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MicroRNA-524-5p suppresses cell proliferation and promotes cell apoptosis in gastric cancer by regulating CASP3

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Abstract. – OBJECTIVE: To investigate miR-524-5p expression in gastric cancer (GC) tissues and the regulatory mechanism of miR-524-5p on biological behaviors of GC cell lines, such as proliferation, cell apoptosis and cycle.

METHODS: The expression of miR-524-5p was detected in 48 paired of GC tissue samples and matched adjacent tissues, and also detected in GC cell lines by quantitative reverse transcription-polymerase chain reaction (qRT-PCR). Using miR-524-5p mimics, we analyzed the effects of overexpressed miR-524-5p on cell proliferation, cell apoptosis and cycle. Bioinformatics analysis, dual-luciferase activity assay and Western blot were recruited to validate the potential target gene of miR-524-5p.

RESULTS: The expression of miR-524-5p was significantly decreased in GC tissue samples and cell lines. Increased miR-524-5p in GC cells significantly decreased cell proliferation capacity, promoted cell apoptosis and induced cell cycle arrest at G0/G1 phase, while decreased miR-524-5p exerted the opposite effects. Dual-luciferase, qRT-PCR and Western blot confirmed CASP3 as a target gene of miR-524-5p. Furthermore, recovery of CASP3 expression attenuated the suppressive effect of miR-524-5p on cell growth.

CONCLUSIONS: MiR-524-5p is participated in the development of GC *via* regulating CASP3, which might provide a new prospect for GC diagnosis and therapy.

Key Words:
MiR-524-5p, Gastric cancer (GC), Proliferation, Apoptosis.

Introduction

Gastric cancer (GC) is one of the most common malignant tumors in the digestive system in the world, whose 5-year survival rate is lower than 25%. Studies have demonstrated that the mortality rate of GC ranks 2nd in the tumor[1,2]. The occurrence and development of GC is a multi-gene and multi-factor biological process, whose basic characteristic is the uncontrolled proliferation of GC cells[3]. Its primary cause is the destruction of regulatory mechanism of GC cell proliferation, including the inactivation of tumor suppressor genes, activation of proto-oncogenes and interaction of related metastasis genes, leading to the occurrence and development of GC[4,5]. Unfortunately, the early clinical symptoms of GC patients are not obvious, and there is a lack of biological indexes for early diagnosis of GC, so most patients have been complicated with metastatic middle-advanced GC when diagnosed[6]. The mechanism of the occurrence and development of GC has been reported in a large number of studies, but its pathophysiological changes at onset remain unclear. Therefore, understanding the biological mechanism of the occurrence and development of GC more deeply and searching new biological targets in targeted therapy are important breakthroughs in the early diagnosis and treatment of GC and the decline in its mortality rate. Micro ribonucleic acid (miRNA) is a group of endogenous conserved single-stranded non-coding RNA molecule with about 18-22 bases in length. Studies have demonstrated that miRNA exerts effects through binding to the 3'-untranslated region (3'UTR) of messenger RNA (mRNA), which can regulate the expression of one mRNA alone or regulate the expressions of multiple mRNAs simultaneously, inhibit the translation of mRNA or degrade mRNA, thereby affecting the cellular metabolism-related functions[7]. The discovery of

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functional diversity of miRNA is of important significance in studying its influence on the occurrence and development of cancer. MiRNA is involved in a series of biological behaviors, such as cell proliferation, metabolism, differentiation and apoptosis, in which miRNA also plays an important role[8,9]. Increasingly studies have demonstrated that the abnormal expression of miRNA is closely related to the occurrence and development of GC. According to Zhuang et al[10], miR-524-5p, a miRNA on chromosome 19q13.42 discovered in recent years, can promote the proliferation of osteosarcoma cells through regulating phosphatase and tensin homolog deleted on chromosome ten (PTEN), thus playing a role as a cancer-promoting gene. Zhen et al[11] have also reported that miR-524-5p can inhibit the proliferation of thyroid cancer through regulating SPAG9, thereby playing a role as a cancer suppressor gene. However, the role of miR-524-5p in GC and its mechanism have not been explored yet. Therefore, it is of great importance to further investigate the role of miR-524-5p in the occurrence and development of GC for clarifying its function.

