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Original article

Relationship between expression of CXCR7 and NF-κB in breast cancer tissue and occurrence of breast cancer and lymphatic metastasis

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ABSTRACT

It is designed to discuss the relationship between the expression of chemokine receptor 7 (chemokine receptor 7, CXCR7) and nuclear transcription factor-κB (nuclear factor kappa B, NF-κB) and the occurrence of the breast cancer and lymphatic metastasis. Method: 80 samples were excised and confirmed as breast cancer through our hospital pathology from January 2014 to December 2016 and tumor tissues and normal mammary tissues 2 cm from the tumor edges were taken as an experimental group and a control group, respectively. The method of immunohistochemical is utilized to test the expression of CXCR7 and NF-κB in the breast cancer tissue, compared with the para-carcinoma tissue, and analyze its relevance with the clinicopathologic features of the breast cancer tissue, such as tumor size, TNM staging, lymphatic metastasis and other conditions. Results: both CXCR7 and NF-κB were highly expressed in the breast cancer tissue, the positive rate was significantly higher that that of paracancerous normal tissues, and the difference was statistically significant. And the expressions of CXCR7 and NF-κB were related to TNM staging of the breast cancer and lymphatic metastasis and unrelated to the tumor size, age, and expressions of ER, PR and HER2. Conclusion: both CXCR7 and NF-κB are related to the malignant grade of the breast cancer and lymphatic metastasis, which may be regarded as in important indicator to judge the prognosis of the breast cancer and be expected to be the new target of curing part of the breast cancer.

Breast cancer is one of the commonest malignant tumors, of which morbidity is increasing year by year, and it has seriously endangered the life health of numerous women. The study on the tumor invasion and metastasis mechanism has always been hot currently, and blocking the growth and metastasis of tumor cells is the key to control the occurrence and development of the tumor. The research indicates that nuclear transcription factor-κB (nuclear factor kappa B, NF-κB) participates in regulating cell proliferation, apoptosis and malignant transformation, while chemokine and its receptor play an very important role in the occurrence, development and transformation of the tumor; chemokine receptor 7 (chemokine receptor 7, CXCR7) (Tang et al., 2016) is receptor of chemokine 12 (CXCL12), and it also participates in regulating the tumor invasion and metastasis. The current research analyzed the relationship between the expression of CXCR7 and NF-κB in breast cancer tissue through detection and the occurrence of breast cancer and lymphatic metastasis.

1. Materials and methods

1.1. General information

80 samples excised and confirmed as breast cancer through our hospital pathology from January 2014 to December 2016 were taken, and tumor tissues and normal mammary tissues 2 cm from the tumor edges were taken as an experimental group and a control group, respectively. The samples excised were placed into liquid nitrogen for storage immediately. Meet the following inclusion criteria: female, aged from 18 to 80, neither chemotherapy or endocrine therapy was done, neither in pregnancy or lactation; all patients underwent the surgical resection in our hospital with histopathological results. The cases with the following conditions were excluded: lack of complete histopathological data; patients with other systematic dysfunction. The average age of the patients

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included is (50.22 ± 2.19) years old; according to *Pathology & Genetics Tumors of the Breast and Female Genital Organs* (Xiaowei and Jun, 2012) standards of WHO, there were 40 cases of invasive ductal carcinoma, 30 cases of invasive lobular carcinoma and 10 cases of other types. Clinical staging is conducted according to TNM: 23 cases of stage I– II, and 48 cases of stage III– IV, including 35 patients with lymphatic metastasis and 45 without.

1.2. Primary reagent

Mouse Anti-human CXCR7, NF-κB Polyclonal Antibody (American R&D Company); PV-6000 kit, ER, PR, HER2 ready-to-use antibody (Fuzhou Maixin Biotechnology Co., Ltd.).

1.3. Immunohistochemical detection on expression of CXCR7 and NF-κB

Pathological tissue paraffin-embedded sections were taken, dewaxing with xylene and ethyl alcohol and restoring with citric acid for 2 min 30 s; develop PBS films for 3 times; after developing PBS films, add the primary antibodies diluted and incubate in water bath at 37 °C for 20 min; rinse with PBS for 3 times; drop with DAB color-substrate solution and observe under the microscope; redye with hematoxylin, 0.1% hydrochloride differentiation and rinse with running water; transparent xylene, rubber sealing piece. The positive piece with satisfied dying in pre-experiment was adopted as the standard for positive control, and PBS substituted the primary antibodies as negative control.

