2* expression and clinicopathological factors showed that the lower *CPEB2* level was related to the GC pTNM stage ($P < 0.05$, stages I-II and III-IV, FIG. 4B). In addition, GC patients with lower *CPEB2* expression showed lower survivability and improved prognosis (Fig. 4C). Immunohistochemical analysis showed that *CPEB2* staining increased significantly in adjacent normal tissues (Fig. 4D). H & E staining showed more metastatic nodules in GC tissues compared with adjacent normal tissues (Fig. 4D). *CPEB2* was found highly expressed in GC cells.

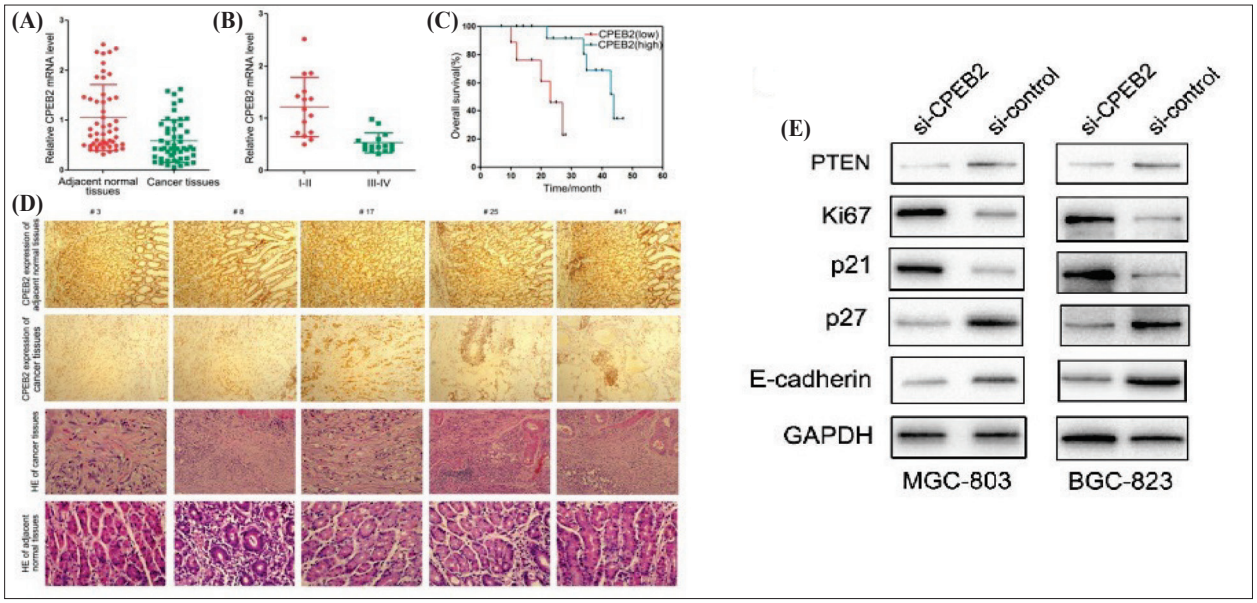

Figure 4. (A)Expression of *CPEB2* mRNA in GC tissues and adjacent normal tissues, (B) Nonparametric test results of the relationship between *CPEB2* expression and clinicopathological factors, (C)Overall survival and disease-free survival in GC patients with lower *CPEB2* expression, (D) immunohistochemical analysis results; (E) Results of immunoblotting.

***CPEB2* promoted the PTEN pathway**

Western blot results showed that the expression of PTEN protein decreased significantly after reducing *CPEB2* in 803 and 823 cell lines. At the same time, the expression of tumor suppressor genes p27 and E-cadherin was inhibited, while the expression of Ki67 and p21 reflecting tumor activity index increased significantly (Fig. 4E).

Table 4. Correlation between clinicopathological features and *miR-147* expression in gastric cancer

Variance	Number of samples	*miR-147b* expression Low(n%)	High(n%)	$\chi 2$	P value
Age					
<60	22	4 (18.1%)	18 (81.9%)	0.081	*P* >0.05
≥ 60	28	6 (21.4%)	22 (78.6%)		
Gender					
male	29	7 (24.1%)	22 (75.9%)	0.176	*P* >0.05
female	21	3 (14.3%)	17 (85.7%)		
Tumor size					
<4 cm	33	6 (18.2%)	27 (81.8%)	0.006	*P* >0.05
≥4 cm	17	4 (23.5%)	13 (76.5%)		
TNM Stage					
I-II	20	7 (35%)	13 (65%)	3.755	*P* =0.048
III-IV	30	3 (10%)	27 (90%)		
Lymph node metastasis					
No	23	8 (34.8%)	15 (65.2%)	4.232	*P* =0.040
Yes	27	2 (7.4%)	25 (92.6%)		

