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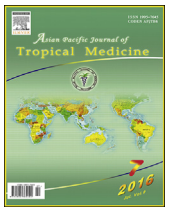

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Experimental study on the inhibition effect of miR-106a inhibitor on tumor growth of ovarian cancer xenografts mice

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ABSTRACT

Objective: To study the inhibition effect of miR-106a inhibitor on tumor growth of ovarian cancer xenografts mice.

Methods: BALB/c mice were selected as experimental animals, ovarian cancer SKOV-3 cells transfected with miR-106a inhibitor and its negative control were inoculated subcutaneously, intratumoral injection of miR-106a inhibitor and its negative control were continued after tumor formation, and they were enrolled as treatment group and model group, respectively. Tumor volume and weight as well as Ki-67 and programmed cell death 4 (PDCD4) expression were determined; miR-106a inhibitor and its negative control as well as miR-106a mimic and its negative control were transfected into SKOV-3 cells, and expression of PDCD4 in cells was determined.

Results: Tumor tissue volume and weight as well as mRNA expression and protein expression of Ki-67 in treatment group were significantly lower than those in the model group while mRNA expression and protein expression of PDCD4 were significantly higher than those in the model group; transfection of miR-106a mimic could decrease mRNA expression and protein expression of PDCD4 in SKOV-3 cells, and transfection of miR-106a inhibitor could increase mRNA expression and protein expression of PDCD4 in SKOV-3 cells.

Conclusions: Transfection of miR-106a inhibitor can inhibit the growth of tumor in ovarian cancer xenografts mice through increasing the expression of PDCD4.

1. Introduction

Ovarian cancer is one of the common malignant tumors of female reproductive system, and the death caused by ovarian cancer ranks first in gynecologic malignant tumors. Ovarian cancer patients can obtain complete remission after cytoreductive surgery and adjuvant chemotherapy, but the overall prognosis is still poor, and there will be local recurrence and distant metastasis in vast majority of patients [1,2]. Ovarian cancer involves many links, genes and steps, and the inactivation of tumor suppressor genes and activation of proto-oncogene as well as abnormal expression of apoptosis-related genes and invasion-related genes can all cause tumor recurrence and metastasis [3,4]. However, the upstream signals regulating the expression of above malignant tumor-related genes are still not clarified at present, and accurate targets for the clinical treatment of ovarian cancer are also scarce. MicroRNA (miRNA) is a family of evolutionarily highly conserved non-coding small molecule RNA found in recent years, which is combined with target gene mRNA 3′ untranslated region (3′UTR) so as to regulate the expression of multiple genes [5,6]. Studies have confirmed that miR-106a has the characteristics of proto-oncogene, and participates in the occurrence and development of lung cancer [7], gastric cancer [8], colon cancer [9] and many other kinds of malignant tumors, thus it was speculated in the study whether miR-106a was also involved in the occurrence and development of ovarian cancer, and whether inhibiting the biological functions of miR-106a could inhibit the growth of ovarian cancer. In the following study, transfection of miR-106a inhibitor was adopted to inhibit the biological effect of miR-106

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in tumor cells, and then the tumor growth in ovarian cancer xenografts mice was analyzed.

2. Materials and methods

2.1. Experimental materials

Human ovarian cancer SKOV3 cell lines were bought from the cell center of Union Medical College, RPMI1640 medium and fetal bovine serum were purchased from Gibco Company, miR-106a mimic and negative control as well as miR-106a inhibitor and negative control were synthesized by Genepharma Company (Shanghai), and Lipofectamine2000 transfection reagents were purchase from Invitrogen Company (USA). RNA extraction kits, reverse transcription kits and fluorescence quantitative PCR kits were bought from Tiangen Biotech Company (Beijing), and Elisa kits were purchased from Westang Biotech Company (Shanghai).

