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Research Paper

Polymorphisms in XRCC5, XRCC6, XRCC7 genes are involved in DNA double-strand breaks(DSBs) repair associated with the risk of acute myeloid leukemia(AML) in Chinese population ☆

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Abstract

Objective: To investigate the association between the X-ray repair cross complementing(XRCC) group 5, XRCC6 and XRCC7 polymorphisms and risk of acute myeloid leukemia(AML). **Methods:**This hospital-based case-control study included 120 AML patients and 210 cancer-free controls in a Chinese population. Three polymorphisms of XRCC5, XRCC6 and XRCC7 were genotyped using the polymerase chain reaction(PCR) or polymerase chain reaction-restriction fragment length polymorphism(PCR-RFLP) method. **Results:** We found that there was a significant decrease in risk of AML associated with the XRCC6 -61 CG/GG genotype(adjusted odd ratio (OR) = 0.55; 95% confident interval(CI) = 0.34-0.89) compared with the -61CC genotype. For the novel tandem repeat polymorphism (VNTR) in the XRCC5 promoter, we found when the XRCC5 six genotypes were dichotomized(i.e., 2R/2R, 2R/1R versus 2R/0R, 1R/1R, 1R/0R and 0R/0R), the latter group was associated with increased risk of AML(adjusted OR = 1.67; 95% CI = 1.00~2.79) compared to 2R/2R+2R/1R genotype. However, the XRCC7 6721G>T polymorphism had no effect on risk of AML. **Conclusion:**The XRCC6 -61C > G and XRCC5 2R/1R/0R polymorphisms, but not XRCC7 6721G > T polymorphism, could play an important role in the development of AML. Larger scale studies with more detailed data on environment exposure are needed to verify these findings.

Keywords: XRCC5; XRCC6; XRCC7; single nucleotide polymorphism; tandem repeat polymorphism; acute myeloid leukemia

INTRODUCTION

Acute myeloid leukemia(AML) is the most common acute leukemia(AL) in adults, with a median incidence of 2.4 cases per 100,000 inhabitants in the western world[1]. In China, AML accounts for approximately 25% of all leukemias in adults, and is the most frequent form of AL. Epidemiologic and genotypic data showed that AML cells possess more than one recurring mutation, either as point mutations, gene rearrangements and/or chromosomal translocations[2]. Epidemiological studies have shown that many different environmental and occupational factors, such as exogenous agents, ionizing radiation(IR) and topoisomerase inhibitors, and some endogenous agents encompassing anticancer drugs and reactive oxygen species(ROS), are responsible for the development of AML[3-7]. Although many people are exposed to these dangerous factors, only a fraction of exposed individuals develop AML, suggesting an individual susceptibility to exposure-related carcinogenesis.

Each individual's genetic material contains a unique

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single nucleotide polymorphism(SNP) pattern on the human genome map that is made up of many different genetic variants. Although, most SNP do not produce a disease per se, SNP serve as biological markers for genetic determinants to identify a disease, as they are usually located near a gene found to be associated with diseases. SNP may be associated with the development of a disease and therefore, used to search for and isolate the disease-causing gene[8].

DNA damage, induced by ROS and chemo-or radiotherapeutic agents, generally includes base modifications, single and double-strand DNA breaks(SSB and DSB_S), and DNA strands crosslink in the cell [9,10], and the DNA damage may lead to genome instability, which in turn may promote the development of cancer. Some evidence indicates that the incidence of leukemia in mouse and man is associated with exposure to chemo-or radiotherapeutic agents, which cause DSB_S[11]. There are two DSB_S repair pathways in mammalian cells: the homologous recombination(HR) and non-homologous end-joining(NHEJ)[12]. The latter plays a predominant role in repairing DSB_S in mammals[13]. A key component of the NHEJ apparatus is the DNA-dependent Protein Kinase(DNA-PK), which consists of a heterodimeric DNA targeting subunit(i.e., KU70/KU80, encoded by *XRCC6/ XRCC5* genes) and a catalytic subunit DNA-PKcs(encoded by *XRCC7* gene)[14].

