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The expression of SFRP2 in gastric cancer and its mechanism of inhibiting gastric cancer invasion and metastasis

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Abstract: *To investigate the effects and mechanisms of SFRP2 on the proliferation and invasion of human gastric cancer cell line BGC823, gastric cancer cell line BGC823 was used, which was resuscitated by liquid nitrogen, added with DMEM medium (containing 10% fetal bovine serum and 1% penicillin-streptomycin), and cultured at 37 ℃ with 5% CO2. When the cell attachment rate exceeded 80%, three passages were performed. The blank control plasmid pc-DNA and SFRP2 overexpression plasmid were transfected with Lipo-fectamine 2000 reagent. BGC823 cells in logarithmic growth phase were divided into control group, blank plasmid group and SFRP2 overexpression group. The cells in each group were cultured for 24 hours, and the proliferation of the cells in each group was detected by MTT assay and clone formation assay. The expression of E-cadherin, β-catenin and SFRP2 was detected by Western blot. The results showed that the proliferative activity of cells in the SFRP2 overexpression group was significantly lower than that of cells in the control and blank plasmid groups (P<0 05); there was no significant difference in the proliferative activity of cells in the control and blank plasmid groups. The numbers of cell clones in the control, blank plasmid and SFRP2 overexpression groups were (255.69 ± 29.71), (243.18 ± 5.82) and (178.28 ± 23.42), respectively; among them, the number of cell clones in the SFRP2 overexpression group was significantly lower than that of the control group and the blank plasmid group (P<0 05).The relative numbers of E-cadherin The relative expression of E-cadherin and SFRP 2 proteins in the SFRP 2 overexpression group was significantly higher than that in the control and blank plasmid groups (P<0.05), and the relative expression of β-catenin protein in the SFRP 2 overexpression group was significantly lower than that in the control and blank plasmid groups (P<0.05). Cells in each experimental group were detected by Transwell invasion assay. The results showed that the number of cells in the SFRP 2 overexpression group was significantly lower than that in the control group and the blank plasmid group (P < 0. 05), and there was no significant difference between the control group and the blank plasmid group.SFRP2 protein can inhibit the WNT pathway and inhibit the occurrence and invasion and metastasis of gastric cancer EMT.*

Keywords: *Gastric cancer, SFRP2, E-cadherin, WNT pathway, EMT*

Gastric cancer is one of the major health challenges worldwide. According to GLOBOCAN's 2020 estimate, stomach cancer causes approximately 800000 deaths (accounting for 7.7% of all cancer deaths)[1]. In recent years, the understanding of tumors has been deepening, and tumor diagnosis and treatment plans have made rapid progress. However, the overall prognosis of gastric cancer patients is still poor, and the clinical efficacy of advanced gastric cancer is extremely poor. Therefore, studying the regulatory mechanisms of malignant growth in gastric cancer has important scientific significance and clinical value.

The SFRP2 gene belongs to a member of the coding SFRP family, which contains a homologous Frizzled protein rich in cysteine. SFRPs are inhibitory molecules of the WNT pathway that can negatively regulate the Wnt signaling pathway[2], inhibit the proliferation, invasion and metastasis of many tumors, and increase tumor apoptosis[3]. Therefore, we speculate that the expression of SFRP2 may also change in gastric cancer tissue and may be involved in regulating the invasion and metastasis of gastric cancer cells. To verify the above hypothesis, this study aims to detect the expression changes of SFRP2 in gastric cancer and adjacent tissues, explore the impact and mechanism of SFRP2 on the

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invasion and metastasis of gastric cancer cells, and provide clinical targets for gastric cancer treatment.

1. Materials and methods

1.1 Materials

Human gastric cancer cell MHCC97H was purchased from the Chinese Academy of Sciences Shanghai Cell Bank. Antibody E-cadherin β- Catenin protein and SFRP2 protein were purchased from abcam in the UK. The SFRP2 overexpression plasmid pc-SFRP2 and blank control plasmid pc-DNA were purchased from Guangzhou Ruibo Biotechnology Co., Ltd.

