Int J Clin Exp Pathol 2014;7(9):6231-6244
www.ijcep.com /ISSN:1936-2625/IJCEP0001459

Original Article

Interaction of DNA repair gene polymorphisms and aflatoxin B1 in the risk of hepatocellular carcinoma

Jin-Guang Yao[1*], Xiao-Ying Huang[1*], Xi-Dai Long[1,2]

[1]Department of Pathology, Youjiang Medical College for Nationalities, Baise 533000, P. R. China; [2]Department of Liver Surgery, Ren Ji Hospital, School of Medicine, Shanghai Jiao Tong University (RJHSJTUM), Shanghai 200127, P. R. China. [*]Equal contributors.

Received July 18, 2014; Accepted August 23, 2014; Epub August 15, 2014; Published September 1, 2014

Abstract: Aflatoxin B1 (AFB1) is an important environmental carcinogen and can induce DNA damage and involve in the carcinogenesis of hepatocellular carcinoma (HCC). The deficiency of DNA repair capacity related to the polymorphisms of DNA repair genes might play a central role in the process of HCC tumorigenesis. However, the interaction of DNA repair gene polymorphisms and AFB1 in the risk of hepatocellular carcinoma has not been elucidated. In this study, we investigated whether six polymorphisms (including rs25487, rs861539, rs7003908, rs28383151, rs13181, and rs2228001) in DNA repair genes (XPC, XRCC4, XRCC1, XRCC4, XPD, XRCC7, and XRCC3) interacted with AFB1, and the gene-environmental interactive role in the risk of HCC using hospital-based case-control study (including 1486 HCC cases and 1996 controls). Genotypes of DNA repair genes were tested using TaqMan-PCR technique. Higher AFB1 exposure was observed among HCC patients versus the control group [odds ratio (OR) = 2.08 for medium AFB1 exposure level and OR = 6.52 for high AFB1 exposure level]. Increasing risk of HCC was also observed in these with the mutants of DNA repair genes (risk values were from 1.57 to 5.86). Furthermore, these risk roles would be more noticeable under the conditions of two variables, and positive interactive effects were proved in the followed multiplicative interaction analysis. These results suggested that DNA repair risk genotypes might interact with AFB1 in the risk of HCC.

Keywords: Aflatoxin B1, DNA repair gene, interaction effect, polymorphism, hepatocellular carcinoma

Introduction

Hepatocellular carcinoma (HCC) is the most common malignant tumor in the Guangxi Zhuang Autonomous Region of China [1, 2]. Epidemiological evidence has shown that exposure to aflatoxin B1 (AFB1), an important chemical carcinogen, is the most important cause of the high rate of HCC in this area [1, 2]. This carcinogen was an important member of aflatoxin family highly substituted coumarins containing a fused dihydrofurofuran moiety [1-3]. It is mainly produced by some strains of the moulds Aspergillus parasiticus and Aspergillus flavus, and is structurally characterized by fusion of a cyclopentanone ring to the lactone ring of the coumarin moiety [1, 2]. AFB1 was discovered as a contaminant of human and animal food, especially peanuts (ground nuts), core, soya sauce, and fermented soy beans in tropical areas such as the Southeastern China as a result of fungal contamination during growth and after harvest which under hot and humid conditions. This type of toxin has three toxicological effects: *a.* genotoxicity, mainly inducing the formation of AFB1-DNA adducts and the hot-spot mutation of p53 gene; *b.* the attraction of specific organs, especially liver; and *c.* carcinogenicity, primarily causing hepatocellular carcinoma (HCC) [1-9]. Today, AFB1 has been classified as a known human carcinogen by the International Agency for Research on Cancer [1-3]. Increasing evidence has suggested that AFB1 can induce the formation of AFB1-DNA adducts and cause DNA strand breakage, DNA base damage, and oxidative damage that may ultimately lead to cancer. AFB1-induced DNA damage can be repaired by base excision repair, strand break repair, and nucleotide excision repair (NER) [1, 2]. Recent studies have shown

Table 1. Characteristics of polymorphisms in the DNA repair genes

Polymorphism	Gene	Chr:bp	Alleles		Genotypes			Codon No.	Amino acid	Genotyping
			Wild	Mutant	Wild	Heterozygotes	mutant			
rs25487	XRCC1	19:44055726	C	T	CC	CT	TT	399	Arg/Gln	TaqMan-PCR
rs861539	XRCC3	14:104165753	G	A	GG	GA	AA	241	Thr/Met	TaqMan-PCR
rs7003908	XRCC7	8:48770702	A	C	AA	AC	CC	/	/	TaqMan-PCR
rs28383151	XRCC4	5:82406873	G	A	GG	GA	AA	56	Ala/Thr	TaqMan-PCR
rs13181	XPD	19:45854919	T	G	TT	TG	GG	751	Lys/Gln	TaqMan-PCR
rs2228001	XPC	3:14187449	T	G	TT	TG	GG	939	Lys/Gln	TaqMan-PCR

that DNA damage by AFB1 plays the central role of carcinogenesis of HCC-related to this toxin in the toxic studies [1, 2, 10-17]. Furthermore, AFB1-induced HCC risk value might be more noticeable under the conditions of more amounts of DNA damage [1, 2, 11, 12, 14-21]. This indicates DNA repair capacity (limited to DNA repair genes) might be able to interact with AFB1 exposure and this interaction might strengthen the risk effects of AFB1 exposure on HCC risk. Here, we investigated the interactive effects of AFB1 exposure and genetic polymorphisms in DNA repair genes XRCC1, XRCC3, XRCC4, XRCC7, XPD, and XPC (including rs25487, rs861539, rs7003908, rs28383151, rs13181, and rs2228001) on HCC risk.

Materials and methods

Study subject

This was a hospital-based case-control study, including 1486 HCC cases and 1996 controls [10]. All cases and controls were recruited from affiliated hospitals of the two main medical colleges in southwestern Guangxi (Guangxi Medical University and Youjiang Medical College for Nationalities) from January 2004 to December 2012. All cases and controls were residents of the Guangxi Zhuang Autonomous Region from AFB1 exposure areas and accepted enrollment in this study. The cases included in this study, representing a significant proportion (> 90%) of HCC patients in the Guangxi population, were identified by histopathological diagnosis in 100% of the HCC cases. During the same period of HCC investigation, controls without any evidence of liver disease were randomly selected from a pool of healthy volunteers who visited the general health check-up centers of the same hospitals for their routinely scheduled physical examinations supported by local governments.

To control the effects of confounders which were associated with the distribution of genotypes or the exposure of AFB1, controls were individually matched (1:1 or 2:1) to cases based on ethnicity (Han, Minority), sex, age (± years), and hepatic B virus (HBV) and hepatic C virus (HCV) infection. All patients and controls gave informed consent for participation and were interviewed uniformly before surgery by a well-trained interviewer. The questionnaire used in the interview sought detailed information on current and past living habits, occupational history, family disease history, dietary history and general demographic data. Demographic information (including age, sex, race, medical history, family disease history, dietary history, and living history), α-fetoprotein (AFP), hepatitis virus B (HBV) and hepatitis virus C (HCV) infection information, and therapeutic data were collected from medical records in the hospitals by a Youjiang Cancer Institution staff member. At the same time, 4 mL of peripheral blood was obtained for the extraction of genomic DNA. In this study, those hepatitis B surface antigen (HBsAg) positive and anti-HCV positive in their peripheral serum were defined as groups infected with HBV and HCV.

The study protocol was been carried out in accordance with *Ethical Principles for Medical Research Involving Human Subjects* (World Medical Association Declaration Of Helsinki, 2004) and approved by Institutional review boards from Guangxi Cancer Institute, and the Medical Research Council from the corresponding hospitals.

Nucleic acid isolation

Leukocytes from peripheral venous blood samples were isolated by standard procedures. DNA was then extracted from leukocyte samples by standard phenol-chloroform extraction and ethanol precipitation. DNA was stored at -20°C until additional analysis.

