European Review for Medical and Pharmacological Sciences 2020; 24: 1177-1185

MicroRNA-625-3p inhibits gastric cancer metastasis through modulating EZH2

Y. LI[1], H.-C. ZHOU[2], Y. ZHANG[1], H. HUANG[3]

[1]Department of Clinical Laboratory, The Fourth Hospital, Xi'an, China
[2]Department of Clinical Laboratory, Taikang Xianlin Drum Tower Hospital, Nanjing, China
[3]Department of Clinical Laboratory, Xijing Hospital, Xi'an, China

Abstract. – OBJECTIVE: The aim of this study was to investigate whether microRNA-625-3p participated in the malignant progression of gastric cancer and inhibited GCa metastasis by regulating EZH2 (Enhancer of zeste homolog 2).

PATIENTS AND METHODS: Quantitative Real Time-Polymerase Chain Reaction (qRT-PCR) was performed to examine the expression of microRNA-625-3p in 36 pairs of GCa tissues and para-cancerous tissues. The interplay between microRNA-625-3p level and clinical indexes or prognosis of GCa patients was analyzed. MicroRNA-625-3p mimics and inhibitors, as well as their negative controls, were transfected into GCa cell lines to establish microRNA-625-3p overexpression and down-regulation models in vitro, respectively. QRT-PCR was applied to further verify the transfection efficiency. Cell counting kit-8 (CCK-8), colony formation, and transwell assays were performed to analyze the impact of microRNA-625-3p on the proliferative and invasiveness abilities of GCa AGS and SGC-7901 cells. Finally, the regulatory mechanism of microRNA-625-3p on the downstream gene EZH2 was explored by cell reverse experiment.

RESULTS: QRT-PCR results revealed that microRNA-625-3p expression level in GCa tissues was remarkably lower than that of adjacent tissues, and the difference was statistically significant ($p<0.05$). Compared with patients with high expression of microRNA-625-3p, the incidence of lymph node or distant metastasis was significantly higher in patients with low expression of miR-625-3p, whereas the overall survival rate was lower ($p<0.05$). Compared with GCa cells in NC inhibitor group, the proliferative ability and invasiveness of cells in microRNA-625-3p inhibitor group were remarkably promoted ($p<0.05$). However, the opposite results were observed in microRNA-625-3p mimics group. Our findings further demonstrated that the expression of EZH2 increased remarkably in GCa cell lines and tissues ($p<0.05$). Meanwhile, its expression level was negatively correlated with microRNA-625-3p level. Cell reverse experiment showed that EZH2 could offset the influence of microRNA-625-3p on the proliferation and metastasis GCa cells, thereby affecting the malignant progression of GCa.

CONCLUSIONS: MicroRNA-625-3p was remarkably correlated with lymph node or distant metastasis and poor prognosis of GCa patients. In addition, microRNA-625-3p might inhibit the malignant progression of GCa via modulating EZH2.

Key Words:
MicroRNA-625-3p, EZH2, Gastric cancer (GCa), Metastasis.

Introduction

Currently, the incidence and mortality of gastric cancer (GCa) remain high worldwide, seriously threatening human health. GCa ranks fourth among all malignant tumors, whose mortality ranks second among all cancer death rates, second only to lung cancer[1-3]. GCa is a malignant tumor originating from gastric mucosa, most of which is adenocarcinoma. It has been confirmed that the onset of GCa is concealed, with no evident symptoms in the early stage. A large number of patients are already in advanced stage when diagnosed, leading to poor prognosis[4-6]. Due to the lack of early diagnosis, the poor effect of treatment, and high incidence of relapse and metastasis in GCa, as well as the overall survival of these patients is far from satisfactory[6,7]. Therefore, there is an urgent need to search for biomarkers and therapeutic targets for early diagnosis and clinical treatment of GCa[8,9].

MicroRNA (miRNA) is a kind of small RNA belonging to non-coding RNA. It has about 22 nucleotides in length and is involved in post-transcriptional regulation. MiRNA can bind to specific target sites, resulting in the degradation of target mRNAs or inhibition of target gene

Corresponding Author: Hai Huang, MM; e-mail: 38647846@qq.com

translation[10,11]. MiRNAs regulate the expression of a variety of tumor-associated genes in cells. Meanwhile, they play a pivotal role in regulating cell cycle, apoptosis, cell differentiation, tissue inflammatory response, and tumor invasion and metastasis[12,13]. In recent years, the occurrence and development of tumors have been found accompanied by abnormal expression of certain specific miRNAs. However, the types of miRNAs in different tumor tissues are different. Even in the same tumor, the types and expressions of miRNAs in different stages of disease development are not the same[14,15]. Therefore, according to the tissue and time specificity of miRNA, miRNA is expected to be a molecular marker for early diagnosis various of malignant tumors. In addition, it has been pointed out that microRNA-625-3p can be used as an important targeted therapy for malignant tumors[16,17]. Enhancer of zeste homolog 2 (EZH2) is a human homolog of the Drosophila zeste gene enhancer, which is also an important member of the Polycomb group gene family. EZH2 is the 27th lysine-specific methyltransferase of histone H3[18-20]. Through miRNA database prediction, it has been found that EZH2 may be the target gene of microRNA-625-3p.