1.4. Result judgment

Immunohistochemistry was adopted for all pathological specimens, completed by the doctor of pathology department in our hospital. Observe under light microscope at 400 times; 3 sections were selected for each sample, and 5 were selected for each section for visual records of the dying situation of tumor cells; the percentage of the positive cell occupying the total tumor cells observed within each field of vision was calculated, and the average value was regarded as the percentage of the positive cell. CXCR7 is mainly expressed in cell membrane, and the brown and brownish yellow expressions are positive; NF-κB is expressed in cell nucleus, and the sepia expression is positive. It is divided into 5 grades according to the percentage of positive cells: 0: no positive cell, 1: <25%, 2: 25–50%, 3: >50–75% and 4: >75%. According to the positive coloring of the cell, it is divided into 0: no color; 1: the cell is lightly colored and in light yellow; 2: the cell is colored and in brownish yellow; 3: the cell is significantly colored and in tawny. Finally, the product of two is calculated and divided into 4 grades: negative: 0; weakly positive (+): 1–3; medium positive (++): 4–6; strongly positive: 8–12.

1.5. Statistical method

SPSS 19.0 statistical software was adopted for all research data for statistical processing, X^2 inspection was adopted for enumeration data, Spearman hierarchical correlation analysis was adopted for intergroup correlation, and $P < .05$ indicated that the difference is statistically significant.

2. Results

2.1. Expression of CXCR7 and NF-κB in breast cancer tissue

In paracancerous normal mammary tissues, CXCR7 and NF-κB are less expressed; in breast cancer tissue, immumohistochemical staining of CXCR7 and NF-κB varies from completely unstained to widely deep stain, see Fig. 1. In breast cancer tissue, the positive rate of CXCR7 is 85.0% (68/80), and that of paracancerous normal tissues is 12.5% (10/80); through the comparison between the experimental group and the control group, the difference is

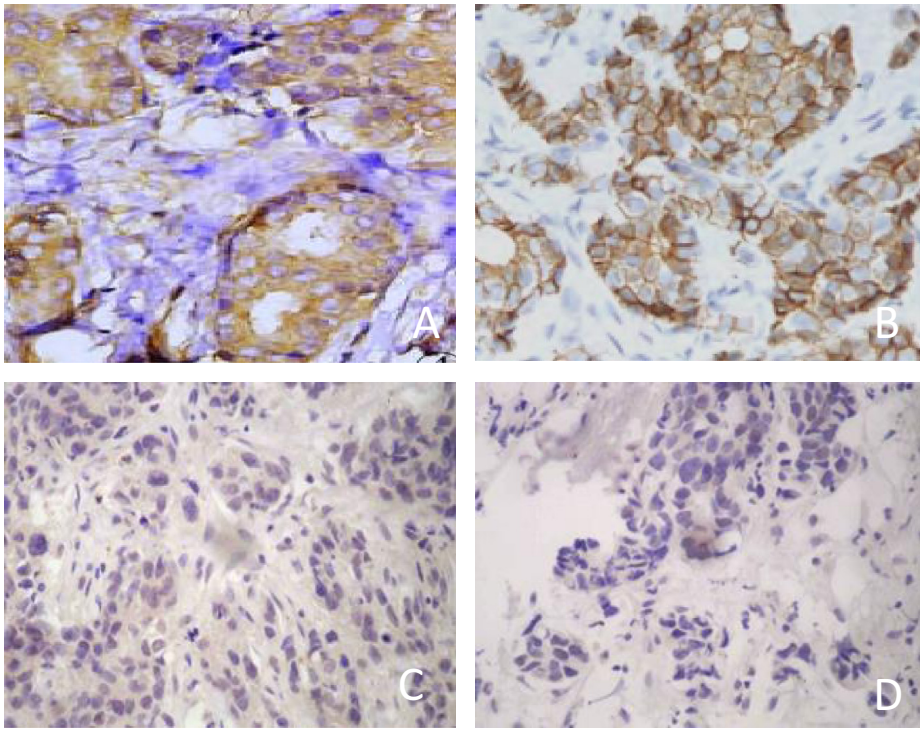

Fig. 1. Expression of CXCR7 and NF-κB in breast cancer tissues and paracancerous normal tissues. A: CXCR7 is positive in breast cancer tissue; B: NF-κB is positive in breast cancer tissue. C: CXCR7 is positive in paracancerous normal tissue; D: NF-κB is positive in paracancerous normal tissue (PV 6000 method *400).

Table 1
Expression of CXCR7 and NF-κB in breast cancer tissue.

