Colorectal Cancer and Genetic Polymorphism of DNA Double-Strand Break Repair Gene *XRCC4* in Taiwan

Da-Tian Bau[2,3], Mei-Due Yang[1], Yung-An Tsou[3], Song-Shei Lin[4], Cheng-Nan Wu[5], Hao-Hsueh Hsieh[2,6], Rou-Fen Wang[2], Chia-Wen Tsai[2,3], Wen-Shin Chang[2], Hsiu-Min Hsieh[2] and Ru-Yin Tsai[2]

Department of [1] Surgery and [2] Terry Fox Cancer Research Lab, China Medical University Hospital, Taichung, Taiwan R.O.C.

[3] Graduate Institute of Chinese Medical Science, and [6] Basic Medical Science, China Medical University, Taichung, Taiwan, R.O.C.

[4] Department of Medical Imaging and Radiological Sciences, Central-Taiwan University of Science and Technology, Taichung, Taiwan, R.O.C.

[5] Institute of Medical Bioscience, Central-Taiwan University of Science and Technology, Taichung , Taiwan , R.O.C.

***Correspondence to**:* Da-Tian Bau and Ru-Yin Tsai, Terry Fox Cancer Research Lab, Department of Medical Research, China Medical University Hospital, 2 Yuh-Der Road, Taichung, 404 Taiwan, R.O.C. Tel: +886 422052121 Ext 1523, Fax: +886 422052121, e-mail: datian@mail.cmuh.org.tw; artbau1@yahoo.com.tw

Running title: Bau *et al*: XRCC4 Polymorphisms in Colorectal Cancer

Abstract The DNA repair gene X-ray repair complementing defective repair in Chinese hamster cells 4 *(XRCC4)* is thought to play a major role in the caretaking of the whole genome via double strand-break repair. However, the association of polymorphic variants of *XRCC4* with colorectal cancer susceptibility has never been reported. In this hospital-based case control study, the association of *XRCC4* polymorphisms C-1622T (rs7727691), G-1394T (rs6869366), G-652T (rs2075685), C-571T (rs2075686), intron 3 DIP (rs28360071), S247A (rs3734091) and intron 7 DIP (rs28360317) with colorectal cancer risk in a Taiwanese population was investigated. The genotypes of *XRCC4* of 370 patients with colorectal cancer and 370 age- and gender-matched healthy controls were determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis. We found significant differences in the genetic and allelic frequencies of the *XRCC4* G-1394T between the colorectal cancer and control groups (P=0.0003 and $8.32^{\times}10^{-5}$, respectively). The distributions of other genetic polymorphisms between cases and the control group were not significantly different. We conclude that the G allele of *XRCC4* G-1394T may contribute to colorectal carcinogenesis and may be useful for early

detection of colorectal cancer.

Key Words: *XRCC4*, polymorphism, colorectal cancer, carcinogenesis.

Colorectal cancer (CRC) is a leading cause of cancer-related morbidity and mortality worldwide. The incidence and age-adjusted mortality of CRC have drastically continued to increase in Taiwan. In recent years, the incidence and mortality of CRC has taken the third place among the common types of cancer. Etiological studies have attributed more than 85% of CRC to environmental factors (1, 2). Meat consumption, cigarette smoking and exposure to carcinogenic aromatic amines are commonly recognized environmental factors for CRC (3-5) and these DNA damage-inducing factors may induce various types of DNA adducts, including double-strand breaks (DSBs) (6). DSBs may lead to severe genome instability, which is closely related to carcinogenesis (7, 8). Two distinct DNA repair pathways are responsible for DSB repair. Homologous recombination (HR) and the non-homologous end-joining (NHEJ) (8). Components involved in NHEJ include the DNA ligase IV—XRCC4 complex, Ku70, Ku80, the catalytic subunit PKcs and artemis (9). Genetic polymorphisms in NHEJ genes influence DNA repair capacity and confer predisposition to several types of cancer, including skin (10), breast (11-13), gastric (14), and oral (15). Among these reports, the study investigating the association of *XRCC4* G-1394T polymorphism with

