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*Original Article*

# microRNA-143 acts as a prognostic marker in gastric cancer and its role in cell proliferation and invasion

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**Abstract:** Background: MiR-143 is frequently downregulated in different cancer types and is involved in the progression of tumorigenesis. However, the molecular mechanism of miR-143 in gastric cancer is still unclear. Methods: RT-PCR was used to test the expression of miRNAs and BACH1 mRNA in 44 gastric cancer tissues and Kaplan-Meier survival analysis to verify the correlation between miR-143 and prognosis of GC patients. The correlation between miR-143 and BACH1 was analyzed by Pearson correlation. *In vitro*, RT-PCR was used to determine the expression of miR-143 in GES-1 cells and other GC cell lines. The biological function of miR-143 was examined using MTT assay, transwell chamber and Western blot in HGC-27 cells. The miR-143 target was validated using luciferase reporter and Western blot. Results: The expression of miR-143 was decreased in gastric cancer ($P < 0.01$). MiR-143 was downregulated in patients with poor differentiation and high clinical stage ($P < 0.01$). Furthermore, patients with high miR-143 expression showed longer survival time after surgery ($P < 0.01$). Besides, BACH1 mRNA, which was significantly increased ($P < 0.01$) in GC tissues, showed a significantly negative correlation with miR143 ($r = -0.6458$, $P < 0.001$). The proliferative and invasive activity of HGC-27 cells was decreased in the miR-143 group ($P < 0.01$); the luciferase activity of the BACH1-3'-UTR plasmid was suppressed following miR-143 binding ($P < 0.01$); BACH1 Overexpression partly reversed the inhibitory effect of miR-143 on HGC-27 cells. Conclusion: The expression of miR-143 was increased in GC tissues and it inhibits HGC-27 cell proliferation and invasion by downregulating BACH1. In addition, MiR-143 may be a prognostic biomarker of GC.

**Keywords:** Gastric cancer, miR-143, prognosis, BACH1, invasion

## Introduction

Gastric cancer (GC) is the fourth most common cancer and the second leading cause of cancer deaths worldwide. More than 950 000 new GC diagnoses are made every year [1]. GC treatment requires a multidisciplinary approach. Surgery is the only curative option available. Tumor progression and metastasis are the leading cause of death in patients with GC [2]. The outlook for patients with metastatic GC is very poor, with median survival ranging from 4 months to around 12 months [3]. However, most patients with early-stage GC are asymptomatic. Despite the progress in understanding the pathogenesis and molecular biology of GC, new treatment strategies and comprehensive management of advanced disease are needed.

The discovery of microRNA (miRNA) is one of the most significant landmarks in modern molecular biology. MicroRNAs can control the expression of their target mRNAs predominantly by binding to the 3' untranslated region (UTR) [4]. They play a critical role in carcinogenesis and have a significant impact on cancer research. The miRNAs are potential cancer biomarkers and act as oncogenes, tumor suppressors as well as regulators of cancer stem cells [5-9]. Indeed, aberrantly expressed miRNAs are potential biomarkers for GC screening, diagnosis, prognosis and disease monitoring, as well as therapeutic targets [10]. Serum miR-106b and miR-93 regulate E2F1 expression and play a key role in the development of TGFβ resistance in gastric cancer [11]. Serum miR-221 and miR-222 regulate radiosensitivity, cell growth and invasion of GC cells, possibly via direct modulation of PTEN expression [12]. Serum miR-378 is a novel noninvasive biomarker in gastric cancer detection [13]. The miR-25 was also found to promote GC progression

by targeting FBXW7 expression [14]. Although miR143 has been reported to act as tumor suppressor in human cancer [15], its exact role in gastric cancer is unclear. We, therefore, investigated the role of miR143 in cancer prognosis and therapeutic monitoring.

*BACH1*, a member of the BTB and CNC Homology (Bach) family, is located on chromosome 21q22.11. It encodes a heme-binding protein. BACH1 was significantly overexpressed in fetal Down's syndrome and suppressed expression of HO-1 [16]. Liang et al. found that BACH1 was a master regulator of several genes involved in bone metastasis of breast cancer [17].

In our study, we found that miR-143 was significantly decreased in GC and was correlated with poor differentiation and high clinical stage. Kaplan-Meier survival analysis revealed that surgery prolonged survival in patients with high miR-143 expression. The miR-143 inhibited GC cell proliferation and invasion. More importantly, we identified *BACH1* as a direct target gene of miR-143 in GC cells and overexpressing *BACH1* partly reversed miR-143-induced inhibition of GC cell proliferation and invasion. We found that miR-143 is a prognostic biomarker of gastric cancer and acted as a tumor suppressor by targeting BACH1.

## Materials and methods

### *Patients and tissues samples*

GC tissues and the corresponding non-tumor tissues were acquired from 44 patients treated with surgery from 2014 to 2016 at the Department of Gastroenterology, South Campus, Ren Ji Hospital. None of these patients received chemotherapy before. All the samples were confirmed pathologically and immediately frozen in liquid nitrogen and stored at -80°C. Written informed consent was obtained from all the patients. All the experimental procedures were approved by the ethics committee of Shanghai Jiaotong University, China.