In this study, we first detected the expression level and potential function of microRNA-625-3p in GCa tissues and cell lines. The expression and functions of its target gene in GCa were investigated as well. Our findings aimed to lay an important theoretical foundation for the early diagnosis and molecular monitoring of GCa.

Patients and Methods

Patients and GCa Samples

In this study, 36 pairs of invasive GCa tumor tissues and corresponding adjacent tissues were collected from surgically treated GCa patients. Collected tissues were, stored at –80°C for use. The collection of clinical specimens was approved by the Ethics Monitoring Committee. Informed consent was obtained from patients and their families before the study.

Cell Lines and Reagents

Human GCa cell lines (including AGS, BGC-823, MKN45, HGC-27, and SGC-7901) and immortalized normal gastric mucosal epithelial cell line (GES-1) were purchased from ATCC (American Type Culture Collection; Manassas, VA, USA). All cells were cultured in DMEM (Dulbecco's Modified Eagle's medium) high glucose medium (Gibco, Rockville, MD, USA) containing 10% fetal calf serum (Gibco, Rockville, MD, USA), penicillin (100 U/mL), and streptomycin (100 μg/mL), and maintained in a 37°C, 5% CO_2 incubator. Cell passage was performed with 1% trypsin + ethylenediaminetetraacetic acid (EDTA) when grown to 80%-90% of confluence.

Cell Transfection

MicroRNA-625-3p inhibitors, microRNA-625-3p mimics, and their corresponding negative controls were purchased from Shanghai Jima Company (Shanghai, China). Cells were first plated into 6-well plates and grown to a cell density of 70%. Cell transfection was performed according to the manufacturer's instructions of Lipofectamine 3000 (Invitrogen, Carlsbad, CA, USA). After 48 h, transfected cells were collected for quantitative Real-Time Polymerase Chain Reaction (qRT-PCR) and cell functional experiments.

Cell Counting Kit-8 (CCK-8) Assay

48 h after transfection, cells were harvested and plated into 96-well plates at a density of 2000 cells per well. After culture for 24, 48, 72, and 96 h, respectively, CCK-8 (Dojindo Laboratories, Kumamoto, Japan) reagent was added in each well, followed by incubation for 2 h in the dark. Optical density (OD) at the absorption wavelength of 490 nm was detected by a micro-plate reader.

Colony Formation Assay

After 48 h of transfection, the cells were collected and seeded into 6-well plates, with 200 cells per well. Then, the cells were cultured in complete medium for 2 weeks. Culture medium was changed after one week and then twice a week. The medium should not be replaced as much as possible in the previous week to avoid cell adhesion. After 2 weeks, formed colonies were fixed with 2 mL of methanol for 20 min and stained with 0.1% crystal violet staining solution for 20 min, followed by washing 3 times with phosphate-buffered saline (PBS). Finally, formed colonies were photographed under a light-selective environment, and the number of colonies was counted.

Transwell Assay

48 h after transfection, the cells were digested, centrifuged, and resuspended in serum-free medium. The density of cells was adjusted to 5 x 10^5 cells/mL. 200 uL of cell suspension (1 x 10^5 cells) was added to the upper chamber. Meanwhile, 700

uL of complete medium containing 20% fetal bovine serum (FBS) was added to the lower chamber. According to different migration abilities, the cells were put back into the incubator and cultured for a specific time. After washing 3 times with 1 x PBS, the cells were fixed with methanol for 15 min and stained with 0.2% crystal violet for 20 min. Cells on the upper surface of the chamber were carefully wiped off with a cotton swab. Perforated cells stained in the outer layer of the basement membrane of the chamber were observed under a microscope. 5 fields of view were randomly selected for each sample.

qRT-PCR

1 mL of TRIzol (Invitrogen, Carlsbad, CA, USA) was used to extract total RNA in tissues and cells. Subsequently, the initially extracted RNA was treated with DNase I to remove genomic DNA and repurify the RNA. RNA reverse transcription was performed according to the instructions of Prime Script Reverse Transcription Kit (TaKaRa, Otsu, Shiga, Japan). Real-time PCR was performed in accordance with SYBR® Premix Ex Taq™ (TaKaRa, Otsu, Shiga, Japan). PCR reaction was performed using the StepOne Plus Real-time PCR System (Applied Biosystems, Foster City, CA, USA). Three replicates were set for each sample, and the assay was repeated twice. Bio-Rad PCR instrument was used to analyze and process the data with software iQ5 2.0 (Bio-Rad, Hercules, CA, USA). β-actin and U6 genes were used as internal references for mRNAs and miRNAs, respectively. Gene expression was calculated by the $2^{-\Delta\Delta Ct}$ method. Primer sequences used in this study were as follows: miR-625-3p, F: 5'-CGGACAGCGGCTACGTGCTA-3', R: 5'-GGCAGCATCTGTTCGCAGCC-3'; EZH2, F: 5'-GCCATTATGGACATCGCGGTGC-3', R: 5'-ACTTCGCGCTCGCGTGCCG-3'; U6: F: 5'-CTCGCTTCGGCAGCACA-3', R: 5'-AACGCTTCACGAATTTGCGT-3'; β-actin: F: 5'-CCTGGCACCCAGCACAAT-3', R: 5'-GCTGATCCACATCTGCTGGAA-3'.

