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MiR-129-5p inhibits proliferation of gastric cancer cells through targeted inhibition on HMGB1 expression

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Abstract. – OBJECTIVE: The aim of this study was to investigate the effects of micro ribonucleic acid (miR)-129-5p on the proliferation and apoptosis of gastric cancer cells via targeted repression on the expression of high mobility group protein B1 (HMGB1).

PATIENTS AND METHODS: Expression levels of miR-129-5p and HMGB1 in gastric cancer tissues (n=25) and adjacent normal tissues were measured *via* reverse transcription-quantitative polymerase chain reaction (RT-qPCR). The regulatory effect of miR-129-5p on the proliferation of gastric cancer MGC-803 and SGC7901 cells was determined through Cell Counting Kit-8 (CCK-8) assay. Flow cytometry was employed to analyze the apoptosis rate of gastric cancer cells. To further discover the mechanism of miR-129-5p in regulating malignant behaviors of gastric cancer cells, the miRDB database was employed to predict the binding targets of miR-129-5p. Finally, binding sites of HMGB1 3'-untranslated region (3'-UTR) to miR-129-5p were discovered. Subsequently, HMGB1 wild-type or mutant 3'-UTR Luciferase reporter vectors were constructed, and transfected to MGC-803 and SGC7901 cells together with miR-129-5p or negative control miRNA. Next, Western blotting was adopted to measure the protein expression level of HMGB1 in MGC-803 and SGC7901 cells transfected with miR-129-5p or negative control miRNA, so as to investigate whether miR-129-5p affected HMGB1 protein expression. Additionally, to determine whether HMGB1 mediated the regulatory effect of miR-129-5p on the proliferation of gastric cancer cells, MGC-803 and SGC7901 cells were transfected with pcDNA-HMGB1 or pcDNA-vector, respectively. The expression level of HMGB1 was measured *via* RT-qPCR, and cell proliferation was determined by CCK-8 assay.

RESULTS: The expression level of miR-129-5p in gastric cancer tissues was significantly lower than that in adjacent normal tissues ($p<0.001$). Meanwhile, the level of miR-129-5p was overtly lower in gastric cancer MGC-803 and SGC7901 cell lines than that in normal gastric mucosal epithelial GES-1 cells ($p<0.001$). These results indicated that miR-129-5p was lowly expressed in gastric cancer tissues and cell lines. Subsequent results demonstrated that the expression of HMGB1 increased remarkably in gastric cancer tissues compared with normal adjacent tissues ($p<0.05$). The proliferation ability of MGC-803 ($p<0.001$) and SGC7901 ($p<0.01$) cells with over-expressed miR-129-5p was remarkably weakened. Overexpression of miR-129-5p distinctly promoted the apoptosis rate of gastric cancer MGC-803 ($p<0.01$) and SGC7901 ($p<0.001$) cells. Moreover, miR-129-5p up-regulation significantly reduced the Luciferase activity of wild-type HMGB1 ($p<0.001$). However, no significant effect was observed on that of mutant HMGB1. The results suggested that overexpression of miR-129-5p significantly down-regulated the level of HMGB1 in gastric cancer cells. In addition, the messenger RNA (mRNA) level of HMGB1 in cells transfected with miR-129-5p also decreased significantly ($p<0.001$). HMGB1 overexpression overtly reversed the inhibitory effect of miR-129-5p on the proliferation of gastric cancer cells ($p<0.05$). All these results demonstrated that the miR-129-5p/HMGB1 axis played a key role in regulating the growth of gastric cancer cells.

CONCLUSIONS: MiR-129-5p suppresses the progression of gastric cancer through targeted inhibition on the expression of HMGB1.

Key Words:
MiR-129-5p, HMGB1, Gastric cancer, Proliferation, Apoptosis.

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Introduction

Gastric cancer, characterized by strong invasiveness and low diagnosis rate, is one of the most common malignant tumors worldwide[1]. Surgical resection combined with chemotherapy and radiotherapy is the main treatment for gastric cancer in recent decades[2,3]. Due to the reason that most patients have already been in advanced stage when diagnosed with extensive invasion and lymphatic metastasis, the prognosis of gastric cancer patients is relatively poor[4,5]. Hence, discovering new pathogenic factors and potential targeted drugs is of great significance for the early diagnosis and effective treatment of gastric cancer.

Micro ribonucleic acids (miRNAs), a kind of small single-stranded non-coding RNAs with approximately 22 nucleotides in length[6], are vital regulators in gene expression and can bind to the 3'-untranslated region (3'-UTR) of target genes to degrade messenger RNAs (mRNAs) or repress the translation of mRNAs[7,8]. MiRNAs play crucial roles in the development and progression of human cancers by modulating the expression of tumor suppressor genes or oncogenes[9-14]. Consistently, Yu et al[15] have shown that miR-6852 inhibits the proliferation and invasion of gastric cancer cells by targeting the forkhead box J1. MiR-543 promotes the development of gastric cancer by downregulating the expression of speckle-type POZ protein[16]. MiR-21-5p is highly expressed in gastric cancer, serving as a promising target for gastric cancer treatment[17-19]. In addition, miR-1179 impedes the growth and invasion of non-small cell lung cancer by modulating the AKT signaling pathway[20].

