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ORIGINAL ARTICLE

Expression of cyclooxygenase-2 and Bcl-2 in breast cancer and their relationship with triple-negative disease

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Summary

Purpose: *Carcinogenesis is a multistep process with many factors being involved. The aim of this study was to investigate the role of cyclooxygenase-2 (COX-2) and Bcl-2 expression in patients with triple-negative breast cancer (TNBC) and, also whether any differences exist between TNBC and non-TNBC patients in relation with these two parameters.*

Methods: *This study included 50 patients with pathologically diagnosed TNBC and followed up at the Medical Oncology Clinic of Antalya Education and Research Hospital between 2008 and 2010. Thirty non-TNBC patients composed the control group. COX-2 and Bcl-2 expression was immunohistochemically investigated in both patient groups.*

Results: *COX-2 expression was positive in 26 (86.7%) of non-TNBC and 18 (90%) of TNBC patients (p=0.722). Compared with non-TNBC, TNBC correlated with higher Bcl-2 expression (p=0.005). Of the non-TBNC patients 86.7% and 50% of TNBC patients showed Bcl-2 expression. When Bcl-2 and COX-2 expression were considered together, a significant difference was found between the two groups (p=0.021).*

Conclusion: *This study showed that increased COX-2 expression correlated with Bcl-2 expression both in TNBC and non-TNBC patients. Analysis of coexpression of Bcl-2 and COX-2 may be meaningful for deciding treatment strategies for TNBC. Treatment strategies targeting Bcl-2 and COX-2 seem to be promising for this aggressive disease with no specific treatment.*

Key words: *Bcl-2, breast cancer, COX-2, triple negative*

Introduction

TNBC is characterized by the absence of expression of prognostic and predictive biomarkers such as oestrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor (HER2). TNBCs constitute 20% of all breast cancers. The molecular pathogenesis and response to therapy of TNBC is different than the other breast cancers. These patients are mostly premenopausal young women with a higher incidence of BRCA-1 mutation [1].

Prostaglandins, the potent inflammatory mediators, play a significant role in cell proliferation and apoptosis. COX is the limiting enzyme in the synthesis of prostaglandins from arachidonic acid. This enzyme exists in two isoforms, COX-1 and COX-2 [2]. The expression of COX-2 is lower in normal tissues, but it increases in neoplastic tissues and inflammatory conditions. The role of COX-2 expression was shown in different malignancies [3,4]. A study with a transgenic mouse model showed that COX-2 overexpression causes breast cancer [5]. COX-2 overexpression is also known as a reason of pancreatic cancer [6]. A study suggested that COX-2 overexpression causes suppression of PTEN and activation of P13/AKT pathway [7].

Bcl-2 gene was identified first in patients with B cell follicular lymphoma with t(14;18) translocation [8], however its expression is not related to this translocation [9]. Bcl-2 increases

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the lifespan of a cell by inhibiting apoptosis, but due to the longer lifespan the possibility the cell come across mutagenic factors increases. A relationship between Bcl-2 expression and survival was demonstrated in most human tumors [10]. The role of COX-2 expression in carcinogenesis is considered to be through apoptotic pathways [11].

Carcinogenesis is a multistep process involving many factors. Prognosis of cancer may be based on certain clinical and laboratory parameters obtained during diagnosis. Laboratory findings may be helpful in deciding the intensity of the treatment for each patient.

The aim of this study was to investigate the role of COX-2 and Bcl-2 expression in patients with TNBC and, if applicable, the difference of expression of COX-2 and Bcl-2 between TNBC and non-TNBC patients.

Methods

Patients

This retrospective study included female patients with histopathologically diagnosed TNBC who were followed up at the Medical Oncology Clinic of Antalya Education and Research Hospital between 2008 and 2010. Twenty patients had TNBC and 30 non-TNBC patients composed the control group. Patients were staged according to the 7th edition of the Staging System of the American Joint Committee on Cancer (AJCC). Data were retrieved from the patient medical records and their analysis included information on age, gender, disease stage and other characteristics, such as hormone receptor status and HER2 expression. Patients without histopathological diagnosis and patients whose initial treatments started at another centre and continued treatment at our centre were excluded from study.

