Expression of RASSF1A in epithelial ovarian cancers

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Abstract. – OBJECTIVE: Ovarian cancer is the third most common cancer in female reproductive system. But ovarian cancer is hard to detect at early phase. It is very urgent to develop effective early diagnosis method for ovarian cancer. RASSF1A (Ras association domain family 1 isoform A) is a tumor suppressor, which modulates multiple apoptotic and cell cycle checkpoint pathways. We aimed to find out the relationship between RASSF1A and ovarian cancer.

METHODS: We compared the expressions of RASSF1A gene in different ovarian cancer cell lines, and also in epithelial ovarian cancer tissues and normal ovarian tissues through RT-PCR (reverse transcription polymerase chain reaction) technique.

RESULTS: RASSF1A was expressed in HO8910 and HO8910PM cells, while RASSF1A mRNA was absent in SKOV-3 and OVCAR-3 cells. RASSF1A was expressed in 10 normal ovarian tissue samples (10/10, 100%), while RASSF1A was only expressed in 2 ovarian cancers tissue samples (2/47, 4.3%). The difference in the frequency was significant in tissue samples (100% vs. 4.3%, p < 0.05).

CONCLUSIONS: RASSF1A could be served as a potential molecular marker for diagnosing ovarian cancer at early phase.

Key Words: Ovarian cancer, RASSF1A, RT-PCR.

Introduction

Malignant ovarian tumor is one of the most malignant tumors in female reproductive system. With hidden onset and rapid development as clinical biological behavior features, 70% of ovarian cancer cases were diagnosed as terminal types1. With the survival rate around 20% within five years, ovarian cancer is one of female malignant tumors with the highest lethality rate. There is no effective diagnostic method at early phase for ovarian cancer so far2. Although the developing surgical techniques could remove most cancer cells and the clinical applications of platinum, paclitaxel and some secondary chemical drugs bring the hopes of reborn to patients3,4, a considerable part of the patients are still undergoing further deterioration or relapse after surgical and chemical treatments.

Recently, with the development of molecular biological techniques and methodologies, gene therapy is emerging as an effective new therapeutic method in genetic disease caused by single gene mutation and gaining worldwide attentions from experts of different fields5. In order to provide much more precise and effective diagnostic, therapeutic, and precaution methods, medical scientists are trying to reveal the relationship between expression level of a certain gene and clinical biological behaviors of ovarian cancer.

As a new member of RAS (rat sarcoma) gene family, tumor repressor gene RASSF1A is regarded to be involved in the regulation of cell proliferation and apoptosis6. Some reports showed that RASSF1A functions as a micro-tubule binding protein and regulates mitosis proceeding6-10. RASSF1A is localized on the cytoplasmic microtubule of interphase cells, especially on the spindle body and centrosome during mitosis. The bind of RASSF1A to microtubule is supposed to regulate mitosis by stabilizing the microtubule structure. Overexpression of RASSF1A could retain the cell at interphase. The mutation of RASSF1A could destabilize the microtubule structure, influence the spindle configuration, and impair the attachment of chromosome to spindle, which could easily lead to gene instability and cell transformation under influential factors. This could be further deteriorated into abnormal cell proliferation and tumor formation by the loss of cell cycle brake and reduced apoptosis by RASSF1A6.

Some scientists also found that RASSF1A could regulate cell cycle directly, repress cell
after being excised from human body, the ovarian samples were quickly frozen in liquid nitrogen and transferred into −80°C freezer. The cell lines of ovarian epithelial tumor (HO8910, HO8910PM, SKOV-3, and OVCAR-3) were purchased from Cell Bank of Chinese Academy of Sciences. The ovarian samples were collected for this study upon the agreements of all the recruited patients.

