Original Article
Effect of mir-186 expression on biological behaviors of gastric cancer cells

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Abstract: This study intends to investigate the effect of mir-186 on the proliferation and invasion of gastric cancer cells. mir-186 mimic (activator) and inhibitor were designed and synthetized according to the sequences of mir-186 followed by transfected into MGC cells to manipulate the expression of mir-186. The effect of mir-186 on cell proliferation and cell migration *in vitro* was detected by CCK8 kit and cell scratch assay, respectively. In addition, transwell assay was performed to evaluate the influence of mir-186 expression on cell invasion and western blot was conducted to measure the expression of TP53 protein for investigating the potential mechanism. Compared with control group, cells transfected with mir-186 inhibitor displayed significantly increased proliferation, migration and invasion with significant differences (P<0.05). However, MGC cell line transfected with mir-186 activator showed decreased proliferation, migration and invasion with significant differences compared with control group (P<0.05). Moreover, the TP53 expression in activator group was significantly lower than that in either inhibitor group or control group, whereas, TP53 expression in inhibitor group was slightly higher than that in control group. In conclusion, mir-186 regulates the proliferation, migration and invasion of gastric cancer cells. In addition, mir-186 might be also involved in the regulation of TP53 signaling pathway in gastric cancer cells.

Keywords: mir-186, gastric cancer cell line MGC, proliferation, migration, invasion

Introduction

Gastric cancer is one of the most common malignant tumors in the digestive tract and about one million new cases are increased each with a high rate of death [1-3]. China has always been a high incidence area for gastric cancer. With the progression of medical treatments, such as radiotherapy, chemotherapy, biological targeted therapy, and cellular immune therapy, treatment efficacy has been improved, leading to the reduced growth rate of gastric cancer. However, the effective treatment is still not achieved in some patients, possibly due to the complicated pathogenesis of the gastric cancer [4-6].

Invasion and migration are the main features of tumors, which are also important factors affecting the tumor treatment. The changes of biological behaviors of tumor cells, such as proliferation, invasion and migration, are affected by the indirect or direct regulation of many genes, for example, the changes of cell adhesion, extracellular matrix degradation [7-10]. A previous study found that the expression of Kail gene was decreased in gastric cancer cells with a stronger metastatic potential, and the expression of Kail gene may inhibit the migration of gastric cancer cells [11, 12].

MiRNA is a non-coding single stranded RNA consisting of about 22 nucleotides, with two different ways to participate in gene regulation: complete complementary and incomplete complementary. The degradation of target gene mRNA can be directly induced by almost complete pairing [7]. At present, the technology of miRNA research is more mature and miRNA has become a marker of early clinical detection of tumors. Some miRNAs have been shown to be the potential targets of clinical drug therapy, for example, miR-21 may participate in the regulation of migration of colon cancer cells by regulating the expression of PTEN protein [8-10]. Therefore, investigations on the regulatory rela-
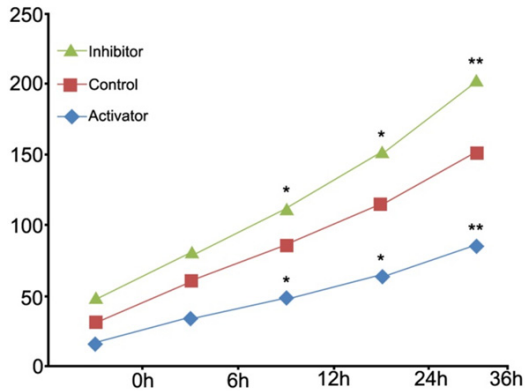
Figure 1. Comparison of cell proliferation in different time of each group. **: Compared with the control group, $P < 0.01$; *: Compared with the control group, $P < 0.05$.

tionship between miRNA and disease-related genes, not only can explain the mechanism of the occurrence of related diseases at the molecular level, but also might provide valuable information on the potential targets for the development of novel drugs [11-13].

TP53 is a classical tumor suppressor gene, whose expression has a certain relationship with the occurrence and malignancy degree of several tumors. In addition, mutations of TP53 gene will increase the risk of cancers, making the study of TP53 gene expression in gastric cancer cells being a good complement to the mechanism of gastric cancer. It is pointed out that mir-186 can effectively inhibit the proliferation and migration of tumor cells in colon cancer. However, whether mir-186 could affect the proliferation and migration of gastric cancer cells as well as related mechanisms remains poorly understood. The aim of the present study was to investigate the role of mir-186 on the proliferation and migration of gastric cancer cells.

