Correlations between the *MEG-A3* gene and incidence of breast cancer

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Abstract. The aim of the present study was to examine the interrelations between *MEG-A3* gene and incidence of breast cancer. The expression of *MEG-A3* gene in the tissue samples of patients with breast cancer and normal controls at RNA and protein levels was determined. Subsequently, the relative expression of RNA for the same patient was measured at different time-points (1, 3, 6, 8, 12 and 24 months), and the protein expression levels were determined using western blotting. The results showed that, the mRNA level in *MEG-A3* gene of samples of patients with breast cancer was significantly higher than that of normal women (p<0.05). The *MEG-A3* gene expression increased apparently with the prolongation and aggravation of the disease. In conclusion, there is a close correlation between *MEG-A3* gene and the incidence of breast cancer; thus, *MEG-A3* gene contributes to the occurrence and deterioration of breast cancer to some extent. It provides a theoretical basis for later disease treatment.

Introduction

According to statistics of the International Union of Counter Cancer, breast cancer has a relatively high morbidity (1). It accounts for almost 28.5% of the total number of female cancers. Approximately 1,600,000 women suffer from breast cancer each year, of whom 780,000 women succumbed to breast cancer (1). The mortality rate is at approximately 48.76%, with an annual increase of 0.42-6.5% (1). The proportion of women with breast cancer in China is also on the increase, accounting for 36.5% of female malignant tumors. The incidence of the disease is 26.4% among women, with a mortality rate of 49.2%, which is slightly higher than global rates (2). Thus, the diagnosis and treatment of breast cancer is crucial.

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Melanoma antigen gene (*MAGE*) is affiliated to cancer and the testis antigen family. A high expression of *MAGE* gene can be detected in many tumors and malignant tissues (3-5). Approximately 60 different types of *MAGE* genes have been identified. According to its expression patterns and differences in gene structure, MAGE can be divided into the subcategories, MAGE-1 and MAGE-2 (6).

In the present study, we first identified genes associated with morbidity of breast cancer that contain the specific conserved domain of the MAGE gene family (7). The interrelation between *MAGE* gene and breast cancer was preliminarily investigated. In addition, we conducted a preliminary discussion on the interrelation between them to provide some theoretical guidance for the later treatment of breast cancer.

Patients and methods

Patient samples. The clinical samples used in the present study were all from surgical specimens of patients with breast cancer admitted to the Second People's Hospital of Liaocheng between June 2012 and April 2014. The patients were aged between 25 and 56 years, and the average age was 45-23.4 years. Normal women were aged 26-58 years, and the average age was 43.3-23.9 years. The study patients were randomly divided into the control and observation groups. The control group included 27 normal women, and the observation group included 27 women with breast cancer.

Experimental drugs. The breast cancer detection kit used in this study was purchased from Roche Diagnostics (Basel, Switzerland). Other relevant drugs were purchased from Thermo Fisher Scientific (Waltham, MA, USA). Relevant fluorescence quantitative primers were produced by Takara Bio (Dalian, China). Primary mouse monoclonal MEG-A3 antibody was provided by PeproTech (Rocky Hill, NJ, USA; cat. no. 60054-1; dilution, 1:500). Peroxidase-conjugated secondary polyclonal goat-anti-mouse antibody (1:1,000) was purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA; cat. no. sc-395763).

RNA cell extraction of breast cancer. Tissue (0.1 g) was dispensed in 0.45 ml of RNA Plus and homogenized in the precooling mortar. Subsequently, the contents were transferred to the 1.5-ml EP tube and 0.45 ml of RNAPlus

was added. Chloroform (200 μl) was added to the tube, followed by centrifugation at 10,000 x g, at 4°C for 15 min. The supernatant was transferred into the EP tube containing dimethyl carbinol and the contents were homogenized on ice for 10 min. The solution was centrifuged at 12,000 rpm, at 4°C for 10 min, and then 750 μl of 75% ethanol was added. The tube contents were centrifuged at 12,000 rpm, 4°C for 10 min. The supernatant was discarded and RNase-free water was added to resuspend the pellet. The extracted RNA was quantified for purity and concentration with a UV-visible spectrophotometer (BioSharp, Hefei, China).

