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Bcl-xl Expression and its Relationships with Clinical Pathological Characteristics, Expression of Estrogen and Progestogen Receptors in Breast Cancer

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ABSTRACT

Objective: To explore an anti-apoptosis factor Bcl-xl expression and its relationships with clinical pathological characteristics, expression of estrogen and progestogen receptors in breast cancer.

Methods: S-P immunohistochemical technique was used to detect the anti-apoptosis factor Bcl-xl expression and its relationships with tumor clinical pathological characteristics as well as expression of estrogen and progestogen receptors in 32 invasive breast cancer tissues, 32 tissues adjacent to cancer, 28 normal and 28 hyperplastic breast tissues.

Results: Compared with the tissue adjacent to cancer, hyperplastic and normal breast tissues, Bcl-xl was over-expressed in breast cancer tissue ($P < 0.01$). Bcl-xl expression in breast cancer tissue increased with the elevation of clinical staging and histological grading ($P < 0.05$ or $P < 0.01$). Its positive expression was higher in the breast cancer tissue with lymphatic metastasis than that in the breast cancer tissue without lymphatic metastasis ($P < 0.01$). Additionally, Bcl-xl expression in breast cancer tissue was negatively associated with the expression of estrogen receptors (ER) and progestogen receptors (PR) ($P < 0.05$).

Conclusion: Bcl-xl expression in breast cancer tissue is associated with tumor staging, histological grading, presence or absence of lymphatic metastasis as well as ER and PR expression, which is of great importance in the occurrence and progression of breast cancer.

Key words: Anti-apoptosis factor Bcl-xl; Breast cancer; Estrogen receptor; Progestogen receptor; Lymphatic metastasis

Introduction

Breast cancer is a commonly-encountered malignant ductal epithelial tumor among women, with the highest incidence in gynecological oncology[1-2]. Its occurrence and progression not only includes activation of proto-oncogenes and inactivation of tumor suppressor genes, but also involves cell proliferation and apoptosis[3]. In recent years, people have paid attention to the relationship between apoptosis and breast cancer, especially the relationship between Bcl-2 protein family (including apoptosis inhibiting and promoting factors) and breast cancer. Bcl-xl, an anti-apoptosis member in Bcl-2 family, is an anti-apoptosis factor stronger than Bcl-2[4-6]. In this study, S-P immunohistochemical technique was used to detect Bcl-xl expression in 32 invasive breast cancer tissues, 32 tissues adjacent to cancer, 28 normal and 28 hyperplastic breast tissues, aiming at investigating Bcl-xl expression in breast cancer and its relationships with tumor clinical pathological characteristics as well as expression of estrogen and progestogen receptors so as to provide objective experimental evidences for the diagnosis, prognostic evaluation and comprehensive treatment of breast cancer.

Materials and Methods

Materials

The breast cancer tissue and tissue adjacent to cancer in 32 patients with breast cancer as well as 28 normal and 28 hyperplastic breast tissues were all from Shiyan Taihe Hospital in Hubei province. All patients were females at the age of 29 ～ 74, with the mean age of 59.3, in which 20 cases suffered from menopause and 12 from pre-menopause. According to TNM staging in the sixth edition of *AJCC Cancer Staging Manual* and tumor severity and invasive range, the patients at phases Ⅰ ～ Ⅱ and Ⅲ～Ⅳ were 17 and 15 cases, respectively, while those at phases Ⅰ ～ Ⅱ and Ⅲ were respectively 16 and 16 cases according to WHO histocytological grading criteria. Eighteen patients were with lymphatic metastasis and 14 without. All diagnoses were confirmed by surgical resection (28 cases) or pathological biopsy (4 cases).