DISCUSSION

MicroRNA (miRNA), a well-known noncoding RNA subtype has recently appeared as gene expression regulators by combining its target messenger 3 ′- Non translation area (3′- UTR) which transcriptionally regulates the gene expression of the messenger RNA (mRNA) [11]. The interaction of miRNA and mRNA generate RNA and induce a silencing complex, which is an event promoting mRNA degradation and translation inhibition [11]. Therefore, miRNA is believed to promote tumorigenesis [12,13]. Various studies have suggested that numerous miRNAs disorders are involved in regulating tumor malignant growth and invasive metastasis; therefore, they act as oncogenes or tumor suppressors [14,15]. In addition, itis used significantly as a biomarker, in prognosis, as well as a therapeutic target [16,17]. *miR-147b* has been also associated with a variety of cancers [18–20].

In this study, differentially expressed miRNAs were screened from 3 pairs of samples by miRNA chip. The cut-off point of multiple changes was 1.50 or 0.67. An miRNA microarray detected that the concentration of 14 miRNAs in GC tissues was up-regulated, among which hsa-*miR-147b* was more significant. Further extraction of *miR-147b* in gastric cancer tissues was established as extensively elevated when compared to neighboring tissues, thus confirming that *miR-147b* was highly expressed in gastric cancer tissues. *miR-147b* was also highly expressed in GC cell lines. Our further functional assay showed that the addition of *miR-147b* inhibitor considerably repressed the propagation and incursion of gastric cancer cells.

Similarly, *miR-147b* is up-regulated in hepatitis-C virus related diffuse large B-cell lymphoma, and patients with elevated *miR-147b* usually have a low prognosis[17]. In addition, the expression of *miR-147b* increased abnormally in hepatocellular carcinoma tissues. This was related to tumor severity, and also thus promoted the growth of hepatocellular carcinoma in vitro [19]. These findings support the carcinogenic effect of *miR-147b*. These results are consistent with this study, but it was also found that the addition of exogenous *miR-147b* inhibitor does not significantly inhibit viability and incursion of gastric cancer cells in *in vitro*, indicating that there are other related microRNAs at the same time.

On the contrary, some studies have shown that *miR-147b* is found to be reduced in tumor samples and functions as a tumor suppressor. The level of *miR-147b* expression was found low in rectal cancer tissues and reduce the propagation and incursion of colorectal cancer cells [19,20]. *miR-147b* can be used as a protecting predictive indicator in patients with ovarian cancer [16], where its function may vary from tumor to tumor [21]. Therefore, the exact role of *miR-147b* in tumorigenesis needs to be further studied in different tumor types.

The gene silencing, and the abnormal expression of downstream genes in tumor tissues may be caused by dysregulated miRNA. It promotes or inhibits eukaryotic mRNA translation by engaging the dynamic change of poly (A) tail length in cytoplasm [22]. In the cytoplasm, the lengthening or shortening of poly (A) is arbitrated by the interaction of the cytoplasmic polyadenylation element (CPE, a cis factor) and RNA binding protein (CPEB, a trans factor). This phenomenon is called cytoplasmic polyadenylation by CPEB protein [23]. *CPEB2*, a member of the CPEB protein family is found concentrated in cytoplasm during transfection and connects to poly (U) RNA oligomers [24]. It functions as a translation regulator. In the human genome database, it was found that the*CPEB2* nucleotide sequence was a potential target gene of *miR-147b* in gastric cancer, which was confirmed by luciferase experiment. Western blotting showed that *miR-147b* negatively regulated *CPEB2* and then affected the expression of the PTEN protein. The results of the study suggest that the expression of *CPEB2* was not considerably associated with gender, age, tumor location, tumor size and histological type, but with tumor cell differentiation, TNM stage and lymph node metastasis. The lower the expression of *CPEB2*, the worse

the tumor differentiation, accompanied by the increase of TNM stage and the aggravation of lymph node metastasis load. Patients with high expression of *miR-147b* and low expression of *CPEB2* had poor 5-year overall survival.

CONCLUSION

The above results suggest that the low expression of *CPEB2* in GC tissue is involved in the promoting the proliferation, migration and invasion of GC cells. Finally, PTEN controls a series of pathophysiological processes related to cell proliferation, differentiation, DNA/chromosome integrity, apoptosis, and invasiveness.

The deficiency of this study is that due to the impact of the epidemic situation, no further animal carrier test can be carried out.

At present, the detection of tumor derived miRNA with abnormal expression in plasma can be used as an important method for early detection of human cancer. As a new non-invasive method, detecting the expression of miRNA in peripheral blood may play a very important role in early tumor detection, clear staging, judging curative effect and evaluating prognosis. Whether *miR-147b* can be used as a meaningful reference index is worthy of further clinical exploration.

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