Hanna Romanowicz-Makowska[1], Beata Smolarz[1], Marcin Makowski[2], Ireneusz Połać[2], Tomasz Pertyński[2]

Ser326Cys Polymorphism in DNA Repair Genes hOGG1 in Breast Cancer Women

[1]Laboratory of Molecular Genetics, Department of Pathology, Institute of Polish Mother's Memorial Hospital, Łódź,
[2]Department of Menopausal, Diseases, Institute of Polish Mother's Memorial Hospital, Łódź

Reduced DNA repair capacity can render a high risk of developing many types of cancer; including breast cancer. Polymorphisms in DNA repair genes may contribute the genetic instability and carcinogenesis. In the present work the distribution of genotypes and frequency of alleles of the Ser326Cys polymorphism of hOGG1 gene in breast cancer women were analysed. Blood samples were obtained from 100 women with breast cancer and control (n=106). The polymorphism was determined by PCR-RFLP methods. No association between Ser326Cys polymorphism of hOGG1 and breast cancer risk was observed. The distribution of the genotypes of the Ser326Cys polymorphism 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 ($p > 0.05$) in genotype distributions and allele frequencies between subgroups assigned to histological stage.

The results suggest that the Ser326Cys polymorphism of hOGG1 gene may not be linked with appearance and development of breast cancer in polish women.

Introduction

Breast cancer is a malignant (cancerous) growth that begins in the tissues of the breast. Over the course of a lifetime, one in eight women will be diagnosed with breast cancer. The genetics of mammary carcinogenesis has not been fully elucidated.

DNA repair plays an important role in tumor development. When DNA damage occurs, DNA repair pathways, cell cycle arrest, and apoptosis may be activated. Single nucleotide polymorphism (SNPs) in genes involved in DNA repair and cell cycle control can affect repair efficiency, increase cancer risk and significantly alter patient responses to cancer treatments [1, 4, 9].

There are five major DNA repairing mechanisms: base excision, nucleotide excision, mismatch repair, photoreactivation (utilizing near UV light as well as an appropriate enzyme system) and recombination repair. BER repairs damage to a single nucleotide caused by oxidation, alkylation, hydrolysis, or deamination. The base is removed with glycosylase and ultimately replaced by repair synthesis with DNA ligase.

Reactive oxygen species may be generated from estrogen metabolism through catechol estrogen redox cycling [10, 13]. If not quenched, these reactive oxygen species may cause oxidative DNA damage and increase breast cancer risk. It has been suggested that 8-hydroxyguanine, a major product of oxidative DNA damage, plays an important role in carcinogenesis given its abundant and highly mutagenic properties [6]. 8-Hydroxyguanine is subjected to base excision repair, especially via the 8-oxoguanine DNA glycosylase (hOGG1) catalyzing the release of 8-hydroxy-2'-deoxyguanosine and the cleavage of DNA at the AP site [2, 6]. A common functional polymorphism (Ser326Cys) in exon 7 of the *hOGG1* gene has been identified [8, 12]. The Cys allele exhibits reduced DNA repair activity [8] and has been reported to be associated with the risk of cancers of the lung, prostate, esophagus, stomach, and orolarynx [12]. Epidemiologic studies evaluating the *hOGG1* polymorphism in relation to breast cancer risk are few and the sample sizes were small [5, 11].

In the present study, the Ser326Cys polymorphism of *hOGG1* gene in breast cancer women was examined.

Materials and Methods

Patients

Blood samples were obtained from 100 postmenopausal women with node-negative (n=58) and node-positive (n=42) ductal breast carcinoma. No distant metastases were found in patients at the time of treatment. The patients ranged in age from 54 to 82 years (median age 58 years). The average tumour size was 20 mm (range 17-32 mm). All tumours were graded by a method based on the criteria of Scarff-Bloom-Richardson. There were 14 tumors of I grade, 64 of II grade and 22 of III grade in total. Blood samples (n=106) from age matched healthy women served as control.

DNA isolation

Genomic DNA was isolated from 200 μL of whole blood, using QIAamp DNA Blood Mini Kits (Qiagen GmbH, Hilden, Germany) according manufacturer instruction.

