Genotype of DNA Double-strand Break Repair Gene *XRCC7* Is Associated with Lung Cancer Risk in Taiwan Males and Smokers

TE-CHUN HSIA[1,2,3*], WEN-SHIN CHANG[2,4*], WEI-CHUN CHEN[1,2*], SHINN-JYE LIANG[1,2], CHIH-YEN TU[1,2], HUNG-JEN CHEN[1,3], JI-AN LIANG[2], CHIA-WEN TSAI[2], CHIN-MU HSU[2], CHANG-HAI TSAI[2] and DA-TIAN BAU[2,4]

[1]Department of Internal Medicine and [2]Terry Fox Cancer Research Laboratory, China Medical University Hospital, Taichung, Taiwan, R.O.C.; [3]Department of Respiratory Therapy and 4Graduate Institute of Clinical Medical Science, China Medical University, Taichung, Taiwan, R.O.C.

Abstract. *Aim: The present study aimed to evaluate the contribution of X-ray repair cross-complementing group 7 (XRCC7) G6721T (rs7003908) genetic polymorphism and smoking habit on the risk of lung cancer in Taiwanese. Materials and Methods: In this hospital-based case–control study, association of single nucleotide polymorphism XRCC7 G6721T with lung cancer risk were examined among 358 patients with lung cancer and 716 age- and gender-matched healthy controls. The genetic–lifestyle interaction was also investigated. Results: The results showed that the percentages of TT, GT and GG genotypes for XRCC7 G6721T were differentially distributed as 60.9%, 34.9% and 4.2% in the group of patients with lung cancer and 48.7%, 43.3% and 8.0% in the non-cancer control group, respectively ($p=3.6*10^{-7}$). We further stratified the populations by gender and smoking behavior to investigate their combinatorial effects with XRCC7 G6721T genotype on lung cancer risk. The results showed that the GG genotype of XRCC7 G6721T had a protective effect on lung cancer susceptibility which was obvious among males and smokers ($p=2.2\times10^{-4}$ and 3.1×10^{-4}, respectively). Conclusion: The GG and GT genotypes of XRCC7 rs7003908 compared to the TT genotype had a protective effect on lung cancer risk in Taiwan, particularly among males and smokers.*

*These Authors contributed equally to this study.

Correspondence to: Da-Tian Bau, Terry Fox Cancer Research Laboratory, China Medical University Hospital, 2 Yuh-Der Road, Taichung, 404 Taiwan, R.O.C. Tel: +886 422052121 Ext. 7534, e-mail: datian@mail.cmuh.org.tw; artbau2@gmail.com

Key Words: Polymorphism, genotype, lung cancer, smoking, Taiwan, XRCC7.

Lung cancer is keeping its throne as the leading cause of cancer death both worldwide and in Taiwan (1). In literature, it is reported that various carcinogens contained in cigarette smoke may produce reactive oxygen species that can induce DNA adducts and strand breaks (2). However, there were also some studies that showed that only 10-15% of all smokers actually develop lung cancer during their lifetime, suggesting that individual susceptibility to carcinogens in cigarette smoke can vary among different populations (3, 4). From the twentieth century, mounting evidence has shown that individual differences in susceptibility may be inherited in genes encoding DNA repair proteins, which may be closely associated with personal cancer risk (5-10).

Homologous recombination (HR) and non-homologous end-joining (NHEJ) are the two important pathways for removing such double-strand breaks (DSBs) induced by endogenous and exogenous carcinogens. HR, which acts during the transition of S to G_2 phases of the cell cycle, entails copying the missing information from an undamaged homologous chromosome. In NHEJ, which acts during all phases of the cell cycle, the broken DNA termini are first processed to make them compatible and then sealed by a ligation step. Among the NHEJ DNA repair proteins, X-ray repair cross-complementing group 7 (*XRCC7*) (MIM: 600899; Genbank accession no: NM_001469) plays a central role, encoding the catalytic subunit of DNA-activated protein kinase (DNA-PKcs) of NHEJ pathway (11). HR and NHEJ are error-free and error-prone pathways, respectively. It should be noted that NHEJ is the dominant subpathway for DSB repair in human cells (11). DNA-PKcs is recruited to the site of DSBs by the KU70/KU80 heterodimer to form an active DNA–PK complex that is essential for the progression of the NHEJ pathway (12). Deficiencies in DNA-PK activity are clinically significant and mice with inactivated components of DNA-PK show severe combined immunodeficiency, as well as