1.2 Cell culture, grouping, and plasmid transfection of human gastric cancer cell line BGC823

After the gastric cancer cell line BGC823 was revived in liquid nitrogen, it was added to DMEM medium (containing 10% fetal bovine serum and 1% penicillin streptomycin dual antibody) and cultured at 37 ℃ and 5% CO2. When the cell adhered to the wall by more than 80%, it was passaged for 3 passages. Transfect blank control plasmids pc-DNA and SFRP2 overexpression plasmids using Lipo - fectamine 2000 reagent, respectively. BGC823 cells in logarithmic growth phase were divided into control group, blank plasmid group, and SFRP2 overexpression group. The cells in each group were further cultured for 24 hours before being used for the experiment.

1.3 Cell proliferation assay of human gastric cancer cell line BGC823

Using MTT experiments. Inoculate each group of cells into a 96 well plate, with 6 re wells each. After cell attachment, culture for 0, 24, 48, and 72 hours, and then add 0 Incubate at 37 ℃ for 4 hours in 5% MTT solution. Extract the culture medium from the plate and add 100% dimethyl sulfoxide μ L/hole, shaking at 37 ℃ for 10 minutes. Use an enzyme-linked immunosorbent assay (ELISA) to detect absorbance at a wavelength of 570 nm, with cell activity expressed as OD value.

1.4 Cloning Formation Experiment of Human Gastric Cancer Cell Line BGC823

Using the flat plate cloning method. Inoculate 800 cells per well into a 6-well plate and culture at 37 ℃ in a 5% CO2 incubator for 14 days. Change the medium every 3 days. Discard the original culture medium after culture, wash with PBS three times, fix the cells with 4% paraformaldehyde for 30 minutes, then stain with 0.1% crystal violet for 15 minutes. After washing, take photos and observe the cell cloning situation. Count the number of cell clones using Image J software.

1.5 Expression detection of E-cadherin, β-catenin and SFRP2 protein

Protein immunoblotting was used. Take the cells of each group that have intervened for 24 hours, extract the protein and load the sample after quantitative analysis, carry out sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) gel electrophoresis, transfer the polyvinylidene fluoride (PVDF) membrane, seal it for 2 hours, and add the primary antibody (E-cadherin β-Catenin protein, SFRP2 protein and GAPDH), incubate at 4 ℃ overnight, add goat anti mouse IgG secondary antibody after TBST washing the next day, incubate for 2 hours, wash for 3 times, finally add developer, and use gel imaging system to take photos and record. The protein grayscale value is represented by G, and the relative expression level of the protein is equal to the target protein/internal reference protein (GAPDH)

1.6 Invasion experiment of human gastric cancer cell line BGC823

Take cells from each group cultured for 24 hours, count cells after trypsin digestion, and resuspend in serum-free culture medium. Dilute Matrigel gel and serum-free medium in a ratio of 1:5 and take a sample of 100 μ Apply L to the upper chamber of the small chamber. Upper chamber drip 4 × 104 (200 μ L suspension cells, add 500 drops to the lower chamber μ RPMI-1640 culture medium containing 10% fetal bovine serum. Incubate for 24 hours and fix staining. Fix with methanol solution for 15-20 minutes, rinse with phosphate buffer three times, gently wipe the upper chamber with a cotton swab to remove cells that have not penetrated the membrane, stain with Giemsa, rinse with double distilled water to dry, observe under a microscope and take photos for counting. The experiment was repeated

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independently three times.

1.7 Statistical methods

Using GraphPad Prism 10.0 software. Quantitative data that conform to a normal distribution are represented by x ± s. Multiple group comparisons are conducted using one-way ANOVA, while pairwise comparisons between groups are conducted using LSD-t-test. The difference is statistically significant with P<0.05.

