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Communication

Analysis of the G/C polymorphism in the 5′-untranslated region of the *RAD51* gene in breast cancer★✪

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The breast cancer suppressor proteins BRCA1 and BRCA2 interact with RAD51, a protein essential for maintaining genomic stability by playing a central role in homology-dependent recombinational repair of the DNA double-strand breaks. Therefore, genetic variability in the *RAD51* gene may contribute to the appearance and/or progression of breast cancer. A single nucleotide polymorphism in the 5′- untranslated region of *RAD51* (a G to C substitution at position 135, the G/C polymorphism) is reported to modulate breast cancer risk. We investigated the distribution of genotypes and frequency of alleles of the G/C polymorphism in breast cancer. Tumor tissues were obtained from postmenopausal women with node-negative and node-positive breast carcinoma with uniform tumor size. Blood samples from age matched healthy women served as control. The G/C polymorphism was determined by PCR-based *Mva*I restriction fragment length polymorphism. The distribution of the genotypes of the G/C polymorphism did not differ significantly ($P > 0.05$) from those predicted by the Hardy–Weinberg distribution. There were no differences in the genotype distribution and allele frequencies between node-positive and node-negative patients. There were no significant differences between distributions of the genotypes in

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subgroups assigned to histological grades according to Scarf–Bloom–Richardson criteria and the distribution predicted by Hardy–Weinberg equilibrium ($P > 0.05$). 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 and so it may not be useful as an independent marker in this disease.

Cancer is associated with genomic instability, which can be a consequence of many unrepaired DNA lesions. Therefore, malfunction of DNA repair can contribute to the instability and to cancer. This malfunction may follow from genetic variability, which could account for alterations of DNA repair proteins. Genetic factors are important in breast cancer and carriers of mutations in the *BRCA1* and/or *BRCA2* genes, whose products are involved in DNA repair, are of increased risk. The penetrance of these mutations can be modified by genetic factors including genes whose products can interact with BRCA1 and/or BRCA2 (Welcsh *et al.,* 2000).

RAD51 is a homologue of bacterial RecA protein, which is required for meiotic and mitotic recombination and plays a central role in homology-dependent recombinational repair of DNA double-strand breaks. It is reported to interact with both BRCA1 and BRCA2 (Chen *et al.,* 1998). Therefore, the problem of genetic variability of the *RAD51* gene in breast cancer is worth studying for at least two reasons: (1) the involvement of RAD51 in the stability of the genome and (2) its potential to modify the penetrance of *BRCA1/BRCA2* mutations, which can increase susceptibility for breast cancer. 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 (Wang *et al.,* 1999; 2001; Levy-Lahad *et al.,* 2001). About two thirds of all breast cancer patients are node-positive and usually receive adjuvant therapy, the other one third, which is node-negative, may or may not receive such therapy depending on the chance of disease recurrence (Kute *et al.,* 1998).

To explore further the association between the *RAD51* G/C polymorphism and breast cancer risk we investigated the distribution of the genotypes and frequencies of alleles of this polymorphism in women with node-negative and node-positive breast cancer. We did not take into account the *BRCA1/BRCA2* genotype of our patients because we wanted to check the potential of the polymorphism as an independent marker.

MATERIALS AND METHODS

Tumor tissues were obtained from 46 postmenopausal women with node-negative (n = 18) and node-positive (n = 28) ductal (n = 34) and non-ductal (n = 12) breast carcinoma treated in 2002 at the Department of Oncological Surgery, N. Copernicus Hospital (Lodz, Poland). No distant metastases were found in the patients at the time of treatment. The patients ranged in age from 48 to 74 years (median age 63 years). The average tumor size was 20 mm (range 8–58 mm). All ductal tumors were graded by a method based on the criteria of Scarff–Bloom–Richardson. Steroid receptor status was not taken into account in this study. Blood samples from age matched healthy women (n = 60) served as control. Genomic DNA from tumor and blood samples was extracted by using standard phenolic procedure. *RAD51* genotyping 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 products. The 135C allele was not digested by the enzyme, resulting in a single 157-bp product. PCR was performed in a MJ Research, INC thermal cycler, model PTC-100 by using the following primers: forward 5′-TGG GAA CTG CAA CTC ATC TGG-3′, reverse 5′-GCG CTC CTC TCT

CCA GCAG-3′) at a final Mg concentration of 1.5 mM and an annealing temperature of 53°C. After digestion with *Mva*I for 4 h at 37°C, samples were run on 3% agarose gel. The allelic frequencies were estimated by gene counting and genotypes were scored. The observed numbers of each *RAD51* genotype were compared with those expected for a population in Hardy–Weinberg equlibrium by using the χ^2 test. The significance of the differences of the observed genotype distributions and allele frequencies between groups was tested using the χ^2 analysis.

