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MicroRNA-494 expression in ovarian cancers and its inhibition on cancer cell proliferation and migration

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Abstract. – OBJECTIVE: The aim of this study is to investigate microRNA-494 (miRNA-494) expression in the plasma and tissue of patients with ovarian cancer and to explore its role in the proliferation and migration of ovarian cancer cells.

PATIENTS AND METHODS: Forty ovarian cancer patients admitted in our institution were included in the study group and 30 healthy subjects seeking health check-up during the same period were included in control group. miRNA-494 expression level in the cancer tissue as well in the paracancerous tissues of ovarian cancer patients were determined using fluorescent quantitative real time PCR (fq RT-PCR). Meanwhile, plasma level of miRNA-494 was compared between ovarian cancer patients and normal controls. Furthermore, miRNA-494 expression was interfered by introducing miRNA-494 mimic and inhibitor respectively into HO-8910 ovarian cancer cells. Cell proliferation and migration were analyzed using CCK-8 assay and cell invasion assay using Transwell method.

RESULTS: miRNA-494 was significantly increased in ovarian cancer tissues than in paracancerous tissues ($p < 0.05$). miRNA-494 was significantly higher in the plasma of ovarian cancer patients than that of normal population ($p < 0.05$). The proliferation and invasion capacity of ovarian cancer cells were significantly reduced in HO-8910 ovarian cancer cells with suppressed expression of miRNA-494 ($p < 0.05$). However, the proliferation and invasion capacity of ovarian cancer cells were significantly enhanced in HO-8910 ovarian cancer cells with overexpression of miRNA-494 ($p < 0.05$).

CONCLUSIONS: miRNA-494 expression is increased in ovarian cancer patients, and miRNA-494 is involved in the progression of ovarian cancer by promoting the proliferation and invasion of ovarian cancer cells.

Key Words:
microRNA-494, Ovarian cancer, Proliferation, Invasion.

Introduction

Ovarian cancer is one of the most common malignant tumors of the female reproductive system. It ranks the third in incidence behind cancers of cervix and uterus. The five-year survival rate for ovarian cancer is only 20%-30%, threatening women's lives[1]. The main risk factor of ovarian cancer is associated with hormone levels. Mutations of the epithelial cells of the ovary stimulated via prolonged exposure to an environment without estrogen is considered to induce the development of ovarian cancer[2]. The early symptoms are atypical due to the complex embryonic development, anatomy and endocrine function of the ovary, making it difficult for preoperative identification of histological classification of ovarian cancer as well as for the differentiation between benign and malignant tumors. In recent years, increasing attention has been drawn to the research on the cellular and molecular mechanisms underlying ovarian cancer, with an effort to discover a more rational and more effective treatment at cellular or molecular level.

MicroRNAs (miRNAs) are a class of 22 nucleotide-long, non-coding, single-stranded RNAs exhibiting a variety of function. In recent years, miRNAs have been shown to express aberrantly in most cancers and is considered to play a crucial role in the development and progression of tumors[3-7]. miRNA-494 is a recently found miRNA and has been shown to highly express in hepatocellular cancer and promote the conversion of tumor cells[8], suggesting that miRNA494 is an important tumor-promoting factor involved in the carcinogenesis of liver cells. However, to date, few studies have been reported on the expression of miRNA-494 in the tissue and plasma of ovarian cancer patients

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as well as the potential function and the implication of miRNA-494 in ovarian cancer.

In the present study, the expression levels of miRNA-494 in the tissue and plasma of ovarian cancer patients were measured and the effect of miRNA-494 on the proliferation and migration of ovarian cancer cells was investigated.

Patients and Methods

Patients

Between January 2012 and January 2014, samples of ovarian cancer harvested from 40 patients with an age range of 23-65 years undergoing surgical resection of the cancer in our institution were included in the study group. *In vitro* manipulation of cancer tissues was controlled within 30 min and paracancerous tissues (with a distance of 4cm from cancerous tissue) were also collected. The control group consisted of 30 normal subjects (an age range of 22-63 years) seeking health check-up in our institution.