There are dozens of genetic variants of the *XRCC5, XRCC6* and *XRCC7* that have been identified. However, to date, there are limited variants that have been determined. Fu *et al.*[15] reported *XRCC6* -61C>G，but not *XRCC6* 43009G>T or *XRCC6* 46922A>G polymorphisms, in the promoter region was associated with an increased risk of breast cancer. In contrast, we found previously that the *XRCC7* but not the *XRCC6* -61C>G appears to be involved in the etiology of human bladder cancer[16]. Two SNPs of *XRCC5*, 69506 G>A(rs3835) and 69632 G>A(rs3834), which are located in the intron of *XRCC5,* are not associated with breast cancer[15]. Recently, we identified a novel variable number of tandem polymorphisms located in the promoter region of *XRCC5*(at -201~-159 nucleotide sites for the initiation of transcription)[17]. This polymorphism contains three different alleles, which are two 21 nucleotides repeats(2R), one 21 nucleotides repeat (1R), and zero repeat unit(0R). We also found that this tandem repeat polymorphism was associated with bladder cancer and could affect *XRCC5* promoter activity and protein expression[18]. Genetic variants of the *XRCC7* 6721G>T locating in the intron 8 might regulate splicing and cause mRNA instability[19], and were also shown to elevate risk of glioma and renal cell carcinoma[20,21]. Some researchers reported that *XRCC6* -61C>G and *XRCC7* 6721G>T polymorphisms are associated with an increased risk of cancers, including breast cancer and glioma[15,20]. To the best of our knowledge, there is no report on the relationship between *XRCC5, XRCC6* and *XRCC7* polymorphisms and risk of AML. As we all know, *XRCC5, XRCC6* and *XRCC7* play an important role in the NHEJ pathway, so we hypothesized that the polymorphisms of the *XRCC5, XRCC6* and *XRCC7* contribute to the etiology of AML. The aim of the present study is to investigate the associations between the *XRCC5*, *XRCC6* and *XRCC7* polymorphisms and the development of AML in a Chinese population.

MATERIALS AND METHODS

Study participants

Study participants included 120 patients who were newly diagnosed with AML and 210 cancer-free controls from a Southern Chinese population, who were Han nationals. The subjects were recruited from the First and the Second Affiliated Hospitals of Nanjing Medical University, Jiangsu Province Hospital of Traditional Chinese Medicine and Suzhou City Hospital, respectively, from 2003 to 2007. All cases had cytogenetically, immunologically and marrow cytomorphologically confirmed AML and were without history of other cancers. All cases were recruited immediately after being diagnosed. The controls were randomly selected from a database used for recording regular physical examinations of cancer-free individuals living in the same residential areas, who were genetically unrelated and frequency-matched to the cases by age and gender. All cases and controls agreed to participate in the present study. After the study subjects provided written informed consent to participate in this study, and an interview was scheduled and conducted by trained interviewers using a structured questionnaire, which elicited information on demographic features and risk factors, such as age, gender. At the end of the interview, all subjects donated a single 5 ml peripheral blood sample that was stored and frozen at -70℃ within 6 h. The institutional review board of Nanjing Medical University approved the research protocol.

Genotyping

Genomic DNA was extracted from leukocyte pellets by Proteinase K digestion, followed by phenol-chloroform extraction and ethanol precipitation. The *XRCC5* 2R/1R/0R polymorphism was identified by polymerase chain reaction(PCR) and the *XRCC6 -61*C>G and *XRCC7* 6721G>T polymorphisms were identified using the polymerase chain reaction-restriction fragment length polymorphism(PCR-RFLP) methods. The following primers were used to amplify the target