RESULTS AND DISCUSSION

From the PCR analysis, all the patients and controls were divided into three genotypes of the *RAD51* 5′-UTR: C/C, G/C and G/G (Fig. 1). There were no differences in the genotype in tumor and normal tissue for each patient. Table 1 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 C and G alleles between patients and controls.

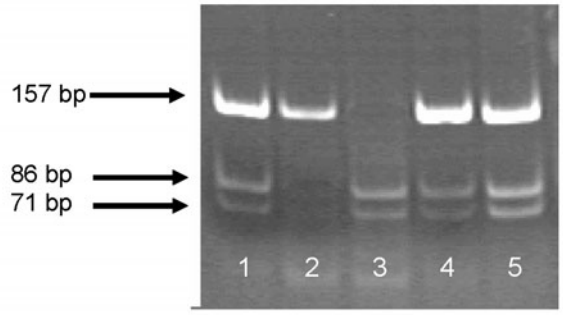

Figure 1. Typical results of restriction fragment length polymorphism polymerase chain reaction performed with genomic DNA extracted from breast cancer tissue giving fragments of the *RAD51* gene 5′-untranslated region analysed by 3% agarose gel electrophoresis and staining with ethidium bromide.

Lanes 1, 4 and 5, display pattern characteristic for genotype G/C; lane 2, for C/C, and lane 3, for G/G.

There were no significant differences between the G/C genotypes and the frequency of the C and G alleles for node-negative and node-positive breast cancer patients (Table 2). The dependency of the distribution of the genotypes and frequencies of alleles on the tumor grade for ductal tumors evaluated according to Scarf–Bloom–Richardson criteria is displayed in Table 3. Non-ductal tumors could not be classified by this criteria. There were no significant differences between the distributions of the genotypes in subgroups assigned to histological grades and the distribution predicted by Hardy–Weinberg equilibrium ($P > 0.05$). There were no differences in the frequencies of the C and G alleles between the subgroups either.

Our results suggest that the G/C polymorphism of the *RAD51* 5′-UTR may not be linked with the appearance and progression of breast cancer. This polymorphism is reported to modify cancer risk in *BRCA2* mutation carriers (Levy-Lahad *et al.,* 2001; Wang *et al.,* 2001), but little is known about its potential general impact on breast cancer. 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% (Gonzales *et al.,* 1999) and reduced RAD51 protein levels in 30% of patients (Yoshikawa *et al.,* 2000). We tried to find a connection between breast cancer onset and the G/C polymorphism of the *RAD51*

Table 1. Distribution of G/C genotypes and frequencies of the G and C alleles of the G/C polymorphism of the *RAD51* gene in patients with breast cancer and controls

	Breast cancer patients (n = 46)		Controls (n = 60)	
	Number	Frequency	Number	Frequency
G/G genotype	11	0.24	21	0.35
G/C genotype	28	0.61	35	0.58
C/C genotype	7	0.15	4	0.07
χ^2	2.363[a]		4.325[b]	
G allele	50	0.54	77	0.64
C allele	42	0.46	43	0.36

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

Table 2. Dependency of the distribution of G/C genotypes and frequencies of the G and C alleles of the G/C polymorphism of the *RAD51* gene in patients with ductal breast cancer on the tumor grade

Grade[a]	I° (n = 1)		II° (n = 15)		III° (n = 18)	
	Number	Frequency	Number	Frequency	Number	Frequency
G/G genotype	0	0	3	0.20	4	0.22
G/C genotype	0	0	11	0.73	12	0.67
C/C genotype	1	1	1	0.07	2	0.11
					χ^2= 0.029[b]	
G allele	0	0	17	0.57	20	0.55
C allele	2	1	13	0.43	16	0.45

[a]According to Scarf–Bloom–Richardson criteria, ductal cancers only; [b]$P > 0.05$ as compared with patients with II°.

Table 3. Distribution of G/C genotypes and frequencies of the G and C alleles of the G/C polymorphism of the *RAD51* gene in patients with node-negative and node-positive breast cancer

	Node negative		Node positive	
	Number	Frequency	Number	Frequency
G/G genotype	1	0.06	10	0.36
G/C genotype	15	0.83	13	0.46
C/C genotype	2	0.11	5	0.18
			χ^2= 2.119[a]	
G allele	17	0.47	33	0.58
C allele	19	0.53	23	0.42

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

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, is needed definitely to establish a correlation or lack thereof between breast cancer and the G/C polymorphism.

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