SINGLE NUCLEOTIDE POLYMORPHISM IN DNA BASE EXCISION REPAIR GENES XRCC1 AND hOGG1 AND THE RISK OF ENDOMETRIAL CARCINOMA IN THE POLISH POPULATION

HANNA ROMANOWICZ-MAKOWSKA 1, BEATA SMOLARZ 1, AMER HOULI 2, KRZYSZTOF SZYŁŁO 3

1Laboratory of Molecular Genetics, Department of Pathology, Institute of Polish Mother’s Memorial Hospital, Lodz, Poland
2Department of Gynaecology, Medical Centre Hospital, Głowno
3Department of Surgical Gynaecology, Institute of Polish Mother’s Memorial Hospital, Lodz, Poland

Background: Polymorphisms in the human oxoguanine glycosylase 1 (hOGG1) and X-ray repair cross-complementing 1 (XRCC1) genes have been extensively studied in the association with various human cancers such as endometrial cancer.

Material and methods: The genotype analysis of hOGG1 Ser326Cys and XRCC1 Arg399Gln gene polymorphisms for 150 endometrial cancer patients and 150 controls of cancer-free subjects, in the Polish population, were performed using PCR-based restriction fragment length polymorphism (PCR-RFLP).

Results: Although there were no significant (p > 0.05) differences in the frequencies of genotypes or alleles of hOGG1 genes between patients and controls, the frequency of the XRCC1 399Gln allele was significantly greater in endometrial cancer patients compared with controls (p = 0.033) with an odds ratio of 1.39 (95% confidence interval 0.99 to 1.95). The distributions of genotypes and alleles of the genes hOGG1 and XRCC1 were not significantly associated with different grades of endometrial cancer (p > 0.05).

Conclusion: In conclusion, these findings indicated that XRCC1 Arg399Gln polymorphism may be a genetic determinant for developing endometrial cancer. The hOGG1 Ser326Cys may not play an important role in susceptibility to endometrial cancer in Polish women.

Key words: endometrial cancer, hOGG1, XRCC1, polymorphism, PCR-RFLP.

Introduction

Endometrial cancer is one of the most common malignant neoplasms which appear in the uterine body. About 80% of cases are diagnosed after menopause. The highest incidence, estimated at 57-58 years, is moving to the 6th and 7th decade of life at present. Endometrial cancer is the fourth most common female carcinoma [1, 2].

Endometrial cancer oncogenesis is not a fully recognized process regarding many risk factors. Neoplasm genesis is a multi-stage process. Carcinogenic factors influencing our organism mostly do not cause neoplasm development directly, but they induce genesis of endogenous intermediates, for example reactive oxygen intermediates (ROI) or substances oxidized by ROI. These substances may damage the DNA structure and cause point or chromosomal mutations. Some of these mutations lead to cell neoplastic transformation and in consequence to neoplasm development. At each of these stages there are actions of some endogenous or exogenous anticarcinogenic factors (e.g. vitamins A, C, E, glutathione,
enzymes acting as free radical scavengers, DNA self-
repairing structures).

For repair of oxidative DNA damage, human cells are supported by five DNA repair systems: direct reversal, mismatch repair, double-strand break repair, nucleotide excision repair (NER) and base excision repair (BER) [3].

The human oxoguanine glycosylase 1 (hOGG1) and X-ray repair cross-complementing 1 (XRCC1) genes are key genes in the BER pathway [3, 4]. All oxidatively induced DNA lesions and single-strand breaks are repaired via the BER pathway [4]. XRCC1 and hOGG1 can be involved in the repair of DNA lesions, which are known to contribute to endometrial cancer.

There have been some reports about the relation between hOGG1 codon 326 and XRCC1 codon 399 polymorphisms and risk for several cancers [4-18]. The hOGG1 G>C transversion at position 1245 of the hOGG1 gene producing a Ser → Cys substitution at codon 326 (the Ser326Cys polymorphism) was associated with the risk of lung [7, 8], gastric [9] and larynx cancer [10]. On the other hand, hOGG1 326Cys allele plays a significant protective effect against breast cancer in European women [11].