The Ethics Committee approved the study protocol and all of the patients enrolled provided oral informed consent.

Immunohistochemistry

Tumor samples obtained immediately after the operation were fixed in 10% formaldehyde and embedded in paraffin. Then, 4 μm-thick histological sections were obtained from paraffin blocks and were initially stained with haematoxylin-eosin for initial assessment.

The histological sections were de-paraffinized and incubated at 60°C for one h. Afterwards, they were kept in xylene for 10 min and in 100% alcohol for 5 min and then washed with distilled water. Slides were kept in solution buffered with 10% citrate solution in microwave oven at maximum power (800 watts) for 15 min. Then, the power was decreased by half and slides were kept in the microwave oven for another 20 min. Slides taken out of the microwave oven were kept at room temperature for 20 min. Endogenous peroxidase activity was blocked by keeping them in 3% hydrogen peroxide for 20 min. Then, the slides, washed with distilled water, were treated with phosphate buffer saline (PBS) for 5 min x 3 times and with protein blocking agent (Novocastra Protein Block, Newcastle, UK). Five min later, COX-2 and Bcl-2 antibodies were dripped onto the slides without washing off the blockage. After being kept in primary antibody for 30 min, they were taken into PBS and washed for 5 min. Then, they were treated with biotinylated secondary antibody for 20 min, washed in PBS for 5 min and kept together with peroxidase conjugate antibody (Novocastra Peroxidase Block, Newcastle, UK) for 20 min. Then, they were washed in PBS for 5 min and kept in chromogen (DAB) for 5 min. Slides washed under tap water were adversely stained with haematoxylin. Then, they were dehydrated, dried and covered with mounting medium.

For the staining of the samples of COX-2 protein, COX-2 mouse monoclonal antibody (clone 4H12, 1:30, DBS, Pleasanton CA) and Bcl-2 lyophilized mouse monoclonal antibody (clone100/D5, 1:50, Thermo Scientific, Fremont, USA) were used. After staining, slides were inspected under Nikon Eclipse 80 (NIKON, USA) microscope.

Immunohistochemistry scoring

Slides were evaluated by two pathologists who were blinded of the patients' clinical characteristics. Each immunohistochemically stained section was examined under optical microscope. Bcl-2 expressions were assessed with respect to the cytoplasmic staining of the cells. Interval value for Bcl-2 and COX-2 staining was accepted as 10%. Bcl-2 or COX-2 staining ≥ 10% was considered as positive expression while staining ≤ 10% was accepted as negative expression (Figures 1,2).

Statistics

Statistical analyses were performed using the SPSS software version 15 (SPSS Inc, Chicago, IL). Differences between groups were assessed by using Chi-square and Mann- Whitney U test. A p value of <0.05 was considered statistically significant.

Results

A total of 50 patients with histopathologically diagnosed breast cancer were included in the study. The median patient age was 42 years (range 25-71). Twenty-six patients (52%) were ER-positive, 22 (44%) PR-positive and 10 (20%) HER2-positive (non-TNBC group). Twenty patients (20%) were ER-negative, PR-negative, and HER2-negative (TNBC group) and 30 patients (60%) without TNBC were identified as ER-positive or HER2-positive. Five patients had metastat-

