**Significant Association of Ku80 Single Nucleotide Polymorphisms with Bladder Cancer Susceptibility in Taiwan**

CHAO-HSIANG CHANG, CHANG-FANG CHIU, SHIU-YUN LIANG, HSI-CHIN WU, CHIA-LIN CHANG, CHIA-WEN TSAI, HWEI-CHUNG WANG, HONG-ZIN LEE and DA-TIAN BAU

Departments of 1Urology, 2Terry Fox Cancer Research Laboratory and 3Hematology Oncology, China Medical University Hospital, Taichung; 4Graduate Institute of Chinese Medical Science and 5School of Pharmacy, China Medical University, Taichung, Taiwan, R.O.C.

**Abstract.** The aim of the present study was to evaluate the association between the polymorphisms of the Ku80 DNA repair gene, which plays an important role in maintaining genome stability, and the risk of bladder cancer in Central Taiwan. Materials and Methods: In this hospital-based case–control study, the association of Ku80 G-1401T rs828907, Ku80 C-319T rs11685387 and Ku80 intron 19 rs9288518 polymorphisms with bladder cancer risk in a central Taiwanese population was investigated. In total, 288 patients with bladder cancer and 288 age- and gender-matched healthy controls recruited from the China Medical Hospital in central Taiwan were genotyped. Results: A significantly different distribution was found in the frequency of the Ku80 G-1401T polymorphism genotypes, but not the Ku80 C-319T or intron 19 polymorphism genotypes, between the bladder cancer and control groups. The T allele of Ku80 G-1401T conferred a significant (p=0.0055) increased risk of bladder cancer. Gene–environment interactions with smoking, but not with alcohol consumption, were significant for the Ku80 G-1401T polymorphism. The Ku80 G-1401T GT and TT genotypes, in association with smoking, conferred an increased risk of 2.053-fold (95% confidence interval=1.232-3.419) for bladder cancer. Conclusion: The first evidence that the T allele of the Ku80 G-1401T may be associated with the development of bladder cancer and may be a novel useful marker for primary prevention and anticancer intervention is provided.

Correspondence to: Da-Tian Bau and Hong-Zin Lee, Terry Fox Cancer Research Laboratory, China Medical University Hospital, 2 Yuh-Der Road, Taichung, 404 Taiwan, R.O.C. Tel: +886 422053366 Ext 3312, Fax: +886 6422053366 Ext 3312, e-mail: datian@mail.cmuh.org.tw; artbau1@yahoo.com.tw

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Bladder cancer is the most serious urinary neoplasm worldwide and the majority (70%) of cases occur in men (1). In the Western world, bladder cancer has become the fourth most common cancer among men, account for 7% of total malignancies (2). In Taiwan, the incidence and mortality of bladder cancer takes seventh place among the common carcinomas (3). Bladder carcinogenesis is a complex, multistep and multifactorial process resulting from the interactions between environmental and genetic factors. The risk factors for bladder cancer include cigarette smoking, exposure to carcinogenic aromatic amines and the uptake of harmful drugs, such as phenacetine, chloroanphrazine and cyclophosphamide (4, 5). Those carcinogens thought of as DNA damage inducers induce various types of DNA adducts, such as DNA base damage, DNA single-strand breaks and double-strand breaks (DSBs) (6). The DSBs may lead to dramatic genome instability, which is closely related to carcinogenesis (7, 8). There are two specific DNA repair pathways responsible for DSBs repair: homologous recombination (HR) repair and the non-homologous end-joining (NHEJ) (7). Most DSBs are repaired by NHEJ and several key components are involved (9). Once DSBs occur in the genomic DNA, they are first recognized by a heterodimeric DNA-binding component KU of a DNA-dependent protein kinase (DNA-PK), which is formed from Ku70 and Ku80 (10). The Ku80 gene is located on chromosome 2q35 and has 21 exons (11). Former studies have indicated that mutation of Ku80 may affect the age at cancer onset (12).

Some single nucleotide polymorphisms (SNPs) have been confirmed as genetic risk factors for cancer (13–17). Recently, the Ku80 gene has been reported to play a role in cancer development (18), but the association of its SNPs with any cancer has not been investigated yet. In this study, for the first time, the role of Ku80 in a central Taiwanese population was investigated by the analysis of SNPs of Ku80 in bladder cancer.
Materials and Methods

Study population and sample collection. The study population consisted of 288 patients and 288 cancer-free control volunteers. The patients, diagnosed with bladder cancer, were recruited at the outpatient clinics of general surgery between 2004 and 2007 at the China Medical University Hospital, Taichung, Taiwan, Republic of China. The clinical characteristics of the patients including their histological details were all graded and defined by expert surgeons. All the patients voluntarily participated, completed a self-administered questionnaire and provided peripheral blood samples. An equal number of cancer-free healthy volunteers, as controls, were selected by matching for age, gender and some habits after initial random sampling, from the Health Examination Cohort of the hospital. The exclusion criteria of the control group included previous malignancy, metastasized cancer from other or unknown origin and any familial or genetic diseases. The study was approved by the Institutional Review Board of the China Medical University Hospital and written-informed consent was obtained from all the participants.

