RELATIONSHIP BETWEEN NEUROTENSIN RECEPTOR 1 EXPRESSION AND CLINICOPATHOLOGICAL FEATURES AND PROGNOSIS IN BREAST CANCER

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ABSTRACT

Objective: *To explore the relationship between the expression of neurotensin receptor 1 (NTR1) in breast cancer and its clinicopathological characteristics and prognosis.*

Methods: *The 132 cases of breast cancer tissues and 30 cases of normal paracancer tissues that were used in this study originated from the surgical resection samples of our hospital between January 2013 to May 2014. The expression of NTR1 in cancer tissues and paracancerous tissues was detected by the immunohistochemistry method, and the correlation between the expression of NTR1 in breast cancer and clinicopathological parameters and prognosis was revealed.*

Results: *The positive expression rate of NTR1 in breast cancer tissues was 70.45% (93/132), and the positive expression rate in the normal adjacent tissues was 0% (0/30), with the difference between the two groups being statistically significant (P<0.05). The expression level of NTR1 correlated with tumour diameter, lymph node metastasis, and tumour grade in patients with breast cancer, but not with the TNM stage, menopausal status, estrogen receptor, progesterone receptor, and human epidermal growth factor receptor 2 (P>0.05). The 5-year survival rate of patients with a positive expression of NTR1 was 68.82% (64/93), and patients with a negative expression of NTR1 had a survival rate of 89.74% (35/39). As such, the difference was statistically significant (P<0.05). The expression of NTR1 and lymph node metastasis can also independently affect the prognosis of breast cancer patients.*

Conclusion: *The expression of NTR1 in breast cancer is significantly increased, suggesting that it plays a significant role in the progression of breast cancer. At the same time, the positive expression of NTR1 is closely related to tumour diameter, lymph node metastasis, and tumour grades in patients with breast cancer, In addition, its expression can independently affect the prognosis of breast cancer patients, which is an important index for clinical evaluation and the future treatment methods of breast cancer.*

Keywords: *NTR1, breast cancer, lymphatic metastasis, neurotensin.*

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Introduction

The epidemiological report shows that the incidence rate of breast cancer in China is increasing gradually, and in a similar fashion, the onset of the disease appears to start younger. As such, breast cancer has become one of the diseases that seriously endanger the lives and health of women[1, 2].

Surgical resection is the main method for the treatment of breast cancer, and while radiotherapy and chemotherapy can kill most of the proliferating tumour cells, this method cannot fundamentally cure the patients. Indeed, mortality still occurs due to the recurrence and distant metastasis, and the 5-year survival rate cannot be improved[3, 4]. At the same time, the emergence of tumours that are drug-resistant can also lead to poor therapeutic effect, and ultimately, lead to a failure in the treatment.

Therefore, the focus of the current research looks at the ways in which they can effectively screen and use the antitumor drugs and evaluate the relationship between tumour cells and drug sensitivity to guide clinical treatment. Under normal circumstances, neurotensin is spread all over the brain and

gastrointestinal tract, and it can bind the neurotensin receptor (NTR) to play a role in multiple centres and peripheral areas[5]. NTR is currently known to have three subtypes: NTR1, NTR2 and NTR3. Among them, NTR1, NTR2 are members of the superfamily of G protein-coupled receptor (GPCRs), and NTR3 is structurally different from NTR1 and NTR2[6]. At present, NRT1, as a high-affinity receptor of neurotensin, is the most studied NTR, and is not sensitive to the H1 receptor antagonist[7]. Indeed, neurotensin can excite NTR1 and combine with it, activating and mediating downstream signals, and transmit the information down through different signal pathways[8].

It has been reported that NTR1 is closely related to the onset, progress, and prognosis of most cancers, but there are relatively few reports on its role in breast cancer[9]. Therefore, the purpose of this study was to investigate the relationship between the expression of NTR1 and the clinicopathological parameters and prognosis of breast cancer by detecting the content of NTR1 in breast cancer tissue.

Data and methods

General information

The 132 cases of breast cancer tissues and 30 cases of normal paracancer tissues which were used in this study were all from the surgical resection samples originating from our hospital and derived from a date range of January 2013 to May 2014.

Inclusion criteria:

• The patients had to be over 18 years old;

• All of them met the diagnostic criteria of breast cancer in the WHO classification of breast tumours, and the clinicopathological data were complete;

• All patients enrolled were informed of the process and signed the consent form;

• An application was also submitted to the ethics committee, and this was passed.