Twenty SPF female BALB/c mice with body mass (18–24) g were bought and raised by the animal center of Hebei University, they were raised under constant temperature (18–22) °C and constant humidity 50%–80%, and they had free water and feeding. Mice were randomly divided into model group and treatment group, 10 in each group. The animal experiment was approved by the ethics committee of Affiliated Hospital of Hebei University.

2.2. Experimental methods

2.2.1. Cell culturing and transfection of miR-106a inhibitor

SKOV3 cell lines were recovered and then cultured in RPMI1640 medium containing 10% fetal bovine serum, cells were digested and sub-cultured with 0.25% trypsin after growing all over, then they were respectively inoculated in culture bottle and Petri dishes, cells in the culture bottles were used for continuous digestion and passage, cells in the Petri dishes were used for processing, and the methods were as follows: miR-106a inhibitor, miR-106a mimic, NC inhibitor and NC mimic powder was configured to 20 μmol/L solution, and the proportion of 6 μL Lipofectamine2000 and 3 μL miR inhibitors or mimics per 1 mL medium was followed for transfection for consecutive 24 h.

2.2.2. Establishment and intervention methods of xenografts mice

SKOV-3 cells transfected with miR-106a inhibitors or NC inhibitors were taken and digested to get cell suspension, the cell density was adjusted to 10^7/mL, model group received subcutaneous injection of 0.5 mL SKOV-3 cell suspension transfected with NC inhibitor into the right upper extremity axillary lateral side, treatment group received subcutaneous injection of 0.5 mL SKOV-3 cell suspension transfected with miR-106a inhibitor into the right upper extremity axillary lateral side, and after 7 d, those with tumor size more than 3 mm^3 were successfully established xenografts models and then received the following intervention: on the 7th day after local injection of SKOV-3 cell suspension, model group received intratumoral injection of 5 μL NC inhibitor + 5 μL Lipofectamine2000 mixture, treatment group received intratumoral injection of 5 μL miR-106a inhibitor + 5 μL Lipofectamine2000 mixture, once every four days, for a total of 10 times.

2.2.3. Assessment of tumor growth

On the 7th day after local injection of SKOV-3 cell suspension as well as the 10th day, 20th day, 30th day and 40th day after local injection of miR-106a inhibitor, vernier caliper was used to measure the maximum major diameter (a) and the maximum transverse diameter (b) of tumor, the maximum transverse diameter referred to the maximum length of the line perpendicular to the maximum major diameter, and tumor tissue volume $V = 0.5 \times a \times b^2$. On the 40th day after local injection of miR-106a inhibitor or NC inhibitor, the volume of tumor tissue was measured, and then the mice were executed and anatomized to obtain and weigh tumor tissue.

2.2.4. Fluorescence quantitative PCR detection methods

Extraction of RNA in cells was by total RNA extraction kit for cells, extraction of RNA in tumor tissue was by total RNA extraction kit for animal tissue, RNA was obtained and then reverse-transcribed into cDNA by reverse transcription Kit TIANScript II RT Kit, then fluorescence quantitative PCR Kit was used to amplify Ki-67 and programmed cell death 4 (PDCD4) genes as well as β-actin, and amplification conditions were as follows: 95 °C initial denaturation 5 min, 95 °C 30 s, specific annealing temperature 30 s, 72 °C 30 s and repeating for 40 cycles, and after amplification curve was obtained, $2^{-\Delta\Delta Ct}$ formula was followed to calculate the relative mRNA levels of Ki-67 and PDCD4.

2.2.5. Elisa detection methods

Treated SKOV3 cells were collected, added in protein lysis buffer, fully broken and centrifuged to get protein suspension; tumor tissue was collected, added in PBS, fully grinded and centrifuged to get protein suspension. Elisa kits for PDCD4 and Ki-67 were used to determine protein content.

2.2.6. Statistical methods

SPSS20.0 software was used to input and analyze data, measurement data comparison between two groups was by *t* test and $P < 0.05$ indicated statistical significance in differences.