Table I. *Distribution of selected demographic data of the 358 patients with lung cancer and the 716 matched controls.*

Characteristic	Controls (n=716)			Patients (n=358)			*p*-Value[a]
	n	%	Mean (SD)	n	%	Mean (SD)	
Age (years)			64.8 (6.8)			64.0 (6.9)	0.5871
Gender							0.3642
Male	488	68.1%		254	70.9%		
Female	228	31.9%		104	29.1%		0.3642
Smoking status							
Ever smokers	563	78.6%		293	81.8%		
Non-smokers	153	21.4%		65	18.2%		0.2282

[a]Based on Chi-square test.

hypersensitivity to ionizing radiation (13, 14). Genetic variation G6721T of *XRCC7* (rs7003908) is located in the intron 8 of the gene. It is speculated that this polymorphism may regulate splicing and cause mRNA instability (12). Recently, an increasing number of studies investigating the G6721T polymorphism of XRCC7 in association with several types of cancer have been published (15-21), however, few have been focused on lung cancer.

As for the overall DSB repair system, some previous reports showed that the genotypes of DSB repair genes, especially genes involved in NHEJ, may interact with personal lifestyle, such as smoking, in determining the relative risk of lung cancer for individual Taiwanese (22-24). Therefore, in this study, we aimed to reveal the genotypic frequencies of genotypes of G6721T polymorphism of *XRCC7*, focusing on the association of *XRCC7* genotypes with lung cancer susceptibility among Taiwanese never and ever smokers.

Materials and Methods

Investigated population and sample collection. Three hundred and fifty-eight patients diagnosed with lung cancer were recruited at the Outpatient Clinics of General Surgery between 2005-2008 at the China Medical University Hospital, Taichung, Taiwan. The clinical characteristics of patients, including histological details, were all graded and defined by expert surgeons. All participants voluntarily completed a self-administered questionnaire and provided their peripheral blood samples. Twice as many healthy volunteers without lung cancer as controls were selected by matching for age, gender and personal habits after initial random sampling from the Health Examination Cohort of our hospital. The exclusion criteria of the controls included previous malignancy, metastasized cancer from other or unknown origin, and any genetic or familial diseases. Our study was approved by the Institutional Review Board of the China Medical University Hospital (DMR100-IRB-284) and written-informed consent was obtained from all participants.

Genotyping conditions. Genomic DNA was extracted from peripheral blood leucocytes using the QIAamp Blood Mini Kit (Blossom, Taipei, Taiwan, ROC) and stored as previously published (25-27). The paired primers used for G6721T polymorphism of *XRCC7* were: forward 5'-TGGTGCTCAGCTTCTGGCTT-3', and reverse 5'-CATCCCTGCCAGCTCTTCTG-3'. The *XRCC7* G6721T polymerase chain reaction (PCR) conditions were 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.

Restriction fragment length polymorphism (RFLP) conditions. After the PCR process of XRCC7 G6721T, the resultant 301 bp PCR product was mixed with 2 U *Taq*I and incubated for 3 h at 65˚C in CutSmart™ Buffer (New England BioLabs, Taipei, Taiwan, ROC). The G form PCR products could be further digested, while the T form could not. Two fragments 235 bp and 66 bp were present if the product was the digestible G form. Then, 10 μl of product was loaded into a 3% agarose gel containing ethidium bromide for electrophoresis. The genotype analysis was performed by two researchers independently and blindly. Five percent of the samples were randomly selected for direct sequencing and the results were 100% concordant.

