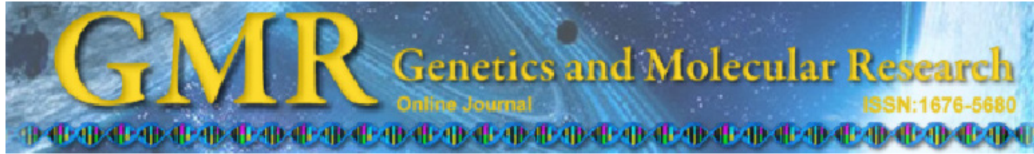

Association among *XRCC1*, *XRCC3*, and *BLHX* gene polymorphisms and chromosome instability in lymphocytes from patients with endometriosis and ovarian cancer

M.S. Monteiro[1], D.B. Vilas Boas[2], C.B. Gigliotti[2] and D.M.F. Salvadori[1]

[1]Laboratório de Toxicogenômica & Nutrigenômica,
Departamento de Patologia, Faculdade de Medicina de Botucatu,
Universidade Estadual Paulista, Botucatu, SP, Brasil
[2]Centro de Ciências da Saúde, Universidade Sagrado Coração,
Bauru, SP, Brasil

Corresponding author: M.S. Monteiro
E-mail: magalysmo@gmail.com

Genet. Mol. Res. 13 (1): 636-648 (2014)
Received January 11, 2013
Accepted May 23, 2013
Published January 28, 2014
DOI http://dx.doi.org/10.4238/2014.January.28.9

ABSTRACT. Endometriosis is a complex disease that has both benign and malignant characteristics. It affects 5-10% of women of reproductive age. Studies have demonstrated the existence of common genetic changes in endometriosis and ovarian cancer, suggesting a possible association between these 2 diseases. However, the mechanisms that lead to the development of cancer from endometriosis remain unknown. In this study, we evaluated 3 groups of women: 72 patients with endometriosis, 70 with ovarian cancer, and 70 healthy individuals (controls). Repair (*XRCC1* codons 194 and 399, *XPD* codons 312 and 751, and *XRCC3* codon 241)- and metabolism (*BLHX* codon 443)-related gene polymorphisms were analyzed using the polymerase chain reaction-restriction fragment length polymorphism technique; the efficiency of DNA damage repair was analyzed *in vitro*

 ©FUNPEC-RP www.funpecrp.com.br

in lymphocytes exposed to bleomycin. The logistic regression model was used to evaluate key associations. The results showed an increased average of chromosome breakage in bleomycin-treated lymphocytes from patients with endometriosis and ovarian cancer compared with healthy women. We also detected significant association between *XRCC1*, *XRCC3*, and *BLHX* polymorphisms and a high frequency of chromosomal damage. Women with endometriosis or ovarian cancer may have an altered mechanism of DNA repair, and these defects may be related to a higher incidence of ovarian cancer.

Key words: Cancer; Chromosome aberration; DNA repair genes; Molecular epidemiology

INTRODUCTION

Endometriosis is characterized by the growth of endometrial tissue outside of the uterine cavity, which often results in pelvic pain, infertility, dysmenorrheal, and dyspareunia. The most common sites for these growths are the ovaries, pelvic peritoneum, and uterus-sacral ligament, but growth can also occur at other sites. This disease is considered benign, although it has malignant characteristics such as rapid invasive growth and metastatic potential (Melin et al., 2006). Literature reports have shown an association between endometriosis or uterine leiomyomas and an increased risk for cancer. Some authors have suggested that the ovary is the principal organ with evidence of a relationship between endometriosis and cancer. It is estimated that the occurrence of ovarian cancer is 0.7 to 5% among all cases of endometriosis (Brinton et al., 2005; Melin et al., 2006; Prowse et al., 2006). Ovarian cancer can affect post-menopausal women and represents 30% of female genital cancers and 50% of deaths due to gynecological cancer. This malignancy is associated with a high mortality rate, mainly due to the difficulty related to an early diagnosis. The absence of symptoms leads most patients to seek specialized services during late clinical stages of the disease (Fehrmann et al., 2007). Recent data have demonstrated the presence of common mutations in the *PTEN*, *P53*, and *BCL2* genes in ovarian cancers and in adjacent endometriotic lesions, thus suggesting a possible malignant genetic transition spectrum (Nezhat et al., 2008).

Individual genetic variability (through single nucleotide polymorphisms) is an important factor in some diseases. Although the functional significance of individual genetic variability is not always known, studies have associated genotypic variants with specific diseases, particularly cancers, and with responses to environmental mutagens and carcinogens (Au and Salama, 2005). Nevertheless, to our knowledge, studies addressing a possible relationship among genetic polymorphisms, endometriosis, and ovarian cancer are rare. Hsieh et al. (2008) and Camargo-Kosugi et al. (2009) showed associations between endometriosis and *XRCC4* (codon 247, promoter 1394) polymorphisms and between endometriosis and the *P27* (codon 109) polymorphism, respectively. Costa et al. (2007) also demonstrated an association of *XPD* codon 312 and 751 polymorphisms with an increased susceptibility to ovarian cancer.

Polymorphisms in DNA repair genes are related to various types of cancer (Au et al., 2003). However, the functions of these polymorphic proteins in carcinogenesis have not yet been elucidated. Because changes in DNA repair mechanisms in patients with endometriosis may be associated with a higher predisposition to ovarian cancer, we investigated the

frequency of chromosomal breaks after bleomycin (BLM)-induced DNA damage *in vitro* in patients with this pathology. BLM is an antibiotic with radiomimetic action that is widely used in toxicogenic studies to induce DNA strand breaks to evaluate DNA repair efficiency (Hsu et al., 1989). This BLM sensitivity test has been used as a model to estimate the risk for cancer development (Wu et al., 1996).

