Effect of taxol on the expression of *FoxM1* ovarian cancer-associated gene

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Abstract. The incidence of ovarian cancer in women has been on the increase in recent years. The aim of the present study was to examine the effects of taxol on the expression of ovarian cancer-associated gene forkhead box transcription factor M1 (*FoxM1)* and its therapeutic effects for ovarian cancer. The expression of *FoxM1* gene was examined in patients with or without ovarian cancer. RNA and protein levels of *FoxM1* gene of ovarian cancer patients were detected at different time periods (1, 3, 6, 8, 12 and 24 months) after treatment with taxol. The results showed that the mRNA level of *FoxM1* gene in patients with ovarian cancer was significantly higher than that in normal women (P<0.05). With time and progression of the disease, the expression of *FoxM1* gene significantly increased in the patients not being administered taxol, whereas the expression of *FoxM1* in the patients administered taxol was significantly lower comparatively (P<0.05). In conclusion, an asssociation was identified between the *FoxM1* gene and ovarian cancer. The *FoxM1* gene therefore promotes the generation and deterioration of ovarian cancer, whereas taxol reduces it. These findings provide a certain theoretical basis for the later treatment of ovarian cancer disease.

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Introduction

The incidence of ovarian cancer in women is on the increase. Statistics obtained in 2013 by the International Anticancer Association (1) showed that ovarian cancer was associated with cervical, uterine, and breast cancer. The high incidence of these diseases accounts for 18.4% of the total number of female cancers (1). At least 137 million women are diagnosed with ovarian cancer annually, of whom approximately 32.4% succumb to ovarian cancer. The number of patients has increased significantly at an annual rate of 0.32-0.48% (1). Statistics have shown that the proportion of women with ovarian cancer in China has increased. It accounts for 17.4% of female malignant tumors, its incidence rate was approximately 26.4% and the mortality rate was approximately 35.4%, slightly higher than the international average level (2). Therefore, the diagnosis and treatment of ovarian cancer has become an important research focus. Previous findings have shown that early detection and appropriate treatment modalities for ovarian cancer are essential to ensure the successful treatment of ovarian cancer (3,4).

Forkhead box transcription factor M1 (FoxM1), a transcription factor identified to be associated with abnormal cell proliferation and cancer (3), was evaluated as the 'Molecule for the Year 2010' by the International Society For Molecular and Cell Biology and Biotechnology Protocols and Researches (4). He *et al* (5) demonstrated that FoxM1 serves as a type of transcription factor in the human body, which may activate downstream target genes regulated by it (such as signaling paths or key proteins relevant to cell proliferation) when the human body received external stimuli or had some internal changes. Wang *et al* (6) demonstrated that FoxM1 was closely associated with the occurrence, development and prognosis of some malignant tumors in the human body. For example, the abnormal expression of *FoxM1* gene was detected in ovarian, liver and lung cancer (7). Zhang *et al* (8) revealed that higher FoxM1 expression level was associated with tumor prognosis, while the 5-year survival rate decreased significantly. Chen *et al* (9) identified that taxol had good curative effects for various types of tumors and cancer. Zhu *et al* (10) suggested that taxol was beneficial in the treatment of colon cancer.

Based on these observations, the aim of the present study was ot examine the mutual association between *FoxM1* gene and ovarian cancer.

Materials and methods

Materials

Clinical samples. The patients with ovarian cancer and their surgical specimens were collected between 2011 and 2014 at the Sichuan Provincial People's Hospital. The patients were aged 37-54 years, with an average age of 43.5±4.6 years. The normal controls were aged 39-55 years, with an average age of 44.3±4.2 years. The subjects were randomly divided into the observation and control groups. The observation group included 36 women with ovarian cancer and the control group included 36 normal women.

Experimental medicines. An ovarian cancer detection kit was purchased from Roche Diagnostics (Indianapolis, IN, USA). Other drugs were purchased from Thermo Fisher Scientific, (Waltham, MA, USA). The fluorescence quantitative primers were produced by Takara Bio (Dalian, China), and FoxM1 antibody was provided by Acris Antibodies (San Diego, CA, USA).

