Expression of MTA2 Gene in Ovarian Epithelial Cancer and Its Clinical Implication*

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Summary: In order to investigate the roles of MTA2 in the pathogenesis of ovarian epithelial cancer, the expression of MTA2 in 4 ovarian cell lines were detected by semi-quantitative RT-PCR and Western-blot assays. MTA2 expression in normal, borderline, benign and malignant epithelial ovarian tissues was immunohistochemically examined. The expression of MTA2 mRNA and protein was detected in all of 4 cell lines of ovarian epithelial cancer. The expression of MTA2 mRNA and protein was higher in strong migration cell lines than in weak migration ones. In borderline and malignant ovarian tissues tested, MTA2 staining was dramatically stronger than in normal and benign tissues ($P<0.01$). The expression levels in malignant ovarian tissues were significantly higher than that in borderline epithelial ovarian tissues ($P<0.01$). The expression of MTA2 was correlated with clinical stage, histopathological grade and lymph node metastasis. It was concluded that the high expression of MTA2 was associated with more aggressive behaviors of epithelial ovarian cancer. MTA2 provides a novel indicator of ovarian cancer.

Key words: MTA2; ovarian cell lines; ovarian tumor; gene expression; immunohistochemistry

Most members of metastasis-associated genes have been identified since 1994. A lot of evidence showed that the expression of MTA1 were elevated in human metastatic breast cell lines and metastatic cancer tissues, such as breast, colorectal, gastric, and esophageal carcinomas. MTA2, which is homologous to MTA1, is a component of the NuRD ATP-dependent chromatin remodeling and histone deacetylase complex. Specifically, MTA2 modulates the enzymatic activity of the histone deacetylase core complex[1]. Although MTA2 sequence is 59.6 % homologous to that of MTA1, no report was available so far concerning tumor invasion and metastasis[2]. To further understand the biological functions of MTA2, we used a variety of assays (RT-PCR, Western blot, Immunohistochemical staining) to probe the expression of the MTA2 in ovarian cell lines and ovarian tumor tissues.

1 MATERIALS AND METHODS

1.1 Cell Lines

Ovarian carcinoma cell lines SW626, A27, CAOV3 and SKOV3 were purchased from ATCC (USA). SW626, A27 and SKOV3 were grown in Dulbecco's modified Eagle's medium with high glucose supplemented with 10 % fetal calf serum, 100 U/mL penicillin and 100 μg/mL streptomycin, but CAOV3 was cultivated in 1640 culture medium. Incubating box was set at 37 ℃ and contained 5 % CO_2.

1.2 Tissue Samples

Tissue samples were collected from patients hospitalized in the Tongji Hospital from 1997 to 2003. All specimens were collected with informed consent obtained from the patients. Ninety-eight tumor samples were pathologically diagnosed as ovarian epithelium cancer, including 68 cases of serous ovarian tumor (23 benign, 8 borderline and 37 malignant ones), 30 cases of mucinous tumor cases (16 benign, and 14 malignant one). Ten normal ovarian tissues, serving as controls, were obtained from patients undergoing ovarian operation. All samples were fixed rapidly by formalin and embedded in paraffin. The age of the 108 patients ranged from 31—78 years with a median age of 52 years. The staging of the ovarian epithelium tumor was based on FIGO (1986). Ten cases were at stage Ⅰ, 10 stage Ⅱ, 20 stage Ⅲ (9 cases had lymphatic metastasis) and 11 stage Ⅳ (11 cases all had lymphatic metastasis). The histological grading was based on cell differentiation, and 16 were at G1, 16 G2 and 19 G3.

1.3 Detection of MTA2 by RT-PCR

Total RNA was extracted from ovarian carcinoma cell (SW626, A27, CAOV3 and SKOV3) by Trizol methods (GIBCO, USA) according to manufacturer's protocol. A total of 4 μg RNA was used for reverse transcription. After cDNA synthesis, amplification was done by using an initial denaturation at 95 ℃ for 30 s, 33 cycles of PCR (94 ℃ 30 s, 45 ℃ 45 s, 72 ℃ 60 s), annealing at 50 ℃ for 2 min, extension at 72 ℃ for 1 min, followed by a final extension at 72 ℃ for 10 min. The reaction system of PCR was 50 μL, including 10× buffer 5 μL, 25 mmol/L $MgCl_2$ 3 μL, 10 mmol/L dNTP 1 μL, primer 1 μL, cDNA template 5 μL and Taq enzyme 2 μL[3]. PCR primer for MTA2 were synthesized by Shanghai Bioa Company, China. The sequences of the MTA2 primers were: 5′-GGAT-

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GAGATGGAGGAATGGT-3′ and 5′-GATAGGAGCATAAGGCCGTC-3′. A 696 bp product for MTA2 was obtained. PCR primer for GAPDH were as follows: 5′-ACGGATTTGGTCGTATTGGG-3′ and 5′-TGATTTTGGAGGGATCTCGC-3′. GAPDH was used as an internal reference and the size of its PCR product was 230 bp. PCR products were separated on 1 % agarose gel.

