



MiR-218 Targeted Regulation of Robol Expression Regulates Proliferation, Invasion and Migration of Glioma Cells

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ABSTRACT

In recent years, more and more researches has focused on "molecular targeted therapy" for basic genes and regulatory cells, but the effect is not ideal. Therefore, the discovery of new molecular targets with diagnostic and therapeutic significance can not only lay a solid foundation for molecular diagnosis and classification of lesions but also contribute to targeted therapy of glioma. This study aimed to discover the molecular mechanism of mir-218 targeting the regulation of robol expression on proliferation, invasion and migration of glioma cells and to provide a theoretical and experimental basis for finding therapeutic targets of glioma. The purpose of this study was to investigate the effect of mir-218 on gliomas by using the method of control experiment. The results showed that the number of gliomas under the action of mir-218 decreased from about 150 to about 80, and the number would tend to a fixed value range over time. In the experiment, the data decreased from about 150 to nearly 20, and compared with the control group, the control of glioma cell proliferation was very excellent.

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Introduction

Glioma is one of the most common local tumors in the brain. Some gliomas (some brain astrocytomas, papillary shapes, etc.) are covered with a capsule, which can be fundamentally displaced and have a good prognosis. Most of the lesions (such as glioblastoma multiforme) are penetrating growth, the end of the lesions is fuzzy, it is difficult to completely resect the lesions, and the recurrence rate is very high (1-2). For patients with a high degree of deterioration, the combination of radiotherapy and chemotherapy is mainly used in the clinic, but the overall treatment effect is not ideal (3). Exploring the effect of mir-218 targeting on the expression of robol on glioma cells has an important reference for the treatment of glioma.

High expression of lncRNA CRNDE is considered to be an oncogene in human glioma (4-5). Allen's findings indicate that CRNDE can be used as a diagnostic marker or a potential target for glioma treatment. The transcripts of colorectal tumor differential expression gene (CRNDE) are believed to be long noncoding RNA (lncRNA) expressed in

specific areas of the human brain, which is the most up-regulated lncRNA in glioma (6-7). The up-regulation of CRNDE has been confirmed by the primary specimens of gliomas and the cell lines cultured in vitro.

Glioblastoma multiforme (GBM) is helpful in finding the potential target of effective treatment to reveal its invasive mechanism (8-9). Wei studied the role of miRNA-373 (mir-373) in GBM cell line U251, which proved that mir-373 did not affect the growth of U251 (10-12). In order to prove the high expression of IKBKE in human glioma, the down-regulation of IKBKE significantly inhibited the proliferation and invasion of glioma cells (13). Tian confirmed the microRNAs targeting IKBKE of let-7b and let-7i by luciferase detection. Western blot analysis showed that let-7b / I analog could down-regulate IKBKE and up-regulate E-cadherin (14-15).

The effects of mir-218 on gliomas were analyzed by comparing low-grade gliomas with high-grade gliomas, high and low expression of mir-218, and luciferase. The collected data are input into excel, and computer software is used to analyze and compare the

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collected data. The statistical methods used are normal distribution and average value.

Materials and Methods

Tissue Sample

According to the medical diagnosis standard, the glioma tissue samples were divided into low-grade glioma group and high-grade glioma group, with 15 cases in each group. All samples were taken from glioma samples which were removed by neurosurgery in Huashan Hospital, and the samples were approved by patients and their families, including 18 males and 12 females aged 15-75 years. Another 12 non-tumor brain tissues from patients with brain contusion and laceration undergoing internal decompression were selected as the normal control group and the glioma cells in these sample groups were observed.

Cell Culture

C6 glioma cells were purchased from the cell bank of Shanghai Ruijin Hospital and cultured according to its culture instructions. Every three to five days, the cells are in the logarithmic growth stage and increase once when the bottom of the culture bottle is about 65% (mainly to ensure the activity of cells). The cells increased in the proportion of 1:2 or 1:3. The control group was treated with robol (50 μ g / L) for about 10 minutes. After the treatment, the cells were collected into the test tube for observation. Then, the glioma cells were measured and packed separately and placed in -100 °C refrigerator for use.

Detection of Glioma Cell Activity

The glioma cells were inoculated into 48-well culture plates (3 \times 42 cells per well). When the cells fused at about 75% after one day, the cells were transfected at the ratio of liposome to DNA of 1.6 μ L: 1.0 μ G. The transfected cells were divided into cell control group and robol group. Each group was set up with two double holes, and the solution was changed after two days of transfection. Culture medium was used to prepare single cell suspension, inoculated to a 64-well plate, and MTT was detected one day later. During the test, 10 μ l of test solution was added to each hole, and the culture was terminated one day later. The supernatant floating on the culture in the abandoned hole was absorbed, and 100 μ l of DMSO was added to each hole. After shaking the tube for

five minutes, the absorbance value at 530nm was measured by the enzyme analyzer.

