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MicroRNA-299-3p regulates proliferation, migration and invasion of human ovarian cancer cells by modulating the expression of OCT4

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ABSTRACT

Ovarian cancer is among the most prevalent and lethal types of cancers in women. Several factors such as late diagnosis, unavailability of the reliable biomarkers, frequent relapses and dearth of efficient therapeutic targets form bottleneck in the treatment of ovarian cancers. In this study we investigated the potential of less studied miR-299-3p as the therapeutic target for the treatment of ovarian cancer. The results of the present investigation revealed that miR-299 is significantly upregulated in the ovarian cancers and suppression of its expression inhibits the proliferation by induction of apoptosis as well suppresses migration and invasion of the SKOV3 cancers cells. Further, OCT-4 was found to be putative target of miR-99-3p in ovarian cancer and inhibition of OCT-4 had similar effects as that of miR-299 inhibition on cell migration and invasion. Intriguingly, even overexpression of miR-299-3p could not rescue the effects of OCT-4 suppression on SKOV3 cell proliferation, migration and invasion. On contrary, overexpression of OCT-4 in SKOV3 cells transfected with miR-299-3p transfected could nullify the effects of miR-200-3p on proliferation, migration and invasion of the SKOV3 cells. Taken together, miR-299-3p regulated cell proliferation and metastasis by modulating the expression of OCT-4 and as such may prove to be an important therapeutic target.

1. Introduction

Of all the gynaecologic cancers, ovarian cancer is responsible for significant mortality in women [1]. Ovarian cancers are mostly detected at advanced stages as there are no apparent symptoms during the development of this disease and hence, it is often difficult to treatment [2]. Nonetheless, ovarian cancers generally respond well to the first line chemotherapy; however there are frequent relapses which are mostly associated with the development of the chemoresistance [3]. Despite recent advancements in the field of cancer research, there is still dearth of reliable markers for the diagnosis of ovarian cancers [4]. Moreover, the currently used chemotherapeutic agents for the treatment of ovarian cancer are associated with lot of side effects [5]. Thus, there is pressing need to develop new treatment strategies, explore reliable and efficient biomarkers and to identify novel therapeutic targets for the management of the ovarian cancers. Over the years microRNAs (miRNAs) have been found to be potential therapeutic targets for the treatment of cancers [6]. MiRNAs include a highly conserved non-coding RNAs that have been found to play vital roles in a diversity of cellular processes [7]. Interestingly, the expression of miRNAs has been found to dysregulated in cancer cells as such they could be utilized as therapeutic targets [8]. MiR-299-3p is one of the important microRNAs that has been reported to regulation the proliferation an metastasis of several types of cancers. For instance, MiR-299-3p has been shown to suppress the proliferation and invasion of human colon carcinoma cells [8]. Similarly, it has been reported to be differentially expressed in malignant mesothelioma cells [9]. Moreover, in a recent study miR-299-3p was reported to regulate the invasiveness of the breast cancer cells [10]. However, the role of miR-299-3p has not been investigated in the ovarian cancer. Against this backdrop, the present study was designed to investigate the potential of miR-299-3p as the therapeutic target for the treatment of ovarian cancer. Consistently, the expression of miR-299 was investigated in four ovarian cancer and one normal ovarian cell lines. It was observed that the expression of miR-299-3p is significantly upregulated in all the ovarian cancer cell lines and suppression of miR-299-3p expression inhibited the proliferation through induction of apoptosis. Moreover, suppression of miR-299-3p could also reduce invasiveness of the ovarian cancer cells by targeting OCT4 (*Octamer-binding factor*) as determined by the bioinformatic analysis. Conversely, suppression of OCT4 had similar effects as that of miR-299-

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3p. Taken together, we propose that miR-299 could prove an important therapeutic target for the treatment of ovarian cancer and deservers further research endeavours.

2. Materials and methods

2.1. Clinical samples

Primary tumor samples (n = 15; Sample S6 to S20) were obtained from untreated ovarian cancer patients (mean age 53 years, range 38–67 years; 5 premenopausal and 10 postmenopausal) who underwent tumor resection surgery. Normal ovarian epithelial tissue samples (n = 5; Sample S1 to S5) were obtained from postmenopausal women undergoing bilateral adnexectomy due to other gynaecological diseases such as uterine prolapse, hysteromyoma, and simple ovarian cysts. All tumors were primary serous ovarian carcinoma. The normal ovarian samples were confirmed to be free of any pathology. Tumor staging and grading was carried out as per the International Federation of Gynecology and Obstetrics standards (FIGO). One of the 15 tumors was classified as stage I, 5 tumors stage II, 7 tumors stage III, and 2 tumors stage IV. All the samples were collected at Department of obstetrics and gynecology, The third people's hospital of Jinan. This study has been approved by the institutional review board of the hospital under approval number HOJ56A/18 and all the informed consents were obtained from patients.

