Association between polymorphisms of the DNA repair gene *RAD51* and ovarian cancer

Beata Smolarz[1], Marianna Makowska[2], Dariusz Samulak[3,4,5], Magdalena M. Michalska[5], Ewa Mojs[6], Hanna Romanowicz[7], Maciej Wilczak[8]

[1]Department of Fetal-Maternal Medicine and Gynecology, Institute of Polish Mother's Memorial Hospital, Lodz, Poland
[2]Regional Hospital in Lodz, Poland
[3]Department of Obstetrics and Gynaecology, Regional Hospital in Kalisz, Poland
[4]Department of Mother's and Child's Health, Poznan University of Medical Sciences, Poznan, Poland
[5]Clinic of Gynaecological Surgery, Poznan University of Medical Sciences, Poznan, Poland
[6]Department of Clinical Psychology, Poznan University of Medical Sciences, Poland
[7]University of Computer Sciences and Skills, Lodz, Poland
[8]Department of Medical Education, Poznan University of Medical Sciences, Poznan, Poland

Genetic polymorphisms in the *RAD51* gene may be associated with increased cancer risk.

The aim of the present study was to evaluate associations between the risk of ovarian cancer and 135G>C (rs1801320) and 172G>T (rs1801321) polymorphisms in the *RAD51* gene.

We analysed the distribution of genotypes and frequency of alleles of the *RAD51* polymorphisms in 210 women with ovarian cancer and 210 healthy controls. Both polymorphisms were genotyped by restriction fragment length polymorphism-polymerase chain reaction (PCR-RFLP).

In the present study only 135G>C polymorphism of the *RAD51* gene was associated with ovarian cancer risk. The distribution of genotypes for 135G>C in ovarian cancer patients vs. controls was: 20% vs. 30% for G/G, 22% vs. 47% for G/C, and 58% vs. 23% for C/C genotype, respectively. We found evidence of an increased ovarian cancer risk in C/C homozygotes but not in heterozygotes. The 135C allele of *RAD51* increased cancer risk.

In the present work we demonstrated a significant positive association between the *RAD51* 135G>C polymorphism and ovarian carcinoma in Poland. However, this gene requires further understanding of its interaction with other genes involved in tumour development.

Key words: *RAD51*, ovarian cancer, gene polymorphism.

Introduction

Ovarian cancer development is not a fully understood process regarding many risk factors. Certain factors may increase risk of ovarian cancer such as age, childbearing status, infertility, dietary factors and gynaecological diseases (endometriosis, ovarian cysts, pelvic inflammatory disease) [1].

It is known that breast cancer-1 (*BRCA1*) and breast cancer-2 (*BRCA2*) gene mutations are responsible for about 5-10% of ovarian cancers [2]. Variants in DNA repair genes that interact biologically with *BRCA1* and/or *BRCA2* may be associated with modified ovarian cancer risk in women who carry *BRCA1/2* mutations [3].

Among all DNA damage such as oxidation of bases, alkylation of bases, hydrolysis of bases, bulky adduct formation, and mismatch of bases, double-strand breaks (DSB) are most mortal to the cell. Unrepaired

DNA damage can lead to mutation, development of various diseases, or cell death. Five systems of DNA repair are known: the pathway of direct reversion of damage, base-excision repair (BER), nucleotide-excision repair (NER), mismatch repair (MMR), homologous recombination (HR) and non-homologous DNA end joining (NHEJ). DSBs in mammalian cells are repaired by two distinct pathways – HRR (homologous recombination) and NHEJ (non-homologous recombination) [4, 5].

During HR-mediated repair of DSB, the sister chromatid is used as a template to copy the missing information into the broken locus. Repair by HRR is mediated by RAD51 protein with the help of other members of the RAD52, RAD54 and RAD55 epistasis group [6]. RAD51 interacts with both BRCA1 and BRCA2 [7].

Two common *RAD51* single nucleotide polymorphisms (SNPs), 135G>C (rs1801320) and 172G>T (rs1801321) in the 5'UTR, have been reported to be associated with altered gene transcription [8]. SNPs are located in the regulatory element of the *RAD51* promoter and are suggested to be associated with messenger RNA expression [8].