Patients and Methods

Clinical Specimens and Cell Lines

A total of 48 pairs of GC and para-carcinoma normal tissue specimens were collected from surgical specimens of GC in Linyi Central Hospital from July 2016 to December 2017. All patients did not undergo radiotherapy and chemotherapy before operation, and they had complete clinical data and signed the informed consent. All GC tissue specimens were stored in RNA later solution and placed in a refrigerator at 4°C overnight. On the next day, specimens were stored in the refrigerator at -80°C for preservation. The postoperative tissue sections were pathologically analyzed by pathologists to confirm the diagnosis with GC. This study was approved by the Ethics Committee of Linyi Central Hospital. Human GC cell lines (MGC803, BGC823, SGC7901 and HGC27), human normal gastric mucosal epithelial cell line (GES-1) and human embryonic kidney cell line (HEK293) were routinely cultured in the Dulbecco's modified Eagle medium (DMEM) (Gibco, Rockville, MD, USA) containing 10% fetal bovine serum (FBS) (Gibco, Rockville, MD, USA) in an incubator with 5% CO_2 at 37°C. All cell lines were purchased from the Cell Lab, Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences (Shanghai, China), and preserved in the liquid nitrogen container in our laboratory. When 80-90% cells fused, they were digested with trypsin, followed by routine passage and culture. The cells in the logarithmic growth phase were selected for subsequent experiments.

RNA Extraction and Quantitative Real-Time PCR

The total RNA was extracted from the target cells according to the instructions of the TRIzol reagent (Invitrogen, Carlsbad, CA, USA), and synthesized into complementary deoxyribonucleic acid (cDNA) in accordance with the instructions of the miRNA reverse transcription kit, followed by PCR amplification according to the instructions of the fluorescence quantitative PCR kit (TaKaRa, Otsu, Shiga, Japan). Primers used as follows: For miR-524-5p, forward, 5'-AAGGGAAGCACTTTCTCTTGTC -3' and reverse, 5'-TCAAACCGTAACACTCCAAAGG -3'; and for U6, forward, 5'-GCACCTTAGGCTGAACA-3' and reverse, 5'-AGCTTATGCCGAGCTCTTGT-3'. For CASP3, forwards, 5'-GGCGGTTGTAGAAGTTAATAAAGGT-3' and reverse, 5'-TTCCAGAGTCCATTGATTCGCT-3'; and for GAPDH, forward, 5'-GAAGGTGAAGGTCGGAGTC -3' and reverse, 5'-GAAGATGGTGATGGGATTTC-3'. The relative quantitative analysis was performed using the $2^{-\cdots CT}$ method.

Cell Transfection

The GC SGC7901 cell lines were divided into miR-524-5p overexpression group (miR-524-5p mimics) and miR-524-5p negative control group (NC-mimics), and transfected using the Lipofectamine 2000 reagent (Invitrogen, Carlsbad, CA, USA) according to the instructions. At 24-48 h after transfection, cells in each group were collected for subsequent experiments.

MTT (3-(4,5-Dimethylthiazol-2-Yl)-2,5-Diphenyl Tetrazolium Bromide) Assay

Cells in the logarithmic growth phase in each group were taken, digested with trypsin, counted and inoculated into a 96-well plate (1×10^3 cells/well), and 5 repeated wells were set in each group. After microscopic observation and adjustment of cell density, cells were cultured in an incubator with 5% CO_2 at 37°C for 4 consecutive days. At 4 h before the termination of culture every day, 20 μL MTT (Sigma-Aldrich, St. Louis, MO, USA) at

a concentration of 0.01 mol/L was added into the well. After 4 h, the culture solution was completely aspirated, and 150 μL dimethyl sulfoxide (DMSO) (Sigma-Aldrich, St. Louis, MO, USA) was added to dissolve the formazan particles, followed by vibration using an oscillator for 5-10 min and determination of optical density at 490 nm (OD_{490}) using a microplate reader. The proliferation times in each group every day relative to that at 1 d were calculated, and the bar graph of cell proliferation was plotted with OD_{490} as the ordinate.

Colony Formation Assay

Cells in the logarithmic growth phase in each group were taken, digested with trypsin and counted, and the cell density was adjusted to 1×10^6 cells/L. The low-melting agarose liquid at concentrations of 1.2% and 0.7% was prepared using the distilled water, and kept liquid at 40°C after autoclaved sterilization. 1.2% agarose and 2×DMEM were mixed at a ratio of 1:1, and 3 mL mixture was poured into a dish (6 cm in diameter), cooled, solidified and placed in the incubator with CO_2 for standby application as the base agar. Then, 0.7% agarose and 2×DMEM were mixed at a ratio of 1:1 in the sterile tube, added with 0.2 mL cell suspension, mixed evenly and poured into the dish with 1.2% agarose base, gradually forming double agar layers. After the upper-layer agar was solidified, the dish was placed for culture in the incubator with 5% CO_2 at 37°C for 10-14 d, and placed under an inverted microscope to observe the number of colony and calculate the formation rate. This experiment was repeated for 3 times.

