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Germline BRCA1 Mutations and G/C Polymorphism in the 5'-Untranslated Region of the RAD51 Gene in Polish Women with Breast Cancer*

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Breast cancer is a significant cause of morbidity and mortality in Western countries. In the present work the distribution of genotypes and frequency of alleles of the RAD51 G/C polymorphism and the frequency of BRCA1 germ-line mutations in patients with breast cancer were investigated. One hundred breast cancer women provided blood for mutation analysis. Blood samples from age matched healthy individuals (n=106) served as control. The G/C polymorphism and BRCA1 mutations were determined by PCR-RFLP methods. The distribution of the genotypes of the G/C polymorphism RAD51 in both control and patients did not differ significantly (P>0.05) from those predicted by the Hardy-Weinberg distribution. There were no significant differences in the genotype distributions and allele frequencies between node-positive and node-negative patients. In the present study one Ex20insC mutation of BRCA1 gene was identified in women with breast cancer. Our study implies that the G/C polymorphism of the RAD51 gene may not be directly involved in the development and/or progression of breast cancer. The lack of detectable BRCA1 germ-line mutations in most cases suggests that there are probably additional, as yet unidentified genes predisposing to this disease.

Introduction

Breast cancer is the most common malignancy in women in Western countries. Approximately 10% of all women in these countries will, during their lifetime, develop breast cancer [9]. Most cases of breast cancer appear to occur without a clear family history of the disease. These sporadic cases account for approximately 95% of all breast cancers. However, about 5% of breast cancers occur clustered within families [2].

The most important prognostic parameters for breast carcinomas are tumor size, nuclear grade, and steroid hormone receptor status [4]. However, these factors present an incomplete picture of the tumor biology. Therefore investigation of other prognostic factors is of special clinical relevance, particularly in view of the unexpectedly progressive course of the disease and frequent relapses in some cases.

The genetics of mammary carcinogenesis has not been fully 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 [8, 11]. Surprisingly, these two genes have been found to participate in the control of homologous recombination, suggesting that they may function as tumor suppressors by regulating genome integrity maintenance functions [13].

Several studies demonstrated the involvement of BRCA1 and BRCA2 in complexes that activate the repair of double-strand breaks and initiate homologous recombination, linking the maintenance of genomic integrity to tumor suppression. BRCA1 and BRCA2 co-localize with RAD51 to form complexes [1]. The RAD51 gene makes a protein also called RAD51, which is essential for the repair of damaged DNA. The protein made by the BRCA2 gene binds to and regulates the RAD51 protein to fix breaks in DNA [7]. These breaks can be caused by natural or medical radiation. They also occur when chromosomes exchange genetic material (when pieces of chromosomes trade places) in preparation for cell division. The BRCA2 protein transports the RAD51 protein to sites of DNA damage in the cell nucleus. RAD51 then binds to the damaged DNA and encases it in a protein sheath, which is an essential first step in the repair process. In addition to its association with BRCA2, the

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RAD51 protein also interacts with the protein made by the BRCA1 gene. By repairing DNA, these three proteins play a role in maintaining the stability of the human genome.

Changes in RAD51 biosynthesis are usually preceded by changes in its gene transcription and mRNA level. Gene variability could contribute to the level of the RAD51 biosynthesis. A single nucleotide polymorphism in the 5'-untranslated region (5'-UTR) of RAD51 (a G to C substitution at position 135, the G/C polymorphism) can influence breast cancer risk among BRCA1/BRCA2 mutation carriers [6, 12]. In view of the potential significant role of RAD51 for tumor development, it is important to know, whether this polymorphism can account for the development and/or progression of breast cancer.

In the present work the distribution of genotypes and frequency of alleles of the G/C polymorphism and BRCA1 mutations in women with breast cancer were investigated.