Methods

All formalin-fixed and parrffin-embedded samples were continuously cut into slices with the depth of 4 μm. 10 ～ 20 pieces of slices were cut for each patient, in which about 10 pieces with abundant tissue were chosen by microscope. The applied immunohistochemical SP staining method was as follows: first, place the sections into xylene Ⅰ (20 min) and Ⅱ (15 min) for dewaxing, and then put them into 100%, 95%, 90%, 80% and 70% alcohol gradient for dehydration (2 min, respectively); second, add 0.01 mmol/L citrate buffer after incubating for 5 ～ 10 min with 3% H_2O_2 at room temperature, and then conduct microwave heating for 10 ～ 15 min at 92 ℃ ～ 98 ℃ ; third, incubating 10 min at room temperature after adding normal goat serum for sealing, dropwise add Bcl-xl (PR, ER) primary antibodies, and then incubate for 2 h at room temperature; fourth, incubating for 30 min at room temperature after dropwise adding biotin-marked secondary antibodies, and then incubate for 30 min at room temperature again after dropwise adding streptavidin working solution marked by horseradish peroxidase; at last, apply DAB color developing agent for coloration and hematoxylin for counterstaining. The known positive sections and phosphate buffer saline (PBS) instead of primary antibodies were regarded as positive and negative controls, respectively.

Judging criteria

According to presence or absence of coloration and intensity, immunohistochemical staining results were divided into 4 ranks: ① no expression of positive cells (－); ② weakly positive, namely positive cell count less than 10% (＋); ③ positive, namely positive cell count occupying 10% ～ 50% (＋ ＋); ④ strongly positive, namely positive cell count more than 50% (＋＋＋). All positive cases in the positive rates of Bcl-xl, ER and PR proposed in the latter part of this study referred to those with coloration (＋) and above.

Statistical data analysis

Statistical software SPSS 17.0 was applied to statistically analyze the data. The enumeration data was compared with χ^2 test. $P < 0.05$ was considered to be statistically significant.

Results

Comparison of Bcl-xl expression in each tissue

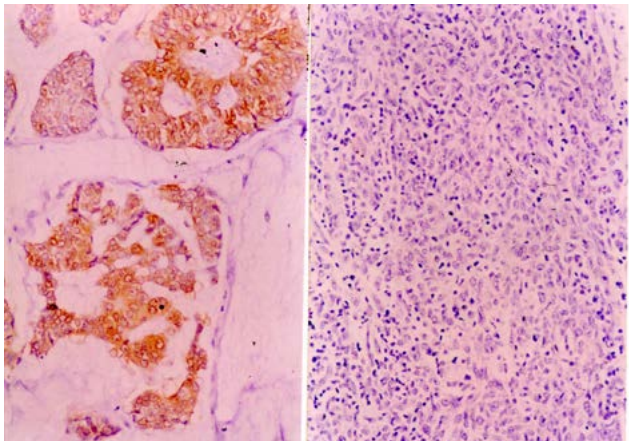
Twenty-one out of 32 breast cancer samples had positive Bcl-xl expression, with 65.6% of positive rate; 2 out of 32 tissues adjacent to cancer had positive expression, with 6.3% of positive rate; 2 out of 28 hyperplastic breast tissues had positive expression, with 7.1% of positive rate, but there was none positive Bcl-xl expression in 28 normal breast cancer tissues. The positive expression rate of Bcl-xl in breast cancer tissues was significantly higher than in the tissue adjacent to cancer, hyperplastic and normal breast tissues, and significant

differences were presented ($P < 0.01$), suggesting that Bcl-xl was over-expressed in breast cancer tissues. (Table 1, Figure 1 ~ 3).

Table 1 Comparison of Bcl-xl Expression in each Tissue[*n*(%)]

