Article is available online at http://www.webio.hu/por/2005/11/1/0022

ARTICLE

Polymorphisms of the XRCC1 DNA Repair Gene in Head and Neck Cancer

Semra DEMOKAN,[1] Deniz DEMIR,[2] Yusufhan SUOGLU,[2] Erkan KIYAK,[2] Ugur AKAR,[3] Nejat DALAY[1]

[1]Oncology Institute, [2]Department of Otolaryngology, Istanbul Medical Faculty, [3]Department of Medical Biology, Istanbul Medical Faculty, Istanbul University, Istanbul, Turkey

Inherited polymorphisms in the genes controlling the cell cycle or functioning in the DNA repair mechanisms may impair their function and contribute to genetic susceptibility. Abnormalities in the DNA repair have been reported in head and neck cancer. The XRCC1 gene functions in single-strand break and base excision repair processes. In this study, two polymorphisms of the XRCC1 gene, Arg194Trp and Arg399Gln were investigated in 95 patients with head and neck carcinoma. The polymorphic regions were amplified by PCR followed by digestion with methylation-specific restriction enzymes, and analyzed electrophoretically. Genotype and allele frequencies were calculated, and association with cancer risk or clinical parameters was investigated. No association was observed between the genotypes and head and neck cancer for either polymorphism. Distribution of the alleles did not significantly differ between the patients and the control group. A significant association was only found for the Trp194 allele among the smoking individuals. Our data indicate that the Arg194Trp and Arg399Gln polymorphisms do not confer a significant risk for head and neck carcinogenesis. (Pathology Oncology Research Vol 11, No 1, 22–25)

Key words: polymorphism, XRCC1, head and neck cancer

Introduction

Head and neck cancer is the sixth most common cancer in the world. Tobacco and alcohol consumption are the main etiological factors in head and neck carcinogenesis.[6] Previous studies have shown that genetic susceptibility plays an important role in the risk of developing this disease.[2,6] Head and neck carcinogenesis is associated with abnormalities in DNA repair, apoptosis, carcinogen metabolism and cell-cycle control,[18,20,21,28] and is one of the best models to investigate the relationships between gene and environment.[11,14,17,27] DNA repair mechanisms function to maintain genome integrity by reducing the mutation rate of cancer-related genes.[2] There is considerable evidence that DNA repair capacity is genetically determined. It has been shown that reduction in this capacity is associated with increased risk of certain cancers. Thus, inherited polymorphisms in the DNA repair genes may impair their function and increase the risk of cancer.[3]

The X-ray repair cross complementary 1 (XRCC1) gene product is implicated in single-strand break repair and base excision repair mechanisms.[12,29] The XRCC1 protein interacts with poly(ADP-ribose) polymerase, DNA polymerase-β and DNA ligase-IIIα participating in the multistep base excision repair and removal of base adducts produced by oxidative damage, methylation, reduction or fragmentation.[4,5,12] Cells with a mutant XRCC1 gene have increased sensitivity to ultraviolet or ionizing radiation and alkylating agents. Two important polymorphisms leading to amino acid substitutions have been identified at the evolutionarily conserved regions of the XRCC1 gene.[22] The Arg194Trp polymorphism in exon 6, codon 194 results from C→T substitution. Another substitution (G→A) at codon 399 in exon 10 leads to the change of arginine to glutamine.[26]

The Arg194Trp substitution resides in the region separating the DNA polymerase-b and polyADP ribose polymerase-interacting domains. The codon 399 polymorphic site is located within the BRCT-1 domain, and is associat-

Received: Nov 24, 2004; *accepted:* Jan 5, 2005
Correspondence: Prof. Dr. Nejat DALAY, I.U. Oncology Institute, 34390 Capa, Istanbul, Turkey. Tel.: 90 212 5313100, fax: 90 216 3387809, e-mail: ndalay@yahoo.com

© 2005 Arányi Lajos Foundation

ed with significant reduction in the repair capacity. It has been suggested that polymorphisms at the active sites involved in protein-protein interactions may result in reduced efficiency to repair DNA damage and confer an increased risk to cancer.[14]

In this case-control study, we aimed to investigate the association between polymorphisms of the XRCC1 gene and risk of head and neck cancer or the clinical parameters.