Cell Apoptosis Analysis

At 48 h after transfection, cells in each group were digested with trypsin without ethylenediaminetetraacetic acid (EDTA), and the digestion was terminated using the supernatant culture solution. After centrifugation at 4°C for 5 min, cells were collected and washed twice with phosphate-buffered saline (PBS), and about $(1\text{-}5)\times10^5$ cells were collected and resuspended with 500 μL 1 × binding buffer. Next, 5 μL Annexin V-FITC and 10 μL Propidium Iodide (PI) were added for incubation in a dark place at room temperature for 15 min. Finally, the apoptosis rate in each group was detected *via* flow cytometry.

Cell Cycle Analysis

At 48 h after transfection, cells in miR-524-5p mimics group and NC-mimics group were collected (1×10^6), washed twice with pre-cooled PBS, fixed with 70% ethanol, placed in the refrigerator at 4°C overnight, and washed twice with pre-cooled PBS again. Then 500 μL (20×) PI staining solution and 100 μL (50×) RNase A were added into each tube, followed by warm bath in a dark place at 37°C for 0.5 h. Finally, the cell cycle was detected *via* flow cytometry.

Dual-Luciferase Assay

It was predicted using the online databases (TargetScan, miRBase, miRanda, etc.) that the target-binding site of miR-524-5p existed in the 3'UTR of CASP3. The wild-type luciferase vector (pGL3-CASP3-3'UTR-WT) and mutant-type luciferase vector (pGL3-CASP3-3'UTR-MUT) of CASP3 3'UTR were constructed. The pGL3-CASP3-3'UTR, pGL3-CASP3-3'UTR-MUT reporter vector and miR-524-5p mimics or NC-mimics were co-transfected into human HEK293 cells using Lipofectamine 2000. At 48 h after transfection, the luciferase activity was detected using the luciferase assay kit.

Western Blotting

Cells in the logarithmic growth phase in each group were taken and digested with trypsin. After the culture solution was discarded, cells were washed with PBS for 3 times and added with 150 μL pre-cooled radioimmunoprecipitation assay (RIPA) lysis buffer (added with 1.5 μL PMSF (phenylmethylsulfonyl fluoride) in advance at a final concentration of 1 mmol/L) (Beyotime, Shanghai, China), and the total protein was extracted on ice. After centrifugation (centrifugal radius: 4 cm) at 10,000 rpm and 4°C for 5 min, the supernatant was taken to determine the protein concentration using bicinchoninic acid (BCA) (Pierce, Rockford, IL, USA), followed by denaturation at 99°C for 10 min. Then, 50 μg proteins were taken for sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE), transferred onto a polyvinylidene difluoride (PVDF) membrane (Millipore, Billerica, MA, USA) and incubated in the blocking solution for 1 h. CASP3 primary antibody (1:100) was added at 4°C overnight. After the membrane was washed with Tris-buffered saline and Tween 20 (TBST) for 3 times (5 min/time), the horseradish peroxidase (HRP)-labeled secondary antibody (1:5000) and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) were added for incubation at 37°C for 2 h.

Statistical Analysis

The experiments in each group were repeated for at least 3 times, and Statistical Product and Service

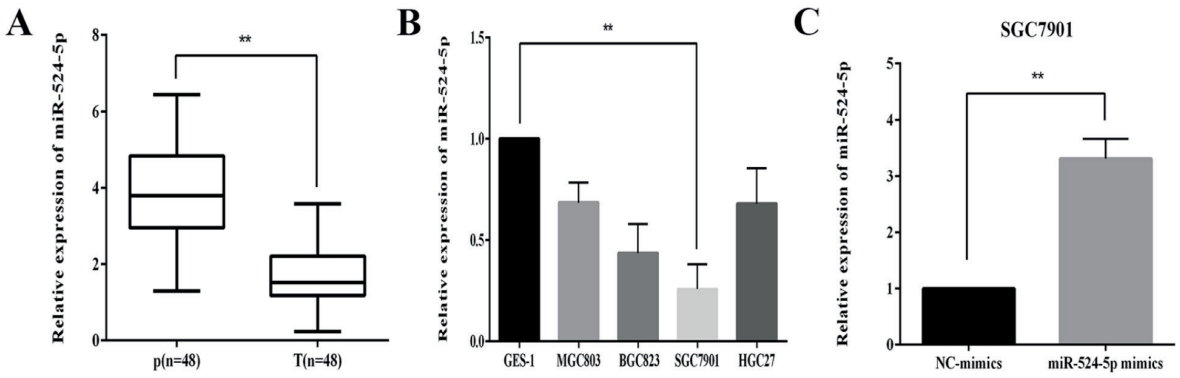

Figure 1. MiR-524-5p expression was decreased in GC tissues and cell lines. **A,** Analysis of miR-524-5p expression in para-carcinoma tissues (P) and tumor tissues (T) (n=48); **B,** Analysis of miR-524-5p expression in several GC cell lines and normal cell line GES-1; **C,** Analysis of transfection efficiency in SGC7901 cells transfected with miR-524-5p mimics and NC-mimics. Total RNA was detected by qRT-PCR and GAPDH was used as an internal control. Data are presented as the mean ± SD of three independent experiments. $^{**}p<0.01$.