**Total RNA Extraction**

After being sheared in 1 ml Trizol (Invitrogen, Carlsbad, CA, USA) in 1.5 ml EP (Eppendorf) tube, 200 mg of ovarian tissue were fractionated under 30 s ultra-sonication and centrifuged at 12000 g, 4°C for 10 min. The supernatant was transferred into the new EP tube containing 200 l chloroform and mixed well by vortex for 15 s. The mixture was placed under room temperature for 3 min. After centrifugation under 12000 g, 4°C for 15 min, colorless supernatant (about 500 l) was transferred into a new EP tube containing 500 µl isopropanol and placed under room temperature for 10 min for RNA precipitation. After centrifugation at 12000 g, 4°C for 10 min, white RNA pellet was recovered and washed with 1 ml 75% ethanol. After centrifugation at 12000 g, 4°C for 5 min, supernatant was discarded and pellet was dried under room temperature for 10-20 min. The dry RNA sample was dissolved in 20 µl diethylpytocarbonate (DEPC) water. After diluting RNA samples 100 times, RNA concentration and purity were determined by measuring OD260 value and OD260/OD280 ratio. The RNA was stored under −80°C. For RNA concentration determination:

RNA concentration (µg/µl) = OD260 ×40×Dilution ratio/1000.

**Materials and Methods**

**Ovarian Tumor Tissues and Cell Lines Collection**

Forty-seven malignant ovarian epithelial tumor patients (forty-three serous cystadenocarcinoma cases and four borderline cases), who received one time treatment of gynecological operation at the hospital from September 2005 to January 2008, were recruited for sample collection. Among these 47 cases, there were 5 cases at clinical stage I, 4 cases at stage II, 37 cases at stage III, and 1 case at stage IV. Ovarian samples from ten patients who underwent ovarian anatomy or prophylactic ovariotomy were collected as control group. All the ovarian samples were collected under sterile condition and pathological validated. After being excised from human body, the ovarian samples were quickly frozen in liquid nitrogen and transferred into −80°C freezer. The cell lines of ovarian epithelial tumor (HO8910, HO8910PM, SKOV-3, and OVCAR-3) were purchased from Cell Bank of Chinese Academy of Sciences. The ovarian samples were collected for this study upon the agreements of all the recruited patients.
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**PCR Reaction**

The PCR reaction was conducted in a 20 µl reaction system containing 4 µl cDNA, 4 µl 5×PCR buffer, 7.9 µl distilled water, 0.1 µl Ex Taq HS, 2 µl forward primer and reverse primer 2 µl. The reaction mixture underwent the program of 2 min 94°C, 30 sec at 94°C, 30 sec at 57°C, and 40 sec at 72°C for 30 cycles. The PCR products were stored at −20°C. Specific primers of RASSF1A was: forward, 5’-CTTCATCTGGGCCGTCG-3’; reverse, 5’-GCATCCCTGGCCAGGTA-3’. Target fragment length for RASSF1A is 420 bp. Specific primers of β-actin was: forward, 5’-TGCGTGACTTAGGAGAAG-3’; reverse, 5’-GAGAGTGGAAGAAGGCC-3’. Primers were synthesized at Saibasheng Biotechnology. Target fragment length for β-actin is 431 bp. Amplification was run on Biometra GmbH (Gottingen, Germany).

**PCR Products Detection**

Total 4 µl PCR products were mixed with 1 µl Loading Buffer and loaded onto 1.5% agarose gel. After running at 150 V, 100 mA for 30 min, the DNA bands were detected via electrophoresis gel imaging system (Chemizager 5500, Alpha Inno Tec Schweiz AG, Altishofen, Switzerland). The 100 bp DNA ladder markers were from Bao Biology Company, Dalian, China and GeneFinderTM dye was purchased from Bio-V.

**Statistical Analysis**

The statistical analysis was carried out with SPSS12.0 software (SPSS Inc., Chicago, IL, USA). When $p < 0.05$, data was considered statistically significant.

**Results**

**The Expression of RASSF1A in Ovarian Cancer Cell Lines**

RASSF1A was expressed in HO8910 and HO8910PM cells and absent in SKOV-3 A and OVCAR-3 cells (Figure 1).