The present study was also performed to investigate the relationship between several gene polymorphisms (*XRCC1*, *XPD*, *XRCC3*, and *BLHX*) and endometriosis and ovarian cancer susceptibility. *XRCC1* encodes a key protein in the base excision repair pathway, which is an important mechanism for the repair of single-strand breaks. Polymorphisms in this gene lead to a decreased ability to repair DNA (Smedby et al., 2006). Two polymorphisms in *XRCC1*, $Arg^{194}Trp$ and $Arg^{399}Gln$, have been implicated in the onset of lung (Ito et al., 2004), bladder (Matullo et al., 2005), breast (Duell et al., 2001), esophageal (Xing et al., 2002), and skin (Nelson et al., 2002) cancers. *XPD* is also involved in DNA repair, specifically in nucleotide excision repair, which is one of the most versatile repair systems; it functions in various types of DNA lesions such as UV-induced pyrimidine dimers and adducts generated by the formation of covalent bonds between DNA bases and hydrocarbons. Polymorphisms in *XPD*, namely the $Lys^{751}Gln$ and $Asp^{312}Asn$ genotypes, are associated with the onset of breast cancer (Dufloth et al., 2005). *XRCC3*, another DNA repair gene, is required for homologous recombination repair and the repair of chromosomal double-strand breaks induced by normal metabolism or exposure to ionizing radiation (Pierce et al., 1999). Carriers of the variant allele *XRCC3* $Thr^{241}Met$ exhibit a significant association with higher levels of DNA adducts, which indicates that this polymorphism is linked to the DNA repair capacity (Melin et al., 2006). Finally, BLM hydrolase (*BLHX*) encodes a protease that is involved in the metabolic inactivation of BLM. It has been shown that the *BLHX* $Val^{443}Iso$ variant results in a modification of the C-terminal region of the enzyme (Bromme et al., 1996).

MATERIAL AND METHODS

Study population and blood sampling

Peripheral blood samples were collected from 212 women: 72 with a positive diagnosis for endometriosis (laparoscopic diagnosis), 70 with ovarian cancer (histological diagnosis), and 70 healthy women (controls). The groups were matched based on age and smoking habits. Women with endometriosis or ovarian cancer were recruited at the Hospital Amaral Carvalho (Jaú, SP, Brazil). The control group was recruited at the Clinical Education for Health (Sagrado Coração University, USC, Bauru, SP, Brazil). All of the study subjects answered a questionnaire to determine hereditary disease predispositions. This study was approved by the Brazilian Ethics Committee for Human Research (CEPFHAC 40/07; CEPUSC 39/07), and informed written consent was obtained from each subject.

Peripheral blood (10 mL) was collected from all women: 5 mL (in heparin) was used in the BLM test, and the remaining 5 mL [in ethylenediaminetetraacetic acid (EDTA)] was used for DNA extraction and genotyping.

BLM test (toxicogenic study)

Lymphocyte cultures were based on the protocol of Wu et al. (2005), with minor modi-

fications. Two cultures from each individual were prepared simultaneously; 1 was treated with BLM. Briefly, 500 µL blood collected in a heparin tube was added to a vial containing 5 mL medium (RPMI-1640, PB-MAX Karyotyping Medium, Invitrogen, Carlsbad, CA, USA) and incubated at 37°C for 72 h. Five hours before harvesting, BLM (Blenoxane, Bristol Laboratories, Princeton, NJ, USA) was added to the sample at a final concentration of 0.03 U/mL. Four hours later, 100 µL 0.016% colchicine (Sigma, Saint Louis, MO, USA) was added. The cultures were homogenized and centrifuged at 1000 rpm for 10 min. The supernatant was discarded, 8 mL 0.075 M KCl (37°C) was added, and the samples were maintained in a water bath (37°C) for 30 min. The cell suspensions were then centrifuged, the supernatant was removed, and 6 mL cold fixation solution (3:1 acetic acid/methanol) was added to the cell pellet. This last step was performed twice. The slides were stained with Giemsa (Merck, Darmstadt, Germany) diluted in phosphate Sӧresen buffer (pH 6.8; Invitrogen) for 5 min. Fifty metaphases from each culture (with and without BLM treatment) were analyzed under a light microscope (100X magnification), and the chromatid breaks were scored. Chromosomes or chromosome groups in which breaks occurred were also assessed. Individuals with values greater than 0.80 breaks per cell were considered to be sensitive to BLM, and values greater than 1.00 indicated hypersensitivity to BLM (Hsu et al., 1989).

DNA extraction and genotyping

Genomic DNA was isolated from whole blood cells (collected using vacutainers containing EDTA) using standard techniques (Miller et al., 1988). Six polymorphic markers were genotyped using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method.

XPD, *XRCC1*, *XRCC3*, and *BLHX* genes

Amplification of the DNA fragments containing *XPD* $Lys^{751}Gln$ and $Asp^{312}Asn$, *XRCC1* $Arg^{399}Gln$ and $Arg^{194}Trp$, *XRCC3* $Thr^{241}Met$, and *BLHX* $Val^{443}Iso$ was performed by PCR (Perkin Elmer thermal cycler). All PCRs comprised a total volume of 50 µL, containing approximately 100 ng genomic DNA, 1.25 U Taq DNA polymerase, 1X PCR buffer, 2.0 mM $MgCl_2$, 0.2 mM dNTPs, and 0.2 mM of each primer. The primer sequences are described in Au et al. (2003) and Tuimala et al. (2002).

The amplification protocol for *XRCC1* codon 194 consisted of an initial denaturation step at 95°C for 2 min, followed by 35 cycles at 95°C for 15 s, 57°C for 45 s, and 72°C for 45 s, with a final extension step at 72°C for 5 min. The amplification conditions for *XRCC1* codon 399 were an initial denaturation step at 95°C for 2 min, followed by 40 cycles at 94°C for 15 s, 55°C for 30 s, and 72°C for 45 s, with a final extension step at 72°C for 5 min. *XPD* codon 312 was amplified using an initial denaturation step at 94°C for 2 min, followed by 35 cycles at 94°C for 30 s, 60°C for 30 s, and 68°C for 1 min, with a final extension at 68°C for 10 min. For *XPD* codon 751, the initial denaturation was performed at 95°C for 2 min, followed by 40 cycles at 94°C for 15 s, 67°C for 30 s, and 72°C for 45 s, with a final extension step at 72°C for 5 min. For *XRCC3* codon 241, the initial denaturation was performed at 94°C for 3 min, followed by 35 cycles at 95°C for 1 min, 60°C for 1 min, and 72°C for 1 min, with a final

extension step at 72°C for 5 min. For *BLHX* codon 443, the initial denaturation was performed at 95°C for 2 min, followed by 35 cycles at 94°C for 30 s, 58°C for 30 s, and 72°C for 15 s, with a final extension step at 72°C for 5 min.