Methods

RNA Isolation and Fluorescent Quantitative RT-PCR (fq RT-PCR)

A total of 5ml of fasting peripheral venous blood sample was collected from each ovarian cancer patient as well as from normal population in the morning. Blood samples were anticoagulated with EDTA followed by centrifugation at 4000 g for 5 min at 4°C within two hours, and the plasma at the upper layer was collected and stored at -80°C. Surgically resected ovarian cancer tissues and paracancerous tissues were immediately placed in liquid nitrogen and stored at -80°C thereafter.

A sample of ~100 mg tissues or 100 µl plasma was homogenized in 1 ml Trizol reagent (Gibco, Franklin Lakes, NY, USA) and total RNAs were isolated from these samples using Trizol RNA isolation kit. The resulting RNAs were dissolved in 20 µl diethylpirocarbonate (DEPC) treated water and fq RT-PCR was performed using RT-PCR kit (cat#11904-018, Invitrogen, Carlsbad, USA). The resulting cDNA products were stored at -20°C. RT-PCR reaction conditions were as follows: denaturation at 95°C for 20s, followed by 35 cycles of 60°C for 20s and 70°C for 1s. The primers for miRNA-494 were as follows: 5'-GAGGTTTCCCGTGTATGTTTCA-3'[9]. The primers for internal reference U6 were F: 5'-CTCGCTTCGGCAGCA CA-3' and R: 5'-AACGCTTCACGAATTTGCGT-3'. QF RT-PCR was performed on ABI 7900 Real-Time PCR system and relative quantitative analysis was performed using $2^{-\Delta\Delta Ct}$ method[10].

Cell Transfection

Transfection of human ovarian cancer cell line HO-8910 was performed using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) to interfere the expression of miRNA-494 by introducing the mimic and inhibitor of the gene respectively into the cells, and interference efficiency was examined after 48h. The primers of the mimic and inhibitor of miRNA-494 as well as U6 were designed and synthesized by Guangzhou RiboBio Co.Ltd., Guangzhou, China.

Cell Counting kit -8 (CCK-8) Assay

Transfected HO-8910 cells were seeded on microplate and incubated with CCK-8 solution (Beyotime Biotechnology, Nantong, China) at a ratio of 1:10 (V/V) in serum-free DMEM at 0, 24, 48 and 72h post-transfection. Optical absorbance at 450nm was determined respectively, and cell growth curve was generated.

Cell Invasion Assay

A cell suspension of transfected HO-8910 cells in logarithmic phase were prepared and cell density was adjusted to ~5×10^5/ml. Cell suspensions were seeded in upper chamber of Transwell at a concentration of 0.1 ml/well and 1ml culture medium containing 10% serum was plated in the bottom chamber. After 24h incubation, Transwell chambers were retrieved and the culture media at the upper layer were aspirated to terminate the assay. Cells were air dried at room temperature. After ethanol fixation, cells were stained with 0.1% crystal violet for 30 min at room temperature. Cells that migrated to the bottom chamber were observed under inverted microscope and cells in the bottom chamber were counted in five randomly selected fields (×200).

Statistical Analysis

Statistical analyses were performed using SPSS software version 19.0 (SPSS Inc., Chicago, IL, USA). Differences between two groups were compared using Student's *t*-test. $p<0.05$ was considered statistically significant.

Results

Level of miRNA-494 Expressed in Ovarian Cancer tissues

Compared with paracancerous tissues, level of miRNA-494 was significantly increased in ovarian cancer tissues ($t = 17.93 < p < 0.05$) (Table I).

Level of miRNA-494 Expressed in the Plasma of Ovarian Cancer Patients

miRNA-494 was significantly higher in the plasma of ovarian cancer patients than in normal population ($t = 14.85$, $p < 0.05$) (Table II).