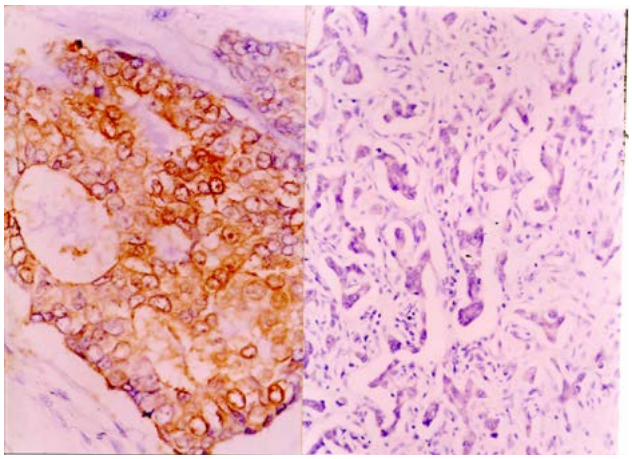
Tissue typing	n	Positive rate
Normal tissue	28	0(0.0)**
Hyperplastic breast tissue	28	2(7.1)**
Tissue adjacent to cancer	32	2(6.3)**
Breast cancer tissue	32	21(65.6)

Compared with breast cancer tissue, $^{**}P < 0.01$.



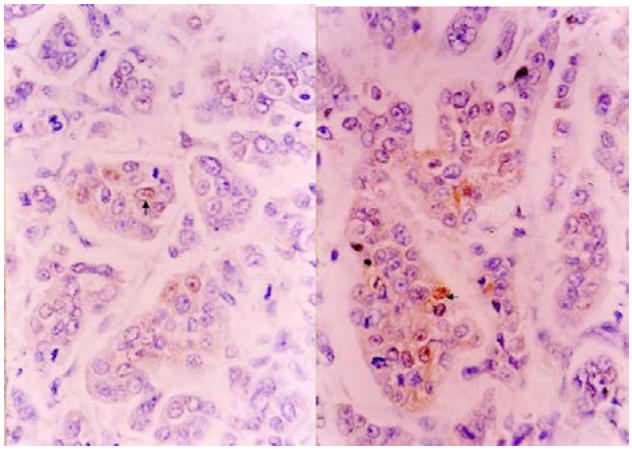
SP staining HE staining

Figure 1 Bcl-xl Staining in Breast Cancer Tissue (×100)



SP staining HE staining

Figure 2 Bcl-xl Staining in Breast Cancer Tissue (×400)



SP staining HE staining

Figure 3 Bcl-xl Staining in the Tissue Adjacent to Cancer (×400)

Table 2 Relationships between Bcl-xl Expression and Clinical Pathological Characteristics of Breast Cancer[*n*(%)]

Bcl-xl expression	Tumor staging		Histological grading		Lymphatic metastasis	
	I ~ II	III~IV	I ~ II	III	Yes	No
Positive (n = 21)	8(38.1)	13(61.9)*	7(33.3)	14(66.7)**	14(66.7)##	7(33.3)
Negative (n = 11)	3(27.3)	8(72.7)**	2(18.2)	9(81.8)**	8(72.7)##	3(27.3)

Compared with I ~ II tumor staging and histological grading, $^{*}P < 0.05$, $^{**}P < 0.01$. Compared with the tissue without lymphatic metastasis, $^{\#\#}P < 0.01$.

Relationships between Bcl-xl expression and clinical pathological characteristics of breast cancer

The positive expression rate of Bcl-xl in phase Ⅲ～Ⅳ breast cancer tissue was markedly higher than in phase Ⅰ ～ Ⅱ breast cancer ($P < 0.05$), suggesting that the later TNM staging was, the higher Bcl-xl expression was. The positive expression rate of Bcl-xl in the breast cancer tissue with histological grading being Ⅲ was notably higher than with histological grading being Ⅰ ～ Ⅱ ($P < 0.01$), showing that the higher the histological grading was, the higher the positive Bcl-xl expression rate was. The positive expression rate of Bcl-xl in the breast cancer tissue with lymphatic metastasis was conspicuously higher than that in the breast cancer tissue without lymphatic metastasis ($P < 0.01$). (Table 2).