Luciferase Reporter Gene Assay

A reporter plasmid was first constructed, in which a specific fragment of the target promoter was inserted in front of the luciferase expression sequence. Subsequently, the transcription factor expression plasmid to be detected was co-transfected with the reporter plasmid into GCa cell lines. If the transcription factor could activate the target promoter, the luciferase gene would be expressed. Meanwhile, the amount of luciferase expression was directly proportional to the intensity of the transcription factor. After the specific luciferase substrate was added, luciferase could react with the substrate to generate fluorescence. By measuring the intensity of the fluorescence, the activity of the luciferase could be used to determine whether the transcription factor interacted with target promoter fragment.

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 22. 0 software (IBM, Armonk, NY, USA) was used for all statistical analysis. Univariate analysis was performed using χ^2-test and exact probability Fisher test. Multivariate analysis was performed using COX regression analysis. Survival analysis was performed using the Kaplan-Meier method. Intergroup curves were compared using the Log-rank test. Experimental data were expressed as mean ± standard deviation. $p<0.05$ was considered statistically significant.

Results

MicroRNA-625-3p Was Down-Regulated in GCa Tissues, and Was Correlated with Advanced Clinical Stage and Poor Prognosis of GCa

First, we detected the expression of microRNA-625-3p in GCa tissues and cell lines by qRT-PCR. The results showed that microRNA-625-3p level was remarkably lower in GCa tissues than that of adjacent tissues (Figure 1A). In addition, microRNA-625-3p was lowly expressed in GCa cell lines compared with GES-1 cells, and the difference was statistically significant (Figure 1B). According to the expression of microRNA-625-3p, GCa patients were divided into high expression group and low expression group. Subsequently, the relation between microRNA-625-3p expression and clinical features and prognosis of GCa patients was explored. As shown in Table I, low expression of microRNA-625-3p was positively correlated with lymph node metastasis (Figure 1C) and distant metastasis (Figure 1D), rather than age, gender, and pathological stage. This suggested that microRNA-625-3p might be a new biological indicator for predicting the malignant progression of GCa. In addition, Kaplan-Meier survival curve indicated that low expression of microRNA-625-3p was remarkably associated with poor prognosis of GCa patients ($p<0.05$; Figure 1E).