Methods

RNA extraction of ovarian carcinoma cells. Frozen tissue samples were removed from ~0.1 g liquid nitrogen, thawed on ice and 0.45 ml RNA Plus (Beijing Ed Biological Technology Co, Ltd., Beijing, China) was added. Subsequently, the tissues were homogenized and 0.45 ml of RNA Plus was added. Chloroform (200 μl) was then added and briefly mixed followed by centrifugation at 8,000 x g for 15 min at <4˚C. The supernatant was transferred into an Eppendorf tube (Beijing Ed Biological Technology Co, Ltd.) with an equal volume of isopropanol and mixed, prior to centrifugation at 8,000 x g for 10 min at <4˚C. The supernatant was removed, 750 μl ethanol (75%) was added to mix gently, and the mixture was centrifuged at 8,000 x g for 10 min at 4˚C. The supernatant and the remainder of the residual ethanol were subsequently removed. An appropriate amount of RNase-free water was added to the RNA pellet.

Fluorogenic quantitative polymerase chain reaction (qPCR). Fluorogenic quantitative polymerase chain reaction was conducted according to the protocol for Takara Bio fluorescence qPCR.

Detection of FoxM1 expression in serum with ELISA. ELISA was carried out according to the manufacturer's instructions (11). The samples were diluted at a ratio of 1:200, and 100 μl serum samples were added into each well. FoxM1 detection solution was then added into the wells with 50 μl/well at 25˚C for 1.5 h. TMB substrate (50 μl) was then added into each well for color development. The optical density was measured at 495 nm using a fluorescent plate reader (Hewlett-Packard Development Company, Palo Alto, USA) to calculate *FoxM1* expression for each sample by comparing with the standard curve.

Detection of FoxM1 in ovarian cancer tissues with immunohistochemistry (IHC). IHC for ovarian tissue samples was performed according to the streptomycin affinity peroxidase (S-P) method (12). Staining was calculated as follows: <10% or negative, negative (-); only stained cell membrane or >10% tumor cells, weak positive (+); >10% tumor cells indicated weak or moderately complete staining, medium strong positive (++); and >10% tumor cells indicated markedly complete membrane staining, strong positive (+++).

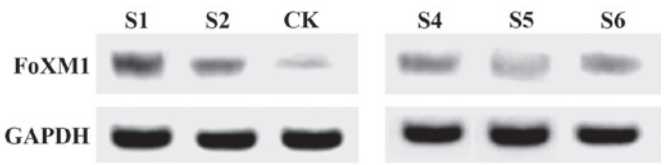

Figure 1. The *FoxM1* expression of the normal women and patients with ovarian cancer. CK, *FoxM1* expression in the normal women; S1-S6, *FoXM1* expression in patients at different time points.

Detection of FoxM1 in ovarian cancer tissues and serum using western blotting. A Thermo Fisher Scientific animal cell protein extraction kit was used to extract the total protein in the samples (particular operation according to the specification) (13). Western blotting was conducted as previously described (14). The primary antibody was anti-FoxM1 (rabbit polyclonal antibody, diluted 1:500, cat.no: sc-502; Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) and secondary antibody was horseradish peroxidase-conjugated anti-rabbit IgG (diluted 1:5,000; Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA).

Statistical analysis. SPSS 20.0 software (IBM SPSS, Armonk, NY, USA) was used to for the statistical analysis. The measurement data were presented as mean ± standard deviation, while χ^2 test was used for the count data.

Results

mRNA expression of FoxM1 in patients with or without ovarian cancer. In the present study, we selected ovarian tissue samples of normal women and ovarian cancer patients. The RNA was extracted from the tissue samples for fluorescence qPCR as mentioned in Materials and methods. The primer sequences used were : Forward 5'-TTTTGCTAGCTC AAGCCCTGTCAACTTTACC-3', and reverse 5'-ATATAA GCTTTTGCTGCATCCCGCTCACCT-3'. Fig. 1 shows the electrophoretic result of the PCR products. *FoxM1* mRNA content in patients with ovarian cancer was higher than that in the normal population (CK), and its expression was not the same at the different time points. By comparing the expression of *FoxM1* mRNA in the experimental and control groups (Fig. 2) the average level of *FoxM1* mRNA expression in patients with ovarian cancer was found to be 4.3- to 5.8-fold significantly higher than that in the normal women. The result identified a certain correlation between *FoxM1* gene and ovarian cancer.