Materials and Methods

Peripheral venous blood samples of 95 newly diagnosed patients (83 men and 12 women, mean age 59.6±12.5, range 12-84) with head and neck cancer, and of 98 healthy individuals (51 men and 47 women, mean age 47.2±8.8, range 30-76) were investigated. Eighty-four percent of the patients had squamous cell carcinoma. The remaining tumors were adenoma (n=5), mixed tumors (n=5), adenoid cystic carcinoma (n=3), Worthin tumors (n=2), and sarcoma (n=2). Thirteen percent had Stage I, 32% Stage II, 36% Stage III and 19% Stage IV disease. Metastasis was not present in 73 (77%) patients. Eighty-three percent of the patients and 40% of the control population were smokers. Twenty-eight patients consumed alcohol regularly.

DNA was isolated by phenol/chloroform extraction after overnight incubation with proteinase K at 37°C. The DNA samples were amplified by using two different primer pairs specific for the two polymorphic regions of XRCC1 gene. Primer sequences (Integrated DNA Technologies, Iowa, USA) were: exon 6, codon 194: 5'-GCC CCG TCC CAG GTA-3' forward and 5'-CCC AAG ACC CTT TCA CT-3' reverse; exon 10, codon 399: 5'-TGC TTT CTC TGT GTC CA-3' forward and 5'-TCC AGC CTT TTC TGA TA-3' reverse.

PCR reactions were performed in 50 ml 1x PCR buffer (MBI, Fermentas, Lithuania) containing 2 mM $MgCl_2$, 0.3 mM of each primer, 200 mM of each dNTP (MBI, Fermentas), 100 ng DNA and 2 U Taq polymerase (MBI, Fermentas). Following initial denaturation at 94°C for 5 minutes, amplification was performed by 32 cycles of denaturation at 94°C for 35 seconds, annealing at 62°C for 35 seconds, and extension at 72°C for 45 seconds. The reaction was terminated by extension at 72°C for 10 minutes. The PCR products were digested with 5 U MspI (MBI, Fermentas) at 37°C. Digested products were resolved on 2% agarose (Arg399Gln) or 8% polyacrylamide (Arg194Trp) gels containing 10% glycerol, 0.5x TEB buffer and 1:10 TEMED/10% ammonium-persulfate at 150 V for 3.5 h, and analyzed in a video gel documentation system (Vilber Lourmat, Cedex, France) after treating with ethidium bromide.

Odds ratios (OR) and 95% confidence intervals (CI) were calculated to evaluate statistical significance. The relative risk of HNSCC was calculated by using the Finetti case-control statistics program (http://ihg.gsf.de/cgi-bin/hw/hwa1.pl). To investigate the associations between the clinical parameters and genotypes, the Chi^2 and Fisher's exact tests were applied using the Practical Stats (www.unc.edu~preacher/index.htm) program package.

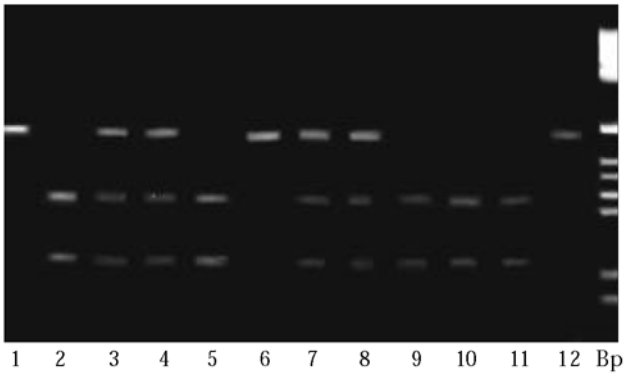

Figure 1. *Analysis of the Arg399Gln polymorphism by digestion of the amplified product with the restriction enzyme MspI. Lanes 2, 5, 9, 10 and 11: wild-type samples; lanes 1, 6 and 12: homozygous variant patients; lanes 3, 4, 7 and 8: heterozygotes; lane 13; molecular weight marker.*

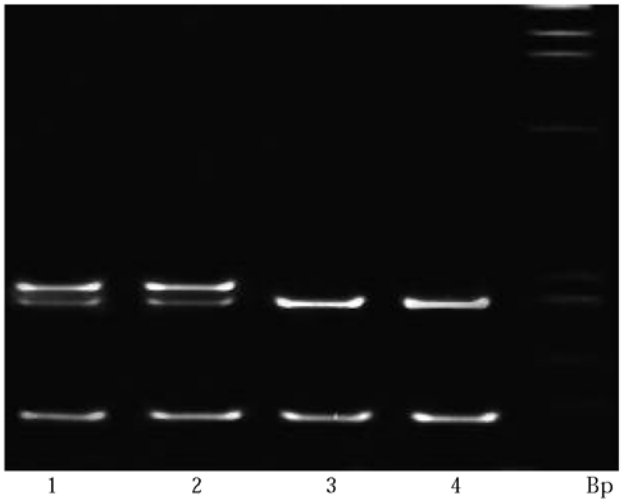

Figure 2. *Analysis of the Arg194Trp polymorphism. Lanes 1 and 2: heterozygous samples; lanes 3 and 4: wild-type samples.*