Material and Methods

Patients and DNA isolation

Blood was obtained from 100 postmenopausal women with node-negative (n=39) and node-positive (n=61) ductal breast carcinoma treated at the Department of Oncology, Institute of Polish Mother's Memorial Hospital. No distant metastases were found in patients at the time of treatment. The patients ranged in age from 40 to 82 years (median age 58 years). Median follow-up of patients still 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 Scarf-Bloom-Richardson. There were 20 tumors of I grade, 45 of II grade and 35 of III grade in total. In node-negative patients these numbers were: I – 9, II – 16, III – 14; in node-positive: I – 11, II – 29, III – 21. Steroid receptors status was not determined in the investigated group. Blood samples from age matched healthy women (n=106) served as control.

DNA was extracted from blood using commercially available QIAmp Kit (Qiagen GmbH, Hilden, Germany) DNA purification kit according to manufacturer's instruction.

Determination of RAD51 genotype

RAD51 genotype was analyzed by PCR amplification of a 175-bp region around nucleotide 135. This region contained a single *Mva*I site that was abolished in the 135C allele. Wild type alleles were digested by *Mva*I resulting in 86- and 71-bp product. The 135C allele was not digested by the enzyme, resulting in a single 157-bp product. The RAD51 genotype was analyzed using the specific primers listed in Table 1.

TABLE 1
Primer sequences

	Primer sequences
forward	5' TGG GAA CTG CAA CTC ATC TGG 3'
reverse	5' GCG CTC CTC TCT CCA GCAG 3'

The PCR was carried out in a Perkin-Elmer/Gene Amp, PCR System 2400 thermal cycler. PCR amplification was performed in a final volume of 25 µl. The reaction mixture contained 5 ng of genomic DNA, 0.2 µmol of each appropriate primer (ARK Scientific GmbH Biosystems, Darmstad, Germany), 2.5 mM $MgCl_2$, 1 mM dNTPs and 1 unit of Taq Polymerase (Qiagen GmbH, Hilden, Germany). The PCR cycle conditions were 94°C for 60 s, 54°C for 30 s then 72°C for 40 s, repeated for 35 cycles. After digestion with *Mva*I for 4 h at 37°C samples were run on 7% polyacrylamide gel and visualized by ethidium bromide staining. Each subject was classified into one of the three possible genotypes: G/G, G/C or C/C (Fig. 1).

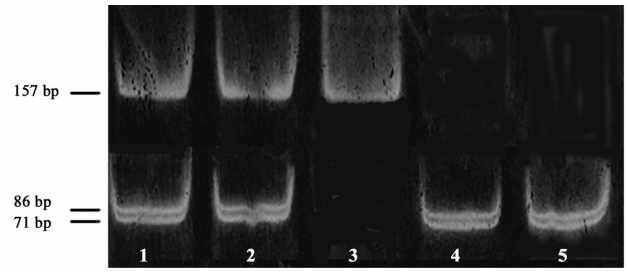

Fig. 1. A typical result of restriction fragment length polymorphism; polymerase chain reaction performed with genomic DNA extracted from breast cancer tissue giving fragments of the RAD51 gene 5'-untranslated region analyzed by 7% polyacrylamide gel electrophoresis and staining with ethidium bromide. Lanes 1 and 2 display pattern characteristic for genotype G/C, lane 3 for C/C and lane 4 and 5 for G/G.

DNA 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) (Fig. 2).

Statistical analysis

The allelic frequencies were estimated by gene counting and genotypes were scored. The observed numbers of each RAD51 genotype were compared with that expected for a population in Hardy-Weinberg equilibrium by using a χ^2 test. The significance of the differences of observed alleles and genotypes between groups was tested using the χ^2 analysis. P-values <0.05 were considered to be significant.