1.4 Western-blot Assay

Protein was extracted from the ovarian carcinoma cells (SW626, A27, CAOV3 and SKOV3) in the exponential phase of growth for Western blot assay. From each sample, 50 μg protein was loaded per gel slot, the samples were then subjected to SDS-polyacrylamide gel electrophoresis. The proteins were transferred to nitrocellulose filters and immunoblotted in a plastic bag. Nitrocellulose was incubated first with blocking solution consisting of 5 % non-fat dry milk, and then with primary polyclonal antibodies of goat against human MTA2 (1: 1000, Santa Cruze, USA) or β-actin (1: 500, Zhongshan Co., China) overnight at 4 ℃ in a shaking water bath, and then incubated with an alkaline phosphatase-conjugated secondary antibody (1: 500) at 37 ℃ for 1 h in shaking water bath. The substrate was developed at room temperature until a purple precipitate formed on the protein band. The reaction was stopped by replacing substrate with 20 mmol/L Tris/HCl (pH = 8.0) and 5 mmol/L EDTA.

1.5 Immunohistochemical Staining

MTA2 protein was detected in ovarian tumor tissues and normal ovarian tissues by using streptavidin peroxidase-labeled secondary antibody immunohistochemical technique according to SP commercial kit (Zymed, USA). Four μm thick serial sections were pre-treated with autoclave heating in 10 mmol/L citric acid buffer for 5 min. The sections were incubated with the primary polyclonal antibodies of goat against human MTA2 (1:200, Santa Cruze, USA) at 4 ℃ overnight. Subsequently, sections were incubated with the secondary antiserum (1:500) for 1 h. The sections were stained by using diaminobenzidine (DAB) and counterstained with hematoxylin. Negative controls, in which PBS was used instead of the first antibody, were used to verify the possibility of false positive results resulting from the secondary antibody. The breast cancer tissues served as positive controls.

1.6 Image Analysis

The images of PCR and Western-blot products were collected by Uvp Grab-it image system and analyzed by Gelworks ID advanced V4d system. The absorbency difference (AD) between the absorbency volume of the target gene and that of internal control was used as the relatively absorbency of the target gene expression. The distribution and intensity dyed of 150 immunohistochemistric sections were checked under the light microscopy. All of the Images were treated by using HPIAS-1000 pathological image analysis system (Qianping, China). 3—5 high power fields (—200) were randomly chosen on every stained section. 50 — 100 epithelial cells were counted per field. The AD between the scanned area and the gray scale was used as the average A value of MTA2.

1.7 Statistical Analysis of Data

The data were expressed as $\bar{x} \pm s$. The A for RT-PCR, Western-Blot and immunohistochemical sections was analyzed using one-way ANOVA with SPSS 11.0 version for windows. The clinic pathological count data were compared by the χ^2-test. But the measure data were compared by t-test and Chi-Square analysis.

2 RESULTS

2.1 Expression of MTA2 mRNA in Ovarian Carcinoma Cell Lines with Different Invasion Potential

The expression of MTA2 mRNA in 4 different ovarian carcinoma cell lines (SW626, A27, SKOV3 and CAOV3) was detectable and relative A was 1.32±0.26, 1.22±0.15, 0.86±0.16 and 0.81±0.12, respectively. The expression of MTA2 mRNA in SW626 and A27 was significantly higher than in SKOV3 and CAOV3 ($P<0.01$, fig. 1).