Marker	Breast cancer tissue				Positive rate (%)	Normal tissue				Positive rate (%)
	Negative	Weakly positive	Positive	Strongly positive		Negative	Weakly positive	Positive	Strongly positive	
CXCR7	12	26	30	12	85.0*	70	10	0	0	12.5
NF-κB	8	20	38	14	90.0*	74	5	1	0	7.5

Compared with the control group,
* P < .05.

Table 2
Correlation between expression of CXCR7 and NF-κB and clinicopathologic characteristics of breast cancer.

Clinicopathologic characteristics		n	CXCR7				*U*	*P*	NF-κB				*U*	*P*
			–	+	++	+++			–	+	++	+++		
Age	≤50	40	8	13	14	5	−0.142	>0.05	4	9	19	8	−0.117	>0.05
	>50	40	4	13	16	7			4	11	19	6		
Tumor size	≤3 cm	43	7	14	15	7	−0.372	>0.05	5	12	18	8	−0.166	>0.05
	>3 cm	37	5	12	15	5			3	8	20	6		
ER	Negative	41	6	11	16	8	−0.271	>0.05	6	13	15	7	−0.141	>0.05
	Positive	39	6	15	14	4			2	7	23	7		
PR	Negative	35	5	13	12	5	−0.125	>0.05	3	9	19	4	−0.322	>0.05
	Positive	45	7	13	18	7			5	11	19	10		
HER2	Negative	41	6	12	17	6	−0.101	>0.05	4	10	21	6	−0.201	>0.05
	Positive	39	6	14	13	6			4	10	17	8		
TNM staging	I–II	32	8	10	10	4	−2.653	<0.05	5	12	10	5	−2.821	<0.05
	III–IV	48	4	16	20	8			3	8	28	9		
Lymphatic metastasis	Y	35	3	4	18	10	−3.11	<0.05	1	2	22	10	−3.01	<0.05
	None	45	9	22	12	2			7	18	16	4		

statistically significant ($P < .05$). The positive expression rate of NF-κb in breast cancer tissue is 90.0% (72/80), and the positive rate of paracancerous normal tissues is 7.5% (6/80); through the comparison between the experimental group and the control group, the difference is statistically significant ($P < .05$) (see Table 1).

2.2. Relationship between expression of CXCR7 and NF-κB and clinicopathologic characteristics of breast cancer

The expression level of CXCR7 and NF-κB has no obvious correlation with the age of breast cancer patients, tumor size and expression of ER, PR and HER2, but it is positively correlated with TNM staging of tumor and lymphatic metastasis ($P < .05$). Through Spearman correlation analysis, it is found that the expression of CXCR7 and NF-κB is positively related, $r = 0.618$, $P < .05$. See Table 2.

3. Discussion

Breast cancer is one of the commonest malignant tumor for the women, and multiple signal path and cell factor are closely related to the occurrence and development of breast cancer. Through the intensive study on biological characteristics of the molecule for breast cancer, searching for therapeutic target has profound significance for improving the effect of breast cancer treatment and prolonging the life. In clinical practice, the tumor invasion and metastasis are usually major influence factors of breast cancer treatment failure. Clarifying the invasion and metastasis mechanism of breast cancer has significant importance on treating breast cancer. Therefore, starting with lymphatic metastasis of breast cancer, the correlation between chemokine receptor 7 (chemokine receptor 7, CXCR7) and nuclear transcription factor-κB (nuclear factor kappa B, NF-κB) and occurrence of breast cancer and lymphatic metastasis was studied in the current research.

Chemokine belongs to secretory protein family, produces chemotaxis on lymphocyte, monocyte, neutrophilic granulocyte and various cells through combination with the corresponding receptor, making it move towards inflammatory position and playing the specific biological effects (Behnam Azad et al., 2016; Wang and Li, 2015; Salazar et al., 2014). At the present, there has been abundant proof demonstrating that multiple chemokines and their receptors participate in the tumor growth, angiogenesis and distant metastasis and other processes. The gene of CXCR7 is located in human chromosome 2q37, expressed in tumor cell, endothelial cell, stellate cell and various cells (Wu et al., 2017; Inaguma et al., 2015). CXCR7 may facilitate angiogenesis, increase the adhesive attraction between tumor cells, and plays a certain role in tumor growth, invasion and distant metastasis. It is found in previous studies on other tumors that in non-small cell lung cancer or thyroid cancer, expression of CXCR7 is related to lymphatic metastasis of the patient and clinical staging. It is also found in the current research that in breast cancer tissue, CXCR7 is highly expressed, and the positive rate is significantly higher than paracancerous normal mammary tissues; through Spearman correlation analysis, it is found that expression of CXCR7 is positively related to lymphatic metastasis and TNM staging, and high expression of CXCR7 usually indicates poor prognosis.