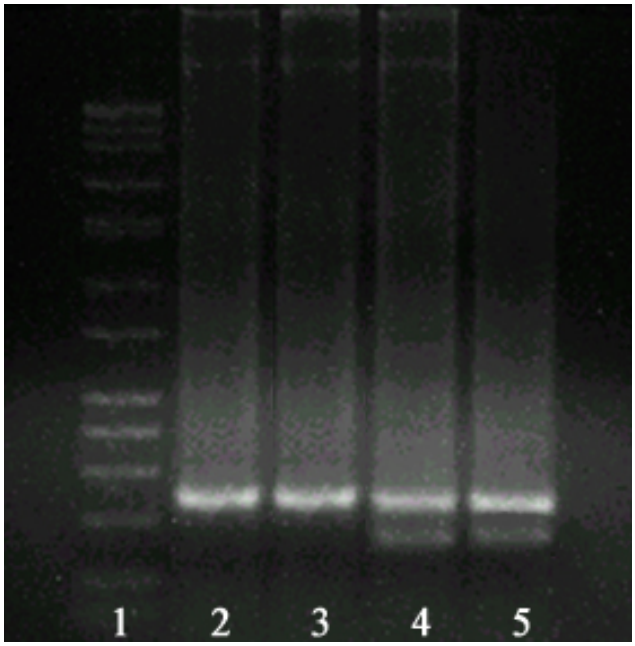

Fig. 2. A typical result of polymerase chain reaction performed with a fragment of the BRCA1 gene and analyzed by 2% agarose gel electrophoresis, staining with ethidium bromide and viewed under ultraviolet light. Lanes 1 – molecular weight markers, 50–2000 bp (Sigma, St. Louis, USA), lanes 2, 3 – display the product of amplification without mutation, lanes 4 – display the product of amplification with the primer pair specific for Ex20insC mutations, lanes 5 – PCR control after restriction endonuclease *Ava*II digestion

Results

From the PCR analysis, all the patients and controls were divided into three genotypes of the RAD51 gene: G/G, G/C and C/C. Table 2 shows genotype distribution between breast cancer patients and controls. Both distributions did not differ significantly (P>0.05) from those predicted by the Hardy-Weinberg distribution. Additionally, there were no differences in the frequencies of the G and C alleles between patients and controls.

Distributions of the G/C genotypes as well as the frequencies of the G and C alleles for node-positive and node-negative breast cancer patients are displayed in Table 3. It can be seen from the Table that there were no significant differences between these two groups in both genotype distribution and allele frequencies (P>0.05).

Dependencies of the distribution of genotypes and frequencies of alleles on the tumor grade evaluated according to Scarf-Bloom-Richardson criteria in patients with node-positive and node-negative breast cancer are shown in Tables 4 and 5, respectively. There were no significant differences between distributions of genotypes in subgroups assigned to histological grades and the distribution predicted by Hardy-Weinberg equilibrium (P>0.05). There were no differences in frequencies of the G and C alleles between subgroups either (P>0.05).

Mutation analysis of BRCA1 gene was performed in all breast cancer patients and control. Out of 100 women with breast cancer one mutation Ex20insC was found.

Discussion

RAD51, RAD52 and RAD54 encode the human homologues of yeast genes that are required for homologous recombination, an essential step in the repair of double-strand DNA breaks. The mechanisms of cancer risk modification by RAD51 are unknown. The discovery that RAD51 associates directly with the product of the breast cancer predisposition gene BRCA2 and indirectly with the BRCA1 product has implicated these tumor suppressor genes in this recombinational pathway for the repair of DNA damage [1]. The human RAD51 gene has been ma-

TABLE 2
Distribution of G/G, G/C and C/C genotypes and frequencies of the G and C alleles in patients with breast cancer (n=100) and controls (n=106)

	Breast cancer patients		Controls	
	Number	Frequency	Number	Frequency
G/G genotype	31	0.31	21	0.20
G/C genotype	40	0.40	48	0.44
C/C genotype	29	0.29	37	0.35
χ^2	3.987[a]		0.591[a]	
G allele	102	0.51[b]	90	0.42
C allele	98	0.49[b]	122	0.58

[a]P>0.05 as compared with Hardy-Weinberg distribution; [b]P>0.05 as compared with the controls

TABLE 3
Distribution of G/G, G/C and C/C genotypes and frequencies of the G and C alleles in patients with node-positive (n = 61) and node-negative (n = 39) breast cancer

	Node-positive breast cancer patients		Node-negative breast cancer patients	
	Number	Frequency	Number	Frequency
G/G genotype	17	0.28	14	0.36
G/C genotype	24	0.39	16	0.41
C/C genotype	20	0.33	9	0.23
χ^2	2.732[a]		1.070[a]	
G allele	58	0.48[b]	44	0.56
C allele	64	0.52[b]	34	0.44

