Effects of silencing MTA1 gene by RNA interference on invasion and metastasis of endometrial carcinoma

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Summary

Objective: The study aimed at the silence effect of MAT1 gene and proteins in Ishikawa cell of human endometrial carcinoma cell line through RNAi mediated by siRNA and also discussed the effect of MTA1 gene silence on the invasion and metastasis of endometrial carcinoma. *Materials and Methods:* According to the principle of designing siRNA sequence, siRNA aiming at MTA1 gene (MTA1-siRNA) and siRNA used for negative control (Control-siRNA) were designed and synthesized and Ishikawa cell was transfected by transfection reagents. RT-PCR method as well as western blot was used, respectively, to detect the MTA1 mRNA and protein expression of stably transfected cells. Transwell method and scarification experiment were adopted to detect the invasion and metastasis of Ishikawa cells. *Results:* The expression results of MTA1 on the levels of mRNA and protein showed that the expression level in transfected MTA1-siRNA group was obviously lower than that in non-transfected group and transfected control-siRNA group ($p < 0.05$), while there was no significant difference between non-transfected group and transfected control-siRNA group ($p > 0.05$). Effective interference on the expression of MTA1 gene remarkably lowered the invasion and metastasis of endometrial carcinoma Ishikawa cells. *Conclusion:* RNAi aiming at MTA1 can effectively inhibit the expression of MTA1 in endometrial carcinoma Ishikawa cells and the effective silence of MTA1 can weaken the invasion and metastasis of Ishikawa cells, which provides a new strategy for gene therapy of endometrial carcinoma and an experimental basis for inhibiting the invasion and metastasis of endometrial carcinoma.

Key words: Endometrial carcinoma; MTA1; RNAi; Invasion and metastasis.

Introduction

Endometrial carcinoma is a kind of malignancy deriving from the endometria and is one of the three malignant tumors among females. The glandular cancer deriving from endometrial gland is most common. In recent years, the incidence of endometrial carcinoma appears to increase and the age of onset becomes younger, while its pathogenesis is still not clear [1]. The occurrence and development of endometrial carcinoma is a complex process involving multiple factors and genes with changes, among which the inactivation of anti-oncogene, abnormal activation of oncogene, as well as the activation of tumor metastasis-related genes and the inactivation of metastasis suppressor genes play an important role. It is also a new direction of tumor therapy [2]. Metastasis-associated gene-1 (MTA1) has been found out to be a new gene related to tumor metastasis in recent years. It was cloned by Pencil *et al.* [3] in 1993 through differential hybridization technique from rat mammary carcinoma cell line 1376NF with the potential for metastasis. Due to the positive relation between the expression of MTA1 and tumor metastasis, it is named as metastasis-associated protein-1. There have been studies proving that MTA1 is a kind of regulatory protein within nuclear and is a component of histone deacetylases. It regulates the growth of cells indirectly through the deacetylation of targeted genes and consequently makes some carcinoma cells to transform into a more invasive phenotype, which is especially closely associated with the invasion and metastasis of malignant epithelial tumor [4]. MTA1 may have physical functions in normal cells, but its expression in tumors appears to be abnormal, which finally leads to the invasion and metastasis of tumors [5]. RNA interference (RNAi) is a new method to silence the gene expression and an important means to study gene function. The present study chose the endometrial carcinoma cell line Ishikawa cells as study objects and used siRNA to discuss the effects of change in the expression of MTA1 on the invasion and metastasis of endometrial carcinoma and provided experimental evidence for the treatment of endometrial carcinoma.

Materials and Methods

Materials

Endometrial carcinoma Ishikawa cells were reserved in the Laboratory of Department of Obstetrics and Gynecology in the

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First Affiliated Hospital of Zhengzhou University. The sense sequence of siRNA in targeted MTA1 gene was 5'-CCAUCGU-CAUCG AGGAC UAdTdT-3' and antisense sequence was 3'-dTdTGGUAGCAGUAGCUCCUGAU-5'; the primer sequence of MTA1 gene: forward sequence: 5'-AGCGTCACC-CTG CTCAACG AGACCG-3'and reverse sequence: 5'-GGTTGGCCTCTGATGCAGACCACTC-3'; the forward sequence and reverse sequence of GAPDH were, respectively, 5'-CTTAGCACCCCTGGCCAAG-3'and 5'-GATGTTCTGGAGA GCCCCG-3'.