RAD51 gene 135G>C and 172G>T polymorphism have been studied as a risk factor for various cancers such as breast, laryngeal, colorectal, and ovarian cancer [9-14]. However, results have been inconsistent.

Little is known about the association of *RAD51* polymorphism and ovarian carcinoma. Therefore the aim of the present study was to evaluate associations between the risk of ovarian cancer and 5' untranslated region polymorphisms in the *RAD51* gene.

Material and methods

Patients

The study was performed on 210 Polish women (n = 210) with recognized ovarian cancer and qualified for tumour debulking surgery at the Department of Gynaecological Surgery of the Institute Polish Mother's Memorial Hospital between 2000 and 2012. All tumours were staged according to the criteria of the International Federation of Gynaecology and Obstetrics (FIGO). The full characteristics of the examined group are presented in Table I. Two hundred ten age and ethnically matched women having unchanged ovaries served as controls. The Local Ethics Committee approved the study and each patient gave written consent.

The ovarian tissue samples (cancerous and non-cancerous) were fixed routinely in formaldehyde, embedded in paraffin, cut into thin slices and stained with haematoxylin/eosin for pathological examination. DNA for analysis was obtained from an archival pathological paraffin-embedded tumour and healthy ovarian samples which were deparaffinized in xylene and rehydrated in ethanol and distilled water. In order to ensure that the chosen histological material was representative for cancerous and non-cancerous tissue, every

Table I. Characteristics of ovarian cancer patients (n = 210)

CHARACTERISTICS	NUMBER OF CASES (%)
Age (years)	
median	54
range	37-80
Histology of tumour	
serous	76 (36.2)
mucinous	6 (2.9)
endometrioid	63 (30.0)
clear cell	7 (3.3)
undifferentiated	53 (25.2)
other	5 (2.4)
FIGO stage	
I	80 (38.1)
II	2 (0.9)
III	120 (57.1)
IV	6 (2.9)
no data	2 (0.9)
Grading	
G1	2 (0.9)
G2	64 (30.4)
G3	100 (47.6)
No data	44 (20.9)
Ascites	
present	95 (45.2)
absent	115 (54.8)
Tumour wall infiltration/injury	
present	115 (54.8)
absent	95 (45.2)
Size of tumour	
< 5 cm	77 (36.7)
> 5 cm	133 (63.3)
Menarche	
< 12 years old	71 (33.8)
> 12 years old	139 (66.2)
Number of pregnancies	
0	40 (19.0)
1	52 (24.8)
2 and more	118 (56.2)
Number of deliveries	
0	46 (21.9)
1	64 (30.5)
2 or more	100 (47.6)

tissue sample qualified for DNA extraction was initially checked by a pathologist. The DNA samples were extracted using the QIAamp Kit (Qiagen GmbH, Hilden, Germany). DNA purification was performed according to the manufacturer's instructions.

Polymorphisms determination

Single nucleotide polymorphism 135G>C of the *RAD51* gene was determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), using primers 5'-TGG GAA CTG CAA CTC ATC TGG-3' (forward) and 5'-GCG CTC CTC TCT CCA GCAG-3' (reverse) [15].

All PCR reactions were carried out in a volume of 25 µl containing 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).

RAD51 135G>C genotyping was analysed 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.

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 visualised by ethidium bromide staining. Each subject was classified into one of the three possible genotypes: G/G, G/C or C/C.

Single nucleotide polymorphism 172G>T of the *RAD51* gene was analysed by PCR-RFLP technique using primers 5'-TGG GAA CTG CAA CTC ATC TGG-3' (forward) and 5'-GCT CCG ACT TCA CCC CGC CGG-3' (reverse) [16].

The PCR profile for 172G>T SNP consisted of an initial melting step at 95°C for 5 min; 30 cycles of 95°C for 30 s, 65°C for 45 s and 72°C for 50 s, and a final extension step of 72°C for 10 min. The product after PCR was digested with *Ngo*MIV (New England BioLabs) overnight. The products were separated in 7% polyacrylamide gel. The G/G genotype produced two bands (110 and 21 bp), whereas the T/T genotype produced only one band (131 bp) and the G/T heterozygote displayed all three bands (131, 110 and 21 bp).

