THE EXPRESSION AND CLINICAL VALUE OF APOPTOSIS CONTROL GENE Bcl-2 AND Bax IN BREAST CANCER

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ABSTRACT

Objective: To study the expression and clinical value of apoptosis control gene bcl-2 and bax in breast cancer. Methods: Protein bax and bcl-2 in 41 breast cancers obtained from operations in our hospital in 1996 were detected using ABC immunohistochemical stain assay and compared with 10 cases with normal breast tissues. Results: The positive rate of bax in normal breast tissue was 90% and in breast cancer was 59%, with a significant statistical difference between them ($P<0.05$), but there was no statistical difference in bcl-2 protein expression. Among the 41 breast cancer, the group with lymph node metastasis (21 cases) had obviously low bax expression (43%) and high bcl-2 expression (76%), showing significant difference to the group without lymph node metastasis ($P<0.05$). Conclusion: The antiapoptosis function of bcl-2 was stronger than bax in breast cancer. Protein bax and bcl-2 assay may be useful in understanding the biological behaviors of breast cancer.

Key words: Breast cancer, Apoptosis control protein, Bax, Bcl-2, Immunohistochemistry.

Bcl-2 (the B-cell leukemia/lymphoma2) gene family are a group of recently found tumor genes which can suppress apoptosis of cells and have significant function in tumor's origin and development.[1] However, another member of bcl-2 gene family bax was found which can regulate the function of bcl-2 and accelerate the death of cells.[2] Joensun[3] found that bcl-2 expression was connected with some advantaged prognosis factors related to clinical pathological characteristics, but bcl-2 was not an independent prognosis factor by multiple factor analysis. Sierra[4] thought that bcl-2 took part in the development of the breast cancer whose diameter was less than 2 cm with well differentiation and PR(+),because bcl-2 family has important effect on the growth, differentiation and development of tumor cells. We analysed bcl-2 and bax proteins in breast cancers obtained from operation in our hospital to explore the expression of this gene family and its clinical value.

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MATERIALS AND METHODS

Materials

Pathological paraffin sections obtained from 41 cases of breast cancer were used for analysis. All cases were certified as invasion carcinoma pathologically. Among them, there were 13 cases in grade I, 22 in II and 6 in III according to TNM grade. Patients age ranged from 32–74 with the average 51.7 years old.

Reagent

Rabbit anti-human polyclonal antibody against bax (from Santa Cruz Co.) was used at a working concentration of 1:40; mouse anti-human monoclonal antibody against bcl-2 (from Santa Cruz Co.) was used with a dilution of 1:20; and ABC Kit (sheep anti-rabbit and rabbit anti-mouse IgG) was bought from Huamei Biological Engineering Co.

Immunohistochemical Assessment

The histological sections were routinely deparaffinized, reacted with 3% H_2O_2 to be got rid of internal peroxide, heated with sodium citrate solution for 10 minutes in a microwave to repair antigen, blocked with horse serum for 20 minutes, and incubated with anti-bax or anti-bcl-2 antibody at 4℃overnight. After washed by PBS, these slides were incubated with sheep anti-rabbit or rabbit anti-mouse antibody at 37℃ for 30 min, then incubated with ABC compound for 30 min, and then DAB-substrate solution was added on the slide at room temperature till positive signs appeared, then re-dyed with hematoxylin and analyzed under light microscope. PBS was used as blank control to replace antibody. Muscle tissue, in which bax is highly expressed, was used as a positive control for bax and the given positive slide as positive control of bcl-2. If cells were colored as yellow-brown, it was regarded as being positive.

Statistical Assesment

Possible value comparing analysis supplied by SAS was used for statistical assay.

RESULTS

Bax and Bcl-2 Expression in Normal Breast Tissue and Breast Cancer

Bax positive rate in normal breast tissue group was 90% (9/10) and in cancer group was 59% (24/41), shown in Figure 1, 2. Bcl-2 positive rate in normal and cancer groups are 40% and 61% respectively, shown as Figure 3, 4. According to possible value comparing analysis, there was a statistically significant difference of bax protein expression with low positive rate in cancer group ($P<0.05$), shown in Table 1.

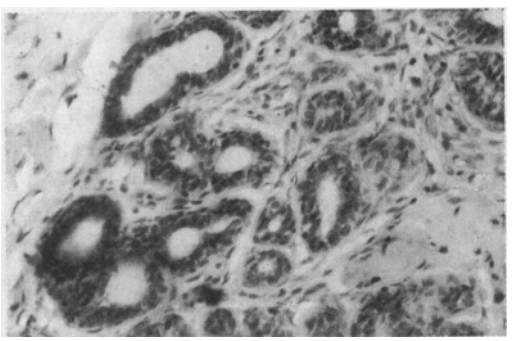

Fig. 1. Positive expression of bax protein in normal mammary epithilium, ABC×200.