Exclusion criteria:

• The patient has prior radiotherapy and chemotherapy;

• The patient had primary cancer, with the exception of breast cancer;

• The patient had heart disease, such as myocardial infarction;

• The clinicopathological data of the patients were incomplete.

Among them, 17 cases were younger than 40 years old, 115 cases were equal to or greater than 40 years old; 64 cases had a tumour diameter equal to or less than 2cm, 68 cases had a tumour diameter greater than 2cm; There were 101 cases with a tumour of grade I~II and 31 cases of grade III; 25 cases were in TNM stage I, 68 cases in stage II, and 39 cases in stage III; 71 cases had lymph node metastasis and 61 cases had no lymph node metastasis; the estrogen receptor was negative in 73 cases and positive in 59 cases; 72 cases were negative for progesterone receptors while 60 cases were positive; 96 cases were negative and 36 cases were positive for human epidermal growth factor receptor 2; There were 61 postmenopausal patients and 71 premenopausal patients; There also were 29 cases with a family history of the illness and 103 cases without family history.

Main reagents and instruments

Reagent

Rabbit anti-human NTR1 monoclonal antibody was purchased from Wuhan Pujian Biology Technology Co., Ltd. Biotin labelled rabbit anti-mouse monoclonal antibody IgG was purchased from the Shanghai Youyu Biotechnology Co., Ltd. Horseradish labelled streptomycin was purchased from Wuhan Chundu Biotechnology Co., Ltd. 3% H2O2 was purchased from Shanghai's Hepeng Biotechnology Co., Ltd. Goat serum blocking buffer was purchased from Anhui Jingke Biotechnology Co., Ltd. Gastric protease was purchased in Shenzhen Kangchuyuan Co., Ltd. Hematoxylin dye was purchased from Shanghai Yubo Biotechnology Co., Ltd. PBS buffer was purchased from Shanghai QianTu Biotechnology Co., Ltd. Citrate buffer was purchased from Jiangxi Jianglanchun Biological Reagent Co., Ltd. Neutral gum was purchased from Beijing Jinkelong Biotechnology Co., Ltd.

Instruments

Optical microscope was purchased from the Beijing Oubotong Optical Technology Co., Ltd. The drawing system was purchased from Olimpus Company (Japan). An automatic tissue processor was purchased in Hubei Oumeilai Medical Technology Co., Ltd. The slicing machine was purchased in Shandong Yinying Cooking Machinery Co., Ltd. The embedding machine was purchased from Hubei Xiaogan Kuohai Medical Science and Technology Co., Ltd. The electric constant temperature incubator was purchased in Hangzhou Huier instrument and equipment Co., Ltd.

Methods

Breast cancer and paracancerous tissues were

collected for immunohistochemical treatment, and the tissues were sliced with a thickness of approximately 5 μm. After slicing, the slices were baked in the oven and then placed in the incubator with the temperature set at 65 °C overnight.

The slices were removed for xylene dewaxing, gradient alcohol debenzol treatment, and then the backwater treatment was carried out. The slices were rinsed with distilled water, then citrate buffer was placed in a pressure cooker and the slices were immersed in liquid for antigen repair for 3 minutes.

After cooling, the slice was put in H_2O_2 for 30 min and rinsed with a PBS buffer. The serum blocking buffer was added and placed at room temperature for 30 min, then the blocking buffer was dumped and rinsed with distilled water. The primary antibody was added and incubated for 20 min and rinsed with PBS buffer. Next, the second antibody was added and incubated for 20 min and rinsed with PBS buffer. The colour reaction was then performed, and the slices were rinsed with distilled water after the reaction was terminated. The slices were re-dyed with hematoxylin, differentiated and returned blue, washed with distilled water, then dewaxed with xylene and treated with gradient alcohol debenzolization, before the slices were finally sealed and observed. All enrolled patients will be followed up with for up to 5 years, and this will be done mainly through telephone and out-of-patient review.

Result determination

NTR1-positive staining was mainly in the cytoplasm, with diffuse or scattered brown-yellow staining. The staining intensity and the percentage of tumour cells were two important scores in determining the results. Under the light microscope, if the slice showed colourlessness, it is regarded as 0 points; if it is light yellow, it is regarded as 1 point; if it is a brownish yellow, it is regarded as 2 points; if it is sepia, it is regarded as 3 points.

The percentage of tumour cells below 10% was regarded as 0 points, 10% to 50% was regarded as 1 point, 50% to 70% was regarded as 2 points, and above 75% was regarded as 3 points. The scores of these two standard colours are multiplied, and if the score is greater than 3 points, it is considered as a positive expression, and if the score is equal to or less than 3 points, it is considered to be a negative expression.

