

Effect of t (11; 19) (q23; p13.1) Gene on Proliferation and Apoptosis of SGC 7901 Gastric Cancer Cell Line

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Abstract

Objective: To investigate the effect of t (11; 19) (q23; p13.1) gene on proliferation and apoptosis of SGC 7901 gastric cancer cell line **Methods:** Gastric cancer cell line SGC 7901 cells were selected to transfect t (11; 19) (q23; p13.1) gene. MRNA levels of t (11; 19) (q23; p13.1) in each group were regulated after 24, 48 and 72h by RT-PCR. Cell proliferation was determined MTT assay. The apoptosis status of the SGC 7901 cells was detected TUNEL method. Immunohistochemical staining evaluated the expression of apoptotic genes Bcl-2 and Bax. **Results:** The MTT assay showed that t (11; 19) (q23; p13.1) decreased the proliferation of SGC 7901 cells. TUNEL method detected t (11; 19) (q23; p13.1) could improve apoptosis of SGC 7901 cells. In addition, t (11; 19) (q23; p13.1) could improve the expression of apoptotic gene Bcl-2 and reduce the expression of apoptotic gene Bax. **Conclusion:** T (11; 19) (q23; p13.1) gene can inhibit gastric cancer cell proliferation and improve apoptosis of gastric cancer cell

Keywords: T (11; 19) (q23; p13.1) gene; Cell proliferation; Cell apoptosis

Introduction

Gastric cancer (GC) is the fourth most common malignancy and the second most common cause of cancer-related mortality.^[1] Gastric cancer is a multi-factorial disease and causes continuous cell damage due to a lifetime of exposure to different carcinogens. Gene therapy as a novel treatment method has achieved improvements over past decades, particularly for treating malignant cancer.^[2-6] However, there are still some unsolved problems, limiting the further development of gene therapy for malignant cancer. Recently, several preliminary studies of targeted genes in gastric cancer have been performed.^[7-11]

The translocation t (11;19) (q23;p13.1) is a recurring abnormality in gastric cancer. In the study, to investigate the effect of t (11; 19) (q23; p13.1) gene silence on proliferation and apoptosis of SGC 7901 gastric cancer cell line.

Material and methods

Designing and constructing expression plasmid

To find the gene sequence of chromosome t (11; 19) (q23; p13.1) from NCBI GenBank, using siRNA design software to find the target sequence with characteristics in the downstream of the starting codon, and manually designing and synthesizing the

siRNA oligonucleotide sequence, End plus corresponding enzyme cut point and protection base, PCR amplification, reaction conditions: 94 °C pre-degeneration 5 min, 94°C degeneration 30s, 65 °C annealing 30s, 70 °C extended 110s, 25 cycles, 70 °C extended 7 min after the end amplification reaction, the product was recycled and purified by glue. Negative control siRNA is a universal unrighteous sequence, and all siRNA chains are purified by high performance liquid phase to remove unpaired single chains.

Cell culture and cell gene transfection

SGC-7901 cells were cultured continuously under the condition of RBMI1640 culture solution containing 10% fetal bovine serum, 37cc, 5%CO and saturated humidity environment. 1 days before transfection, select logarithmic growth SGC-7901 cells. Inoculation of appropriate density cell suspension in cultured utensils (24-hole culture Plate: 2.5x10 cell/fl; 96 Hole culture Plate: 0.4x10 cell/fl; 6 Hole culture Plate: 1.2x10 cell/fl; 6cm Petri dish : 2.5x10; 10cm petri dish: 7x10), culture overnight up to 40%~50% Fusion, the next day for instantaneous transfection. The basic operation refers to the cationic liposome Metafectenem (Biontex Company) Manual. Experimental group: ① negative control group: only inoculated cells, not transfected plasmid; ② Air Carrier control group: transfection Pegfp-c1; ③ Experimental

Group: Transfection pegfp-c1-t (one;) (q23; p13.1). Continue to be cultured to 24, 48, 72h, respectively.