Table 2. Technical details of TaqMan-PCR analysis

Polymorphism	Gene	Primers	Probes
rs25487	XRCC1	5'-GTGGGTGCTGGACTGTC-3'	5'-FAM-CCTCCCGGAGGTAA-MGB-3'
		5'-GCAGGGTTGGCGTGTGA-3'	5'-VIC-CCCTCCCAGAGGTAA-MGB-3'
rs861539	XRCC3	5'-CCAGGGCCAGGCATCTG-3'	5'-FAM-CAGCATGGCCCCCA-MGB-3'
		5'-CAGCACAGGGCTCTGGA-3'	5'-VIC-CAGCGTGGCCCCCA-MGB-3'
rs7003908	XRCC7	5'-CCTACCTCACGAACTCAGCAATT-3'	5'-FAM-CTAAGAGTCCGCTGTTT-MGB-3'
		5'-GCTGCCAACGTTCTTTCCTTATAGT-3'	5'-Hex-CCTAAGAGTCAGCTGTTT-MGB-3'
rs28383151	XRCC4	C_58444701_10[a]	C_58444701_10
rs13181	XPD	5'-AGTCACCAGGAACCGTTTATGG-3'	5'-HEX-CTCTATCCTCTGCAGCG-MGB-3'
		5'-TCTGTTCTCTGCAGGAGG ATC-3'	5'-FAM-TATCCTCTTCAGCGTCT-MGB-3'
rs2228001	XPC	5'-AGCAGCTTCCCACCTGTTC-3'	5'-FAM-CACAGCTGCTCAAAT-MGB-3'
		5'-GTGGGTGCCCCTCTAGTG-5'	5'-Hex-CTCACAGCTTCTCAAAT-MGB-3'

[a]From the Applied Biosystems.

Table 3. Demographic and Etiologic Characteristics of HCC Cases and Controls

	Controls (n = 1996)		Cases (n = 1486)			
	n	%	n	%	c^2	*P*
Sex					0.157	0.692
Male	1516	76.0	1120	75.4		
Female	480	24.0	366	24.6		
Age (years)[a]					4.631	0.705
≤ 34	225	11.3	147	9.9		
35-40	309	15.5	261	17.6		
41-45	292	14.6	219	14.7		
46-50	296	14.8	221	14.9		
51-55	242	12.1	168	11.3		
56-60	218	10.9	160	10.8		
61-65	210	10.5	164	11.0		
≥ 66	204	10.2	146	9.8		
Ethnicity					2.089	0.148
Han	891	44.6	700	47.1		
Zhuang	1105	55.4	786	52.9		
HBV infection					2.424	0.119
HBsAg (-)	588	29.5	402	27.1		
HBsAg (+)	1408	70.5	1084	72.9		
HCV infection					0.312	0.576
anti-HCV (-)	1640	82.2	1210	81.4		
anti-HCV (+)	356	17.8	276	18.6		

[a]The mean ± S.D. ages were 49.32 ± 11.43 and 49.42 ± 11.30 for cases and controls, respectively.

AFB1 exposure assay

In this study, AFB1 exposure levels were ascertained according to serum AFB1-albumin adducts levels of peripheral blood. AFB1-albumin adducts levels were tested using the comparative enzyme-linked immunosorbent assay (ELISA) as previously published [22].

Polymorphisms selection of DNA repair genes and genotyping

In this study, we only selected these single nucleotide polymorphisms (SNPs) in the DNA repair genes that might modify AFB1-related HCC risk. According to our previous results, a total of 6 SNPs, including rs25487 (in the XRCC1), rs861539 (in the XRCC3), rs7003908 (in the XRCC7), rs28383151 (in the XRCC4), rs13181 (in the XPD), and rs2228001 (in the XPC), were finally analyzed in the present study (**Table 1**) [10].

The genotypes of DNA repair genes were genotyped using the previous TaqMan-PCR methods on iCycler iQ™ real-time PCR detection system (iQ5, Bio-Rad Laboratories Inc.). Primer and probe sets and annealing temperatures used for TaqMan-PCR assay are shown in **Table 2** [10]. For quality control, controls were included in each run, and repeated genotyping and sequencing of a random 5% subset yielded 100% identical genotypes.

Statistical analysis

All statistical analyses were done using SPSS version 18 (SPSS, Inc., Chicago, IL). In this

Table 4. AFB1 exposure and the risk of HCC

AFB1 exposure[b]	Controls		HCCs		OR (95% CI)[a]	P_{trend}
	n	%	n	%		
Low[c]	1060	53.1	352	23.7	Reference	
Medium	604	30.3	417	28.1	2.08 (1.75-2.47)	1.36×10^{-16}
High	332	16.6	717	48.3	6.52 (5.46-7.79)	1.26×10^{-94}

[a]OR conditional on matched set. [b]The mean ± S.D. level of serum AFB1-album adducts is 2.98 ± 0.80 and 2.18 ± 0.64 ln fmol/mg for cases and controls, respectively. [c]Adduct levels were: < 2.18 ln fmol/mg for low level; 2.18-2.98 ln fmol/mg for medium; > 2.98 ln fmol/mg for high level.

study, genotype data were analyzed as trichotomous variables, including wild homozygotes (wild genotype), heterozygotes (heterozygotes genotype), and mutant homozygotes (mutant genotype). Frequency tables of independent variables were evaluated for statistical significance by Pearson's χ^2. To analyze the risk for gene mutation and HCC associated with each genotype while adjusting for confounders, multivariable logistic regression was done and odds ratios (OR) along with 95% confidence intervals (95% CI) generated. In this type of the additive model, we treated genotype as an ordinal variable (wild type coded as 0, heterozygote as 1, and homozygotes variant as 2). Based on individually matched design of case-control study, we did conditional logistic regression (with multivariate factors, including known causes of HCC among the Guangxi population) to estimate ORs for risk of HCC and their 95% CIs.

For environment-gene interaction analysis, joint effects between genotypes and AFB1 exposure status on HCC risk were assessed with the full regression model, which included all possible confounders. The interactive effects were evaluated according to the following formula [11, 12, 23]: $OR_{eg} < OR_{eg'} \times OR_{e'g}$ where OR_{eg} is the odds ratio for the presence of both high AFB1 exposure and risk genotypes of DNA repair genes (adjusted OR > 1), $OR_{eg'}$ is the odds ratio for high AFB1 exposure for patients with the low-risk genotypes of DNA repair genes, and Ore'g is the odds ratio for the high risk genotypes of DNA repair genes in patients with low AFB1 exposure. In the present study, a *P*-value of < 0.05 was considered statistically significant.

Results

Demographic characteristics of HCC cases

The 1486 HCC cases and 1996 controls were from high AFB1 exposure areas and included in the final analysis and the demographic data of these subjects is shown in **Table 3**. There were no significant differences between cases and controls in terms of distribution of age, sex, race, and HBV and HCV status as a result of individual matching ($P > 0.05$). These results suggest that HCC patient data were comparable to control data.

AFB1 exposure and HCC risk

The AFB1 exposure information for the entire study population is shown in **Table 4**. We found that HCC cases (28.36 fmol/mg) had higher serum levels of AFB1-albumin adducts than controls (11.55 fmol/mg). For statistical analysis, values were logarithmically transformed and then were divided into three stratus: low (< 2.18 ln fmol/mg), medium (2.18-2.98 ln fmol/mg), and high (> 2.98 ln fmol/mg), according to the mean logit value of serum AFB1-albumin adducts among controls and cases (**Table 4**). Regression analysis showed that HCC risk gradually increased with an increasing number of exposure levels (adjusted OR = 2.08-6.52; $P < 0.01$).