2.2. Cell lines, culture conditions and transfection assays

Ovarian cancer cell lines PA-1, Caov-3, SW-626, TOV-112D, SK-OV-3, OVACAR-3 and immortalized epithelial cell line, SV40 were procured from American Type Culture Collection. All of these cell lines were maintained in Dulbecco's modified Eagle's medium containing fetal 10% bovine serum, antibodies (100 units/mL penicillin and 100 µg/mL streptomycin), and 2 mM glutamine. The Cells were cultured in CO_2 incubator (Thermo Scientific) at 37 °C with 98% humidity and 5% CO2. MiR-299 inhibitor (UAUGUGGGAUGGUAAACCGCUU, Product ID: INH0292), Inhibitor-NC (UUGUACUACACAAAAGUACUG, Product ID: INH9002), miR-299-3p mimics (UAUGUGGGAUGGUAAACCGCUU Product ID: MIM0292) were all procured from Active motif (Shanghai, China). For dual luciferase assay in HEK293T cells with miR-299-3p inhibitor, inhibitor-NC were transfected with OCT4- 3′-UTR wt/OCT4 or 3′-UTR mut. All transfection assays were carried out using Lipofectamine 2000 reagent (Invitrogen) according as per the manufacturer's guidelines.

2.3. cDNA synthesis and quantitative RT-PCR

Total RNA was extracted from all the cell lines as well as the clinical samples by using TRIZOL reagent (Invitrogen). Thereafter, reverse transcription and real time PCR were performed with the help of PrimeScript RT reagent Kit (TaKaRa, Dalian, China) and SYBR Premix Ex TaqII (Tli RNaseH Plus) kit (TaKaRa) respectively. Finally relative expression was determined by $2^{-\Delta\Delta CT}$ method.

2.4. Cell viability assay

The viability of ovarian cancer cells transfected was determined by MTT assay. In brief, the cultured ovarian cancer cells were seeded at the density of 1.5×10^4 in 96-well microtiter plates. This was followed by the addition of MTT solution in all the wells and then the absorbance at 570 nm was taken at 0, 24, 48, 72 and 96 h using an ELISA plate reader.

2.5. Apoptosis assay

The ovarian cancer cells were seeded in 6-well plates (2×10^5 Cells per well). The cells were then DAPI stained to detect the apoptosis by fluorescence microscopy as previously reported [11]. For percentage of the apoptotic cells an FITC-Annexin V/PI Apoptosis detection kit was employed as per the instructions of the manufacturer (Beijing Biosea Biotechnology, China). Alkaline comet assay was also performed as described previously [12].

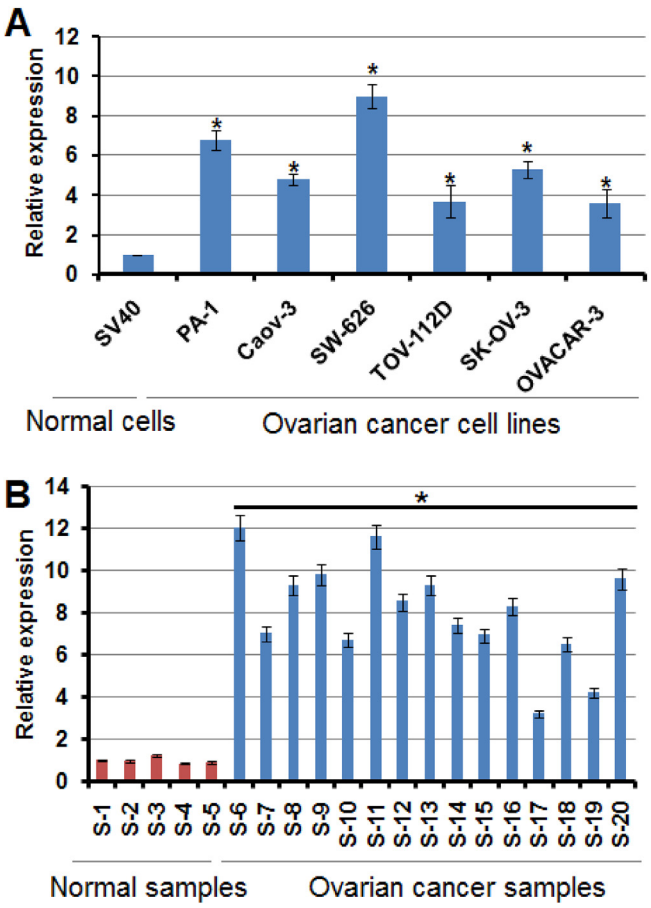

Fig. 1. Expression of miR-299-3p in (A) different ovarian cancer and normal cell lines (B) in normal and ovarian cancer tissues as determined by qRT-PCR. The experiments were carried out thrice in triplicates and expressed as mean ± SD (*$p < 0.05$).

2.6. Cell migration and invasion assay

The cell migration of the ovarian cancer cells was assessed by wound healing assay. The media was removed after 24 h of transfection and the cells were subjected to PBS washing. This was followed by scratching of the wound with a sterile pipette tip. A picture of the wound was captured and then the cells were grown for 48 h in a complete media and another photograph was captured under an inverted microscope. The cell invasion ability of cells the ovarian cancer cells was evaluated by transwell assay. Briefly, the Cells were seeded at 2×10^5 cells/mL density after 48 h transfection. Thereafter, 200 ml cell suspensions were added into the upper chamber and complete medium was added into the bottom wells. After 24 h culturing, the cells in the upper chambers were removed and cells migrated through the chambers were subjected to fixation with methyl alcohol and followed by staining with crystal violet. Finally the number of cells that migrated was determined by counting the cells under an inverted microscope (Magnification 200×, 10 different fields).

2.7. Western blotting

The ovarian cancer cells were harvested lysed with lysis buffer (Tris-