Results

XRCC1 Arg399Gln, XRCC1 Arg194Trp polymorphisms were investigated by PCR/RFLP. The recognition site of the restriction enzyme is present only in the arginine coding allele for the 399Gln polymorphism. Thus, after digestion, two fragments of 241 and 374 bp are obtained for the wild-type Arg/Arg genotype. The homozygous variant genotypes display only one fragment of 615 bp, and the heterozygotes display all three fragments (***Figure 1***).

For the Arg194Trp polymorphic site, after digestion with MspI, two fragments of 174 and 292 bp or 174 and

311 bp are obtained for the wild type (Arg/Arg) or homozygous variant (Gln/Gln) genotypes, respectively. Heterozygous individuals displayed all three fragments of 174, 292 and 311 bp (*Figure 2*).

The genotype frequencies and distribution of the alleles are given in *Table 1*. The data are consistent with the Hardy-Weinberg equilibrium. No significant difference was observed between the controls and patients for either polymorphism, although the risk associated with the Trp allele was close to statistical significance (Arg/Arg vs. Arg/Trp+Trp/Trp: OR (95%CI)=1.92 (0.83-4.43), p=0.12).

Association with the demographic parameters (gender, family history, alcohol and tobacco consumption) was also evaluated as a risk factor for the disease. Both alcohol (p=0.01; Chi^2=5.94; df=1) and tobacco consumption (p=0.000; Chi^2=25.85; df=1) were significant risk factors for SCCHN. Smoking patients had a higher risk of SCCHN than non-smoking patients (codon 194: Arg/Arg vs. Arg/Trp+Trp/Trp: Fisher's p=0.0047 and codon 399: Arg/Arg vs. Arg/Gln+Gln/Gln: Fisher's p=0.029). When smoking patients were compared with the smoking individuals in the control group with respect to the polymorphisms, a statistically significant association was found only for the Trp allele carriers (Arg/Arg vs. Arg/Trp+Trp/Trp: OR (95%CI)=5.2 (0.29-93.3)). The risk of head and neck cancer among this group was 5.2-fold higher. No such association was observed for the codon 399 polymorphism.

We also investigated the association between the homozygote variant genotype for one polymorphism and the homozygote wild-type of the other, and did not observe a meaningful correlation (399 Gln/Gln vs. 194 Arg/Arg, p=0.78, and 194 Trp/Trp vs. 399 Arg/Arg, p=0.72).

Table 1. **Genotype frequencies and allelic distributions**

XRCC1 polymorphisms	*SCCHN (n=95) n (%)*	*Control (n=98) n (%)*	*OR (95% CI)*	*P (Chi^2)*
Exon 10, codon 399				
Arg399Gln (G→A)				
Arg/Arg	42 (44)	39 (40)	1 (reference)	–
Arg/Gln	41 (43)	46 (47)	0.83 (0.45-1.52)	0.54 (0.37)
Gln/Gln	12 (13)	13 (13)	0.86 (0.35-2.10)	0.74 (0.11)
Arg/Gln+Gln/Gln	53 (56)	59 (60)	0.83 (0.47-1.48)	0.53 (0.39)
Alleles				
Gln	65 (34)	72 (37)	0.89 (0.6-1.36)	0.60 (0.27)
Arg	125 (66)	124 (63)	1 (reference)	–
Exon 6, codon 194				
Arg194Trp (G→T)				
Arg/Arg	78 (82)	88 (90)	1 (reference)	–
Arg/Trp	14 (15)	8 (8)	1.97 (0.79-4.96)	0.14 (2.15)
Trp/Trp	3 (3)	2 (2)	1.69 (0.28-10.39)	0.56 (0.33)
Arg/Trp+Trp/Trp	17 (18)	10 (10)	1.92 (0.83-4.43)	0.12 (2.37)
Alleles				
Trp	20 (11)	12 (6)	1.80 (0.86-3.80)	0.11 (2.46)
Arg	170 (89)	184 (94)	1 (reference)	–