2. Results

2.1 Determination of cell proliferation activity in each experimental group

The cell proliferation activity of the SFRP2 overexpression group was significantly lower than that of the control group and blank plasmid group (P<0 05); There was no significant difference in cell proliferation activity between the control group and the blank plasmid group. The comparison of cell OD values at different concentrations and time points among different groups of cells is shown in Figure 1.

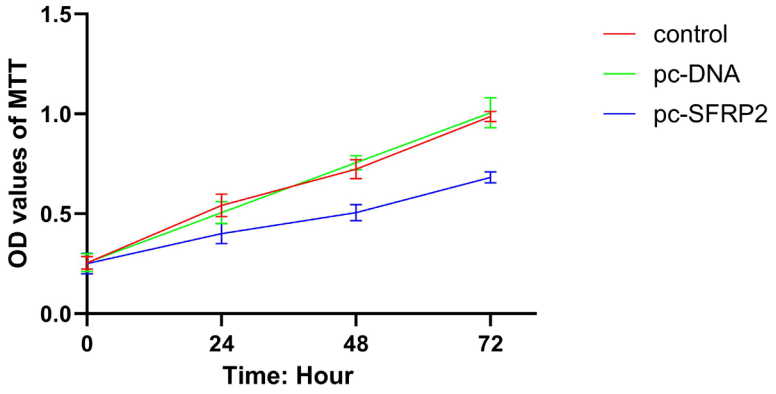

Figure 1: Cell OD values at different time points for each concentration

2.2 Comparison of cell proliferation ability among different experimental groups

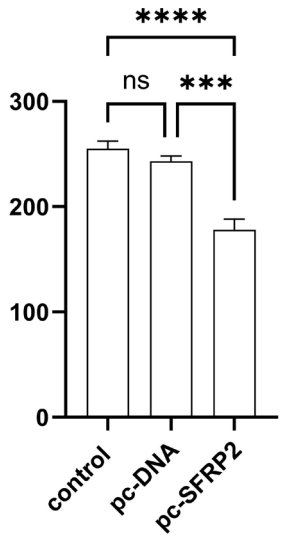

Figure 2: Comparison of proliferation abilities of cells in each experimental group.

The number of cell clones in the control group, blank plasmid group, and SFRP2 overexpression

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group was 255.69 ± 29.71, 243.18 ± 5.82, and 178.28 ± 23.42, respectively; Among them, the number of cell clones in the SFRP2 overexpression group was significantly lower than that in the control group and blank plasmid group (P<0 05); There was no significant difference in the number of cell clones between the control group and the blank plasmid group (Figure 2).

2.3 Comparison of the expression of E-cadherin, β -catenin protein, and SFRP 2 protein in each experimental group

The relative expression of E-cadherin and SFRP 2 protein in SFRP 2 overexpression group was significantly higher than that in control group and blank plasmid group (P <0. 05), and the relative expression of β -catenin protein in SFRP 2 overexpression group was significantly lower than that in control group and blank plasmid group (P <0. 05). (Figure 3).

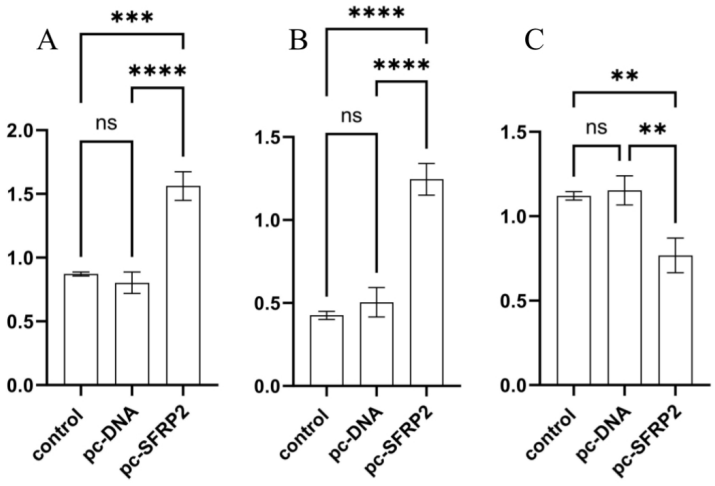

Figure 3: (A) Relative protein expression levels of SFRP2 in each experimental group. (B) Relative protein expression levels of E-cadherin in each experimental group.(C) Relative protein expression levels of β-Catenin in each experimental group.