Relationship between Bcl-xl expression and ER, PR expression

Bcl-xl expression in breast cancer tissues with negative ER and PR were notably higher than those with positive ER and PR ($P < 0.05$), indicating that Bcl-xl expression in breast cancer tissue is negatively associated with ER and PR. (Table 3)

Table 3 Relationship Between Bcl-xl Expression and ER, PR Expression

Groups	Bcl-xl expression		
	(+)～(+++)	(−)	Positive rate (%)
ER(+)	9	10	47.37
ER(−)	9	3	75.00*
PR(+)	9	9	50.00
PR(−)	12	4	75.00*

Compared with positive ER and PR, *$P < 0.05$.

Discussion

Breast cancer with the highest incidence among women threatens the female health seriously, even the life. Although the surgery, chemotherapy and radiotherapy have achieved a dramatic progress, most patients still suffer from recurrence and metastasis at last, leading to treatment failure. One of tumorigenesis mechanisms is imbalanced cell proliferation and apoptosis related to the metastasis and drug resistance of tumor cells[7], in which anti-apoptosis genes play an important role in breaking this balance. Hence, anti-apoptosis genes become a potential target in the treatment of tumors[8]. In human breast cancer, Bcl-xl activation is mainly abnormal amplification of genes and accompanied by over-expression of Bcl-xl proteins[9]. The study demonstrated that high expression of Bcl-xl genes was not only related to malignant severity of breast cancer, but also endowed tumor resistance to chemotherapy, radiotherapy and hormonotherapy[10].

In 1993, with mouse Bcl-2cDNA as a probe, Boise screened a clone named Bcl-xl in cDNA library of chicken lymphocytes[11]. Similar to Bcl-2 in function, Bcl-xl can inhibit cell apoptosis. Feng *et al* applied SP method to detect 70 breast cancer tissues, and the results demonstrated that Bcl-xl expression in the breast cancer group respectively with negative ER (76.92%) and PR (77.42%) was significantly higher than that with positive ER (52.27%) and PR (48.72%), and that Bcl-xl expression in the patients with lymphatic metastasis (64.58%) was obviously higher than those without lymphatic metastasis (36.36%)[12]. All of these findings suggest that for endocrinotherapy, the patients with breast cancer who had Bcl-xl over-expression have a poor reaction, strong invasion and poor prognosis. By researching on 151 cases with phase Ⅲ breast cancer, Lee *et al* found that Bcl-2 could be an independent prognostic factor and provided valuable information for the prognosis of breast cancer[13]. Among 32 breast cancer samples in this study, the positive Bcl-xl expression rate was 65.6% (21/32). Compared with the tissue adjacent to cancer, normal and hyperplastic breast tissues, Bcl-xl showed over-expressed in breast cancer tissue. Further analysis results revealed that Bcl-xl expression had a significant correlation with tumor clinical staging and histological grading, and was elevated with the increase of clinical staging and histological grading. The positive expression rate of Bcl-xl in the breast cancer tissue with lymphatic metastasis was conspicuously higher than that without lymphatic metastasis, indicating that Bcl-xl expression was related to presence or absence of lymphatic metastasis. Additionally, Bcl-xl expression in the breast cancer tissue with negative ER and PR was notably higher than those with positive ER and PR, suggesting that Bcl-xl expression was negatively associated with ER and PR expression. Therefore, Bcl-xl is intimately associated with the clinical staging, histological grading, lymphatic metastasis, ER and PR expression of breast cancer, and plays a crucial role in inhibiting the apoptosis of breast cancer.

At present, the effective therapeutic measures for early breast cancer are radical operation of resecting cancer tissue, while radiotherapy and chemotherapy are frequently used as postoperative palliative treatment regimens for

preventing recurrence and middle-advanced breast cancer, and endocrinotherapy is suitable for hormone-dependant patients with breast cancer. However, a large amount of research work that how many treatment methods can exert effects in inducing mammary cell apoptosis needs to be conducted. Hence, by combing Bcl-xl with ER, PR, P53 and CerbB-2 monitoring, we possibly observe the apoptosis and conduct the study on tumor biological characteristics at the molecular level, which provides new ideas for clinical individualized treatment and development of new drugs by explaining tumor reactions to different treatments.

Declaration

The authors declare that they have no conflict of interest.

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