fragments containing the polymorphisms to be studied: 5′-AGGCGGCTCAAACACCACAC-3′(forward) and 5′-CAAGCGGCAGATAGCGGAAAG-3′(reverse) to amplify the target fragment of *XRCC5* gene. The annealing temperature was 62℃. The fragment size was 266 bp, 245 bp or 224 bp, which were 2R allele, 1R allele or 0R allele,respectively;5′-TCTCCAC-TCGGCTT-TTCTTCCA-3′(forward) and 5′-TCT-CCCTCCGC-TTCGCACTC-3′(reverse) for the *XRCC6* -61C>G polymorphism and 5′-CGGCT-GCCAACGTTC-TTTCC-3′(forward) and 5′-TGCC-CTTAGTGGTT-CCCTGG-3′(reverse) for the *XRCC7* 6721 G > T polymorphism. The annealing temperature was 63℃ for the *XRCC6*-61C>G polymorphism and 61℃ for the *XRCC7* 6721 G > T polymorphism. The fragment size was 320 bp for the *XRCC6* -61C>G polymorphism and 368 bp for the *XRCC7* 6721 G>T polymorphism. The *Ban*Ⅰ and *Pvu*Ⅱ restriction enzymes(New England Biolabs, Beverly, MA) were used to distinguish the *XRCC6* -61C>G and *XRCC7* 6721 G>T, respectively, which resulted in fragments of 182, 80, and 58 bp in the presence of the *XRCC6* -61G allele, 262 and 58 bp in the presence of the *XRCC6* -61C allele, 368 bp in the *XRCC7* 6721 G allele and 274 and 94 bp in the presence of the *XRCC7* 6721 T allele[20]. The polymorphism analysis was performed by two individuals independently in a blind fashion. More than 30% of the samples were chosen randomly selected for repeated assays, and the results were 100% consistent.

Statistical analysis

The χ^2 test was used to evaluate differences in the frequency distributions of selected demographic variables and the frequencies of allele and genotype of the *XRCC5, XRCC6* and *XRCC7* polymorphisms between cases and controls. Both the univariate and multivariate logistic regression analysis were used to obtain the adjusted odds ratios(ORs) and 95% confidence intervals (CIs). The multivariate analysis was performed with adjustment for age and gender. The χ^2 test of statistical significance was conducted using the SAS software (version 9.1.3; SAS Institute Inc., Cary, NC). The criterion for significance was set at $P<0.05$ in two-sided tests.

RESULTS

Characteristics of the study subjects

The frequency distributions of selected characteristics between the cases and controls are shown in ***Table 1***. The mean age was 43.9 years for the cases(±20.8 years) and 43.7 years for controls(± 20.2 years), and the difference was not statistically significant($P = 0.15$). Similarly, the distribution of gender was not significantly different($P = 0.57$), suggesting that age and gender were equally matched between two populations.

Table 1 **Frequency distributions of selected variables in the AML cases**

Variables	Cases(n=120)		Controls(n=210)		P^a
	n	%	n	%	
Age(years)					
≤45	54	44	112	53.3	0.15
>45	66	55	98	46.7	
Gender					
Male	59	49	110	52.4	0.57
Female	61	51	100	47.6	

[a]Two-sided χ^2 test

Genotype distributions and association between *XRCC5, XRCC6* and *XRCC7* polymorphisms and risk of AML

Table 2 shows the genotype and allele frequencies of the *XRCC5, XRCC6* and *XRCC7* polymorphisms among cases and controls and the associations with AML development. For the *XRCC7* 6721G>T polymorphism, the frequencies of the TT, TG, and GG genotypes were 59.2%, 35.8%, and 5.0%, respectively, among AML cases, and 54.3%, 39.5%, and 6.2%, respectively, among controls. However, the difference was not statistically significant(P=0.68). For the *XRCC6* 61C > G polymorphism, the frequencies of the CC, CG, and GG genotypes were 69.2%, 29.2%, and 1.6%, respectively, among AML cases, and 55.2%, 41.0%, and 3.8%, respectively, among controls. Similarly, the frequencies of 2R/2R, 2R/1R, 2R/0R, 1R/1R, 1R/0R and 0R/0R genotypes of the *XRCC5* were 10.0%, 14.2%, 27.5%, 0.8%, 32.5% and 15.0%, respectively, among AML cases and 13.3%, 20.5%, 27.1%, 4.3%, 16.7% and 18.1%, respectively, among controls. These differences were statistically significant(P= 0.04 for *XRCC6* -61C > G and P= 0.01 for *XRCC5* 2R/1R/0R). As shown in ***Table 2***, the genotypes of 2R/2R, 2R/1R were more common (13.3%, 20.5%) and that of 2R/0R was less common (27.1%) among the controls than among the cases (10.0%, 14.2%, and 27.5% respectively). When these six genotypes were dichotomized into two groups(i.e., 2R/2R, 2R/1R vs. 2R/0R, 1R/1R, 1R/0R and 0R/0R), their distributions differed significantly between the cases and controls(P= 0.05). The genotype frequencies of these three polymorphisms among the controls were all in agreement with the Hardy-Weinberg equilibrium.

