Abstract. – OBJECTIVE: This clinical study aimed to explore the expression of miR-195-5p and Yes-associated protein (YAP) in gastric cancer (GC) and their relevant apoptosis mechanism.

PATIENTS AND METHODS: Thirty GC patients treated in our hospital were enrolled as a patient group and 30 normal people who underwent physical examination during the same period were enrolled as a normal group. The purchased GC cells and human gastric mucous membrane cells were used to transfected miR-195-5p-mimics, miR-NC, si-YAP, and si-rRNA into MKN45 and SGC7901 cells. qRT-PCR was used to detect the expression of miR-195-5p and YAP in samples, and WB was used to detect the expression of YAP, Caspase-3, Caspase-9, β-catenin, c-myc, and cyclin D1. CCK-8, the transwell, and the flow cytometry were used to detect cell proliferation, invasion, and apoptosis. The dual fluorescent enzyme reporter was used to determine the relationship between miR-195-5p and YAP.

RESULTS: MiR-195-5p was lowly expressed in the tissues and serum of patients, but YAP was highly expressed, and the area under the miR-195-5p and YAP curves was more than 0.9. MiR-195-5p and YAP were associated with tumor diameter, TNM stage, lymph node metastasis, and differentiation in GC patients. The overexpression of miR-195-5p and the inhibition of YAP expression can inhibit cell proliferation and invasion, and apoptosis. The dual fluorescent enzyme reporter was used to determine the relationship between miR-195-5p and YAP.

CONCLUSIONS: The overexpression of miR-195-5p can inhibit YAP-mediated Wnt/β-catenin signaling pathway and promote cell apoptosis, so it may be a potential therapeutic target for GC.

Key Words: MiR-195-5p, Yes related protein, Wnt/β-catenin signaling pathway, Apoptosis, Gastric cancer (GC).

Introduction

In recent years, with the continuous improvement of the people's living standards, the people's diet structure and habits have changed, which leads to a significant increase in the incidence of the digestive tract tumors. Gastric cancer (GC) is the most common digestive tract cancer in the clinic. The latest cancer statistics showed that there were 1.033 million new cases of gastric cancer and 783 thousand deaths for gastric cancer in the world in 2018, ranking 5th and 3rd in incidence and mortality of all cancers, respectively. Effective treatment and diagnosis are the key to improve the survival of patients. There are no clinical symptoms of GC in the early stage, so most patients have suffered metastasis when diagnosed with GC, which is one of the reasons for the high mortality of GC. Studies have shown that chemotherapy combined with adjuvant therapy was the only effective treatment for patients with advanced GC. However, the causes and potential mechanisms of GC are still unexplored, so it is very important to explore the relevant mechanisms and the molecule of GC.

MicroRNA (miR), a hot research topic in various fields, is a type of non-coding short-chain
MiR-195-5p with Yes-related protein mediated Wnt/β-catenin signaling pathway on apoptosis of G...RNA of 22nt in length in eukaryotes, which can regulate the expression of the target genes by binding to the downstream target gene 3 'UTR, 5' UTR, and coding region, and participate in the regulation of many intercellular signals. Located on 17p13.1, miR-195-5p is an important member of the miR-195 family. Mirzaei et al. pointed out that miR-195-5p can be used as a potential diagnostic marker for GC patients. We predicted that Yes-associated protein (YAP) was a potential target site for miR-195-5p through the online biological prediction software (http://www.targetscan.org/vert_72/). Chitragari et al. have shown that YAP has a regulatory effect on cell growth, organ development, tumorigenesis, and development. Wnt/β-catenin signaling pathway is widely expressed in eukaryotes, extremely lowly expressed in normal mature cells, while overexpressed in tumor cells, and it is closely related to tumor proliferation and apoptosis. Deng et al. have found that the effect of YAP on Wnt/β-catenin signaling pathway could affect cell growth.

Based on the above studies, we speculated that the regulation of miR-195-5p to affect YAP can change the activity of Wnt/β-catenin signaling pathway and thus inhibit the biological function of the GC cells, and we carried out an experiment for it.

Patients and Methods

Patients

Altogether 30 patients (20 males and 10 females) with a mean age of 60.5±3.4 years treated in our hospital from May 2016 to January 2019 were enrolled as a patient group, and other 30 normal people (20 males and 10 females) with a mean age of 59.3±3.3 years who underwent physical examination during the same period were enrolled as a normal group. The laboratory and image detection of the normal group were in line with the normal standard. There was no difference in age and gender between the two groups (both p>0.05). This study was approved by the Ethics Committee.