FoxM1 mRNA expression in ovarian cancer patients at different stages. FoxM1 mRNA expression was detected in ovarian cancer patients at different time points (Fig. 3). The expression of *FoxM1* gene gradually increased with the

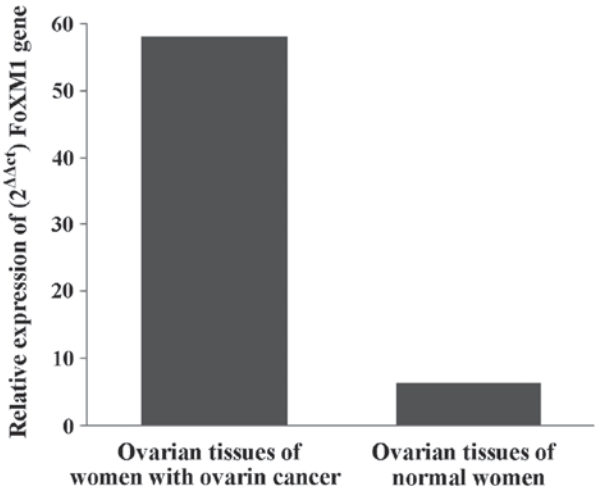

Figure 2. The relative expression of *FoxM1* mRNA in the observation and control groups.

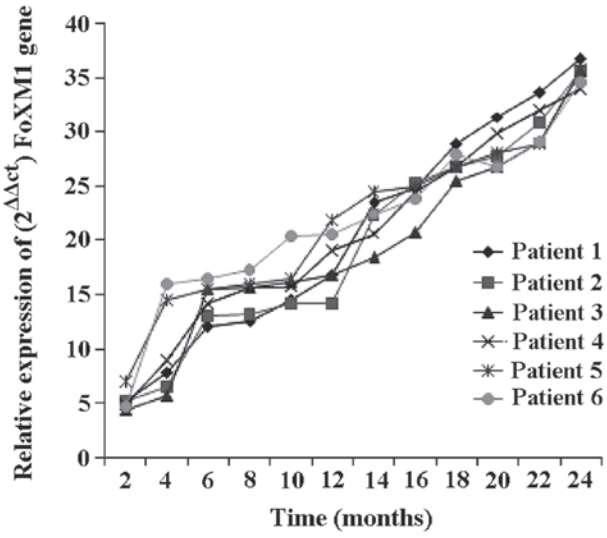

Figure 3. Changes of *FoxM1* expression at different time points in different ovarian cancer patients (Patients 1-6).

progression of disease, the growth was gradual at the early stage of disease and became rapid at the later stage (6 months later). Within 8-18 months of diagnosis of ovarian cancer, the mRNA expression increased, indicating that *FoxM1* gene is associated with ovarian cancer, and there is a positive correlation between the levels of FoxM1 and the severity of ovarian cancer patients.

Expression of FoxM1 mRNA in serum of ovarian cancer patients at different stages. FoxM1 protein expression in the serum of ovarian cancer patients at different time points was detected using western blotting (Fig. 4). The results showed that the expression level of FoxM1 protein in serum gradually increased as the disease progressed and the growth was significantly increased after six months (Fig. 3). The results showed that *FoxM1* gene is associated with ovarian cancer, and there is a positive correlation between the expression levels of FoxM1 in serum and the severity of ovarian cancer patients.

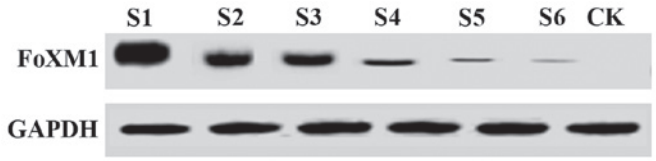

Figure 4. *FoxM1* expression at different time points of one ovarian cancer patient. CK, serum of normal women; 1-6, serum of patients suffering from ovarian cancer at 24, 12, 8, 6, 3 and 1 month.