DNA repair genes polymorphisms and HCC risk

To explore the correlation between these six polymorphisms in DNA repair genes and HCC risk, we conducted a hospital-based case-control study according to our previously published methods [11, 12]. A total of 1486 HCC cases and 1996 individually-matched (based on age, sex, race, and HBV and HCV infection status) controls were included in the present risk analysis (**Table 5**). Higher frequency of mutants of DNA repair genes was observed in the HCC patients than in the controls. Conditional logistic regression analysis exhibited that mutant alleles increased about 2 to 6 fold of HCC risk value. This risk was more noticeable under the conditions of mutant homozygotes (**Table 5**). For example, HCC risk for the genotype with XRCC4 rs28383151-GA was 2.248 (1.857-2.722); whereas risk value was 3.690 (2.708-5.029) for XRCC4 rs28383151-AA genotype. These results suggested the risk of HCC was associated with the number of mutant alleles

Table 5. Polymorphisms in DNA repair genes and HCC risk

			Controls		HCCs				
Gene	Polymorphism	Genotype	n	%	n	%	OR[a]	95% CI	*P*
XRCC1	rs25487	CC	1437	71.99	777	52.29	Reference		
		CT	520	26.05	608	40.92	2.155	1.861-2.495	9.918×10^{-25}
		TT	39	1.95	101	6.80	4.774	3.264-6.981	7.614×10^{-16}
XRCC3	rs861539	GG	1430	71.64	509	34.25	Reference		
		GA	539	27.00	634	42.66	3.321	2.848-3.872	5.671×10^{-53}
		AA	27	1.35	343	23.08	5.846	3.907-13.747	3.399×10^{-67}
XRCC7	rs7003908	AA	1141	57.16	363	24.43	Reference		
		AC	608	30.46	663	44.62	3.434	2.921-4.037	1.664×10^{-50}
		CC	247	12.37	460	30.96	5.867	4.828-7.129	7.478×10^{-71}
XRCC4	rs28383151	GG	1717	86.02	1047	70.46	Reference		
		GA	217	10.87	300	20.19	2.248	1.857-2.722	1.034×10^{-16}
		AA	62	3.11	139	9.35	3.690	2.708-5.029	1.341×10^{-16}
XPD	rs13181	TT	1214	60.82	549	36.94	Reference		
		TG	611	30.61	607	40.85	2.193	1.884-2.551	3.216×10^{-24}
		GG	171	8.57	330	22.21	4.270	3.458-5.273	1.841×10^{-41}
XPC	rs2228001	TT	988	49.50	546	36.74	Reference		
		TG	804	40.28	696	46.84	1.570	1.357-1.817	1.388×10^{-9}
		GG	204	10.22	244	16.42	2.185	1.764-2.706	8.231×10^{-13}

[a]Adjusted by age, sex, race, HBV status, and HCV status.

of DNA repair genes XRCC1, XRCC3, XRCC7, XRCC4, XPD, and XPC.

To assess possible interactive effects of matching factors and polymorphisms in the DNA repair genes on HCC risk, we performed a series of bivariate stratified analyses by matching factors, such as HBV and HCV infection, age, race, and sex, on this polymorphism and did not find that these factors modulated the effect of these polymorphisms on HCC risk ($P_{interaction} > 0.05$; Data not shown). This implied that these matching factors should be effectually manipulated and should not modify the association between these six polymorphisms and HCC in the present study.

Joint effects of DNA repair genes polymorphisms and AFB1 exposure on HCC risk

To study the correlation between the polymorphisms in the DNA repair genes and AFB1 exposure in the risk for HCC, we first analyzed the joint effects of AFB1 exposure levels and DNA repair genes genotypes on HCC risk (**Table 6**). In this analysis, we used as a reference the lowest risk group: those who had wild genotypes of DNA repair genes and low AFB1-exposure levels. We observed that increasing the number of exposure levels consistently increased HCC risk; moreover, this risk was more pronounced among subjects with the risk genotypes of DNA repair genes XRCC1, XRCC3, XRCC7, XRCC4, XPD, and XPC (OR, > 1). For example, risk value was 1.41 (1.02-1.95) for these individuals with risk genotypes with XRCC4-GA/AA (Stratum 2); whereas corresponding value was 8.81 (6.95-11.17) under the conditions of high AFB1 exposure levels (Stratum 4). We next analyzed the possible environment exposure-risk genotypic interaction using multiplicative models (**Table 7**) and found significantly evidence of multiplicatively interactive effects of genotypes and AFB1 exposure on HCC risk according to the previously published formula ($OR_{eg} < OR_{eg'} \times OR_{e'g}$) [23].

To elucidate the environment-gene interactive effects on HCC risk, we calculated the interactive coefficient between AFB1 exposure and genotypes of DNA repair genes using logistic regression models with the environment-gene interactive variables (**Table 8**). Results showed that the corresponding interactive coefficients were about 1.6 for between AFB1 exposure and DNA repair genes XRCC1, XRCC3, XRCC4, and XRCC7 ($P_{interaction} < 0.01$). Taken together, these results exhibited AFB1 exposure significantly and multiplicatively interacted with the poly-

Table 6. Joint effects of Polymorphisms in DNA repair genes and AFB1 exposure on HCC risk

	Controls		HCCs				
Variable	n	%	n	%	OR[a]	95% CI	*P*
AFB1/XRCC1							
Stratum 1[b]	769	38.5	200	13.5	Reference		
Stratum 2[c]	291	14.6	152	10.2	2.01	1.56-2.58	4.77×10^{-8}
Stratum 3[d]	668	33.5	577	38.8	3.32	2.74-4.02	1.10×10^{-34}
Stratum 4[e]	268	13.4	557	37.5	7.98	6.44-9.87	4.64×10^{-81}
AFB1/XRCC3							
Stratum 1	782	39.2	185	12.5	Reference		
Stratum 2	278	13.9	167	11.2	2.54	1.98-3.26	2.72×10^{-13}
Stratum 3	648	32.5	324	21.8	2.12	1.72-2.61	1.95×10^{-12}
Stratum 4	288	14.4	810	54.5	11.9	9.65-14.88	1.14×10^{-118}
AFB1/XRCC7							
Stratum 1	592	29.7	103	6.9	Reference		
Stratum 2	468	23.4	249	16.8	3.06	2.36-3.97	2.99×10^{-17}
Stratum 3	549	27.5	260	17.5	2.72	2.11-3.52	1.79×10^{-14}
Stratum 4	387	19.4	874	58.8	12.96	10.19-16.50	2.40×10^{-96}
AFB1/XRCC4							
Stratum 1	916	45.9	288	19.4	Reference		
Stratum 2	144	7.2	64	4.3	1.41	1.02-1.95	3.66×10^{-2}
Stratum 3	801	40.1	759	51.1	3.09	2.55-3.55	8.24×10^{-39}
Stratum 4	135	6.8	375	25.2	8.81	6.95-11.17	3.36×10^{-72}
AFB1/XPC							
Stratum 1	516	25.9	152	10.2	Reference		
Stratum 2	544	27.3	200	13.5	1.25	0.98-1.60	0.07
Stratum 3	472	23.6	394	26.5	2.83	2.26-3.55	1.14×10^{-19}
Stratum 4	464	23.2	740	49.8	5.41	4.36-6.71	1.79×10^{-53}
AFB1/XPD							
Stratum 1	654	32.8	143	9.6	Reference		
Stratum 2	406	20.3	209	14.1	2.36	1.84-3.01	9.15×10^{-12}
Stratum 3	560	28.1	406	27.3	3.32	2.66-4.14	2.74×10^{-26}
Stratum 4	376	18.8	728	49.0	8.87	7.11-11.05	4.27×10^{-84}

[a]OR conditional on matched set. [b]Stratum 1 refers to the combination of low AFB1 exposure and wild genotypes of DNA repair genes. [c]Stratum 2 refers to the combination of low AFB1 exposure and genotypes of DNA repair genes with mutant alleles. [d]Stratum 3 refers to the combination of medium-high AFB1 exposure and wild genotypes of DNA repair genes. [e]Stratum 4 refers to the combination of medium-high AFB1 exposure and genotypes of DNA repair genes with mutant alleles.

morphisms in the DNA repair genes in the process of HCC carcinogenesis.