3. Results

3.1. Tumor tissue growth

On the 7th day after local injection of SKOV-3 cell suspension (the time of tumor formation), tumor tissue volume in treatment group was (10.32 ± 1.86) mm^3 *vs.* (34.28 ± 6.61) mm^3 and significantly lower than that in the model group; on the 10th day, 20th day, 30th day and 40th day after treatment, tumor volume of both groups gradually increased, and tumor tissue volume in treatment group at various points in time were significantly lower than those in the model group; mice were executed and anatomized to get tumor tissue, and tumor tissue weight in treatment group was (103.56 ± 17.78) mg *vs.* (523.25 ± 79.26) mg and significantly lower than that in the model group, shown in Table 1.

3.2. Ki-67 expression in tumor tissue

Detection of Ki-67 mRNA expression in tumor tissue of model group and treatment group by fluorescence quantitative PCR showed that mRNA level of Ki-67 in tumor tissue of

Table 1
Volume and weight of tumor tissue on the 10th day, 20th day, 30th day and 40th day after treatment.

Groups	Tumor tissue volume (mm^3)					Tumor tissue weight (mg)
	0	10	20	30	40	
Treatment group	10.32 ± 1.86**	33.51 ± 4.61**	49.42 ± 5.18**	65.20 ± 8.62**	82.35 ± 11.64**	103.56 ± 17.78**
Model group	34.28 ± 6.61	94.29 ± 11.34	176.55 ± 24.18	258.24 ± 41.35	379.13 ± 52.48	523.25 ± 79.26

Compared with model group at the same point in time, $^{**}P < 0.01$.

treatment group was (0.26 ± 0.05) *vs.* (1.00 ± 0.17) and significantly lower than that of the model group; detection of Ki-67 protein expression in tumor tissue of model group and treatment group by Elisa showed that protein level of Ki-67 in tumor tissue of treatment group was (35.96 ± 6.13) *vs.* (127.75 ± 21.45) μg/mg total protein and significantly lower than that of the model group.

3.3. PDCD4 expression in tumor tissue

Detection of PDCD4 mRNA expression in tumor tissue of model group and treatment group by fluorescence quantitative PCR showed that mRNA level of PDCD4 in tumor tissue of treatment group was (2.86 ± 0.61) *vs.* (1.00 ± 0.19) and significantly higher than that of the model group; detection of PDCD4 protein expression in tumor tissue of model group and treatment group by Elisa showed that protein level of PDCD4 in tumor tissue of treatment group was (186.36 ± 31.56) *vs.* (42.84 ± 7.12) μg/mg total protein and significantly higher than that of the model group.

3.4. PDCD4 expression in SKOV-3 cells

After transfection of miR-106a mimic and NC mimic, analysis of PDCD4 expression in SKOV-3 cells was as follows: compared with cells transfected with NC mimic, mRNA content of PDCD4 (0.48 ± 0.08) *vs.* (1.00 ± 0.11) and protein content of PDCD4 (15.61 ± 2.32) *vs.* (34.47 ± 5.28) μg/mg total protein significantly decreased in cells transfected with miR-106a mimic. After transfection of miR-106a inhibitor and NC inhibitor, analysis of PDCD4 expression in SKOV-3 cells was as follows: compared with cells transfected with NC inhibitor, mRNA content of PDCD4 (2.52 ± 0.42) *vs.* (1.00 ± 0.14) and protein content of PDCD4 (70.25 ± 10.38) *vs.* (37.23 ± 6.13) μg/mg total protein significantly increased in cells transfected with miR-106a inhibitor.