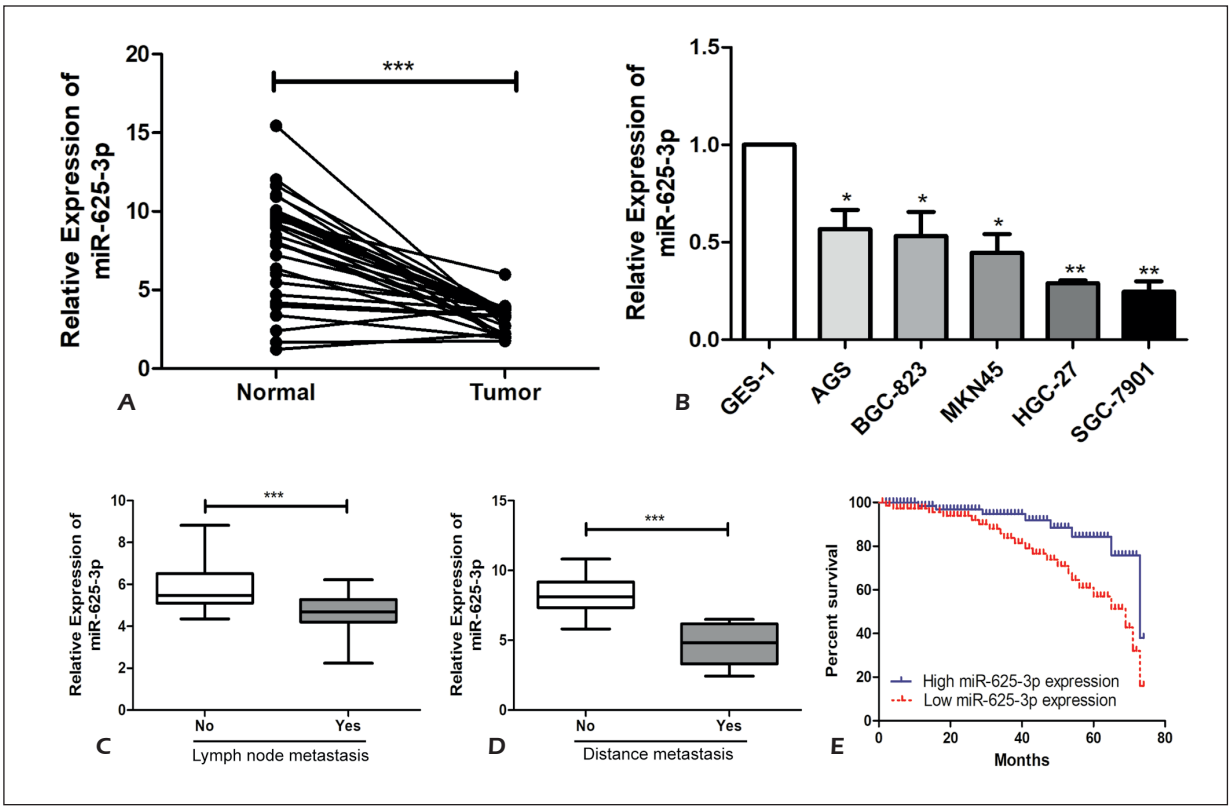

Figure 1. MiR-625-3p was lowly expressed in GCa tissues and cell lines. **A**, QRT-PCR was used to observe the differential expression of miR-625-3p in GCa tissues and adjacent tissues; **B**, QRT-PCR was used to detect the expression level of miR-625-3p in GCa cell lines; **C**, QRT-PCR was used to detect the expression level of miR-625-3p in GCa patients with or without lymph node metastasis; **D**, QRT-PCR was used to reveal the expression level of miR-625-3p in GCa patients with distant metastasis; **E**, Kaplan-Meier survival curve of GCa patients based on miR-625-3p expression. Data were expressed as mean ± SD, *p<0.05, **p<0.01, ***p<0.001.

Table I. Association of miR-625-3p expression with clinicopathologic characteristics of gastric cancer.

Parameters	No. of cases	MiR-625-3p expression		*p*-value
		High (%)	Low (%)	
Age (years)				0.587
<60	13	8	5	
≥60	23	12	11	
Gender				0.463
Male	17	11	6	
Female	19	10	9	
T stage				0.877
T1-T2	23	13	10	
T3-T4	13	7	6	
Lymph node metastasis				0.009
No	22	16	6	
Yes	14	4	10	
Distance metastasis				0.009
No	24	17	7	
Yes	12	3	9	

Up-regulation/Down-regulation of MicroRNA-625-3p Inhibited/Promoted Cell Proliferation and Metastasis

To explore the effect of microRNA-625-3p on the cytological function of GCa, microRNA-625-3p mimics model was first constructed *in vitro*. Transfection efficacy was verified by qRT-PCR (Figure 2A). Subsequently, cell proliferation, plate cloning, and transwell experiments were performed in AGS and SGC-7901 cell lines, respectively. The results showed that the proliferative, invasion, and migration abilities of AGS cells in microRNA-625-3p inhibitor group were remarkably promoted when compared with cells in NC inhibitor group. Similarly, the proliferative, invasion, and migration abilities of SGC-7901 cells in microRNA-625-3p mimics group were remarkably inhibited compared with cells in NC mimics group (Figure 2B-2D). These results suggested that miR -625-3p regulated the proliferation and metastasis of GCa cells.

EZH2 Was Highly Expressed in GCa Tissues and Cell Lines

To further explore the ways in which microRNA-625-3p promoted the malignant progression of GCa, TargetScan, miRbase, and MiRcode databases were searched to assess the mutual expression of miRNAs and mRNA. Bioinformatics predicted that there might be a certain association between microRNA-625-3p and EZH2 in GCa (Figure 3A). To further verify the targeting effect of microRNA-625-3p on EZH2, microRNA-625-3p and EZH2 were co-transfected into AGS and SGC-7901 cell lines. Subsequent luciferase reporter gene assay demonstrated that