Statistical analysis

Statistical significance of the differences between the patient and control groups were estimated by χ^2 tests. For each polymorphism, deviation of the genotype frequencies in the controls from those expected under Hardy-Weinberg equilibrium was assessed using the standard χ^2 test. The genotypic-specific risks were estimated as odds ratios (ORs) with associated 95% confidence intervals (CIs) by unconditional logistic regression. Statistical significance was set at p values < 0.05.

Results

Association between *RAD51 135G>C* gene polymorphism and ovarian cancer

Table II shows the genotype distribution of *RAD51* 135G>C polymorphism between ovarian cancer patients and controls. Genotype and allele frequencies of 135G>C were significantly different between the two

Table II. Frequency of *RAD51* gene polymorphisms among ovarian cancer patients and control subjects

***RAD51* 135G>C**	**OVARIAN CANCER PATIENTS (N = 210)**		**CONTROLS (N = 210)**		**OR (95% CI)[a]**	**p[b]**
	NUMBER	**(%)**	**NUMBER**	**(%)**		
G/G	43	20	63	30	1.00 Ref	
G/C	45	22	99	47	0.66 (0.393-1.12)	0.164
C/C	122	58	48	23	**3.72 (2.23-6.21)**	**< .0001**
G	131	31	225	54	1.00 Ref	
C	289	69	195	46	**2.54 (1.92-3.37)**	**< .0001**
RAD51 172G>T	Number	(%)	Number	(%)	OR (95% CI)[a]	p[b]
G/G	53	25	59	28	1.00 Ref	
G/T	92	44	88	42	1.16 (0.72-1.86)	0.610
T/T	65	31	63	30	1.14 (0.69-1.90)	0.689
G	198	47	206	49	1.00 Ref	
T	222	53	214	51	1.07 (0.82-1.41)	0.631

Data in boldface are statistically significant.
[a]Crude odds ratio (OR), 95% CI = confidence interval at 95%,
[b]χ^2

Table III. Frequency of *RAD51* haplotypes among ovarian cancer patients and control subjects

HAPLOTYPES *RAD51*-135-172	PATIENTS (N = 210) N (%)	CONTROLS (N = 210) N (%)	OR (95% CI)[a]	P[b]
G/G-G/G	15 (7.1)	25 (11.9)	1.00 Ref.	
G/G-G/T	16 (7.6)	17 (8.1)	1.56 (0.61-3.99)	0.479
G/G-T/T	15 (7.1)	20 (9.5)	1.25 (0.49-3.15)	0.806
G/C-G/G	17 (8.1)	24 (11.7)	1.18 (0.48-2.88)	0.887
G/C-G/T	15 (7.1)	38 (11.4)	0.66 (0.27-1.57)	0.475
G/C-T/T	15 (7.1)	25 (11.9)	1.00 (0.40-2.47)	0.823
C/C-G/G	30 (14.3)	19 (9.0)	2.63 (1.11-6.22)	0.043
C/C-G/T	55 (26.2)	23 (10.9)	3.98 (1.78-8.90)	0.001
C/C-T/T	32 (15.2)	19 (9.0)	2.80 (1.19-6.60)	0.029

Data in boldface are statistically significant.
[a]Crude odds ratio (OR), 95% CI = confidence interval at 95%, [b]χ^2

investigated groups. We observed an association between ovarian carcinoma occurrence and presence of the C/C genotypes. Variant 135C allele of *RAD51* increased cancer risk. In patients the observed frequencies of the G/G, G/C and C/C genotypes differed significantly ($p < 0.05$) from the distribution expected from the Hardy-Weinberg equilibrium.

Association between *RAD51 172G>T* gene polymorphism and ovarian cancer

Table II illustrates the distributions of the frequency of genotypes and alleles in ovarian cancer patients in comparison to controls. No significant difference was observed between the two study groups. The distribution of genotypes of 172G>T polymorphism in ovarian cancer patients did not differ significantly ($p > 0.05$) compared to that predicted by the Hardy-Weinberg distribution.

Association between *RAD51* haplotypes and ovarian cancer

The haplotype analysis according to wild-type of G135G-G172G showed a strong association with ovarian cancer (Table III). The findings indicated that a statistically significantly increased risk of ovarian cancer was associated with the combined C/C-G/G genotype and C/C-T/T genotype ($p < 0.05$). A higher risk of ovarian cancer occurrence was associated with the combined C135C-G172T genotype but no altered risk was associated with other haplotypes.