gastric cancer found that those who had G/T or G/G at G-1394T showed a 3 to 79-fold increased risk of gastric cancer compared to those with T/T (15). Since both the stomach and intestine originate from endoderm during embryonic development, we proposed that this genetic polymorphism may also be associated with CRC susceptibility. To the best of our knowledge, there is no report studying the association of *XRCC4* with CRC risk. The present study aimed at investigating the potential association of *XRCC4* polymorphisms, including C-1622T (rs7727691), G-1394T (rs6869366), G-652T (rs2075685), C-571T (rs2075686), intron 3 DIP (rs28360071), S247A (rs3734091) and intron 7 DIP (rs28360317), with CRC susceptibility.

Materials and Methods

Study population and sample collection. The study population consisted of 370 case patients and 370 cancer-free control volunteers. Three hundred and seventy patients diagnosed with CRC were recruited at the outpatient clinics of general surgery during 2002-2009 at the China Medical University Hospital, Taichung, Taiwan. The clinical characteristics of patients include histological details were all graded and

defined by expert surgeons (Dr. Yang's team). All patients voluntarily participated, completed a self-administered questionnaire and provided peripheral blood samples. An equal number of healthy volunteers as controls were selected by matching for age, gender and some indulgences after initial random sampling from the Health Examination Cohort of the hospital. The exclusion criteria for the control group included previous malignancy, metastasized cancer from other or unknown origin, and any familial or genetic diseases. Our study was approved by the Institutional Review Board of the China Medical University Hospital and written-informed consent was obtained from all participants.

Genotyping conditions. Genomic DNA was prepared from peripheral blood leukocytes using a QIAamp Blood Mini Kit (Blossom, Taipei, Taiwan, R.O.C.) and further processed according to our previous papers (15-19). The polymerase chain reaction (PCR) cycling conditions were: one cycle at 94^{o}C for 5 min; 35 cycles of 94^{o}C for 30 s, 55^{o}C for 30 s, and 72^{o}C for 30 s; and a final extension at 72^{o}C for 10 min. Pairs of PCR primer sequences and restriction enzymes for each DNA product are all listed in Table I.

Statistical analyses. Only those matches with all DNA polymorphism data (case/control=370/370) were selected for 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 *XRCC4* single nucleotide polymorphism (SNPs) in the controls 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 *XRCC4* genotypes between cases and controls. Data were recognized as significant when the statistical *P*-value was less than 0.05.

Results

The frequencies of the genotypes for *XRCC4* C-1622T, G-1394T, G-652T, C-571T, intron 3 DIP, S247A and intron 7 DIP for controls and CRC patients are shown in Table II. Genotype distribution of various genetic polymorphisms of G-1394T was significantly different between the CRC and control groups (P=0.0003), while those for C-1622T, G-652T, C-571T, S247A and intron 3 DIP and intron 7 DIP were not different

(P>0.05) (Table II).

The frequencies of the alleles for the *XRCC4* C-1622T, G-1394T, G-652T, C-571T, intron 3 DIP, S247A and intron 7 DIP between controls and CRC patients is shown in Table III. Allelic frequency distribution of the *XRCC4* promoter -1394 was significantly different between the CRC and control groups ($P=8.32^{\times}10^{-5}$), while that of the other were not (Table III). The conclusion deduced from Table II and III is that *XRCC4* promoter -1394 G allele seems to be associated with a higher risk for CRC.

Discussion

To our knowledge, there are no studies investigating the role of *XRCC4*, which plays an important role in the NHEJ pathway, in CRC. According to our results, the G-1394T polymorphism of the *XRCC4* gene was found for the first time to be associated with CRC (Tables II and III). According to previous study, XRCC4 is such an essential protein in taking care of our genome that critical mutations of *XRCC4* will lead to cell apoptosis and possibly fatality during the embryonic stage (20). The variant polymorphism of G-1394T of *XRCC4* may slightly alter the normal

expression level of XRCC4. These changes may be very subtle initially, and cause no obvious pathological change in the human body without alerting the cell via its apoptosis checking system, such as the cell cyclins and their related kinase system. If the cells undergo severe DNA damage, the cell cycle checking points will arrest resulting in either DNA repair or apoptosis. The effects may only lead to a lower capacity of the NHEJ in which the *XRCC4* gene is involved. After birth, exposure to environmental carcinogens increases the possibility for genomic instability, and the need for the intracellular repair system to stem this DNA damage will increase too. In those people whose NHEJ repair system cannot perform at its normal efficiency, the raised instability of their genome can be expected. With time, these genetic deficiencies will increase the number of abnormal cells, and thus raise the risk of CRC.