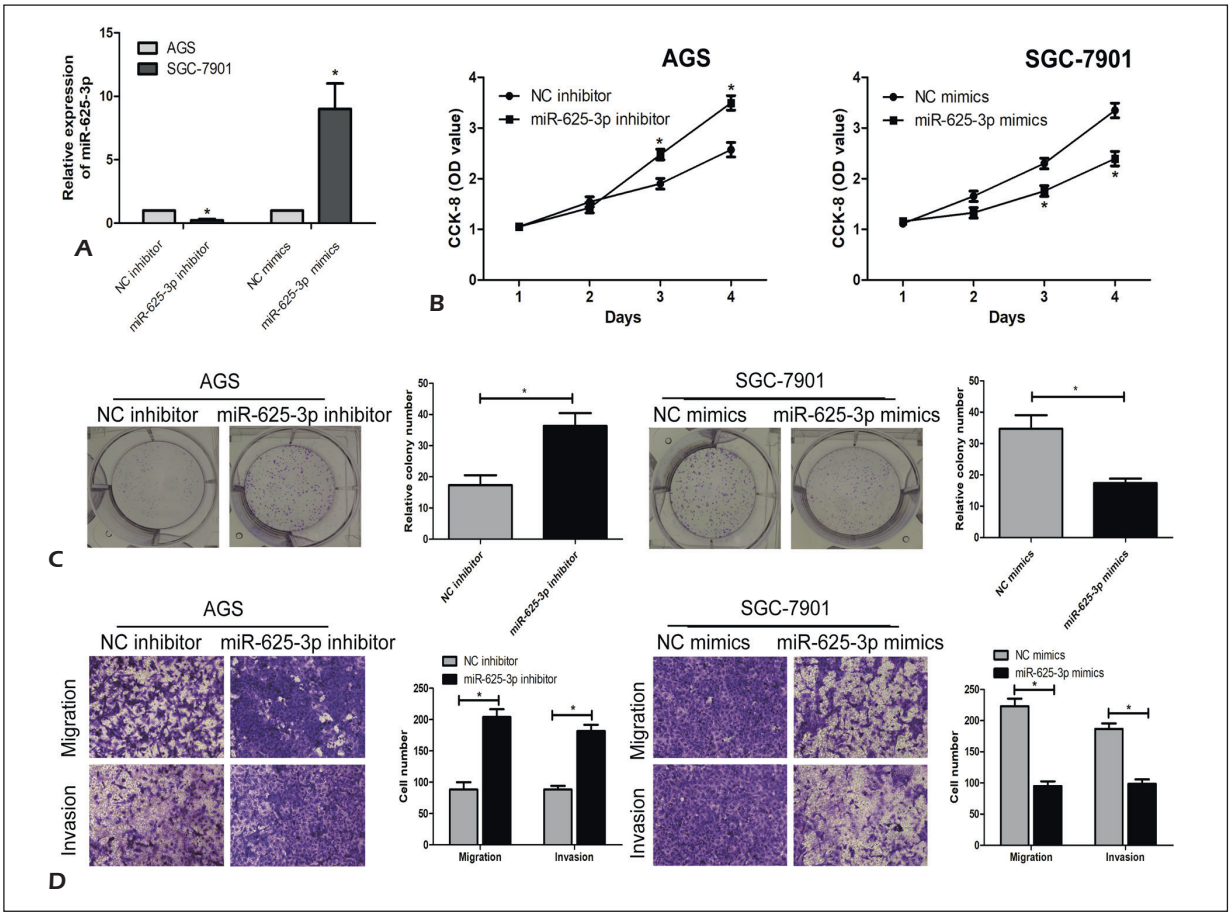

Figure 2. MiR-625-3p mimics/inhibitor inhibited/promoted GCa cell proliferation, invasion and migration. **A**, QRT-PCR verified the transfection efficiency of miR-625-3p after transfection of miR-625-3p in AGS and SGC-7901 cell lines; **B**, CCK-8 assay detected the effect of miR-625-3p on the proliferation of AGS and SGC-7901 cell lines; **C**, Colony formation assay was used to observe the effect of miR-625-3p on the colony formation ability of AGS and SGC-7901 cell lines (magnification x 10); **D**, Transwell assay disclosed the effect of miR-625-3p on the invasion and migration of AGS and SGC-7901 cell lines (magnification x 20). Data were expressed as mean ± SD, **p*<0.05.

microRNA-625-3p could be targeted by EZH2 through this binding site (Figure 3B). In addition, the expression level of EZH2 decreased remarkably after overexpression of microRNA-625-3p in GCa cell lines. Meanwhile, the expression level of EZH2 was significantly up-regulated after silencing microRNA-625-3p (Figure 3C). Besides, qRT-PCR showed that microRNA-625-3p expression was negatively correlated with EZH2 expression in GCa tissues (Figure 3D).

MicroRNA-625-3p Exactly Inhibited EZH2 Expression

To further explore the way in which microRNA-625-3p inhibited the malignant progression of GCa, EZH2 was silenced in AGS cells transfected with NC inhibitor and microRNA-625-3p inhibitor, and was overexpressed in SMC-7901 cells transfected with NC mimics and microRNA-625-3p mimics, respectively. Subsequently, the role of microRNA-625-3p and EZH2 in GCa was investigated. QRT-PCR was used to verify the transfection efficiency of EZH2 (Figure 4A). CCK-8 assay, colony formation, and transwell assays showed that EZH2 was able to counteract the effect of microRNA-625-3p on the proliferation, invasiveness, and migration of GCa cells (Figure 4B-4C). In sum, microRNA-625-3p regulated the proliferation and migration of GCa cells *via* inhibiting EZH2 expression.