Effect of miRNA-494 Interference on the Proliferation and Migration of Ovarian Cancer Cells

miRNA-494 mRNA levels were significantly decreased in HO-8910 ovarian cancer cells transfected with miRNA-494 inhibitor (Figure 1A), indicating that gene interference was achieved. Meanwhile, proliferation of HO-8910 cells was significantly decreased (Figure 1B) and migration of these cells was reduced significantly in these transfected cells (Figure 1C) ($p < 0.05$).

Effect of miRNA-494 Overexpression on the Proliferation and Migration of Ovarian Cancer Cells

miRNA-494 mRNA levels were significantly increased in HO-8910 cells transfected with miRNA-494 mimic (Figure 1A). Besides, in these transfected cells with increased expression of miRNA-494, cell proliferation was significantly increased (Figure 1B) and migration of the cells was significantly enhanced (Figure 1C) ($p < 0.05$).

Table I. MicroRNA-494 expression in ovarian cancer tissues.

Group	microRNA-494 mRNA	*t/p*
Paracancerous tissues	1.24 ± 0.31	
Ovarian cancer tissues	3.18 ± 0.61*	17.93/0.000

*Vs. paracancerous tissues, $t = 17.93$, $p < 0.05$.

Discussion

Ovarian cancer is one of the three most common gynecological malignancies with its incidence behind cervical cancer and uterine cancer. Nevertheless, ovarian cancer ranks first in cancer related-mortality, seriously damaging women's health[11,12]. Ovarian cancer frequently occurs in

Table II. microRNA-449a mRNA expression in the plasma of ovarian cancer patients.

Group	n	microRNA-494 mRNA	*t/p*
Healthy population	30	1.33 ± 0.42	
Ovarian cancer patients	40	3.74 ± 0.81*	14.85/0.000

*Vs. normal population, $t = 14.85$ $p < 0.05$.

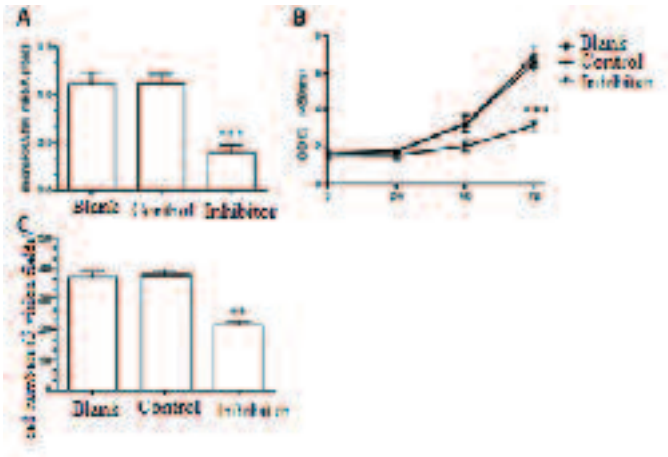

Figure 1. Impact of suppressed expression of microRNA-494 on the proliferation and migration of ovarian cancer cells. ***Vs. controls, $p<0.000$

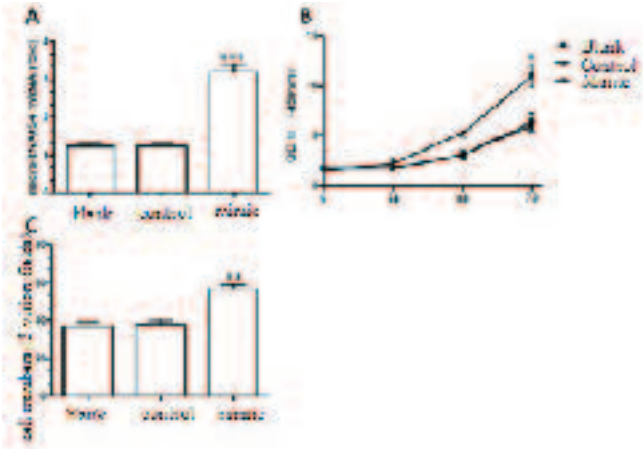

Figure 2. Impact of microRNA-494 on the proliferation and migration of ovarian cancer cells. ***Vs. controls, $p < 0.000$.