Discussion

Evaluation of AFB1-DNA repair genes interaction

The main toxicological effect of AFB1 is to induce DNA damage, consisting of AFB1-DNA adducts and the hot-spot mutation of tumor suppressor gene p53 at codon 249 (TP53M) [5, 20, 24, 25]. Our previous studies have shown the low DNA repair capacity from genetic polymorphisms in the DNA repair genes increases the toxicological effects of AFB1 exposure [10]. This suggests that there be environment-gene interaction in the process of HCC carcinogenesis induced by AFB1 exposure. In this study, we systematically investigated the possible interaction of this toxin and genotypes of DNA repair genes via three models: (1) joint effects model, (2) environment-gene interactive model, and (3) interactive coefficient model.

Because previous reports have exhibited that both environmental variable AFB1 exposure and genic variable DNA repair genes increase HCC risk [10-17], we analyzed the combination effects of AFB1 exposure and polymorphisms in the DNA repairgenes XRCC1, XRCC3, XRCC4, XRCC7, XPD, and XPC in the joint effects model. In this model, environmental variable and genotype variable were combined and divided into four subgroups: (1) Stratum 1, the combination of low AFB1 exposure and wild genotypes of DNA repair genes; (2) Stratum 2, the combination of low AFB1 exposure and genotypes of DNA repair genes with mutant alleles; (3) Stratum 3, the combination of medium-high AFB1 expo-

Table 7. Interactive effects of Polymorphisms in DNA repair genes and AFB1 exposure on HCC risk

Variable	OR_{eg}	$OR_{e'g}$	$OR_{eg'}$	$OR_{e'g} \times OR_{eg'}$	AFB1 exposure-Gene interaction
AFB1/XRCC1	7.98	2.01	3.32	6.67	Multiplication interaction
AFB1/XRCC3	11.9	2.54	2.12	5.38	Multiplication interaction
AFB1/XRCC7	12.96	3.06	2.72	8.32	Multiplication interaction
AFB1/XRCC4	8.81	1.41	3.09	4.36	Multiplication interaction
AFB1/XPC	5.41	1.25	2.83	3.54	Multiplication interaction
AFB1/XPD	8.87	2.36	3.32	7.84	Multiplication interaction

Table 8. Interactive coefficient of Polymorphisms in DNA repair genes and AFB1 exposure on HCC risk

Interactive variable	$OR_{interaction}$	95% CI	$P_{interaction}$
AFB1 × XRCC1	1.61	1.53-1.69	2.31×10^{-87}
AFB1 × XRCC3	1.74	1.66-1.82	2.21×10^{-133}
AFB1 × XRCC7	1.59	1.53-1.65	2.08×10^{-127}
AFB1 × XRCC4	1.64	1.55-1.73	6.41×10^{-73}
AFB1 × XPC	1.36	1.29-1.43	2.75×10^{-33}
AFB1 × XPD	1.14	1.08-1.20	1.00×10^{-6}

sure and wild genotypes of DNA repair genes; and (4) Stratum 4, the combination of medium-high AFB1 exposure and genotypes of DNA repair genes with mutant alleles. For the estimate of cumulative risk value of the combination of AFB1 exposure and DNA repair genotypes, this combinative variable was treated as an ordinal variable (Stratum 1 coded as 0, Stratum 2 as 1, Stratum 3 as 2, and Stratum 4 as 3) and incorporated into multivariable logistic regression. To calculate risk value, we used as a reference the lowest risk group: those who had wild genotypes of DNA repair genes and low AFB1-exposure levels, namely "Stratum 1". This model suggests possible additive or multiplicative effects of environmental and genic factors.

Based on the results in the first model, we obtained risk value of environmental variable AFB1 exposure and genic variables DNA repair genotypes. According to previous in interactive formula [23]: $OR_{eg} < OR_{eg'} \times OR_{e'g}$.

We can analyze whether environmental variable AFB1 exposure and genic variables DNA repair genotypes are likely to act simultaneously at the same mutation sites. If the value of OR_{eg} is more than that of $OR_{eg'} \times OR_{e'g}$, higher AFB1 exposure should interact with risk genotypes of DNA repair genes and they might simultaneously exert influence on HCC carcinogenesis [23]. Furthermore, interactive coefficient can reflect the strength of the interaction of environmental variable AFB1 exposure and genic variables DNA repair genotypes using. Because of the aforementioned reasons, the interactive effects of AFB1 exposure and DNA repair genes were evaluated through the following three methods: (1) joint effects, (2) interactive effects; and (3) interactive coefficient, in this study. Our results also exhibited that there is significantly interaction of environmental variable AFB1 exposure and genic variables DNA repair genotypes in the process of HCC carcinogenesis.

Interactive effects of XRCC1 polymorphism and AFB1 exposure on HCC

XRCC1 gene is one of three submits of DNA repair complex in the SSBR pathway (Gene dbase from PubMed). This gene spans about 32 kb on chromosome 19q13.2 and contains 17 exons and 16 introns. Its' encoding protein (633 amino acids), consists of three functional domains: N-terminal domain (NTD), central breast cancer susceptibility protein-1 homology C-terminal (BRCT I), and C-terminal breast cancer susceptibility protein-1 homology C-terminal (BRCT II) [26-28]. This protein is directly associated with Pol β, DNA ligase III, and PARP, via their three functional domains and is implicated in the core processes in single-strand break repair (SSBR) and base excision repair (BER) pathway [26-28]. More than 50 SNPs in the coding region of XRCC1 gene that lead to amino acid substitution have been described (SNP database). Among these polymorphisms, rs25487 polymorphism (also called codon Arg399Gln polymorphism) is of special concern, because this polymorphism resides in functionally significant regions (BRCT II) and may be related to decreasing DNA repair activity [26, 29-31].

In the several decade years, increasing evidence has shown that XRCC1 rs25487 polymorphism interacts with environmental factors such as HBV infections, cigarettes and alcohol, and plays an important role in the carcinogenesis of cancers [29-31]. However, these studies about XRCC1 rs25487 polymorphism were reported with the results being contradictory in the several decades. To investigate the association between XRCC1 genotypes and HCC-related to AFB1 exposure and possible gene-environmental interaction, we designed and conducted a hospital-based case-control study in the high AFB1 exposure areas. We first evaluated the risk role of this polymorphism on the AFB1-related HCC. Results showed that the HCC patients with XRCC1 genotypes with rs25487 T alleles (namely: rs25487-CT or rs25487-TT) faced a significantly increasing risk of HCC than those with the wild-type homozygote of XRCC1 [namely, rs25487-CC, OR = 2.155, 95% CI = 1.861-2.495 for rs25487-CT; OR = 4.774, 95% CI = 3.264-6.981 for rs25487-TT, respectively]. Our results showed this polymorphism could modulate HCC risk. Interactive analysis of both environmental and genotypic variables showed a significant interactive effects on HCC risk (interactive risk value = 1.61, $P_{interaction} < 0.01$). Previous several meta-analysis based on different AFB1 exposure levels supported our conclusion [13, 30-32]. These results suggest that the decreasing capacity of DNA repair resulting from XRCC1 rs25487 polymorphism is able to interact with higher AFB1 exposure in the process of HCC carcinogenesis.