Little is known about hOGG1 and XRCC1 polymorphism in endometrial cancer risk. In the available literature not many researchers have investigated the association of hOGG1 and XRCC1 polymorphism and endometrial carcinoma [20-22]. Therefore, the aim of this study was to determine the relationship between XRCC1 (Arg399Gln) and hOGG1 (Ser326Cys) and endometrial cancer.

Material and methods

Endometrial cancer patients

150 patients with a histologically proven diagnosis of endometrial cancer were included in the study. Table 1 shows characteristics of endometrial cancer subjects. Tumour tissues were obtained from women with endometrial carcinoma treated at the Department of Surgical Gynaecology, Institute of Polish Mother’s Memorial Hospital during 2004-2009. The endometrial cancer tissue samples were fixed routinely in formalin and embedded in paraffin. All tumours were graded according to the criteria of the International Federation of Gynaecology and Obstetrics (FIGO). DNA from normal endometrial tissue (n = 150) served as a control.

DNA isolation

Archival paraffin-embedded tumour sections on slides were deparaffinized in xylene and rehydrated in ethanol and distilled water. DNA was extracted from material using commercially available QIAmp DNA Mini Kit (Qiagen GmbH, Hilden, Germany) DNA purification kit according to the manufacturer’s instructions.

Determination of hOGG1 genotype

Polymorphism Ser326Cys of the hOGG1 gene was determined by PCR-RFLP, using primers (5’-GGAAGGTGCTTGGGGAAT-3’ and 5’-ACT- GTCACTAGTCTCACCAG-3’). The 25 μl PCR mixture contained about 100 ng of DNA, 12.5 pmol of each primer, 0.2 mmol/l of dNTPs, 2 mmol/l of MgCl2 and 1 U of Taq DNA polymerase. PCR products were electrophoresed in a 7% polyacrylamide gel (PAGE) and visualised by ethidium bromide staining. Only one 100-bp fragment was seen in subjects with the Cys/Cys genotype. In subjects with the Ser/Cys genotype, two bands of 100 and 200 bp were seen, whereas in those subjects homozygous for the Ser variant (Ser/Ser), only one 200-bp PCR fragment is seen. All PCR was carried out in a DNA
Thermal Cycler (GeneAmp PCR System 2400; Perkin-Elmer, Norwalk, CT, U.S.A.). After an initial denaturation at 95°C for 5 min, 35 cycles of amplification with denaturation at 95°C for 30 sec, annealing at 56°C for 30 sec, and extension at 72°C for 30 sec were performed, followed by a final extension step of 7 min at 72°C. The PCR product was digested overnight with 1 U of \( \text{TaqI} \) at 37°C.

**Determination of XRCC1 genotype**

Genotypic analyses of the XRCC1 gene were carried out by multiplex PCR-RFLP, using primers for codon 399 (5'-TTGTGCTTTCTCTGTGGCA-3' and 5'-TCCTCCAGCTTTTCTGATA-3'), which generate a fragment of 615 and 491 bp. Briefly, PCR was performed in 25 μl reaction buffer containing 12.5 pmol of each primer, 0.2 mmol/l of dNTPs, 3 mmol/l of MgCl₂, about 100 ng of DNA and 1 U of Taq DNA polymerase. The PCR products were digested overnight with 10 U of \( \text{MspI} \) at 37°C.

For codon 399, the presence of two bands of 375 and 240 bp, respectively, identifies the wild-type Arg allele, while the uncut 615 bp band identifies the mutant Gln allele (indicative of the absence of the \( \text{MspI} \) cutting site).