Detection of Protein Expression Level

The total protein was extracted and quantified from the samples of the low-grade glioma group and the high-grade glioma group. After 15% gel electrophoresis analysis, the films were transferred to PVDF films. 5% skimmed milk was sealed at room temperature for 2 hours, added with corresponding anti-4 °C for one night, and washed with tbs three times. The second antibody was added and cultured at 37 °C for half an hour. Finally, the reagent box was added for development and the film was exposed.

Luciferase Experiment

The cells were divided into two groups: mir-218 expression group, which were transfected with fluorescence report plasmid wild type and mutant, and empty plasmid control group; mir-218 inhibition group, which were transfected with fluorescence report plasmid wild type and mutant, and empty plasmid control group. One day after transfection, the luciferase activity was detected by a double fluorescence detection kit.

Statistical Methods

The number of cells in this project was calculated by computer software. The results showed that the average value and standard deviation in mathematics were used, which were expressed as mean \pm SEM, respectively. The comparison between the experimental group and the control group was tested by independent samples, and the comparison between multiple data sets was tested by one-way ANOVA. When the p-value is less than 0.05, it is considered that there is a statistical difference between the data.

Results and discussion

Relationship between Glioma and MiR-218

The occurrence of glioma involves gene abnormality, so understanding the gene variation in the process of glioma occurrence and development can provide a theoretical basis for targeted treatment of glioma. The high and low expression of mir-218 have different effects on the survival time of glioma and have a close relationship with the survival time of glioma patients.

It can be seen from Figure 1 that the survival time of patients with high expression is significantly shorter than that with low expression. At the beginning of time, the two forms have little effect on the survival time. With the increase of time, the difference between the two forms is larger.

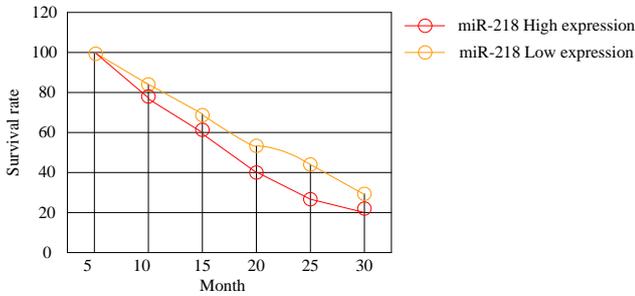


Figure 1. Survival rate of glioma patients

The Effect of Mir-218 on Different Glioma Cells

The abnormal expression of mir-218 will lead to a change in biological behavior. Some miRNAs play an important role in the proliferation, invasion, migration and chemical sensitivity of mucosa. Mir-218 wild type and mir-218 inhibited type have different effects on the expression of low-grade glioma group and high-grade glioma group, as shown in Figure 2:

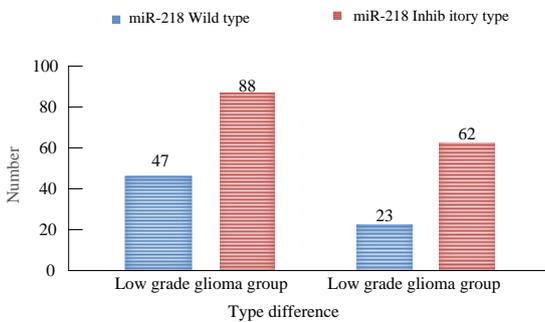


Figure 2. Effect of mir-218 on different glioma cells

It can be seen from the data in the figure that the effect of mir-218 on low-grade glioma is higher than that of high-grade glioma. Under the same cell condition, mir-218 wild type had a more significant control effect on glioma cells than the mir-218 inhibitory type.

Comparison of Glioma Cell Proliferation

Through the colony-forming experiment, the control group was glioma cells without mir-218, and the experimental group was glioma cells with mir-

218. The effect of mir-218 on the proliferation of glioma cells can be evaluated by comparing the observed approximate cell number, as shown in Table 1:

Table 1. Cell growth rate

Group	Number of cells passing through the matrix
Control group	142-163
Experience group (One day)	86-98
Experience group (A week)	71-84
Experience group (A month)	25-31
Experience group (Three months)	22-25

The results showed that the number of stromal cells in the experimental group was significantly less than that in the control group, indicating that the experimental group had a significant ability to inhibit cell invasion indicating that mir-218 played an important role in the occurrence of glioma, and could control the deterioration and mutation of patients. And with the passage of time, the effect is more significant. Finally, the glioma cells are controlled in a certain range. For the data in the above table, a more obvious contrast will appear when making a radar map, as shown in Figure 3.