PCR-RFLP

Polymorphism Ser326Cys of the *hOGG1* gene was determined by PCR-RFLP, using primers (5'- GGAAGGTGCTTGGGGAAT-3' and 5'- ACTGTCACTAGTCTCACCAG- 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 $MgCl_2$ 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 *Sat*I at 37°C.

Statistical analysis

The allelic frequencies were estimated by gene counting and genotypes were scored. The observed numbers of each *hOGG1* 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.

Results

Table 1 shows Ser and Cys genotype distribution between breast cancer patients and control. Both distribution 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 Ser and Cys alleles between patients and controls.

Distribution of the Ser/Cys polymorphism as well as frequencies of the Ser and Cys alleles for node-positive and node-negative breast cancer patients are displayed in Table 2. 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 of Ser326Cys polymophism of *hOGG1* gene on the tumour stage evaluated according to Bloom-Scarf-Richardson criteria of patients with breast cancer was investigated (Table 3). There were no significant differences between distributions of genotypes in subgroups assigned to histological stage and the distribution predicted by Hardy-Weinberg equilibrium ($p > 0.05$). There were no differences in frequencies of the all alleles between subgroups either ($p > 0.05$).

Discussion

Base excision repair (BER) is a very important mechanism for repairing oxidative DNA damage. There are many enzymes involved in BER. Human oxoguanine glycosylase 1 (hOGG1) is enzyme gene of BER.

A C/G polymorphism at position 1245 in exon 7 of the *hOGG1* gene is associated with the substitution of cysteine for serine at codon 326 [8]. This genetic polymorphism is frequently observed in the Japanese population, in both healthy individuals and patients with lung cancer [8]. The same polymorphism is also observed, at a similar frequency, among European patients with head and neck tumors or kidney tumors [3]. Moreover, the allelic frequencies of *hOGG1* 1245G are higher in the Chinese population (64.1%), than in the Japanese (40.5 to 43.3%), European (40%), and Caucasian (24 to 26.5%) populations, suggesting ethnic variations [3, 7, 8].

TABLE 1
Distribution of Ser/Ser, Ser/Cys and Cys/Cys genotypes and frequencies of the Ser and Cys alleles of *hOGG1* polymorphism in patients with breast cancer and controls

	Breast cancer [n=100]		Control [n=106]	
	number	frequency	number	frequency
Ser/Ser	32	0.32	20	0.19
Ser/Cys	34	0.34	52	0.49
Cys/Cys	34	0.34	34	0.32
χ^2	1,764[a]		0,013	
Ser	98	0.49[b]	92	0.43
Cys	102	0.51[b]	120	0.57

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

TABLE 2
Distribution of Ser/Ser, Ser/Cys and Cys/Cys genotypes and frequencies of the Ser and Cys alleles of *hOGG1* polymorphism in patients with node-negative and node-positive breast cancer

	Node-negative [n=58]		Node-positive [n=42]	
	number	frequency	number	frequency
Ser/Ser	17	0.29	10	0.23
Ser/Cys	21	0.36	21	0.50
Cys/Cys	20	0.34	11	0.26
χ^2	0,371[a]		0,040	
Ser	55	0,47[b]	41[b]	0.49
Cys	61	0,53[b]	43	0.51

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

TABLE 3
Dependency of genotypes and frequencies of the alleles of *hOGG1* gene polymorphism on the tumour stage in patients with breast cancer[a]

stage[b]	I n=14		II n=64		III n=22	
	number	frequency	number	frequency	number	frequency
Ser/Ser	4	0,29	23	0,36	5	0,23
Ser/Cys	7	0,50	16	0,25	11	0,50
Cys/Cys	3	0,21	25	0,39	6	0,27
χ^2	0,003		0,999		0,076	
Ser	15	0,54[c]	62	0,48	21	0,48
Cys	13	0,46	66	0,52	23	0,52

[a]n = 100; [b]according to Scarf-Bloom-Richardson criteria; [c]$p > 0.05$ as compared with Hardy-Weinberg distribution

In this work conducted on 100 ductal breast carcinoma patients we did not find any correlation between Ser326Cys polymorphism and occurrence of cancer. Moreover we did not detect any significant difference between genotypes of node-positive and node-negative patient, that suggests a lack of association between polymorphisms and breast cancer invasiveness.