Aliquots of the amplified products were digested with *Pvu*II (*XRCC1* codon 194), *Nci*I (*XRCC1* codon 399), *Sty*I (*XPD* codon 312), *Pst*I (*XPD* codon 751), *Nco*I (*XRCC3* codon 241), and *Mfe*I (*BLHX* codon 443). The digestion products were subjected to electrophoresis on 1 and 3% agarose gels and stained with ethidium bromide. The stained gels were visualized under UV light and photographed using an AlphaImager for subsequent analysis. The gel electrophoresis results after digestion were as follows: *XRCC1* 194 Arg allele with a 490-bp band and Trp allele with 294- and 196-bp bands; *XRCC1* 399 Arg allele with 159- and 89-bp bands and Gln allele with a 248-bp fragment; *XPD* 312 homozygous Asp allele with 2 bands (507 and 244 bp), the homozygous Asn/Asn genotype with 3 bands (474, 244, and 33 bp), and heterozygous Asp/Asn with 4 bands (507, 474, 244, and 33 bp); *XPD* 751 Lys allele with a 161-bp band and Gln allele with 120- and 41-bp bands; *XRCC3* 241 Thr allele with a single 136-bp product and Met allele with 97- and 39-bp bands; and *BLHX* 443 Val with a 130-bp band and the Iso allele with 106- and 24-bp bands.

Statistical analysis

Data were analyzed using a multiple logistic regression model (SAS version 9.1.3 for Windows) to determine associations among the studied groups with respect to tobacco habits and cancer history and to evaluate interactions between genotypes and the frequency of breaks per cell in cultures treated or not treated with BLM. A negative binomial distribution was used to compare the values determined for breaks per cell in cultures with and without BLM treatment. The Hardy-Weinberg equilibrium was assessed using the Pearson χ^2 test. Allele frequencies were calculated according to Rodriguez et al. (2009).

RESULTS

Table 1 shows the characteristics of the study population. Significant associations were detected between endometriosis and dysmenorrhea (P = 0.0025) and between dyspareunia (P = 0.0036) and irregular menstrual cycles (P = 0.0036) in endometriosis patients. A past smoking habit (>5 years) was associated with higher frequencies of BLM-induced chromosomal breaks only in ovarian cancer patients (P = 0.0384). These patients also demonstrated an association between spontaneous chromosomal breaks and dysmenorrhea (P = 0.0385). No other associations were detected among the characteristics surveyed and spontaneous chromosomal breaks in the study groups.

The average breaks per cell in cultures treated or not treated with BLM are presented in Table 2. Untreated cultures demonstrated the highest frequency of chromatid breaks in patients with endometriosis. However, a significant difference was detected only between patients with endometriosis *vs* those with ovarian cancer (χ^2 = 4.64, P = 0.0313). BLM induced a significant increase in strand breaks in all 3 groups (P < 0.01), and the frequency differences among them were statistically significant (endometriosis *vs* control, χ^2 = 35.78, P < 0.0001; cancer *vs* control, χ^2 = 10.35, P = 0.0013; cancer *vs* endometriosis, χ^2 = 7.80, P = 0.0052).

Table 1. Frequencies (means ± SD) of chromosome breaks and demographic and clinical characteristics of the study population.

	N	Controls		N	Endometriosis patients		N	Ovarian cancer patients	
		Chromatid breaks			Chromatid breaks			Chromatid breaks	
		No BLM	With BLM		No BLM	With BLM		No BLM	With BLM
Age (years)									
≤35	27	0.01 ± 0.02	0.45 ± 0.21	29	0.03 ± 0.05	0.79 ± 0.26	10	0.00 ± 0.01	0.57 ± 0.24
>35	43	0.02 ± 0.04	0.54 ± 0.26	43	0.02 ± 0.03	0.78 ± 0.32	60	0.02 ± 0.03	0.65 ± 0.24
Dysmenorrhea									
Yes	29	0.02 ± 0.03	0.54 ± 0.25	39[a]	0.03 ± 0.04	0.79 ± 0.26	18	0.03 ± 0.04[b]	0.67 ± 0.26
No	41	0.02 ± 0.03	0.48 ± 0.24	33	0.03 ± 0.03	0.77 ± 0.34	52	0.01 ± 0.03	0.63 ± 0.23
Dyspareunia									
Yes	10	0.02 ± 0.04	0.48 ± 0.23	27	0.02 ± 0.03	0.77 ± 0.27	10	0.01 ± 0.03	0.57 ± 0.24
No	60	0.02 ± 0.03	0.51 ± 0.25	45	0.03 ± 0.04	0.79 ± 0.31	60	0.01 ± 0.03	0.65 ± 0.24
Menstrual cycle									
Regular cycle	57	0.02 ± 0.03	0.50 ± 0.25	44	0.03 ± 0.04	0.79 ± 0.31	58	0.01 ± 0.03	0.65 ± 0.24
Irregular cycle	13	0.03 ± 0.04	0.52 ± 0.26	28	0.03 ± 0.03	0.77 ± 0.27	12	0.02 ± 0.03	0.60 ± 0.22
Smoker									
Yes	10	0.02 ± 0.03	0.54 ± 0.18	14	0.04 ± 0.06	0.80 ± 0.24	10	0.00 ± 0.01	0.48 ± 0.21
Never	55	0.02 ± 0.03	0.51 ± 0.26	52	0.03 ± 0.03	0.76 ± 0.30	55	0.01 ± 0.03	0.66 ± 0.23
Ex-smoker									
<5 years	3	0.01 ± 0.01	0.41 ± 0.18	2	0.00 ± 0.00	1.00 ± 0.57	0	0.00 ± 0.00	0.00 ± 0.00
>5 years	2	0.00 ± 0.00	0.25 ± 0.01	4	0.01 ± 0.01	0.87 ± 0.33	5	0.04 ± 0.03	0.73 ± 0.32
Cancer history in family									
Yes	32	0.02 ± 0.03	0.48 ± 0.21	39	0.02 ± 0.04	0.72 ± 0.26	35	0.02 ± 0.03	0.68 ± 0.26
No	38	0.02 ± 0.03	0.53 ± 0.28	33	0.03 ± 0.04	0.86 ± 0.32	35	0.01 ± 0.03	0.60 ± 0.21
Endometriosis									
Yes	0	-	-	72	0.03 ± 0.04	0.78 ± 0.29	3	0.00 ± 0.00	0.56 ± 0.25
No	70	0.02 ± 0.03	0.51 ± 0.25	0	-	-	67	0.02 ± 0.03	0.65 ± 0.24