Materials and methods

Experimental materials

The MGC cell line selected in the study was purchased from Sunbio company and was frozen in liquid nitrogen by Weifang People's Hospital; RPMI1640 culture medium, trypsin, calf serum, Phosphate buffer and so on were purchased from Gibco; consumable materials such as cell culture dish were purchased from Corning

Incorporated; double resistant for cell culture, CCK8 kit and western blot reagents were purchased from Beyotime Biotechnology Co. Ltd., Martrigel gel was purchased from BD company, the synthesis of primer sequences of TP53, activator and inhibitor of mir-186 were conducted by Shanghai Baolige Biological Technology Co. Ltd., and the sequences of activator were: CCAAAGAAUUCUCCUUUUGGGC, the sequences of inhibitor were: GGUUUUUUAAGUG-GAAACCCG, anti-TP53 antibody was purchased from Abcam, fluorescence inverted microscope with type of TE2000 was purchased from Niko company; Electric rotary instrument and electro transfer reagent were the products of Loza company.

Cells transfection and the detection of TP53 protein

The frozen MGC cells were removed from liquid nitrogen and transferred to 37°C in a water bath. The test tube with MGC cells were taken from water bath to wipe off the water droplets in the frozen storage tube after 5 min. The cells were transferred into the 1.5 ml sterilized EP tube, and centrifuged at 1000 rpm/min for 3 min followed by transferring cells into RPMI 1640 culture medium containing 10% calf serum. Cell passage was performed after 24 h followed by digestion using 1 ml 0.25% trypsin. After cells (1×10^5) adhesion for 16 h, the mimics (activator) were mixed (10 μg:100 μl) and transfected by electric transfection kit according to manufacturer's instructions. After 36 h transfection, cells were collected, and the expression of TP53 protein was detected by western blot with β-actin as an internal control.

Changes of cell proliferation ability by CCK-8 method

The transfected cells were transferred into the 60 mm cell culture dish with the number of 1×10^5 cells per dish at 37°C 5% CO_2 and cultured for 6 h, 12 h, 24 h and 36 h in an incubator with saturated humidity. When the abundance of cells wall and the bottom reached approximate 90%, cells were counted after digestion, 1×10^4 cells per 100 μl each well were added to 96-well plate. Then 10 μl CCK8 solution was added into each well after 12 h followed by culturing 3 h. The cell growth rate and
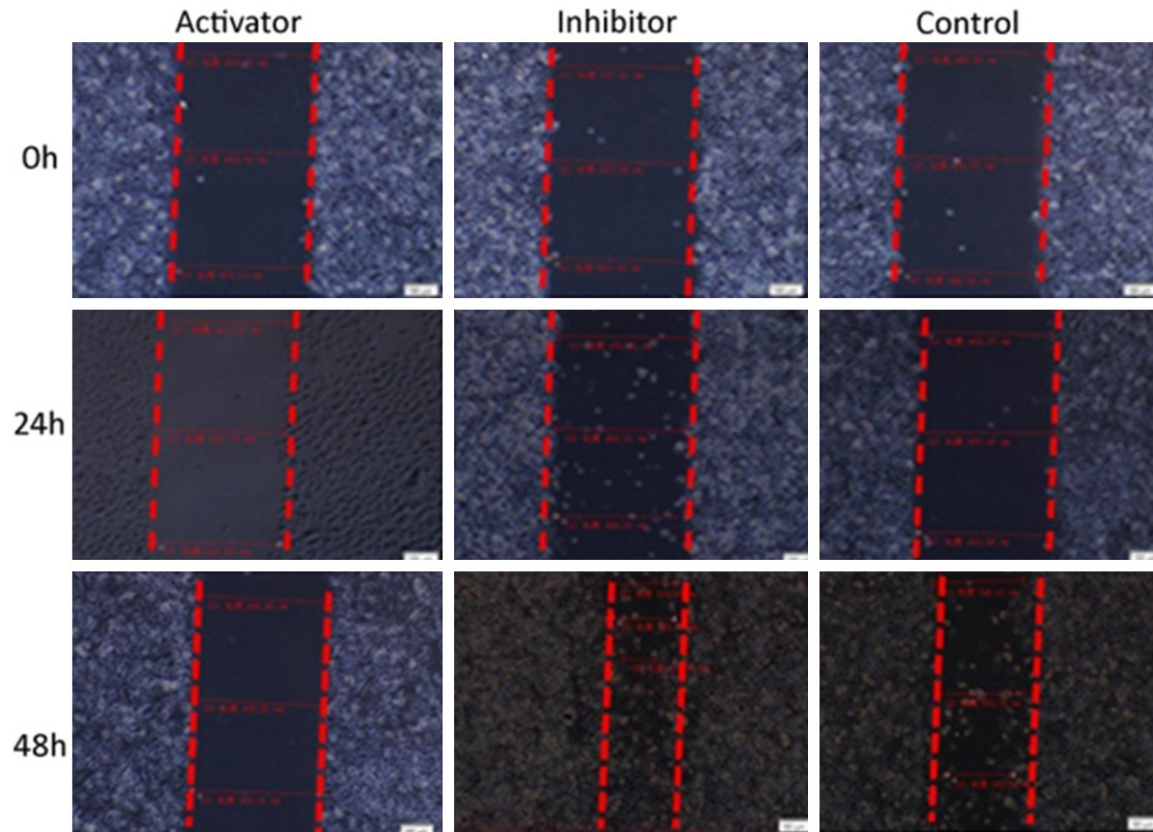
Figure 2. The results of scratch test.