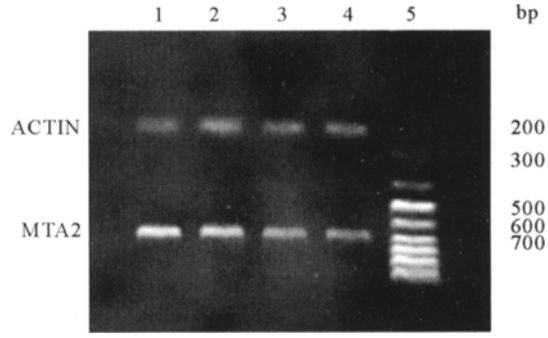

Fig. 1 Expression of MTA2 mRNA in different cell lines
1: SW626; 2: A27; 3: SKOV3; 4: CAOV3; 5: GAPDH (marker)

2.2 Expression of MTA2 Protein in Ovarian Carcinoma Cell Lines with Different Attack Potential and Different Metastasis Potential

The expression of MTA2 protein in 4 different ovarian carcinoma cell lines (SW626, A27, SKOV3 and CAOV3) was detectable and relative A was 0.80±0.12, 0.76±0.14, 0.48±0.15 and 0.46±0.06 respectively. The expression of MTA2 protein was significantly associated with the invasion and metastasis potential of the ovarian carcinoma cell lines. Statistical analyses showed that SW626 and A27 expressed higher levels of MTA2 protein than SKOV3 and CAOV3 ($P<0.01$, fig. 2).

2.3 Correlation between the Expression of MTA2 and Clinicopathological Parameters

2.3.1 The Expression of MTA2 in Different Histo-

logical Tissue The expression of MTA2 was located in the nuclear (fig. 3). In normal ovarian samples and ovarian tumor samples, the cells were all positive for MTA2 staining. Table 1 showed that the expression of MTA2 in cystadenocarcinoma samples or Borderline cystadenoma samples was higher than that in normal samples or cystadenoma samples. There was significantly statistical difference ($P<0.01$). The expression of MTA2 in mucinous cystadenocarcinoma was dramatically higher than that in normal samples or cystadenoma samples ($P<0.01$). Meanwhile, there was no statistical difference between the ovarian benign samples and normal ovarian samples ($P>0.05$). The difference was also not detected between the main histological types.

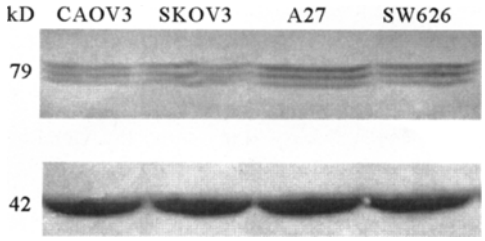

Fig. 2 The expression of MTA2 protein in 4 different ovarian carcinoma cell lines, β-actin as control. The molecular weight of MTA2 and β-actin was 79 and 42 kD (1 kD = 0.9921 ku) respectively

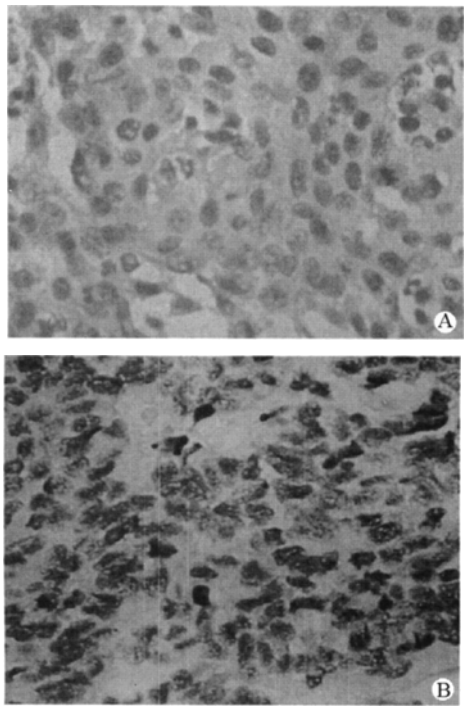

Fig. 3 The expression of MTA2 protein in human ovarian epithelial tumor cells (SP 200)
A: Serous cystadenocarcinoma;
B: Mucinous cystadenocarcinoma

Table 1 Correlation between the expression of MTA2 protein and clinicopathological Parameters

Parameters	*n*	Positive (%)	$\bar{x}\pm s$
Histology types			
Serous cystadenocarcinoma	37	81.08 (30/37)	6.93±1.03
Mucinous cystadenocarcinoma	14	50 (7/14)	6.14±1.92
Borderline serous cystadenoma	8	37.5 (3/8)	4.30±2.17
Serous cystadenoma	23	21.74 (5/23)	2.60±1.02
Mucinous cystadenoma	16	18.75 (3/16)	2.89±0.69
Normal	10	10 (1/10)	3.01±0.88
Clinical stages			
I, II	20	65 (13/20)	3.69±1.72
III, IV	31	83.87 (26/31)	6.79±0.97
Grades			
1, 2	32	53.13 (17/32)	4.11±2.01
3	19	94.74 (18/19)	6.97±1.01
Involving in lymph nodes			
No	30	46.67 (14/30)	5.79±1.18
Yes	21	76.2 (16/21)	8.04±2.83

