Immunohistochemical Expression of Bcl3 in Human Breast Carcinoma

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Abstract
B-Cell lymphoma 3 is a putative proto-oncogene that involved in central oncogenic pathways that regulate cell death, apoptosis and metastatic of tumor cells so it could be important as a target to validation as a diagnostic or prognostic marker in these tumors. This study revealed positive expression of Bcl3 in (76.6%) of 47 cases infiltrating ductal carcinoma and (61.7%) of positive were strong positive. These results showed no significant association of immunohistochemical expression of Bcl3 with clinicopathological features as well no relation with immunohistochemical expressions of estrogen rreceptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER-2/*neu*).
Keyword: Breast cancer, Bcl3, Tissue microarray.

التعبير الكيميائي النسيجي المناعي لـ Bcl3 في سرطان الثدي بالانسان

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الخلاصة:
يعتبر Bcl3 ذو اهمية في المسارات الجينية الورمية المسؤولة عن الموت المبرمج للخلية وانتشار الخلايا الورمية وهذا يمكن اعتباره دليل لتشخيص او لتكهن الورم السرطاني للمرض ، اذا توصلت هذه الدراسة الى ان التعبير الكيميائي النسيجي المناعي الموجب للمعلم الورمي اعلاه Bcl3 في 76,6% من مجموع 47 عينة مصابة بمرض سرطان الثدي، اذ ان 61,7% من النماذج موجبة التعبير المناعي ذات تعبير مناعي قوي . كما اظهرت هذه الدراسة عدم وجود علاقة معنوية بين الصفات السريرية المرضية والتعبير الكيميائي النسيجي المناعي الموجب لـ Bcl3 وكذلك عدم وجود علاقة بين التعبير الكيميائي النسيجي المناعي الموجب لـ Bcl3 والتعبير الكيميائي النسيجي المناعي الموجب لمستقبلات الاستروجين والبروجستيرون و متلقي عامل النمو البشري النوع الثاني.

Introduction

Bcl3 was originally discovered through its involvement in the t(14;19)(q32.3;q13.2) chromosomal translocation found in a subset of patients with B-cell chronic lymphocytic leukaemia [1]. Subsequent analysis revealed it to be a member of the IκB family of proteins on the basis of its structure [1-3]. However, unlike classical IκB proteins which act to sequester NF-κB subunits to the cytoplasm, Bcl3 is predominantly a nuclear protein which is involved in regulating NF-κB-mediated gene transcription in a cell-type and stimulus-specific manner [4-6]. Because no previous study assessed this marker in

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breast cancer, this study aims to assess expression of Bcl3 in breast tumor and association of results with clinicopathological features and estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER-2/*neu*).

Materials and Methods

From March 2011 to Feburary 2012, fifty four paraffin blocks of breast tumors were randomly selected from archive files of Histopathology and Cytology Unit in Tikrit Teaching Hospital-Salah Al-Din-Iraq and Private lab in Baghdad- Iraq. Forty seven were malignant breast tumors and seven were benign. For each case, an initial hematoxylin and eosin-stained control section was reviewed to confirm an adequate tissue in donor block for transfer to the tissue microarray (TMA) block and to select and mark the location points for cores to be taken. Beecher TMA instrument (Beecher Instrument, Sun Prairie, WI 53590) was used to remove 2 cores of 0.6 mm from each donor block and transferred them to a recipient block. Cores were arranged in sectors, each containing 12 rows with 12 cores per row, the distance between each two cores 1mm and each two rows 1mm. TMA block was cut at a thickness of 5μm on a microtome cutter (Leica RM2135). Sections were placed on poly-L-lysine (PLL) coated slides (polysine, Thermo Fisher) and heated at 58°C for 24 hours after that the melting paraffin wax was added on the top of TMA section to prevent loss of cores. Slides were deparaffinized and rehydrated in graded alcohols, heat-induced epitope retrieval were done by immersing them in a 0.01-mol/L concentration of citrate buffer (pH 6.0) preheated to more than 90°C and left for 20 minutes, followed by 20-minutes cool down period at 25-28°C. Then slides were then incubated with Bcl3, ER, PR and HER-2 antibodies markers.