Fluorescence quantitative polymerase chain reaction (PCR). The procedure was conducted according to the protocol of the kit (Takara fluorescence quantitative PCR specification).

Detection of MEG-A3 expression in serum by enzyme-linked immunosorbent assay (ELISA). The procedure was conducted according to the specification of the ELISA kit (8). The standards were diluted according to the proportion of 1:25 with assay buffer, and the standard curve was designed. The sample to be tested was first diluted at a 1:100 ratio and then TMB chromogenic substrate was added to each well. After incubation for 2 h at 20°C, the absorbance was determined at 495 nm using a microplate reader (BioTek Instruments, Inc., Winooski, VT, USA). The content and concentration of MEG-A3 in each sample were calculated according to the standard curve.

Detection of MEG-A3 in breast cancer tissue by immunohistochemistry. The control and test samples were incubated using streptavidin-peroxidase (SP) as previously described (8). The criteria for the evaluation of the immune system were as follows (9): If capsule staining was <10% or tumor cells exhibited a negative reaction after staining, it was determined as negative. If only the cell membrane was stained or capsule staining showed >10% content of tumor cells, it was determined as positive. Over 10% of tumor cells showing a weak or medium complete stain was considered as strongly positive (++), whereas >10% of tumor cells showing strong complete membrane staining was determined as strongly positive (++).

Detection of MEG-A3 in breast cancer tissue and serum with western blotting. A Roche animal cell protein extraction kit was used to extract the total protein of samples (10). Western blotting was carried out as previously described (10).

Statistical analysis. SPSS 20.0 statistical software (Chicago, IL, USA) was used for data analysis. The correlative measurement results were indicated as mean ± SD. P<0.05 was considered statistically significant.

Results

MEG-A3 mRNA expression level of normal patients and patients with breast cancer. The expression of MEG-A3 mRNA in patients with breast cancer was higher than that in controls, and the expression level was different in patients with a different course of disease (Fig. 1). Comparison of the *MEG-A3* mRNA levels for normal and observation group
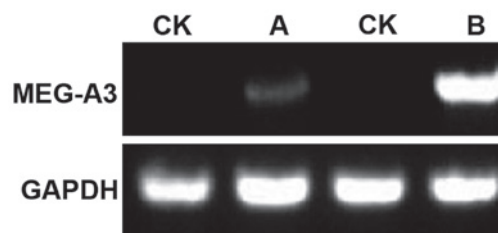

Figure 1. MEG-A3 gene expression level for controls and patients of breast cancer; (A) MEG-A3 gene expression level of female patients for 3 months; (B) MEG-A3 gene expression level of female patients for 18 months.

Table I. Relative expression level of MEG-A3 mRNA of patients in the observation and control groups.

Group	Example number	Relative expression level of MEG-A3 mRNA	χ^2	P-value
Control	27	5.6-6.3	95.3	<0.05
Observation	27	33.6-44.1		

p<0.05 with significant difference.

showed that the average expression level of *MEG-A3* gene in patients with breast cancer was 6- to 7-fold significantly higher than that for controls (p<0.05). Thus, there is a certain correlation between *MEG-A3* gene and breast cancer.

Expression of MEG-A3 in serum of patients with breast cancer and controls. The ELISA results showed that the average expression of *MEG-A3* in serum of healthy women was ~7.14-5.76 ng/ml. The results of *MEG-A3* in serum of 27 female patients with breast cancer showed that its average level of *MEG-A3* in serum was 12.47-10.17 ng/ml. It indicated that levels in serum of female patients with breast cancer was significantly higher than those in normal controls (Table I).

Immunohistochemistry. The immunohistochemical staining of breast cancer tissues for MEG-A3 revealed that the positive staining of MEG-A3 expression was mainly concentrated in the breast cancer cell membrane (A/B) (Fig. 2). The main feature was yellow brown small particles of non-uniform sizes, which was not identified in the breast tissue of normal women.

