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Research Article

Expression of MxA Protein in Triple Negative Breast Cancer and its Relationship with Prognosis

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Abstract: ***Objective:*** To explore the expression of human myxovirus resistance protein A (MxA) in triple negative breast cancer (TNBC) and its relationship with prognosis. ***Methods:*** 144 cases of TNBC confirmed by pathology before or after surgery from January 2014 to January 2017 in the First Central Hospital of Baoding City were retrospectively collected. Western blotting was used to detect the expression of MxA protein in TNBC and adjacent breast tissues. According to the expression of MxA protein, 144 TNBC patients were divided into low MxA protein expression group (n = 91) and MxA protein high expression group (n = 53) for subsequent comparison of prognosis of patients in between these two groups. ***Results:*** The expression of MxA protein in TNBC tissue was lower than that of adjacent breast tissue, and the difference was statistically significant (P<0.05). The patients in high MxA expression group had higher loco-regional recurrence-free rate, disease-free survival (DFS) rate, and overall survival (OS) rate than those in low MxA expression group for 3 years. On the other hand, the distant metastasis rate was lower in the high expression group compared to that in the low MxA expression group, and the difference was statistically significant (P<0.05). ***Conclusion:*** In triple-negative breast cancer, high MxA expression is a potential predictor of TNBC prognosis.

Keywords: Triple negative breast cancer; Human myxovirus resistance protein A; Prognosis

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Triple negative breast cancer (TNBC) refers to a special type of breast cancer that is negative for estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER-2) TNBC is characterized by large tumor load, high malignancy, early recurrence and metastasis, limited treatment methods, and poor prognosis[1]. Therefore, discovery of biological indicators to predict and interfere with the occurrence and development of TNBC, and to break through the dilemma of traditional radiotherapy and chemotherapy and endocrine therapy, is of great significance for delaying the progression of TNBC and improving the prognosis of patients. Human myxovirus resistance protein A (MxA) is an antiviral protein produced by human cells that have been induced by type I interferon (IFNα/β) and this protein participates in the innate immune system's response to viral infections[2]. This study explored the expression of MxA in TNBC tissues and the relationship with the prognosis of patients, providing new clues to the clinical diagnosis and targeted therapy of TNBC.

1 Materials and methods

1.1 Baseline characteristics

144 cases of TNBC confirmed by pathology before and after surgery in the Baoding No. 1 Central Hospital from January 2014 to January 2017 were collected retrospectively. During the operation, cancer tissues and normal tissues 5 cm away from the edge of the cancer tissues were obtained. Intraoperative specimens were

taken from the body in vitro, quickly placed in liquid nitrogen for quick freezing, and frozen at -80°C in the freezer. The patients were 26 – 61 years old, with an average age of 43.26 ± 3.28 years. Inclusion criteria of the present study include: The patients were all newly diagnosed with TNBC; Case data and follow-up data of each patient were complete; Endocrine therapy, chemotherapy, and immune therapy were not received before surgery. Exclusion criteria of this study include: The patient was also diagnosed with other tumors; Patient cases and follow-up data are incomplete.

1.2 Method

The tissue sample blocks that were frozen in the -80°C freezer were used to extract tissue protein, and the total protein was quantified using the BCA method. Western blotting was used to detect protein expression. The electrophoresis process entailed the use of 10% separating gel and 4% stacking gel that were placed in an electrophoresis tank added with distilled water. The gels were vertically loaded. After electrophoresis, membrane was transferred using wet transfer method. After blocking, the membrane was incubated the primary antibody overnight at 4°C (the primary antibody rabbit anti-human MxA polyclonal antibody was diluted to 1:500; the membrane was then washed with TBST for 3 times, 5 min each time). After incubating with secondary antibody, the membrane with washed TBS solution for 3 times, 10 min each time. The membrane was then subject to ECL chemiluminescence Liquid color exposure, development, and final fixation. Images were taken and data were analyzed. The reagents were purchased from Fuzhou Maixin Biotechnology Development Co., Ltd., and the experiments were carried out strictly in accordance with the reagent instructions.

1.3 Observation indicators

According to the relative expression of MxA protein, 144 patients were divided into two groups, namely the low expression group (MxA expression < 1.50) and high expression group (MxA expression ≥ 1.50). The follow-up date was from the date of discharge from the hospital until January 2020 or the death of the patient. The follow-up was carried out in the form of clinic consultation and via phone calls to record no local recurrence, distant metastasis, disease-free survival (DFS) and overall survival (OS) of patients for 3 years.