The inclusion criteria were as follows: patients more than 18 years old who were diagnosed with GC by pathological examination, and met the diagnostic criteria of the TNM staging issued by the American Joint Committee on Cancer (AJCC) in 2017; patients and their families who agreed to participate in the study and signed the informed consent form and those with complete clinical data. The exclusion criteria were as follows: patients with other malignant tumors, liver and kidney function disease, infection before admission, history of previous treatment (surgery, chemotherapy, radiotherapy, or antibiotic treatment), and those whose estimated survival time was less than 3 months.

Source of Cells

Human gastric mucosa cells GES1 and the human gastric carcinoma cells MKN45, SGC7901, BGC823, and MGC803 (ATCC subordinate agent-Culture Collection, Beijing, China) (337970, 337682, 100674, 337689, and 100665).

Reagents and Instruments

The main reagents and instruments were: Cell Counting kit-8 (CCK-8; Beyotime Biotechnology, Shanghai, China, C0037), Transwell kit, Dulbecco's Modified Eagle's Medium (DMEM), Phosphate-Buffered Saline (PBS), bovine serum, and Penicillin-Streptomycin (Gibco, Rockville, MD, USA; 1142802, 10566024, 1001002323, 2640004, 15070063); Radio Immunoprecipitation Assay (RIPA), bicinchoninic acid (BCA) protein kit, Electrochemiluminescence (ECL) luminous kit, trypsin, and Lipofoctamine™ 2000 Transfection Reagent (Thermo Fisher Scientific, Waltham, MA, USA; 89900, 23250, 32209, 90059, 11668019); YAP, Caspase-3, Caspase-9, β-catenin, c-myc, cyclin D1, β-actin, and horseradish peroxidase (HRP)-labeled goat anti-mouse IgG secondary antibodies (R&D Systems, Minneapolis, MN, USA; MAB8094, AF835, MAB8301, AF1329, MAB3696, AF4196, MAB8929, HAF0707); TransScript Green miRNA Two-Step qRT-PCR SuperMix, TransScript II Green Two-Step qRT-PCR SuperMix (TransGen Biotechnology, Beijing, China, AQ202-01, AQ301-01); Annexin V/PI apoptosis detection kit (Yeasen Biotechnology Co., Ltd., Shanghai, China, 40302ES20); Dual-Luciferase Reporter Gene detection kit (Roche, China, D0010); PCR instrument (ABI, Foster City, CA, USA); flow cytometry (BD, ABI, Franklin Lakes, NJ, USA); Microplate reader (BioTek, Winooski, VT, USA; PerkinElmer, Waltham, MA, USA). All primers were designed and synthesized by GenPharma Co., Ltd. (Shanghai, China). More details are shown in Table I.

Collections of Tissues, Blood, and Serum

The cancer tissues and adjacent tissues were collected from the patient group and preserved in
liquid nitrogen during the operation. The peripheral blood (5 ml) was collected from the control group and the patient group, respectively (before operation), placed for 30 min, and centrifuged at a speed of 3000 rpm for 10 min. The serum was collected from them for the follow-up experiment.

**Cell Culture and Transfection**

All the cells were transferred to DMEM low glucose medium (Penicillin streptomycin double antibody, 10% FBS), cultured in a constant temperature incubator with 5% of CO₂ at 37°C. MiR-195-5p-mimics, miR negative control (miR-NC), target inhibitor YAP RNA (si-YAP), target overexpression YAP RNA (sh-YAP), and negative control RNA (Si-NC) were transfected with Lipofectamine™ 2000 kit in strict accordance with the instructions of the kit. All primers were transfected into the cells with the largest difference in expression.