Statistical analyses. All 716 of the controls and 358 cases with genotypic and clinical data were analyzed. Pearson's Chi-square test was used to compare the distribution of the genotypes between cases and controls. Data were recognized as significant when the statistical *p*-value was less than 0.05. The lung cancer risk associated with the genotypes was estimated as an odds ratio (ORs) and 95% confidence intervals (CIs) by unconditional logistic regression with adjustment for the effect of possible confounders such as age and gender.

Results

The frequency distributions of selected characteristics for the 358 lung cancer patients and 716 non-cancer controls are summarized in Table I. Since we applied frequency matching to select the healthy controls, distributions of age and gender were comparable between the cases and the controls (Table I). The cases had a slightly higher percentage of smokers (81.8%) than don the controls (78.6%) ($p>0.05$) (Table I).

The distributions of the *XRCC7* G6721T genotypes among the controls and the lung cancer patients are provided and analyzed in Table II. The genotypes of *XRCC7* G6721T were differently distributed between lung cancer and healthy

Table II. *Distribution of X-ray repair cross-complementing group 7 (XRCC7) genotypes among the 358 patients with lung cancer and the 716 matched controls.*

Genotype	Controls		Patients		OR (95% CI)	*p*-Value[a]
	n	%	n	%		
rs7003908						
TT	349	48.7%	218	60.9%	1.00 (reference)	
GT	310	43.3%	125	34.9%	0.65 (0.49-0.84)	0.0016*
GG	57	8.0%	15	4.2%	0.42 (0.23-0.76)	0.0040*
P_{trend}						$3.6*10^{-7}$*

CI: Confidence interval; OR: odds ratio. aBased on Chi-square test.

control groups ($p=3.6\times10^{-7}$) (Table II). Statistically speaking, the *XRCC7* G6721T heterozygous GT and homozygous GG genotypes were significantly associated with decreased lung cancer risk (OR=0.65, 95% CI=0.49-0.84, $p=0.0016$; OR=0.42, 95% CI=0.23-0.76, $p=0.0040$, respectively).

The gender ratio of patients with lung cancer in Taiwan is about two males to one female. We are interested in the genotypic contribution of *XRCC7* G6721T to gender difference in lung cancer susceptibility. After stratification by the gender, it was found that the genotypes of *XRCC7* G6721T were differently distributed among males ($p=2.2\times10^{-4}$) but not females ($p=0.6134$) (Table III).

The interaction of the genotype of *XRCC7* G6721T and the smoking behavior of the participants was of great interest since lung cancer is a smoking-related cancer. The results in Table IV show that the genotypic distribution of the variant genotypes of *XRCC7* G6721T was significantly different between lung cancer and control groups who were ever smokers ($p=3.1\times10^{-4}$), but not different in the case among the non-smokers ($p=0.6885$) (Table IV). Overall, it seemed that there was an interaction between *XRCC7* G6721T genotype and smoking lifestyle in regard to lung cancer susceptibility.

Table III. *Distribution of X-ray repair cross-complementing group 7 (XRCC7) genotypes among patients with lung cancer after stratification by gender.*

Variable	*XRC77* G6721T genotype			*p*-Value[a]
	TT (%)	GT (%)	GG (%)	
Males				
Controls	234 (48.0%)	212 (43.4%)	42 (8.6%)	
Cases	160 (63.0%)	84 (33.1%)	10 (3.9%)	2.2×10^{-4}
Females				
Controls	115 (50.4%)	98 (43.0%)	15 (6.6%)	
Cases	58 (55.8%)	41 (39.4%)	5 (4.8%)	0.6134

[a]Based on Chi-square test.