Solutions (SPSS) 17.0 software (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. The normal measurement data were expressed as mean ± standard deviation ($\bar{\chi} \pm s$). *t*-test was adopted for the comparison of two sample means, and one-way analysis of variance and q test were adopted for the comparison among groups. $p<0.05$ suggested that the difference was statistically significant

Results

MiR-524-5p Expression was Decreased in GC Tissues and Cell Lines

First, we detected the relative expression of miRNA-524-5p in gastric cancer tissues by qPCR. The results show that miR-524-5p exhibited a general low expression in GC tissue samples, whereas the expression of which in para-carcinoma normal tissue specimens was approximately three times higher (Figure 1A). This result might indicate that miR-524-5p played a role in the malignant progression of GC. In addition, in order to further illustrate, we examined the expression of miR-524-5p in GC cell lines (MGC803, BGC823, SGC7901 and HGC27) and human normal gastric mucosal epithelial cell line (GES-1) by qPCR method as well. Which hit our hypothesis was that the expression of miR-524-5p in GC cells was also significantly suppressed by comparing with GES-1 cell line (Figure 1B), with SGC7901 expressed relatively the lowest. Therefore, we chose SGC7901 cells to overexpress miR-524-5p (Figure 1C), and the transfected cells were subjected to the subsequent experiments.

MiR-524-5p Inhibited GC Cell Proliferation in Vitro

To validate the effect of miR-524-5p on cell proliferation, we first analyzed using MTT assay. Up-regulation of miR-524-5p expression in SGC7901 cells, compared with the negative control (NC-mimics) group, could decrease cell proliferation at 48 h and 72 h (Figure 2A). In addition, we also used the clone formation experiment to further clarify. Compared with the control group, the miRNA-524-5p mimics group significantly inhibited the formation of tumor cell clones (Figure 2B).

MiR-524-5p Inhibited Cell Apoptosis and Promoted Cell Cycle Arrest at G0/G1 Phase

Flow cytometry was performed to detect the apoptosis of SGC7901 cells transfected with miR-524-5p mimics and NC-mimics. The results showed that the apoptotic rate was significantly increased in the miR-524-5p mimics group compared with the NC-mimics group. It indicated that miR-524-5p overexpression could increase the apoptosis rate of SGC7901 and increase apoptosis (Figure 3A). At the same time, we also used flow cytometry to detect changes in the cell cycle of SGC7901 after transfection of miR-524-5p mimics and NC-mimics. The data showed that compared with the NC-mimics group, the ratio of G0/G1 phase in the cell cycle distribution of the miR-524-5p mimics group was increased, and the proportion of the S phase was decreased. These results revealed that miR-524-5p overexpression could block the cell cycle of SGC7901 in G0/G1 phase, preventing it from being excessive to S

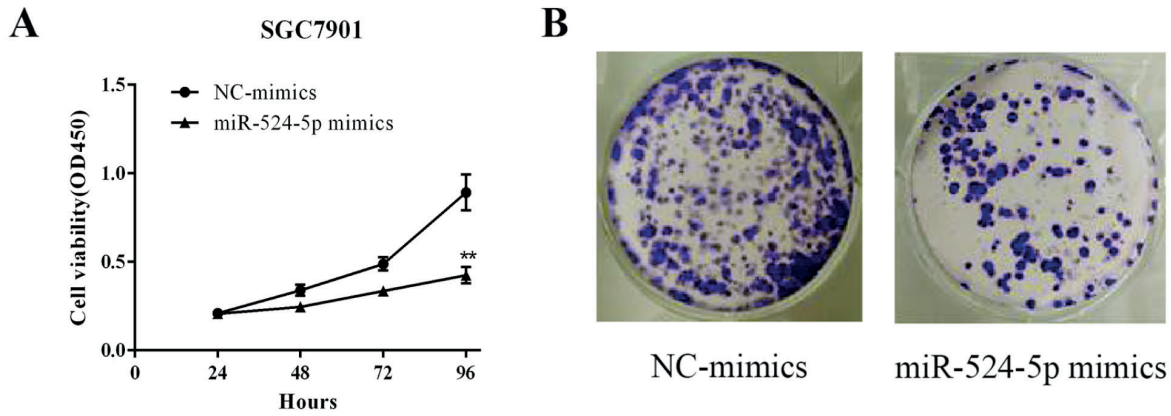

Figure 2. MiR-524-5p inhibited GC cell growth *in vitro*. **A,** MTT assay was performed to determine the viability of transfected cells; **B,** Clone formation assay was performed to determine the viability of transfected cells. *p<0.05, **p<0.01.

phase (Figure 3B). From the above results, miR-524-5p could regulate cell proliferation by inducing the distribution of cell cycle in G0/G1 phase and promoting apoptosis ability.

