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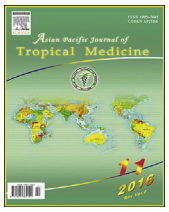

Original Research http://dx.doi.org/10.1016/j.apjtm.2016.09.002

Expression and mechanism of action of miR-196a in epithelial ovarian cancer

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ARTICLE INFO

Article history:
Received 16 Jul 2016
Received in revised form 17 Aug 2016
Accepted 16 Sep 2016
Available online 15 Oct 2016

Keywords:
MiR-196a
Epithelial ovarian cancer
Migration
Invasion
HOXA10

ABSTRACT

Objective: To explore the expression, biological function and possible mechanism of action of microRNA molecular-196a (miR-196a) in epithelial ovarian cancer.

Methods: RT-PCR was used to detect the expression quantities of epithelial ovarian tissue, benign ovarian tissue, normal ovary epithelial tissue, ovarian cancer cell lines and miR-196a in normal ovarian epithelial cells to analyze the relationship between the expression of miR-196a and the clinical pathologic parameters of ovarian cancer. Among those cell lines, the cell line of which miR-196a expressed the most or least was selected and transfected the ovarian cancer cell line by using negative control plasma and miR-196a inhibitor. After transfection, RT-PCR was used to test the expression quantity of miR-196a, Transwell chamber method was applied to determine the migration and invasion abilities of ovarian carcinoma cells and Western blot was employed to detect the expression of HOXA10 protein.

Results: The relative expression quantities of miR-196a in ovarian cancer tissue and benign ovarian tissue were significantly higher than that in normal ovarian epithelial tissue, and the expression quantity of miR-196a in ovarian cancer tissue was distinctively higher than that in benign ovarian tissue ($P < 0.05$). Among 78 cases of epithelial ovarian cancer, the expression quantities of miR-196a in patients with low differentiation were all significantly higher than those in patients with high differentiation ($P < 0.05$). The expression of miR-196a showed no significant relation with age, clinical stage and whether CA125 was positive or not in patients ($P > 0.05$). Compared with normal ovarian epithelial cell line IOSE80, the expression quantities of miR-196a of all ovarian cancer cell lines increased obviously and differences were statistically significant ($P < 0.05$). Among them, the expression of miR-196a of ovarian cancer cell line SKOV3 was the highest, while it decreased significantly (4.678 ± 0.785 *vs.* 2.131 ± 0.345, $t = 2.938$, $P < 0.05$) after the ovarian cancer cell line SKOV3 was transfected by miR-196a inhibitor. The results of Transwell chamber method showed that the migration and invasion abilities of ovarian cancer cells SKOV3 were declined significantly after the expression of miR-196a was down-regulated and the difference showed statistical significance ($P < 0.05$). The results of Western blot revealed that the relative expression of HOXA10 decreased distinctly after the expression of miR-196a was down-regulated and also the difference showed statistical significance ($P < 0.05$).

Conclusions: The miR-196a might serve as a cancer-promoting gene to promote the migration and invasion of epithelial ovarian cancer by downstream target gene HOXA10.

1. Introduction

The fatality rate of epithelial ovarian cancer has topped the list of gynecologic malignant tumors. Epithelial ovarian cancer is a common malignant tumor with concealed onset, difficult for early diagnosis, high rate of recurrence and migration, intractable treatment and poor prognosis, which seriously threatens women's life and health [1]. At present, researchers on the pathogenesis of epithelial ovarian cancer are still in the stage of exploration. Current studies mostly concentrate on levels of gene regulation such as histone modification, DNA methylation and non-coding RNA [2–4]. Non-RNA is different from protein-coding genes. It regulates and controls network to regulate cell differentiation, disease occurrence and the nature of biological evolution and heredity by new RNA-mediated genetic massage expression, which provides new thoughts for the

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Peer review under responsibility of Hainan Medical University.
Foundation project: It was supported by the General Project of Provincial Natural Science Research of the Anhui Higher Education Institutions (Grant No. KJ2015B096by).

pathogeneses and treatments of multiple important human diseases with more and more attentions.

MicroRNA molecular-196a (miR-196a) is a member of the non-code RNA family with a depth of 21–23 nucleotides, which combines with downstream target gene mRNA 3′ untranslated regions (3′-UTR) adequately or deficiently to specifically explain or inhibit the translation of mRNA [5]. Many important life activities such as the growth and differentiation of cells on organisms are related to miRNA. It is supposed that miRNA regulates and controls about 1/3 genes of all human genomes, and it has been found that many diseases have relations with the abnormal expression of miRNA [6–8]. Since half of the miRNA are located in cancer-regulated-related chromosomal domain fragile sites such as the regions of homozygous deletion, loss of heterozygosity, oncogene and around tumor suppressor gene, breakpoint area and amplified region, the occurrence and development of tumors are closely related to miRNA. At the moment, it has been proved that the expression or loss of function of miRNA is closely associated with various tumors such as breast cancer [9], liver cancer [10], non-small cell lung cancer [11], gastric cancer [12], *etc*.