RT-PCR detects mRNA expression

After transfection, collected each group of cells from the 6-hole culture plate at 24, 48 and 72h, extracted the total RNA, ultraviolet spectrophotometry and agarose gel electrophoresis to detect its purity and concentration in accordance with the Trizol reagent (Invitrogen Company) manual, according to reverse transcription System Kit Fpromega Company operated synthetic cDNA for PCR amplification. Reaction conditions: 94 °C Pre-degeneration 5 Min, 94°C degeneration S, 65 °C annealing 30 S, 70 °C extended S, 25 cycles, 70 °C extended 7 min after the end amplification reaction, amplification products by 1.5% agarose gel electrophoresis detection.

MTT assay for cell proliferation

After transfection, MTT assay was applied to evaluate the proliferation of both introduced MGC-803, AGS, and SGC-7901 cells and normal GES-1 cells. A total of 2×10^4 cells per well were plated in 96-well plates that were cultured for 24, 48, and 72 h. Before removal from incubation, the cells were incubated with 200 μ l of 0.5 mg/ml MTT (Sigma, Guangzhou, China) for an additional 4 h. The medium was then replaced with 200 μ l of dimethyl sulfoxide (Sigma-Aldrich, Guangzhou, China) to resolve the crystals. The absorbance at 490 nm was then measured for each well.

TUNEL assay

Apoptosis cells were detected in a MEBSTAIN Apoptosis Kit Direct (MBL International, Woburn, MA, USA) for Tunel assay. After transfection for 24 to 72 h, POD-horseradish peroxidase was added. Then cells were incubated with TdT buffer for 1 h. The apoptosis cells were counted under an Olympus fluorescent microscope (Olympus Corp). The Apoptotic Index (AI) was (number of apoptotic cells/total number) \times 100%.

Immunohistochemical staining

After transfection for 24 to 72 h, cells grew in six-well glass slides and were fixed with acetone. After PBS washing, the cells grew in 0.3% H₂O₂ solution at room temperature for 5 min. After 1:300 diluted anti-Bcl-2 or anti-Bax was added, the cells grew overnight. After washed by PBS, the cells grew with the second antibody at room temperature for 1 hour. After washed by PBS, the cells grew with ABC compound at room temperature for 10 min. DAB was added as the chromagen for 10 min. The brown color representing the binding of antigens to the antibodies was detected under an Olympus fluorescent microscope and photographed at \times 200. Controls were absent of primary antibody. The Positive Rate (PR) was (number of positive cells/total number) \times 100%.

Statistical analysis

The paired two-tailed Student t test was used to analyze the results by Clinical Physician Statistics Assistant 5.0 (Book Sword Software Online, Hangzhou, China) and significance was assumed at $P < 0.05$.

Results

Expression of transfection cell mRNA

The cells of transfection t (11; 19) (q23; p13.1) siRNA group and other groups of cells were collected at 24, 48 and 72h respectively. The total RNA were extracted. RT-PCR test results showed the relative expression of t (11; 19) (q23; p13.1) mRNA was lower than that of the control group, respectively, The difference was statistically significant ($P < 0.05$); In the transfection siRNA group, the relative expression of mRNA at different points of time was also statistically significant, and the expression of mRNA decreased with the prolongation of transfection time ($P < 0.05$). There was no statistically significant difference in the expression of mRNA between control groups at each point in time ($P > 0.05$). This showed that the role of siRNA is specific and time-dependent. (Table 1)

Table 1: Expression of t (11; 19) (q23; p13.1) mRNA in each group of cells

Group	24h	48h	72h
blank control	0.883 \pm 0.055	0.866 \pm 0.043	0.872 \pm 0.057
blank liposomes	0.875 \pm 0.035	0.869 \pm 0.036	0.851 \pm 0.037
negative control	0.869 \pm 0.025	0.871 \pm 0.052	0.863 \pm 0.029
transfection	0.623 \pm 0.014 ¹⁾	0.553 \pm 0.031 ¹⁾²⁾	0.269 \pm 0.024 ¹⁾²⁾³⁾