CASP3 is Directly Targeted by miR-524-5p

We used online databases to predict target genes of miR-524-5p, and selected CASP3 as a potential target gene based on the predicted results (Figure 4A). Besides, the dual luciferase reporter activity assay showed that the luciferase activity of the miR-524-5p + WT 3' UTR group was significantly lower than that of the miR-524-5p +mutated 3' UTR group and the NC-mimics group (Figure 4B). It suggested that when the CASP3-3'UTR was transferred into a vector carrying the luciferase gene, the addition of miR-524-5p could inhibit the activity of luciferase, while after the mutation of the binding site of CASP3-3'UTR to miR-524-5p, the activity of luciferase was no longer inhibited, indicating miR-524-5p significantly inhibited the activity of the luciferase reporter gene containing the CASP3-3'UTR WT region. Further qPT-PCR and western blot showed that the expression of CASP3 protein was decreased in the miR-524-5p mimics group compared with the NC-mimics group (Figure 4C and 4D). The above results indicated that CASP3 was a target gene of miR-524-5p in GC cells.

Restoration of CASP3 Impaired the Antitumor Effect of miR-524-5p

We first detected the expression of CASP3 in GC tissues and found that CASP3 was highly ex-

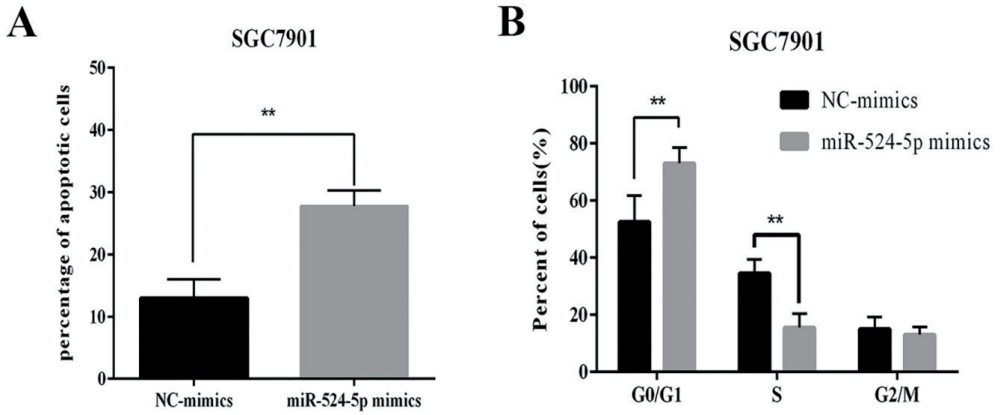

Figure 3. MiR-524-5p inhibited cell apoptosis and promoted cell cycle arrest at G0/G1 phase. **A,** Flow cytometric analysis was performed to detect the apoptotic rates of transfected cells; **B,** Flow cytometric analysis was performed to detect cell cycle progression of transfected cells.