perimenopausal women. Its etiology remains obscure, which may be closely associated with the immune function, endocrine system, genetics and mental factors. Ovarian cancer is an occult disease showing no symptoms or signs at early stage, and effective screening as well as diagnostic methods are still unavailable. As a result, most patients were in advanced stage at diagnosis. Therefore, specific diagnosis and treatment strategies are urgently required in the management of ovarian cancer. To this end, discovering specific molecular marker (including cellular markers, serum or plasma markers and genetic markers) for early diagnosis of ovarian cancer as well as novel gene targeted therapy have become the focus of both basic and clinical research of ovarian cancer.

miRNAs are a class of 22 nucleotide-long, non-coding, single-stranded RNAs, which are widely present in tissues, plasma, serum or other body fluids. They control the expression of target genes at posttranscriptional level mainly through degrading mRNA or suppress the contranslation of target genes. Studies have shown that miRNAs play important roles in various biological processes (such as cell proliferation, differentiation and apoptosis, as well as individual development, metabolism and viral infections)[3-7]. In addition, increasing evidence has demonstrated aberrant expression of miRNAs in majorities of human cancers, and tumor-related miRNAs have been shown to play important roles in the development, progression, diagnosis and prognosis of cancers[13,14]. Zhang et al[15] have shown that miRNA-145 expression is significantly up-regulated in ovarian cancer cell lines, inhibiting cell proliferation and promoting cell apoptosis. Furthermore, Yeh et al[16] have revealed that miRNA-138 expression is down-regulated in ovarian cancer tissues and it can suppress cell migration and invasion. However, to date, it has not been clearly elucidated in terms of miRNA-494 expression in ovarian cancer patients as well as its potential role in cancer cell proliferation and migration.

In the present study, the expression of miRNA-494a in the cancer tissues and plasma of ovarian cancer patients were investigated with an effort to explore the function and implication of miRNA-494 in these patients. The results showed that miRNA-494 was significantly higher in ovarian cancer tissues than in paracancerous tissues, and miRNA-494 expression was higher in the plasma of ovarian cancer patients than those of normal population. These results indicated that the increase of miRNA-494 may play an important role in the development and progression of ovarian cancer. Lim et al[8] have shown that miRNA-494 was increased in the tissues of hepatocellular carcinoma (HCC) and promoted the metastasis of HCC cells. Moreover, Wang et al[17] have demonstrated that miRNA-494 was highly expressed in non-small cell lung cancer tissues and was significantly correlated with the staging of lung cancer. All above evidence support the results of the present study.

In addition, miRNA-494 mRNA was interfered in ovarian cancer cell lines in the present study, in an effort to study the role of miRNA-494 in the proliferation and migration of ovarian cancer cells. Results showed that cell proliferation and migration were significantly reduced in ovarian cancer cells with low expression of miRNA-494 but were significantly enhanced in the cells with overexpression of miRNA-494. These

results suggested that miRNA-494 may be involved in the progression of ovarian cancer by affecting the proliferation and migration of ovarian cancer cells. Ohdaria et al[18] have demonstrated that miRNA-494 can significantly inhibit cell proliferation in A549 lung cancer cells supporting the findings of the present study from another angle. However, further studies are required to elucidate the cellular mechanisms by which miRNA-494 affects the proliferation and migration of ovarian cancer cells.

Conclusions

miRNA-494 may serve as a tumor-promoting factor involved in the progression of ovarian cancer mainly by affecting the proliferation and migration of ovarian cancer cells. Detection of miRNA-494 in the plasma and tissue of ovarian cancer patients may be of a certain value in guiding the diagnosis, treatment and prognostic evaluation of ovarian cancer.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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