4. Discussion

Metastasis, recurrence rate and mortality rate are high in ovarian cancer patients after cytoreductive surgery and adjuvant chemotherapy, and the inactivation of tumor suppressor genes and the activation of proto-oncogene as well as abnormal expression of apoptosis-related genes and invasion-related genes are all closely related to the recurrence and metastasis of ovarian cancer. miRNA is a class of small non-coding RNA with length of (22–25) bp, and can be combined with target gene mRNA 3′UTR region to lead to mRNA degradation or inhibit mRNA translation [10,11]. In the occurrence and development of malignant tumor, some fragile area in chromosomes can change, and more than 50% of the miRNA genes are in the fragile area of chromosomes and with abnormal expression, resulting in changes in the expression of a variety of downstream target genes and causing corresponding changes in cell biological behavior [12,13].

miR-106a is a class of miRNA with proto-oncogene properties found in recent years, and it shows the trend of high expression in lung cancer [7], gastric cancer [8], colon cancer [9] and many other malignant tumor tissues. Experimental cell research of domestic LI Min and others [14] confirms that miR-106 is highly expressed in metastatic ovarian cancer cells SKOV-3 and miR-106a mimic can promote ovarian cancer cell migration and invasion *in vitro*. So it was speculated that miR-106a had promoting effect on the occurrence and development of ovarian cancer, and antagonizing the biological function of miR-106a in cells could inhibit the development of ovarian cancer. But relevant study in vivo on the relationship between miR-106a and the occurrence and development of ovarian cancer is still scarce at present.

In this study, transfection of miR-106a inhibitor was adopted to antagonize the biological effect of miR-106a in ovarian cancer cells, and after subcutaneous injection of ovarian cancer cells transfected with miR-106a inhibitor and NC inhibitor, observation of the tumor formation showed that at the time of tumor formation, tumor tissue volume of treatment group was significantly less than that of model group. That means that after subcutaneous injection of ovarian cancer cells transfected with miR-106a inhibitor into mice, tumor volume is smaller at the time of tumor formation. In the process of tumor growth, continuous intratumoral injection of miR-106a inhibitor can further inhibit tumor growth, and tumor tissue volume of treatment group on the 10th day, 20th day, 30th day and 40th day after injection were significantly less than those of model group and the weight of tumor tissue from anatomizing executed mice was significantly lower than that of model group. Ki-67 is a nuclear antigen located in the nucleus and can reflect the cell proliferation activity, and analysis of Ki-67 expression in tumor tissue in the research confirmed that mRNA expression and protein expression of Ki-67 in tumor tissue of treatment group were significantly lower than those of the model group. Thus it further confirms that transfection of miR-106a inhibitor can inhibit the growth of ovarian cancer, reduce tumor volume and reduce tumor weight.

The way for miRNA to regulate gene expression is to be combined with target gene mRNA 3′UTR to induce mRNA degradation and inhibit mRNA translation. Studies have reported that PDCD4 is an important tumor suppressor gene in the body [15–17] and also a target gene regulated by miR-106a [18]. Protein encoded by PDCD4 contains two highly conserved α-helical MA-3 domains, can interact with RNA helicase and eIF4A protein, and can also adjust the phosphorylation status of a variety of downstream signaling molecules, thus inhibiting cell proliferation and invasion [19,20]. Research of Wei N [21] shows

that PDCD4 expression decreases significantly and anticancer activity is lost in ovarian cancer tissue. In the research, analysis of PDCD4 expression in tumor tissue confirmed that after transfection of miR-106a inhibitor, PDCD4 expression in tumor tissue increased significantly. Thus it demonstrates that miR-106a can adjust the expression of PDCD4 in ovarian cancer tissue, thereby affecting the growth of tumor. In order to further define whether miR-106a directly regulated PDCD4 expression, miR-106a mimic and inhibitor were transfected into ovarian cancer cells respectively in the study, and analysis of mRNA and protein expression of PDCD4 showed that miR-106a mimic could inhibit PDCD4 expression and the inhibitor could increase PDCD4 expression. It indicates that miR-106a can target and regulate PDCD4 expression in ovarian cancer cells.

To sum up, transfection of miR-106a inhibitor can inhibit the growth of tumor in ovarian cancer xenografts mice through increasing the expression of PDCD4.

Conflict of interest statement

We declare that we have no conflict of interest.

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