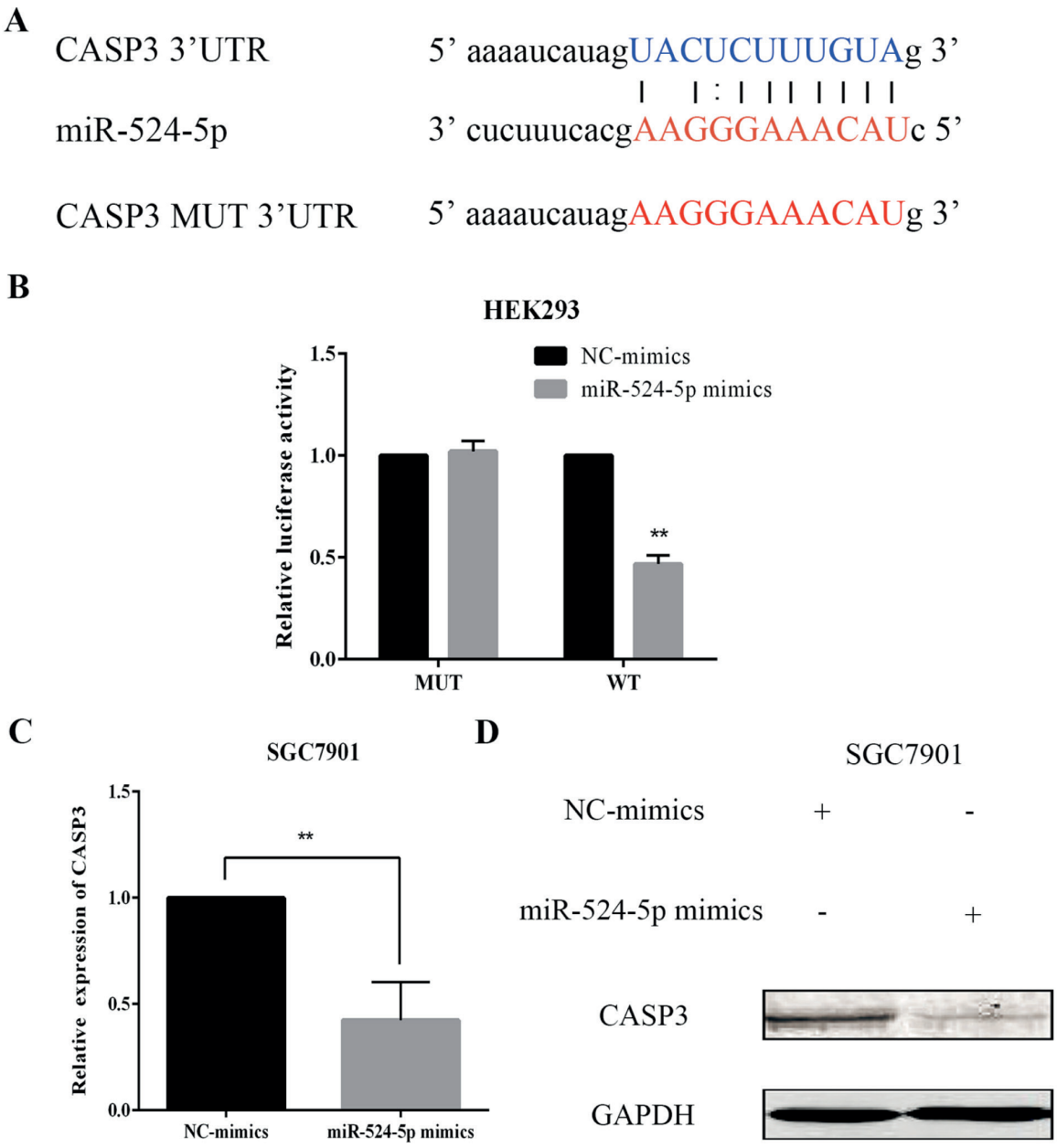

Figure 4. CASP3 is directly targeted by miR-524-5p. **A,** CASP3 was selected as the potential downstream of miR-524-5p using bioinformatics analysis; **B,** Luciferase activities of HEK293 cells transfected with the wild-type or the mutated CASP3 3'UTR together with miR-524-5p mimics or NC-mimics; **C,** Analysis of CASP3 mRNA expression level of transfected cells; **D,** Analysis of CASP3 protein expression level of transfected cells. Data are presented as the mean ± SD of three independent experiments. $^{**}p<0.01$.

pressed compared with the adjacent tissues (Figure 5A). Subsequently, we manifested the relationship between miR-524-5p and CASP3 expression in GC tissues. The results verified that the expression of miR-524-5p was negatively correlated with the expression of CASP3 in GC tissues (Figure 5B). Meanwhile, we manifested whether CASP3 was responsible for the functional roles of miR-524-5p in GC development. We overexpressed CASP3 by transfected with LV-CASP3 in miR-524-5p-increased SGC7901 cells (Figure 5C). The finding showed that CASP3 upregulat-