Table IV. *Distribution of X-ray repair cross-complementing group 7 (XRCC7) genotypes among patients with lung cancer after stratification by personal smoking habit.*

Variable	*XRCC7* G6721T genotype			*p*-Value[a]
	TT (%)	GT (%)	GG (%)	
Smokers				
Controls	274 (48.7%)	246 (43.7%)	43 (7.6%)	
Cases	182 (62.1%)	101 (34.5%)	10 (3.4%)	3.1×10^{-4}
Non-smokers				
Controls	75 (49.0%)	64 (41.8%)	14 (9.2%)	
Cases	36 (55.4%)	24 (36.9%)	5 (7.7%)	0.6885

[a]Based on Chi-square test.

Discussion

As the catalytic subunit of the DNA-PK complex, XRCC7 play a role in NHEJ *via* recognition and repair of DNA DSBs (28). It was reported that mice with inactivated components of DNA-PK had severe combined immunodeficiency and hypersensitivity to ionizing radiation (13, 14). In addition, cells defective in DNA-PK components are hypersensitive to ionizing radiation killing due to an inability to repair DSBs effectively (29). The polymorphic site *XRCC7* G6721T (rs.7003908), located in intron 8, may regulate alternative splicing and cause mRNA instability (12), associated with altered cancer risk (15-18, 20, 21).

In the present study, we report that the GT and GG genotypes were associated with reduced lung cancer risk in a moderate (controls:cases=716:358) Taiwanese population (Table II). The prevalence of the major T allele of the *XRCC7* G6721T polymorphism in our controls (70.4%) was similar to those reported from other Han populations (15-18, 20, 21). There is no report regarding the role of *XRCC7* genotype as a genetic marker for lung cancer. In Taiwan, lung cancer is the most prevalent type of cancer and of highest mortality.

There is a gender difference and there were about twice as many males as females suffering from lung cancer. Smoking is also a risk factor for lung cancer worldwide. Therefore, the present study was also aimed to investigate the interaction of *XRCC7* genotypes with gender and smoking lifestyle on lung cancer risk in Taiwan. We found that the association between *XRCC7* genotype and lung cancer risk was obvious among the males (Table III). As for the females, the frequency of wild-type TT genotype was also higher in the case group (55.8%) than the control group (50.4%), but the difference did not reach the statistically significant level (Table III). As for the smoking lifestyle, the association between *XRCC7* genotypes with lung cancer risk was obvious, especially among ever smokers (Table IV). However, there was no such differential genotypic distribution for the non-smokers. Although this *XRCC7* genetic variation was not found to directly result in an amino acid coding change, it might influence the expression level or stability of XRCC7 protein, as well as its function in NHEJ and genome-protecting capacity. Overall, males carrying a T allele for *XRCC7* G6721T may have lower capacity than those carrying G allele in DSB-removal capacity, leading to higher susceptibility to smoking-induced lung cancer development.

The current study has several limitations. Firstly, the power of the hospital-based case–control study could be enhanced by enlarging the sample size. Secondly, other confounding factors, such as obesity, alcohol drinking, lifestyle and pollutant exposure status, were not taken into consideration and adjusted for. Further studies could be performed to reveal the contribution of *XRCC7* to lung carcinogenesis such as measuring the expression alterations at mRNA and protein levels for *XRCC7* in tumor sites from patients with lung cancer, comparing with the non-tumor sites.

In conclusion, our findings suggested that the G allele of XRCC7 G6721T was associated with lower lung cancer risk, especially among males and smokers.

Acknowledgements

The Authors declare no interest conflict with any person or company. We appreciate Hong-Xue Ji, Liang-Yi Lin, Chieh-Lun Hsiao, Chia-En Miao and Tissue-bank of China Medical University Hospital for their excellent technical assistance. This study was supported by research grants from Terry Fox Cancer Research Foundation and Taiwan Ministry of Health and Welfare Clinical Trial and Research Center of Excellence (MOHW103-TDU-B-212-113002).

References

1 Hecht SS: Cigarette smoking and lung cancer: chemical mechanisms and approaches to prevention. Lancet Oncol *3*: 461-469, 2002.