Our result was supported by the reports from a case-control study conducted in Korean and Japanese populations [5] and a nested case-control study conducted in Denmark [11]. The sample sizes of these two previous studies were small. The Ser326Cys polymorphism has been well documented to be related to major functional changes in the *hOGG1* gene [8]. The *hOGG1* gene has been well characterized and no other major functional single-nucleotide polymorphisms have been found in the Chinese population. Therefore, it is unlikely that other polymorphisms in this gene would be related to a substantial risk of breast cancer.

In summary, the functional Ser326Cys polymorphism in the *hOGG1* gene may not play a substantial role in the risk of breast cancer among polish women. Larger, well-designed functional and epidemiologic studies are needed to clarify these relationships, especially with respect to interactions with other DNA repair enzymes and interactions with environmental factors that increase carcinogenic load.

References

1. *Allan JM, Smith AG, Wheatley K:* Genetic variationin XPD predicts treatment outcome and risk of acute myeloid leukemia following chemotherapy. Blood 2004, 104, 3872-3877.
2. *Boiteux S, Radicella P:* The human OGG1 gene: structure, functions, and its implication in the process of carcinogenesis. Arch Biochem Biophys 2000, 377, 1–8.
3. *Blons H, Radicella JP, Laccourreye O, Brasnu D, Beaune P, Boiteux S, Laurent-Puig P:* Frequent allelic loss at chromosome 3p distinct from genetic alternations of the 8-oxoguanine DNA glycosylase 1 gene in head and neck cancer. Mol Carcinogen 1999, 26, 254 –260.
4. *Chang-Claude J, Popanda O, Tan XL:* Association between polymorphisms in the DNA repair genes XRCC1, APE1 and XPD and acute side effects of radiotherapy in breast cancer patients. Clin Cancer Res 2005, 11, 4802-4809.
5. *Choi JY, Hamajima N, Tajima K:* hOGG1 Ser326Cys polymorphism and breast cancer risk among Asian women. Breast Cancer Res Treat 2003, 79, 59–62.
6. *Floyd RA:* The role of 8-hydroxyguanine in carcinogenesis. Carcinogenesis 1990, 11, 1447–1450.
7. *Hardie LJ, Briggs JA, Davidson LA, Allan JM, King RFGJ, Williams GI, Wild CP:* The effect of hOGG1 and glutathione peroxidase I genotypes and 3p chromosomal loss on 8-hydroxydeoxyguanosine levels in lung cancer. Carcinogenesis 2000, 21, 167 –172.
8. *Kohno T, Shinmura K, Tosaka M:* Genetic polymorphisms and alternative splicing of the hOGG1 gene, that is involved in the repair of 8-hydroxyguanine in damaged DNA. Oncogene 1998, 16, 3219–3225.
9. *Price N:* Impact of genetic polymorphisms in DNA repair enzymes on drug resistance in lung cancer. Clin Lung Cancer 2004, 6, 79-82.
10. *Roy D, Liehr JG:* Estrogen, DNA damage and mutation. Mutat Res 1999, 424, 107–115.
11. *Vogel U, Nexo BA, Olsen A:* No association between OGG1 Ser326Cys polymorphism and breast cancer risk. Cancer Epidemiol Biomarkers Prev 2003, 12, 170–171.
12. *Weiss JM, Goode EL, Ladiges WC, Ulrich CM:* Polymorphic variation in hOGG1 and risk of cancer: a review of the functional and epidemiologic literature. Mol Carcinog 2005, 42, 127–141.
13. *Yoshie Y, Ohshima H:* Synergistic induction of DNA strand breakage by catechol-estrogen and nitric oxide: implications for hormonal carcinogenesis. Free Radic Biol Med 1998, 24, 341–348.

Address for correspondence and reprint requests to:
Hanna Romanowicz-Makowska,
Laboratory of Molecular Genetics, Department of Pathology
Institute of Polish Mother's Memorial Hospital
Ul. Rzgowska 281/289
93-338 Łódź
Email: smolbea@wp.pl