MEG-A3 mRNA expression level of patients with different stage of breast cancer. The result of MEG-A3 mRNA expression levels of patients with different stages of breast cancer is shown in Fig. 3. The data analysis showed that MEG-A3 mRNA expression levels of patients was increased with the prolongation of the disease. The increase had a downward-ascend-downward trend, especially between 8 and 18 months of disease. The mRNA expression level was significantly increased, which indicated that there was an interrelation between *MEG-A3* gene and breast cancer. Thus, there is a positive correlation between the level of *MEG-A3* gene expression and the severity of breast cancer patients.
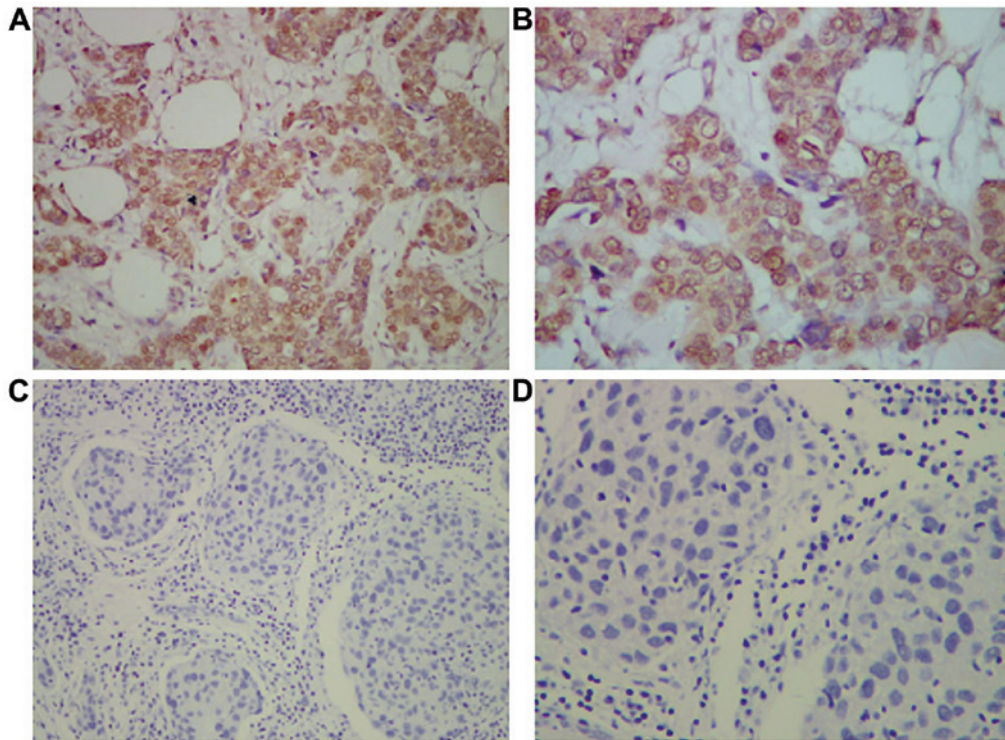
Figure 2. Immunochemical staining of MEG-A3 in breast tissue. (A) Breast cancer tissue (magnification, x200), (B) breast cancer tissue (magnification, x400), (C) normal breast tissue (magnification, x200) and (D) normal breast tissue (magnification, x400).
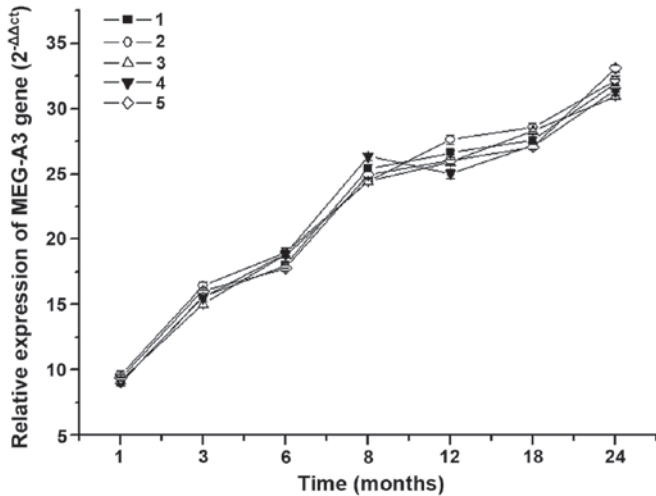

Figure 3. Relative expression of *MEG-A3* gene at different time points in breast cancer patients. 1-5, different breast cancer patients.
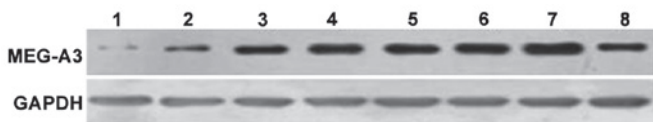

Figure 4. MEG-A3 expression level of the same patient with breast cancer in different period. Lane 1, serum of normal women; lanes 2-8, serum of patients with breast cancer 1, 3, 6, 8, 12, 18 and 24 months after diagnosis with breast cancer.