**Statistical analysis**

For each polymorphism, deviation of the genotype frequencies in the controls from those expected under Hardy-Weinberg equilibrium was assessed using the standard \( \chi^2 \)-test. Genotype frequencies in cases and controls were compared by \( \chi^2 \)-tests. The genotypic-specific risks were estimated as odds ratios (ORs) with associated 95% confidence intervals (CIs) by unconditional logistic regression. P-values < 0.05 were considered to be significant.

**Results**

To verify the association of risk and the genetic change in the base excision repair (BER) pathway in endometrial cancer development, the polymorphisms of XRCC1 and \( hOGG1 \) in patients and control groups were analysed.

The results of the genotypes of XRCC1 (Arg399Gln) and \( hOGG1 \) (Ser326Cys) in the endometrial cancer and control groups are shown in Table II. It can be seen from the table that there were significant differences (\( p < 0.05 \)) between the two investigated groups. The women with endometrial cancer showed an incidence of 52 and 48%, respectively, for the Arg and Gln allele of the XRCC1 gene, whereas the control group showed 65 and 34% for the same alleles. The frequencies of XRCC1-399Gln allele in the case group were higher than that of the control group (\( p < 0.05 \)). The variant 399Gln allele was significantly increased in endometrial cancer patients compared with the control group (OR = 1.39, 95% CI: 0.99-1.95; \( p = 0.033 \)) (Table II).

We did not find any significant differences for \( hOGG1 \) genotype frequencies in patients with cancer and controls (Table III). Additionally, there were no differences in the frequencies of alleles between both distributions (\( p > 0.05 \)).

To understand whether the genetic polymorphisms of XRCC1 (Arg399Gln) and \( hOGG1 \) (Ser326Cys) increased the risk of endometrial cancer development, the different genotypes and the tumour grade evaluated according to FIGO criteria were compared (Table III).

The histological grade was evaluated in all cases (\( n = 150 \)). 67 cases were grade I, 77 cases were grade II and 6 cases were grade III. Grade II and III were grouped together for the purposes of statistical analysis.

There were no significant differences between distributions of XRCC1-Arg399Gln and \( hOGG1 \)-Ser326Cys genotypes in subgroups assigned to histological grades (\( p > 0.05 \)) (Table IV).

No statistically significant differences were observed in the alleles or in the genotype frequencies of the XRCC1-Arg399Gln and \( hOGG1 \)-Ser326Cys gene polymorphisms between risk factors of endometrial cancer such as body mass index (BMI), hormone

**Table II. Distribution of XRCC1 genotype frequencies in patients with endometrial cancer and control group**

<table>
<thead>
<tr>
<th></th>
<th><strong>ENDOMETRIAL CANCER PATIENTS</strong> (( n = 150 ))</th>
<th><strong>CONTROLS</strong> (( n = 150 ))</th>
<th>OR (95% CI) a</th>
<th>p b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arg/Arg</td>
<td>41 (27)</td>
<td>64 (43)</td>
<td>0.64 (0.36-1.12)</td>
<td>0.158</td>
</tr>
<tr>
<td>Arg/Gln</td>
<td>73 (49)</td>
<td>68 (45)</td>
<td>1.07 (0.67-1.70)</td>
<td>0.8628</td>
</tr>
<tr>
<td>Gln/Gln</td>
<td>36 (24)</td>
<td>18 (12)</td>
<td>2.0 (0.93-4.29)</td>
<td>0.110</td>
</tr>
<tr>
<td>Arg</td>
<td>155 (52)</td>
<td>196 (65)</td>
<td>0.79 (0.58-1.07)</td>
<td>0.079</td>
</tr>
<tr>
<td>Gln</td>
<td>145 (48)</td>
<td>104 (34)</td>
<td>1.39 (0.99-1.95)</td>
<td>0.033</td>
</tr>
</tbody>
</table>

Data in boldface are statistically significant.

aCrude odds ratio (OR), 95% CI = confidence interval at 95%, \( \chi^2 \).
replacement therapy (HRT), uterine bleeding, endometrial transvaginal ultrasound, diabetes and hypertension and the women with endometrial cancer.