High mobility group protein B1 (HMGB1) is a protein in the high mobility group box superfamily[21,22]. As a chromatin component common in mammalian cells, HMGB1 plays an important role in various cellular processes, such as inflammation, cell differentiation and tumor cell migration. Meanwhile, it has been found to participate in transcriptional regulation[23]. HMGB1 is of vital importance in the progression of cancers by regulating the transcription of cancer-related genes[24]. HMGB1 has also been confirmed to be overexpressed in many human cancers[25,26]. As a result, blocking the expression of HMGB1 inhibits cancer progression. However, few reports have elucidated the effects of miR-129-5p on the proliferation of gastric cancer cells through HMGB1. In this study, our findings showed that miR-129-5p was significantly down-regulated in patients with gastric cancer. Overexpression of miR-129-5p significantly inhibited the proliferation and promoted the apoptosis of gastric cancer cells by targeting HMGB1. All these results revealed the key role of the miR-129-5p/HMGB1 axis in modulating the progression of gastric cancer.

Patients and Methods

Clinical Specimens

A total of 50 pairs of gastric cancer tissues and adjacent normal tissues were collected from patients who underwent surgery in Shanxi Provincial People's Hospital from January 2018 to October 2019. The selection of patients was based on the guideline proposed by the Union for International Cancer Control (UICC). All collected tissues were immediately frozen in liquid nitrogen at -80°C for use. No patient received radiation or chemotherapy before surgery. Informed consent was obtained from all subjects before the study. This investigation was approved by the Ethics Committee of Shanxi Provincial People's Hospital.

Cell Culture

Human gastric cancer MGC-803 and SGC7901 cell lines were cultured in Dulbecco's Modified Eagle's Medium (DMEM; Thermo Fisher Scientific, Waltham, MA, USA) in a 5% CO_2 incubator at 37°C.

Plasmid Construction

pcDNA-HMGB1 plasmid was constructed as follows. HMGB1 complementary deoxyribose nucleic acid (cDNA) full length sequence was amplified *via* polymerase chain reaction (PCR) and inserted into pcDNA™3.1 (+) mammalian expression vectors (V79020, Thermo Fisher Scientific, Waltham, MA, USA). EcoRI and XhoI were used as endonucleases. The expression of pcDNA-HMGB1 was detected by Western blotting.

Reverse Transcription-Quantitative Polymerase Chain Reaction (RT-qPCR)

Total RNA in tissues and cells was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). The concentration of RNA was determined using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). A reverse transcription kit (SuperScript II cDNA synthesis kit, Thermo Fisher Scientific, Waltham, MA, USA) was utilized. QPCR was

Table I. Primer sequences.

Gene	Primer sequences
MiR-129-5p	F: 5'-CUUUUUGCGGUCUGGGCUUGC-3′ R: 5′-AGCAAGCCCAGACCGCAAAAA-3′
HMGB1	F: 5′- CCAGCGATAGTCCCACTGAT-3′ R: 5′-CCTTCTCCTTGGCAGACATC-3′
U6	F: 5'- CTCGCTTCGGCAGCACA -3' R: 5'- AACGCTTCACGAATTTGCGT -3'
GAPDH	F: 5'- CGCTCTCTGCTCCTCCTGTTC -3' R: 5'-ATCCGTTGACTCCGACCTTCAC-3'

performed using SYBR Green dye (Applied Biosystems, Foster City, CA, USA) and an ABI Prism 7900 detection system (Thermo Fisher Scientific, Waltham, MA, USA). U6 was used as the internal reference in the quantitative analysis of miR-129-5p expression, and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as the internal reference in the quantitative analysis of the HMGB1 expression. Primer sequences used in this study were shown in Table I.

Cell Proliferation Assay

Cell Counting Kit-8 (CCK-8; Dojindo Molecular Technologies, Kumamoto, Japan) was adopted to detect the proliferation of gastric cancer cells. Briefly, gastric cancer cells were first transfected with corresponding expression vectors for 24 h. Next, CCK-8 reagent was added in cells, followed by incubation at 37°C for 2 h in the dark. Absorbance of each well at 450 nm was determined using a micro-plate reader (Bio-Rad Laboratories, Hercules, CA, USA).

Western Blotting

Total proteins were extracted from gastric cancer cells with NP-40 lysis buffer (Beyotime Institute of Biotechnology, Shanghai, China). The concentration of proteins was determined *via* bicinchoninic acid (BCA) method (Beyotime, Shanghai, China). 30 μg of proteins were loaded, subjected to electrophoresis and transferred onto polyvinylidene difluoride (PVDF) membranes. After blocking with 5% skimmed milk, the membranes were incubated with primary antibodies at room temperature for 2 h. Next, the membranes were incubated with horseradish peroxidase (HRP)-labeled secondary antibody for 1 h. Immunoreactive bands were finally observed *via* enhanced chemiluminescence (ECL) Western blotting substrate (Thermo Fisher Scientific, Waltham, MA, USA).

Cell Apoptosis Through Flow Cytometry

The cells were first digested with trypsin. The supernatant of the culture was collected and centrifuged at 2000 rpm for 6 min at room temperature. Subsequently, the cell pellet was collected and washed twice with phosphate-buffered saline (PBS). 300 μL of binding buffer was then added to suspend cells, followed by addition of two fluorescent probes (Annexin V-FITC and PI). After incubation in dark at room temperature for 15-20 min, cell apoptosis was determined by flow cytometry.