inhibition rate were calculated through measuring the absorbance value of 450 nm by UV spectrophotometer.

Detection the effect of transfection on cells invasion by transwell

According to the manufacturer's instruction, Matrigel was coated on the polycarbonate membrane (8 μm aperture) in the upper chamber in the 24-well Transwell chamber and 70 μl Matrigel were added and cultured at 37°C for 60 min to make the matrix glue reform and become the basement structure of chamber. Then cell suspensions (1×10^5/ml) prepared with serum-free medium containing 10% BSA were added to upper chamber and down chamber each well together with complete culture medium and cultured for 12 h, 24 h and 36 h in the incubator at 37°C 5% CO_2 and saturated humidity. Then the trans-membrane cells were labeled with 0.1% crystal violet staining and observed under the fluorescence inverted microscope (100×). 3 horizons were randomly selected and the numbers of trans-membrane cell were recorded.

Cell migration was measured by cell scratch assay in different treatment groups

The cells after transfection were selected to prepare the suspension followed by placing in an incubator overnight at 37°C 5% CO_2. The cell masses in the plate were washed with PBS for 3 times, and then serum-free medium was added and cultured for 24 h and 48 h at 37°C 5% CO_2 followed by observation under a microscope. Image J software was used to analyze scratch width with 6 lines in the scratch drawn. Meanwhile, the migration distance was calculated.

Data analysis

SPSS11.0 software was used to analyze data. Data were represented as mean ± standard deviation (SD). Two-way ANOVA was performed to evaluate the significant difference. P<0.05 was considered as statistically significant.
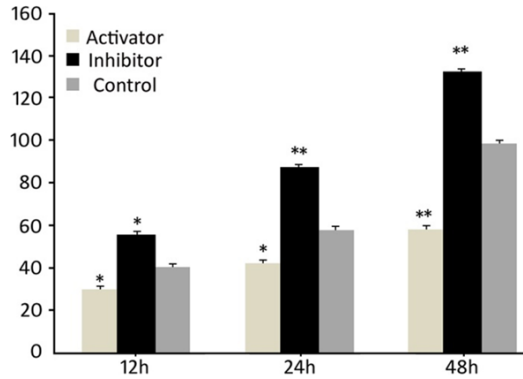
Figure 3. Determination of the number of transmembrane cells after transfection in Transwell Boyden chamber model. ****: Compared with control group, P<0.01; *: Compared with control group, P<0.05.
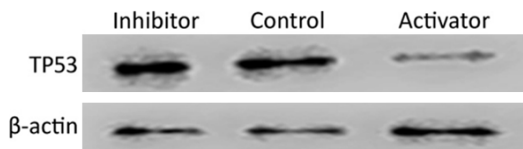
Figure 4. Detection of TP53 expression in each group by Western blot.