### *Cell culture*

Human GC cell lines BGC-823, HGC-27, SGC-7901 and MGC-803 and immortalized normal gastric epithelial cell line GES-1 were purchased from American Type Culture Collection (ATCC, Rockville, Maryland, USA). Cells were cultured in DMEM medium (Hyclone; Thermo Fisher Scientific, Waltham, MA, USA) with 10% fetal bovine serum (Gibco, Invitrogen Inc., Carlsbad, CA, USA), and 1% of 100 U/mL penicillin and streptomycin sulfates. All the cell lines were incubated in humidified incubators with at 37°C in 5% $CO_2$ and 95% air.

### *Construction of vectors and cell transfection*

Oligonucleotides encoding the pre-miR-143 sequence were synthesized as follows: 5'-CUGUAGCUCAGGAAGAGAGAAGUUGUUCUGCAGAGCA-3' and 5'-CGCAGCGCCCUGUCUCCCAGCCUGAGGUGC-3'. The oligonucleotides were cloned into pCDH-MSCV-MCS-EF1-copGFP-T2A-Puro (SBI, Mountain View, CA, USA) between the EcoRI and BamHI sites. BACH13'-UTR sequences were: 5'-GACAGTGCCAGTCAGACATA-3' (forward) and 5'-CATTTTCAGACCTTTCATTT-3' (reverse). The PCR products were cloned into the pcDNA3.0 vector (Addgene, MA, USA). All the constructs were verified by sequencing. HGC-27 cells were added to the 24 well-plates at a concentration of $2 \times 10^5$ per well. After 24 h, a nonspecific control siRNA (NC) or a specific siRNA for BACH1 was transfected into the wells at a final concentration 50 nM using Hiperfect transfection reagent according to the manufacturer's instructions (Qiagen, Germany). Si-BACH1 primers were as follows: sense (5'-GACAGTGCCAGTCAGACATA-3') and anti-sense (5'-CAGGCATTCATTTTCAGACCTTTCA-3'). HGC-27 cells were transiently transfected with miR-143 mimics, or negative controls (Ambion, Austin, TX, USA) using Lipofectamine™ 2000 (Invitrogen, CA) according to the manufacturer's instructions.

### *RNA preparation and quantitative real-time PCR*

The RNA from clinical specimens and GC cells was extracted using TRIzol (Invitrogen, USA) following the manufacturer's instructions. The miRNAs were reverse transcribed to cDNA using reverse transcription kit (Thermo, USA). TaqMan human MiRNA assay kit (Invitrogen) was used for PCR amplification of miR-143 and U6. The primer sequences used were as follows: 5'-TCAGTTGGGAGTCTGAGATG-3' (forward); 5'-CTTCCTGAGCTACAGTGCTT-3' (reverse); β-actin 5'-CCCAGATCATGTTTGAGACCT-3' (forward); β-actin 5'-GAGTCCATCACGATGCCAGT-3' (reverse); U6 RNA 5'-CTCGCTTCGGCAGCACA-3' (forward); U6 RNA 5'-AACGCTTCACGAATTTGCGT-3' (reverse).

Relative quantification of miR-143 and BACH1 expression was calculated using the 2-ΔΔCT

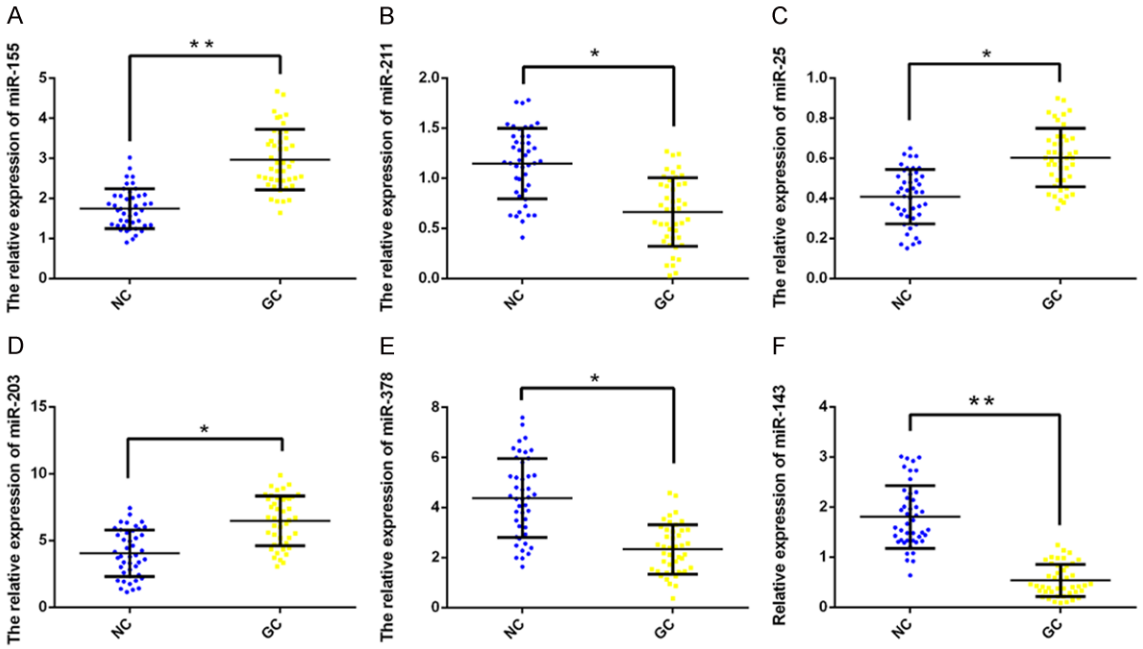


**Figure 1.** Expression of miRNAs in GC tissues. RT-PCR showed that miR-155, -25, and -203 were upregulated, with a fold change of 1.72, 1.51, 1.58 respectively, while miR-211, -378, and -143 were downregulated, with a fold change of 1.72, 1.88, 3.71, respectively, in GC compared with normal gastric tissues (*P < 0.05; **P < 0.01 *vs*. normal tissues).

method. All experiments were conducted in triplicate.

