Effect of siRNA Targeting MTA1 on Metastasis Malignant Phenotype of Ovarian Cancer A2780 Cells*

Yu-mei RAO (饶玉梅)¹, Mei JI (纪 纪)¹, Cai-hong CHEN (陈彩虹)², Hui-rong SHI (史惠蓉)¹ #
¹Department of Obstetrics and Gynecology, the First Affiliated Hospital of Zhengzhou University, Zhengzhou 450052, China
²Center of Reproductive Medicine, the First Affiliated Hospital of Zhengzhou University, Zhengzhou 450052, China

© Huazhong University of Science and Technology and Springer-Verlag Berlin Heidelberg 2013

Summary: Ovarian cancer is the fifth lethal gynecologic malignancy. Metastasis-associated gene 1 (MTA1) is overexpressed in many malignant tumors with high metastatic potential. This study investigated whether down-regulation of MTA1 expression by RNAi in A2780 ovarian cancer cells could affect proliferation, anoikis, migration, invasion and adhesion of the cells and to research the potential for MTA1 gene therapy of ovarian cancer. After transfection with effective Mta1 gene siRNA, the effects on proliferation, anoikis, migration, invasion and adhesion of A2780 cells were tested by MTT assay, flow cytometry, wound-healing assay, Transwell assay and adhesion assay. Expression levels of PTEN, beta 1 integrin, MMP-9, phosphor-AKT (Ser473), and total AKT activity were evaluated in control and transfected cells. The results showed that inhibition of MTA1 mediated by Mta1-siRNA transfection decreased the cell invasion, migration and adhesion, and induced the increased cell anoikis, but no significant difference was found in proliferation of A2780 cancer cells. In addition, beta 1 integrin, MMP-9, and phosphor-AKT protein levels were significantly down-regulated, while PTEN was significantly up-regulated. These results demonstrated that MTA1 played an important role in the cell metastasis in ovarian cancer. MTA1 could serve as another novel potential therapeutic target in ovarian cancer.

Key words: metastasis-associated gene 1; ovarian cancer; invasion; migration; anoikis

Ovarian cancer is the fifth lethal gynecologic malignancy. Despite advances in chemotherapy, the five-year survival rate of advanced ovarian cancer patients with peritoneal metastasis remains around 30%. The leading cause of the gynecologic malignancies is characterized by rapid progression, late metastases and poor prognosis[1]. On account of the absence of early diagnosed after the cancer has spread. Therefore, the events leading to metastatic disease are poorly understood. Traditionally, surgical therapy and combination chemotherapy using a platinum analogue as the key drug are performed, but the long-term prognosis is still poor[2]. Gene therapy targeting the metastasis-related biochemical pathways might be a useful additive treatment for women with advanced cancer in order to prolong their life.

Metastasis-associated gene 1 (MTA1) was first cloned from the highly metastatic and non-metastatic rat mammary adenocarcinoma cell line by differential cDNA library screening[3]. It is a component of histone deacetylase 1 (HDAC1) involved in chromatin remodeling[4]. Overexpression of MTA1 plays an important role in tumorigenesis and tumor aggressiveness through deacetylation of the estrogen receptor (ER) α, hypoxia-inducible factor-1α (HIF-1α) and p53 proteins[5]. However, the cellular mechanisms of MTA1 function are still not completely understood. Its overexpression is associated with the invasion and metastasis of some malignant tumors, including breast, cervical and non–small cell lung cancer[6-8], etc. Recent studies have shown that ovarian cancer high expression of MTA1[9] was found to be associated with advanced stage (I/II versus III/IV) and with worse response to first-line treatment. Gene therapy with MTA1 would provide a successful approach for treating human ovarian cancer. However, roles of MTA1 in the genesis, development and metastasis of ovarian cancer cell line and the molecular mechanisms have rarely been reported. The accurate function of MTA1 in ovarian cancer progression has not been completely studied. Thus, to test the potential for MTA1 gene therapy of ovarian cancers, we investigate the influence of MTA1 on the malignant phenotype of ovarian cancer cells and the possible mechanism by using small interfering RNA (siRNA).

1 MATERIALS AND METHODS

1.1 Cell Culture and Reagents

Human ovarian epithelial carcinoma cell line A2780 was obtained from the European Collection of Cell Cultures (ECACC, UK), cells were maintained in RPMI-1640 complete medium supplemented with 2 mmol/L L-glutamine and 10% FBS at 37°C in a humidified atmosphere containing 5% CO₂. The cells were...
1.2. Chemicals and Antibodies
RPMI-1640, fetal bovine serum, and Lipofectamine2000 reagent were purchased from Life Technologies Inc. (USA). MTT was obtained from Sigma (USA). MTA1, PTEN, beta 1 integrin and beta-actin antibodies were obtained from Santa Cruz Biotechnology (USA). Phosphor-AKT (Ser473) and total AKT antibodies were obtained from Cell Signaling Biotechnology, Inc. (USA). MMP-9 antibody was from Boster Bio-engineering Ltd. Co. (China).

