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The Role of BRCA1 Gene Mutations and Apoptosis Phenomenon in Sporadic Breast Cancer*

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BRCA1 tumor suppressor gene encodes an 1863-amino acid gene product that is implicated in many cellular pathways including transcription, cell-cycle checkpoint control, apoptosis and DNA repair. A role of apoptosis and BRCA1 germ-line mutation in breast cancer appearance was investigated in this study by both apoptosis frequency analysis and mutation screening of BRCA1 among breast cancer cases. Blood was obtained from 40 women with node-negative and node-positive ductal breast carcinomas with uniform tumor size. The blood samples from age matched healthy women (n=42) served as control. BRCA1 gene mutations were determined by PCR-RFLP methods. The apoptotic peripheral blood cells were detected by agarose gel electrophoresis. The apoptotic cells were identified in 30% (12/40) of the patients. There were no significant differences in apoptosis frequencies between patients and controls ($P>0.05$). Three mutations of BRCA1 gene were identified in apoptosis positive samples from breast cancer women; one Ex20insC and two ExII17delA. Our study implies that apoptosis may be involved not only in sporadic breast carcinoma without BRCA1 mutations, but also in BRCA1-associated breast carcinoma.

Introduction

Breast cancer is one of the major killers worldwide. The molecular mechanisms involved in breast carcinogenesis, however, remain to be elucidated. Women who inherit a mutated form of breast cancer susceptibility genes such as BRCA1 and BRCA2 possess a high risk of developing breast cancer [6, 14, 21, 22]. A number of observations have also linked BRCA1 to DNA damage response pathways [23, 12]. Recent studies suggest that BRCA proteins are required for maintenance of chromosomal stability, thereby protecting the genome from damage [20]. New data also show that BRCAs regulate transcriptionally some genes involved in DNA repair, the cell cycle, and apoptosis [5].

Apoptosis is a phenomenon that mediates physiological processes such as embryogenesis, metamorphosis, endocrine-dependent tissue atrophy and normal tissue turnover [10, 11]. The BCL-2 family with its anti-apoptotic members BCL-2, BCL-XL, MCL-1, and A1 and the growing subfamily of death-promoting members BAX, BCL-Xs, BAK, BAD, BIK, BID, BIM, BOK, HRK, MTD and BOO play a central role in the regulation of apoptotic cell death [18, 19].

The BCL-2 family and the p53 tumor suppressor gene have been extensively studied in breast cancer. In breast cancer a defect in expression of the BAX mRNA and the BAX protein is a key promoter of apoptosis [1]. p53 can act as transcriptional regulator of the BAX gene, and part of the tumor suppressor properties of the p53 gene can be mediated by transcriptional activation of the BAX gene [15]. p53 tumor suppressor gene that controls cellular growth and differentiation is also known to be mutated in more than 50% of human cancers including breast cancer. Several properties of BRCA1 and p53 suggest that these two proteins may functionally interact. BRCA1 increases p53-dependent transcription from the p21WAF1/CIP1 and BAX promoters [25]. BRCA1 and p53 proteins interact both *in vitro* and *in vivo* and cooperatively induce apoptosis of cancer cells.

*This work was supported by the grants No. 3P05C06624 and 3P05E07124 from the State Committee of Scientific Research (KBN)

Increased apoptosis with increased proliferation is associated with malignant tumors. High levels of apoptosis in a breast tumor have been correlated with worse survival [17].

One of the features of apoptotic cells is fragmentation of chromatin DNA at nucleosome level. Usually this fragmented DNA is detected with laddering in gel electrophoresis. In the present work, we addressed the question as to whether presence of apoptotic peripheral blood cells detected by agarose gel electrophoresis may be a risk factor of breast cancer appearance.

In addition, we screened for mutations in the DNA of the BRCA1 gene, since BRCA1 has been implicated in the apoptosis.

Material and Methods

Patients

Blood was obtained from 40 women with node-negative (n=28) and node-positive (n=12) ductal breast carcinomas treated at Department of Oncology of the Institute of Polish Mother's Memorial Hospital between 2004 and 2005. No distant metastases were found in patients at the time of treatment. Blood samples were collected from 18 premenopausal women (mean age ±SD 41.33±4.72 years) and from 22 postmenopausal women (mean age ±SD 65.33±7.66 years). Median follow-up of the patients still alive at the time of analysis was 39 months (range: 2–71 months). The average tumor size was 20 mm (range 17–32 mm). All tumors were graded by a method based on the criteria of Scarff-Bloom-Richardson. There were 17 tumors of I grade, 16 of II grade and 7 of III grade in total. Steroid receptor status was not determined in the investigated group. Blood samples from age matched healthy women (n=42) served as control.

Apoptosis detection

Apoptosis analysis was performed in DNA from blood extracted using commercially available ApopLadder Ex™ (TaKaRa BIO INC., Japan) according to manufacturer's instruction.

BRCA1 mutation analysis

Mutation analysis of BRCA1 gene was performed in DNA from peripheral blood lymphocytes obtained from all patients using commercially available kit according to manufacturer's instruction (Pomeranian Medical University, Szczecin, Poland).

Statistical analysis

For statistical analysis, the χ^2 test was used, $P<0.05$ was considered as significant.

Results

Genomic DNA was extracted from 40 blood samples obtained from breast cancer patients and 42 healthy volunteer blood samples. The presence of apoptosis in blood cells for breast cancer patients and control is shown in Table 1. Twelve out of 40 carcinoma samples (30%) were positive for the presence of apoptosis in blood cells and 14 out of 42 control samples (33%) were positive for the presence of apoptosis in blood cells. There were no significant differences between apoptotic effect between patients and control ($P>0.05$).