**QRT-PCR Detection**

The total RNAs were extracted from the collected cells, tissues, and serum using the TRIzol kit (Invitrogen, Carlsbad, CA, USA). The purity, concentration, and integrity of the RNAs were detected using an ultraviolet spectrophotometer and agarose gel electrophoresis. MiR-195-5p and YAP reverse transcription were carried out strictly according to the instructions of the kit. MiR-195-5p amplification was carried out in a 20-µL reaction volume containing 1 µL of cDNA, 0.4 µL of upstream and downstream primer, respectively, 10 µL of 2× TransTaq® Tip Green qPCR SuperMix, and 0.4 µL of Passive Reference Dye (50×), and ddH₂O (added to make up to 20 µL in total). The PCR conditions were as follows: pre-denaturation at 94°C for 30 s, denaturation at 94°C for 5 s, annealing, and extension at 60°C for 30 s, for a total of 40 cycles. YAP amplification was carried out in a 20-µL reaction volume containing 1 µL of cDNA, 0.4 µL of upstream and downstream primer, respectively, 10 µL of 2× TransScript®Tip Green qPCR SuperMix, and 0.4 µL of Passive Reference Dye (50×), and Nuclease-free Water (added to make up to 20µL in total). The amplification conditions were as follows: pre-denaturation at 94°C for 30 s, denaturation at 94°C for 5 s, annealing, and extension at 60°C for 30 s, for a total of 40 cycles. Each sample was tested in 3 repeat wells, and the experiment was carried out 3 times. In this study, U6 was used as an internal reference of miR, and GAPDH was used as an internal of mRNA, and the 2^-∆∆CT method was used to analyze the data.

**Western Blot (WB) Detection**

The total protein was extracted from the cultured cells in each group using the RIPA lysis method, and its concentration was detected using the bicinchoninic acid method and adjusted to 4 µg/µL. The proteins were separated by 12% SDS-PAGE and then transferred to a polyvinylidene difluoride (PVDF) membrane. The membrane was stained with Ponceau S working solution, immersed in PBST for 5 min, and then washed, blocked with 5% skimmed milk powder for 2 h, and finally incubated overnight at 4°C with the primary antibodies (YAP, Caspase3, Bcl-2, Bax, β-catenin, c-myc, and cyclin D1) (1:1000). Then, the membrane was washed with phosphate-buffered saline (PBS) to remove the unbound primary antibodies. The HRP-labeled goat anti-mouse secondary antibody (1:5000) was added to the membrane, incubated for 1 h at 37°C. After that, the membrane was rinsed 3 times with PBS, 5 min each time. The protein bands on the membrane were developed in a dark room using the enhanced chemiluminescence (ECL) reagent, and the excess liquid on the membrane was absorbed with a filter paper. The luminescent protein bands were scanned, and the gray value was analyzed using Quantity One software. The relative expression level of each protein = the gray value of the target protein band / the gray value of the β-actin protein band.

**Cell Proliferation Detection (CCK-8)**

After 24 hours of transfection, the cells were collected, adjusted to 4×10⁶, and inoculated in 96-well plates, and then cultured for 24 h, 48 h, 72 h, 96 h, and 10 µL of CCK solution and 90 µL of basal medium (DMEM) were added to each well. The cells were cultured at 37°C for 2 h, and then the optical density (OD) value of each group was measured using a microplate reader with the absorbance of 570 nm.

**Cell Invasion Detection of Transwell**

After 24 hours of transfection, the cells were collected, adjusted to 5×10⁴, inoculated in 6-well plates, rinsed 2 times with PBS, and then transferred to the upper chamber. DMEM (200 µL) was added to the upper chamber and 500 mL of DMEM (containing 20% FBS) was added to the lower chamber. The matrix and the cells that did not pass through the surface of the membrane in the upper chamber were wiped after being cultured at 37°C for 48 h, rinsed three times with PBS, fixed with paraformaldehyde.
for 10 min, rinsed 3 times with double distilled water, and dried with 0.5% crystal violet after drying. The cell invasion was observed using a microscope.

**Apoptosis Detection (Flow Cytometry)**

The cells after transfection were digested with 0.25% trypsin and rinsed twice with PBS. A total of 100 μL of binding buffer was added to prepare 1×10⁶ cells/mL suspension, followed by addition of Annexin V-fluorescein isothiocyanate (FITC) and propidium iodine (PI), and incubated at room temperature for 5 min in the dark. The FC500M-CL flow cytometry system was used for detection, and the experiment was repeated 3 times to get the average value.

**Target Gene Detection**

The downstream target gene of miR-195-5p was predicted using Targetscan 7.2. The pmirGLO-YAP-3’UTR wild type (Wt), the pmirGLO-YAP-3’UTR mutant (Mut) miR-195-5p-mimics, and miR-NC were transferred to HEK293T cells using the Lipofectamine™ 2000 kit. The Luciferase reporter activity assay showed that pmirGLO-YAP-3’UTR Wt, pmirGLO-YAP-3’UTR Mut, miR-195-5p-mimics, and miR-NC were co-transfected into HEK293T. The Luciferase activity was detected using a Dual-Luciferase Reporter Assay kit.