Luciferase Reporter Gene Assay

HMGB1 3'-UTR wild-type (5'-TTT TGT TGA TCAT CAT TCT GAA TGCTT) and mutant (5'-TTT TGT TGA TCAT CAT TCT CCC TAATC) sequences were inserted into the pGL3 Luciferase reporter vectors (GenScript; Piscataway, NJ, USA). Gastric cancer cells were inoculated into 24-well plates and transfected with 100 ng of pGL3-HMGB1 or pGL3-HMGB1 mutant and miR-129-5p mimic or control vector in accordance with the instructions of Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA). 48 h later, Luciferase activity was determined using a Dual-Luciferase reporter kit (Promega, Madison, WI, USA).

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 15.0 (SPSS Inc., Chicago, IL, USA) was employed for statistical analysis. Data were expressed as mean ± standard deviation of three independent experiments. The differences between the two groups were analyzed *via* *t*-test. $p<0.05$ was considered statistically significant.

Results

MiR-129-5p Was Significantly Downregulated in Patients with Gastric Cancer

To evaluate the role of miR-129-5p in gastric cancer, the expression of miR-129-5p in gastric cancer tissues and adjacent normal tissues was detected *via* RT-qPCR. The results (Figure 1) showed that miR-129-5p was lowly expressed in gastric cancer tissues and MGC-803 and SGC7901 cell lines when compared with adjacent normal tissues and gastric mucosal epithelial GES-1 cells. These results suggested the down-regulation of miR-129-5p in gastric cancer.

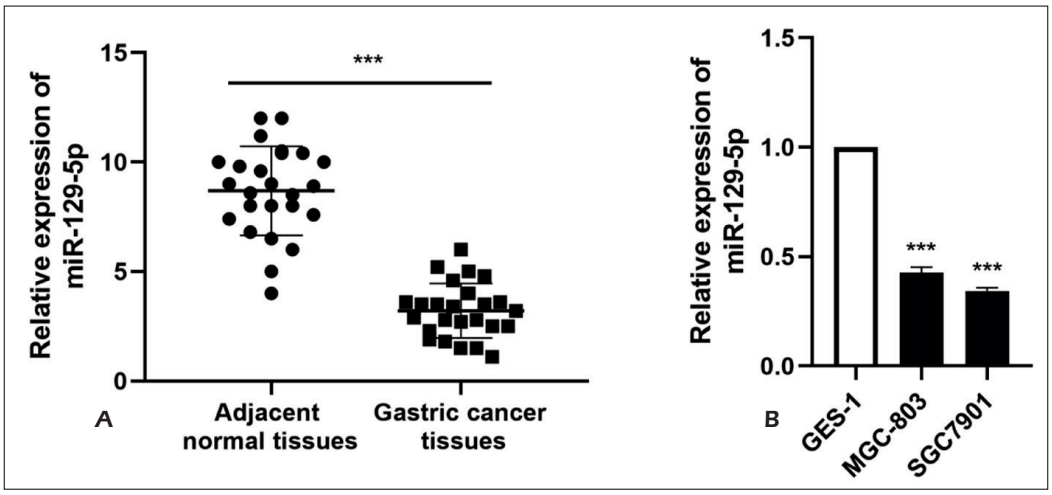

Figure 1. The expression level of miR-129-5 is overtly higher in adjacent normal tissues than gastric cancer tissues (p<0.001), and is distinctly lower in gastric cancer cell lines than gastric mucosal epithelial cells (p<0.001). **A**, Expression level of miR-129-5 in gastric cancer tissues and adjacent normal tissues, and **B**, Expression level of miR-129-5 in gastric cancer cell lines and normal gastric mucosal epithelial cells).

HMGB1 Was Highly Expressed In Patients With Gastric Cancer

Subsequently, the role of HMGB1 in gastric cancer was evaluated. QRT-PCR results demonstrated that the expression of HMGB1 was (3.16±0.12) in adjacent normal tissues and (7.82±0.24) in gastric cancer tissues, respectively. It could be seen that the expression of HMGB1 was remarkably higher in gastric cancer tissues than that of adjacent normal tissues (Figure 2).

Mir-129-5p Was Negatively Correlated With HMGB1 Expression In Gastric Cancer

The expression levels of miR-129-5p and HMGB1 in gastric cancer tissues were statistically analyzed. The results (Figure 3) revealed that there was a significant negative correlation between miR-129-5p expression and HMGB1 expression (r^2=0.9578, p=0.0085), implying that there might be a mutual relationship between the expressions of miR-129-5p and HMGB1.

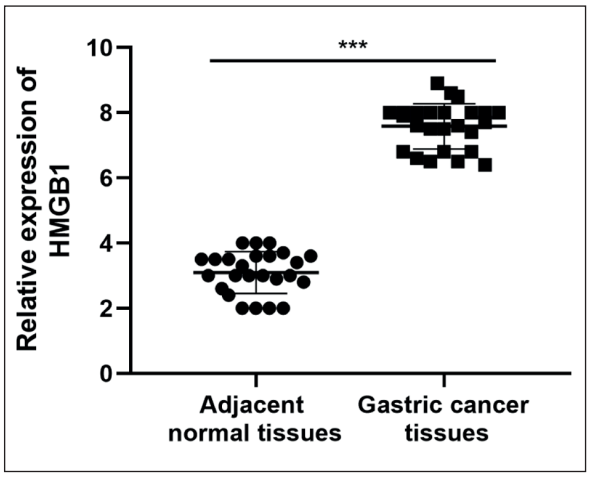

Figure 2. Expression level of HMGB1 in gastric cancer tissues and adjacent normal tissues. The expression level of HMGB1 is overtly lower in adjacent normal tissues than that in gastric cancer tissues (p<0.001).