As shown in ***Table 2***, logistic regression analysis revealed that compared with the *XRCC6* -61CC wild-type homozygote, subjects carrying the -61CG heterozygote had a significant 43% decreased risk of AML (adjusted OR=0.57; 95% CI=0.35~0.92) and those carrying-61CG/GG variant genotypes had 45% decrease in risk of AML(adjusted OR=0.55; 95% CI=0.34~0.89).

For the *XRCC5* 2R/1R/0R polymorphism, we found that *XRRC5* 1R/0R genotype was associated with a 2.60-fold increase in risk of AML(95% CI=1.42~5.92) compared with the 2R/2R genotype. When compared with the combined genotypes(2R/2R+2R/1R), the(2R/0R+1R/1R+1R/0R+0R/0R) genotypes had a 1.67-fold elevated risk with AML(95% CI=1.00~2.79). In this study, we found that *XRCC7* 6721G >T polymorphism was not associated with the risk of AML(***Table 2***).

Table 2 **Genotype and allele frequencies of XRCC5, XRCC6 and XRCC7 polymorphisms among AML cases and associations with odds of AML**

Genotypes	Controls(N=210)[a]		AML(N=120)		P[b]	Adjusted OR(95% CI)[c]
	N	%	N	%		
XRCC7 6721G > T						
TT	114	54.3	71	59.2		1.00(reference)
TG	83	39.5	43	35.8	0.68	0.81(0.50~1.30)
GG	13	6.2	6	5		0.73(0.26~2.01)
TG+GG	96	45.7	49	40.8		0.80(0.50~1.26)
XRCC6 –61C > G						
CC	116	55.2	83	69.2		1.00 (reference)
CG	86	41	35	29.2	0.04	0.57 (0.35~0.92)
GG	8	3.8	2	1.6		0.36 (0.07~1.74)
CG+GG	94	44.8	132	30.8		0.55 (0.34~0.89)
XRCC5 2R/1R/0R						
2R/2R	28	13.3	12	10		1.00 (reference)
2R/1R	43	20.5	17	14.2		0.91 (0.38~2.20)
2R/0R	57	27.1	33	27.5	0.01	1.42 (0.63~3.19)
1R/1R	9	4.3	1	0.8		0.25 (0.03~2.17)
1R/0R	35	16.7	39	32.5		2.60 (1.42~5.92)
0R/0R	38	18.1	18	15		1.16 (0.48~2.82)
Dichotomized groups						
2R/2R, 2R/1R	71	33.8	29	24.2		1.00 (reference)
2R/0R, 1R/1R, 1R/0R, 0R/0R	139	66.2	91	75.8	0.05	1.67 (1.00~2.79)

The observed genotype frequency among the control subjects was in agreement with the Hardy-Weinberg equilibrium($p^2+2pq+q^2=1$) (χ^2=0.17, P=0.68 for XRCC7 6721G>T, and 2.71,0.10, for *XRCC6*-61C>G, χ^2=1.55, P=0.21 for *XRCC5* 2R>1R, χ^2=0.55, P=0.46 for XRCC5 2R>0R). [b]Two-sided χ^2 test for either genotype distribution; [c]Obtained from logistic regression models with adjustment for age, gender.

Association and stratification analyses between genotypes of the *XRCC5*, *XRCC6* and *XRCC7* polymorphisms and risk of AML

The association between the *XRCC5* 2R/1R/0R, *XRCC6* -61C>G and *XRCC7* 6721G>T variant genotypes and risk of AML was further examined by stratifying potential confounding variables, such as age and gender. As shown in ***Table 3*** and ***Table 4,*** males and aged ⩽ 45 years subjects carrying the *XRCC6* -61 (CG+GG) genotypes were associated with 51% (adjusted OR=0.49; 95% CI=0.25~0.98), 54% (adjusted OR=0.46; 95% CI=0.23~0.93), respectively, decrease in risk of AML. However, corresponding females and aged >45 years subjects were not associated with risk of AML. In the case of *XRCC5*(2R/0R+1R/1R+1R/0R+0R/0R) genotypes, we observed highly significant risks among males(adjusted OR=2.17; 95% CI=1.06~4.43) and aged >45 years subjects (adjusted OR=3.31; 95% CI=1.59~6.88), whereas they were not statistically significant among females and aged ⩽ 45 years individuals. However, there were no significant associations between the XRCC7 6721G>T polymorphism and AML risk stratified by age and gender.