It is known that DSBs are one of the most severe types of DNA damage, and if the DSBs cannot be repaired before the duplication of the genome, they will result in irreversible cellular injuries, which increases the possibility of CRC carcinogenesis, and perhaps of other types of cancer too. Some reports showed that variations of the *XRCC4* gene are associated with many other types of cance**r (11-15, 21)**. The finding that

the G allele of *XRCC4* gene is a risk factor for CRC is consistent with the case for gastric cancer (14). A previous study showed that intestinal-type gastric carcinoma shared common pathways with CRC, indicating that the clinical subtypes of gastric cancer and CRC are both important in genetic study of cancer (22). Moreover, defects in XRCC4 function and failures of the DNA DSB repair pathway may serve as common biomarkers for early carcinogenesis detection. The phenotypes related to DNA DSB repair capacity, and the correlation between genotype and phenotype need further investigation.

In this study, we have screened seven SNPs of the *XRCC4* gene and investigated their associations with susceptibility to CRC. We found that the G-1394T polymorphism of the *XRCC4* gene appears to be associated with CRC risk and that the G allele is a risk factor. However, the effects of these SNPs of *XRCC4* gene need further study, such as promoter assays, to reveal the role of each section or even each nucleotide in the subtle regulation of the transcriptional, translational, and post-translational expression of the *XRCC4* gene. In addition, a larger population size for stratification of factors such as gender, anatomic distributions of CRC, and genomic-environmental combinatorial studies

could provide a better understanding of the complex relationship between *XRCC4* and CRC carcinogenesis.

Acknowledgements

This study was supported by research grants from the China Medical University and Hospital (DMR-99-049 and CMU-97-333), Terry Fox Cancer Research Foundation and the National Science Council (NSC 98-2320-B-039-010-MY3).

References

1 Doll R and Peto R: The causes of cancer: quantitative estimates of avoidable risks of cancer in the United States today. J Natl Cancer Inst *66*: 1191-1308, 1981.

2 Thomas HJ: Familial colorectal cancer. BMJ *307*: 277-278, 1993.

3 Giovannucci E, Colditz GA, Stampfer MJ, Hunter D, Rosner BA, Willett WC and Speizer FE: A prospective study of cigarette smoking and risk of colorectal adenoma and colorectal cancer in U.S. women. J Natl Cancer Inst *86*: 192-199, 1994.

4 Giovannucci E, Rimm EB, Stampfer MJ, Colditz GA, Ascherio A, Kearney J and Willett WC: A prospective study of cigarette smoking and risk of colorectal adenoma and colorectal cancer in U.S. men. J Natl Cancer Inst *86*: 183-191, 1994.

5 Heineman EF, Zahm SH, McLaughlin JK and Vaught JB: Increased risk of colorectal cancer among smokers: results of a 26-year follow-up of US veterans and a review. Int J Cancer *59*: 728-738, 1994.

6 Pryor WA, Hales BJ, Premovic PI and Church DF: The radicals in cigarette tar: their nature and suggested physiological implications. Science *220*: 425-427, 1983.

7 Goode EL, Ulrich CM and Potter JD: Polymorphisms in DNA repair genes and associations with cancer risk. Cancer Epidemiol Biomarkers Prev *11*: 1513-1530, 2002.

8 Karran P: DNA double-strand break repair in mammalian cells. Curr Opin Genet Dev *10*: 144-150, 2000.

9 Burma S, Chen BP and Chen DJ: Role of non-homologous end joining (NHEJ) in maintaining genomic integrity. DNA Repair (Amst) *5*: 1042-1048, 2006.