*Western blot*

Total protein was extracted from cells using 1% RIPA Lysis Buffer (Beyotime, China). Equal amounts of protein were separated by SDS-PAGE and blotted to PVDF membranes (Millipore, Billerica, MA). The membranes were probed with antibodies against BACH1 (1:400, Abcam, England), PCNA (1:1,000, Abcam), vimentin (1:500, Abcam), and GAPDH (1: 1000, Abcam) overnight at 4°C followed by incubation with secondary antibody (zsgb-bio, Beijing, China) for 1 h at room temperature. The specific proteins were visualized with Odyssey™ Infrared Imaging System (Gene Company, Lincoln, NE, USA).

*Proliferation assay*

Cells were seeded at a density of 1 × $10^4$ cells per well and allowed to grow for 24, 48, and 72 h. After incubation, 20 μL of 5 mg/mL MTT reagent was added to each well, and incubated for 4 h. Absorbance was measured at 490 nm using a microtiter plate reader and a growth curve was drawn. The results represent the mean of three replicates under the same conditions.

*Cell invasion assay*

Transwell 24-well plates coated with diluted Matrigel were used to determine cell invasion. The medium was supplemented with 1% heat-inactivated FBS in the upper chamber, and the lower chamber was filled with 20% FBS as a chemoattractant. After incubation for 48 h at 37°C, non-invading cells in the upper chamber were gently removed. Cells migrating to the lower surface were fixed and stained, and five random fields in each chamber were photographed and counted at × 100 magnification.

*Luciferase reporter assay and target gene identification*

Wild-type (WT) 3'-UTR of BACH1 and the mutated sequence were inserted into the pGL3 control vector (Promega Corporation, Madison, WI, USA) to construct a WT *BACH1*-3'-UTR vector and a mutant *BACH1*-3'-UTR vector, respectively. In the luciferase reporter assay, HGC-27 cells were added to 96-well plates at 10,000 cells per well on the day before transfection with the corresponding vectors. After 48 h post-transfection, the dual-luciferase reporter assay system (Promega, Madison, WI, USA) with the luminometer (Promega) was used to measure the luciferase activity. The firefly luciferase ac-

**Table 1.** Clinical characteristics of 44 patients and the expression of miR-143 in GC tissues

| Parameters | Case | MiR-143 median (range) | *P* |
|---|---|---|---|
| Age (years) | | | 0.457 |
| < 50 | 18 | 0.74 (0.25-1.77) | |
| ≥ 50 | 26 | 0.59 (0.16-1.08) | |
| Tumor size | | | 0.036 |
| < 5 cm | 29 | 0.89 (0.15-2.03) | |
| ≥ 5 cm | 15 | 0.42 (0.16-1.48) | |
| Lymph node metastasis | | | 0.028 |
| Negative | 10 | 1.04 (0.37-1.76) | |
| Positive | 34 | 0.85 (0.22-1.48) | |
| Clinical stage | | | < 0.001 |
| T1-T2 | 21 | 0.92 (0.15-2.15) | |
| T3-T4 | 23 | 0.58 (0.18-1.98) | |
| Differentiation | | | < 0.001 |
| Well and moderate | 19 | 0.78 (0.15-2.04) | |
| Poor | 25 | 0.48 (0.26-2.98) | |
| Liver metastasis | | | 0.013 |
| Negative | 18 | 0.92 (0.15-1.81) | |
| Positive | 26 | 0.78 (0.21-1.68) | |
| Peritoneal dissemination | | | 0.037 |
| Negative | 28 | 1.09 (0.45-1.93) | |
| Positive | 16 | 0.77 (0.32-1.78) | |

tivities were used as an internal control for transfection efficiency.

*Statistical analysis*

All the data were expressed as mean ± standard deviation based on three independent experiments. Differences between the groups were analyzed using Student's t-test. Statistical analyses were performed using SPSS20.0 software. The significance level was $P < 0.05$.

## Results

*Identification of miRNA expression in GC tissues*

As miRNAs play an important role in tumorigenesis, our attention was focused on the six popular miRNAs including miR-143, -155, -211, -25, -378, -203 in GC tissues and corresponding non-tumor tissues. The differentially expressed miRNAs (**Figure 1**) were detected by real-time quantitative reverse transcription-PCR (qRT-PCR). The miR-155 exhibited the largest degree of upregulation (1.72-fold) ($P < 0.01$), whereas miR-143 showed the largest downregulation (3.71-fold) in GC tissues ($P < 0.01$). Other miR-25 and miR-203s were upregulated, and miR-211 and miR-378 were downregulated significantly ($P < 0.05$).