DISCUSSION

In our study, three polymorphisms, *XRCC5* 2R/1R/0R, *XRCC6* -61C>G and *XRCC7* 6721G>T were selected to investigate the associations between the polymorphisms and risk of AML in a hospital-based case-control study in a southern Chinese population. We found a significant association with the polymorphisms of *XRCC5* 2R/1R/0R, *XRCC6* -61C>G and the risk of AML. However, there was no evidence for an association between the *XRCC7* 6721G>T variants and AML. To the best of our knowledge, this is the first report that the *XRCC5* 2R/1R/0R, *XRCC6* -61C>G and *XRCC7* 6721G>T polymorphisms are associated with the risk of AML.

Given the critical roles of the NHEJ pathway in DNA repair[22-24], it is biologically plausible that the *XRCC5, XRCC6* and *XRCC7* variants may modulate the risk of cancer, including leukemia. It has been shown that increased NHEJ activity is due to the presence of *XRCC5* and *XRCC6* protein, which result in genomic instability in myeloid leukemia cells[25-26].

Table 3 **Distribution of *XRCC5*, *XRCC6* and *XRCC7* polymorphisms in AML cases and controls stratified for gender**

Genotypes	Male		P^a	Adjusted OR (95% CI)[b]	Female		P^a	Adjusted OR (95% CI)[b]
	Cases (*n*=59)	Controls (*n*=110)			Cases (*n*=61)	Controls (*n*=100)		
XRCC7 6721G > T								
TT	34	57		1.00(reference)	37	57		1.00(reference)
TG+GG	25	53	0.42	0.77(0.40~1.46)	24	43	0.66	0.86(0.45~1.66)
XRCC6 –61C>G								
CC	41	60		1.00(reference)	42	56		1.00(reference)
CG+GG	18	50	0.04	0.49(0.25~0.98)	19	44	0.10	0.57(0.29~1.12)
XRCC5 2R/1R/0R								
2R/2R, 2R/1R	16	45		1.00(reference)	13	26		1.00(reference)
2R/0R, 1R/1R 1R/0R, 0R/0R	43	65	0.03	2.17(1.06~4.43)	48	74	0.50	1.30(0.61~2.77)

[a]Two-sided χ^2 test for the genotypes distribution between the cases and controls; [b]Obtained from a logistic regression model with adjustment for age

Table 4 **Distribution of *XRCC5*, *XRCC6* and *XRCC7* polymorphisms in AML cases and controls stratified for age**

Genotypes	Age(≤ 45 years)		P^a	OR(95% CI)[b]	Age(> 45 years)		P^a	OR(95% CI)[b]
	Cases (*n*=54)	Controls (*n*=112)			Cases (*n*=66)	Controls (*n*=98)		
XRCC7 6721G > T								
TT	32	66		1.00(reference)	39	48		1.00(reference)
TG+GG	22	46	0.84	1.07(0.55~2.10)	27	50	0.22	0.67(0.36~1.26)
XRCC6 -61C > G								
CC	38	61		1.00(reference)	45	55		1.00(reference)
CG+GG	16	51	0.03	0.46(0.23~0.93)	21	43	0.10	0.57(0.29~1.11)
XRCC5 2R/1R/0R								
2R/2R, 2R/1R	15	27		1.00(reference)	14	44		1.00(reference)
2R/0R, 1R/1R 1R/0R, 0R/0R	39	85	0.54	0.79(0.38~1.67)	52	54	0.001	3.31(1.59~6.88)

[a]Two-sided χ^2 test for the genotypes distribution between the cases and controls; [b]Obtained from a logistic regression model with adjustment for gender.