Results

Analysis the change of proliferation ability of MGC cells in different group by CCK-8 method

The change of proliferation ability of MGC cells in different group was analyzed by CCK-8 method and the results were shown in **Figure 1**. The absorbance value was higher in cells transfected with inhibitor than that transfected with control. At 12 h after transfection, the inhibitor group began to have obvious difference compared with control group (P<0.05), suggesting the ability of proliferation was significantly enhanced at the beginning of 12 h transfection with dramatically significant difference at 36 h transfection compared with control group (P<0.01). However, cells transfected with activator displayed decreased absorbance value compared with control group with dramatically significant difference at 36 h after transfection (P<0.01). Taken together, these results demonstrated that the activator and inhibitor begin to play a role at 12 h after transfection in a time-dependent manner.

The change of cell migration after transfection

The results of cell scratch test were showed in **Figure 2**. After 24 h transfection, the migration

distances of inhibitors, activators and control groups were 24.23±1.13 μm, 6±3.85 μm and 10.32±3.13 μm, respectively. After 48 h transfection, the migration distances were 37.23± 4.46 μm, 11±7.14 μm, 16.21±4.43 μm, respectively. Results showed that after transfection with mir-186 inhibitor, the migration ability of cells was increased with significant difference compared with the other two groups (P<0.05). However, the migration ability of cells was significantly decreased after transfection mir-186 activator with significant difference compared with inhibitor group and control group (P<0.05). These results showed that mir-186 had a certain regulation effect on the migration ability of MGC cell.

Effect of Transwell Boyden cell model for determination of mir-186 on the expression of cell invasion

The treated chamber was continuously cultured for 12 h, 24 h and 36 h and then was stained by 0.1% crystal violet for 10-15 min and was observed under the fluorescence inverted microscope. The results were shown in **Figure 3**. Compared with control group, the number of cells in the activator group and the inhibitor group were significantly higher and lower than that in the control group (P<0.01) respectively.

Detection TP53 expression after transfection 36 h

As shown in **Figure 4**, the expression of TP53 protein in activator group was obviously lower than that in control group and inhibitor group. However, the expression of TP53 protein in inhibitor group was slightly higher than that in the control group.

Discussion

The malignant degree and prognosis of the tumor have been shown to have a direct relationship with the proliferation, migration and invasion of tumor cells with most tumor suppressor genes playing a role in the various stages of tumor cells through regulation of cell cycles [14, 15]. Abnormal expression of some genes, especially the tumor suppressor genes, will directly induce cancer cell proliferation and metastasis, causing cancer cells spreading, which results in grave consequences [16, 17]. Therefore, studies on the regulation mechanism of tumor suppressor genes have become

a hotspot, such as mir-RNA, lnc-RNA and so on. At present, there are many tumor related genes such as TP53 and PTEN, which are also considered to be the significant genes involving in tumor related gene expression pathway, and the mechanism of the majority of tumors is related to the abnormal signaling pathways induced by these two genes.

Previous studies showed that there was different expression of mir-186 in cancer tissues and their corresponding adjacent cancer tissues [18, 19]. However, the effects of mir-186 on the mechanism of proliferation and invasion in gastric cancer cells and its signaling pathway remain poorly understood. This study aimed to investigate the effect of mir-186 on the proliferation and invasion of gastric cancer cells through manipulating the expression of mir-186 in MCG cells transfected with activator and inhibitor. Results showed that after transfection with mir-186 inhibitor, the ability of cell proliferation, migration and invasion were significantly enhanced, with significant differences compared with activator group and control group (P<0.05). However, after transfected with the activator of mir-186, cell proliferation, invasion and migration ability were decreased significantly with significant difference compared with the control group (P<0.05). These findings are consistent with the role of mir-186 in colon cancer cells [20]. In addition, TP53 expression in different time periods was detected after transfection of mir-186 activator and inhibitor into MGC cell line and results showed that there was a negative correlation between the expression of mir-186 and TP53, demonstrating mir-186 may regulate the proliferation and invasion of gastric cancer cell line MGC through regulating TP53 signaling pathway.

Conclusion

Our study demonstrated that the expression of mir-186 in gastric cancer affects the proliferation and invasion of gastric cancer cells. In addition, mir-186 may involve in the regulation TP53 signaling pathway in gastric cancer cells.

Acknowledgements

Disclosure of conflict of interest

None.

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