Statistical method

The relationship between the expression of NTR1 and clinical parameters in patients with breast cancer was tested by χ^2. The relationship between the expression of NTR1 and the clinicopathological parameters and prognosis of breast cancer patients was determined by the Spenman correlation coefficient.

The survival curve of patients with breast cancer was drawn by the Kaplan-Meier method, and the data of patients with positive expression of NTR1 and patients with negative expression of NTR1 were compared by a log-rank method.

The COX proportional hazards regression model was established to analyse the independent factors affecting breast cancer. SPSS23.0 software was used to process all the data, and $P<0.05$ indicated that the difference was statistically significant.

Results

The expression of NTR1 in breast cancer and paracancer tissue

The positive expression rate of NTR1 in the breast cancer tissues was 70.45% (93/132), and the positive expression rate in the normal adjacent tissues was 0% (0/30), with the difference between the two groups being statistically significant ($P<0.05$). The results are shown in Figure 1.

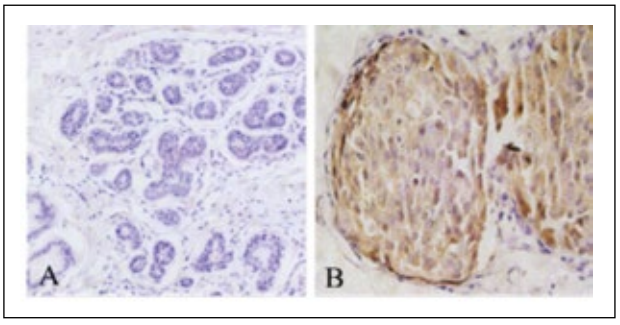

Figure 1: The expression of NTR1 in breast cancer and paracancer tissue.
A: The negative expression of NTR1 in the paracancer tissues. B: The positive expression of NTR1 in breast cancer.

Relationship between NTR1 expression and clinicopathological parameters in patients with breast cancer

The expression level of NTR1 was correlated with tumour diameter, lymph node metastasis, and tumour grades in patients with breast cancer, but was not correlated with the TNM stage, menopausal status, estrogen receptor, progesterone receptor, and human epidermal growth factor receptor 2 ($P>0.05$). The results are shown in Table 1.

Clinical parameter	n	NTR1 expression		χ^2	*P*
		Negative expression	Positive expression		
Age (years old)				0.621	0.748
<40	17	6	11		
≥40	115	33	82		
Tumour diameter(cm)				3.936	0.047
≤2	64	24	40		
>2	68	15	53		
Tumour grade				4.178	0.039
I~II	101	34	67		
III	31	5	26		
TNM stage				1.071	0.599
I	25	7	18		
III	68	23	45		
III	39	19	20		
Lymphatic metastasis				5.621	0.021
Without	71	27	44		
With	61	12	49		
Estrogen receptor				3.095	0.091
Negative	73	26	47		
Positive	59	13	46		
Progesterone receptor				2.494	0.841
Negative	72	22	50		
Positive	60	17	43		
Human epidermal growth factor receptor 2				3.852	0.339
Negative	96	31	65		
Positive	36	8	28		
Menopausal state				0.181	0.691
post-menopause	61	17	44		
Pre-menopause	71	22	49		
Family history				1.808	0.191
Yes	29	6	23		
No	103	33	70		

Table 1: Relationship between NTR1 expression and clinicopathological parameters in patients with breast cancer.

Relationship between NTR1 and prognosis of patients with breast cancer

The 5-year survival rate of patients with positive expression of NTR1 was 68.82% (64/93), and that of patients with a negative expression of NTR1 was 89.74% (35/39). As such, the difference was statistically significant (P<0.05). The results were shown in figure 2.

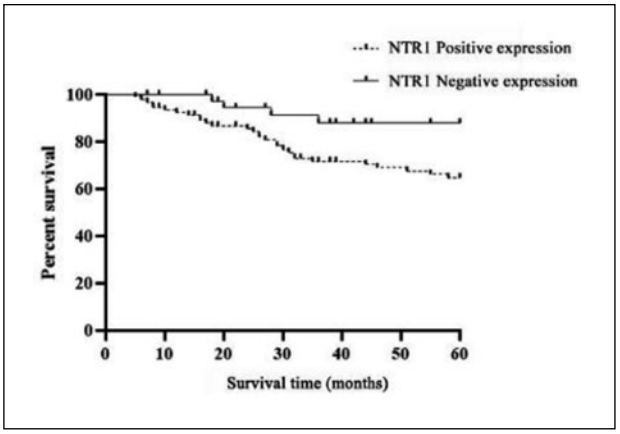

Figure 2: Relationship between NTR1 and prognosis of patients with breast cancer

Multivariate analysis of COX in patients with breast cancer

Through COX multivariate analysis, it was found that the expression of NTR1 and lymph node metastasis can independently affect the prognosis of breast cancer patients. The results are shown in Table 2.