*Decreased miR-143 expression is correlated with poor tumor differentiation and poor prognosis*

The clinicopathological characteristics of 44 GC patients are shown in **Table 1**. The miR-143 was significantly downregulated in 37 cancer tissues compared with matched tissues (**Figure 2A**). Notably, we correlated miR-143 expression with the clinicopathological factors (**Figure 2B**, **2C**). The miR-143 downregulation was correlated with poor tumor differentiation ($P < 0.0001$) and advanced pathological stage ($P < 0.0001$). Kaplan-Meier survival analysis was used to further correlate the expression of miR-143 with prognosis of GC patient. The 44 GC patients were divided into high-miR-143 and low-miR-143 groups according to the median ratio of relative miR-143 expression (1.18). As a result, we found that patients with high miR-143 expression ($n = 24$) lived longer than those with low miR-451 expression ($n = 20$) ($P < 0.001$, log-rank test; **Figure 3**). Hence, we concluded that the downregulation of miR-143 in GC tissues was significantly correlated with differentiation and pathological stages, and served as a prognostic biomarker in GC patients.

*Upregulation of BACH1 in GC*

To further elucidate the underlying molecular mechanisms of miR-143 in gastric tissues, we used open access software programs including TargetScan, miRBase and PicTarget, to search for potential downstream targets of miR-143. BACH1 was our underlying target. Therefore, we tested the expression of BACH1 mRNA in GC tissues and found that BACH1 mRNA expression was upregulated and negatively correlated with control, as shown in **Figure 4A**, **4B** ($P < 0.01$). In addition, we found that the expression of PCNA and Vimentin, the proliferation and

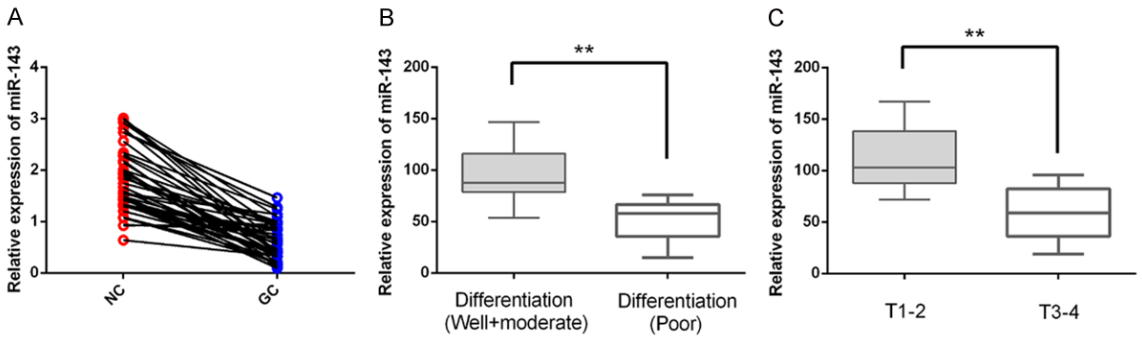


**Figure 2.** Expression of miR-143 in gastric tissues and its significance. A: Relative expression of miR-143 in GC tissues compared with normal tissues (n = 44). B: The expression of miR-143 was significantly lower in poorly differentiated GC tissues compared with normal tissues (**P < 0.01). C: The miR-143 expression was significantly lower in patients at a higher pathological stage (T3-4) (**P < 0.01).

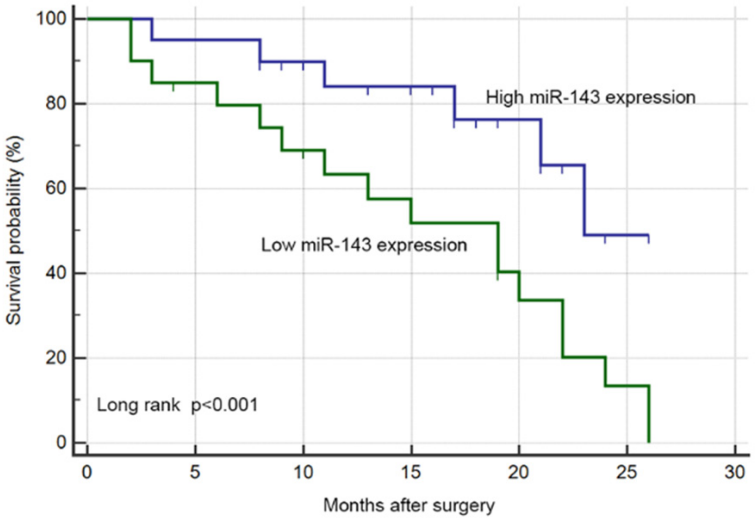


**Figure 3.** Kaplan-Meier survival curve in GC patients. The expression level of miR-451 was determined by qRT–PCR assay in 20 GC patients divided into two groups according to the expression level of miR-143. After a follow-up of 26 months, the survival data were compared using log-rank test. GC patients with high miR-451 expression showed shorter survival compared with patients exhibiting low miR-451 expression (P < 0.001).

invasion related protein, was higher in GC than in normal tissues (**Figure 4C**, **4D**, P < 0.01), prompting *in vitro* studies.

*MiR-143 suppresses HGC-27 cell proliferation and invasion in vitro*

We used RT-PCR to detect the levels of miR-155 in immortalized normal gastric epithelial cell line GES-1 and human GC cell lines BGC-823, HGC-27, SGC-7901 and MGC-803. The miR-143 was down-regulated in GC cell lines (**Figure 5A**, P < 0.01). To further study the role of miR-143 in GC, miR-143 mimics and the corresponding negative control were transfected into HGC-27 cells. Compared with miR-negative control group, the expression of miR-143 in HGC-27 cells was significantly decreased after transfection with miR-143 mimics (P < 0.01, **Figure 5B**).