Interactive effects of XRCC3 polymorphism and AFB1 exposure on HCC

The protein encoded by XRCC3 gene is one of identified paralogs of the strand-exchange protein RAD51 in human beings. This protein associates directly with DNA breaks and facilitates of the formation of the RAD51 nucleoprotein filament, which is crucial both for homologous recombination and HRR [33, 34]. Previous studies have shown that a common polymorphism (rs861539) at codon 241 of XRCC3 gene (Thr to Met) modifies the function of this gene [35-44]. Two reports from high AFB1-exposure areas all of world supported above-mentioned conclusions [16, 45]. In the first frequent case-control study in Guangxiese, we observed that the higher-frequency of genotypes with XRCC3 codon 241 Met alleles (namely Thr/Met and Met/Met) was observed in controls (33.01%) than HCC cases (61.48%, $P < 0.001$). Regression analysis showed that Met alleles increases about 2- to 10-fold risk of HCC and this running-up risk is modulated by the number of Met alleles (adjusted OR 2.48 and 10.06 for one and two this alleles) [45]. The followed relative size analysis and the present study not only found similar risk value of AFB1-related HCC [16], but also found this polymorphism significantly interacted with AFB1 exposure [19]. Our present study supported aforementioned results. These data exhibit that the polymorphism at codon 241 of XRCC3 gene is a genetic determinant in AFB1-induced HCC. Its deficient type strengthens the carcinogenic role of higher AFB1 exposure, and showed a gene-environment interactive effect in the process of HCC tumorigenesis induced by AFB1 exposure.

Interactive effects of XRCC7 polymorphism and AFB1 exposure on HCC

DNA repair gene XRCC7, also called DNAPK, DNPK1, HYRC, HYRC1, or p350) (Genbank ID. 5591), spans about 197 kb on chromosome 8q11 and contains 85 exons and 86 introns (Gene dBase in PubMed). The protein encoded by XRCC7 acts as DNA-dependent protein kinase catalytic subunit (DNA-PKcs) that constitutes the large catalytic subunit of the DNA-PK complex [46]. When DNA-PKcs is recruited to the site of DSBs by the Ku70/Ku80 heterodimer, DNA-PK complex changes into its active form and subsequently initiates the non-homologous end joining (NHEJ) repair, an important DSBR pathway [47]. Murine mutants defective in the XRCC7 have non-detectable DNA-PK activity, suggesting that XRCC7 is required for NHEJ pathway protein [48, 49]. More than 100 polymorphisms have been reported in the XRCC7 gene, some of which are correlated with malignant tumors such as bladder cancer (dbSNP in NCBI Database). Of these genetic polymorphisms in XRCC7 gene, we only investigated the interactive relation between rs7003908 polymorphism and AFB1 toxicological effects, and found this polymorphism might be an important interactive variable for AFB1 toxic role. Supporting our findings, a previous study was also found that these individuals with XRCC7 rs7003908 G alleles increased HCC risk compared the homozygote of XRCC7 rs7003908 T alleles (XRCC7-TT), with OR value

3.45 (2.40-4.94) for XRCC7-TG and 5.04 (3.28-7.76) for XRCC7-GG, respectively. Furthermore, this genetic mutation was correlated with higher the levels of AFB1-DNA adducts ($r = 0.142$, $P < 0.001$) [18], suggesting an environment-gene interactive effect. Taken together, these results explored that genetic polymorphism of XRCC7 rs7003908 might decrease AFB1-related DSBR capacity and result in an increasing toxicological capacity of AFB1. This interaction of environmental and genic variables progressed risk value of themselves on HCC tumorigenesis, however, it inquires more studies to support this conclusion.

Interactive effects of XRCC4 polymorphism and AFB1 exposure on HCC

XRCC4, located on chromosome 5q14.2, is an important the nonhomologous end-joining (NHEJ) gene [50, 51]. The encoded protein of this gene consists of 336 amino acid residues (DDBJ/EMBL/Genbank accession no. AAD472-98) and interacts directly with Ku70/Ku80 in the NHEJ pathway [50, 51]. It is hypothesized that XRCC4 serves as a flexible join between Ku70/Ku80 and its associated protein, Ligase IV [50, 51]. XRCC4 is required for precise end-joining of blunt DNA DSBs in mammalian fibroblasts, and the mutant, XRCC4, results in more-deficient NHEJ capacity. A gene-targeted mutation study has also shown that differentiating neurons and lymphocytes strictly require XRCC4 end-joining proteins. The targeted inactivation of this gene leads to late embryonic lethality accompanied by defective neurogenesis and defective lymphogenesis. These results demonstrate that XRCC4 is essential for the DNA repair capacity of NHEJ [52-54]. More than 100 polymorphisms have been reported in the XRCC4 gene (SNP database), some of which are correlated with DNA adducts, gene mutation, and malignant tumors (such as oral, gastric, liver, and bladder cancers) [11, 12, 55-60]. In this study, we only analyzed rs28383151 polymorphism in the coding region of this gene because this polymorphism localizes at conserved sites of this gene. It changes the coded amino acids and may be associated with a decreased DNA repair capacity and an increased cancer risk [11, 12]. Our previous and present studies exhibited that this polymorphisms increased AFB1-related HCC risk, especially under the conditions of higher AFB1 exposure. This is possibly associated with the fact that this polymorphism is associated with increasing AFB1-DNA adducts levels and the hot-spot mutation risk of TP53 gene. Given that the amount of AFB1-DNA adducts and TP53 gene mutation reflects the toxic capacity of AFB1 exposure, there is reason to believe that the interaction of AFB1 exposure and XRCC4 genotypes exerts an important effects on HCC carcinogenesis. Supporting aforementioned hypothesis, our present study showed a significantly interactive risk value OR of 1.64 (1.55-1.73), therefore, this role should not be neglected.

Interactive effects of XPD polymorphism and AFB1 exposure on HCC

XPD gene, also called excision repair cross-complementing rodent repair deficiency complementation group 2 (ERCC2), COFS2, EM9, or TTD.) (Genbank ID. 2068), spans about 20 kb on chromosome 19q13.3 and contains 23 exons and 22 introns [1, 61]. Its encoded-protein is one of seven central proteins in the NER pathway and act as a DNA-dependent ATPase/helicase. This protein is associated with the TFIIH transcription-factor complex, and plays a role in NER pathway. During NER, XPD participates in the opening of the DNA helix to allow the excision of the DNA fragment containing the damaged base [61]. There are four described polymorphisms that induce amino acid changes in the protein: at codons 199 (Ile to Met), at codon 201 (His to Tyr), at codon 312 (Asp to Asn) and at codon 751 (Lys to Gln) [62]. Among these polymorphisms, we only analyzed codon 751 polymorphism (rs13181) in this study, mainly because our previous studies [10, 15] found the variant XPD codon 751 genotypes (namely Lys/Gln and Gln/Gln) detected by TaqMan-MGB PCR was significantly different between HCC cases (35.9% and 20.1% for Lys/Gln and Gln/Gln, respectively) and controls (26.3% for Lys/Gln and 8.6% for Gln/Gln, $P < 0.001$). Individuals having variant alleles had about 1.5- to 2.5-fold risk of developing the cancer (adjusted OR 1.75 and 95% CI 1.30-2.37 for Lys/Gln; adjusted OR 2.47 and 95% CI 1.62-3.76 for Gln/Gln). Our present study (based on relative large sample size) suggested that the genetic polymorphisms at conserved sequence of XPD gene such as at codon 751 may have potential effect on AFB1-related HCC susceptibility. Furthermore, risk role would be more noticeable under the conditions of higher

AFB1 exposure (OR = 8.87, 95% CI = 7.11-11.05). Interactive analysis showed significant AFB1-XPD joint effects on HCC risk (corresponding $P_{interaction}$ < 0.01). This supports HCC risk might modified by the interactive effects of genetic polymorphisms at codon 751 in DNA repair gene XPD and AFB1 exposure.

