Effects of siRNA-mediated silencing of Bmi-1 gene expression on proliferation of gastric cancer cells

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Abstract
This study was designed to investigate the effects of siRNA-mediated silencing of Bmi-1 gene expression on proliferation of AGS gastric cancer cell. siRNA Bmi-1 was transfected into human AGS gastric cancer cells by liposome (as siRNA Bmi-1 group) with negative control (as control group); the expressions of Bmi-1 and apoptosis-related genes like P21, Bax, and Bcl-2 in AGS cells were determined by Western blot method; the apoptosis of AGS cells was detected by flow cytometry double staining and Hoechst staining; and cell cycle was measured by flow cytometry. Compared with the control group, the expression of Bmi-1 in the siRNA Bmi-1 group was significantly decreased ($P < 0.05$), the apoptosis rate was increased ($P < 0.05$), and cell cycles were arrested at G1 phase ($P < 0.05$); the expression level of P21 and Bax in cells was significantly up-regulated while that of Bcl-2 down-regulated ($P < 0.05$). The down regulation of Bmi-1 can inhibit the proliferation of AGS gastric cancer cell and promote its apoptosis, which takes such effects mainly by up-regulating P21 as well as Bax and down-regulating Bcl-2.

Keywords
AGS cell, apoptosis, Bmi-1, gastric cancer, proliferation

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tumors and the tumors can be treated by targeted regulation of Bmi-1. The purpose of this study was to investigate the effect of siRNA-mediated silencing of AGS Bmi-1 gene expression on proliferation of AGS gastric cancer cell and to explore its possible mechanism so as to provide a reference for the treatment of gastric cancer.

Materials and methods

Cell strain

AGS gastric cancer cell was supplied by cell bank of Chinese Academy of Sciences.

Reagents and instruments

The following reagents and instruments were used: rabbit anti-Bmi-1 polyclonal antibody (Shanghai Ke min Biotechnology Co. Ltd.); rabbit anti-P21, Bax, Bcl-2, and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) monoclonal antibody (Beijing Alice Shenzhou Company Limited); Annexin V-PI flow cytometry double staining detection kit and cell cycle detection kit (Shanghai Xinle Biotechnology Co. Ltd.); siRNA Bmi-1 (Beijing Boer Mai Biotechnology Co. Ltd.); Dulbecco's Modified Eagle Medium (DMEM; Beijing Ya'an Biotechnology Co. Ltd.); electrophoresis apparatus (Beijing Kaiyuan Xinrui Instruments Co. Ltd.); microplate reader (BioTek instruments Limited); gel imaging system (Shanghai Peiqing Science & Technology Co Ltd); fluorescence microscopy (Shanghai Ba Tuo Instrument Factory); and flow cytometry (Suzhou pharmatech Co. Ltd).

Transfection of siRNA Bmi-1

The AGS gastric cancer cells were inoculated on 96- and 24-pore culture plates and siRNA Bmi-1 was transfected into human AGS gastric cancer cells by liposome (as siRNA Bmi-1 group) with negative control (as control group). The transfection effect was determined 6 h after transfection.

The measurement of cell apoptosis by Annexin V-PI flow cytometry double staining

Cells were collected at 48 h after the transfection by siRNA Bmi-1 and stained for 30 min followed by the measurement operation in accordance with the instructions of Annexin V-FITC/PI apoptosis detection kit.

Hoechst staining test

AGS cells were collected at 48 h after the transfection by siRNA Bmi-1 followed by the operation according to instructions of Hoechst 33258 staining kit.

The detection of cell cycle by flow cytometry

AGS cells were collected at 48 h after the transfection by siRNA Bmi-1 followed by the operation according to instructions of cell cycle test kit for the detection by flow cytometry.

The detection of expression of Bmi-1, P21, Bax, and Bcl-2 by Western blot

AGS cells were collected at 48 h after the transfection by siRNA Bmi-1. The lysate was added to split cells to extract total protein. The protein concentration was detected by bicinchoninic acid assay (BCA) kit. Transmembrane was conducted at 2 h after sodium dodecyl sulfate (SDS) electrophoresis. First antibodies (Bmi-1, P21, Bax, Bcl-2, GAPDH, 1: 100 in working concentration) were added followed by incubation at 4°C for the overnight and second antibodies were added followed by incubation for 2 h at room temperature, which was observed by electrophoresis gel imaging system with the gray values of each protein strip analyzed by Quantity one software.

Statistical analysis

SPSS 20 was used for statistical analysis of data and the t test was applied for assessment; \( P < 0.05 \) suggested there was statistical significance.

Results

Expression of Bmi-1 protein in AGS cells after transfection of siRNA Bmi-1

The level of Bmi-1 in siRNA Bmi-1 group was significantly lower than that in the control group of statistical value \( (P < 0.05) \), as shown in Figure 1.