NF-κB is a kind of extensive nuclear transcriptional regulatory element, which exists nearly in all cells, and it is able to combine with nucleic acid sequence in many gene starting subrange and initiate the gene transcription (Zhao et al., 2017). NF-κB also plays a key role in tumor occurrence and development; when NF-κB is abnormally activated, located in cell nucleus and cannot return to cytoplasm, it can change the normal cell signaling to reduce apoptosis and prolong the survival time to make the tumor cells proliferate indefinitely (Darvishi et al., 2017). It is found in the current research that NF-κB is expressed in both breast cancer tissue and normal tissue, but the expression rate of NF-κB protein in breast cancer tissue is increased significantly compared with the normal tissue, and there is significant difference. It has been observed by the research that NF-κB is expressed in high activity prior to malignant transformation of mammary gland cell (Mi et al., 2017); NF-κB promotes the malignant progression of the

breast cancer, which is the same as the results of the current research, and the positive rate of NF-κB in the breast cancer tissue with higher histological grade is higher. In addition, expression of NF-κB is also positively related to lymphatic metastasis. It is indicated through the previous studies that NF-κB is closely related to HER-2 and ER, they affect each other, and expression of HER-2 is also regulated by NF-κB. However, in some breast cancers, signal HER-2 may result in activation of NF-κB signal path (Kawiak, 2016). ER may restrain the connectivity of NF-κB DNA and the activity of NF-κB. But the correlation between NF-κB and HER-2 and ER is not showed in the current research, which may be resulted from less samples and complex biological behaviors of breast cancer.

In conclusion, both CXCR7 and NF-κB are related to the malignant grade of breast cancer and lymphatic metastasis, it can be regarded as an important indicator to judge the prognosis of the breast cancer, and it is expected to be a new target of curing part of breast cancer.

References

Behnam Azad, B., Lisok, A., Chatterjee, S., et al., 2016 Jun. Targeted imaging of the atypical chemokine receptor 3 (ACKR3/CXCR7) in human cancer xenografts. J. Nucl. Med. 57 (6), 981–988.

Darvishi, B., Farahmand, L., Eslami-S, Z., et al., 2017 Jun. NF-κB as the main node of resistance to receptor tyrosine kinase inhibitors in triple-negative breast cancer. Tumour Biol. 39 (6). 1010428317706919.

Inaguma, S., Riku, M., Ito, H., et al., 2015. GLI1 orchestrates CXCR4/CXCR7 signaling to enhance migration and metastasis of breast cancer cells. Oncotarget. 6 (32), 33648–33657.

Kawiak, A., Domachowska, A., 2016 Oct 11. Plumbagin suppresses the invasion of HER2-overexpressing breast cancer cells through inhibition of IKKα-mediated NF-κB activation. PLoS One 11 (10), e0164064.

Mi, X.K., Liu, Q.R., Zhu, L., et al., 2017 May. Mechanism of the high coagulation state of breast cancer tissue factor. Eur. Rev. Med. Pharmacol. Sci. 21 (9), 2167–2171.

Salazar, N., Muñoz, D., Kallifatidis, G., et al., 2014 Aug. The chemokine receptor CXCR7 interacts with EGFR to promote breast cancer cell proliferation. Mol. Cancer 28 (13), 198.

Tang, X., Li, X., Li, Z., et al., 2016 Oct. Downregulation of CXCR7 inhibits proliferative capacity and stem cell-like properties in breast cancer stem cells. Tumour Biol. 37 (10), 13425–13433.

Wang, R., Li, J.C., 2015. TRAIL suppresses human breast cancer cell migration via MADD/CXCR7. Asian Pac. J. Cancer Prev. 16 (7), 2751–2756.

Wu, W., Qian, L., Dai, J., et al., 2017. Expression of chemokine CXCL12 and its receptor (CXCR4 and CXCR7) in different molecular subtypes of human breast carcinoma and the clinical significance. Zhong Nan Da XueXueBao Yi Xue Ban 42 (2), 147–153.

Xiaowei, Qi, Jun, Jiang, 2012. Introduction to [J], the 4th edition of 2012. Chin. J. Breast Dis. (Electron. Ed.) 05, 586–591.

Zhao, W., Ma, N., Wang, S., et al., 2017 Jun 28. RERG suppresses cell proliferation, migration and angiogenesis through ERK/NF-κB signaling pathway in nasopharyngeal carcinoma. J. Exp. Clin. Cancer Res. 36 (1), 88.