Discussion

DNA repair mechanisms play an important role in maintaining genomic integrity in response to mutations, replication errors and environmental effects. The DNA repair enzyme, XRCC1 functions in the base excision repair of genomic damage caused by exposure to carcinogens such as tobacco or alcohol. Amino acid substitutions in the active protein binding domains may impair the efficiency to repair DNA damage and affect the function of this enzyme. Individual differences in the DNA repair capacity or metabolic activation and detoxification processes may affect the acquired host status and influence the risk of developing cancer. Association of the XRCC1 polymorphisms with different cancer types such as lung,[3,8] breast,[9,24] gastric,[25] colon,[1] head and neck cancers[17,26] and esophageal carcinomas[2 9] has been investigated. However, the results are not consistent. Our aim was to evaluate the association between the XRCC1 Arg399Gln and Arg194Trp polymorphisms and risk of SCCHN in the Turkish population.

In our study we did not observe a significant difference between Gln allele frequencies in the patients and the controls (Arg/Arg vs. Arg/Gln+Gln/Gln: OR (95%CI)=0.83 (0.47-1.48), p=0.53). Although some reports in the literature support an association between the Gln allele and other types of cancer,[8,9,23,26] our results are more in line with studies suggesting lack of association.[3,7,10,15]

The frequency of the Trp allele was not significantly different in the patients. In contrast to a report on patients with colon cancer,[1] these data are in concordance with studies in which the Arg194Trp polymorphism was not found to confer a significant risk for head and neck,[1 7] lung,[3,19] or breast[9] cancer in the Caucasian population. The conflicting results may stem from the complexity of cancer etiology with regard to exposure to car-

cinogens, DNA repair genotypes or other genetic factors, and to the small sample sizes.

On the other hand, we observed a highly significant positive association between the frequency of the variant alleles for both polymorphisms and the risk of head and neck cancer among the patients with regard to smoking. This data is consistent with a recent study on breast cancer,[9] and supports the role of tobacco consumption in the etiology of head and neck carcinoma.

It has been hypothesized that the homozygote variant genotype of one polymorphism is significantly associated with the wild-type homozygote of the other, e.g. subjects carrying the 194 Trp/Trp genotype had exclusively the 399 Arg/Arg genotype or vice versa.[29] In our study we did not observe such an association, nor has it been confirmed by any other study, suggesting that such an association is spurious.

Since polymorphisms in the XRCC1 gene have been linked to reduced capacity for removal of DNA damage, our results indicating lack of association between the polymorphisms and head and neck cancer may imply that the base excision pathway may not be involved in the repair of damaging effects playing a role in the initiation of head and neck carcinogenesis.

Acknowledgement

This work was supported by the Research Fund of Istanbul University (T-149/06032003). We thank to Prof. Dr. Thomas F. Wienker for providing the statistics program.