2.3.2 Expression of MTA2 in Different Clinical Stage or Histopathological Grade Tissues The correlation between MTA2 expression and clinical stages/grades was also summarized in table 1. The higher expression level ($\bar{x}\pm s$, 6.79±0.97) was frequently found in stage Ⅲ and stage Ⅳ than that ($\bar{x}\pm s$, 3.69±1.72) in stage Ⅰ and Ⅱ. In 19 cases of poor differentiation, the positive signal was stronger than that of 32 cases of well and middle differentiation ($P<0.05$). MTA2 expression was also closely related to lymphatic invasion. The expression level of MTA2 ($\bar{x}\pm s$, 8.04±2.83) in samples with lymph node metastasis was significantly higher than that ($\bar{x}\pm s$, 5.79±1.18) without lymph node metastasis ($P<0.05$).

3 DISCUSSION

MTA2 is encoded by 18 exons that span 10 kb. Similar (identical 59.6 %) to the candidate metastasis associated protein MTA1, an elevated level of MTA2 correlates with cellular proliferation[4]. Dynamic Changes in chromatin structure through nucleosome remodeling and core histone tail acetylation play important roles in transcriptional regulation. The purification and functional characterization of a nucleosome remodeling and histone deacetylase complex, NuRD, has suggested that nucleosome remodeling and core histone tail modification are potentially linked processes. MTA2, a component of the NuRD complex, plays an important role in regulating histone deacetylase activity of the NuRD complex. NuRD is a multisubunit protein complex that possesses both nucleosome remodeling and histone deacetylase activities. In addition to the four-subunit histone deacetylase core, HDAC1/2 and RbAp46/48, also

present in the Sin3 histone deacetylase complex, NuRD contains at least three more subunit: Mi2, MBD3 (methyl-CpG-binding domain-containing protein) and MTA2. The histone deacetylase activity of the core complex is severely compromised[1]. MTA2 modulates the enzymatic activity of the histone deacetylase core complex. MBD3 mediates the association of MTA2 with the core histone deacetylase complex. MBD3 does not directly bind methylated DNA but is highly related to MBD2, a polypeptide that binds to methylated DNA and has been reported to possess demethylase activity. MBD2 interacts with the NuRD complex and directs the complex to methylated DNA. NuRD may provide a means of gene silencing by DNA methylation. PID, a p53 target protein in the deacetylase complexes, identical to MTA2, has been identified as a component of the NuRD complex. The p53 tumor suppressor is a transcriptional factor whose activity is modulated by protein stability and post-translational modifications including acetylation. Luo[5] reported that the deacetylation of p53 was mediated by an histone deacetylase-1 (HDAC1)-containing complex. PID specifically interacts with p53 both *in vitro* and *in vivo*, and its expression reduces significantly the steady-state levels of acetylated p53. PID expression strongly represses p53-dependent transcriptional activation, and, notably, it modulates p53-mediated cell growth arrest and apoptosis. These results showed that MTA2 potentially involved in cancer.

The present study demonstrated that there was higher expression of MTA2 mRNA and protein in strong invasion and migration cell lines than in weak ones. The results revealed that MTA2 were altered in a way characteristic for invasive and migration tumor cells.

In the current study of ovarian tissues, it was found that the expression of MTA2 in malignant or borderline samples was higher than that in benign and normal controls. Meanwhile, there was no statistical difference between the ovarian benign samples and normal ovarian samples. Maybe MTA2 took part in the early formation of ovarian epithelial cancer and play an important role in maintaining the malignant cell growth. It was also found that MTA2 expression had a stronger correlation with clinical stage, histological grade and lymphoid metastasis. The difference was also not detected between the main histological types. The results indicated that MTA2 was related to the malignant biological behavior of ovarian epithelia cancer and it could be used an indicator evaluating the state of patients. The further study on the function of MTA2 was benefit to elucidate the mechanism of the development and progression of ovarian epithelial cancer and provide a new therapy strategy for it.

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