Cell culture and transfection

Ishikawa endometrial carcinoma cell line was placed in the DMEM culture media which contained 10% fetal bovine serum and cultivated under the condition of 37°C and 5%CO_2. Every day or each two days, a generation appeared. It was cultivated to log phase and then reserved for further use. Transfection reagent was used to operated according to the instructions. After the conventional digestion of well-grown cells, these cells were inoculated onto the orifice plate with six bores with the density of 50%, and transfected after the adherence. DMEM culture media was used to dilute siRNA to five mmol/L. Then after the mixing, 12 μL reagent was added and then the mixture was added into the culture bores, which was qualified to 2.2 ml by blood serum media. Without changing the media, it continued to be cultivated in the incubator (37°C, 5% CO_2) for 48 hours. Then, the detection of MTA1 gene expression was carried out. In addition, there were non-transfected group and transfected control siRNA group to be as the contrast.

Detection of expression of MTA1 on the levels of mRNA and protein

The total RNA in each group was abstracted. According to the instruction of RT-PCR kit, cDNA was synthesized through reverse transcription. PCR amplification was carried out with the contrast of GAPDH and the products were detected by agarose gel electrophoresis. The total proteins of cells were abstracted. After the detection of protein concentration, 50 μg protein samples were analyzed through SDS-PAGE electrophoresis and then transferred to PVDF membrane by electricity. Five percent skim milk powder was used to seal the sample for one hour at the temperature of 37°C. MTA1 primary antibody (dilution 1:1000) was added and then it was hatched at the temperature of 4°C overnight. After the wash by TBST, HRP labelled secondary antibody was added. Then it was hatched for one hour at room temperature. Finally, DAB was used to stain and photos were taken.

Experiment of transwell matrigel invasion

Thirty μL of matrigel (1.0 mg/mL) was placed in the upper chamber of transwell. The cell concentration in each group was adjusted to 1×10^6/mL and 200 μL respectively collected from each group was added in upper chamber, and 600 μL complete culture solution containing three μL / hole fibronectin was added into down chamber. Then it was cultured routinely for 24 hours and crystal violet stained for ten min. Then it was cleaned by water three times and then air-dried. The filter membrane was taken down and placed on the glass slide. Then the glass slide was sealed and transmembrane cells were calculated. Regarding the number of transmembrane cells without the matrigel cover as the base (100%), the numbers of transmembrane cells in each group were compared with that and the relative percentages were calculated, which was used to symbolize the matrigel invasion of cells. Each experiment was repeated in the same way for three times.

Table 1. — *Comparison of the relative percentage of cells invading into matrigel.*

Groups	Relative percentage of cells invading into matrigel (%)	*t* value	*p*
MTA1-siRNA	25.97 ± 2.13		
Control- siRNA	55.08 ± 5.25	2.640	0.015
Non-transfected	52.54 ± 4.66	3.541	0.011

Table 2. — *Comparison of the cell metastasis rate after scratched for 48 hours.*

Groups	Cell metastasis rate (%)	*t* value	*p*
MTA1-siRNA	19.8 ± 0.5		
Control- siRNA	68.6 ± 0.7	9.556	0.021
Non-transfected	72.4 ± 0.3	11.069	0.017

Scarification experiment

Transfected cells in each group were inoculated on orifice plate with six bores and cultivated conventionally. When the cells grew to full monolayer, the culture media was abandoned. Sterile transferpettor head was used to draw a straight line on the plate and the cells were washed three times by PBS and cultivated for 48 hours. The growth situation in the scratched place was observed under the microscope and the metastasis rate of cells was calculated. The adopted formula was as the following: metastasis rate of cells=(1-48 h the width of scratch/initial width of scratch)×100%.

Statistical analysis

The data were all processed by MATLAB7.0 or software SPSS17.0. All the measurement data are represented as mean ± SD and the difference among groups was compared and analyzed by *t*-test and one-way ANOVA. The difference would be statistically significant when $p < 0.05$.