References

1. *Abdel-Rahman SZ, Soliman AS, Bondy ML, et al:* Inheritance of the 194Trp and the 399Gln variant alleles of the DNA repair gene XRCC1 are associated with increased risk of early-onset colorectal carcinoma in Egypt. Cancer Lett 159: 79-86, 2000
2. *Bohr VA:* DNA repair fine structure and its relations to genomic instability. Carcinogenesis 16: 2885-2892, 1995
3. *Butkiewicz D, Rusin M, Enewold L, et al:* Genetic polymorphisms in DNA repair genes and risk of lung cancer. Carcinogenesis 22: 593-597, 2001
4. *Caldecott KW, Aoufouchi S, Johnson P, Shall S:* XRCC1 polypeptide interacts with DNA polymerase beta and possibly poly (ADP-ribose) polymerase, and DNA ligase III is a novel molecular 'nick-sensor' in vitro. Nucleic Acids Res 24: 4387-4394, 1996
5. *Cappelli E, Taylor R, Cevasco M, et al:* Involvement of XRCC1 and DNA ligase III gene products in DNA base excision repair. J Biol Chem 272: 23970-23975, 1997
6. *Crowe DL:* Molecular pathology of head and neck cancer. Histol Histopathol 17: 909-914, 2002
7. *David-Beabes GL, London SJ:* Genetic polymorphism of XRCC1 and lung cancer risk among African-Americans and Caucasians. Lung Cancer 34: 333-339, 2001
8. *Divine KK, Gilliland FD, Crowell RE, et al:* The XRCC1 399 glutamine allele is a risk factor for adenocarcinoma of the lung. Mutat Res 461: 273-278, 2001
9. *Duell EJ, Millikan RC, Pittman GS, et al:* Polymorphisms in the DNA repair gene XRCC1 and breast cancer. Cancer Epidemiol Biomarkers Prev 10: 217-222, 2001
10. *Goode EL, Ulrich CM, Potter JD:* Polymorphisms in DNA repair genes and associations with cancer risk. Cancer Epidemiol Biomarkers Prev 11: 1513-1530, 2002
11. *Hu JJ, Mohrenweiser HW, Bell DA, et al:* Symposium Overview: Genetic polymorphisms in DNA repair and cancer risk. Toxicol Appl Pharmacol 185: 64-73, 2002
12. *Kubota Y, Nash RA, Klungland A, et al:* Reconstitution of DNA base excision-repair with purified human proteins: interaction between DNA polymerase beta and the XRCC1 protein. EMBO J 15: 6662-6670, 1996
13. *Lee SG, Kim B, Choi J, et al:* Genetic polymorphisms of XRCC1 and risk of gastric cancer. Cancer Lett 187: 53-60, 2002
14. *Miller MC, Mohrenweiser HW, Bell DA:* Genetic variability in susceptibility and response to toxicants. Toxicol Lett 120: 269-280, 2001
15. *Misra RR, Ratnasinghe D, Tangrea JA, et al:* Polymorphisms in the DNA repair genes XPD, XRCC1, XRCC3, and APE/ref-1, and the risk of lung cancer among male smokers in Finland. Cancer Lett 191:171-178, 2003
16. *Nelson HH, Kelsey KT, Mott LA, Karagas MR:* The XRCC1 Arg399Gln polymorphism, sunburn, and non-melanoma skin cancer: evidence of gene-environment interaction. Cancer Res 62:152-155, 2002
17. *Olshan AF, Watson MA, Weissler MC, Bell DA:* XRCC1 polymorphisms and head and neck cancer. Cancer Lett 178: 181-186, 2002
18. *Park JY, Muscat JE, Ren Q, et al:* CYP1A1 and GSTM1 polymorphisms and oral cancer risk. Cancer Epidemiol Biomarkers Prev 6: 791-797, 1997
19. *Ratnasinghe D, Yao SX, Tangrea JA, et al:* Polymorphisms of the DNA repair gene XRCC1 and lung cancer risk. Cancer Epidemiol Biomarkers Prev 10:119-123, 2001
20. *Scully C, Field JK, Tanzawa H:* Genetic aberrations in oral or head and neck squamous cell carcinoma (SCCHN): 1. Carcinogen metabolism, DNA repair and cell cycle control. Oral Oncol 36: 256-263, 2000
21. *Scully C, Field JK, Tanzawa H:* Genetic aberrations in oral or head and neck squamous cell carcinoma: 2. Chromosomal aberrations. Oral Oncol 36: 311-327, 2000
22. *Shen MR, Jones IM, Mohrenweiser H:* Nonconservative amino acid substitution variants exist at polymorphic frequency in DNA repair genes in healthy humans. Cancer Res 58: 604–608, 1998
23. *Shen H, Xu Y, Qian Y, et al:* Polymorphisms of the DNA repair gene XRCC1 and risk of gastric cancer in a Chinese population. Int J Cancer 88:601-606, 2000
24. *Shu XO, Cai Q, Gao YT, et al:* A population based case control study of the Arg399Gln polymorphism in DNA repair gene XRCC1 and risk of breast cancer. Cancer Epidemiol Biomarkers Prev 12: 1462-1467, 2003
25. *Stern MC, Umbach DM, van Gils CH, et al:* DNA repair gene XRCC1 polymorphisms, smoking, and bladder cancer risk. Cancer Epidemiol Biomarkers Prev 10:125-131, 2001
26. *Sturgis EM, Castillo EJ, Li L, et al:* Polymorphisms of DNA repair gene XRCC1 in squamous cell carcinoma of the head and neck. Carcinogenesis 20: 2125-2129, 1999
27. *Sturgis EM, Wei Q:* Genetic susceptibility-molecular epidemiology of head and neck cancer. Curr Opin Oncol 14: 310-317, 2002
28. *Taningher M, Malacarne D, Izzotti A, et al:* Drug metabolism polymorphisms as modulators of cancer susceptibility. Mutat Res 436: 227–261, 1999
29. *Xing D, QI J, Miao X, et al:* Polymorphisms of DNA repair genes XRCC1 and XPD and their associations with risk of esophageal squamous cell carcinoma in a Chinese population. Int J Cancer 100: 600-605, 2002