Genotyping assays. Genomic DNA was prepared from peripheral blood leucocytes using a QIAamp Blood Mini Kit (Blossom, Taipei, Taiwan) and further processed according to previous methods (19, 20). Briefly, the following primers were used: for Ku80 G-1401T rs828907, 5’-TAGCTGACAACCTCACAGAT-3’ and 5’-ATTCAG AGGTGCTCATAGAG-3’; for Ku80 C-319T rs11685387, 5’-TCTAACTCCAGAGCTCTGAC-3’ and 5’-AACTCTGAGCAT GCACGAT-3’, and for Ku80 intron 19 rs9288518, 5’-GGTGT GAAGACCTATCAATC-3’ and 5’-TTACAGAACAAGCCTTGAC-3’. The following cycling conditions were performed: one cycle at 94˚C for 5 min; 35 cycles of 94˚C for 30 s, 55˚C for 30 s and 72˚C for 30 s and a final extension at 72˚C for 10 min. The PCR products were studied after digestion with BfaⅠ, SpeⅠ, and BsrⅠ restriction enzymes for Ku80 G-1401T (cut from 252 bp G type into 81+171 bp T type), and for Ku80 C-319T (cut from 311 bp C type into 108+203 bp T type) and Ku80 intron19 rs9288518 (cut from 275 bp A type into 110+165 bp G type), respectively.

Statistical analyses. Only those matches with all the SNP data were selected for the final analysis. To ensure that the controls used were representative of the general population and to exclude the possibility of genotyping error, the deviation of the genotype frequencies of Ku80 SNPs in the control subjects from those expected under the Hardy-Weinberg equilibrium was assessed using the goodness-of-fit test. Pearson’s chi-square test or Fisher’s exact test (when the expected number in any cell was less than five) was used to compare the distribution of the Ku80 genotypes between cases and controls. The data were recognized as significant when the statistical p-value was less than 0.05.

Results

The frequency distributions of the selected characteristics of the bladder cancer patients and the controls are shown in Table I. The characteristics of the patients and controls were all well matched and none of the differences between the groups was statistically significant (p>0.05) (Table I).

The frequency distributions of the Ku80 G-1401T, C-319T and intron 19 genotypes in the controls and the bladder cancer patients are shown in Table II. The genotype distribution of the genetic polymorphisms of Ku80 G-1401T was significantly different between the bladder cancer and control groups (p=0.0294), while those for C-319T and intron 19 were not significant (p>0.05) (Table II). The heterozygous Ku80 G-1401T genotype was significantly associated with bladder cancer susceptibility. The representative PCR-based restriction analyses for the Ku80 G-1401T polymorphisms are shown in Figure 1.

The frequency distributions of the alleles for Ku80 G-1401T, C-319T and intron 19 in the controls and bladder cancer patients are shown in Table III. The distributions of all these polymorphisms were in Hardy-Weinberg equilibrium and were similar between controls and bladder cancer patients.
The T allele of the Ku80 G-1401T polymorphism was significantly associated with bladder cancer (p=0.0055) (Table III). The genotype distribution of the various genetic polymorphisms of Ku80 G-1401T was significantly different between the bladder cancer and control groups who had a smoking habit (p=0.0053) (Table IV). In the smoking groups, the T allele clearly raised the bladder cancer risk (Table IV).

According to these findings, the T allele of the Ku80 G-1401T polymorphism may play a role in carcinogenesis. Those people carrying the T allele may have similar efficiency in removing DSBs if non-smokers, but in smokers, DNA damage increases significantly and individuals carrying the T allele may not have enough capacity to remove all the DSBs promptly and efficiently, thus increasing their bladder cancer risk.

In this study, a novel potential biomarker of bladder cancer, Ku80 G-1401T, was found and the importance of smoking in bladder cancer was also shown. Carcinogenesis is indeed a complex and multistep process, and it is difficult to explain the causes of bladder cancer with simply one hypothesis. Thus, the findings in this study only reveal part of the process of bladder carcinogenesis, but we strongly believe that the findings can help the fight against bladder cancer and help to lower its prevalence.

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References


Figure 1. PCR-based restriction analysis of the Ku80 G-1401T polymorphism shown on 2.5% agarose electrophoresis. M: 100 bp DNA size marker, G/G: enzyme indigestible homozygote, G/T: heterozygote, and T/T: enzyme digestible homozygote.


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