We determined the effect of miR-143 on the proliferation and invasion of HGC-27 cells. As shown in **Figure 5C**, **5D**, MTT and Transwell invasion assays, cell proliferation and invasion were distinctly inhibited in miR-143 mimics compared with the other two groups (P < 0.01). Furthermore, the proliferation and invasion-related protein expression was also suppressed by miR-143 mimics (P < 0.01), as shown by the Western blot in **Figure 5E**, **5F**. Overall, these data indicated that miR-143 inhibited the proliferation and invasion of HGC-27 cells *in vitro*.

*BACH1 is a downstream target of miR-143 in HGC-27 cells*

BACH1 expression was significantly upregulated in GC tissues as shown in **Figure 4**. To confirm whether miR-143 modulate the expression of BACH1, we determined the expression of BACH1 in miR-143 mimics, miR-NC and control groups, using WB. We found that the level of

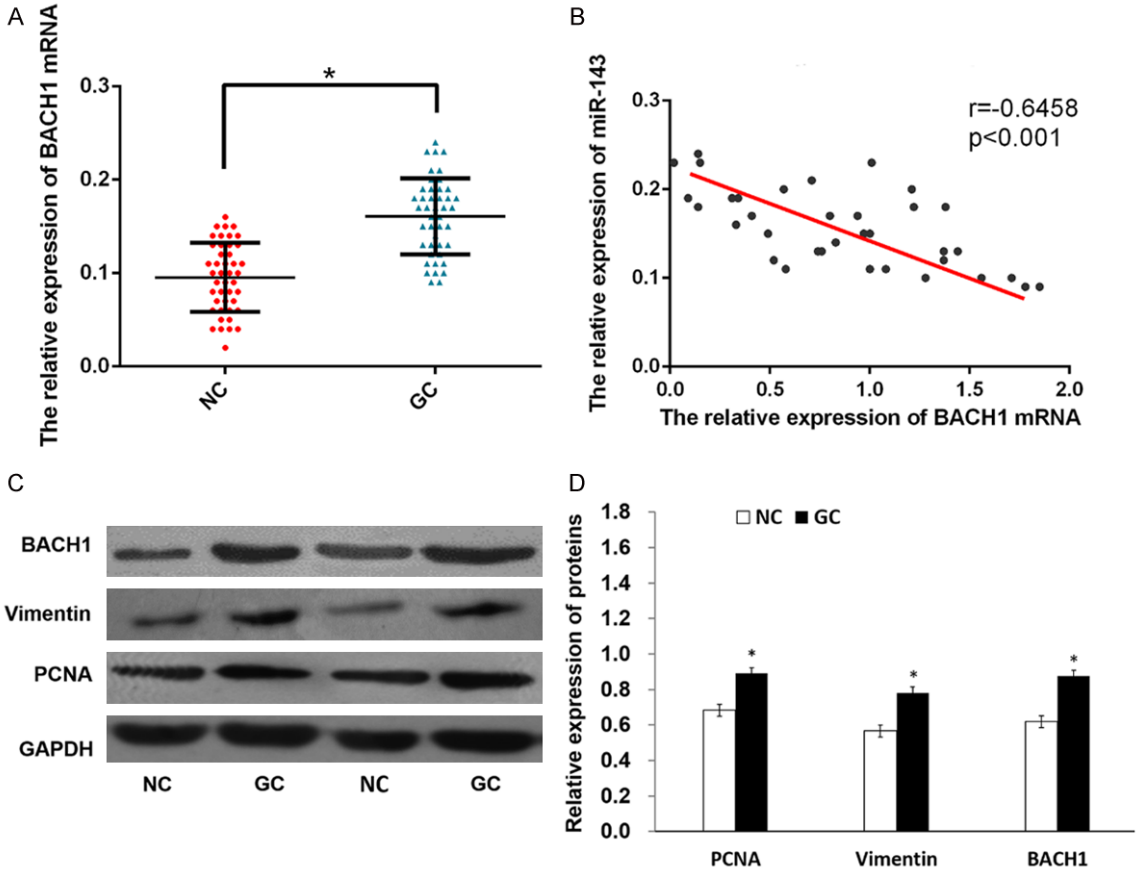


**Figure 4.** BACH1 expression and proliferation-related proteins in GC patients. A: Analysis of the expression of BACH1 mRNA in normal gastric tissue and GC tissues by qRT-PCR. The expression values were normalized to U6 RNA levels. The expression of BACH1 mRNA was significantly increased ($P < 0.01$). B: Pearson correlation was used to analyze the relationship between miR-143 and BACH1 mRNA. The result showed that miR-155 was negatively correlated with BACH1 ($P < 0.01$). C, D: Proliferation and invasion-related proteins were also overexpressed in GC tissues tested by Western blot ($P < 0.01$), using GAPDH as an internal control.

BACH1 was downregulated when miR-143 was overexpressed, compared with the other two groups (**Figure 6A**, **6B**, $P < 0.01$). As suggested in **Figure 6C**, the complementary sequence of miR-143 was found in the 3'-UTR of *BACH1* mRNA. To further validate *BACH1* as the putative target gene of miR-143, the luciferase reporter assay was used. The results demonstrated that the luciferase activity was suppressed by miR-143 mimics in the WT vector ($P < 0.01$), and the effect due to miR-143 disappeared in the MUT vector (**Figure 6D**). All the data suggested that BACH1 might be a target of miR-143 in HGC-27 cells.