Association between *RAD51* polymorphism and histological staging

FIGO staging was related to the *RAD51* 135G>C and 172G>T polymorphisms. The histological stage was evaluated in all cases (n = 210). No differences were observed in those groups, regarding either *RAD51* genotype or allele distributions. There were no correlations between genotypes of the polymorphisms and ovarian cancer invasiveness.

Association between *RAD51* polymorphism and ovarian cancer risk factors

No statistically significant differences were observed in the alleles or in the genotype frequencies of the *RAD51* gene polymorphisms between risk factors of ovarian cancer such as age, number of pregnancies, number of deliveries, menarche or size of tumour.

Discussion

In the present work we analysed the role of 135G>C and 172G>T single nucleotide polymorphisms (SNPs) in the homologous recombination repair gene *RAD51* and risk of ovarian cancer.

Literature data suggest that the genes involved in DNA repair and in the maintenance of genome integrity play a crucial role in providing protection against mutations that may lead to cancer [17]. It is known that *RAD51* is an important component of double-stranded DNA-repair mechanisms. The *RAD51* gene has been mapped to chromosome 15q14-15 and is highly polymorphic [18, 19].

RAD51 polymorphisms have been investigated in various malignancies. Numerous studies confirm the significant role of the influence of specific genetic variants on repair phenotype and cancer risk [9-14, 20-22].

From a review of the literature we learned that *RAD51* is the first gene to be reliably identified as a modifier of risk among BRCA1/2 mutation carriers [23-26].

Several studies confirm the significant role of the influence of specific genetic variants of *RAD51* on repair phenotype and breast and ovarian cancer risk [23-26].

Literature data suggest that *RAD51* paralogues such as *RAD51C* involved in homologous recombination mu-

tations may be associated with ovarian cancer predisposition [27].

However, no association was detected between ovarian cancer risk and *RAD51* polymorphism [14, 28]. Auren et al. reported that 135G>C and 172G>T variants of *RAD51* are not correlated with ovarian carcinoma [14]. Similar results were obtained by Webb et al. in the Australian population [28].

By contrast, in the Polish population, the variability of DNA repair gene *RAD51* may play a role in ovarian cancer risk. In our earlier study we found that 135C allele frequency is highest in the ovarian cancer group, suggesting a possible significant role of *RAD51* 135G>C polymorphism in ovarian cancer occurrence [13]. In the Polish population, C/C genotype of *RAD51* polymorphism slightly increased the risk of ovarian cancer patients. However, a small number of patients were included in that study. Therefore we suggested that further studies, conducted on a larger group, were needed to clarify this point.

The current study was performed on an ethnically homogeneous population of 210 women with ovarian cancer. The study revealed no significant association between ovarian carcinoma and 172G>T polymorphism. However, the results for women from Poland indicated that the second investigated polymorphism, 135G>C, is significantly associated with ovarian cancer. Our results for this polymorphism showed a significant association between the C-allele carriers (C/C genotype) and ovarian cancer. We identified the combined genotype of C135C-G172G, C135C-G172T and C135C-T172T that was associated with ovarian cancer risk and may have an impact on identification of a high-risk population.

The relationship between the 172G>T and 135G>C polymorphisms of the *RAD51* gene and clinical-pathological characteristics of ovarian cancer patients was also studied. Ovarian cancer cases were divided according to histological grade and tumour histology. We did not find any association between FIGO staging or tumour size and either polymorphism of the *RAD51* gene.

Our results should be treated as preliminary, because the number of patients and controls enrolled in our study does not guarantee high statistical power. However, we feel that our findings may be an important step towards appreciating the role of *RAD51* in the pathogenesis of ovarian cancer and further studies on this subject.

In conclusion, the results obtained suggest the potential role of the *RAD51* 135G>C polymorphism in ovarian cancer occurrence in Poland.

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Address for correspondence

Beata Smolarz MD, PhD
Laboratory of Molecular Genetics
Department of Pathology
Institute of Polish Mother's
Memorial Hospital
Rzgowska 281/289
93-338 Lodz, Poland
tel. +48 42 271 11 12
e-mail: smolbea@wp.pl