**Statistical Analysis**

In this study, GraphPad 7 software package was used to analyze the collected data statistically and the plot-related figures. The K-S test was used to analyze dose data distribution. The data in line with normal distribution were expressed as mean ± standard deviation (mean±SD). An independent sample 𝑡-test was used for the comparison between the groups. Data not in line with normal distribution were expressed as quartile [Means (P₂₅-P₇₅)], analyzed by non-parametric test, and represented by Z. One factor variance analysis was used for the comparison among multiple groups, and F was used to represent it. Bonferroni was used for post-hoc test. ROC was used to plot the diagnostic value of miR-195-5p and YAP in GC. Statistical significance was indicated by 𝑝<0.05.

**Results**

**Clinical Value of MiR-195-5p and YAP**

By detecting the expression of miR-195-5p and YAP in the tissues and serum of the patients, it was found that the expression of miR-195-5p in the serum and tissues from the patient group was significantly lower than that from the normal group, but the expression of YAP in the serum of the patient group was significantly higher than that from the normal group (both 𝑝<0.05). The Pearson analysis showed that the expression of miR-195-5p and YAP in tissues were positively correlated with their expression in serum (𝑝<0.05). By drawing the ROC curve, it was found that the area under the miR-195-5p and YAP curves were 0.958 and 0.906, respectively. Further analyses of the relationship between the two indexes and pathological data showed that miR-195-5p and YAP were closely related to tumor diameter, TNM stage, lymph node metastasis, and differentiation (𝑝<0.05) (Table I, Figure 1).

**Effect of MiR-195-5p on Biological Function of Gastric Cancer Cells**

By detecting the miR-195-5p expression in the cells of each group, it was found that the expression of miR-195-5p in gastric cancer cells MKN45, SGC7901, BGC823, MGC803 in each group was significantly lower than that in GES1 (𝑝<0.05). SGC7901 and MKN45 were selected for transfection. The expression of miR-195-5p was increased after transfection with miR-195-5p-mimics (𝑝<0.05). Proliferation, invasion, and apoptosis of cells after transfection showed that the proliferation and invasion of SGC7901 and MKN45 cells transfected with miR-195-5p-mimics were significantly inhibited, while their apoptosis rate was significantly increased (𝑝<0.05). WB analysis showed

<table>
<thead>
<tr>
<th>Gene</th>
<th>Upstream primer</th>
<th>Downstream primer</th>
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<tbody>
<tr>
<td>miR-195-5p</td>
<td>5′-GATTCGCCCTCAAGAGACAAAGTGGAG-3′</td>
<td>5′-AGATCCCATGCGGCTAGCCCT-3′</td>
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<tr>
<td>YAP</td>
<td>5′-TACGGCAGAAGAGCTGAAATAC-3′</td>
<td>5′-GAGGATAAATAATCCACCAGAC-3′</td>
</tr>
<tr>
<td>GAPDH</td>
<td>5′-GGAGGAGATCCTCCCAGAAAATAT-3′</td>
<td>5′-GGCTGTGTTCACTTCTATGG-3′</td>
</tr>
<tr>
<td>U6</td>
<td>5′-AAGGAGAGGAGGAGGACAC-3′</td>
<td>5′-GCAAATTCGTGAGCAGGTCCT-3′</td>
</tr>
</tbody>
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that the expression of β-catenin, c-myc, cyclinD1 protein in SGC7901, MKN45 cells transfected with miR-195-5p-mimics was decreased significantly, but the expression of Caspase-3 and Caspase-9 was increased remarkably (p<0.05) (Figure 2).

**Effect of YAP on Biological Function of Gastric Cancer Cells**

By detecting the YAP expression in the cells of each group, it was found that the expression of YAP in gastric cancer cells MKN45, SGC7901, BGC823, and MGC803 in each group was significantly lower than that in GES1 (p<0.05). SGC7901 and MKN45 were selected for transfection. The expression of YAP was increased after transfection with si-YAP (p<0.05). Proliferation, invasion, and apoptosis of the cells after transfection showed that the proliferation and invasion of SGC7901 and MKN45 cells transfected with si-YAP were significantly inhibited, while their apoptosis rate was significantly increased (p<0.05). WB analysis showed that the expression of β-catenin, c-myc, cyclinD1 protein in SGC7901, MKN45 cells transfected with miR-195-5p-mimics was decreased significantly, but the expression of Caspase-3 and Caspase-9 was increased remarkably (p<0.05) (Figure 2).
protein in SGC7901 MKN45 cells transfected with si-YAP was decreased significantly, but the expression of Caspase-3 and Caspase-9 was remarkably increased (p<0.05) (Figure 3).