BLM = bleomycin treatment. [a]Positive association between dysmenorrhea and endometriosis; [b]positive association between dysmenorrhea and chromatid breaks.

Table 2. Frequencies of chromatid breaks in lymphocytes cultured with or without bleomycin (BLM) treatment.

Groups	Number of individuals	Age (means ± SD)	Cells scored	Breaks/cell	
				No BLM	With BLM
Control	70	41.0 ± 14.5	50	0.02 ± 0.03	0.51 ± 0.25[a]*
Endometriosis patients	72	38.1 ± 10.2	50	0.03 ± 0.04[#]	0.78 ± 0.29[b]*
Ovarian cancer patients	70	53.9 ± 15.0	50	0.01 ± 0.03	0.64 ± 0.24[c]*

BLM - 0.03 U/mL (final concentration); *P < 0.01 (compared with “No BLM”); [#]P < 0.05 (compared with “No BLM” cancer patients), [a,b,c]P < 0.01 (different letters denote statistical significance for each row in the column “With BLM”).

The distribution of genotypes was in Hardy-Weinberg equilibrium in the endometriosis group. In ovarian cancer patients, *XPD* codon 751 and *BLHX* genotypes were not in Hardy-Weinberg equilibrium (χ^2 = 6.313, P = 0.012 and χ^2 = 15.706, P = 0.00007, respectively). Similarly, *BLHX* genotypes were not in Hardy-Weinberg equilibrium in the control group (χ^2 = 12.857, P = 0.0003). When the frequencies of gene polymorphisms were compared among the 3 groups, a statistically significant difference was detected only for *BLHX*. The Val/Val variant occurred more frequently in individuals with endometriosis than in those in the ovarian cancer and control groups (P = 0.026). Furthermore, the frequency of the Val/Iso genotype was low (P = 0.009) in endometriosis patients (Table 3).

No associations were detected between the frequencies of chromosomal breaks and genotypes within the study groups (ovarian cancer, endometriosis, and control). However, an

Table 3. Frequencies of *XRCC1* (Arg^{194}Trp; Arg^{399}Gln), *XPD* (Asp^{312}Asn; Lys^{751}Gln), *XRCC3* (Thr^{241}Met), and *BLHX* (Val^{443}Iso) genotypes in the study population.

Polymorphisms	Genotypes	Controls [N (%)]	Control allele frequencies	Endometriosis patients [N (%)]	Endometriosis allele frequencies	Ovarian cancer patients [N (%)]	Cancer allele frequencies	P value
XRCC1	Arg/Arg	57 (81.4%)	C: 0.90	65 (90.3%)	C: 0.83	61 (87.1%)	C: 0.94	P = 0.2991
codon 194	Arg/Trp	12 (17.1%)	T: 0.10	6 (8.3%)	T: 0.17	9 (12.9%)	T: 0.06	P = 0.2894
(C>T exon 6)	Trp/Trp	1 (1.4%)		1 (1.4%)		0		P = 0.6078
XRCC1	Arg/Arg	35 (50%)	G: 0.71	43 (59.7%)	G: 0.77	35 (50%)	G: 0.70	P = 0.4054
codon 399	Arg/Gln	30 (42.8%)	A: 0.29	25 (34.7%)	A: 0.23	28 (40%)	A: 0.30	P = 0.6012
(G>A exon 10)	Gln/Gln	5 (7.2%)		4 (5.6%)		7 (10%)		P = 0.5977
XPD	Arg/Arg	41 (58.5%)	A: 0.73	37 (51.4%)	A: 0.73	33 (47.1%)	A: 0.68	P = 0.3919
codon 312	Arg/Gln	20 (28.7%)	G: 0.27	30 (42.2%)	G: 0.27	29 (41.4%)	G: 0.32	P = 0.1846
(A>G exon 23)	Gln/Gln	9 (12.8%)		4 (5.7%)		8 (11.5%)		P = 0.3024
XPD	Arg/Arg	37 (52.8%)	A: 0.74	33 (45.8%)	A: 0.69	33 (47.1%)	A: 0.73	P = 0.6744
codon 751	Arg/Gln	30 (42.8%)	C: 0.26	33 (45.8%)	C: 0.31	36 (51.4%)	C: 0.27	P = 0.5869
(A>C exon 23)	Gln/Gln	3 (4.4%)		6 (8.4%)		1 (1.5%)		P = 0.1490
XRCC3	Thr/Thr	32 (45.7%)	C: 0.69	37 (51.4%)	C: 0.74	32 (45.7%)	C: 0.69	P = 0.7955
codon 241	Thr/Met	33 (47.1%)	T: 0.31	32 (44.4%)	T: 0.26	33 (47.1%)	T: 0.31	P = 0.5782
(C>T exon 7)	Met/Met	5 (7.2%)		3 (4.2%)		5 (7.2%)		P = 0.1539
BLHX	Val/Val	28 (40.0%)[a]	A: 0.70	41 (56.9%)[b]	A: 0.77	25 (35.7%)[a]	A: 0.68	P = 0.0262
codon 443	Val/Iso	42 (60.0%)[a]	G: 0.30	29 (40.3%)[b]	G: 0.23	45 (64.3%)[a]	G: 0.32	P = 0.0089
(A>G exon 11)	Iso/Iso	0		2 (2.8%)		0		P = 0.1404

[a,b]Different letters indicate statistically significant differences among values in the line.