Comparing the transfected siRNA group with other control groups, 1) $P < 0.05$; compared with the same group of H, 2) $P < 0.05$; compared with the same group, 3) $P < 0.05$

Expression of t (11; 19) (q23; p13.1) gene inhibits SGC-7901 cell proliferation

T (11; 19) (q23; p13.1) after transfection into SGC-7901 cells for 24, 48 and 72h, the proliferative ability of cells decreased significantly compared with the control group ($P < 0.05$), and the

growth inhibition rate increased with the prolongation of transfection time. There was no statistically significant difference in cell growth rate between points in the control group ($P > 0.05$), which suggested that siRNA might have the ability to inhibit cell proliferation. (Table 2)

Table 2 Inhibitory effect of siRNA on cell proliferation

Group	24h		48h		72h	
	OD inhibitive % Rate		OD inhibitive % Rate		OD inhibitive % Rate	
blank control	0.516 \pm 0.021	0.0	0.523 \pm 0.037	0.0	0.528 \pm 0.023	0.0
blank liposomes	0.502 \pm 0.016	2.4	0.517 \pm 0.018	3.2	0.519 \pm 0.014	2.6
negative control	0.508 \pm 0.018	0.6	0.509 \pm 0.028	0.8	0.515 \pm 0.018	0.7
transfection	0.356 \pm 0.016 ¹⁾	5.8	0.286 \pm 0.023 ¹⁾²⁾	27.3	0.201 \pm 0.012 ¹⁾²⁾³⁾	47.9

Comparing the transfected siRNA group with other control groups, 1) $P < 0.05$; compared with the same group of H, 2) $P < 0.05$; compared with the same group, 3) $P < 0.05$

Detection of apoptosis by TUNEL method

Positive staining was in the nucleus (Figure 1). After transfection of 24, 48 and 72, the number of apoptosis of SGC-7901 cells increased significantly with time. The apoptosis index of 24h group

was $4.95 \pm 0.29\%$. The apoptosis index of 48h group was $16.97 \pm 0.34\%$. The apoptosis index of 72h Group was $27.56 \pm 0.39\%$, and the difference was significant ($P < 0.05$) after examination.

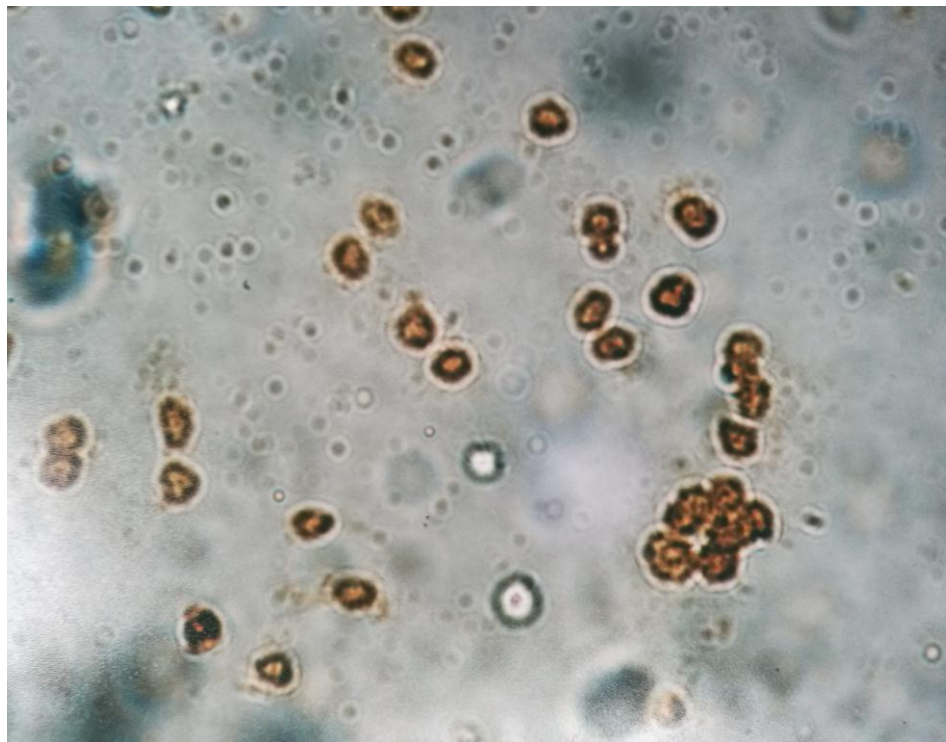


Figure 1: Apoptotic cells as shown by the TUNEL assay. Positive staining located in the nucleus. Magnification $\times 200$

