Association between XRCC3 Gene Polymorphism and the Risk of Head and Neck Squamous Cell Carcinoma

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Abstract

Capacity for DNA repair is essential in maintaining human cellular functions and homeostasis. Repair capacity can be altered by XRCC3 gene polymorphisms that affect an individual's susceptibility to carcinogenesis. The present study aimed to investigate the potential association between the XRCC3 C722T (rs861539) gene polymorphism and the risk of head and neck squamous cell carcinoma (HNSCC) in an Indonesian population. Using the polymerase chain reaction-restriction fragment length polymorphism method, the genomic DNA of 81 patients with oral squamous cell carcinoma and 95 healthy control participants was genotyped to identify the status of XRCC3 C722T polymorphism. For statistical analysis, chi-square test was applied. The TT genotype and T allele of the XRCC3 C722T polymorphism was significantly associated with the risk of HNSCC (*P*=0.001). The C722T (rs861539) polymorphism of the XRCC3 gene may be associated with the risk of HNSCC. Moreover, this polymorphism might be used as a predictive indicator of precancerous lesions and HNSCC in an Indonesian population.

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Introduction

Head and neck squamous cell carcinomas (HNSCCs) are often observed in patients with cancer after platinum-based chemotherapy.[1] Recently, there has been increasing evidence on the incidence of HNSCC in individuals in Indonesia.[2]

Genetic consideration influences the outcome of HNSCC. Among the genetic factors, DNA repair is important. That is, DNA repair pathways, including nucleotide excision repair, base excision repair, and double-strand break repair (DSBR), have an important role in maintaining genetic stability throughout the different pathways.[3] Among the various DNA repair pathways, DSBR plays a key role in X-ray repair cross-complementing group 3 (XRCC3) and polymorphism.[4,5] The survival of patients with HNSCC may be affected by DNA repair capacity.

Failure of DNA repair capacity causes DNA damage, which is important in carcinogenesis and in maintaining the integrity of cellular DNA.[6] Evidence from epidemiological studies has suggested that susceptibility to precancerous lesions and cancer is affected by both genetic and environmental factors.[7] Altered cancer susceptibility could arise due to polymorphisms in DNA repair genes, with the modified activity of corresponding proteins. Over 130 genes are involved in DNA repair through various pathways.[8]

Studies on the association between genes and the risk for cancer have mostly focused on single-nucleotide polymorphisms of the candidate genes. Such genes include those for DNA repair, which are increasingly assessed due to their essential role in maintaining the integrity of genomic DNA.[9] Even when the polymorphisms may be not be significantly associated with the risk of cancer, they can be more prevalent and can contribute to the risk of cancer at the population level.[10] Recent studies

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have confirmed the associations between DNA repair gene variants as well as oral, pharyngeal, and laryngeal cancer.[11]

Genes that influence DNA repair include *XRCC1*, *XRCC2*, *XRCC3*, *XPC*, *XPD/ERCC2*, *XPF*, and *RAD51.*[12,13] In particular, the XRCC3 gene is involved in the homologous recombination repair of double-strand breaks and cross-links.[14] A particular variant is a C to T substitution in exon 7 at position 18067 of XRCC3, which results in threonine to methionine substitution at codon 241, and this C722T (rs861539) polymorphism of XRCC3, or its TT genotype, has been associated with the increased risk of cancer, such as HNSCC.[15,16]

Recently, there has been increasing evidence showing that DNA repair capacity resulting from genetic polymorphisms of various DNA repair genes is associated with improved survival with platinum-based chemotherapy.[17] To the best of our knowledge, only few studies have examined the effect of these polymorphisms on the outcome of cancer in other populations. To determine the significance of these polymorphisms, we focused on the relationship between the different XRCC3 C227T (rs861539) genotypes and HNSCC susceptibility in an Indonesian population.

Methods

Study participants

This laboratory study conducted a descriptive analysis on 176 DNA samples extracted from the blood serum of 81 patients with HNSCC and 95 healthy control participants. This study was approved by the Ethical Committee of the University of Indonesia.

DNA Isolations

The DNA isolation procedures were taken from 3 mL of peripheral blood of the 176 subjects, placed in 15 mL tubes containing 9 mL of red blood lysis solution (1.45M NH4Cl, 5mM anhydrous EDTA, and 0.1M KHCO3) and incubated at room temperature for 10 min. The sample was then centrifuged at 1500 rpm for 10 min at room temperature, and the supernatant was removed to leave a precipitate of mononuclear leukocytes. These steps were repeated to obtain a white pellet and a supernatant containing no red blood cells. To this pellet 2 mL of cell lysis solution was added and pipetted until homogeneous and incubated in a water bath at 37 °C for 30-60 min until completely homogeneous. Then 1.3 mL of protein precipitation solution (5M ammonium acetate) was added, vortex mixed for 15-20 s and centrifuged at 3000 rpm for 15 min at 4 °C, producing a light brown precipitate (proteins) and the supernatant containing DNA. The supernatant was poured into a new Falcon tube with 2.3 ml of cold isopropanol. The tube was inverted up to 20-30 times until showing a collection of DNA strands. The supernatant was removed and 1.3 mL of 70% ethanol was added for washing, and the DNA solute on was centrifuged at 3000 rpm for 5 min at 4 ^{o}C. After discarding supernatant, the DNA was dried in open air by reversing the tube, then DNA was rehydrated with a solution of 200-300 uL TE (Tris-HCl EDTA) and incubated in a water bath at 37 ^{o}C for 2 h. The solution was transferred into 1.5 mL sterile microcentrifuge tubes and stored at -20 °C in the Oral Biology Laboratory of the Faculty of Dentistry, University of Indonesia.[18-20]