*BACH1 regulated miR-143-induced inhibition*

To further determine whether BACH1 is associated with the regulation of miR-143-induced inhibition of HGC-27 cells, the expression of BACH1 protein in HGC-27 cells transfected with PcDNA3.1+HA-BACH1, PcDNA3.1+HA empty vector and miR-143 mimics was analyzed (**Figure 7A**). The results revealed that the proliferative and invasive capacity of cells transfected with BACH1 was enhanced compared with miR-143 mimics+NC and miR-143 mimics group ($P < 0.01$) (**Figure 7B**, **7C**). Immunoblot data in the present study also showed an upswing in the expression of proliferative and invasive proteins PCNA and vimentin following overexpression of BACH1 ($P < 0.01$) (**Figure 7D**, **7E**). In brief, studies strongly suggested that the target gene of miR-143 *BACH1* regulated miR-143-induced inhibition of HGC-27 cells.

## Discussion

GC is a complex disorder characterized by marked global variation in etiology, incidence, nat-

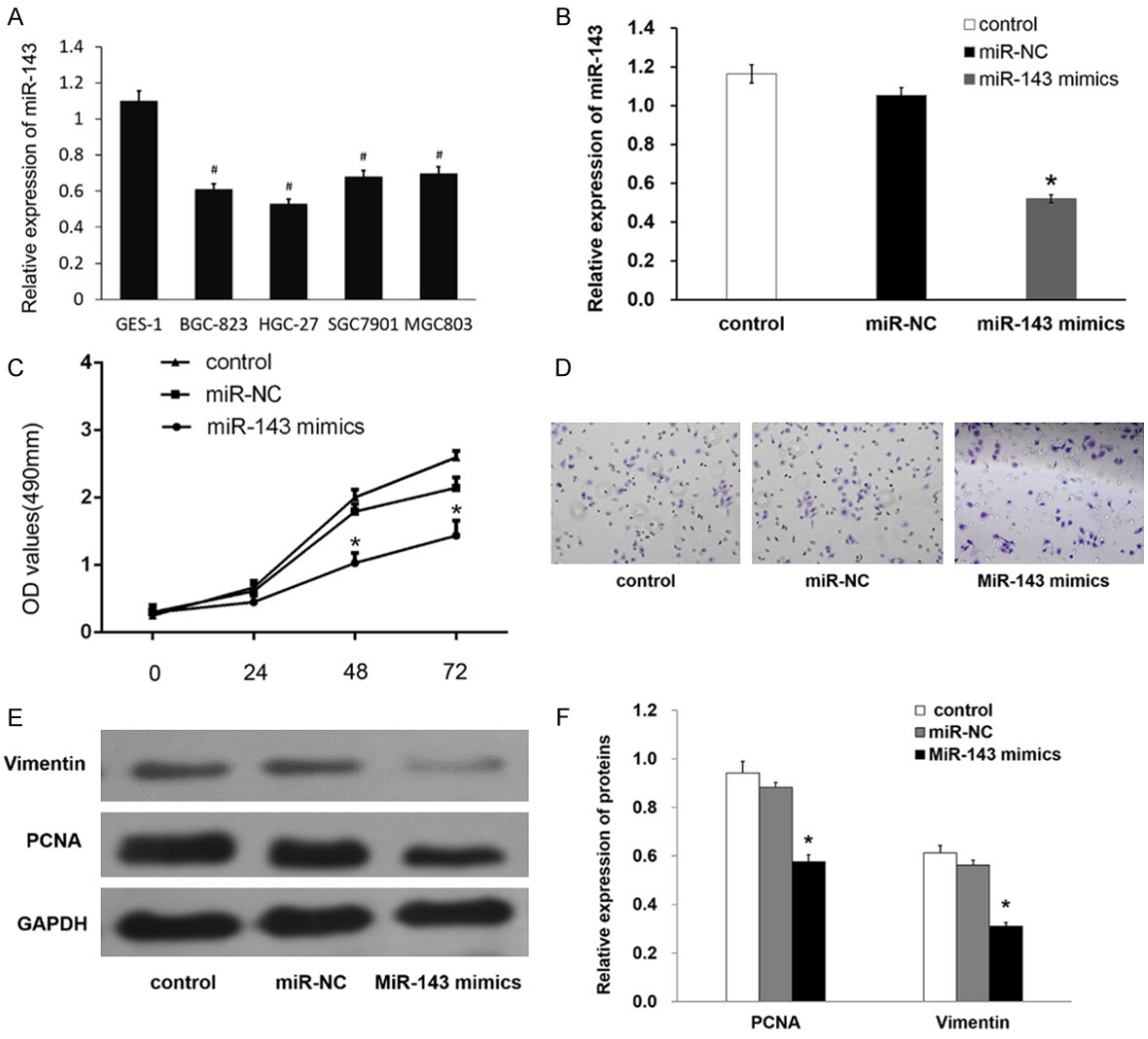


**Figure 5.** MiR-143 inhibited HGC-27 cell proliferation and invasion *in vitro*. A: miR-143 was significantly downregulated in different GC cell lines compared with immortalized normal gastric epithelial cell line GES-1 ($P < 0.01$). B: miR-143 mimics were successfully transfected into HGC-27 cells. C: MTT assay showed that overexpression of miR143 inhibited HGC-27 cell proliferation compared with miR-NC and control groups ($P < 0.01$). D: Transwell migration assay revealed that miR-143 remarkably inhibited cell invasion ($P < 0.01$). E, F: Western blot showed that the proliferation and invasion-related proteins PCNA and Vimentin were distinctly suppressed in the miR-143 group compared with the other two groups. *$P < 0.01$ compared with miR-NC, #$P < 0.01$ compared with GEC-1 cells.