10 Chang CH, Chiu CF, Wang HC, Wu HC, Tsai RY, Tsai CW, Wang RF, Wang CH, Tsou YA and Bau DT: Significant association of *ERCC6* single nucleotide polymorphisms with bladder cancer susceptibility in Taiwan. Anticancer Res *29*: 5121-5124, 2009.

11 Bau DT, Fu YP, Chen ST, Cheng TC, Yu JC, Wu PE and Shen CY: Breast cancer risk and the DNA double-strand break end-joining capacity of nonhomologous end-joining genes are affected by *BRCA1*. Cancer Res *64*: 5013-5019, 2004.

12 Bau DT, Mau YC, Ding SL, Wu PE and Shen CY: DNA double-strand break repair capacity and risk of breast cancer. Carcinogenesis *28*: 1726-1730, 2007.

13 Chiu CF, Wang HC, Wang CH, Wang CL, Lin CC, Shen CY, Chiang SY and Bau DT: A new single nucleotide polymorphism in *XRCC4* gene is associated with breast cancer susceptibility in Taiwanese patients. Anticancer Res *28*: 267-270, 2008.

14 Chiu CF, Wang CH, Wang CL, Lin CC, Hsu NY, Weng JR and Bau DT: A novel single nucleotide polymorphism in *XRCC4* gene is associated with gastric cancer susceptibility in Taiwan. Ann Surg Oncol *15*: 514-518, 2008.

15 Chiu CF, Tsai MH, Tseng HC, Wang CL, Wang CH, Wu CN, Lin CC and Bau DT: A novel single nucleotide polymorphism in *XRCC4* gene is associated with oral cancer susceptibility in Taiwanese patients. Oral Oncol *44*: 898-902, 2008.

16 Bau DT, Tseng HC, Wang CH, Chiu CF, Hua CH, Wu CN, Liang SY, Wang CL, Tsai CW and Tsai MH: Oral cancer and genetic polymorphism of DNA double-strand break gene *Ku70* in Taiwan. Oral Oncol *44*: 1047-1051, 2008.

17 Chiu CF, Tsai MH, Tseng HC, Wang CL, Tsai FJ, Lin CC and Bau DT: A novel single nucleotide polymorphism in *ERCC6* gene is associated with oral cancer susceptibility in Taiwanese patients. Oral Oncol *44*: 582-586, 2008.

18 Hsu CF, Tseng HC, Chiu CF, Liang SY, Tsai CW, Tsai MH and Bau DT: Association between DNA double-strand break gene *Ku80* polymorphisms and oral cancer susceptibility. Oral Oncol *45*: 789-793, 2009.

19 Tsai MH, Tseng HC, Liu CS, Chang CL, Tsai CW, Tsou YA, Wang RF, Lin CC,

Wang HC, Chiu CF and Bau DT: Interaction of *EXO1* genotypes and smoking habit in oral cancer in Taiwan. Oral Oncol *45*: e90-94, 2009.

20 Gao Y, Ferguson DO, Xie W, Manis JP, Sekiguchi J, Frank KM, Chaudhuri J, Horner J, DePinho RA and Alt FW: Interplay of p53 and DNA-repair protein XRCC4 in tumorigenesis, genomic stability and development. Nature *404*: 897-900, 2000.

21 Han J, Colditz GA, Samson LD and Hunter DJ: Polymorphisms in DNA double-strand break repair genes and skin cancer risk. Cancer Res *64*: 3009-3013, 2004.

22 Shimada S, Tashima S, Yamaguchi K, Matsuzaki H and Ogawa M: Carcinogenesis of intestinal-type gastric cancer and colorectal cancer is commonly accompanied by expression of brain (fetal)-type glycogen phosphorylase. J Exp Clin Cancer Res *18*: 111-118, 1999.

Table I. The primer sequences, polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) conditions for *XRCC4* gene polymorphisms.