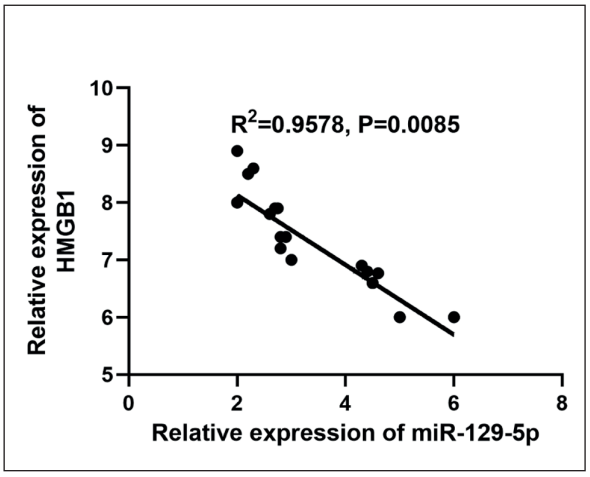

Figure 3. MiR-129-5p expression is negatively correlated with HMGB1 expression in gastric cancer tissues (r2=0.9578, p=0.0085).

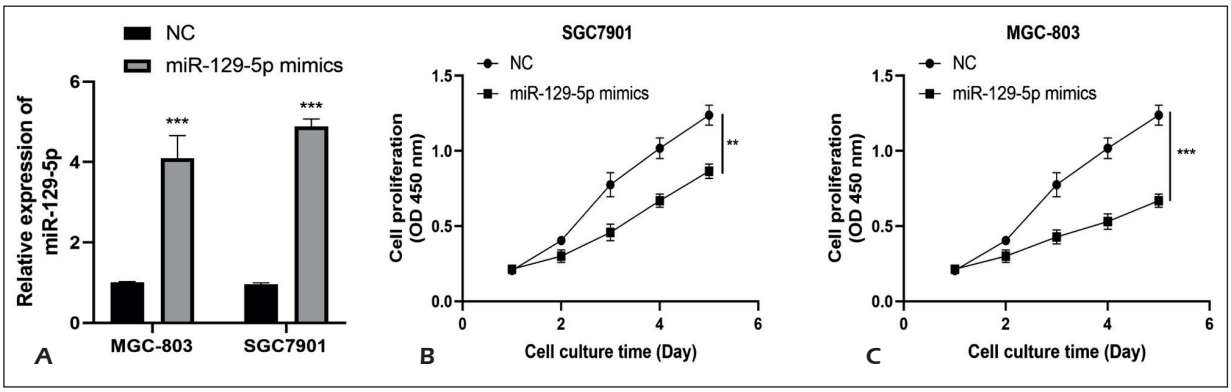

Figure 4. MiR-129-5p overexpression inhibits the proliferation of gastric cancer cells. **A**, Expression level of miR-129-5p in MGC-803 and SGC7901 cells transfected with miR-129-5p and control plasmids detected by qPCR, **B**, Viability of SGC7901 cells determined *via* CCK-8 assay (**p<0.01: a significant difference vs. control group), and **C**, Viability of MGC-803 cells determined through CCK-8 assay (***p<0.001: an extremely significant difference vs. control group).

Mir-129-5p Overexpression Inhibited Gastric Cancer Cell Proliferation

Since miR-129-5p was lowly expressed in gastric cancer tissues, its effect on the progression of gastric cancer was detected using MGC-803 and SGC7901 cell lines with low expression of miR-129-5p. RT-qPCR was conducted to verify the expression of miR-129-5p in MGC-803 and SGC7901 cells transfected with miR-129-5p or negative control miRNA. The results showed that miR-129-5p expression increased significantly after transfection of miR-129-5p mimic (Figure 4A). The regulation of miR-129-5p on the proliferation of gastric cancer cells was determined *via* CCK-8 assay. It was discovered that the proliferation ability of MGC-803 and SGC7901 cells with overexpressed miR-129-5p was remarkably inhibited (Figure 4B and 4C).

Overexpression of Mir-129-5p Promoted Apoptosis of Gastric Cancer Cells

Flow cytometry was employed to determine the apoptosis rate of gastric cancer cells. As shown in Figure 5, overexpression of miR-129-5p markedly promoted the apoptosis rate of MGC-803 and SGC7901 cells. The above results demonstrated that miR-129-5p overexpression repressed the proliferation and induced the apoptosis of gastric cancer cells.