MiR-196a belongs to the family of homeotic genes (HOX) and plays different roles in different cells and tissues. Researches in recent years has found that the over-expression of miR-196a in cervical cancer [13], oral cancer [14] and gastric cancer [15] might play a role of cancer gene in regulating and controlling the growth, differentiation and metastasis of cancer cells. HOX is a kind of highly conserved homeobox DNA sequence consisting of 39 transcription factors which influence the growth and development of cells, embryos and tissues according to anterior–posterior axis (A–P) in the embryonic development period. It is found that the over-expression of HOXA10, a family number of HOX, in ovarian cancer is closely related to the occurrence of ovarian cancer [16].

At present, although the expression and biological function of miR-196a in ovarian cancer remain unknown, it is found through bioinformatics finding aid that HOXA10 is one of the downstream targets of miR-196a. Therefore, this study aimed to discuss the expression and biological function of miR-196a in epithelial ovarian cancer and to study whether miR-196a has a role to play by regulating and controlling the target gene HOXA10.

2. Materials and methods

2.1. Clinical tissue samples

A total of 78 epithelial ovarian cancer tissue samples acquired from December 2013 to December 2015 in our hospital were collected. Among them, 36 cases were benign ovarian tissues and the other 36 were normal pathological sections of ovary epithelial tissues. All samples were diagnosed definitely by pathological method. Patients with epithelial ovarian cancer received no radiotherapy, chemotherapy or immunotherapy before operation. The ages of the 78 patients ranged from (29–64) years with the average age of (47.75 ± 15.63) years. Out of those patients, 33 cases were diagnosed with mucinous cystadenocarcinoma and 45 suffered from serous papillary cystadenocarcinoma. As for pathological grades, 43 cases were of poorly differentiated stage and 35 were of highly to moderately differentiated stage. Clinical stages consisted of 8 cases of I stage, 26 cases of II stage, 32 cases of III stage and 12 cases of IV stage. The age of the 36 patients with benign ovarian tumors ranged from 25 to 66 years with the average age of (46.28 ± 16.28) years, and 16 cases of them were diagnosed with mucinous cystadenoma and the other 20 cases were with papillary cystadenoma. The other 36 cases of normal ovarian epithelial tissues were all normal tissues collected from hysterectomies with ages of (27–63) years and the average age of (46.69 ± 16.10) years. The tissues were all quick-frozen by placing them in liquid nitrogen once they were excised for further application.

2.2. Cell culture

Ovarian cancer cell lines (SKOV3, OVCAR3, A2780, ES2) and normal ovarian epithelial cells (IOSE80) were all purchased from Shanghai Huiying Biological Technology co., Ltd. and cultured in 10% fetal bovine serum medium with saturated humidity and 5% CO_2 at 37 °C. Cells growing in the logarithmic phase were selected and digested by 0.25% trypsin. The subsequent experiments were conducted after 2–3 stable passages.

2.3. RT-PCR

About 150 mg of the pathological tissue samples were selected, thawed, mixed, dissociated and centrifuged. RT-PCR was used to test the expression quantity of miR-196a of each pathological tissue sample and cell line. Firstly, TRIzol kits (Invitrogen, USA) was applied to extract the total thyroid RNA. The reverse transcription reaction system was set for RNA reverse transcription to compound the first-strand of modified miRNA cDNA. PCR amplification was conducted with the amplification system of RNU6B serving as the internal reference. The amplification procedure were 50 °C, 15 min, 95 °C, 10 min, 95 °C, 5 s, 60 °C, 30 s which circulated for 40 times. The recurring numbers reaching the cycle threshold value (Ct value) in the tube were recorded. RNU6B correction was used to acquire $^{\Delta}Ct$ = CtmiR − 196a − CtRNU6B. $2^{-\Delta\Delta Ct}$ method was used to calculate the relative expression quantity of miR-196a.

2.4. Plasma transfection

Negative control plasma (NC) and miR-196a inhibitor were bought from Shanghai GenePharma Co., Ltd. The ovarian cancer cell line of which miR-196a expressed the most or least was selected and inoculated in six-well plates of 2×10^6/well with DMEM medium to culture until the cell confluence exceeded 50%. According to the instructions of lipidosome LipofectamineTM200 (Promega, USA), ovarian cancer cell lines growing in the logarithmic phase were transfected by NC and miR-196a inhibitor plasmas respectively and cultured until the final volume reached 2 mL.

2.5. Transwell chamber method used to detect the migration and invasion of ovarian carcinoma cells

RT-PCR was repeated to detect the changes of the expression quantities of miR-196a in ovarian cancer cell lines after they were transfected by miR-196a inhibitor plasmas. Transwell chamber method was applied to determine the migration and