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Short Communication

Polymorphisms of DNA repair genes are risk factors for prostate cancer

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ABSTRACT

DNA repair gene alterations have been shown to cause a reduction in DNA repair capacity. We hypothesised that DNA repair gene polymorphisms may be risk factors for prostate cancer (PC). To test this hypothesis, DNA samples from 165 cases of prostate cancer and healthy controls were analyzed by PCR-RFLP to determine the genotypic frequency of three DNA repair genes (*XRCC1*, *XPC* and *XRCC7*). We found that the frequency of 939Gln variant at *XPC Lys939Gln* was significantly lower in PC cases (OR = 0.39, P = 0.016). Haplotype analysis of *XRCC1 Arg194Trp (C/T)* and *Arg399Gln (G/A)* revealed that the frequency of the T–A haplotype was significantly higher in PC patients. This is the first report on the studies of XPC and XRCC1 *Arg194Trp* polymorphisms in PC, and our present data suggest that *XPC Lys939Gln* and the T–A haplotype of *XRCC1 Arg194Trp* and *Arg399Gln* may be risk factors for PC in Japanese.

1. Introduction

Prostate cancer (PC) is one of the most common malignancies in men in the United States.[1] The aetiology of prostate cancer is largely unknown. Although several risk factors have been shown, ethnicity, family history and age have been associated with the increased risk.[2] In addition, diet has been linked to prostate cancer risk and prevention.[3]

Human DNA repair mechanisms protect the genome from DNA damage caused by endogenous and environmental agents. Genetic polymorphisms of DNA repair genes have been reported to lead to amino acid substitution in various cancers. Base excision repair (BER) is the repair mechanism for small lesions such as single-strand breaks, non-bulky adducts, oxidative damage, alkylation, or methylation. The *hOGG1* gene, which is in this category, encodes a DNA glycosylase/AP lyase. It suppresses the mutagenic effects of 8-hydroxyguanine by catalysing its removal from reactive oxygen species (ROS). In this regard, Xu *et al.*[4] found two sequence variants of this gene and showed an association between these polymorphisms and prostate cancer risk.[4] The X-ray cross-complementing group 1 (XRCC1) is also one of the enzymes participating in the BER pathway and acts as a scaffolding intermediate by interacting with Ligase III, DNA polymerase-β and poly (ADP-ribose) polymerase.[5] Shen *et al.* described two polymorphisms in XRCC1 (*Arg194Trp*

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and *Arg399Gln*), which are non-conservative amino acid changes.[6] The polymorphism of the *XRCC1* codon 399 was shown to be correlated with DNA repair activity[7] and associated with susceptibility to various cancers.[7]

Recently, we reported an association between the polymorphism of *XRCC1 Arg399Gln* and the risk of renal cell carcinoma.[8] Van Gils *et al.*[9] investigated the *XRCC1* codon 399 polymorphism in prostate cancer. They found no discernible difference between PC cases and controls for the *XRCC1* codon 399 variants, but found a remarkable risk for the combination of low dietary intake of vitamin E and the *XRCC1* codon 399 *Gln/Gln* genotypes.[9] Although Ribicki *et al.*[10] and Ritchey *et al.*[11] also investigated the *XRCC1* codon 399 polymorphism in prostate cancer, a significant association was found only in the latter study. To our knowledge, there have been no studies of the XRCC1 Arg194Trp polymorphism in prostate cancer.

Among DNA repair systems, the nucleotide-excision repair (NER) pathway repairs bulky DNA adducts and includes xeroderma pigmentosum group D (*XPD*) and xeroderma pigmentosum group C (*XPC*) repair genes. The *XPD* gene encodes a DNA helicase, its product being one of the integral members of the transcription factor TFIIH.[12] The XPC protein, involved in the NER pathway, binds to HR23B to form the XPC-HR23B complex and is thought to be an early damage detector and initiatior of NER.[13] The XPC codon 939 polymorphism (A–C transition, exon 15) results in a Lys to Gln alteration, which has been found to be associated with increased risk of bladder and lung cancer.[14,15] Among non-homologous end joining double-strand break repair genes, the *XRCC7* gene encodes the catalytic polypeptide of DNA-activated protein kinase: Wang *et al.*[16] found that the *XRCC7* gene polymorphism was associated with glioma. No studies have examined the risk of the polymorphisms of this group of DNA repair genes, including *XRCC1 Arg194Trp*, *XPC Lys939Gln*, and *XRCC7* in prostate cancer.