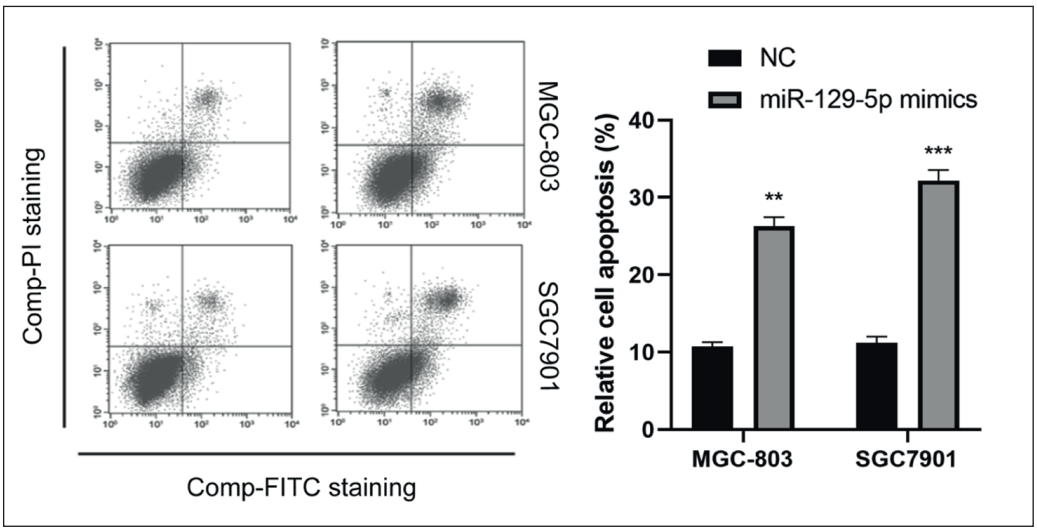

Figure 5. Overexpression of miR-129-5p facilitates the apoptosis of gastric cancer cells. The apoptosis rate of MGC-803 and SGC7901 cells transfected with miR-129-5p and control plasmids is detected *via* flow cytometry. The results show that the apoptosis rate is markedly elevated in gastric cancer cells compared with control group (**p<0.01: a significant difference, ***p<0.001: an extremely significant difference).

HMGB1 Was a Target Of Mir-129-5p in Gastric Cancer

To further discover the mechanism of miR-129-5p in regulating malignant behaviors of gastric cancer cells, the binding targets of miR-129-5p were predicted using the miRDB database. It was found that there were binding sites of HMGB1 3'-UTR to miR-129-5p. To further verify the prediction results, HMGB1 wild-type or mutant 3'-UTR Luciferase reporter vectors were then constructed. Meanwhile, MGC-803 and SGC7901 cells were transfected with miR-129-5p or negative control miRNA and Luciferase reporter vectors, respectively. Subsequent results showed that overexpressing miR-129-5p significantly reduced the Luciferase activity of wild-type HMGB1 (p<0.001) (Figure 6A). However, no significant effect was observed on mutant HMGB1 (Figure 6B). Next, Western blotting was adopted to measure the protein expression level of HMGB1 in MGC-803 and SGC7901 cells transfected with miR-129-5p or negative control miRNA (Figure 6C). The results uncovered that overexpression of miR-129-5p significantly down-regulated the protein expression level of HMGB1 in gastric cancer cells. Besides, the mRNA level of HMGB1 in cells transfected with miR-129-5p also decreased remarkably (Figure 6D).