ural course, and management. According to the World Health Organization GLOBOCAN database, GC affected an estimated 952,000 people and resulted in almost 723,000 deaths [18]. GC is one of the most common cancers in China and is associated with poor survival [19]. Approximately two-thirds of newly diagnosed GC patients show metastatic disease at some point during the course of their illness and require systemic therapy [20]. Currently, in addition to surgery, several randomized clinical trials of chemotherapy are investigating the role of molecular targeting in advanced GC [21, 22] and individualized treatment strategies using adaptive cell therapies, cancer vaccines, and antibody therapies also showed promising outcomes [23]. Despite the several treatment options available, the median survival in advanced GC remains a dismal 8 to 10 months.

MicroRNAs (miRNAs) are short (20-24nt) noncoding RNAs that are involved in post-transcriptional regulation of gene expression. Several miRNAs promote tumorigenesis and cancer progression [24, 25]. They are dysregulated in cell growth, invasion, metastasis, and apopto-

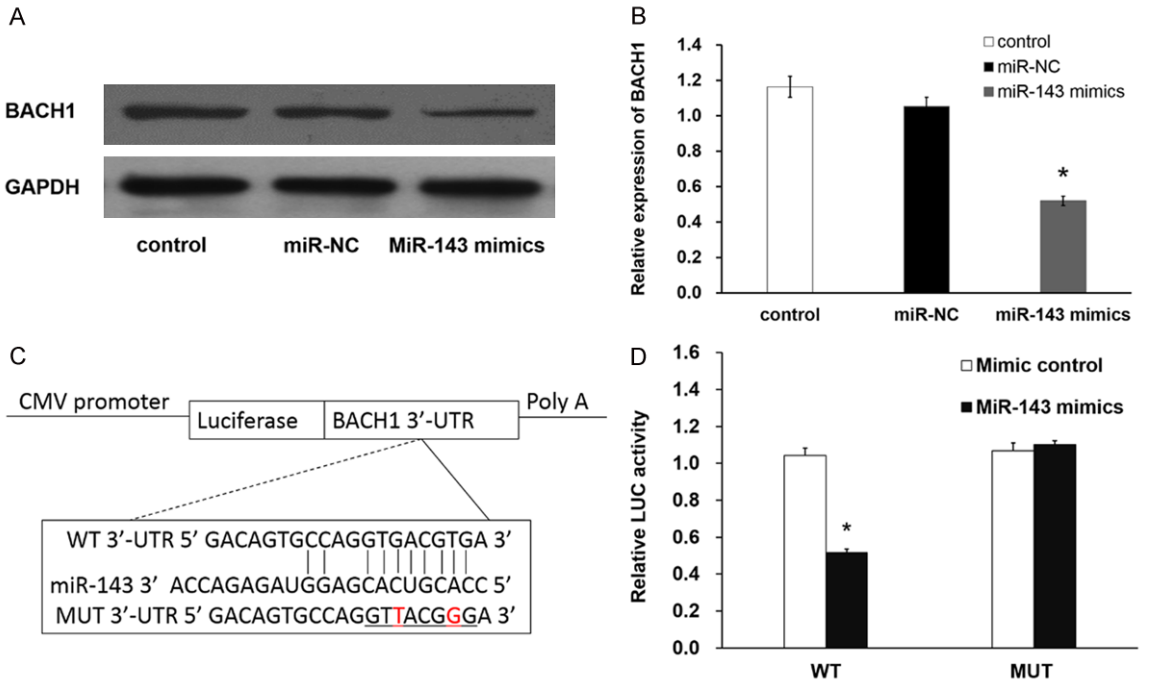


**Figure 6.** miR-143 directly targeted BACH1 in HGC-27 cells. A, B: Western blot of BACH1 expression in each group. BACH1 was significantly suppressed in miR-143 mimics group ($P < 0.01$). C: A human BACH1 3'-UTR fragment containing wild type or mutant miR-143-binding sequence was cloned downstream of the luciferase reporter gene. Putative miR-143-binding sequence in the 3'-UTR of BACH1 mRNA is shown. D: Luciferase reporter assay showed that miR-143 inhibited luciferase activity in BACH1 with WT-binding sites but not with the MUT-binding sites in 3'UTR of BACH1 mRNA ($P < 0.01$).

sis underlying GC tumorigenesis and progression [26-28]. Recent data indicated that miR-143 showed distinct expression profiles and played a crucial role in adipogenesis, macrosomia, cancer, and cardiovascular diseases [29]. In this study, we discovered that the expression of miR-143 was downregulated in GC patients. Further, miR-143 downregulation was correlated with poor tumor differentiation and advanced pathological stage. We further found that patients with high miR-143 expression manifested significantly longer survival than those with low miR-143 expression, suggesting that miR-143 might play a critical role in GC progression and development. Naito et al. reported that miR-143 may support the progression of scirrhous GC through fibrillar formation [30]. Dimitrova et al. found that miRNA143 acted as a key modulator of endothelial function in lung cancer development and cancer biology by regulating neoangiogenesis [31]. Du et al. found that miRNA143 was a potent inhibitor of autophagy by targeting GABARAPL1 and miR-143 improved the efficacy of Quercetin by inhibiting autophagy in GC cell lines [32]. PCNA and Vimentin are involved in cell migration and invasion [33, 34], and are increased in the GC tissue. We, therefore, investigated the function of miR-143 and found that it might be an efficient tumor inhibitor in the genesis and progression of GC.