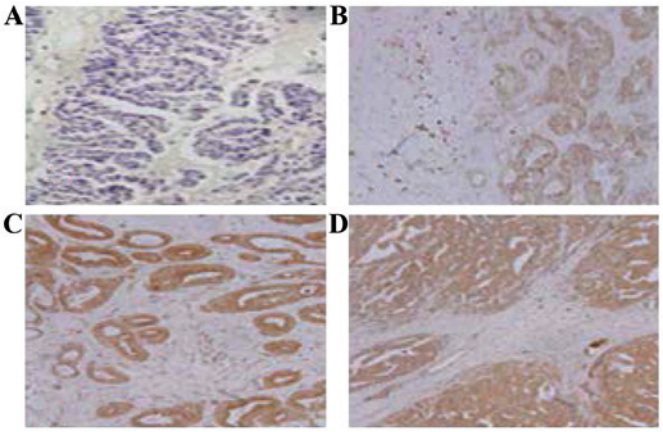

Figure 5. Immunohistochemical staining observation results of *FoxM1* in human ovarian cancer tissues. (A) normal ovarian tissues (x200); (B) ovarian cancer tissues (x200); (C) ovarian cancer tissues (x200); (D) ovarian cancer tissues (x200).

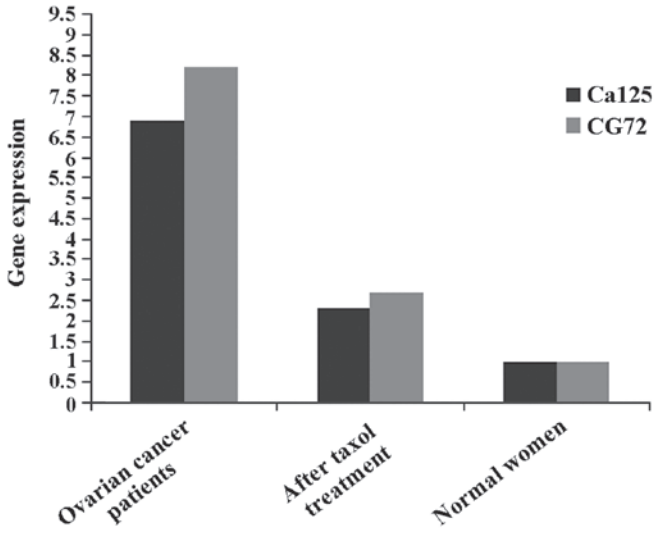

Figure 6. Recovery of ovarian cancer patients prior to and following taxol treatment.

IHC results of FoxM1 in ovarian cancer tissues (Fig. 5) demonstrated that positive staining of *FoxM1* expression was mainly concentrated in the ovarian cancer cell membrane (B-D), and the expression was shown as: i) Negative (-); ii) weak positive (+); iii) moderately strong positive (++); and iv) strong positive (+++). The main features of positive staining were the uneven size of brown small particles, while there were not

found in the normal ovarian tissues. The content of FoxM1 in ovarian cancer patients was higher than that in the normal population and mainly concentrated in the ovarian tissue.

Curative effects of taxol on ovarian cancer patients. In the present study, we preliminarily examined the curative effects of taxol on ovarian cancer patients. The results revealed corresponding sensitive markers of ovarian cancer (CA125, AG72), and contents of the above sensitive markers in ovarian cancer patients of the experimental group administered taxol significantly decreased, suggesting that taxol has curative effects on ovarian cancer to a certain extent (Fig. 6).