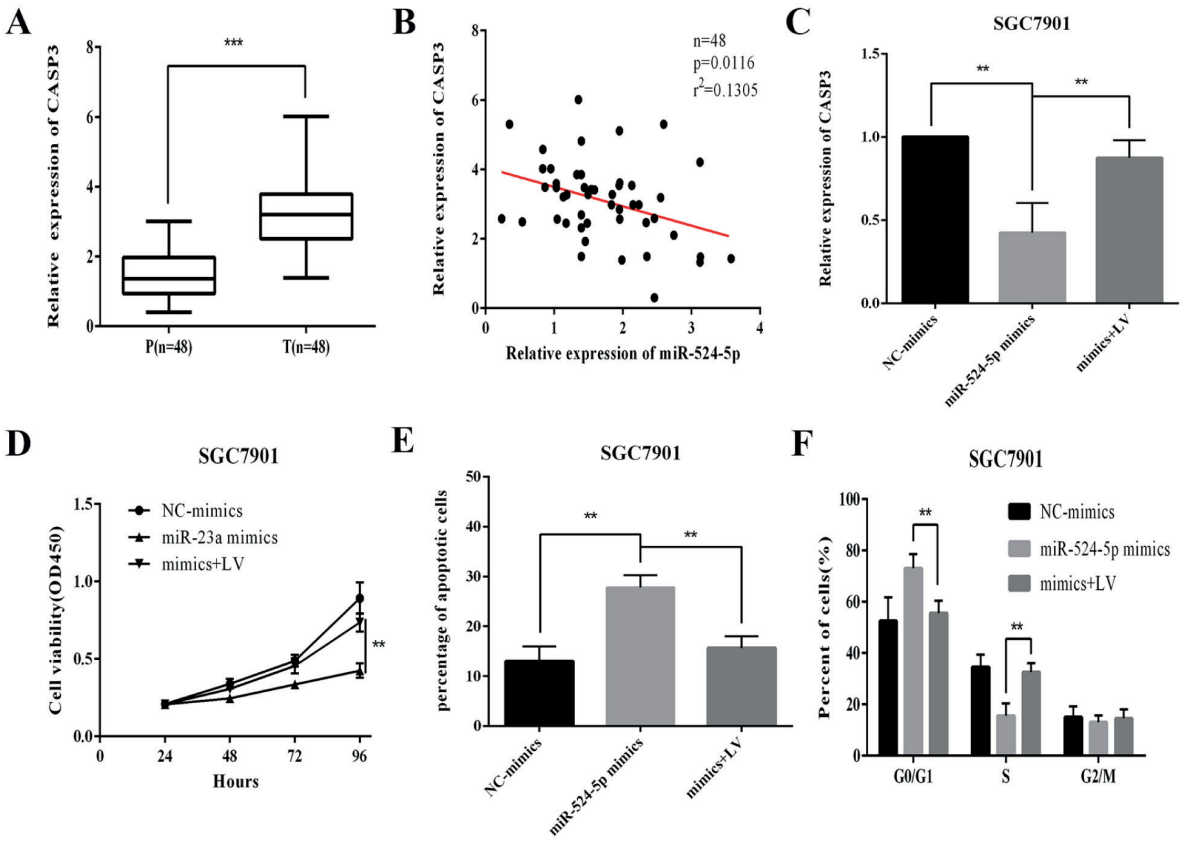

Figure 5. Restoration of CASP3 impaired the antitumor effect of miR-524-5p. **A,** Analysis of CASP3 expression level in GC tissues (T) and matched paracarcinoma tissues (N) (n=48); **B,** Correlation between miR-524-5p and CASP3 expression in GC tissues (n=70); **C,** Analysis of transfection efficiency of CASP3 in transfected cells; **D,** Analysis of cell proliferation in transfected cells with CASP3 dysregulation; **E,** Analysis of cell apoptosis in transfected cells with CASP3 dysregulation; **F,** Analysis of cell cycle distribution in transfected cells with CASP3 dysregulation. Data are presented as the mean ± SD of three independent experiments. *p<0.05, **p<0.01, ***p<0.001.

ing not only enhanced cell proliferation capacity (Figure 5D), but also attenuated cell apoptosis and cell cycle distribution at G0/G1 phase (Figure 5E & 5F). These results indicated that miR-524-5p suppressed GC tumorigenesis by regulating CASP3 partially.

Discussion

Gastric cancer (GC) is one of the most common malignant tumors in the world, whose incidence rate ranks 4th and mortality rate ranks 2nd in malignant tumors around the world. China is one of the countries with the highest morbidity and mortality rates of GC[12]. According to statistics, there were 952,000 new cases of GC in the world in 2012, 42% of whom were in China. In recent years, the incidence rate of GC has declined year by year through improving dietary habits, radical treatment of Helicobacter pylori and reducing risk factors, but it is still one of the most common malignant tumors. The expected incidence rate of GC in 2014 in China was 477.7/100,000/year in male and 201.4/100,000/year in female, ranking 1st in the malignant tumors of digestive tract, and the expected mortality rate of GC (498/100,000/year) was second only to lung cancer[13]. The occurrence and development of GC, similar to other tumors, is a complex biological process involving such factors as environment, genetics and epigenetics, which is closely related to the activation of proto-oncogenes, inactivation of cancer suppressor genes and numerous changes at gene and molecular levels[14, 15]. Most patients have been in