[a]P>0.05 as compared with Hardy-Weinberg distribution; [b]P>0.05 as compared with node-negative patients

TABLE 4
Dependency of the distribution of G/G, G/C and C/C genotypes and frequencies of the G and C alleles on the tumor grade in patients with node-positive breast cancer[a]

Stage[b]	I (n=11)		II (n=29)		III (n=21)	
	Number	Frequency	Number	Frequency	Number	Frequency
G/G genotype	3	0.27	9	0.31	3	0.14
G/C genotype	5	0.46	12	0.41	9	0.43
C/C genotype	3	0.27	8	0.28	9	0.43
χ^2	0.360[c]		0.222[c]		0.886[c]	
G allele	11	0.50	30	0.52	15	0.36
C allele	11	0.50	28	0.48	27	0.64

[a]n=61; [b]according to Scarf-Bloom-Richardson criteria; [c]P>0.05 as compared with Hardy-Weinberg distribution

TABLE 5
Dependency of the distribution of G/G, G/C and C/C genotypes and frequencies of the G and C alleles on the tumor grade in patients with node-negative breast cancer[a]

Stage[b]	I (n=9)		II (n=16)		III (n 14)	
	Number	Frequency	Number	Frequency	Number	Frequency
G/G genotype	2	0.22	4	0.25	7	0.50
G/C genotype	5	0.55	7	0.43	5	0.35
C/C genotype	2	0.22	5	0.32	2	0.15
χ^2	0.360[c]		0.222[c]		0.886[c]	
G allele	9	0.50	15	0.47	19	0.68
C allele	9	0.50	17	0.53	9	0.32

[a]n=39; [b]according to Scarf-Bloom-Richardson criteria; [c]P>0.05 as compared with Hardy-Weinberg distribution

pped to chromosome 15q14-15, where loss of heterozygosity is found frequently in breast carcinomas [3, 14]. These findings suggest that loss of RAD51 function may contribute to mammary carcinogenesis. BRCA1-associated RING domain protein 1 was identified as a protein that interacts with the BRCA1 RING domain [15]. The phenotypic changes induced in mammary epithelial cells by antisense oligonucleotide-mediated inhibition of BARD1 suggest that

BARD1 functions as a growth suppressor [5]. In addition the formation of BRCA1/BARD1/RAD51 complexes on damaged replicating DNA suggests that these proteins might help maintaining genomic integrity [10].

In light of substantial evidence that the progression of breast cancer can be associated with RAD51 and BRCA1 protein, it seems reasonable to check a possible correlation between the G/C polymorphism, BRCA1 mutations and clinical status of breast cancer patients.

In this work conducted on 100 ductal breast carcinoma we did not find any correlation between G/C genotypes, BRCA1 mutations and appearance of cancer. Moreover, we did not detect any significant difference between genotypes of node-positive and node-negative patients that suggests a lack of association between G/C genotypes and breast cancer invasiveness. This polymorphism is reported to modify cancer risk in BRCA2 mutation carriers, but little is known about its potential general impact on breast cancer [6, 12].

The biological effect of the polymorphism is yet to be elucidated and will be important to investigate. As mentioned above it is located in the 5'-untranslated region of the RAD51 gene and could affect mRNA stability and/or translation efficiency, leading to altered product levels, which could affect the function of a multi-protein DNA-repair complex consisting of BRCA1, BRCA2 and RAD51. Because mutations in BRCA1 and BRCA2 are directly linked with breast cancer, genetic variations in the RAD51 gene may be believed to play a role in this disease. Indeed in breast cancer a loss of heterozygosity at the RAD51 locus has been reported in 32% [3] and reduced RAD51 protein levels in 30% of patients [16].

We tried to find a connection between breast cancer onset and the G/C polymorphism of the RAD51 gene without linkage with its other mutations and the results obtained suggest that this polymorphism may not be useful as an independent marker in breast cancer. However, our study had a preliminary character only and further research, performed on a larger group, and is needed definitely to establish a correlation or lack of it between breast cancer and the G/C polymorphism.

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