Results

Detection results of MTA1 expression

RT-PCR method was used respectively to analyze the expression of MTA1 on the level of mRNA and the results showed that the expression level in transfected MTA1-siRNA group (0.03 ± 0.01) was obviously lower than that in non-transfected group (0.52 ± 0.06) and transfected control-siRNA group (0.45 ± 0.11) ($p < 0.05$) while there was no significant difference between non-transfected group and transfected control-siRNA group ($p > 0.05$) (Figures 1a, 1b). Western blot method was used to detect the expression of MTA1 on the level of protein, showing that the expression level in transfected MTA1-siRNA group (0.15 ± 0.23) was obviously lower than that in non-transfected group (3.06 ± 0.45) and transfected control-siRNA group (2.12 ± 0.33) ($p < 0.05$), while there was no significant difference between non-transfected group and transfected control-siRNA group ($p > 0.05$) (Figures 1c, 1d).

Experimental results of transwell matrigel invasion

The relative percentage of cells invading into matrigel in transfected MTA1-siRNA group was (25.97 ± 2.13%), significantly lowering than that in non-transfected group (52.54

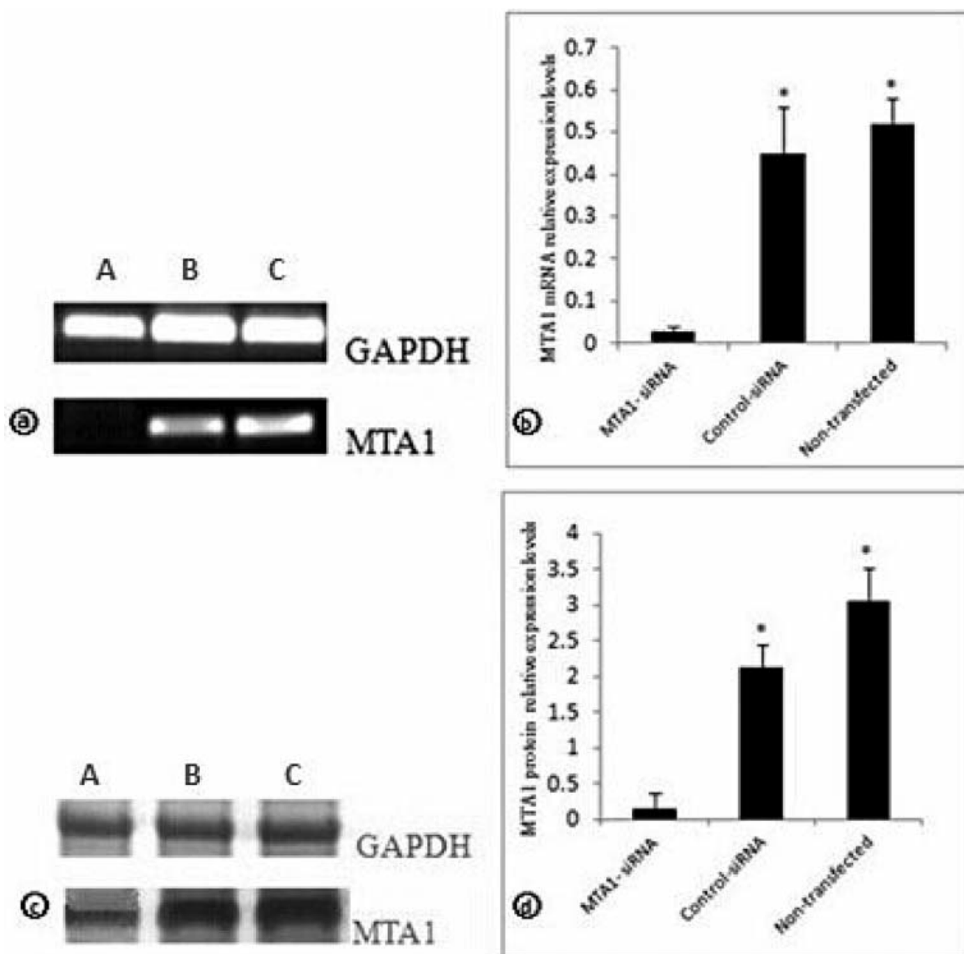

Figure 1. — Influence of RNAi on expression of MTA1 (A, B, C, respectively represent MTA1- siRNA group, control-si group, and non—siRNA group).