There are several reports that support our finding. For example, Ku is best known for its crucial role in DNA repair, especially in NHEJ. NHEJ is a homology-independent mechanism that rejoins broken ends irrespective of sequence, and this may result in genomic disorders[22]. *XRCC6* was elevated in bladder tumor tissues and neck cancer cell line[27]; and Holgersson *et al.*[28] reported increased expression of *XRCC5* and *XRCC7* protein in high-grade lymphoma patients. *XRCC5* and *XRCC6* may function as a caretaker gene for the development of T-cell lymphomas[29]. *XRCC7* encodes DNA-PKcs, which also may have a caretaker role in colon carcinogenesis[30]. Therefore, the polymorphisms of the *XRCC5, XRCC6, XRCC7*, if functional, could be expected to have an effect on DSB repair, and thus, on carcinogenesis.

We previously reported that fewer repeats(1R/1R,1R/0R) of *XRCC5* in the promoter region enhance the transcriptional activity and were associated with increased risk of bladder cancer, and this finding was supported by other research studies in gastric cancer[31], esophageal cancer[24], colorectal cancer[32], and head and neck cancer[23]. This over-activity of *XRCC5* leads to excess DNA repair, which can increase the resistance of cells to genotoxic agents and interfere with normal apoptosis, and thus increase the likelihood for the development of neoplasia. In the present study, we observed that *XRCC5* 2R/1R/0R polymorphism had a 1.67-fold increased risk of AML, which was consistent with our previous findings in bladder cancer[18].

The associations between *XRCC6*-61C>G and *XRCC7* 6721G>T polymorphisms and susceptibility to cancer have been extensively studied in some cancers. However, the results are conflicting. For example, *XRCC6* -61C>G and *XRCC7* 6721G>T were associated with an increased risk of breast cancer[15], and glioma[20], respectively, while recent studies reported that there were no significant associations between the *XRCC7* 6721G>T polymorphism and risk of renal cell carcinoma and differentiated thyroid cancer[21-33]. Our findings further support that the polymorphisms of the *XRCC6* -61C>G (not *XRCC7* 6721G>T polymorphism) may contribute to genetic susceptibility to AML. Compared with the published data, our findings indicate that the genotype distributions of *XRCC7* 6721G>T polymorphisms vary with ethnicity. In the present study, the MAF of 6721G allele was 0.29(data not shown), which is consistent with the report by Hiroshi *et al.*[21] among Japanese, but differs from American Caucasians (0.39)[20]. However, the frequency of the *XRCC6* -61

G allele was 0.24(data not shown) in this study, which is similar to a study of Taiwanese[16]. However, these findings require further investigation because of the small number of cases.

It is well known that *XRCC5* ,*XRCC6* and *XRCC7* play critical roles in the same causal pathway involved in DNA double-strand breaks(DSBs) repair. Liu *et al.*[34] showed that the interaction of SNPs of *XRCC5, XRCC6, XRCC7* is the best model for predicting risk of glioma, and the association between glioma and three genes was stronger for gene-gene interactions than for a single gene. In our study, we found the combined genotypes of these three genes were associated with a significantly increased risk of AML(data not shown), suggesting that these three polymorphisms may contribute a joint effect to the etiology of AML

In this study, subgroups of male and aged > 45 years subjects with regard to *XRCC5* polymorphism appeared to have higher risk of AML. However, the protective effect of *XRCC6* on risk of AML was more pronounced among the aged ⩽45 years and male individuals. This finding may reflect a high level of genetic susceptibility, possibly owing to various DNA repair capability in these groups[35]. Similar results have been found by Lin *et al.*[36] showing that ALL was associated with age and gender in polymorphisms of *ERCC1* However, this finding may be by chance, owing to the small number of observations in the stratification analysis. Also, in the present study we did not make the classification of the AML because of limitary information, and it may be a potential confounder in the evaluation of polymorphism attributed to the development of AML.

In conclusion, the study suggests that functional *XRCC6* -61C>G and *XRCC5* 2R/1R/0R polymorphisms could play an important role in the development of AML. However, the *XRCC7* 6721G>T polymorphism had no main effect on risk of AML. Larger scale studies, including more detailed environmental exposure status and more detailed patient clinical information, are needed to verify these findings.

