Bax protein, the proapoptotic member of Bcl-2 protein family, plays the key role in apoptosis pathway.

The aim of the study was to assess the expression of Bax protein in breast cancer cells.

Material and methods. Sixty-two breast cancer patients were included in the study. The control group encompassed 11 fibroadenoma patients. Single cells were isolated from defrosted samples and prepared for flow cytometry measurement.

Results. Median expression of Bax protein in study group was 7.9% (range: 0-49.4%) and was significantly lower than in control (median expression 15.8%; range 4.9-30.9%; p=0.034). Expression of Bax correlated with expression of p53 and caspase-3 proteins (p<0.01, rank Spearman test). In patients under 70 years old and with positive estrogen receptors status the expression of Bax protein was significantly higher (p=0.03 and p=0.01 respectively).

Conclusions. Lower expression of Bax protein in breast cancer cells may suggest the potential way of apoptosis avoidance of tumor cells. Correlations among Bax protein, p53 and caspase-3 are likely associated with active apoptotic mechanism in breast cancer cells expressing Bax protein. Further investigation with long time follow-up should be performed to establish the prognostic role of Bax protein expression in breast cancer patients.

Key words: breast cancer, apoptosis, Bax protein

Apoptosis is a physiological process leading to elimination of unwanted or damaged cells from the body (1). There are two molecular pathways of apoptosis activation – an external pathway using a receptor of tumor necrosis factor (TNF) and an internal pathway, called also a mitochondrial pathway. When apoptosis is activated through a mitochondrial pathway, significant DNA damage leads to increased synthesis of p53 protein and dependant Bax protein. Bax protein, as proteins Bcl-x, Bad, Bak, Bik/Nbk, Bid, Bag-1 from Bcl-2 family, has proapoptotic activity (2, 3). Other proteins from Bcl-2 family, such as Bcl-2, Bcl-x, Bcl-w, A1, Mcl-1, inhibit apoptosis. Bax goes into a mitochondrion, associates with its internal...
membrane which leads to loss of membrane potential and formation of pores; cytochrome c is released through these pores into the cytoplasm (4). Then cytochrome c along with procaspase-9 and Apaf protein form a complex called apotosome. Activation of procaspase-9 leads to activation of caspase-3, which inadvertently results in apoptosis of such cell (fig. 1).

Bax expression was evaluated in patients with three types of malignancies. Clinical studies of patients with colorectal cancer demonstrated that reduced Bax expression in tumor cells was associated with poorer response to chemotherapy and shorter patient survival time (5). On the other hand, in patients with esophageal cancer increased Bax expression was a factor of favorable prognosis (6). However results of studies conducted in breast cancer patients were equivocal. Study by Krajewski et al. found that patients with metastatic breast cancer and low Bax expression had poorer response to chemotherapy and shorter overall survival (7). No differences in treatment outcome related to Bax expression in the tumor cells were found in patients with early breast cancer (8).

In view of equivocal results of studies of prognostic significance of Bax expression in breast cancer patients, we decided to conduct a prospective study in our own material. We determined Bax expression using a cytometric method in breast cancer cells and compared it to Bax expression in breast fibroadenoma cells. To assess Bax effect on apoptosis in breast cancer cells, we compared its expression with expression of two key apoptosis proteins: p53 protein and caspase-3. Then we evaluated association between the protein expression and prognostic factors in breast cancer.

MATERIAL AND METHODS

Material

The study group consisted of 62 female breast cancer patients that underwent surgical treatment at Department of Surgical Oncology, Medical University of Łódź, in years 2007 – 2009. Median age in the study group was 59 years (range 41 – 88 years). The control group consisted of 11 patients that underwent surgical treatment for breast fibroadenoma (median age 30 years, range 24-53 years). Table 1 presents characteristics of the study group.

Methods

The study material was collected during a surgical procedure. Unpreserved tumor tissue was stored at -80°C. Single cells were isolated from thawed tissues for assessment in a flow cytometer according to methods presented by Ehemann et al. (9). Briefly it involves fragmentation of frozen tissue and its suspension in a 2.1% citric acid solution and Tween 20 and then preservation in 70% ethanol solution and PBS solution (phosphate buffered saline, Sigma Aldrich Chemie Gmbh, Steinheim, Germany). Then the cellular suspension was washed in PBS, centrifuged for 5 minutes at 1100 rpm and incubated in 0.01% saponin solution for 1 minute. Before addition of a primary antibody, the suspension was again washed in PBS solution and centrifuged. Incubation was performed at 4°C for approximately 8-12 hours. Then a secondary antibody was added. After 2-hour incubation, the cellular suspension was prepared for assessment in a flow cytometer (fig. 2).