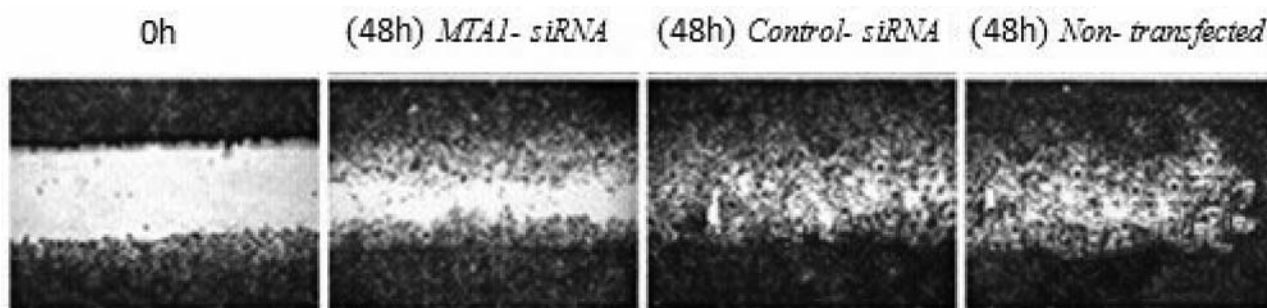

Figure 2. — Results of wound-healing assay (×200).

± 4.66%) and transfected control-siRNA group (55.08 ± 5.25%), which showed that effective interference on the expression of MTA1 gene by siRNA could weaken the invasion of endometrial carcinoma Ishikawa cells (Table 1).

Results of scarification experiment

The growth of cells after being scratched for 48 hours was observed under the microscope. The results showed that the cell metastasis rate in transfected MTA1-siRNA group was (19.8 ± 0.5%), significantly lower than that in non-transfected group (72.4 ± 0.3%) and transfected control-siRNA group (68.6 ± 0.7%) ($p < 0.05$). The difference was statistically significant (Table 2, Figure 2). This indicated that effective interference on the expression of MTA1 gene by siRNA could weaken the metastasis of endometrial carcinoma Ishikawa cells.

Discussion

Endometrial carcinoma is one of the common gynecological malignancies. With the improvement of daily life, the aging population, and replacement of treatment by hormone, the incidence of endometrial carcinoma has been increasing continuously. Some studies showed that 15%-20% of patients with endometrial carcinoma would suffer carcinoma metastasis and relapse within five years after operation [6]. Invasion and metastasis are important factors leading to the death of patients. Therefore, the discussion of the mechanism of invasion and metastasis at the molecular level and the necessary means to control it are significant for the treatment of endometrial carcinoma.

MTA1, as a factor related to tumor metastasis, participates in the process of invasion and metastasis of malignant carcinoma such as lung, breast, prostate, pancreatic, ovarian cancer, and so on [7-11]. Mahoney *et al.* [12] found that MTA1 can combine with some apoptosis-related proteins, mediating its regulation of cell apoptosis. In recent years, the role of MTA1 in endometrial carcinoma has also attracted attention. Some studies showed that the positive expression of MTA1 in endometrial carcinoma tissues was significantly higher than that in healthy endometrial tissues [13, 14]. The present study used siRNA to directly transfect specific siRNA in order to silence the expression of MTA1. The results showed that effective interference on the expression of MTA1 could lower the invasion and metastasis of endometrial carcinoma Ishikawa cells, which was similar to the report of Zhang *et al.* [15] that used expression vector psilencer2.0-MTA1-siRNA of constructed specific siRNA through liposome mediated method to transfect HEC-1-A cell line. These all indicate that MTA1 plays an important role in the invasion and metastasis of endometrial carcinoma and probably becomes a potential target of gene treatment of endometrial carcinoma.

Given that MTA1 is closely related to the occurrence and clinical pathologic features of endometrial carcinoma [16] and plays an important role in the invasion and metastasis of endometrial carcinoma, further discussion regarding the concrete mechanism would provide more exact evidence for it to become an effective biological indicator of judging the invasion and metastasis of endometrial carcinoma, monitoring relapse and judging prognosis. In future studies, the use of proper interference to cut off the function of MTA1 or lower its expression level to weaken the invasion and metastasis of endometrial carcinoma would provide a new model for the treatment of endometrial carcinoma.

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