©FUNPEC-RP www.funpecrp.com.br

association was detected between the gene variant and the frequency of chromosome breaks induced by BLM when the total population was considered. Women carrying the *XRCC1* codon 194 homozygous genotype Arg/Arg exhibited a greater number of breaks than did those carrying the Arg/Trp and Trp/Trp genotypes (P = 0.0395). In contrast, women carrying Arg/Arg at codon 399 exhibited a lower frequency of breaks compared with those with the Arg/Gln and Gln/Gln genotypes (P < 0.0001). Furthermore, a higher frequency of chromosome breaks was detected in BLM-untreated lymphocytes from subjects with Gln/Gln compared with those with the Arg/Arg and Arg/Gln genotypes (P = 0.0007; Table 4). Conversely, individuals carrying the *BLHX* Val/Val genotype presented fewer spontaneous breaks per cell (P < 0.0001). Similarly, this genotype was also associated with a lower frequency of chromosome breaks after BLM treatment (Table 4). *XRCC3* codon 241 variants presented a higher frequency of BLM-induced chromosome breaks in individuals with Thr/Thr compared with those with the Thr/Met and Met/Met genotypes (P = 0.0005).

Table 4. Frequencies of chromatid breaks for lymphocytes exposed or unexposed to bleomycin (BLM) *in vitro* according to the genotypes of the study population.

Genotypes	Chromatid breaks (mean ± SD)[1]					
	Controls		Endometriosis patients		Ovarian cancer patients	
	No BLM	With BLM	No BLM	With BLM	No BLM	With BLM
XRCC1 (Arg^{194}Trp)	0.02 ± 0.03	0.51 ± 0.24	0.03 ± 0.04	0.78 ± 0.28	0.02 ± 0.03	0.64 ± 0.24
Arg/Arg	(57)	(57)	(62)	(61)	(61)	(61)
Arg/Trp + Trp/Trp	0.02 ± 0.03	0.50 ± 0.29	0.04 ± 0.05	0.80 ± 0.42	0.00 ± 0.00	0.60 ± 0.24
	(13)	(13)	(7)	(7)	(9)	(9)
XRCC1 (Arg^{399}Gln)	0.02 ± 0.04	0.48 ± 0.26	0.03 ± 0.03	0.76 ± 0.24	0.01 ± 0.02	0.59 ± 0.23
Arg/Arg	(35)	(35)	(40)	(39)	(35)	(35)
Arg/Gln + Gln/Gln	0.02 ± 0.03	0.54 ± 0.23	0.03 ± 0.05	0.82 ± 0.36	0.02 ± 0.04	0.68 ± 0.24
	(35)	(35)	(29)	(29)	(35)	(35)
XPD (Asp^{312}Asn)	0.02 ± 0.03	0.48 ± 0.24	0.03 ± 0.04	0.79 ± 0.31	0.02 ± 0.04	0.68 ± 0.23
Asp/Asp	(41)	(41)	(35)	(35)	(33)	(33)
Asp/Asn + Asn/Asn	0.02 ± 0.03	0.55 ± 0.26	0.03 ± 0.04	0.78 ± 0.28	0.01 ± 0.03	0.60 ± 0.24
	(29)	(29)	(33)	(33)	(37)	(37)
XPD (Lys^{751}Gln)	0.02 ± 0.03	0.46 ± 0.23	0.03 ± 0.04	0.83 ± 0.31	0.02 ± 0.03	0.68 ± 0.24
Lys/Lys	(37)	(37)	(31)	(30)	(33)	(33)
Lys/Gln + Gln/Gln	0.02 ± 0.03	0.55 ± 0.26	0.02 ± 0.04	0.75 ± 0.28	0.01 ± 0.03	0.61 ± 0.23
	(33)	(33)	(38)	(38)	(37)	(37)
XRCC3 (Thr^{241}Met)	0.02 ± 0.03	0.55 ± 028	0.03 ± 0.03	0.77 ± 0.32	0.02 ± 0.04	0.69 ± 0.23
Thr/Thr	(34)	(34)	(35)	(35)	(32)	(34)
Thr/Met + Met/Met	0.02 ± 0.03	0.47 ± 0.21	0.02 ± 0.04	0.79 ± 0.27	0.01 ± 0.02	0.60 ± 0.24
	(36)	(36)	(34)	(33)	(38)	(36)
BLHX (Val^{443}Iso)	0.02 ± 0.03	0.50 ± 0.27	0.02 ± 0.02	0.73 ± 0.27	0.01 ± 0.02	0.62 ± 0.20
Val/Val	(28)	(28)	(39)	(38)	(25)	(28)
Val/Iso + Iso/Iso	0.02 ± 0.03	0.51 ± 0.23	0.04 ± 0.05	0.85 ± 0.32	0.02 ± 0.04	0.65 ± 0.26
	(42)	(42)	(30)	(30)	(45)	(42)

[1]Fifty cells per individual were scored. [2]Number of individuals is shown in parentheses.

DISCUSSION

Cancer development is usually associated with cumulative changes in the DNA of normal cells. Recent data demonstrated, for example, an association between *PTEN*, *P53*, and *BCL2* gene mutations and human endometriosis and ovarian cancer (Nezhat et al., 2008). According to the authors, these mutations might correlate with a genetic transition to malignancy. Nevertheless, the pathogenesis of endometriosis and ovarian cancer remains to be clarified.

Chromosomal aberrations have usually been investigated in patients with cancer or other related pathologies (Mateuca et al., 2006; Bonassi et al., 2008). In the present study, we observed an increased number of chromatid breaks in patients with endometriosis compared with those with ovarian cancer. Endometriosis is considered an important site of origin for ovarian cancer, and many molecular events observed in endometriosis (*PTEN* silencing, *P53* alteration, and *KRAS* mutations) occur during ovarian cancer development [reviewed by Mandai et al. (2009)]. Prowse et al. (2006) analyzed the association between endometriosis and ovarian cancers using 82 microsatellite markers spanning the genome, to examine the loss of heterozygosity (LOH) in coexisting disease samples. Sixty-three LOH events were observed in carcinoma samples, and among these, 22 events were detected in the corresponding endometriosis samples. In each case, the same allele was lost in the endometriosis and cancer samples. Therefore, the authors suggested that endometriosis and cancer are related events.