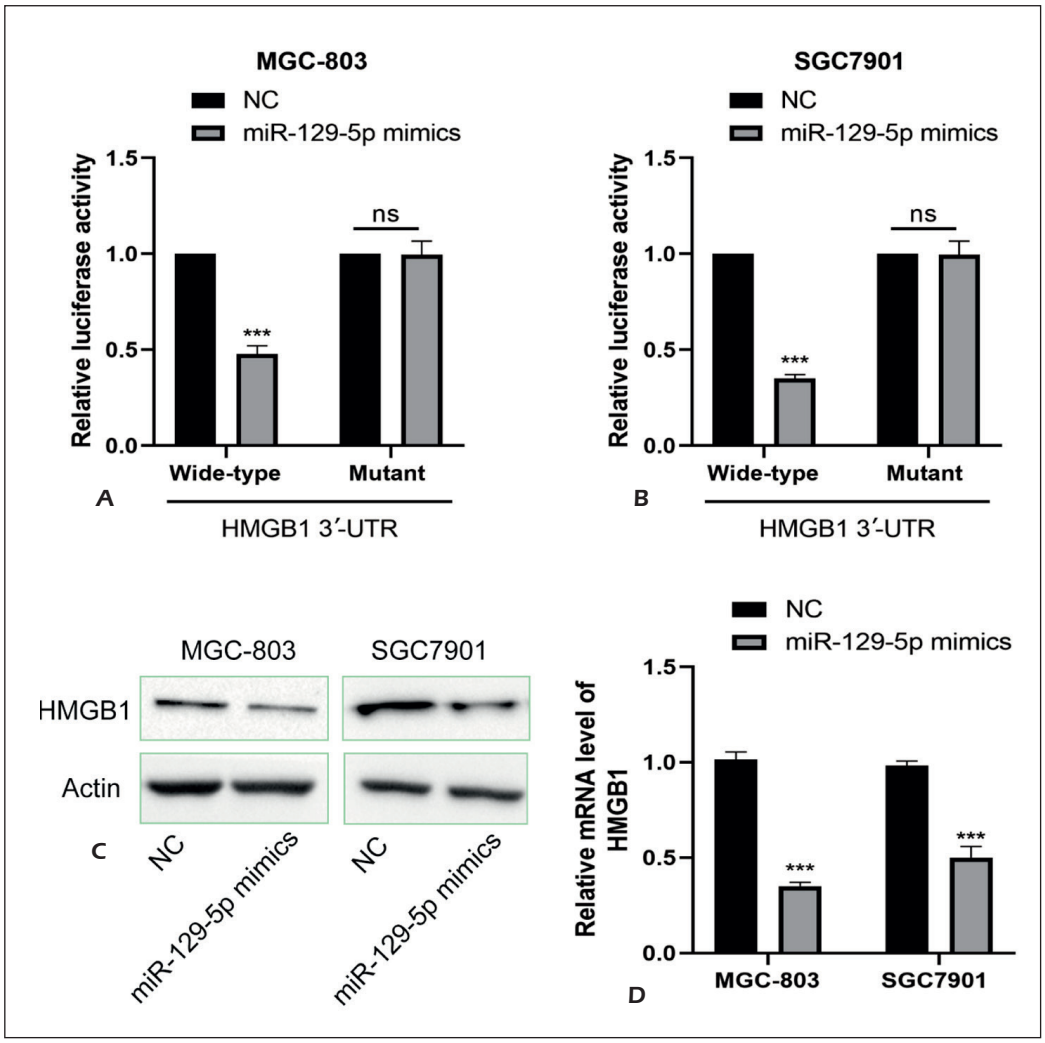

Figure 6. HMGB1 is a target of miR-129-5p in gastric cancer. **A**, In MGC-803 cells, overexpressing miR-129-5p markedly reduces the Luciferase activity of wild-type HMGB1 (p<0.001), but has no significant effect on mutant HMGB1. **B**, In SGC7901 cells, miR-129-5p overexpression overtly lowers the Luciferase activity of wild type HMGB1 (p<0.001), but has no significant effect on mutant type. **C**, MGC-803 and SGC7901 cells transfected with miR-129-5p or negative control miRNA. Western blotting indicates that overexpression of miR-129-5p significantly decreases the protein expression level of HMGB1 in gastric cancer cells. **D**, The mRNA level of HMGB1 in MGC-803 and SGC7901 cells is also down-regulated after transfection of miR-129-5p (p<0.001).

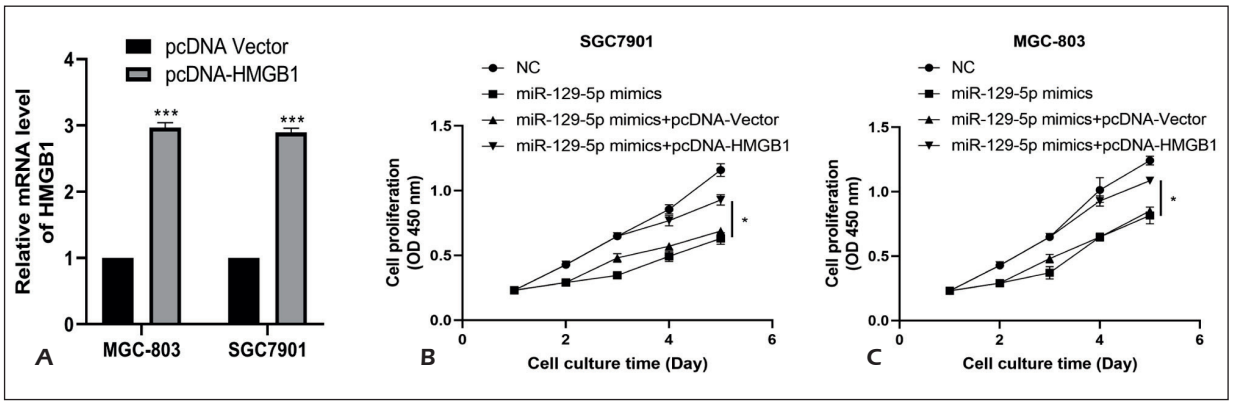

Figure 7. HMGB1 overexpression overtly reverses the inhibitory effect of miR-129-5p on the growth of gastric cancer cells (**A**, Expression level of HMGB1 in MGC-803 and SGC7901 cells measured *via* RT-qPCR, and **B**, and **C**, proliferation of MGC-803 and SGC7901 cells transfected with pcDNA-HMGB1 determined through CCK-8 assay.). HMGB1 overexpression offsets the inhibitory effect of miR-129-5p on the proliferation of gastric cancer cells, and the proliferation capacity of cells rises again.

HMGB1 Overexpression Overtly Reversed the Inhibitory Effect of MiR-129-5p on the Growth of Gastric Cancer Cells

To determine whether HMGB1 mediated the regulatory effect of miR-129-5p on the proliferation of gastric cancer cells, both MGC-803 and SGC7901 cells were transfected with pcDNA-HMGB1 or pcDNA-vector. The expression level of HMGB1 therein was measured *via* RT-qPCR, and cell proliferation was determined by CCK-8 assay (Figure 7A). It was discovered that HMGB1 overexpression markedly reversed the inhibitory effect of miR-129-5p on the proliferation of gastric cancer cells (Figure 7B and 7C). All these findings suggested that the miR-129-5p/HMGB1 axis was a key player in regulating the growth of gastric cancer cells.