TABLE 1
Number of patients presenting or not apoptotic peripheral blood cells as compared with controls

Patients (n=40)		Control (n=42)	
Apoptosis		Apoptosis	
Positive	Negative	Positive	Negative
12 (0.30)a	28 (0.70)	14 (0.33)	28 (0.67)

[a]$P>0.05$ as compared with controls

Among apoptosis-positive samples 7 were from premenopausal and 5 from postmenopausal women. Analysis of apoptosis in cancer samples obtained from premenopausal and postmenopausal women showed no differences (Mann-Whitney U test, $P=0.054$).

The studies on the presence of apoptosis in the peripheral blood cells for node-positive and node-negative breast cancer patients are summarized in Table 2. In lymph node-positive tumors, the presence of apoptotic cells was lower than in lymph node-negative tumors; 8/28 (28%) node-negative subjects exhibited apoptosis. However there were no significant differences between investigated groups ($P>0.05$).

TABLE 2
The presence of apoptotic peripheral blood cells in patients with node-positive and node-negative breast cancers

Node-positive breast cancer patients (n=12)		Node-negative breast cancer patients (n=28)	
Apoptosis		Apoptosis	
Positive	Negative	Positive	Negative
4 (0.33)a	8 (0.67)	8 (0.28)	20 (0.72)

[a]$P>0.05$ as compared with node-negative patients

The histological analysis of tumor grade (Scarf-Bloom-Richardson grade) showed a lack of correlation between grade of tumors and the number of patients presenting apoptotic peripheral blood cells (Table 3).

TABLE 3
Number of breast cancer[a] patients presenting or not apoptotic peripheral blood cells in relation to tumor grade

grade[b]	I (n=17)		II (n=16)		III (n=7)	
Apoptosis	Number	Frequency	Number	Frequency	Number	Frequency
Negative	13	0.77	11	0.69	4	0.57
Positive	4	0.23	5	0.31	3	0.43

[a]n=40; [b]according to Scarf-Bloom-Richardson criteria

Mutation analysis of BRCA1 gene was performed in breast cancer women. In 40 samples three mutations of BRCA1 were found. There were: one Ex20insC and two ExII17delA**.** These mutations of BRCA1 gene were identified in apoptosis-positive samples.

Discussion

Breast cancer is the commonest malignancy in women and comprises 18% of all cancers in women. Sporadic cases account for approximately 95% of all breast cancers. However, about 5% of breast cancers occur clustered within families [8]. Importantly, cancer in these familial syndromes characteristically manifests at a younger age than sporadic cancer.

Recent experimental results suggest that BRCA1 plays a role in the regulation of apoptosis. A number of observations have also linked BRCA1 to DNA damage response pathways [3]. The analysis of both hereditary and sporadic breast cancers demonstrated that most of BRCA1-associated carcinomas belong to the group of ER-, HER2-negative tumors that express basal cell markers and/or p53 and have higher expression of activated caspase 3 [16]. The cell cycle proteins associated with these tumors were E2F6, cyclins A, B1 and E, SKP2 and Topo IIalpha. In contrast, most of BRCA2-associated carcinomas grouped in a branch composed by ER/PR/BCL2-positive tumors with a higher expression of the cell cycle proteins: cyclin D1, cyclin D3, p27, p16, p21, CDK4, CDK2 and CDK1. The study on hereditary breast cancer tumors defines the molecular differences between BRCA1 and BRCA2 tumors with respect to hormonal receptors, cell cycle, apoptosis and basal cell markers.

During carcinogenesis in epithelial tissue, genetic mutations accumulate and loss of cellular functions occurs. The phenotype of the cells changes from normal through a series of malignant lesions to superficial cancers and finally invasive disease. In the premalignant stages there are major alterations in apoptosis, proliferation, and regulatory biomarkers of the cell cycle.

Apoptosis is increased in ductal carcinoma *in situ* and invasive breast cancer [7, 13].

High levels of apoptosis in tumors have been correlated with worse survival [2, 24] and have been reported by others to be an independent variable when all other prognostic indicators are considered [4, 9].

Because the appearance of breast cancer can be associated with apoptosis it seems reasonable to check a possible correlation between apoptosis and high-risk of breast cancer. In this work conducted on 40 breast cancer women we did not find any correlation between apoptosis and risk of breast cancer appearance

In an attempt to determine whether patients are under control of apoptosis, genomic DNA was extracted from blood samples obtained from patients diagnosed at the Polish Mother's Memorial Hospital. DNA analysis by agarose gel electrophoresis using peripheral blood cells from the patients showed the characteristic pattern of DNA fragmentation. Thirty per cent of the patients presented such profile, which is a hallmark of the apoptosis process; while 14 blood samples from 42 healthy individuals (33%) showed this pattern.

The detection of apoptosis by gel electrophoresis initially involved the use of static gel electrophoresis for assessing DNA fragmentation to nucleosomes and nucleosome multimers [9]. In this work we demonstrated that apoptosis might be successfully detected by agarose gel electrophoresis in blood samples.

In group of 40 patients with breast cancer family, apoptosis was found in 12. These 12 cases plus 28 other subjects whose tumors were apoptosis-negative were studied for BRCA1 germline mutations. Germline mutation in BRCA1 was found in three cases (7.5%). These mutations in breast cancer samples suggested their role in appearance of this cancer. Moreover, the analysis showed that BRCA1 mutations are present in women with apoptotic DNA ladder. It is suggested that apoptosis may be associated with high risk of BRCA1- associated breast cancer.

Our studies suggest that genetic alterations such as BRCA1 mutation and apoptosis can be detected in sporadic breast cancer. Present work implies that it is possible that the apoptosis process may be involved in the appearance and/or progression of the breast cancer with and without BRCA1 mutations. Further studies, conducted on a larger population, are required to clarify this point.

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