Parameter	Hazard value	95%CI	*P*
NTR1 expression	3.065	1.083~8.711	0.039
Tumour grade	1.174	0.619~2.237	0.659
Lymphatic metastasis	2.633	1.302~5.33	0.009
TNM stage	0.815	0.507~1.313	0.385
Family history	1.273	0.608~2.681	0.553
Menopausal state	1.151	0.493~2.711	0.776
Age	1.205	0.620~2.314	0.608

Table 2: Multivariate analysis of COX in patients with breast cancer.

Discussion

Breast cancer has seriously endangered the lives and health of female patients on the premise of various factors, such as changes in life and diet structure, increased pressure, and other factors. The prognosis for young women is worse, but specific reasons have yet to be fully clarified[10].

In many countries globally, breast cancer classification and genetic classification have been used for clinical guidance and treatment. In the case of China, the prognosis of breast cancer is mainly evaluated by clinical and pathological prognostic factors. With the deepening of research on the molecular typing of breast cancer, NTR1 has become the focus of clinical workers[11]. Therefore, the purpose of this study was to explore the correlation between NTR1, pathological parameters and the prognosis of breast cancer,

allowing a provision of reference for the treatment of breast cancer in the future. NTR1 has 424 amino acids, and the human NTR1 is similar, to a certain extent, to the NTR1 found in mice. Indeed, NTR1 can mediate many biological effects of neurotensin[12]. In the study of breast cancer, some scholars have found that NTR1 can bind to the neurotensin and then promote the activation of B-cell lymphoma factor 2 (Bcl-2), before finally playing a role in promoting the growth of cancer cells[13]. It has been shown that the activation of neurotensin and its receptor NTR1 can increase the expression of Interleukin-8 (IL-8), and then activate the PI3K/Akt signalling pathway, before eventually inducing the proliferation of tumour cells[14, 15]. Clinical studies have shown that NTR1 is not only closely related to tumours but is also involved in the onset and progression of certain neurological diseases, such as strokes. The above studies have also indicated that NTR1 is closely related to the progression of breast cancer. However, the specific mechanics behind this are unclear.

The collected specimens were stained and it was found that the positive staining of NTR1 was mainly in the cytoplasm. The positive expression rate of NTR1 in the breast cancer tissues was 70.45% (93/132), the positive expression rate in the normal adjacent tissues was 0% (0/30), and the difference between the two groups was statistically significant ($P<0.05$). The results suggested that NTR1 was involved in the progress of breast cancer, and the effect was significant. The expression level of NTR1 was correlated with tumour diameter, lymph node metastasis, and tumour grade in patients with breast cancer. However, this was not with the TNM stage, menopausal status, estrogen receptor, progesterone receptor, and human epidermal growth factor receptor 2 ($P>0.05$). The 5-year survival rate of patients with a positive expression of NTR1 was 68.82% (64/93), and that of patients with a negative expression of NTR1 was 89.74% (35/39), with the difference being statistically significant ($P<0.05$). The expression of NTR1 and lymph node metastasis can independently affect the prognosis of breast cancer patients. The results showed that positive expression of NTR1 could predict the poor prognosis of patients with breast cancer and increase the risk of death amongst breast cancer patients. These results further suggest that NTR1 expression level can be detected and used to evaluate the malignant degree of breast cancer in patients to a certain extent, a prospect which is of great significance to the targeted therapy of breast cancer.

In conclusion, the expression of NTR1 in breast cancer tissue was significantly increased, indicating that it played an important role in the progress of breast cancer. At the same time, the positive expression of NTR1 was closely related to tumour diameter, lymph node metastasis, and tumour grade in patients with breast cancer, and its expression can independently affect the prognosis of these patients. This is an important index for clinical evaluation and treatment of breast cancer in the future. However, this study does not further elucidate the role of NTR1 in the pathogenesis and development of breast cancer. In future studies, the function and molecular mechanism of NTR1 will be studied and will provide a marker reference for evaluating the prognosis of breast cancer patients.

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