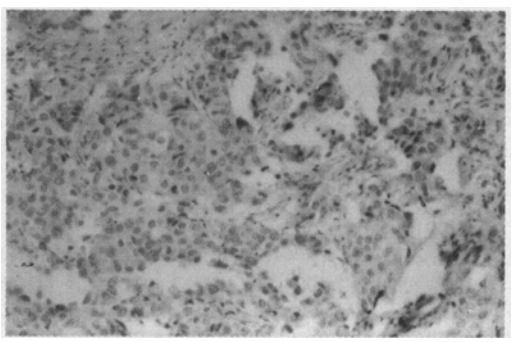

Fig. 2. Weakly positive expression of bax protein in breast cancer, ABC×200.

Table 1. Bax and bcl-2 expression in normal breast tissue and breast cancer groups

Group	Number	Positive ratio (%)	
		Bax	bcl-2
Normal breast tissue	10	9 (90%)	4 (40%)
Breast cancer	41	24 (59%)*	25 (61%)

Note: *Compared with normal group, $P<0.05$

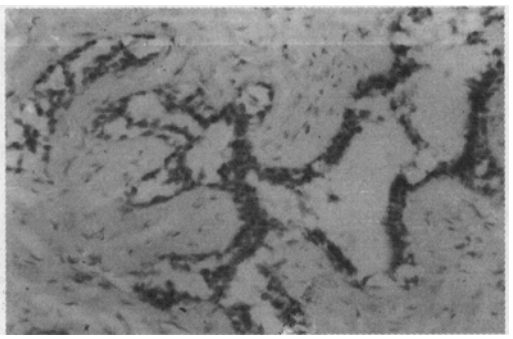

Fig. 3. Positive expression of bcl-2 protein in normal mammary wpithelium ABC× 200.

Table 2. Relationships between bax & bcl-2 expression and clinic-pathological characteristics

Pathological characteristics	Numbers	Positive rate (%)	
		Bax	Bcl-2
Age			
<60	31	23 (74%)	18 (58%)
≥60	10	4 (40%)	5 (50%)
Clinical stage			
I	13	9 (69%)	4 (31%)•
II	22	15 (68%)	16 (72%)
III	6	3 (50%)	4 (66%)
Histological stage			
I	16	12 (75%)	10 (63%)
II	13	7 (54%)	8 (62%)
III	12	7 (58%)	5 (42%)
ER			
+	23	15 (65%)	15 (65%)
-	18	11 (61%)	8 (44%)
PR			
+	14	9 (64%)	7 (50%)
-	27	16 (59%)	15 (56%)
Lymph node metastasis			
+	21	9 (43%)*	16 (76%)*
-	20	16 (80%)	9 (45%)

Note: • compared with stage II, $P<0.05$
*Compared with lymph node negative, $P<0.05$

Relationships between Bcl-2 Expression and Clinic-pathological Characteristics in Breast Cancer Group

There were no difference of bax and bcl-2 expression in regard to the clinical stage, histological stage, ER and PR of the tumor, but there was a decline trend in bax protein expression in those with the age ≥ 60 years old (P=0.052), and bcl-2 in stage I was lower than stage II. However, positive rate of bax protein expression in those with positive lymph node metastasis was significantly lower than those without lymph node metastasis

($P<0.05$); while bcl-2 protein expression in the former group was obviously higher than later ($P<0.05$), shown in Table 2.

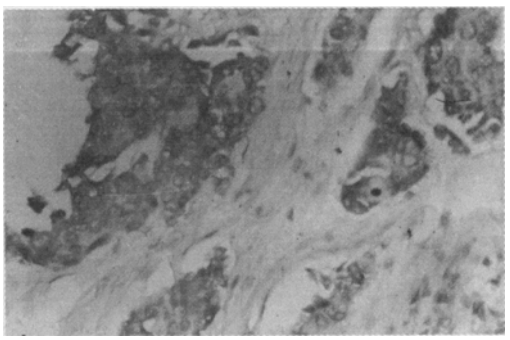

Fig. 4. Positive expression of bcl-2 protein in breast cancer ABC× 200.

DISCUSSION

Recent researches indicated that the functions of anti-tumor drugs and radiotherapy were based on inducing tumor cell apoptosis,[5] This process is regulated by multiple genes among which the critical ones are bcl-2 gene family.[6] Zoltan indicated that bax promotes cell apoptosis by forming homodimers with other bax proteins.[7] Our study showed that there was no difference of bcl-2 protein expression between normal mammary epithelium and cancer cells, but bax protein which promotes apoptosis was significantly declined in breast cancers. We also found that bax protein expression declined as the patients' age increased and in the 21 cases with lymph node metastasis, bax positive rate was 43% and bcl-2 was 76%. These inferred that the functions of anti-apoptosis genes in breast cancer were stronger than those of apoptosis promotion genes, so it is beneficial for tumor cell proliferation and metastasis. Therefore, it is suggested that therapy which can strengthen bax expression as well as suppress bcl-2 expression would promote tumor cell apoptosis, decrease metastasis and improve therapy effect.

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