Genotyping

The polymerase chain reaction (PCR)-restriction fragment length polymorphism (RLFP) method was used to determine the genotypes of the C722T (rs861539) polymorphism of the XRCC3 gene. The primers used in this study were F:5'-GCCTGGTGGTCATCGCTC-3' and R:5'-ACAGGGCTCTGGAAGGCACTGCTCA-3'.[21] The PCR reaction was carried out in 20 μl of reaction volume containing 0.3 μl of genomic DNA, 10 μl of Taq polymerase (MyTaq), 0.5 μl of forward primers (IDT), 0.5 μl of reverse primers (IDT), and 8.7 μl of ddH_2O. Thermal cycling conditions for the fragment containing the XRCC3 gene were as follows: an initial denaturation at 94 °C for 3 min, followed by 30 cycles of 94 °C for 30 s at an annealing temperature of 57.7 °C for 30 s, and at 72 °C for 50 s. The final extension was performed at 72 °C for 7 min. The PCR products were electrophoresed in 1.5% agarose gel (Thermo Fisher Scientific) at 75 V, 400 mA, for 45 min with 50 bp DNA ladder and were visualized using Gel Doc.

Using the RLFP method, the resulting 136 bp PCR product (10 μl) was digested by 1 U of Nia III enzyme (Thermo Fisher Scientific) in 2 μl of 10x Buffer Tango and 5.9 ddH_2O, then

incubated at 37 °C for 16 h, and inactivated at 65 °C for 20 min. The RLFP products were electrophoresed in 2% agarose gel (Thermoscientific) set at 70 V, 400 mA, for 40 min with 50 bp DNA ladder, then stained with GelRed (Biotium Inc., the USA), and visualized using Gel Doc 200 (Bio-Rad, the USA).

The Nla III digestion yields fragments of 136 bp (homozygote CC: 35 and 101 bp [homozygote TT] or 35, 101, and 136 bp [heterozygote CT]) (Figure 1).

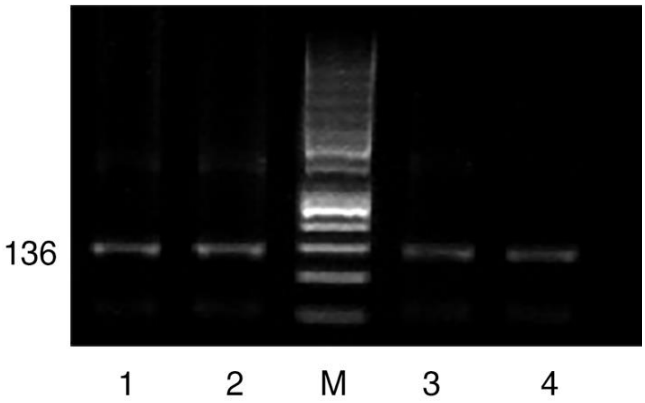

Figure 1. Representative PCR-RFLP results of the XRCC3 C227T polymorphism. Lanes 1, 2, 3, and 4: 136 bp bands for CC genotype; lane M: 50 bp ladder marker.

Statistical analysis

Statistical analyses were performed using the Statistical Package for the Social Science software version 25 (IBM, NY, USA). A chi-square test was used to analyze statistical difference between the control and experimental groups. Fisher's exact test was used to analyze the Hardy–Weinberg equilibrium. The results were expressed as odds ratio and 95% confidence interval. Statistical significance was set at $P<0.05$.

Results

This present study consisted of 176 participants from the Indonesian population, of which 81 presented with HNSCC and 95 were healthy controls. The genotype distribution between those with HNSCC and healthy controls for the XRCC3 C227T polymorphism was significantly different ($p<0.05$, chi-square test) (Table 1).

Group/type		HNSCC	Control	*P* value
Genotype	CC	36 (44.5)	74 (77.9)	0.001
	CT	4 (4.9)	2 (2.1)	
	TT	41 (50.6)	19 (20.0)	
	Total	81 (100)	95 (100)	
Allele	C	76 (46.9)	150 (78.9)	0.001
	T	86 (53.1)	40 (21.1)	

Table 1. Distribution of the genotypes and alleles of XRCC3 C227T polymorphism in the HNSCC and control groups.

Discussion

In this study, we assessed the XRCC3 gene polymorphism that may influence DNA repair capacity and its association with the outcome of HNSCC. The polymorphism chosen for this study has functional significance, and it may be responsible for a low DNA repair capacity phenotype that is a characteristic of patients with cancer.[22,23] The XRCC3 might be an independent prognostic factor of squamous cell carcinoma. Thus, DNA repair gene polymorphism affects the outcome of typical squamous cell carcinoma.

Genomic stability, integrity, and carcinogenesis in principle are caused by various DNA damage lesions. The homologous recombinant repair pathway plays an important role in repairing DSBR in mammalian cells, and the XRCC3 complex plays a role in end-joining reactions, and it may contribute to carcinogenesis.[24,25] In our study, we considered the role of XRCC3 C722T (rs186539) polymorphisms in the occurrence of HNSCC. Moreover, the CC genotype of XRCC3 was correlated to the increased risk of HNSCC in an Indonesian population.

Since genetic variation has caused different DNA repair capabilities in the human population, genetic polymorphism has an important role in cancer.[26] Individuals with these factors and with higher-stage disease may have too many genetic alterations during tumor growth that develop into malignancy.

We analyzed the association between polymorphisms of XRCC3 DNA repair genes and the outcome of HNSCC in an Indonesian population. Results showed that the XRCC3 C227T gene polymorphism may play an important role in the development of HNSCC. The XRCC3 gene may be a prognostic factor of HNSCC.

Conclusion

This study shows that the polymorphism of XRCC3 C722T gene was associated with the risk of HNSCC. This finding can be used as a predictive indicator of precancerous lesions and HNSCC patients.

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