Analyses of chemically induced chromosomal breaks in cultured blood lymphocytes have been used as an indirect tool to evaluate the efficiency of DNA repair systems. Numerous epidemiological studies suggested that sensitivity to mutagens is a genetic factor in a variety of epithelial cancers (Wu et al., 2007). A study of twins (148 monozygotic and 82 dizygotic pairs) confirmed this association and provided a heritability estimate of 40.7% (Wu et al., 2006). In the present study, we observed that the mean number of chromatid breaks induced by BLM was significantly higher in patients with endometriosis and ovarian cancer than in controls. This result is consistent with that reported by Lin et al. (2003), who analyzed the frequency of breaks induced by the same mutagen in the peripheral blood lymphocytes of 46 women with endometriosis and 19 controls.

To investigate the association between DNA repair capability and an increased risk for ovarian cancer, we performed a molecular epidemiology evaluation. We analyzed the frequencies of polymorphisms in 3 DNA repair-related genes (*XRCC1*, *XPD*, and *XRCC3*) and investigated the relationship among these gene variants and the mean number of chromatid breaks. No association was detected between these gene variants and endometriosis or ovarian cancer. Although the distribution of *XPD* codon 751 genotypes was not in Hardy-Weinberg equilibrium in our highly selective population of ovarian cancer patients, we do not believe that the significance of our investigation was affected.

Our data highlighted the interactions between some gene polymorphisms and the mean number of chromosome breaks. A significant relationship was observed between *XRCC1* codon 194 genotypes and BLM-induced chromosome damage. Likewise, Wang et al. (2003) also showed that individuals carrying the *XRCC1* Arg/Arg variant have significantly higher levels of breaks induced by this mutagen compared with those carrying other allelic variants. These findings may be biologically relevant because the *XRCC1* polymorphism at codon 194 occurs in the N-terminal protein-protein interaction domain that is important for the recognition by DNA polymerase β. Both *XRCC1* and *POLB* are involved in maintaining chromosomal stability, and indicators of genetic damage such as elevated levels of sister chromatid exchange have been associated with *XRCC1* and *POLB* deficiency (Horton et al., 2008). In addition to the association with a decreased ability to repair DNA (Smedby et al., 2006), *XRCC1* polymorphisms have been linked to increased susceptibility to breast (Duell et al., 2001) and skin (Nelson et al., 2002) tumors. Importantly, we noted 33 endometriosis patients carrying the Arg/Arg genotype with reported family histories of pancreas, kidney, leukemia, prostate, liver, lung, intestine, stomach, bladder, breast, neck and head, ovarian, and uterine cancers; among the ovarian cancer patients, 30 reported previous histories of skin, esophageal, leukemia, pros-

tate, breast, ovarian, uterine, kidney, liver, lung, intestine, stomach, bladder, neck, and head cancers. A previous incidence of familial cancer was lower in the control group, in which 22 individuals reported a family history of skin, lung, breast, uterine, intestine, esophageal, leukemia, stomach, neck, and head cancer. For the *XRCC1* codon 399 polymorphism, although some studies have demonstrated associations between the Arg/Gln and Gln/Gln genotypes and the occurrence of bladder (Melin et al., 2006), breast (Duell et al., 2001), esophageal (Xing et al., 2002), and skin (Nelson et al., 2002) cancers, we did not find any association between these variants and ovarian cancer in our study population. However, we detected a significant relationship between the Arg/Gln and Gln/Gln genotypes and an increased frequency of BLM-induced chromatid breaks as well as between the Gln allele and spontaneous breaks per cell. Our results are consistent with other studies that demonstrated associations between the Gln399 allele and sensitivity to mutagens such as BLM and aflatoxin B1 (Wang et al., 2003; Melin et al., 2006). Nevertheless, no relationship between *XRCC1* codon 399 and 194 variants and sensitivity to BLM has been detected in healthy individuals (Tuimala et al., 2002).

Although our analysis showed an association between the Lys/Gln and Lys/Lys genotypes and a family history of cancer in the subjects, we did not observe associations between *XPD* codon 312 and 751 polymorphisms and endometriosis or ovarian cancer, or with the frequencies of chromosome breaks in lymphocytes that were treated or not treated with BLM *in vitro*. Our results are inconsistent with a previous report that demonstrated an association between *XPD* codon 312 and an increased susceptibility to ovarian cancer during early stages of the disease (Costa et al., 2007). In addition, an interaction between the *XPD* codon 312 Asp/Asn and Asn/Asn genotypes and X-ray- and UV-light-induced chromosome aberrations in healthy individuals has also been reported (Au et al., 2003). However, a different study did not detect a significant relationship between these polymorphisms and X-ray-induced chromatid breaks in healthy subjects (Lunn et al., 2000). With respect to *XPD* codon 751 polymorphisms, the data are also conflicting. While Melin et al. (2006) reported a reduced capacity for DNA repair in healthy 751 Lys homozygous individuals who had never smoked, Duell et al. (2000) did not find such an association. In summary, these data indicate a necessity for further studies to elucidate the role of *XPD* in induced mutations.

For the *XRCC3* codon 241 variants, we again did not detect a significant association with endometriosis or ovarian cancer. Similar results have been reported by Lopez-Cima et al. (2007), who found that polymorphisms in this gene are not associated with the onset of cancer. In contrast, in the present study, we observed a significant interaction between *XRCC3* codon 241 variants and the frequency of induced chromatid breaks. Individuals carrying the Thr/Thr genotype exhibited higher values for BLM-induced breaks compared with those carrying the Thr/Met genotype. However, there was no association between spontaneous chromosome damage and the genotypic variants. While Au et al. (2003) reported that the *XRCC3* Met allele is associated with increased X-ray-induced deletions in healthy individuals, Angelini et al. (2008) did not find a significant association between *XRCC3* codon 241 polymorphisms and the frequency of BLM-induced micronuclei in healthy subjects.

