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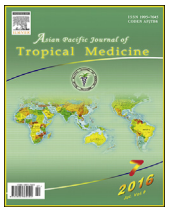

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Experimental study on the inhibition effect of miR-106a inhibitor on tumor growth of ovarian cancer xenografts mice

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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