Expression of FoxM1 in ovarian cancer patients prior to and following taxol treatment. In the present study, the patients with ovarian cancer were treated with taxol. Prior to and following therapy, the expression of FoxM1 in the lesion tissues of the experimental samples was measured by ELISA, and results revealed that the average content of FoxM1 in the serum of normal women was approximately 4.19±0.63 ng/ml. The results for the 36 female ovarian cancer patients revealed that the average content of FoxM1 in the serum prior to treatment was 12.1±21.21 ng/ml, and that after treatment with taxol the serum level was approximately 5.73±0.39 ng/ml. The content in the serum of ovarian cancer patients (12.12±1.21 ng/ml) was significantly higher than that in the serum of the controls (4.19±0.63 ng/ml). Compared with conditions prior to treatment, the expression of FoxM1 in ovarian cancer patients treated with taxol was significantly decreased, indicating that taxol exerts certain effects on ovarian cancer. Therefore, the taxol may be used to treat ovarian cancer by reducing the *FoxM1* gene expression in patients.

Discussion

Previous findings by Francis *et al* (15) revealed that FoxM1 is an important transcription factor, and is closely associated with tumor occurrence and development. The study by Yoshida *et al* (16) demonstrated that FoxM1 comprised 10 exons located at 12 p13-3 positions of the 20 kb chromosome. The expression amount of *FoxM1* genes in cancer cells has been found to be significantly higher than that in the normal cells, and it can be taken as a marker for certain types of tumor and cancer (16). The percentage of female ovarian cancer in China has increased annually (17,18), and the rapid diagnosis of ovarian cancer has become a hot research topic. Chen *et al* (19) suggested that genes associated with the occurrence and deterioration of ovarian cancer can be classified as those associated with ovarian cancer at the mRNA level and those associated with ovarian cancer at the protein level. Gong *et al* (20) identified 61 genes possibly associated with ovarian cancer, although those authors did not examine the mechanism involved in mRNA and protein expression levels in a detailed manner.

Taxol was identified in 1971 by Wani *et al* (21) who separated it from the bark of short-leaf taxus brevifolia. Wang *et al* (22) previously demonstrated that it taxol may serve as a broad-spectrum antitumor drug. Schiff *et al* proved that taxol had a unique anticancer mechanism (23,24). For instance, it acted on cell microtubules and to a certain extent induced protein separation in relevant proteins by interplaying with the amino acid at the 31st position at the N end of the microtubule and the amino acid at the 217th-231st position. Further blocking cells in the G2PM period and ultimately causing abnormality or ceasing of mitosis, apoptosis of cancer cells, owing to the inability of the cells to multiply, can be used for treatment of the cancer (24). In the present study, we found that *FoxM1* gene was associated with the onset of some tumors and cancer, and demonstrated that it was correlated with ovarian cancer. The expression of *FoxM1* in ovarian cancer patient serum and ovarian tissue was significantly higher than that in normal women. To the best of our knowledge, for the first time, we confirmed by experiment that, taxol also had certain therapeutic effects on female ovarian cancer. Compared with conditions prior to treatment, contents of relevant sensitive markers (CA125 and AG72) of ovarian cancer in patients decreased significantly, By comparing the expression amount of *FoxM1* gene in ovarian cancer patients prior to and following treatment with taxol, we found that the expression amount of *FoxM1* gene significantly decreased following treatment.

In conclusion, we preliminarily evaluated treatment of female ovarian cancer with taxol, and subsequently, to the best of our knowledge, identified a new gene, *FoxM1*, which was associated with female ovarian cancer. By comparing the expression amount of *FoxM1* gene in the observation and control groups, it was found that in comparison to the normal women, the FoxM1 levels in patients with ovarian tumor significantly increased ($P<0.05$). Additionally, the results showed that *FoxM1* gene expression was reduced in ovarian cancer patients after treatment with taxol. Thus, the curative effects of female ovarian cancer were preliminarily evaluated. Compared with the control group without taxol treatment, contents of relevant sensitive markers of ovarian cancer (CA125 and AG72) in ovarian patients decreased significantly, suggesting that taxol exerted a therapeutic effect on ovarian cancer. Measuring the expression amount of *FoxM1* gene in ovarian cancer patients prior to and following taxol treatment showed that the expression of FoxM1 with taxol significantly decreased ovarian cancer patients. The finding suggests that taxol is capable of reducing FoxM1 levels in ovarian cancer patients. Therefore, taxol has promising implications for the treatment of ovarian cancer and may be developed as a therapeutic agent.

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