**Dual Fluorescent Enzyme Report**
In order to further verify the relationship between miR-195-5p and YAP, the target binding sites between YAP and miR-195-5p were predicted by the
downstream target genes of miR-195-5p with TargetScan 7.2. The results of the double Luciferase activity showed that the Luciferase activity of pmirGLO-YAP-3'UTR Wt was significantly decreased after the overexpression of miR-195-5p (p<0.05), but there was no effect on pmirGLO-YAP-3'UTR Mut (p>0.05). WB analysis showed that the expression of YAP protein in MKN45 and SGC7901 cells was significantly decreased after transfection with miR-195-5p-mimics (p<0.05) (Figure 4).

**Effect of Overexpression of MiR-195-5p and YAP on Biological Function of Gastric Cancer Cells**

In the rescue experiment, the proliferation, invasion, and apoptosis of MKN45 and SGC7901 cells were detected after the transfection with miR-195-5p-mimics and pmirGLO-YAP. The results showed that there was no difference in proliferation, invasion, and apoptosis between the cells transfected with miR-195-5p-mimics+

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**Figure 3.** Effect of YAP on biological function of gastric cancer cells. **A,** Expression of YAP in cells of each group. **B,** Expression of YAP in MKN45 and SGC7901 cells after transfection. **C,** Proliferation of cells after transfection. **D,** Invasion of cells after transfection. **E,** Apoptosis of cells after transfection. **F,** Expression of β-catenin, c-myc, cyclinD1, Caspase-3, and Caspase-9 protein after transfection. * indicates that p<0.05.
MiR-195-5p with Yes-related protein mediated Wnt/β-catenin signaling pathway on apoptosis of G6493

pmirGLO-YAP and cells transfected with miR-NC. On the other hand, compared with cells transfected with miR-195-5p-mimics, the ability of the invasion and apoptosis of those transfected with miR-195-5p-mimics+pmirGLO-YAP were significantly increased, and their apoptosis was significantly decreased. WB showed that the expression of β-catenin, c-myc, cyclinD1, Caspase-3, and Caspase-9 protein in the cells transfected with miR-195-5p-mimics+pmirGLO-YAP was not different from that with miR-NC. On the other hand, compared with the cells transfected with miR-195-5p-mimics, the expression of β-catenin, c-myc, cyclinD1 protein of those transfected with miR-195-5p-mimics+pmirGLO-YAP was significantly increased, and their expression of Caspase-3 and Caspase-9 protein was significantly decreased (Figure 5).

**Discussion**

Because of the high migration and invasion ability of GC, the clinical treatment of GC has always been a thorny problem. In recent years, miR has outstanding performance in various fields. As a short-chain non-coding RNA, miR is involved in cell growth, proliferation, apoptosis, and other biological functions. MiR-195-5p is an important miR in the miR family. Mirzaei et al have shown that miR-195-5p is closely related to the diagnosis and prognosis of GC, but the specific function of miR-195-5p in GC is not complete. Therefore, this study further explored the relationship between miR-195-5p and GC biological function.

In this study, we first verified the expression of miR-195-5p in GC. By detecting the expression of miR-195-5p in the serum and tissues of patients, it was found that the expression of miR-195-5p in cancer tissues was significantly lower than that in adjacent tissues, and the expression in serum was consistent with that in the tissues. It was found by the correlation analysis that the expression of miR-195-5p was positively correlated with the expression in serum. The area under the curve of miR-195-5p was found to be more than 0.95 by the ROC curve analysis, suggesting that miR-195-5p was a very excellent diagnostic index of GC. Guo et al found that the expression of miR-195 in GC tissues was significantly lower than that in non-cancer tissues. By detecting the expression of miR-195-5p in the plasma of patients with GC, Gorur et al observed that the expression of miR-195-5p in the serum of GC patients was also significantly lower than that of normal subjects. The results agree with ours, and we further analyzed the ROC curve to show that miR-195-5p has a high
diagnostic value in GC. Finally, we analyzed the relationship between miR-195-5p and the pathological data of patients and found that there was a close relationship between tumor diameter, TNM stage, lymph node metastasis, differentiation degree, and miR-195-5p. We speculated that miR-195-5p participated in the development of GC.