References

[1] Lowenberg B, Downing JR, Burnett A. Acute myeloid leukemia. *N Engl J Med* 1999; 341: 1051-62.

[2] Kelly LM, Gilliland DG. Genetics of myeloid leukemias. *Annu Rev Genomics Hum Genet* 2002; 3: 179-98.

[3] Elliott B, Jasin M. Double-strand breaks and translocations in cancer. *Cell Mol Life Sci* 2002; 59: 373-85.

[4] Olive PL. The role of DNA single- and double-strand breaks in cell killing by ionizing radiation. *Radiat Res* 1998; 150: S42-51.

[5] West RB, Yaneva M, Lieber MR. Productive and nonproductive complexes of Ku and DNA-dependent protein kinase at DNA termini. *Mol Cell Biol* 1998; 18: 5908-20.

[6] Pedersen-Bjergaard J, Rowley JD. The balanced and the unbalanced chromosome aberrations of acute myeloid leukemia may develop in different ways and may contribute differently to malignant transformation. *Blood* 1994; 83: 2780-6.

[7] Chou WC, Jie C, Kenedy AA, Jones RJ, Trush MA, Dang CV. Role of NADPH oxidase in arsenic-induced reactive oxygen species formation and cytotoxicity in myeloid leukemia cells. *Proc Natl Acad Sci U S A* 2004; 101: 4578-83.

[8] Shen Q, Tang WY, Li CP, Chen R, Zhu YJ, Huang S, *et al.* Functional variations in the JWA gene are associated with increased odds of leukemias. *Leuk Res* 2007; 31: 783-90.

[9] Kopjar VG-VN, Milas I. Assessment of chemotherapy-induced DNA damage in peripheral blood leukocytes of cancer patients using the alkaline comet assay. *Carcinog Mutagen* 2002; 22: 13-30.

[10] Singh MTM NP, Tice RR, Schneider EL. A simple technique for quantitation of low levels of DNA damage in individual cells. *Exp Cell Res* 1988; 175 184-91.

[11] Preston DL, Kusumi S, Tomonaga M, Izumi S, Ron E, Kuramoto A, *et al.* Cancer incidence in atomic bomb survivors. Part III. Leukemia, lymphoma and multiple myeloma, 1950-1987. *Radiat Res* 1994; 137: S68-97.

[12] Khanna KK, Jackson SP. DNA double-strand breaks: signaling, repair and the cancer connection. *Nat Genet* 2001; 27: 247-54.

[13] Hopfner KP, Putnam CD, Tainer JA. DNA double-strand break repair from head to tail. *Curr Opin Struct Biol* 2002; 12: 115-22.

[14] Smith GC, Jackson SP. The DNA-dependent protein kinase. *Genes Dev* 1999; 13: 916-34.

[15] Fu YP, Yu JC, Cheng TC, Lou MA, Hsu GC, Wu CY, *et al.* Breast cancer risk associated with genotypic polymorphism of the nonhomologous end-joining genes: a multigenic study on cancer susceptibility. *Cancer Res* 2003; 63: 2440-6.

[16] Wang SY, Peng L, Li CP, Li AP, Zhou JW, Zhang ZD, *et al.* Genetic variants of the XRCC7 gene involved in DNA repair and risk of human bladder cancer. *Int J Urol* 2008; 15: 534-9.

[17] Ludwig DL, Chen F, Peterson SR, Nussenzweig A, Li GC, Chen DJ. Ku80 gene expression is Sp1-dependent and sensitive to CpG methylation within a novel cis element. *Gene* 1997; 199:181-94.

[18] Wang S, Wang M, Yin S, Fu G, Li C, Chen R, *et al.* A novel variable number of tandem repeats (VNTR) polymorphism containing Sp1 binding elements in the promoter of XRCC5 is a risk factor for human bladder cancer. *Mutat Res* 2008; 638: 26-36.

[19] Sipley JD, Menninger JC, Hartley KO, Ward DC, Jackson SP, 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 U S A* 1995; 92: 7515-9.

[20] Wang LE, Bondy ML, Shen H, El-Zein R, Aldape K, Cao Y, *et al.* Polymorphisms of DNA repair genes and risk of glioma. *Cancer Res* 2004; 64: 5560-3.