Interactive effects of XPC polymorphism and AFB1 exposure on HCC

XPC gene (Genbank accession No. AC090645) spans 33kb on chromosome 3p25, and consists of 16 exons and 15 introns. This gene encodes a 940-amino acid protein, an important DNA damage recognition molecule which plays an important role in NER pathway [63-65]. XPC protein binds tightly with another important NER protein HR23B to form a stable XPC-HR23B complex, the first protein component that recognizes and binds to the DNA damage sites. XPC-HR23B complex can recognize a variety of DNA adducts formed by exogenous carcinogens such as AFB1 and binds to the DNA damage sites [63-65]. Therefore, it may play a role in the decreasing toxic effects of AFB1 and its deficiency may interact with AFB1 exposure. Recent some studies have shown that low DNA repair capacity resulting from the genetic mutation of XPC rs2228001 can progress AFB1-induced HCC [63-65]. In the past several decade years, a total of three studies reported the interaction of XPC rs2228001 polymorphism and environmental variables including HBV and AFB1 exposure, involving in HCC tumorigenesis [14, 66, 67]. The first study is from Shunde area, Guangdong Province which is characterized by high AFB1 exposure and high incidence rate of HCC. In this study, researchers explored the correlation between this polymorphism and risk of HCC via an 1-1 case-control study (including 78 HCC patients and 78 age- and sex-matching controls) method, and found the mutation of XPC modified HCC risk (adjusted odds ratios [ORs] were 6.78 with 95% CI 2.03-22.69) [67]. They furthermore analyze the mutant genotype interacted with HBV infection status, suggesting this polymorphism could interact with such environmental variables as HBV infection status. Although they did not directly evaluated the interactive effects of XPC rs2228001 polymorphism and AFB1 exposure on HCC risk, study population in their study is from both high HBV infection areas and high AFB1 exposure areas and high risk of HCC for XPC mutant. The other two studies was conducted by our teams and showed XPC codon 939 Gln alleles increased about 2-times risk of HCC, this risk would be more noticeable under the conditions of higher AFB1 exposure [14, 66]. In our present study, we not only observed this mutation increased AFB1-related HCC risk, but found more direct evidence of interactive effects of XPC polymorphism and AFB1 exposure on HCC risk. As a result, these data suggest that genetic polymorphism at codon 939 of XPC gene is a genetic determinant in the DNA repair process of DNA damage induced by AFB1 exposure, and its low activity might interact with different AFB1 exposure status in the carcinogenetic process of HCC.

Limitation and conclusions

This study had several limitations. First, because the present study is the hospital-based case-control study, potential selection bias might have occurred. Second, because the liver disease itself may affect the metabolism of AFB1 and modify the levels of AFB1-DNA adducts, the increased risk with AFB1 exposure status noted in this study was probably underestimated. Third, although six genetic mutations in DNA repair genes were analyzed, some other genes might interact with AFB1 exposure in the process of HCC carcinogenesis. Therefore, more genes deserve further elucidation based on a large sample and the combination of genes and AFB1 exposure.

In conclusion, to the best of our knowledge, this is the first report to systematically investigate the interactive effects of AFB1 exposure and polymorphisms in DNA repair genes XRCC1, XRCC3, XRCC4, XRCC7, XPC, and XPD on HCC risk among Guangxi population from a high AFB1-exposure area. We find that both AFB1 exposure and the genetic mutations in the DNA repair genes XRCC1, XRCC3, XRCC4, XRCC7, XPC, and XPD might increase the risk of HCC, and their environment-gene interaction would furthermore strengthens this risk value. Given that AFB1 is am important genic agent and a kind of I type carcinogen, our findings might have prevention implications through identifying population with low DNA repair capacity, once these findings are replicated by other studies based on a larger scale or prospective studies.

Acknowledgements

We thank Dr. Qiu-Xiang Liang, Dr. Yun Yi, and Dr. Yuan-Feng Zhou for sample collection and management; Dr. Hua Huang for molecular biochemical technique. We also thank all members of Department of Medical Test and Infective Control, Affiliated Hospital of Youjiang Medical College for Nationalities for their help. This study was supported in part by the National Natural Science Foundation of China (No. 81372639 and 81160255), the Innovation Program of Guangxi Municipal Education Department (No. 201204LX674), Innovation Program of Shanghai Municipal Education Commission (No. 13YZ035), the Natural Science Foundation of Guangxi (No. 2013GXNSFAA019251), Key Discipline and Specialty Foundation of Shanghai Municipal Commission of Health and Family Planning, and "Shu Guang" project (supported by Shanghai Municipal Education Commission and Shanghai Education Development Foundation, No. 13SG19).

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Jin-Guang Yao, Department of Pathology, AHYMCN, 98 Chengxiang Road, Baise City, Guangxi Zhuang Autonomous Region 533000, China. Tel: +86 776 2849481; Fax: +86 776 2849481; E-mail: yyyaojg001@163.com; Dr. Xi-Dai Long, Department of Liver Surgery, RJHSJTUM, No. 1630, Dongfang Road, Shanghai 200127, China. Tel: +86 776 2849481; Fax: +86 776 2849481; E-mail: sjtulongxd@263.net

References

[1] Xia Q, Huang XY, Xue F, Zhang JJ, Zhai B, Kong DC, Wang C, Huang ZQ and Long XD. Genetic polymorphisms of DNA repair genes and DNA repair capacity related to aflatoxin b1 (AFB1)-induced DNA damages. In: Chen C, editors. New Research Directions in DNA Repair. Rijeka: InTech; 2013. pp. 377-412.

[2] Long XD, Yao JG, Zeng Z, Huang CH, Huang ZS, Huang YZ, Ban FZ, Huang XY, Yao LM, Fan LD and Fu GH. DNA repair capacity-related to genetic polymorphisms of DNA repair genes and aflatoxin B1-related hepatocellular carcinoma among Chinese population. In: Kruman I, editor. DNA Repair. Rijeka: InTech; 2011. pp. 505-524.

[3] Kensler TW, Roebuck BD, Wogan GN and Groopman JD. Aflatoxin: a 50-year odyssey of mechanistic and translational toxicology. Toxicol 2011; 120 Suppl 1: S28-48.

[4] Fiala JL, Egner PA, Wiriyachan N, Ruchirawat M, Kensler KH, Wogan GN, Groopman JD, Croy RG and Essigmann JM. Sulforaphane-mediated reduction of aflatoxin B-N-guanine in rat liver DNA: impacts of strain and sex. Toxicol 2011; 121: 57-62.

[5] Golli-Bennour EE, Kouidhi B, Bouslimi A, Abid-Essefi S, Hassen W and Bacha H. Cytotoxicity and genotoxicity induced by aflatoxin B1, ochratoxin A, and their combination in cultured Vero cells. J Biochem Mol Toxicol 2010; 24: 42-50.

[6] Paget V, Sichel F, Garon D and Lechevrel M. Aflatoxin B1-induced TP53 mutational pattern in normal human cells using the FASAY (Functional Analysis of Separated Alleles in Yeast). Mutat Res 2008; 656: 55-61.

[7] Tong WM, Lee MK, Galendo D, Wang ZQ and Sabapathy K. Aflatoxin-B exposure does not lead to p53 mutations but results in enhanced liver cancer of Hupki (human p53 knock-in) mice. Int J Cancer 2006; 119: 745-749.

[8] Reddy L, Odhav B and Bhoola K. Aflatoxin B1-induced toxicity in HepG2 cells inhibited by carotenoids: morphology, apoptosis and DNA damage. Biol Chem 2006; 387: 87-93.

[9] Habib SL, Said B, Awad AT, Mostafa MH and Shank RC. Novel adenine adducts, N7-guanine-AFB1 adducts, and p53 mutations in patients with schistosomiasis and aflatoxin exposure. Cancer Detect Prev 2006; 30: 491-498.

[10] Long XD, Yao JD, Yang Q, Huang CH, Liao P, Nong LG, Tang YJ, Huang XY, Wang C, Wu XM, Huang BC, Ban FZ, Zeng LX, Ma Y, Zhai B, Zhang JQ, Xue F, Lu CX and Xia Q. Polymorphisms of DNA Repair Genes and Toxicological Effects of Aflatoxin B1 Exposure. In: Faulkner AG, editor. Aflatoxins: Food Sources, Occurrence and Toxicological Effects. New York: Nova Science Publishers; 2014. pp. 107-124.