Expression of Bcl-2 protein

Positive staining was in the cytoplasm. After transfection of 24, 48 and 72, the PRs of Bcl-2 protein increased with the prolongation of

treatment time ($P < 0.05$) (Table 3). This showed that visfatin could improve Bcl-2 expression.

Table 3: Positive rate of Bcl-2 expression in SGC 7901 cells (%)

	Treatment time (h)			
	Control	24	48	72
Positive rate	12.36 ± 0.58	39.01 ± 0.46	47.89 ± 0.51	57.66 ± 0.71
T		129.22	163.18	165.09
P value		$P < 0.001$	$P < 0.001$	$P < 0.001$

vs control group.

Expression of Bax protein

Positive staining was in the cytoplasm. After transfection of 24, 48 and 72, the PRs of Bax protein decreased with the prolongation

treatment time ($P < 0.05$) (Table 4). This showed that visfatin could decrease Bax expression.

Table 4: Positive rate of Bax expression in SGC 7901 cells

	Treatment time (h)			
	Control	24	48	72
Positive rate	38.12 ± 0.67	25.09 ± 0.28	12.21 ± 0.49	9.68 ± 0.07
T		53.83	18.74	126.69
P value		$P < 0.001$	$P < 0.001$	$P < 0.001$

vs control group.

Discussion

Gastric cancer (GC) is the fourth most common malignancy and the second most common cause of cancer-related mortality. Gastric cancer is a multi-factorial disease and causes continuous cell damage due to a lifetime of exposure to different carcinogens. Gene therapy as a novel treatment method has achieved improvements over past decades, particularly for treating malignant cancer.

However, there are still some unsolved problems, limiting the further development of gene therapy for malignant cancer. Recently, several preliminary studies of targeted genes in gastric cancer have been performed. Some studies that the t (11; 19) (q23;p13.1) translocation results in many cancers.^[12-13] The translocation t (11;19)(q23;p13.1) is a recurring abnormality in gastric cancer. In the study, to investigate the effect of t (11; 19) (q23; p13.1) gene silence on proliferation and apoptosis of SGC 7901 gastric cancer cell line.

This study showed the relative expression of t (11; 19) (q23; p13.1) mRNA transfection t (11; 19) (q23; p13.1) siRNA group was lower than that of the control group, respectively. In the transfection siRNA group, the relative expression of mRNA at different points of time was also statistically significant, and the expression of mRNA decreased with the prolongation of transfection time. This showed that the role of siRNA is specific and time-dependent.

This study showed after transfection into SGC-7901 cells for 24, 48 and 72h, the proliferative ability of cells decreased significantly compared with the control group, and the growth inhibition rate increased with the prolongation of transfection time. It suggested that siRNA might have the ability to inhibit cell proliferation. After transfection into SGC-7901 cells for 24, 48 and 72h, the AIs decreased with the prolongation of treatment time. After transfection of 24, 48 and 72, the PRs of Bcl-2 protein increased with the prolongation of treatment time and the PRs of Bax protein decreased with the prolongation treatment time. This showed that t (11; 19) (q23; p13.1) gene could improve Bcl-2 expression and decrease Bax expression.

Conclusion

We found that t (11; 19) (q23; p13.1) gene can inhibit gastric cancer cell proliferation and improve apoptosis of gastric cancer cell

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Author contributions

Zhou HB designed and coordinated the research and wrote the paper; Zhou HB, Ma WQ and Shao LM performed the majority of experiments

Conflict-of-interest statement

The authors declare no conflicts of interest.

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