**The Expression of RASSF1A in Normal Ovarian and Ovarian Tumor Tissues**

The expression of RASSF1A was detected in all the 10 normal ovarian tissues (100%, 10/10), while was detected in 2 cases among 47 ovarian tumor samples (4.3%, 2/47). Figure 2 listed PCR results of some ovarian normal and cancer tissue samples. There was significant difference ($p < 0.05$) for frequency (100% vs. 4.3%). The two RASSF1A positive ovarian tissues were pathologically diagnosed as serous cystadenocarcinoma cases, one of which was clinical stage I and the other was stage III, which indicated that the phenomenon is not connected with cancer staging.

**Discussion**

The anti-tumor roles of RASSF1A haven’t been fully revealed so far. Some reports have shown that RASSF1A is a micro-tubule binding protein and involved in regulation of mitosis pro-

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**Figure 1.** The expression of RASSF1A in different ovarian cancer cell lines. Lane 1 was the DNA marker; lane 2, 4, 6, and 8 represent RASSF1A band, while lane 3, 5, 7, 9 represent β-actin band.

**Figure 2.** The expression of RASSF1A in normal ovarian and ovarian tumor tissues. Lane 1 was the DNA marker; The even lanes represented RASSF1A band, while the odd lanes represented β-actin band. Lane 2-9 were samples from normal ovarian tissues, while lane 10-25 were samples from ovarian tumor tissues.
ceeding. RASSF1A could regulate cell cycle directly, repress cell growth in vitro and in vivo, and also induce apoptosis. Previous results showed that cells transformed with RASSF1A could repress the accumulation of cyclin D1 and further prevent cell proliferation by hindering the cells at G1/S phase. Many evidences RASSF1A/Mst-1 complex could maintain high Mst-1 activity and assist Mst-1 to be correctly localized inside cell, and further synergistically induce cell apoptosis.

Previous results from breast cancer, lung cancer, and gastric cancer have discovered the absence of RASSF1A expression is connected with tumor development. In this study, we found that RASSF1A expression was absent from ovarian epithelial tumor tissues, which is inconsistent with previous report which stated that RASSF1A was overexpressed in the ovarian tumor tissues. But our results are consistent with RASSF1A as tumor suppressor as previously reported. However, small number of samples may be the reason why these differences appeared. Thus, large number of ovarian cancer tissues and normal samples is needed to confirm our results in the future and functional studies are needed to find out whether RASSF1A functions as a tumor suppressor through regulating cell cycle and apoptosis in ovarian tumor as in other cancer types.

Our findings also provide informative clues for developing new diagnostic method for ovarian cancer. As we all know, there is no effective diagnostic method at early phase for ovarian cancer so far because of its hidden onset and rapid development as clinical biological behavior features. For example, the serum BHCG (beta human chorionic gonadotropin) level should be measured in a female in whom pregnancy is a possibility. In addition, serum alpha-fetoprotein (AFP) and lactate dehydrogenase (LDH) should be measured in young girl and adolescents with suspected ovarian tumors because the younger the patient, the greater the likelihood of a malignant germ cell tumor. But these indicators are non-specific and the use of them is little in diagnosis. Our results show that RASSF1A was expressed in all 10 tested normal ovarian tissues, while among the 47 ovarian tumor tissues, only two cases showed RASSF1A expression. And the absence of RASSF1A mRNA happened at each clinical stages. So RASSF1A could be served as one potential molecular marker for diagnosing ovarian cancer at early phase.

Conclusions

The expressions of RASSF1A gene is absent in ovarian cancer tissues, while there is considerable mRNA level of RASSF1A in normal ovarian tissues. These findings suggest that RASSF1A, as a molecular marker, could be instrumental in developing new effective prognostic method for ovarian cancer at early phase.

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Conflict of Interest

The Authors declare that there are no conflicts of interest.

References

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