the progressive stage when diagnosed in China due to the low detection rate of early GC. Surgical resection and adjuvant chemotherapy have been dominated in the treatment of GC in China for many years, but the efficacy is poor for advanced patients, which is a main reason for the death in patients with GC[16, 17]. Therefore, it is of great significance in improving the survival rate and reducing the mortality rate of GC to clarify the molecular mechanism of the occurrence and development of GC and search the effective and sensitive specific tumor markers, providing new methods for early diagnosis and targeted therapy. Hwang and Mendel[18] have found that miRNAs are widely involved in a variety of life processes, such as body growth and development, and play important roles in cell differentiation, proliferation, apoptosis, invasion and metastasis. Under normal physiological conditions, miRNAs mainly participate in the differentiation of hematopoietic cells, skeletal muscles, myocardium and nervous system. With the deepening of research on the correlation between miRNAs and tumors, increasingly evidence has suggested that the abnormally expressed miRNAs in tumors are important molecules regulating the proliferation and apoptosis of tumor cells. Most of these miRNAs inhibit cell proliferation or induce apoptosis, thus playing a role as cancer suppressor genes[19-22], while a few miRNAs promote cell proliferation and inhibit apoptosis, thus playing a role as oncogenes[23]. More and more researches show that miRNAs are also involved in the malignant progression of GC[24]. For example, the expression level of miR-449 declines in GC, thus promoting the cell cycle progression in G1/S and M/G1 and cell proliferation. MiR-21, a cancer-promoting molecule, promotes proliferation and inhibits apoptosis of GC cell lines through direct targeting on the expression of tumor suppressor gene RECK[25]. MiR-524-5p is a miRNA discovered recently that is abnormally expressed in tumors. Nevertheless, the role of miR-524-5p in GC has not been identified yet. This study focused on the interactions between miRNA and GC development. In the present study, we were the first to find that miR-524-5p was down-regulated in GC tissues when compared to adjacent tissues, indicating that miR-524-5p might play a potential role in the development of GC. Meanwhile, over-expression of miR-524-5p could inhibit GC cell proliferation, promote apoptosis and induce cell cycle arrest at G0/G1 phase, suggesting that miR-524-5p has an inhibitory effect on the growth of GC cells. MiRNA is a small RNA transcribed from DNA without being translated, but it can regulate other genes, whose biosynthesis process is highly complicated. The action mode of miRNA reported the most in recent years is that the mRNA degradation is promoted or its translation is inhibited through the binding between miRISC and 3'UTR sequence of the target gene, thereby inhibiting the expression of target gene. In tumors, the expressions of some miRNAs are inhibited, and the binding between miRISC and 3'UTR of the target gene is reduced, thus terminating the inhibited translation of the downstream target gene[26]. Studies have reported that miR-524 can regulate the PTEN and SPAG9 gene expressions in a targeted manner, thus affecting the tumor occurrence and development[10,11]. Bioinformatics software is an important means of predicting target genes of miRNA. To further investigate the molecular mechanism of miR-524-5p in the invasion and metastasis of GC, three databases (TargetScan, miRBase and miRanda) were selected to predict the target genes of miR-524-5p, and the common target gene (CASP3) predicted by the three databases was taken as the object of study. CASP3, also known as caspase-3, is a member of the cysteine-aspartic acid protease (caspase) family. It is generally believed that caspase-3 is the most important terminal cleavage enzyme in the apoptosis process and an important component in the killing mechanism of cytotoxic lymphocyte, as well as an important apoptosis-related gene[27]. The CASP3 gene is located on chromosome 4q35.1, and its encoded precursor protein contains 277 amino acids. CASP3 is an effector of the apoptotic cascade pathway of caspase, and CASP3 is activated after the initiation of both extrinsic apoptotic pathway and intrinsic apoptotic pathway, so it serves as a pivot in the entire apoptotic pathway and plays an important role in the execution of apoptosis program[28, 29]. At the same time, CASP3 is also involved in the regulatory process of many miRNAs in tumors. For example[30,31] miR-143 can promote the apoptosis of osteosarcoma through activating CASP3, and miR-224 is involved in the pathogenesis of lung cancer through targeting caspase-3 and caspase-7. However, upstream miRNAs that interact with CASP3 in GC have not been studied. In the present report, we initially found that CASP3 was directly targeted by miR-524-5p, and CASP3 expression was negatively correlated with miR-524-5p expression in GC tissue

samples. Meanwhile, restoration of CASP3 expression attenuated the inhibitory effect of miR-524-5p on GC cell growth. These results above validated that miR-524-5p might be the upstream of CASP3 involved in GC tumorigenesis.

Conclusions

miR-524-5p is participated in the development of GC *via* regulating CASP3, which might provide a new prospect for GC diagnosis and therapy.

Conflict of interest

The authors declare no conflicts of interest.

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