In the present study, *BLHX* Val/Val and Val/Iso were the only polymorphisms that were differentially distributed among the 3 groups. Patients with endometriosis presented a higher frequency of Val/Val and a lower frequency of Val/Iso genotypes compared with the control and ovarian cancer groups. Papassotiropoulos et al. (2000) described an association between *BLHX* variants (Iso/Iso genotype) and Alzheimer's disease. Subsequently, the Iso/Iso genotype was also associated with a reduced survival and a higher prevalence of early relapses

 ©FUNPEC-RP www.funpecrp.com.br

in testicular cancer patients treated with BLM (de Haas et al., 2008). We also detected an association between the Val/Iso genotype and an increased number of BLM-induced chromosome breaks. Similar results have been reported previously (Maffei et al., 2008). Healthy subjects carrying the Val/Val genotype showed lower frequencies of BLM-induced micronuclei compared with those carrying Val/Iso or Iso/Iso genotypes. In contrast, Tuimala et al. (2002) reported that smokers carrying the Val/Val genotype presented a greater number of BLM-induced chromosomal breaks. *BLHX* variants of codon 443 map to the C-terminal region of the encoded protein, and a deletion of 18 amino acids in this region has been associated with a reduced aminopeptidase activity, thereby impairing BLM inactivation. Therefore, the substitution of Val for Ile at this polymorphic site may increase the chromosome damage induced by BLM in lymphocytes (Maffei et al., 2008).

Nevertheless, chromosomal instability induced by BLM is likely a multigenic process that involves both mismatch repair- and metabolism-related genes. In addition to strengthening the use of the BLM assay for the detection of abnormal DNA repair systems in healthy and diseased individuals, our data confirmed the influence of *XRCC1* codon 194, *XRCC3* codon 241, and *BLHX* codon 443 polymorphisms on the sensitivity to this mutagen in peripheral lymphocytes.

Endometriosis patients may present abnormal DNA repair mechanisms, and such deficiencies may be related to the development of ovarian cancer. It is also plausible that variations or mutations of *XRCC1* and *XRCC3* affect the ability to repair DNA damage, thus facilitating the development of cancer in healthy individuals who are exposed to mutagens. However, further studies are necessary to elucidate the association among polymorphisms in DNA repair genes, chromosomal instability, and endometriosis and ovarian cancer.

ACKNOWLEDGMENTS

We are particularly grateful to Dr. Lenira Maria Queiroz Mauad and Ana Martam B.A. Prado Auler (Hospital Amaral Carvalho, Jaú, São Paulo, Brasil) for assistance with selecting patients and collecting blood samples, respectively. Research supported by Fundação de Amparo à Pesquisa do Estado de São Paulo - FAPESP (Grant #2006/60417-9).

REFERENCES

Angelini S, Kumar R, Carbone F, Bermejo JL, et al. (2008). Inherited susceptibility to bleomycin-induced micronuclei: correlating polymorphisms in GSTT1, GSTM1 and DNA repair genes with mutagen sensitivity. *Mutat. Res.* 638: 90-97.

Au WW and Salama SA (2005). Use of biomarkers to elucidate genetic susceptibility to cancer. *Environ. Mol. Mutagen.* 45: 222-228.

Au WW, Salama SA and Sierra-Torres CH (2003). Functional characterization of polymorphisms in DNA repair genes using cytogenetic challenge assays. *Environ. Health Perspect.* 111: 1843-1850.

Bonassi S, Norppa H, Ceppi M, Stromberg U, et al. (2008). Chromosomal aberration frequency in lymphocytes predicts the risk of cancer: results from a pooled cohort study of 22,358 subjects in 11 countries. *Carcinogenesis* 29: 1178-1183.

Brinton LA, Sakoda LC, Sherman ME, Frederiksen K, et al. (2005). Relationship of benign gynecologic diseases to subsequent risk of ovarian and uterine tumors. *Canc. Epidemiol. Biomarkers Prev.* 14: 2929-2935.

Bromme D, Rossi AB, Smeekens SP, Anderson DC, et al. (1996). Human bleomycin hydrolase: molecular cloning, sequencing, functional expression, and enzymatic characterization. *Biochemistry* 35: 6706-6714.

Camargo-Kosugi CM, da Silva ID, Sato H, D'Amora P, et al. (2009). The V109G polymorphism in the p27 gene is associated with endometriosis. *Eur. J. Obstet. Gynecol. Reprod. Biol.* 145: 180-183.

Costa S, Pinto D, Pereira D, Vasconcelos A, et al. (2007). Importance of *Xeroderma pigmentosum* group D polymorphisms in susceptibility to ovarian cancer. *Canc. Lett.* 246: 324-330.

de Haas EC, Zwart N, Meijer C, Nuver J, et al. (2008). Variation in bleomycin hydrolase gene is associated with reduced survival after chemotherapy for testicular germ cell cancer. *J. Clin. Oncol.* 26: 1817-1823.

Duell EJ, Wiencke JK, Cheng TJ, Varkonyi A, et al. (2000). Polymorphisms in the DNA repair genes XRCC1 and ERCC2 and biomarkers of DNA damage in human blood mononuclear cells. *Carcinogenesis* 21: 965-971.

Duell EJ, Millikan RC, Pittman GS, Winkel S, et al. (2001). Polymorphisms in the DNA repair gene XRCC1 and breast cancer. *Canc. Epidemiol. Biomarkers Prev.* 10: 217-222.

Dufloth RM, Costa S, Schmitt F and Zeferino LC (2005). DNA repair gene polymorphisms and susceptibility to familial breast cancer in a group of patients from Campinas, Brazil. *Genet. Mol. Res.* 4: 771-782.

Fehrmann RS, Li XY, van der Zee AG, de Jong S, et al. (2007). Profiling studies in ovarian cancer: a review. *Oncologist* 12: 960-966.