Discussion

The present study examined whether polymorphism in XRCC1-Arg399Gln and hOGG1-Ser326Cys gene is related to the development of endometrial cancer. In our present study, the polymorphism of X-ray repair cross complementary 1 (XRCC1), a major gene in the BER system, is associated with endometrial cancer, but the polymorphism of hOGG1 is not associated.

Although there are other SNPs in the XRCC1 gene, the three XRCC1 polymorphisms (Arg194Trp, Arg280His and Arg399Gln) have been evaluated as risk factors for cancers in a number of studies.

It was suggested that SNPs in the XRCC1 gene may alter the ability of XRCC1 to repair damaged DNA, especially SNPs at codon 399.

The XRCC1-Arg399Gln gene polymorphism has been studied as a risk factor for various cancers. XRCC1-Arg399Gln has been associated with increased risk for lung cancer [23, 24], head and neck cancer [25] and possibly stomach cancer [26].

In contrast, no increased risk was observed for bladder cancer [27], oesophageal cancer [28] and non-melanoma skin cancer [29].

In breast cancer no association between the XRCC1-399 Gln/Gln genotype and this carcinoma was found [30]. However, other studies showed an increased risk of breast cancer with this polymorphism [31-33].

In the literature little is known about XRCC1 Arg399Gln polymorphism in endometrial cancer risk.

Only De Ruyck et al. showed that SNPs in XRCC1 with a combination of different polymorphisms in DNA repair genes (XRCC3 and hOGG1) is associated with an enhanced clinical radiosensitivity in endometrial cancer patients treated with late radiotherapy (RT) [21].

In our work the 399Gln allele was associated with increased risk for the development of endometrial cancer compared with 399Arg. Our study demonstrated that the 399Gln allele may be a risk factor for this cancer in the Polish population. It is possible

Table III. Distribution of hOGG1 genotype frequencies in patients with endometrial cancer and control group

<table>
<thead>
<tr>
<th>hOGG1-Ser326Cys</th>
<th>ENDOMETRIAL CANCER PATIENTS (N = 150)</th>
<th>CONTROLS (N = 150)</th>
<th>OR (95% PU)*</th>
<th>p**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ser/Ser</td>
<td>94 (63)</td>
<td>105 (70)</td>
<td>0.89 (0.67-1.19)</td>
<td>0.458</td>
</tr>
<tr>
<td>Ser/Cys</td>
<td>46 (31)</td>
<td>39 (26)</td>
<td>1.18 (0.87-1.62)</td>
<td>0.271</td>
</tr>
<tr>
<td>Cys/Cys</td>
<td>10 (6)</td>
<td>6 (4)</td>
<td>1.66 (0.82-3.38)</td>
<td>0.154</td>
</tr>
<tr>
<td>Ser</td>
<td>234 (78)</td>
<td>249 (83)</td>
<td>0.94 (0.79-1.12)</td>
<td>0.497</td>
</tr>
<tr>
<td>Cys</td>
<td>66 (22)</td>
<td>51 (17)</td>
<td>1.27 (0.99-1.66)</td>
<td>0.057</td>
</tr>
</tbody>
</table>

*aCrude odds ratio (OR), 95% CI = confidence interval at 95%, bχ²

Table IV. Dependency of the distribution of genotype frequencies on the tumour grade in patients with endometrial cancer