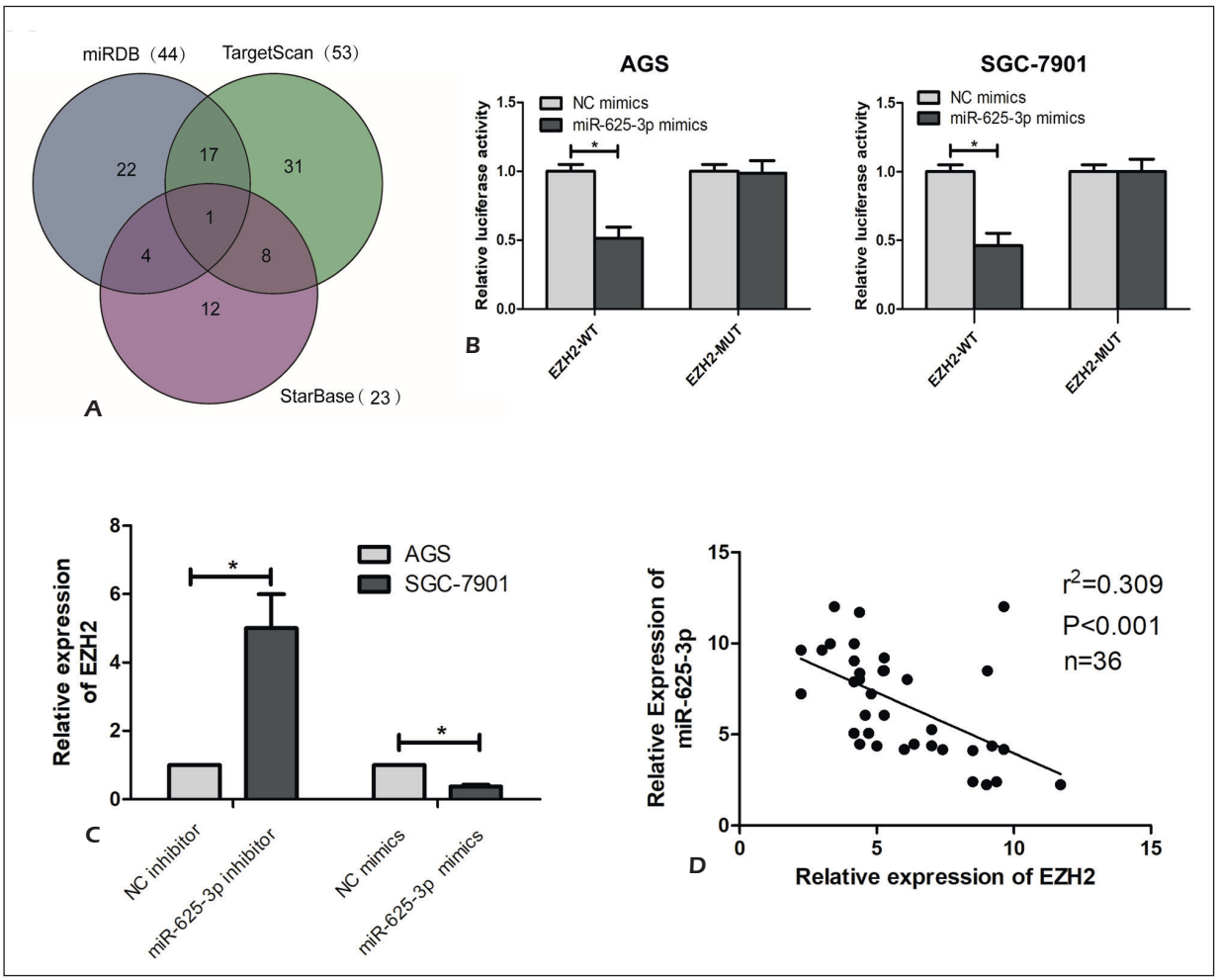

Figure 3. EZH2 was highly expressed in GCa tissues and cell lines. **A**, TargetScan, miRbase, and MiRcode suggested that miR-625-3p could target miR-625-3p; **B**, Dual-Luciferase reporter gene assay verified the direct targeting of miR-625-3p and EZH2; **C**, QRT-PCR revealed the expression level of EZH2 after transfection of miR-625-3p in AGS and SGC-7901 cell lines; **D**, There was a significant negative correlation between miR-625-3p and EZH2 expression in GCa tissues. Data were expressed as mean ± SD, *p<0.05, **p<0.01, ***p<0.001.