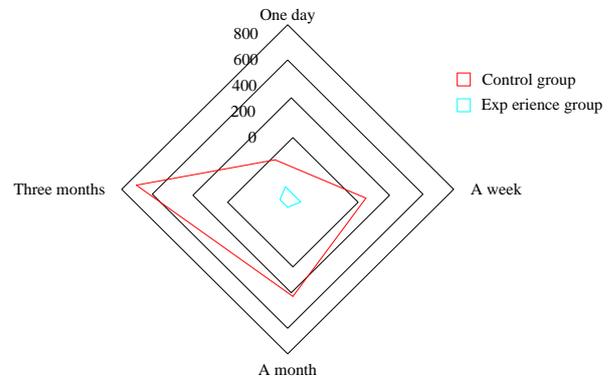


Figure 3. Glioma cell proliferation

It can be seen from the figure that mir-218 has an obvious effect on the inhibition of glioma cell proliferation. Compared with the control group, the colony formation of the mir-218 group was significantly smaller than that of the control group after one day of culture. The difference was statistically significant. Compared with the control group, the growth rate of glioma cells in the mir-218 group decreased significantly.

The Relative Activity of Luciferase in Each Co Transfection Group

A large number of studies on glioma and other

tumors have shown that gene expression is different, gene mutation and gene modification can be used as tumor molecular markers. MiRNA can regulate common tumor processes, such as labeling tyrosine kinase receptor, invasion, differentiation inhibition, cell cycle enhancement and apoptosis inhibition, so as to play a regulatory role in glioma.

As shown in Figure 4, by quantitative detection of luciferase activity, the luciferase activity of the p-wt + SCR CO transformation group or p-wt alone transfection group in the two cell lines is higher than that of the p-wt + miR-137 CO transformation group in the same cell line. However, there was no significant difference in luciferase activity between the p-mt + SCR co-transfer group or the p-mt alone transfection group and the p-mt + miR-137 group. The results showed that mir-218 could negatively regulate the expression of robol, and the mechanism was that the seed sequence of mir-218 inhibited the expression of its target protein by binding to robol.

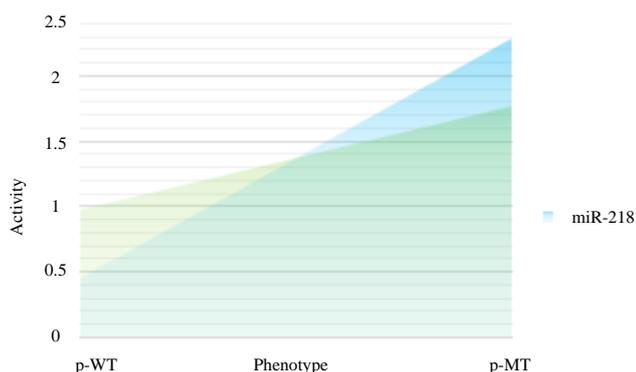


Figure 4. Relative activity of luciferase in transfection group

The invasion and migration of glioma cells are the root cause of the formation of mucosa, which is difficult to be completely removed. The world health organization divides the lesions into four histopathological grades: astrocytoma, astrocytoma, glioblastoma multiforme and oligodendroglioma. Among them, glioblastoma is the most common malignant glioblastoma, increasing with age (16-18). Although the treatment of gliomas has improved significantly, it is still difficult to treat (19). Elucidate the regulatory mechanism of glioma development has become one of the hot spots in medical research in recent years (20). In this study, the targeted robol expression of mir-218 regulated the proliferation, invasion and migration of glioma cells, providing further research reference for mucosal therapy and

drug selection. I believe that in the future, we will make more outstanding achievements in further exploration and provide a better theoretical basis for the future development of medicine. Subsequent functional tests showed that the recovery of mir-218 led to a significant decrease in cell proliferation, indicating that mir-218 has the effect of inhibiting tumors and participating in glioma proliferation. In order to study the effect of mir-218 on the proliferation of glioma cells, the transfection experiment was also carried out. In the colony formation experiment, the yield of stably transfected glioma cells decreased significantly in the short term and in the long term. Finally, it tends to a relatively stable level.

The occurrence of glioma may lead to gene abnormality, so understanding the gene variation in the process of glioma occurrence and development can provide a good theoretical basis for targeted treatment of glioma (21-23). However, the exact molecular mechanism of the occurrence and development of gliomas has not yet been determined, and a large number of studies are still needed to fully understand how cells regulate in the process of pathological changes so as to facilitate the use of new biomarkers and new technologies to improve the treatment of gliomas (24-27).

Acknowledgments

Not applicable.

Interest conflict

The authors declare that they have no conflict of interest.

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