2 Xu J, Zheng SL, Turner A, Isaacs SD, Wiley KE, Hawkins GA, Chang BL, Bleecker ER, Walsh PC, Meyers DA and Isaacs WB: Associations between hOGG1 sequence variants and prostate cancer susceptibility. Cancer Res *62*: 2253-2257, 2002.

3 Shields PG and Harris CC: Cancer risk and low-penetrance susceptibility genes in gene-environment interactions. J Clin Oncol *18*: 2309-2315, 2000.

4 Shields PG: Molecular epidemiology of smoking and lung cancer. Oncogene *21*: 6870-6876, 2002.

5 Chen WC, Tsai CW, Hsia TC, Chang WS, Lin LY, Liang SJ, Tu CY, Cheng WE, Chen HJ, Wang SM and Bau DT: The Contribution of DNA apurinic/apyrimidinic endonuclease genotype and smoking habit to Taiwan lung cancer risk. Anticancer Res *33*: 2775-2778, 2013.

6 Hsu CM, Yang MD, Chang WS, Jeng LB, Lee MH, Lu MC, Chang SC, Tsai CW, Tsai Y, Tsai FJ and Bau DT: The contribution of XRCC6/Ku70 to hepatocellular carcinoma in Taiwan. Anticancer Res *33*: 529-535, 2013.

7 Tsai CW, Ho CY, Shih LC, Ying TH, Hsieh YH, Chen YC, Chang WS, Huang CY, Pan SB, Shui HA, Chen CP, Wang PS and Bau DT: The joint effect of HOGG1 genotype and smoking habit on endometriosis in Taiwan. Chin J Physiol *56*: 263-268, 2013.

8 Wang HC, Liu CS, Chiu CF, Chiang SY, Wang CH, Wang RF, Lin CC, Tsai RY and Bau DT: Significant association of DNA repair gene *Ku80* genotypes with breast cancer susceptibility in Taiwan. Anticancer Res *29(12)*: 5251-5254, 2009.

9 Hsu NY, Wang HC, Wang CH, Chiu CF, Tseng HC, Liang SY, Tsai CW, Lin CC and Bau DT: Lung cancer susceptibility and genetic polymorphisms of EXO1 gene in Taiwan. Anticancer Res *29*: 725-730, 2009.

10 Liu CJ, Hsia TC, Tsai RY, Sun SS, Wang CH, Lin CC, Tsai CW, Huang CY, Hsu CM and Bau DT. The joint effect of *HOGG1* single nucleotide polymorphism and smoking habit on lung cancer in Taiwan. Anticancer Res *30(10)*: 4141-4145, 2010.

11 Valerie K and Povirk LF: Regulation and mechanisms of mammalian double-strand break repair. Oncogene *22*: 5792-5812, 2003.

12 Sipley JD, Menninger JC, Hartley KO, Ward DC, Jackson SP and Anderson CW: Gene for the catalytic subunit of the human DNA-activated protein kinase maps to the site of the *XRCC7* gene on chromosome 8. Proc Natl Acad Sci USA *92*: 7515-7519, 1995.

13 Ferguson DO, Sekiguchi JM, Chang S, Frank KM, Gao Y, DePinho RA and Alt FW: The nonhomologous end-joining pathway of DNA repair is required for genomic stability and the suppression of translocations. Proc Natl Acad Sci USA *97*: 6630-6633, 2000.

14 Singleton BK, Priestley A, Steingrimsdottir H, Gell D, Blunt T, Jackson SP, Lehmann AR and Jeggo PA: Molecular and biochemical characterization of *XRS* mutants defective in *Ku80*. Mol Cell Biol *17*: 1264-1273, 1997.

15 Gangwar R, Ahirwar D, Mandhani A and Mittal RD: Do DNA repair genes *OGG1, XRCC3* and *XRCC7* have an impact on susceptibility to bladder cancer in the North Indian population? Mutat Res *680*: 56-63, 2009.