Polymorphism (location)	**Primer sequence (5'->3')**	**Restriction enzyme**	**SNP sequence**	**DNA fragment size (bp)**
C-1622T	F: AAGATACTGAGACACTAATC	*Fnu4H* I	T	218 bp
rs77277691	R: CACAACATAACTAAGGATGA		C	32 + 186 bp
G-1394T	F: GATGCGAACTCAAAGATACTGA	*Hinc* II	T	300 bp
rs6869366	R: TGTAAAGCCAGTACTCAAACTT		G	200 + 100 bp
G-652T	F: GCTAGACACCACTCCAATAA	*Mbo* II	T	326 bp
rs2075685	R: GGCTACGTAGATTATGTGTG		G	127 + 199 bp
C-571T	F: GGCTACTGACTAAACAGATG	*Mnl* I	C	197 bp
rs2075686	R: TAACACGTTGGCTACGTAGA		T	69 + 128 bp
Intron 3	F: TCCTGTTACCATTTCAGTGTTAT		Insertion	139 bp
rs28360071	R: CACCTGTGTTCAATTCCAGCTT		Deletion	109 bp
Codon 247	F: GCTAATGAGTTGCTGCATTTTA	*Bbs* I	C	308 bp
rs3734091	R: TTTCTAGGGAAACTGCAATCTGT		A	204 + 104 bp

Intron 7	F1 (CCT-positive):	CCT	239 bp
rs28360317	ATACTGTGTTTGGAACTCCT	—	No product
	F2 (CCT-negative):		
	ATACTGTGTTTGGAACTAGA		
	R: TATCCTATCATCTCTGGATA		

F and R indicate forward and reverse primers, respectively.

Table II. Distribution of *XRCC4* genotypes among colorectal cancer patient and control groups.

Genotype	Controls		Patients		*P*-value[a]
	n	%	n	%	
C-1622T rs7727691					0.6352
CC	299	80.8%	305	82.4%	
CT	71	19.2%	65	17.6%	
TT	0	0.0%	0	0.0%	
G-1394T rs6869366					**0.0003**
GG	0	0.0%	3	0.8%	
GT	44	11.9%	81	21.9%	
TT	326	88.1%	286	77.3%	
G-652T rs2075685					0.8140
GG	191	51.6%	183	49.5%	
GT	165	44.6%	171	46.2%	
TT	14	3.8%	16	4.3%	
C-571T rs2075686					0.6529
CC	216	58.4%	204	55.1%	
CT	135	36.5%	144	38.9%	
TT	19	5.1%	22	6.0%	
Intron3 DIP rs28360071					0.4096
II	246	66.5%	229	61.9%	
ID	113	30.5%	127	34.3%	
DD	11	3.0%	14	3.8%	

Codon247 rs3734091						0.2597
AA	Ser/Ser	0	0.0%	0	0.0%	
AC	Ser/Ala	67	18.1%	74	20.0%	
CC	Ala/Ala	303	81.9%	269	80.0%	
Intron7 DIP rs28360317						0.5605
II		182	49.2%	168	45.4%	
ID		154	41.6%	163	44.1%	
DD		34	9.2%	39	10.5%	

[a] Based on chi-square test.

Table III. Distribution of *XRCC4* alleles among the colorectal cancer patient and control groups.

Allele	Controls		Patients		*P*-value[a]
	n	%	n	%	
C-1622T rs7727691					0.5893
Allele C	669	90.4%	675	91.2%	
Allele T	71	9.6%	65	8.8%	
G-1394T rs6869366					**$8.32^{\times}10^{-5}$**
Allele G	44	6.0%	87	11.8%	
Allele T	696	94.0%	653	88.2%	
G-652T rs2075685					0.5571
Allele G	547	73.9%	537	72.6%	
Allele T	193	26.1%	203	27.4%	
C-571T rs2075686					0.3639
Allele C	567	76.6%	552	74.6%	
Allele T	173	23.4%	188	25.4%	
Intron3 DIP rs28360071					0.1903
Insertion	605	81.8%	585	79.1%	
Deletion	135	18.2%	155	20.9%	

Codon247 rs3734091					0.5954
Allele A (Ser)	67	9.1%	74	10.0%	
Allele C (Ala)	673	90.9%	666	90.0%	
Intron7 DIP rs28360317					0.2868
Insertion	518	70.0%	499	67.4%	
Deletion	222	30.0%	241	32.6%	

[a] Based on chi-square test