[11] Long XD, Zhao D, Wang C, Huang XY, Yao JG, Ma Y, Wei ZH, Liu M, Zeng LX, Mo XQ, Zhang JJ, Xue F, Zhai B and Xia Q. Genetic polymorphisms in DNA repair genes XRCC4 and XRCC5 and aflatoxin B1-related hepatocellular carcinoma. Epidemiology 2013; 24: 671-681.

[12] Long XD, Yao JG, Zeng Z, Ma Y, Huang XY, Wei ZH, Liu M, Zhang JJ, Xue F, Zhai B and Xia Q. Polymorphisms in the coding region of X-ray repair complementing group 4 and aflatoxin B1-related hepatocellular carcinoma. Hepatology 2013; 58: 171-181.

[13] Chen BP, Long XD and Fu GH. Meta-analysis of XRCC1 Codon 399 Polymorphism and Susceptibility of Hepatocellular Carcinoma. Journal of Shanghai Jiao Tong University (Medical Science) 2011; 31: 1588-1602.

[14] Long XD, Ma Y, Zhou YF, Ma AM and Fu GH. Polymorphism in xeroderma pigmentosum complementation group C codon 939 and afla-

toxin B1-related hepatocellular carcinoma in the Guangxi population. Hepatology 2010; 52: 1301-1309.
[15] Long XD, Ma Y, Zhou YF, Yao JG, Ban FZ, Huang YZ and Huang BC. XPD codon 312 and 751 polymorphisms, and AFB1 exposure, and hepatocellular carcinoma risk. BMC Cancer 2009; 9: 400.
[16] Long XD, Ma Y, Qu de Y, Liu YG, Huang ZQ, Huang YZ, Lin ZH, Wei NB and Zhou SC. The polymorphism of XRCC3 codon 241 and AFB1-related hepatocellular carcinoma in Guangxi population, China. Ann Epidemiol 2008; 18: 572-578.
[17] Long XD, Ma Y, Wei YP and Deng ZL. The polymorphisms of GSTM1, GSTT1, HYL1*2, and XRCC1, and aflatoxin B1-related hepatocellular carcinoma in Guangxi population, China. Hepatol Res 2006; 36: 48-55.
[18] Long XD, Yao JG, Huang YZ, Huang XY, Ban FZ, Yao LM and Fan LD. DNA repair gene XRCC7 polymorphisms (rs#7003908 and rs#1010-9984) and hepatocellular carcinoma related to AFB1 exposure among Guangxi population, China. Hepatol Res 2011; 41: 1085-1093.
[19] Long XD, Ma Y and Deng ZL. GSTM1 and XRCC3 polymorphisms: Effects on levels of aflatoxin B1-DNA adducts. Chin J Cancer Res 2009; 21: 177-184.
[20] Long XD, Ma Y, Huang HD, Yao JG, Qu de Y and Lu YL. Polymorphism of XRCC1 and the frequency of mutation in codon 249 of the p53 gene in hepatocellular carcinoma among Guangxi population, China. Mol Carcinog 2008; 47: 295-300.
[21] Long XD, Ma Y, Wei YP and Deng ZL. X-ray repair cross-complementing group 1 (XRCC1) Arg 399 Gln polymorphism and aflatoxin B1 (AFB1)-related hepatocellular carcinoma (HCC) in Guangxi population. Chin J Cancer Res 2005; 17: 17-21.
[22] Chen CJ, Wang LY, Lu SN, Wu MH, You SL, Zhang YJ, Wang LW and Santella RM. Elevated aflatoxin exposure and increased risk of hepatocellular carcinoma. Hepatology 1996; 24: 38-42.
[23] Brennan P. Gene-environment interaction and aetiology of cancer: what does it mean and how can we measure it? Carcinogenesis 2002; 23: 381-387.
[24] Wild CP and Montesano R. A model of interaction: aflatoxins and hepatitis viruses in liver cancer aetiology and prevention. Cancer Lett 2009; 286: 22-28.
[25] Pineau P, Marchio A, Battiston C, Cordina E, Russo A, Terris B, Qin LX, Turlin B, Tang ZY, Mazzaferro V and Dejean A. Chromosome instability in human hepatocellular carcinoma depends on p53 status and aflatoxin exposure. Mutat Res 2008; 653: 6-13.
[26] Thompson LH and West MG. XRCC1 keeps DNA from getting stranded. Mutat Res 2000; 459: 1-18.
[27] Marintchev A, Robertson A, Dimitriadis EK, Prasad R, Wilson SH and Mullen GP. Domain specific interaction in the XRCC1-DNA polymerase beta complex. Nucleic Acids Res 2000; 28: 2049-2059.
[28] Lakshmipathy U and Campbell C. Mitochondrial DNA ligase III function is independent of Xrcc1. Nucleic Acids Res 2000; 28: 3880-3886.
[29] Saadat M and Ansari-Lari M. Polymorphism of XRCC1 (at codon 399) and susceptibility to breast cancer, a meta-analysis of the literatures. Breast Cancer Res Treat 2009; 115: 137-144.
[30] Geng J, Zhang Q, Zhu C, Wang J and Chen L. XRCC1 genetic polymorphism Arg399Gln and prostate cancer risk: a meta-analysis. Urology 2009; 74: 648-653.
[31] Geng J, Zhang YW, Huang GC and Chen LB. XRCC1 genetic polymorphism Arg399Gln and gastric cancer risk: A meta-analysis. World J Gastroenterol 2008; 14: 6733-6737.
[32] Coursaget P, Depril N, Chabaud M, Nandi R, Mayelo V, LeCann P and Yvonnet B. High prevalence of mutations at codon 249 of the p53 gene in hepatocellular carcinomas from Senegal. Br J Cancer 1993; 67: 1395-1397.
[33] Qiu LX, Mao C, Yao L, Yu KD, Zhan P, Chen B, Liu HG, Yuan H, Zhang J, Xue K and Hu XC. XRCC3 5'-UTR and IVS5-14 polymorphisms and breast cancer susceptibility: a meta-analysis. Breast Cancer Res Treat 2010; 122: 489-493.
[34] Kajita M, Magari M, Todo K, Kanayama N and Ohmori H. Conditional transformation of immunoglobulin mutation pattern from gene conversion into point mutation by controlling XRCC3 expression in the DT40 B cell line. J Biosci Bioeng 2010; 109: 407-410.
[35] Zhou C, Ren S, Zhou S, Zhang L, Su C, Zhang Z, Deng Q and Zhang J. Predictive effects of ERCC1 and XRCC3 SNP on efficacy of platinum-based chemotherapy in advanced NSCLC patients. Jpn J Clin Oncol 2010 Oct; 40: 954-60.
[36] Wang J, Zhao Y, Jiang J, Gajalakshmi V, Kuriki K, Nakamura S, Akasaka S, Ishikawa H, Suzuki S, Nagaya T and Tokudome S. Polymorphisms in DNA repair genes XRCC1, XRCC3 and XPD, and colorectal cancer risk: a case-control study in an Indian population. J Cancer Res Clin Oncol 2010; 136: 1517-1525.
[37] Sun H, Qiao Y, Zhang X, Xu L, Jia X, Sun D, Shen C, Liu A, Zhao Y, Jin Y, Yu Y, Bai J and Fu S. XRCC3 Thr241Met polymorphism with lung cancer and bladder cancer: a meta-analysis. Cancer Sci 2010; 101: 1777-1782.

[38] Silva SN, Tomar M, Paulo C, Gomes BC, Azevedo AP, Teixeira V, Pina JE, Rueff J and Gaspar JF. Breast cancer risk and common single nucleotide polymorphisms in homologous recombination DNA repair pathway genes XRCC2, XRCC3, NBS1 and RAD51. Cancer Epidemiol 2010; 34: 85-92.