By detecting miR-195-5p in normal gastric cells and GC cells, it was found that the expression of miR-195-5p in GC cells was significantly lower than that in normal gastric cells, which provided mutual authentication to our previous experiment. Then, we selected a cell line with more significant differences for transfection to observe the biological function of the cell. The proliferation and invasion of MKN45 and SGC7901 cells transfected with miR-195-5p-mimics were significantly inhibited, and the apoptosis rate was significantly increased, which suggested that miR-195-5p could be the potential target for GC treatment. The overexpression of miR-195-5p could inhibit cell proliferation, invasion, and promote apoptosis, but the way in which miR-195-5p caused apoptosis was not clear. We found that YAP and miR-195-5p have binding targets through the online target gene prediction website. The YAP was located on the human 11q13 chromosome, and the encoded protein was closely associated with the

Figure 5. Rescue experiment. A, Proliferation ability of cells after transfection with miR-195-5p-mimics+pmirGLO-YAP. B, Invasion ability of cells after transfection with miR-195-5p-mimics+pmirGLO-YAP. C, Apoptosis ability after transfection with miR-195-5p-mimics+pmirGLO-YAP. D, Expression of β-catenin, c-myc, cyclinD1, Caspase-3, and Caspase-9 protein after transfection with miR-195-5p-mimics+ pmirGLO-YAP. * indicates that p<0.05.

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transcriptional activation. Previous studies have found that YAP was overexpressed in breast cancer, lung cancer, and pancreatic cancer, and Zhou et al. revealed that YAP was highly expressed in gastric cancer tissues and was related to the tumorigenesis of nude mice. This study showed that the expression of YAP was increased in the serum and tissue of GC patients, and the area of ROC curve was more than 0.9, which was also a potential diagnostic index of GC. The cell detection showed that the expression of YAP in GC cells was also increased, which was consistent with the expression in clinical patients. By transfecting si-YAP plasmid into MKN45 and SGC7901 cells, it was found that the inhibition of YAP expression could lead to proliferation, significantly inhibited invasion ability, and significantly increased apoptosis rate, which indicated that YAP could also be used as a potential target of GC. We also found through rescue experiments that after the overexpression of miR-195-5p and YAP, the proliferation and invasion of MKN45 and SGC7901 significantly increased, while the apoptosis rate significantly decreased, suggesting that there was a close relationship between miR-195-5p and YAP. Therefore, we further verified the relationship between miR-195-5p and YAP by dual fluorescent enzyme report. The results showed that the Luciferase activity of pmirGLO-YAP-3'UT Wt significantly decreased after over-expression, but there was no effect on the Luciferase activity of pmirGLO-YAP-3'UTR Mut. The expression of YAP protein was significantly decreased after transfection with miR-195-5p-mimics, which indicated that there was a targeted regulatory relationship between miR-195-5p and YAP. Through the above experiments, we can preliminarily prove that the upregulation of miR-195-5p can inhibit the expression of YAP, thus affecting the biological function of cells. However, it is not clear by which pathway it is regulated.

As a classical Wnt signaling pathway, Wnt/β-catenin signaling pathway has a very low intracellular expression level under normal conditions. When the cells are stimulated by related signals, β-catenin converges into the nucleus, thus affecting the transcription of the downstream genes. Reis et al. have revealed that YAP activated Wnt/β-catenin signaling pathway in glioma to stimulate tumor cell growth. In this study, we detected the expression of Wnt/β-catenin signaling pathway-related proteins in the cells transfected with miR-195-5p-mimics, and found that the expression of YAP, β-catenin, c-myc, and cyclinD1 protein was significantly inhibited, while the expression of Caspase-3 and Caspase-9 was significantly increased in MKN45 and SGC7901 cells transfected with miR-195-5p-mimics, suggesting that miR-195-5p can promote apoptosis by regulating the change of YAP and affecting the regulation of Wnt/β-catenin signaling pathway.

This study preliminarily proved that miR-195-5p can accelerate apoptosis by regulating the expression of YAP and affecting Wnt/β-catenin signal pathway. However, there are still some limitations to this study. We did not carry out tumorigenesis experiment in nude mice. Whether miR-195-5p-mimics injection can improve the tumorigenesis of rats is one of the criteria for the clinical trial. Secondly, the survival curve could not be drawn because of the short time of collection and the failure to follow up for a long time, and whether miR-195-5p can be used as a potential prognostic marker of GC has not been verified. Therefore, we hope to observe the survival of patients in future studies and to carry out more basic experiments to supplement our research results.

**Conclusions**

We observed that miR-195-5p promotes the apoptosis of GC cells by inhibiting YAP-mediated Wnt/β-catenin signaling pathway, so it is expected to become a potential target for clinical practice.

**Conflict of Interests**

The Authors declare that they have no conflict of interests.

**References**