Discussion

Cancer has always been a great threat to human society, bringing huge economic burden and suffering to humans. Gastric cancer is one of the most common cancers worldwide. Latest global cancer statistics have shown there are 1.03 million patients with gastric cancer in 2019, ranking fifth among all cancers. As the most common malignancy of the digestive system, morbidity and mortality rates of gastric cancer are relatively high. Therefore, understanding the molecular mechanism of gastric cancer is of great significance to discover new therapeutic targets. MiRNAs are endogenous inhibitors of genes, which can bind to 3'-UTR to trigger translation inhibition or mRNA degradation. MiRNAs have been confirmed to be closely correlated with the development and progression of cancers. In gastric cancer, various carcinogenic miRNAs or tumor suppressor miRNAs are deregulated, which play important roles in the proliferation, apoptosis and invasion of tumor cells. In this study, it was discovered that miR-129-5p was significantly downregulated in gastric cancer tissues and cell lines. Down-regulation of miR-129-5p was closely related to tumor enlargement, increased tumor stage and lymph node metastasis. Our findings provided new insights into the vital role of miR-129-5p in the progression of gastric cancer. MiRNAs act as oncogenes or tumor suppressor genes in the development and progression of cancers in humans. MiRNAs may be promising targets in the treatment of malignancies. MiR-1179 is lowly expressed in glioblastoma, which can also inhibit the proliferation of glioblastoma cells by targeting E2F5. Moreover, miR-1179 represses the proliferation of gastric cancer cells by targeting HMGB1. Therefore, it is necessary to further discover the inhibitory effect of miR-129-5p on the progression of gastric cancer, and to verify the inhibitory effect of miR-129-5p on the malignant behavior of gastric cancer *in vivo*.

As a protein in the high mobility group box superfamily, HMGB1 is a chromatin component common in mammalian cells and it plays an important role in various cellular processes, such as inflammation, cell differentiation, tumor cell migration, as well as transcriptional regulation[23]. HMGB1 is of great importance in the progression of cancers by regulating the transcription of cancer-related genes. Overexpression of HMGB1 is associated with poor

prognosis of patients with cancers, which may be a promising biomarker for survival prediction. Furthermore, HMGB1 can also be a target for miRNAs to interfere in the progression of cancers[27]. MiR-1284 enhances the sensitivity of cervical cancer cells to cisplatin by down-regulating HMGB1. HMGB1 is a target of miR-505 in hepatocellular carcinoma, which enhances doxorubicin-induced cytotoxicity[28]. It has also been observed[29] that miR-505 exerts a suppressive effect on gastric cancer by targeting HMGB1. Wu et al[30] have denoted that miR-449a targets HMGB1 to repress the migration and invasion of non-small cell lung cancer. Therefore, the potential regulatory effects of miR-449a and HMGB1 in gastric cancer may be interesting topics worthy of further study. In this research, it was uncovered that miR-129-5p could bind to the 3'-UTR of HMGB1 and reduce the expression of HMGB1 in gastric cancer cells. Overexpressing HMGB1 reversed the inhibitory effect of miR-129-5p on the proliferation of gastric cancer cells. These results indicated that HMGB1 mediated the regulatory effect of miR-129-5p on the progression of gastric cancer. The function of miR-129-5p/HMGB1 axis in other types of cancer warrants further studies in the future. Blocking the expression of HMGB1 may inhibit cancer progression. However, the repression of miR-129-5p on the proliferation of gastric cancer cells through HMGB1 is rarely reported.

Conclusions

Taken all together, the results of this study miR-129-5p is lowly expressed in gastric cancer tissues and cell lines. Overexpressed miR-129-5p suppresses the proliferation and induces the apoptosis of gastric cancer cells by directly targeting HMGB1. The novelty of this research was it uncovered the molecular mechanism of miR-129-5p in modulating the progression of gastric cancer, making miR-129-5p a potential target in newly developed treatment strategies for gastric cancer.

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Conflict of Interests

The authors declare that they have no conflict of interests.