MEG-A3 expression level of patients with different stage of breast cancer. MEG-A3 protein expression was detected in the patients with breast cancer at different time points using western blotting (Fig. 4). It was identified that MEG-A3 protein expression level in the serum of patients increased with the prolongation of the disease, and the increments increased apparently since six months after diagnosis with disease. Thus, there was a positive correlation between the level of MEG-A3 in serum and the severity of breast cancer patients.

Correlation between MEG-A3 expression level in tissue and serum of patients with breast cancer. The detection result of MEG-A3 expression level in the tissue of 27 patients with breast cancer showed that the level was 24.62-9.24 ng/ml. The MEG-A3 content in tissue of normal women was 11.7-5.22 ng/ml, and significant differences were detected (P<0.05). The detection of MEG-A3 expression level in serum of 27 patients with breast cancer showed that the level was 12.47-10.17 ng/ml. The MEG-A3 content in tissue of normal women was 7.14-5.76 ng/ml, and significant differences were observed (P<0.05). There was a positive correlation between the content of MEG-A3 in tissue and serum (r=0.401). The serological detection method was consistent with the histological detection method (κ consistency check κ=0.392).

Discussion

MAGE is a cancer/testis antigen family member that has no or little expression in other tissues except testis and placenta (11,7). However, *MEGA* gene can be detected in some tumor and cancer cells. It is believed that MEGA protein can be used for the detection and treatment of certain tumors (12,13). *MAGE* gene has different degrees of expression in melanoma, lung, breast, liver, and ovarian cancer, as

well as other diseases (14-18). Additionally, in the same type of tumor disease, there were many different subtypes of MAGE genes involved. In recent years, as the proportion of female breast cancer has been on the increase (19), the rapid diagnosis of breast cancer has become a research hot spot. The genes associated with breast cancer morbidity and deterioration can be divided into two categories, the one associated with breast cancer at the mRNA level and the other associated with breast cancer at the protein level (20,21). The experiment of Canzian *et al* identified 61 genes that may be associated with breast cancer (22). However, no detailed study is available on the mechanism of mRNA and its protein level.

In the current study, we found that the conserved domains were commonly used as epitopes of MAGE and protein interaction sites by nucleotide and amino acid sequences. Therefore, the *MEG-A3* gene may be partially associated with morbidity of tumors and cancers. We confirmed that there was a certain correlation between *MEG-A3* gene and breast cancer through the study, the results of which indicated that the expression of MEG-A3 was higher in the serum and breast tissues of patients with breast cancer and was significantly different compared to normal women. However, the molecular mechanism for MEG-A3 in patients with breast cancer remains to be elucidated.

In conclusion, to the best of our knowledge, in the present study for the first time, we identified the association of a new gene known as *MEG-A3* with breast cancer in women. Using sequence alignment we found that, it has similar conserved domains with MAGE. By comparing the expression of *MEG-A3* gene in normal women and women with breast cancer, we found that the *MEG-A3* gene mRNA and protein levels were significantly increased in the patients (P<0.05). Further research revealed that there was a positive correlation between the levels of MEG-A3 in serum and the course of disease for breast cancer patients. The levels of *MEG-A3* gene in serum of patients was enhanced with the prolongation of the disease. By comparing the expression level of MEG-A3 in serum and breast tissue, it was identified that the method of serum detection and histological examination has a good consistency for MEG-A3. Thus, there was a positive correlation between *MEG-A3* gene and breast cancer, which can be used for the detection of breast cancer and relevant treatment to a certain extent. Additionally, the findings provided a theory and experimental basis for the later treatment of breast cancer.

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