Effect of siRNA Bmi-1 on apoptosis of AGS cells

Compared with the control group, the apoptosis rate of AGS in siRNA Bmi-1 group was obviously increased \( (P < 0.05) \), as shown in Table 1 and Figure 2(a) and (b).
The effect of siRNA Bmi-1 on cell cycle of AGS cells and the expression of P21, Bax, and Bcl-2

Compared with the control group, the cell cycle of siRNA Bmi-1 group was arrested mainly at G1 phase as shown in Figure 3(a).

In order to further confirm the possible mechanism for inhibition of the AGS cell apoptosis after silencing of Bmi-1 gene expression, we detected the expression of P21, Bax, and Bcl-2 in this study. Compared with the control group, the expression levels of P21 and Bax were significantly up-regulated in the siRNA Bmi-1 group, but the anti-apoptotic protein Bcl-2 down-regulated significantly \((P < 0.05)\) as shown in Figure 3(b).

Discussion

Like some malignant tumors, gastric cancer is one characterized by excessive proliferation and hyperactivity of cells.\(^7\)\(^-\)\(^9\) The growth of gastric cancer is very fast with the degree of malignancy continuing to increase, which is closely related to activation of oncogenes and inactivation of anti-oncogenes. In recent years, Bmi-1 has been defined as a gene that actively participates in cell self-renewal and cell senescence, and it is proved by studies to show high expression in many malignancies and is closely related to development as well as progression of tumors.\(^10\) Some studies have suggested that\(^11\) Bmi-1 is tightly associated with various pathologic processes of gastric cancer like pathogenesis, progression, metastasis, and prognosis. Over expression of Bmi-1 can facilitate the invasion and metastasis of gastric cancer cells. Moreover, Bmi-1 may be one of potential targets for inhibition of malignant tumors like gastric cancer. At present, however, the effects and molecular mechanism of Bmi-1 gene on biological function of gastric cancer cells are still unknown.

The results of this study show that compared with the control group, the expression of Bmi-1 in the siRNA Bmi-1 group was significantly decreased \((P < 0.05)\), the apoptosis rate was increased \((P < 0.05)\), and cell cycles were arrested at G1 phase \((P < 0.05)\), suggesting that the down regulation of Bmi-1 expression with RNA-mediation can effectively inhibit the growth and promote the apoptosis of AGS cells. This further demonstrates that Bmi-1, as a proto oncogene in gastric cancer, can inhibit the proliferation and progression of gastric cancer cells by targeted down regulation of its expression. It is hypothesized that Bmi-1 regulates the proliferation of gastric cancer cells by inducing cell cycle arrest and apoptosis.

It is indicated in related study that\(^8\) P21 can induce cell growth arrest by inhibiting cyclin-dependent kinase (CDK) and promote the chemosensitivity of cancer cells. If DNA damage occurs in cells, Bcl-2/Bax would be a key factor in determining the apoptosis or otherwise of cancer cells.\(^12\) The results of this study show that the expression level of P21 and Bax was significantly up-regulated while that of Bcl-2 down-regulated in the siRNA Bmi-1 group (Bax/Bcl-2 was increased).

Table 1. Effects of siRNA Bmi-1 on apoptosis in AGS cells (n=6).

| Group                  | Results of flow cytometry detection (%) | Hoechst staining result (%) |
|------------------------|-----------------------------------------|-----------------------------|
|                        | Early apoptosis                          | Late apoptosis              |
| Control group          | 2.05 ± 0.32                              | 4.27 ± 0.21                 | 6.03 ± 0.78                 |
| siRNA Bmi-1 group      | 5.67 ± 0.51                              | 36.04 ± 2.58                | 38.72 ± 2.96                |
| \(t\)                  | 6.702                                    | 8.092                       | 8.164                       |
| \(P\)                  | \(<0.05\)                                | \(<0.05\)                   | \(<0.05\)                   |
suggesting that P21, Bax, and Bcl-2 take part in proliferation of AGS gastric cancer cells induced by Bmi-1. It also illustrates that down regulation of Bmi-1 expression can significantly inhibit the proliferation and induce apoptosis of AGS gastric cancer cells.

As a conclusion, oncogene Bmi-1 plays an important role in the development of gastric cancer. Down regulation of Bmi-1 can significantly inhibit the proliferation and induce apoptosis of AGS gastric cancer cells possibly by up-regulating P21 as well as Bax and down-regulating Bcl-2.

Declaration of conflicting interests
The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethical approval
This study was approved from the institutional ethical review board of Jilin University, Changchun, Jilin, China (reference no. 108/IRB/JU/2015).

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