RESULTS

Bax expression

Bax expression in the study group was found in 51 of 62 patients (82%). Bax expression was found in any patient from the control group (11/11; 100%). Median expression in the
study group was 7.9% (range 0-49.4%) and was significantly lower than in the control group (median Bax expression 15.8%; range 4.9-30.9; p = 0.034).

Bax expression and expression of p53 protein and caspase-3

Expression of p53 protein was present in 85% of patients from the study group (53/62 patients). Median expression was 5.8% (range 0–55.9%). In cases with increased Bax expression in the study group there was a correlation with higher p53 protein expression (p < 0.01. Spearman rank test).

Expression of caspase-3 was found in 98% of patients (61/62 patients), while its median expression was 8.1% (range 0 – 42.3). A correlation was found between Bax and caspase-3 expression (p < 0.01).

Analysis of correlation between Bax expression and clinical and pathological characteristics of the study group

In patients over 70 years of age, Bax expression was lower than in patients under 70 years of age (p = 0.03).
Increased Bax expression was observed in patients with positive estrogen receptor ($p = 0.01$). No correlation was found between presence of progesterone receptors and Her-2 ($p=0.59$, $p=0.69$, respectively; insignificant differences). However, a trend was found towards lower Bax expression in so called triple negative patients, i.e. patients without estrogen, progesterone receptors and Her-2 ($p = 0.06$).

Furthermore, no correlation was found between Bax expression and tumor size (T1 vs. T2, T3; $p=0.1$) or tumor grade (G1 vs G2, G3; $p=0.15$). No changes in Bax expression were found between patients with and without metastases in the axillary lymph nodes (N1,N2 vs. N0; $p=0.86$).

**DISCUSSION**

Our study showed that Bax expression was lower in breast cancer patients than in fibroadenoma patients. This could indicate that it is one of the mechanisms of apoptosis avoidance by tumor cells. This is supported by experimental studies by Shibat et al. (10). In a breast cancer model in transgenic mice that were completely or partially unable to synthesize Bax protein, they showed that apoptosis was inhibited and the disease progressed rapidly in cases of invasive cancer, which was not observed for precancerous lesions. Immunochemistry studies conducted in patients with advanced breast cancer demonstrated reduced Bax expression in 1/3 of patients (7). On the contrary, Zhang et al found increased Bax expression in breast cancer cells collected from 54 patients versus cells from benign breast masses (11). This indicates that these observations need to be continued in larger patient groups.

We found that breast cancer cells that expressed Bax, also expressed p53 protein and caspase-3. This supports activation of apoptosis in these cells. Experimental studies demonstrated that in transgenic mice for the brain tumor model, Bax expression related to p53 caused suppression of tumor development via apoptosis. However other studies did not find correlation between Bax and p53 expression in breast cancer patients that was observed by us (12, 13). There was no correlation between Bax and caspase-3 expression in breast cancer cells in our study.

When we analyzed correlation between Bax expression and clinical and pathological characteristics, we found that expression of this protein was lower in patients over 70 years of age than in younger patients. We suspected that this could be related to inhibition of defense mechanisms against tumor development in the elderly. This observation was not confirmed by other authors (8, 13).

Presence of estrogen and progesterone receptors in breast cancer cells is a predictor of favorable prognosis (14). We observed a correlation between increased Bax expression and presence of estrogen receptors. Furthermore we found a trend towards reduced Bax expression in so called “triple negative” patients, i.e. patients without estrogen, progesterone receptors and Her-2. Such receptor profile is a predictor of poor prognosis since it is associated with more aggressive disease and limited response to ancillary therapy (15). Our study indicates a potential association between apoptotic pathways and receptor profile in breast cancer cells. This observation is not supported by studies of other authors. Study by Martinez-Arribas et al. demonstrated a trend towards a presence of Her-2 expression and reduced Bax expression (13). This correlation should be assessed in a larger patient group.

In conclusion, our study indicates a significant participation of Bax protein in the apoptosis in breast cancer cells. Finding an association between Bax expression, patient age and presence of estrogen receptors and potential association with “triple negative” profile of the breast cancer encourages to continuation of these investigations. Long-term follow up of our patient may allow establishment of prognostic significance of Bax protein.

**REFERENCES**