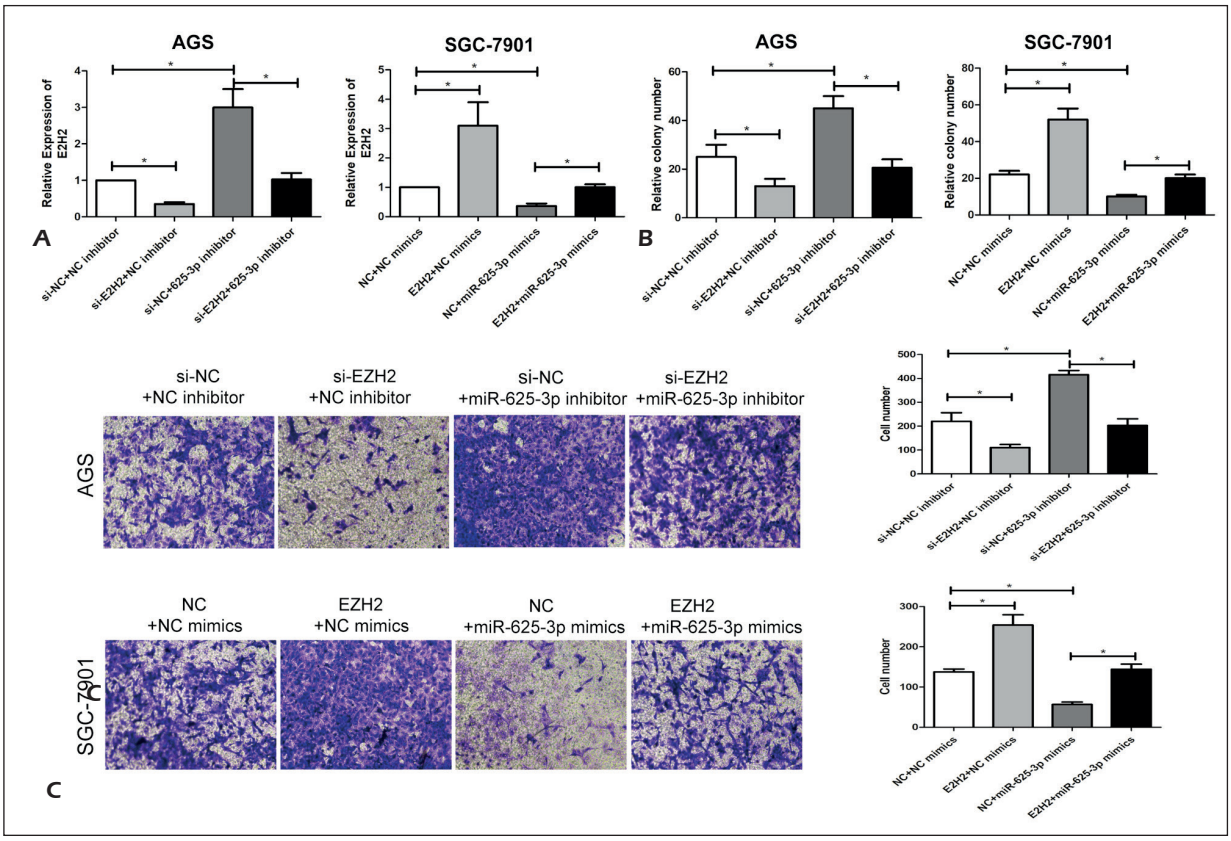

Figure 4. MiR-625-3p regulated EZH2 inhibition of gastric carcinogenesis. **A**, EZH2 expression level in miR-625-3p and EZH2 co-transfected cell lines was detected by qRT-PCR; **B**, Colony formation assay detected the proliferation of GCa cells after co-transfection of miR-625-3p and EZH2; **C**, Transwell assay verified the invasion and migration of GCa cells after co-transfection of miR-625-3p and EZH2 (magnification X 20). Data were expressed as mean ± SD, *$p<0.05$.

Discussion

GCa is a malignant tumor originating from gastric mucosa, whose incidence remains highest among various malignant tumors in China[1-3]. The incidence of GCa is remarkably higher in men than that in women (the incidence of GCa in men is about twice that of women). Meanwhile, it is remarkably higher in developing countries and rural areas than in developed countries and urban areas[4,5]. China is a country with high GCa incidence and mortality, showing an upward trend in recent years[5]. Currently, surgical treatment is the only way to cure GCa. However, the overall survival rate of GCa patients treated with surgery is only about 20%[4-6]. Therefore, clinical and surgical treatments combined with adjuvant chemotherapy and radiotherapy are often used to optimize the treatment strategy of patients with GCa[6,7]. Other treatments for GCa include traditional Chinese medicine treatment, immunotherapy, and targeted drug therapy. However, most of these treatments for GCa patients have limited efficacy due to the lack of effective therapeutic targets and low sensitivity to radiotherapy and chemotherapy drugs. Therefore, there is still an urgent need to discover new prediction strategies and therapeutic targets[7-9].

As one of the hottest indicators in recent years, miRNA plays an important regulatory role in various tumors. It is believed that miRNA maintains or regulates gene expression through post-transcriptional regulation, thus promoting physiological and pathological changes in the body[10,11]. Existing studies have shown that a single miRNA can regulate multiple target genes, while a single target gene can also be regulated by multiple miRNAs. This complex regulatory network allows miRNAs to play a pivotal role in gene expression[12-15]. Moreover, some miRNAs can be stably present in body fluids such as blood and urine, showing great potential as clinical diagnostic markers in the future[16,17]. To explore the role of microRNA-625-3p in the development of GCa, qRT-PCR was first used to detect the expression

of microRNA-625-3p in paired GCa tissues and adjacent tissues. The results indicated that the expression of microRNA-625-3p was remarkably down-regulated in GCa and was correlated with lymph node metastasis and distant metastasis. This suggested that microRNA-625-3p might act as a tumor suppressor gene in GCa. Subsequently, to further explore the effect of microRNA-625-3p on the biological function of GCa, microRNA-625-3p mimics model was constructed *in vitro*. CCK8, colony formation, and transwell experiments showed that microRNA-625-3p inhibited the proliferation, invasiveness, and migration of GCa cells, thus playing a pivotal role in GCa development.