Scoring system of IHC

Bcl3 scoring system was suggested by (Cardiff School of Bioscience Laboratory, Cardiff University, UK), two parameters evaluated in this score; proportion of stained tumor cells and intensity of stain, cut off value is 30%. ER and PR were scored according to Allred *et al.* [7]. HER-2/*neu* was scored according to Dako score. Chi square and Fisher's Exact test were used and ANOVA. P value < 0.05 was considered as significant.

Results

Patient's age ranged from 29-85 years with a mean of 50.7±11.8 years. The peak age frequency was in the age category 40-49 years. All those cases were invasive ductal carcinoma. Of the tissue specimens, 3(6.4%) were equal or less than 2 cm in largest diameter and 44 (93.6%) were more than 2cm, positive lymph node metastasis were in 32 (68.1%). Grade I infiltrative ductal carcinoma formed 6 (12.8%) grade II 32(68.1%) and grade III 9(19.1%). 3(6.4%) were stage I, 20(42.5%) were stage II and 24 (51.1%) were stage III.

From 7 benign cases (71.4% and 42.9%) positive expression for ER and Bcl3 respectively and these cases were negative for PR and HER-2.

From 47 infiltrative ductal carcinoma 30 (63.8%), 11 (23.4%) and 12 (25.5%) were positive for ER, PR and HER-2 respectively. Thirty six out of 47 (76.7%) were positive for Bcl3. The majority of those 36 positive Bcl3 cases, 61.7% were strong positive, 6.4% were moderate and 8.5% were weak positive expressions for Bcl3.

Positive immunostaining with Bcl3 was observed in the cytoplasm of tumor cells as abrown diffusion pigmentation (Figure1). Association of Bcl3 with clinicopathological features was shown in Table 1- and association of Bcl3 with ER, PR and HER-2/*neu* table 2.

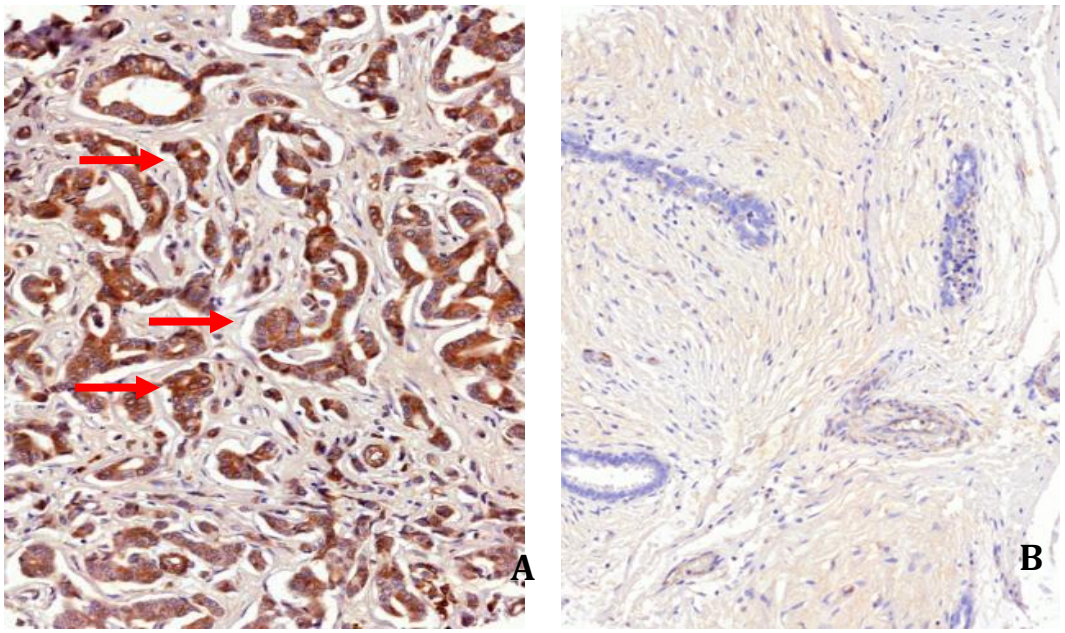

Figure 1-Immunohistochemical staining of Bcl3 in breast tumor.(A)Strong Bcl3 expression in a grade III invasive ductal carcinoma. (B) Negative Bcl3 expression in the epithelial cells of a fibroadenoma. Red arrows indicated for positive stained cells. Original magnification, X10.