Recently, Cantor et al. described BACH1 as a nuclear DNA helicase, which interacted directly with BRCA1 and contributed to DNA repair [35]. Two germline missense mutations were found in 65 breast cancer patients lacking *BRCA1* and *BRCA2* alterations [36], suggesting that *BACH1* may be a breast cancer susceptibility gene. Lei et al. confirmed that the bipartite NLS1 was critical for the nuclear translocation of BACH1 suggesting the role of functional polymorphism in breast cancer susceptibility [37]. In 2011, *BACH1* was reported as a pro-metastatic gene and a direct target of the tumor suppressive microRNA Let-7 in breast cancer [38]. Additionally, it was found that BACH1 was increased in PCa and HGPIN, and was a reliable target of miR-155, suggesting a complex regulatory mechanism [39]. In this experiment, after finding that BACH1 was upregulated against miR-143 expression, we overexpressed BACH1

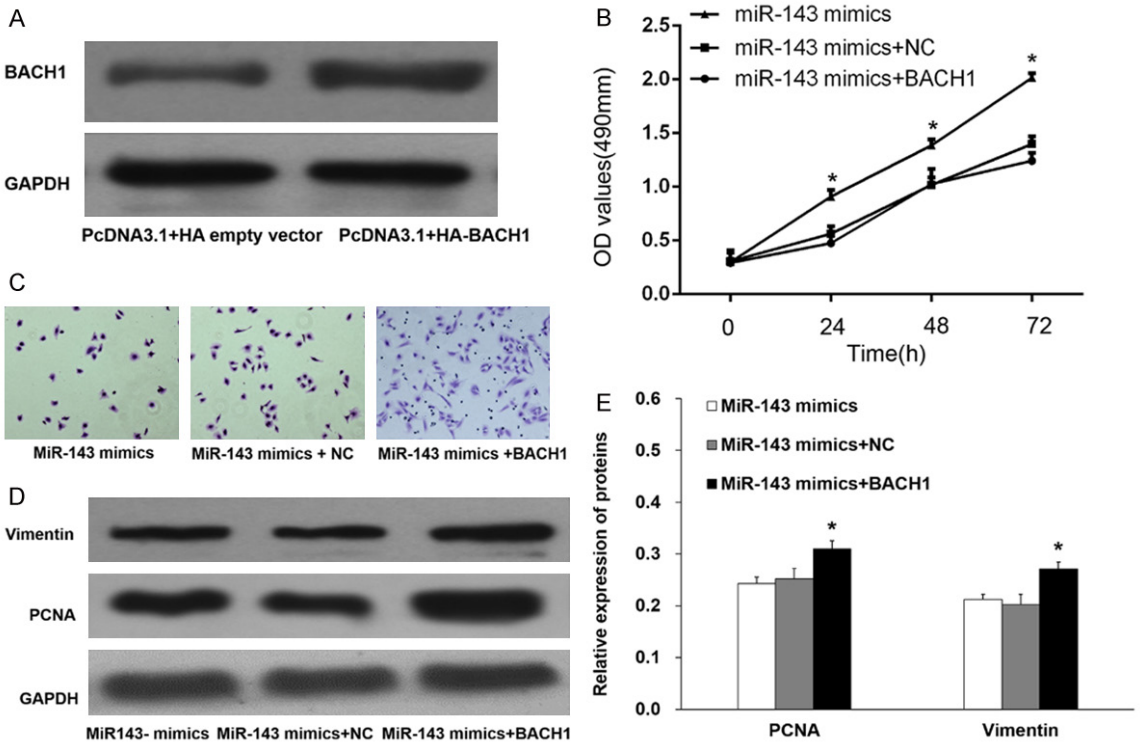


**Figure 7.** Overexpression of BACH1 partly abrogated miR-143-induced inhibitory effects on HGC-27 cells. A: The expression of BACH1 detected by Western blot was higher in PcDNA3.1+HA-BACH1 group than in the matched group. B: MTT assay showed that the cell proliferation of HGC-27 cells transfected with miR-143 mimics+BACH1 was stronger than in other groups ($P < 0.01$). C: Transwell migration assay was performed to detect invasion of HGC-27 cells. The results revealed that overexpression of BACH1 enhanced the invasive ability of HGC-27 cells ($P < 0.01$). D, E: Western blot showed that PCNA and Vimentin were highly expressed in the presence of BACH1 overexpression.

to partly reverse miR-143 induced-inhibition of proliferation and invasion of HGC-27 cells. Therefore, we believed that miR-143 directly targeted BACH1 in HGC-27 cells via interaction with the 3'-UTRs of *BACH1* gene.

In conclusion, our study show for the first time, that miR-143 acted as a prognostic biomarker and tumor suppressor in GC cells. BACH1 is a target of miR-143, and regulated miR-143-induced inhibition of HGC-27 cells. Our findings suggest a novel therapeutic strategy targeting miR-143/BACH1 interaction in GC.

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## Disclosure of conflict of interest

None.

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