1.4 Statistical analysis

The data was analyzed using Statistical Package for Social Sciences (SPSS) version 20.0 software. The measurement data that conformed to normal distribution were expressed in $\overline{X} \pm s$. Two independent samples t test were used for comparison between groups. Count data were analyzed using Chi-squared (χ^2) test. Kaplan-Meier method was used to analyze the survival rate, and the survival time was compared using Log-rank χ^2 test. $P<0.05$ was considered statistically significant.

2 Results

2.1 Expression of MxA protein in TNBC and adjacent breast tissues

The expression of MxA protein in TNBC tissue was lower than that of the adjacent breast tissue, and the difference was statistically significant ($P<0.05$). See Table 1.

Table 1. MxA protein expression in TNBC and the adjacent normal breast tissue ($\overline{X}\pm s$)

Group	MxA protein
TNBCgroup (n = 144)	0.48±0.15
Adjacent normal breast tissue (n = 144)	2.42±0.56
t	18.930
P	0.000

2.2 Relationship between MxA protein expression and prognosis in cancer tissues

According to the relative expression of MxA protein, 144 patients were divided into high expression group (n = 53) and low expression group (n = 91). The TNBC patients of high MxA expression group had a 3-year loco-regional recurrence-free rate (75.47%) higher than those of the low MxA expression group (29.67%), distant metastasis rate (11.32%) was lower than high MxA expression group (22.64%), the differences were statistically significant ($P<0.05$). Kaplan-Meier curve showed that the 3-year DFS rates of low MxA expression group and high MxA expression group patients were 50.13% and 71.35%, whereas the OS rates

were 58.23% and 83.27%, respectively. The differences were statistically significant ($P< 0.05$).

3 Discussion

Breast cancer is a complex disease that is jointly affected by various factors such as environment and genetics. Breast cancer is ranked first as the most common cancer in Chinese females, and the sixth for mortality. From 2005 to 2013, the standardized rate of female breast cancer deaths increased from 5.00 / 100,000 to 6.55 / 100,000, corresponding to an increase of 31.00%[3]. TNBC accounts for about 20% of breast cancer, and its prognosis is generally poor. To date, dinding a suitable TNBC treatment and specific targets is still a problem that clinicians need to solve.

MxA plays a role in cellular protein synthesis and apoptosis, and it can inhibit the replication of various RNA viruses. Although MxA is mainly associated with viral infection, it may be a mediator of IFN's regulation on normal and tumor cell movement. MxA can act as a tumor suppressor, and its expression level can be used as a predictor of metastatic potential[4]. IFN-α is widely used in anti-viral, anti-tumor cell proliferation and immunomodulatory therapy. Type I or type III IFN signaling pathways can induce the expression of MxA gene, leading to an increased level of MxA protein. Studies have shown[5] that Mx1 gene mutations in the MxA coding region have been found in many types of cancer, and seven severe MxA mutations (L95P, P96S, G392V, V449G, P218S, R522C, and E632K) may play important roles in the development and progression of human cancers. Thus, they have been established as effective tumor-related biomarkers. There is a strong link between skin squamous cell carcinoma and MxA, and the related mutations (L95P, P96S, and P218S) cause significant destruction of protein structure. Immunohistochemical examination showed that the expression of MxA protein in prostate cancer was lower than that in normal tissues. The low expression of MxA led to increased invasion and metastasis of tumor cells and decreased sensitivity to docetaxel[6].

The expression of MxA in HER2-positive breast cancer tumor cells is associated with high levels of tumor-infiltrating lymphocytes (TIL)[7]. Our previous study showed that MxA was highly expressed in basal cell-like breast cancer and might interact with tumor-infiltrating lymphocytes, and MxA plays a certain role in suppressing cancer during tumor progression[8].

The vast majority of basal cell-like breast cancers lack the expression of ER, PR and HER-2, but there are still some differences in gene expression profile and immune expression between basal cell-like breast cancer and TNBC. At present, the research on the expression and prognostic value of MxA protein in TNBC has not been reported in the literature in China. In this experiment, the Western blotting method was used to detect the expression of MxA in TNBC tissues. The results showed that the expression of MxA in TNBC tissues was lower than that of normal breast tissues. The expression of MxA in TNBC was also different, and low MxA expression indicates a poor prognosis. The 3-year loco-regional recurrence-free rate , DFS rate, and OS rate of the patients were low, and the distant metastasis rate was high. MxA can be used as a potential tumor marker for TNBC, providing a reference index for evaluating the prognosis of TNBC. Nonetheless, its mechanism of action in TNBC warrants further study.

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