Table 1- Association of Bcl3 expression with clinicopathological features

Parameters	Bcl3 positive	Bcl3 Negative	*P* value
Tumor largest diameter			
>2 cm	34(77.3%)	10 (22.7)	1.0
≤ 2cm	2(66.7%)	1(33.3%)	
Lymph node status			
Negative	11(73.3%)	4(26.7%)	1.0
Positive	25(78.1%)	7(21.9%)	
Histological grade			
I	5(83.3%)	1(16.7%)	0.5
II	24(75%)	8 (25%)	
III	7(77.8%)	2(22.2%)	
Pathological stage			
I	2(66.7%)	1(33.3%)	0.8
IIA	6(66.7%)	3(33.3%)	
IIB	10(90.9%)	1(9.1%)	
IIIA	12(70.6%)	5(29.4%)	
IIIB	6(85.7%)	1(14.3%)	

Table 2- Association of Bcl3 Expression with ER, PR and HER-2/*neu*

ER status	Bcl3 Negative	Bcl3 Positive	*P* value
ER -	4(23.5%)	13(76.5%)	0.1
ER+	7(23.3%)	23(76.7%)	
PR status			
PR-	8(22.2%)	28(77.8%)	0.4
PR+	3(27.3%)	8(72.7%)	
HER-2 status			
HER-2/*neu* -	8(22.9%)	27(77.1%)	0.7
HER-2/*neu* +	3(25%)	9(75%)	

Discussion

This study revealed no significant accociation between Bcl3 positive expression and clinicopathological parameters such as (patien's age, tumor largest diameter, nodal status, histological grade and pathological stage). No previous study assessed Bcl3 in breast tumors by immunohistochemistry technique, but by RT-PCR technique. The role of Bcl3 in breast cancer development has not been extensively investigated. There are, however, a small number of studies describing Bcl3 expression and function in human breast tumor, normal mammary epithelial cells and breast cancer cell lines. Cogswell *et al*. [8] was the first to analyse Bcl3 expression in primary human breast tumors and human breast cancer cell lines. Overexpression of Bcl3 is characteristic of a growing cancer [8]. Rocha *et al*. [9] demonstrated that Bcl3 can contribute to cyclin D1 transcription, a key component in driving cell cycle progression. However, analysis of samples from four breast cancer cases revealed that the protein levels of Bcl3, along with p50, p52 and c-Rel, were increased in all tumor samples in comparison with normal adjacent tissue. This was found to be regulated at the RNA level in two further samples, which also showed increases in *cyclin D1* expression. In support of these data, a more recent report found that 9 out of 12 human breast cancer tumors had higher Bcl3 protein levels than their corresponding adjacent tissue [10] . In contrast with the study showed that Bcl3 protein levels were elevated in breast cancer cell lines in comparison with a normal breast cell line [8]. Another study revealed that the deletion of the Bcl3 gene in ErbB2 positive mice resulted in a 75% reduction in metastatic tumor burden in the lungs with a 3.6-fold decrease in cell turnover index in these secondary lesions with no significant effect on primary mammary tumor growth, cyclinD1 levels or caspase 3 activity. Direct inhibition of Bcl3 by siRNA in a transplantation model of an Erbb2-positive mammary tumor cell line confirmed the effect of Bcl3 in malignancy suggesting that the effect of Bcl3 was intrinsic to the tumor cells. Bcl3 knockdown resulted in a 61% decrease in tumor cell motility and a concomitant increase in the cell migration inhibitors Nme1, Nme2, Nme3 and the metalloproteinase inhibitors Timp1 and Timp2. Independent knockdown of Nme1 and Nme2 partially rescued the Bcl3 motility phenotype. These results indicate for the first time a cell-autonomous disease-modifying role for Bcl3 in vivo, affecting metastatic disease progression rather than primary tumor growth [11]. Our results reported high expression of Bcl3 in invasive ductal carcinoma this may be give impression of Bcl3 role in motility of cancer cells as well this study showed no significant association of Bcl3 expression with ER, PR and HER-2. No correlation between positive expressions of hormone receptors and Bcl3 may be considering a bad prognosis. It is recommended to study larger number of breast cancer specimens in Iraq to validate the result of current study.

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