In this study, we mainly focused on the role of the microRNA-625-3p/EZH2 family axis during the development and progression of GCa. The results suggested that the microRNA-625-3p/EZH2 axis might be a new diagnostic and therapeutic target for GCa. Consistent with other types of cancer research, EZH2 has been shown to be a key role in GCa suppression[18-20]. Subsequently, we performed a functional verification of the microRNA-625-3p/EZH2 interaction. QRT-PCR showed that microRNA-625-3p expression was negatively correlated with EZH2 expression in GCa tissues. In addition, we detected the expression level of EZH2 in GCa and adjacent tissues as well. QRT-PCR results found that the expression level of EZH2 in GCa tissues increased remarkably. After overexpression of microRNA-625-3p in GCa cells, the expression level of EZH2 was remarkably down-regulated. Meanwhile, the proliferation and invasiveness of GCa cells were significantly inhibited, which was similar to the phenotype of EZH2 silencing. All our findings suggested that the microRNA-625-3p/EZH2 adjustment shaft might play a pivotal role in the occurrence and development of GCa.

Conclusions

We first identified that microRNA-625-3p was closely related to lymph node metastasis, distant metastasis, and poor prognosis of GCa patients. Additionally, microRNA-625-3p inhibited malignant progression of GCa by regulating EZH2.

Funding acknowledgments

The study was granted by Shaanxi General Project of Scientific Development-Social Development (No. 2017SF-265).

Conflict of Interests

The Authors declared that they have no conflict of interests.

References

1) Choi YJ, Kim N. Gastric cancer and family history. Korean J Intern Med 2016; 31: 1042-1053.
2) Yu L, Zhou GQ, Li DC. MiR-136 triggers apoptosis in human gastric cancer cells by targeting AEG-1 and BCL2. Eur Rev Med Pharmacol Sci 2018; 22: 7251-7256.
3) Ren J, Liu J, Sui X. Correlation of COX-2 and MMP-13 expressions with gastric cancer and their effects on prognosis. J BUON 2018; 23: 665-671.
4) Ding Z, Jiang L, Zhang K, Huang R. Short- and long-term outcomes of conversion in laparoscopic gastrectomy for gastric cancer. J BUON 2018; 23: 1004-1012.
5) Xie H, Lu Q, Wang H, Zhu X, Guan Z. Effects of probiotics combined with enteral nutrition on immune function and inflammatory response in postoperative patients with gastric cancer. J BUON 2018; 23: 678-683.
6) Uno Y. Prevention of gastric cancer by Helicobacter pylori eradication: a review from Japan. Cancer Med 2019; 8: 3992-4000.
7) Song Z, Wu Y, Yang J, Yang D, Fang X. Progress in the treatment of advanced gastric cancer. Tumour Biol 2017; 39: 1010428317714626.
8) Tahara T, Arisawa T. DNA methylation as a molecular biomarker in gastric cancer. Epigenomics 2015; 7: 475-486.
9) Necula L, Matei L, Dragu D, Neagu AI, Mambet C, Nedeianu S, Bleotu C, Diaconu CC, Chivu-Economescu M. Recent advances in gastric cancer early diagnosis. World J Gastroenterol 2019; 25: 2029-2044.
10) Wang QX, Zhu YQ, Zhang H, Xiao J. Altered MiRNA expression in gastric cancer: a systematic review and meta-analysis. Cell Physiol Biochem 2015; 35: 933-944.
11) Shin VY, Chu KM. MiRNA as potential biomarkers and therapeutic targets for gastric cancer. World J Gastroenterol 2014; 20: 10432-10439.
12) Shirjang S, Mansoori B, Asghari S, Duijf P, Mohammadi A, Gjerstorff M, Baradaran B. MicroRNAs in cancer cell death pathways: apoptosis and necroptosis. Free Radic Biol Med 2019; 139: 1-15.
13) Subramaniam S, Jeet V, Clements JA, Gunter JH, Batra J. Emergence of microRNAs as key players in cancer cell metabolism. Clin Chem 2019; 65: 1090-1101.
14) Tang XJ, Wang W, Hann SS. Interactions among lncRNAs, miRNAs and mRNA in colorectal cancer. Biochimie 2019; 163: 58-72.
15) Ruggieri V, Russi S, Zoppoli P, La Rocca F, Angrisano T, Falco G, Calice G, Laurino S. The role of microRNAs in the regulation of gastric cancer stem cells: a meta-analysis of the current status. J Clin Med 2019; 8. pii: E639.

16) Bahiraee A, Ebrahimi R, Halabian R, Aghabozorgi AS, Amani J. The role of inflammation and its related microRNAs in breast cancer: a narrative review. J Cell Physiol 2019; 234: 19480-19493.
17) Micolucci L, Akhtar MM, Olivieri F, Rippo MR, Procopio AD. Diagnostic value of microRNAs in asbestos exposure and malignant mesothelioma: systematic review and qualitative meta-analysis. Oncotarget 2016; 7: 58606-58637.
18) Yamagishi M, Uchimaru K. Targeting EZH2 in cancer therapy. Curr Opin Oncol 2017; 29: 375-381.
19) Yan KS, Lin CY, Liao TW, Peng CM, Lee SC, Liu YJ, Chan WP, Chou RH. EZH2 in cancer progression and potential application in cancer therapy: a friend or foe? Int J Mol Sci 2017; 18. pii: E1172.
20) Kim KH, Roberts CW. Targeting EZH2 in cancer. Nat Med 2016; 22: 128-134.