1.3. Design, Synthesis, and Transfection of MTA1 siRNAs
The expression of MTA1 was monitored in the cell lines transfected with specific siRNAs. A series of siRNA sequences targeting MTA1 were designed and chemically synthesized for screening (Ruibo Co., Shanghai, China). The siRNAs had the following sequences: si-1 (sense: 5’-GACCCUCUGCAGAUAAAdTdT-3’, antisense: 3’-dTdTCUGGGACGACCCGUCUAUUU-5’); si-2 (sense: 5’-CCCUAGUGCUAGCUAUAdTdT-3’, antisense: 3’-dTdTCGGAGACGACCUAGCUAUUU-5’); si-3 (sense: 5’-CCAUCGUCUAUGCAGACGAAdTdT-3’, antisense sequence: 3’-dTdTGGUGACGUAGCUAGCUAGCUAUUU-5’). The MTA1-siRNAs or control siRNA were dissolved in sterilized and RNase-free water. The lipofectamine 2000 was used to transfect the A2780 cell line according to the manufacturer’s instruction. Briefly, siRNAs and liposomes were separately added to 250 μL serum-free DMEM in the two Eppendorf’s tubes, and placed at room temperature for 5 min. Then, the two solutions were mixed together and incubated at room temperature for another 20 min. After incubation, siRNA-lipid complexes were transferred to the A2780 cell line and grown at 5% CO2 humidified incubator at 37°C. Then they were washed again in TBST three times with three washes with TBST for 15 min each at room temperature. Protein was visualized with NBT/BCIP/buffer (1:1:50). Protein loading was assessed by blotting the same membrane with an antibody against beta-actin.

1.5. MTT Assay
The transfected and control A2780 cells were detached with 0.25% trypsin. A total of 100 μL single cell suspensions, approximately containing 5000 cells, were added into each well of 96-well plates, and cultured in corresponding medium containing 10% serum for the different time courses of 1, 2, 3, 4, 5, and 6 days. Then 15 μL of thiazolyl blue (5 mg/mL) (Sigma Chemical Co., USA) was added to each well and incubated at 37°C for 4 h until formazan was formed. After the supernatants were discarded, 100 μL DMSO was added into each well. The plates were then gently agitated for 20 min to dissolve the crystals. Finally, absorbance (A) was examined at 570 nm using a microplate reader. All experiments and measurements were repeated three times in order to calculate the growth curves. The average A value and culture days were used for the Y-axis and X-axis of growth curve graph, respectively.

1.6. Assessment of Anoikis
The transfected and control A2780 cells were collected at 48 h after transfection. For detachment culture, 50,000 cells/well were seeded in Ultra-Low attachment 6-well plates (Corning, USA). After 24 h of growth in suspension, cells were harvested and washed in ice cold PBS, then stained using propidium iodide and Annexin V-FITC (BD Biosciences, USA) for 15 min at room temperature in the dark. Samples were analyzed for cell apoptosis using a flow cytometer.

1.7. Cell Invasion Assays
Cell invasion was assessed by a Matrigel invasion assay, using Transwell chambers (Costar, Corning Inc., USA) with 8-μm pore polycarbonate filters. The filters were coated with 50 μL Matrigel (BD Co., USA). Before assay, the filters were hydrated with 200 μL of serum-free medium at room temperature for 60 min. The A2780 cells were harvested by trypsinization and washed in serum-free RPMI medium. The cells were suspended in RPMI 1640 medium. 2×10⁶ Cells were added into the top chambers of 24-well plates, and the lower chambers were filled with 0.5 mL of DMEM containing 10% FBS as chemoattractant. The plates were then incubated in a 5% CO2 humidified incubator at 37°C for 48 h. The invaded cells on the bottom surface of the membrane were fixed with 95% alcohol for 15 min and stained with 0.5% crystal violet in 20% methanol for 15 min. The cells on the upper surface were gently removed with a cotton swab, and those on the lower surface of the filters were quantified under a microscope at 200× magnification.

1.8. Wound Healing Assay
Post-transfected A2780 cells were seeded into 6-well tissue culture dishes at 95% confluence in complete tissue culture medium. The confluent cell monolayer was scraped with a micropipette tip with the same diameter. For each dish, 3 wounds were made, and then washed three times with phosphate buffer saline (PBS) to remove cell debris. The medium was immediately replaced with serum-free DMEM, and spontaneous cell migration was propagated once every 1 to 2 day(s) and used for following experiments in its logarithmic phase.