References

1) Zheng Y, Zhu XQ, Ren XG. Third-line chemotherapy in advanced gastric cancer: a systematic review and meta-analysis. Medicine (Baltimore) 2017; 96: e6884.
2) Coburn N, Cosby R, Klein L, Knight G, Malthaner R, Mamazza J, Mercer CD, Ringash J. Staging and surgical approaches in gastric cancer: a systematic review. Cancer Treat Rev 2018; 63: 104-115.
3) Shimizu D, Kanda M, Kodera Y. Review of recent molecular landscape knowledge of gastric cancer. Histol Histopathol 2018; 33: 11-26.
4) Orditura M, Galizia G, Sforza V, Gambardella V, Fabozzi A, Laterza MM, Andreozzi F, Ventriglia J, Savastano B, Mabilia A, Lieto E, Ciardiello F, De Vita F. Treatment of gastric cancer. World J Gastroenterol 2014; 20: 1635-1649.
5) Song Z, Wu Y, Yang J, Yang D, Fang X. Progress in the treatment of advanced gastric cancer. Tumour Biol 2017; 39: 1393375038.
6) Mohr AM, Mott JL. Overview of microRNA biology. Semin Liver Dis 2015; 35: 3-11.
7) Fabian MR, Sonenberg N, Filipowicz W. Regulation of mRNA translation and stability by microRNAs. Annu Rev Biochem 2010; 79: 351-379.
8) Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 2004; 116: 281-297.
9) Ambros V. The functions of animal microRNAs. Nature 2004; 431: 350-355.
10) Xie M, Ma L, Xu T, Pan Y, Wang Q, Wei Y, Shu Y. Potential regulatory roles of microRNAs and long noncoding RNAs in anticancer therapies. Mol Ther Nucleic Acids 2018; 13: 233-243.
11) Kwak PB, Iwasaki S, Tomari Y. The microRNA pathway and cancer. Cancer Sci 2010; 101: 2309-2315.
12) Farazi TA, Spitzer JI, Morozov P, Tuschl T. MiRNAs in human cancer. J Pathol 2011; 223: 102-115.
13) Qu H, Xu W, Huang Y, Yang S. Circulating miRNAs: promising biomarkers of human cancer. Asian Pac J Cancer Prev 2011; 12: 1117-1125.
14) Gentilin E, Degli UE, Zatelli MC. Strategies to use microRNAs as therapeutic targets. Best Pract Res Clin Endocrinol Metab 2016; 30: 629-639.
15) Yu H, Zhang J, Wen Q, Dai Y, Zhang W, Li F, Li J. MicroRNA-6852 suppresses cell proliferation and invasion via targeting forkhead box J1 in gastric cancer. Exp Ther Med 2018; 16: 3249-3255.
16) Xu J, Wang F, Wang X, He Z, Zhu X. MiRNA-543 promotes cell migration and invasion by targeting SPOP in gastric cancer. Onco Targets Ther 2018; 11: 5075-5082.
17) Park SK, Park YS, Ahn JY, Do EJ, Kim D, Kim JE, Jung K, Byeon JS, Ye BD, Yang DH, Park SH, Hwang SW, Jung HY, Myung SJ. MiR 21-5p as a predictor of recurrence in young gastric cancer patients. J Gastroenterol Hepatol 2016; 31: 1429-1435.
18) Kao HW, Pan CY, Lai CH, Wu CW, Fang WL, Huang KH, Lin WC. Urine miR-21-5p as a potential

non-invasive biomarker for gastric cancer. Oncotarget 2017; 8: 56389-56397.

19) Li Q, Li B, Li Q, Wei S, He Z, Huang X, Wang L, Xia Y, Xu Z, Li Z, Wang W, Yang L, Zhang D, Xu Z. Exosomal miR-21-5p derived from gastric cancer promotes peritoneal metastasis via mesothelial-to-mesenchymal transition. Cell Death Dis 2018; 9: 854.

20) Song L, Dai Z, Zhang S, Zhang H, Liu C, Ma X, Liu D, Zan Y, Yin X. MicroRNA-1179 suppresses cell growth and invasion by targeting sperm-associated antigen 5-mediated Akt signaling in human non-small cell lung cancer. Biochem Biophys Res Commun 2018; 504: 164-170.

21) Chen J, Li G. MiR-1284 enhances sensitivity of cervical cancer cells to cisplatin via downregulating HMGB1. Biomed Pharmacother 2018; 107: 997-1003.

22) Lu L, Zhang D, Xu Y, Bai G, Lv Y, Liang J. miR-505 enhances doxorubicin-induced cytotoxicity in hepatocellular carcinoma through repressing the Akt pathway by directly targeting HMGB1. Biomed Pharmacother 2018; 104: 613-621.

23) Kumari T, Kumar B. High-mobility group box 1 protein (HMGB1) gene polymorphisms and cancer susceptibility: a comprehensive meta-analysis. Clin Chim Acta 2018; 483: 170-182.

24) Huang CY, Chiang SF, Ke TW, Chen TW, Lan YC, You YS, Shiau AC, Chen WT, Chao K. Cytosolic high-mobility group box protein 1 (HMGB1) and/or PD-1+ TILs in the tumor microenvironment may be contributing prognostic biomarkers for patients with locally advanced rectal cancer who have undergone neoadjuvant chemoradiotherapy. Cancer Immunol Immunother 2018; 67: 551-562.

25) Xu Y, Chen Z, Zhang G, Xi Y, Sun R, Chai F, Wang X, Guo J, Tian L. HMGB1 overexpression correlates with poor prognosis in early-stage squamous cervical cancer. Tumour Biol 2015; 36: 9039-9047.

26) Wu T, Zhang W, Yang G, Li H, Chen Q, Song R, Zhao L. HMGB1 overexpression as a prognostic factor for survival in cancer: a meta-analysis and systematic review. Oncotarget 2016; 7: 50417-50427.

27) Chen J, Li G. MiR-1284 enhances sensitivity of cervical cancer cells to cisplatin via downregulating HMGB1. Biomed Pharmacother 2018; 107: 997-1003.

28) Lu L, Zhang D, Xu Y, Bai G, Lv Y, Liang J. MiR-505 enhances doxorubicin-induced cytotoxicity in hepatocellular carcinoma through repressing the Akt pathway by directly targeting HMGB1. Biomed Pharmacother 2018; 104: 613-621.

29) Tian L, Wang ZY, Hao J, Zhang XY. MiR-505 acts as a tumor suppressor in gastric cancer progression through targeting HMGB1. J Cell Biochem 2018 Dec 7. doi: 10.1002/jcb.28082. [Epub ahead of print]

30) Wu D, Liu J, Chen J, He H, Ma H, Lv X. MiR-449a suppresses tumor growth, migration, and invasion in non-small cell lung cancer by targeting a HMGB1-mediated NF-kappaB signaling pathway. Oncol Res 2019; 27: 227-235.