Horton JK, Watson M, Stefanick DF, Shaughnessy DT, et al. (2008). XRCC1 and DNA polymerase beta in cellular protection against cytotoxic DNA single-strand breaks. *Cell Res.* 18: 48-63.

Hsieh YY, Bau DT, Chang CC, Tsai CH, et al. (2008). XRCC4 codon 247*A and XRCC4 promoter -1394*T related genotypes but not XRCC4 intron 3 gene polymorphism are associated with higher susceptibility for endometriosis. *Mol. Reprod. Dev.* 75: 946-951.

Hsu TC, Johnston DA, Cherry LM, Ramkissoon D, et al. (1989). Sensitivity to genotoxic effects of bleomycin in humans: possible relationship to environmental carcinogenesis. *Int. J. Canc.* 43: 403-409.

Ito H, Matsuo K, Hamajima N, Mitsudomi T, et al. (2004). Gene-environment interactions between the smoking habit and polymorphisms in the DNA repair genes, APE1 Asp148Glu and XRCC1 Arg399Gln, in Japanese lung cancer risk. *Carcinogenesis* 25: 1395-1401.

Lin J, Zhang X and Chen Y (2003). Mutagen sensitivity as a susceptibility marker for endometriosis. *Hum. Reprod.* 18: 2052-2057.

López-Cima MF, González-Arriaga P, García-Castro L, Pascual T, et al. (2007). Polymorphisms in XPC, XPD, XRCC1, and XRCC3 DNA repair genes and lung cancer risk in a population of northern Spain. *BMC Cancer* 7: 162.

Lunn RM, Helzlsouer KJ, Parshad R, Umbach DM, et al. (2000). XPD polymorphisms: effects on DNA repair proficiency. *Carcinogenesis* 21: 551-555.

Maffei F, Carbone F, Angelini S, Forti GC, et al. (2008). Micronuclei frequency induced by bleomycin in human peripheral lymphocytes: correlating BLHX polymorphism with mutagen sensitivity. *Mutat. Res.* 639: 20-26.

Mandai M, Yamaguchi K, Matsumura N, Baba T, et al. (2009). Ovarian cancer in endometriosis: molecular biology, pathology, and clinical management. *Int. J. Clin. Oncol.* 14: 383-391.

Mateuca R, Lombaert N, Aka PV, Decordier I, et al. (2006). Chromosomal changes: induction, detection methods and applicability in human biomonitoring. *Biochimie* 88: 1515-1531.

Matullo G, Guarrera S, Sacerdote C, Polidoro S, et al. (2005). Polymorphisms/haplotypes in DNA repair genes and smoking: a bladder cancer case-control study. *Canc. Epidemiol. Biomarkers Prev.* 14: 2569-2578.

Melin A, Sparén P, Persson I and Bergqvist A (2006). Endometriosis and the risk of cancer with special emphasis on ovarian cancer. *Hum. Reprod.* 21: 1237-1242.

Miller SA, Dykes DD and Polesky HF (1988). A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res.* 16: 1215.

Nelson HH, Kelsey KT, Mott LA and Karagas MR (2002). The XRCC1 Arg399Gln polymorphism, sunburn, and non-melanoma skin cancer: evidence of gene-environment interaction. *Canc. Res.* 62: 152-155.

Nezhat F, Datta MS, Hanson V, Pejovic T, et al. (2008). The relationship of endometriosis and ovarian malignancy: a review. *Fertil. Steril.* 90: 1559-1570.

Papassotiropoulos A, Bagli M, Jessen F, Frahnert C, et al. (2000). Confirmation of the association between bleomycin hydrolase genotype and Alzheimer's disease. *Mol. Psychiatr.* 5: 213-215.

Pierce AJ, Johnson RD, Thompson LH and Jasin M (1999). XRCC3 promotes homology-directed repair of DNA damage in mammalian cells. *Genes Dev.* 13: 2633-2638.

Prowse AH, Manek S, Varma R, Liu J, et al. (2006). Molecular genetic evidence that endometriosis is a precursor of ovarian cancer. *Int. J. Canc.* 119: 556-562.

Rodriguez S, Gaunt TR and Day IN (2009). Hardy-Weinberg equilibrium testing of biological ascertainment for Mendelian randomization studies. *Am. J. Epidemiol.* 169: 505-514.

Smedby KE, Lindgren CM, Hjalgrim H, Humphreys K, et al. (2006). Variation in DNA repair genes ERCC2, XRCC1, and XRCC3 and risk of follicular lymphoma. *Canc. Epidemiol. Biomarkers Prev.* 15: 258-265.

Tuimala J, Szekely G, Gundy S, Hirvonen A, et al. (2002). Genetic polymorphisms of DNA repair and xenobiotic-metabolizing enzymes: role in mutagen sensitivity. *Carcinogenesis* 23: 1003-1008.

©FUNPEC-RP www.funpecrp.com.br

Wang Y, Spitz MR, Zhu Y, Dong Q, et al. (2003). From genotype to phenotype: correlating XRCC1 polymorphisms with mutagen sensitivity. *DNA Repair* 2: 901-908.

Wu X, Zheng YL and Hsu TC (2005). Mutagen-induced chromatid breakage as a marker of cancer risk. *Methods Mol. Biol.* 291: 59-67.

Wu X, Spitz MR, Amos CI, Lin J, et al. (2006). Mutagen sensitivity has high heritability: evidence from a twin study. *Canc. Res.* 66: 5993-5996.

Wu X, Gu J and Spitz MR (2007). Mutagen sensitivity: a genetic predisposition factor for cancer. *Canc. Res.* 67: 3493-3495.

Wu XF, Spitz MR, Delclos GL, Connor TH, et al. (1996). Survival of cells with bleomycin-induced chromosomal lesions in the cultured lymphocytes of lung cancer patients. *Canc. Epidemiol. Biomarkers Prev.* 5: 527-532.

Xing D, Qi J, Miao X, Lu W, et al. (2002). Polymorphisms of DNA repair genes XRCC1 and XPD and their associations with risk of esophageal squamous cell carcinoma in a Chinese population. *Int. J. Canc.* 100: 600-605.

 ©FUNPEC-RP www.funpecrp.com.br