We hypothesised that the polymorphisms of DNA repair genes could be risk factors for prostate cancer (PC). To test this hypothesis, we examined whether the *XRCC1* (*Arg194Trp*, *Arg399Gln*), *XPC Lys939Gln* and *XRCC1G6721T* polymorphisms are risk factors for PC through analysis for SNPs of various DNA repair genes in normal and prostate cancer samples. Since smoking has been proven to be a risk factor for PC,[2] we also investigated the relationship between polymorphisms of DNA repair genes and smoking status in PC cases.

2. Materials and methods

2.1. Samples

A total of 165 patients with pathologically confirmed prostate cancer (PC), and 165 age-matched control individuals were enrolled in this study. The mean ages of the patient and control groups were 68 ± 5 and 67 ± 15 years, respectively.

Genomic DNA was obtained from the peripheral blood of healthy controls and patients. All of the patients tested were diagnosed with prostate cancer on the basis of histopathological findings from radical prostatectomy at Shimane University Hospital (Izumo Japan). They were classified according to the WHO criteria and staged according to the tumour-node-metastasis (TNM) classification and the Gleason grading system. Healthy controls consisted of volunteers with no apparent abnormal findings upon medical examination at Shimane University Hospital. The smoking status were investigated through interviews with doctors or nurses. The current smokers were defined as those who smoked within 12 months of tumour development. The former smokers were those who had quit smoking more than 12 months before tumour development. None of these patients had received androgen deprivation therapy before radical prostatectomy. The participation rates were 99% and 80% for the patients and controls, respectively.

There were no significant differences between patients and control groups with regard to family history of cancer and body mass index.

2.2. PCR-RFLP genotyping

Each PCR was carried out in a total volume of 20 μl consisting of 0.3 μl of a 10 μM solution of each primer, 1.5 mM $MgCl_2$, 0.8 mM dNTP, 0.5 U RedTaq DNA polymerase (Sigma, St. Louis, MO), 1 μl of genomic DNA (80 ng/μl) and 15.6 μl H_2O using a PTC 200 Thermal Cycler (MJ Research). Primer sets and annealing temperatures used for the PCR-RFLP assay are shown in Table 2.[8,15,16] The PCR program had an initial denaturation step of 7 min at 94 °C followed by 35 cycles of 30 s at 94 °C, 45 s of annealing at 57–62 °C based on the primers and 45 s at 72 °C.

For *XRCC1 Arg194Trp*, nested PCR was done (Table 2).

For RFLP analysis, PCR products were digested with *Pvu*II, *Msp*I, *Pvu*II and *Pvu*II (New England Biolabs, Beverly, MA) for *XRCC1 Arg194Trp*, *XRCC1 Arg399Gln*, *XPC Lys939Gln* and *XRCC7 G6721T*, respectively, by the manufacturer's protocols. The PCR products were separated by electrophoresis in a 2.0% agarose gel, and subsequently stained with ethidium bromide (Fig. 1).

Table 1 – Characteristics of PC patients and controls

	Cases (n = 165)	Controls (n = 165)
Age (means ± S.D.)[a]	68 ± 5	67 ± 15
pT		
1	0 (0%)	
2	108 (65%)	
3	53 (32%)	
4	4 (2%)	
Gleason sum		
<7	82 (50%)	
7	54 (32%)	
>7	29 (18%)	
Preoperative serum		
<4	24 (15%)	
4–10	75 (45%)	
>10	66 (40%)	
Smoking status		
Current smokers	43 (26%)	
Former smokers	29 (18%)	
Never smokers	93 (56%)	

a Cases *versus* controls, P = 0.20.