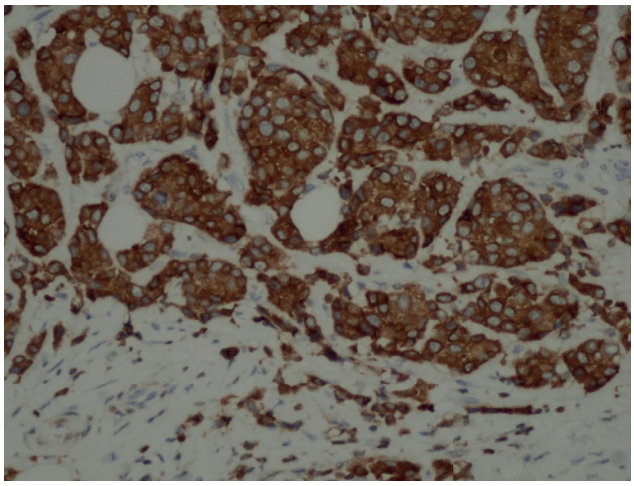

Figure 1. Bcl-2 expression in >10% of the tumor cells (Bcl-2 immunohistochemistry, x200).

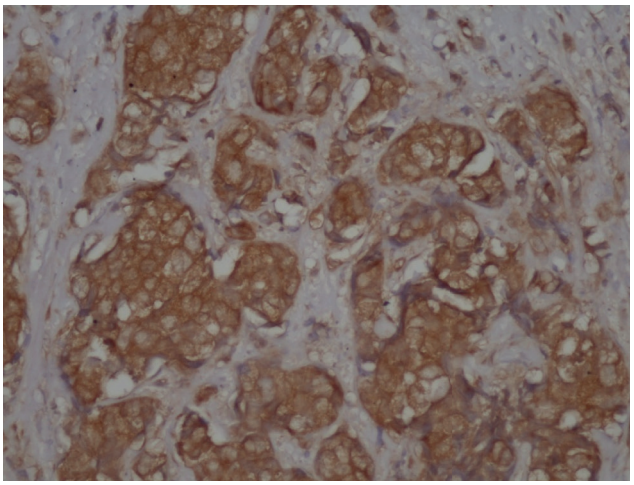

Figure 2. COX-2 expression in >10% of the tumor cells (COX-2 immunohistochemistry, x400).

ic disease (vertebra in 3 patients and liver in 2 patients). Only one of the metastatic patients had TNBC. Eight (17.8%) of the non-metastatic patients underwent breast-conserving surgery and 37 (82.2%) modified radical mastectomy.

No difference was found between non-TNBC and TNBC groups in terms of age (p=0.072). Compared with non-TNBC, TNBC was correlated with higher histological and nuclear grade (p=0.003).

COX-2 expression was positive in 44 (88%) patients and negative in 6 (12%) patients. COX-2 expression was positive in 26 (87.7%) non-TNBC patients and in 18 (90%) TNBC patients (p=0.722). Bcl-2 expression was positive in 36 (88%) patients. Compared with non-TNBC, TNBC was correlated with significantly higher Bcl-2 expression (p=0.005). Bcl-2 expression was positive in 26 (87.7%) of non-TNBC and in 10 (50%) of TNBC patients (Table 1).

When Bcl-2 and COX-2 expressions were considered together, a significant difference was found between the two groups (p=0.021). Coexpression of Bcl-2 and COX- 2 was shown in 24 (80%) of the non-TNBC group and 10 (50%) of TNBC group cases (p=0.018; Table 2).

Table 1. Patient characteristics according to TNBC or non-TNBC status

Characteristics	*TNBC N (%)*	*non-TNBC N (%)*	*p-value*
Age, years±SD	48.9±12.3	42.8±12.6	0.072
Grade			0.003
1	0	2 (6.7)	
2	4 (20)	21 (70)	
3	11 (55)	6 (20)	
Missing	5 (25)	1 (3.3)	
COX-2 expression			0.722
Negative	2 (10)	4 (13.3)	
Positive	18 (90)	26 (86.7)	
Bcl-2 expression			0.005
Negative	2 (10)	2 (10)	
Positive	18 (90)	18 (90)	