<table>
<thead>
<tr>
<th>POLYMORPHISM</th>
<th>GRADE I (%) (N = 67)</th>
<th>GRADE II + III (%) (N = 83)</th>
<th>OR (95% PU)*</th>
<th>p**</th>
</tr>
</thead>
<tbody>
<tr>
<td>XRCC1-Arg399Gln</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arg/Arg</td>
<td>17 (25)</td>
<td>32 (38)</td>
<td>0.48 (0.24-0.94)</td>
<td>0.051</td>
</tr>
<tr>
<td>Arg/Gln</td>
<td>37 (55)</td>
<td>35 (42)</td>
<td>1.34 (0.77-2.3)</td>
<td>0.292</td>
</tr>
<tr>
<td>Gln/Gln</td>
<td>15 (20)</td>
<td>16 (18)</td>
<td>2.04 (0.84-4.90)</td>
<td>0.113</td>
</tr>
<tr>
<td>Arg</td>
<td>71 (52)</td>
<td>99 (60)</td>
<td>0.87 (0.67-1.12)</td>
<td>0.296</td>
</tr>
<tr>
<td>Gln</td>
<td>63 (48)</td>
<td>67 (40)</td>
<td>1.19 (0.90-1.56)</td>
<td>0.204</td>
</tr>
<tr>
<td>hOGG1-Ser326Cys</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ser/Ser</td>
<td>41 (61)</td>
<td>53 (64)</td>
<td>0.93 (0.62-1.41)</td>
<td>0.751</td>
</tr>
<tr>
<td>Ser/Cys</td>
<td>22 (33)</td>
<td>24 (29)</td>
<td>1.12 (0.73-1.72)</td>
<td>0.596</td>
</tr>
<tr>
<td>Cys/Cys</td>
<td>4 (6)</td>
<td>6 (7)</td>
<td>1.14 (0.46-2.79)</td>
<td>0.777</td>
</tr>
<tr>
<td>Ser</td>
<td>104 (77)</td>
<td>130 (78)</td>
<td>0.96 (0.76-1.23)</td>
<td>0.791</td>
</tr>
<tr>
<td>Cys</td>
<td>30 (23)</td>
<td>36 (22)</td>
<td>1.12 (0.79-1.58)</td>
<td>0.497</td>
</tr>
</tbody>
</table>

*aCrude odds ratio (OR), 95% CI = confidence interval at 95%, bχ²
that the presence of the 399Gln allele is in linkage disequilibrium with another, so far unknown, mutation located outside the coding region in the XRCC1 gene, which may be of importance for the XRCC1 concentration in plasma.

However, in our study we did not find any association between bOGG1 gene Ser326Cys polymorphisms and endometrial carcinoma occurrence. This is in line with the reports which indicate that the amino acid replacement of bOGG1-326Ser to Cys might lead to lack of association with risk of endometrial carcinoma [21, 22]. Krupa et al. suggested that the Ser326Cys polymorphism of the bOGG1 gene might not be directly involved in the development and/or progression of endometrial cancer in the Polish population; therefore, it may not be useful as an independent marker of this disease [22].

Moreover, we did not find any association of the SNPs in the patient group with cancer progression assessed by BMI, number of pregnancies, HRT, uterine bleeding, endometrial transvaginal ultrasound, diabetes and hypertension and the women with endometrial cancer.

Our results show that the polymorphism of the XRCC1 gene but not bOGG1 may be associated with the occurrence of endometrial cancer in Poland.

Therefore, we suggest that different DNA repair systems may play different roles in endometrial carcinoma. These repair systems could be the basis of future surveys. Further studies on the polymorphisms of other genes in NER, BER, or other DNA repair systems are necessary for the detection of a genetic predisposition to endometrial cancer formation and for the investigation of the roles of NER, BER, or other DNA repair genes in cancer formation. In conclusion, XRCC1 Arg399Glu is correlated with endometrial carcinoma and might become a potential marker for the prediction of endometrial carcinoma susceptibility. It also provides valuable insight into the pathogenesis of endometrial carcinoma.

References

Address for correspondence
Beata Smolarz
Laboratory of Molecular Genetics, Department of Pathology, Institute of Polish Mother’s Memorial Hospital, Rzgowska 281/289, 95-338 Łódź, Poland
tel. +48 42 271 20 71
e-mail: smolbea@wp.pl