[21] Hirata H, Hinoda Y, Matsuyama H, Tanaka Y, Okayama N, Suehiro Y, *et al.* Polymorphisms of DNA repair genes are associated with renal cell carcinoma. *Biochem Biophys Res Commun* 2006; 342: 1058-62.

[22] Gullo C, Au M, Feng G, Teoh G. The biology of Ku and its potential oncogenic role in cancer. *Biochim Biophys Acta* 2006; 1765: 223-34.

[23] Chang HW, Kim SY, Yi SL, Son SH, Song DY, Moon SY, *et al.* Expression of Ku80 correlates with sensitivities to radiation in cancer cell lines of the head and neck. *Oral Oncol* 2006;42: 979-86

[24] Tonotsuka N, Hosoi Y, Miyazaki S, Miyata G, Sugawara K, Mori T, *et al.* Heterogeneous expression of DNA-dependent protein kinase in esophageal cancer and normal epithelium. *Int J Mol Med* 2006; 18: 441-7.

[25] Gaymes TJ, Mufti GJ, Rassool FV. Myeloid leukemias have increased activity of the nonhomologous end-joining pathway and concomitant DNA misrepair that is dependent on the Ku70/86 heterodimer. *Cancer Res* 2002; 62: 2791-7.

[26] Brady N, Gaymes TJ, Cheung M, Mufti GJ, Rassool FV. Increased error-prone NHEJ activity in myeloid leukemias is associated with DNA damage at sites that recruit key nonhomologous end-joining proteins. *Cancer Res* 2003; 63: 1798-805.

[27] Sturgis EM, Clayman GL, Guan Y, Guo Z, Wei Q. DNA repair in lymphoblastoid cell lines from patients with head and neck cancer. *Arch Otolaryngol Head Neck Surg* 1999; 125: 185-90.

[28] Holgersson A, Erdal H, Nilsson A, Lewensohn R, Kanter L. Expression of DNA-PKcs and Ku86, but not Ku70, differs between lymphoid malignancies. *Exp Mol Pathol* 2004; 77: 1-6.

[29] Li GC, Ouyang H, Li X, Nagasawa H, Little JB, Chen DJ, *et al.* Ku70: a candidate tumor suppressor gene for murine T cell lymphoma. *Mol Cell* 1998; 2: 1-8.

[30] Ochiai M, Ubagai T, Kawamori T, Imai H, Sugimura T, Nakagama H. High susceptibility of Scid mice to colon carcinogenesis induced by azoxymethane indicates a possible caretaker role for DNA-dependent protein kinase. *Carcinogenesis* 2001; 22: 1551-5.

[31] Lim JW, Kim H, Kim KH. Expression of Ku70 and Ku80 mediated by NF-kappa B and cyclooxygenase-2 is related to proliferation of human gastric cancer cells. *J Biol Chem* 2002; 277: 46093-100.

[32] Hosoi Y, Watanabe T, Nakagawa K, Matsumoto Y, Enomoto A, Morita A, *et al.* Up-regulation of DNA-dependent protein kinase activity and Sp1 in colorectal cancer. *Int J Oncol* 2004; 25: 461-8.

[33] Sturgis EM, Zhao C, Zheng R, Wei Q. Radiation response genotype and risk of differentiated thyroid cancer: a case-control analysis. *Laryngoscope* 2005; 115: 938-45.

[34] Liu Y, Zhang H, Zhou K, Chen L, Xu Z, Zhong Y, *et al.* Tagging SNPs in non-homologous end-joining pathway genes and risk of glioma. *Carcinogenesis* 2007; 28: 1906-13.

[35] Wei Q, Cheng L, Amos CI, Wang LE, Guo Z, Hong WK, *et al.* Repair of tobacco carcinogen-induced DNA adducts and lung cancer risk: a molecular epidemiologic study. *J Natl Cancer Inst* 2000; 92: 1764-72.

[36] Wang SL, Zhao H, Zhou B, Chen YL, Zou Y, Zhu XF, *et al.* Polymorphisms in ERCC1 and susceptibility to childhood acute lymphoblastic leukemia in a Chinese population. *Leuk Res* 2006; 30: 1341-5.