TNBC: triple negative breast cancer, SD: standard deviation

Table 2. Bcl-2/COX-2 expression in non-TNBC and TNBC groups

Bcl-2/COX-2	*Non-TNBC N (%)*	*TNBC N (%)*	*Total N (%)*
Bcl-2(+)/COX-2(-)	2 (6.7)	0	2 (4)
Bcl-2(-)/COX-2(-)	2 (6.7)	2 (10)	4 (8)
Bcl-2(+)/COX-2(+)	24 (80)	10 (50)	34 (68)
Bcl-2(-)/COX-2(+)	2 (6.7)	8 (40)	10 (20)
Total	30 (100)	20 (100)	50 (100)

Discussion

As is the case in the world, breast cancer is still a major cause of morbidity and mortality for women of our country. Breast cancer is a heterogeneous disease in terms of clinical and pathological characteristics and response to therapy. Conventional histological classification of breast cancer does not offer adequate information about the clinical outcome. Histological type, grade and size of the tumor, expression of ER, PR and HER2 receptors, lymph node and metastatic statuses are considered as the important classical prognostic factors [12].

The molecular subgroups of breast cancer revealed the heterogeneity of the disease with respect to prognosis and response to therapy. Among the subgroups of breast cancer, TNBC is associated with worse prognosis and early age. There is no specific systemic treatment for TNBC [13-15]. In our study, when Bcl-2 and COX-2 ex-

pression were considered together, it was found significantly higher in non-TNBC patients than in TNBC patients.

Bcl-2 expression has been searched in many types of cancer and in most of these studies it has been shown as a good prognostic factor.

There are studies pointing out that Bcl-2 expression has a prognostic significance whereas some other studies show the opposite [16,17]. Variable expression of Bcl-2 in normal ductal epithelium, intraductal carcinoma and invasive ductal carcinoma was observed in different studies.

In a study by Zhang et al. Bcl-2 expression was 96% in normal ductal epithelium, 79% in intraductal carcinoma and 45% in invasive carcinoma, showing decreasing expression of Bcl-2 during the development of carcinoma from the normal ductal epithelium [18].

Silvestrini et al. reported that Bcl-2 expression was associated with well differentiation and positive ER expression in breast cancer [19]. In a study by Yu et al. evaluating the differences between very young patients and elderly patients with breast cancer, a positive correlation between expression of Bcl-2, ER and PR was found [20]. The results were similar to those of our study. Rhee et al. demonstrated that Bcl-2 expression was significantly lower in the TNBC group and this group manifested a more aggressive behavior [21].

Tawfik et al. observed that Bcl-2 positivity was correlated with less aggressive tumor histology and better overall survival in the non-TNBC group. Interestingly, they found that, in TNBC group, Bcl-2 positivity was correlated with poor survival [22].

In breast cancer, COX-2 expression is associated with poor prognostic factors. As COX-2 expression increases, metastatic capacity increases too [23,24]. Kim et al. found positive COX-2 expression in 62.3% of the patients with TNBC and suggested that COX-2 expression was a factor of poor prognosis [25]. A similar expression rate was found in a study evaluating the response to neoadjuvant therapy [26]. In our study, we found a higher rate of COX-2 expression in TNBC patients. We believe that this may be associated with the low number of patients.

The reason of the increased of COX-2 expression in breast cancer cells is not exactly known. It was suggested that tumor suppressor genes such as p53 and oncogenes such as HER2 induced COX-2 expression in malignant cells [27,28]. In our study, COX-2 expression was found increased in all of the subgroups with Bcl-2 expression, as shown in Table 1. These results show that there is a coexpression of COX-2 and Bcl-2 in breast cancer.

In the present study we showed the increase of COX-2 expression was correlated with Bcl-2 expression both in TNBC and non-TNBC patients. There may be a pathway leading to this coexpression of Bcl-2 and COX-2. Analysis of coexpression of Bcl-2 and COX-2 may be significant in deciding the treatment strategies for TNBC. Treatment strategies targeting Bcl-2 and COX-2 seem promising for this aggressive disease with no specific treatment.

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