16 Hirata H, Hinoda Y, Matsuyama H, Tanaka Y, Okayama N, Suehiro Y, Zhao H, Urakami S, Kawamoto K, Kawakami T, Igawa M, Naito K and Dahiya R: Polymorphisms of DNA repair genes are associated with renal cell carcinoma. Biochem Biophys Res Commun *342*: 1058-1062, 2006.

17 Hirata H, Hinoda Y, Tanaka Y, Okayama N, Suehiro Y, Kawamoto K, Kikuno N, Majid S, Vejdani K and Dahiya R: Polymorphisms of DNA repair genes are risk factors for prostate cancer. Eur J Cancer *43*: 231-237, 2007.

18 Liu Y, Zhang H, Zhou K, Chen L, Xu Z, Zhong Y, Liu H, Li R, Shugart YY, Wei Q, Jin L, Huang F, Lu D and Zhou L: Tagging SNPs in non-homologous end-joining pathway genes and risk of glioma. Carcinogenesis *28*: 1906-1913, 2007.
19 Mandal RK, Kapoor R and Mittal RD: Polymorphic variants of DNA repair gene *XRCC3* and *XRCC7* and risk of prostate cancer: a study from North Indian population. DNA Cell Biol *29*: 669-674, 2010.
20 Wang LE, Bondy ML, Shen H, El-Zein R, Aldape K, Cao Y, Pudavalli V, Levin VA, Yung WK and Wei Q: Polymorphisms of DNA repair genes and risk of glioma. Cancer Res *64*: 5560-5563, 2004.
21 Wang SY, Peng L, Li CP, Li AP, Zhou JW, Zhang ZD and Liu QZ: Genetic variants of the *XRCC7* gene involved in DNA repair and risk of human bladder cancer. Int J Urol *15*: 534-539, 2008.
22 Hsia TC, Tsai CW, Liang SJ, Chang WS, Lin LY, Chen WC, Tu CY, Tsai CH and Bau DT: Effects of ataxia telangiectasia mutated (*ATM*) genotypes and smoking habits on lung cancer risk in Taiwan. Anticancer Res *33*: 4067-4071, 2013.
23 Hsia TC, Liu CJ, Chu CC, Hang LW, Chang WS, Tsai CW, Wu CI, Lien CS, Liao WL, Ho CY and Bau DT: Association of DNA double-strand break gene XRCC6 genotypes and lung cancer in Taiwan. Anticancer Res *32*: 1015-1020, 2012.
24 Hsu NY, Wang HC, Wang CH, Chang CL, Chiu CF, Lee HZ, Tsai CW and Bau DT: Lung cancer susceptibility and genetic polymorphism of DNA repair gene *XRCC4* in Taiwan. Cancer Biomark *5*: 159-165, 2009.
25 Tsai CW, Chang WS, Liu JC, Tsai MH, Lin CC and Bau DT: Contribution of DNA double-strand break repair gene *XRCC3* genotypes to oral cancer susceptibility in Taiwan. Anticancer Res *34*: 2951-2956, 2014.
26 Tsai CW, Chang WS, Lin KC, Shih LC, Tsai MH, Hsiao CL, Yang MD, Lin CC and Bau DT: Significant association of Interleukin-10 genotypes and oral cancer susceptibility in Taiwan. Anticancer Res *34*: 3731-3737, 2014.
27 Huang CY, Chang WS, Shui HA, Hsieh YH, Loh CH, Wang HC, Ji HX, Hsiao CL, Hsu CM, Tsai CW and Bau DT: Evaluation of the contribution of methylenetetrahydrofolate reductase genotypes to taiwan breast cancer. Anticancer Res *34*: 4109-4115, 2014.
28 Gottlieb TM and Jackson SP: The DNA-dependent protein kinase: requirement for DNA ends and association with Ku antigen. Cell *72*: 131-142, 1993.
29 Jackson SP: DNA-dependent protein kinase. Int J Biochem Cell Biol *29*: 935-938, 1997.

Received August 21, 2014
Revised September 23, 2014
Accepted September 26, 2014