[39] Mangoni M, Bisanzi S, Carozzi F, Sani C, Biti G, Livi L, Barletta E, Costantini AS and Gorini G. Association between genetic polymorphisms in the XRCC1, XRCC3, XPD, GSTM1, GSTT1, MSH2, MLH1, MSH3, and MGMT genes and radiosensitivity in breast cancer patients. Int J Radiat Oncol Biol Phys 2011; 81: 52-8.

[40] 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 2010; 29: 669-74.

[41] Jiang Z, Li C, Xu Y and Cai S. A meta-analysis on XRCC1 and XRCC3 polymorphisms and colorectal cancer risk. Int J Colorectal Dis 2010; 25: 169-180.

[42] Fang F, Wang J, Yao L, Yu XJ and Yu L. Relationship between XRCC3 T241M polymorphism and gastric cancer risk: a meta-analysis. Med Oncol 2011; 28: 999-1003.

[43] Economopoulos KP and Sergentanis TN. XRCC3 Thr241Met polymorphism and breast cancer risk: a meta-analysis. Breast Cancer Res Treat 2010; 121: 439-443.

[44] Zhou K, Liu Y, Zhang H, Liu H, Fan W, Zhong Y, Xu Z, Jin L, Wei Q, Huang F, Lu D and Zhou L. XRCC3 haplotypes and risk of gliomas in a Chinese population: a hospital-based case-control study. Int J Cancer 2009; 124: 2948-2953.

[45] Long XD, Ma Y, Deng ZL, Huang YZ and Wei NB. [Association of the Thr241Met polymorphism of DNA repair gene XRCC3 with genetic susceptibility to AFB1-related hepatocellular carcinoma in Guangxi population]. Zhonghua Yi Xue Yi Chuan Xue Za Zhi 2008; 25: 268-271.

[46] Chen BP, Li M and Asaithamby A. New insights into the roles of ATM and DNA-PKcs in the cellular response to oxidative stress. Cancer Lett 2012; 327: 103-110.

[47] Pardo B, Gomez-Gonzalez B and Aguilera A. DNA repair in mammalian cells: DNA double-strand break repair: how to fix a broken relationship. Cell Mol Life Sci 2009; 66: 1039-1056.

[48] Shrivastav M, De Haro LP and Nickoloff JA. Regulation of DNA double-strand break repair pathway choice. Cell Res 2008; 18: 134-147.

[49] Jolly CJ, Cook AJ and Manis JP. Fixing DNA breaks during class switch recombination. J Exp Med 2008; 205: 509-513.

[50] Mizuta R, Cheng HL, Gao Y and Alt FW. Molecular genetic characterization of XRCC4 function. Int Immunol 1997; 9: 1607-1613.

[51] Li Z, Otevrel T, Gao Y, Cheng HL, Seed B, Stamato TD, Taccioli GE and Alt FW. The XRCC4 gene encodes a novel protein involved in DNA double-strand break repair and V(D)J recombination. Cell 1995; 83: 1079-1089.

[52] Mari PO, Florea BI, Persengiev SP, Verkaik NS, Bruggenwirth HT, Modesti M, Giglia-Mari G, Bezstarosti K, Demmers JA, Luider TM, Houtsmuller AB and van Gent DC. Dynamic assembly of end-joining complexes requires interaction between Ku70/80 and XRCC4. Proc Natl Acad Sci U S A 2006; 103: 18597-18602.

[53] Jones KR, Gewirtz DA, Yannone SM, Zhou S, Schatz DG, Valerie K and Povirk LF. Radiosensitization of MDA-MB-231 breast tumor cells by adenovirus-mediated overexpression of a fragment of the XRCC4 protein. Mol Cancer Ther 2005; 4: 1541-1547.

[54] van Heemst D, Brugmans L, Verkaik NS and van Gent DC. End-joining of blunt DNA double-strand breaks in mammalian fibroblasts is precise and requires DNA-PK and XRCC4. DNA Repair (Amst) 2004; 3: 43-50.

[55] Garcia-Leston J, Roma-Torres J, Vilares M, Pinto R, Prista J, Teixeira JP, Mayan O, Conde J, Pingarilho M, Gaspar JF, Pasaro E, Mendez J and Laffon B. Genotoxic effects of occupational exposure to lead and influence of polymorphisms in genes involved in lead toxicokinetics and in DNA repair. Environ Int 2012; 43: 29-36.

[56] Mandal RK, Singh V, Kapoor R and Mittal RD. Do polymorphisms in XRCC4 influence prostate cancer susceptibility in North Indian population? Biomarkers 2011; 16: 236-242.

[57] Lee JE, Heo JI, Park SH, Kim JH, Kho YJ, Kang HJ, Chung HY, Yoon JL and Lee JY. Calorie restriction (CR) reduces age-dependent decline of non-homologous end joining (NHEJ) activity in rat tissues. Expe Gerontol 2011; 46: 891-6.

[58] Huang L, Zheng M, Zhou QM, Zhang MY, Jia WH, Yun JP and Wang HY. Identification of a gene-expression signature for predicting lymph node metastasis in patients with early stage cervical carcinoma. Cancer 2011; 117: 3363-3373.

[59] Wu KH, Wang CH, Yang YL, Peng CT, Lin WD, Tsai FJ, Lin DT and Bau DT. Significant association of XRCC4 single nucleotide polymorphisms with childhood leukemia in Taiwan. Anticancer Res 2010; 30: 529-533.

[60] Wang Y, Wang L, Li X, Liu B, Zhao Q, Chen P, Wang T, Li T, Ji J, Yang F, Wang Q, Wang J, Xiao Y, Xu Y, Feng G, Peng Z, He L and He G. Polymorphisms of XRCC4 are involved in reduced colorectal cancer risk in Chinese schizophrenia patients. BMC Cancer 2010; 10: 523.

[61] Manuguerra M, Saletta F, Karagas MR, Berwick M, Veglia F, Vineis P and Matullo G. XRCC3 and XPD/ERCC2 single nucleotide polymorphisms and the risk of cancer: a HuGE review. Am J Epidemiol 2006; 164: 297-302.

[62] Bienstock RJ, Skorvaga M, Mandavilli BS and Van Houten B. Structural and functional characterization of the human DNA repair helicase XPD by comparative molecular modeling and site-directed mutagenesis of the bacterial repair protein UvrB. J Biol Chem 2003; 278: 5309-5316.

[63] Strom SS, Estey E, Outschoorn UM and Garcia-Manero G. Acute myeloid leukemia outcome: role of nucleotide excision repair polymorphisms in intermediate risk patients. Leuk Lymphoma 2010; 51: 598-605.

[64] Khan SG, Yamanegi K, Zheng ZM, Boyle J, Imoto K, Oh KS, Baker CC, Gozukara E, Metin A and Kraemer KH. XPC branch-point sequence mutations disrupt U2 snRNP binding, resulting in abnormal pre-mRNA splicing in xeroderma pigmentosum patients. Hum Mutat 2010; 31: 167-175.

[65] Gangwar R, Mandhani A and Mittal RD. XPC gene variants: a risk factor for recurrence of urothelial bladder carcinoma in patients on BCG immunotherapy. J Cancer Res Clin Oncol 2010; 136: 779-786.

[66] Long XD, Ma Y, Zhou YF, Ma AM and Fu GH. The polymorphism at codon 939 of xeroderma pigmentosum C gene and hepatocellular carcinoma among Guangxi population. Zhonghua Xiaohua Zazhi 2010; 30: 846-848.

[67] Cai XL, Gao YH, Yu ZW, Wu ZQ, Zhou WP, Yang Y, Xu Y, Song SF and Chen SD. [A 1:1 matched case-control study on the interaction between HBV, HCV infection and DNA repair gene XPC Ala499Val, Lys939Gln for primary hepatocellular carcinoma]. Zhonghua Liu Xing Bing Xue Za Zhi 2009; 30: 942-945.