2.4 Comparison of cell invasion ability among different experimental groups

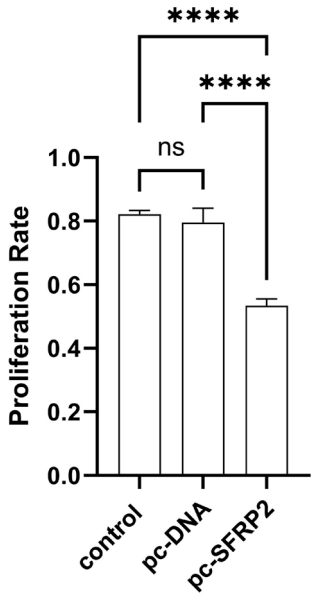

Figure 4: Cell invasion ability among different experimental groups

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Detect cells in each experimental group through Transwell invasion assay. The results showed that the number of cells passing through the matrix gel in the SFRP2 overexpression group was significantly lower than that in the control group and blank plasmid group ($P<0.5$). There was no significant difference in the number of cells passing through the matrix gel between the control group and the blank plasmid group. (Figure 4).

3. Conclusion

The SFRP2 protein inhibits the WNT pathway, inhibits the occurrence of EMT and invasion and metastasis in gastric cancer.

4. Discussion

The molecular regulatory mechanisms of tumor invasion and metastasis and their role in the occurrence and development of malignant tumors have become key scientific issues in the field of cancer research. As an important molecular regulatory pathway, the Wnt pathway is involved in a series of important physiological processes such as cell proliferation, invasion, metastasis, self-renewal, and differentiation[4]. SFRP2, as one of the inhibitory proteins of the Wnt pathway, is involved in the occurrence and development of various diseases such as tumors. However, its role in tumor epithelial mesenchymal transition (EMT) and invasion and metastasis is still unclear[5].

SFRP2 belongs to a group of secreted curl related protein families, SFRP1-5, which are Wnt/ β-Proteins and that inhibit catenin[6,7]. The Wnt signaling pathway plays an important role in tumor development, embryonic development, and neurodegenerative diseases, and has been widely studied in human cancer research[8]. It is reported that it is abnormally activated in various human tumors, such as non-small cell lung cancer (NSCLC)[9], colon cancer[10], melanoma[11], and leukemia[12]. There are studies reporting that SFRP2 increases the expression of epithelial cell marker E-cadherin through three transcription factors of epithelial mesenchymal transition (EMT)[13]: SLUG, TWIST, and SNAIL, inhibiting the invasion and metastasis of cervical cancer. In esophageal cancer, low expression of SFRP2 has been found in cancer tissue, and SFRP2 can inhibit esophageal cancer proliferation by inhibiting the WNT pathway[14].

At present, few reports report the effect of SFRP 2 on invasion and metastasis of gastric cancer cells. In order to preliminarily explore the possible important role of SFRP2 in the occurrence and development of gastric cancer, the expression of SFRP2 was detected using immunohistochemical clinical specimens in the early stage of this study, and it was found that SFRP2 was low expressed in gastric cancer tissue; The abnormal expression of SFRP2 is likely to be a tumor suppressor factor, involved in the occurrence and development of gastric cancer. In this study, it was demonstrated that SFRP2 inhibited the WNT pathway, inhibited the occurrence of EMT and invasion and metastasis in gastric cancer. The research results are expected to provide new ideas for the biological behavior of inhibiting malignant growth of gastric cancer, and provide new targets and experimental evidence for inhibiting gastric cancer invasion and metastasis.

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