

The Polymorphism of XRCC3 Codon 241 and the Hotspot Mutation in the TP53 Gene in Hepatocellular Carcinoma Induced by Aflatoxin B1

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ABSTRACT

AIM: DNA repair gene X-ray repair cross-complementary group 3 (XRCC3) Thr241Met polymorphism (rs861539) is related to AFB1-DNA adducts and hepatocellular carcinoma (HCC). However, the association between this polymorphism and the hotspot mutation in codon 249 of TP53 gene (TP53M) is unclear.

METHODS: A total of 585 HCCs were included in this study to investigate the role of the XRCC3 codon 241 polymorphism on TP53M risk. The genotypes of XRCC3 codon 241 and p53 codon 249 were examined by TaqMan-PCR technique.

RESULTS: We found AFB1-DNA adduct levels were significantly correlated with increasing TP53M risk [adjusted odds ratio (OR)=3.30, 95% confidence interval (CI)=2.26-4.82]. The HCC patients with XRCC3 genotypes with 241 Met (namely: XRCC3-TM/MM) exhibited a significantly higher risk of TP53M than those with the wild-type homozygote of XRCC3 (namely: XRCC3-TT, OR=4.96, 95% CI=3.46-7.11). Compared with those individuals who did express XRCC3-MM as reference (OR=1), moreover, individuals featuring XRCC3-TM/MM and AFB1-DNA adducts did experience a significantly increasing risk of TP53M (OR=19.70, 95% CI=9.90-39.18, $P<0.01$).

DISCUSSION: This study suggests that the XRCC3 Thr241Met

polymorphism and AFB1-DNA adducts are associated with the increased TP53M risk.

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Key words: XRCC3; TP53M; Polymorphism; HCC

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INTRODUCTION

Hepatocellular carcinoma (HCC) is the most common cancer type in Guangxi Zhuang Autonomous Region, China, with an incidence rate of 53/10,000 per year and a death rate of 37-55/10,000 annually^[1-3]. Clinic-epidemiological evidence has shown that aflatoxin B1 (AFB1) exposure and hepatitis B virus (HBV) infection are two major risk factors^[4-6]. AFB1 is mainly metabolized by cytochrome P450 (CYP) into the genotoxic metabolic 8, 9-epoxide-AFB1 (AFB1-epoxide). This metabolite can bind to DNA, which results in the formation of AFB1-DNA adducts and causes DNA damage that may ultimately lead to gene mutations^[6-10]. Increasing evidence has exhibited that AFB1 is responsible for the spot mutation in codon 249 of the p53 gene (namely, hotspot mutation, TP53M)^[8]. Accordingly, the frequency of this mutation varies greatly depending on the country of origin of cancer patients, and it has been related to the degree of exposure to AFB1. While DNA repair enzymes coded by DNA repair genes can repair DNA damage such as AFB1-DNA adducts and may prevent TP53M^[11,12].

DNA repair gene X-ray cross-complementing group 3 (XRCC3) is required for the efficient repair of double-strand breaks (DSBs)^[11-13]. Recent studies have shown that a common polymorphism in codon 241 (Thr241Met, rs861539) of this gene may be associated with lower DNA repair capacity, more gene mutations, and higher cancer tumors^[11,11-24]. However, there have been no studies to investigate the association between this polymorphism and AFB1-related TP53M, especially from AFB1 exposure areas. Here, we specifically

conducted a hospital-based study to examine whether XRCC3 codon 241 polymorphism influenced the risk of TP53M in HCC among Guangxi population from an AFB1 exposure area.

METHODS

Study Population

During the period from January 2004 through December 2008, all incident cases with HCC were recruited at the affiliated hospitals of Youjiang Medical College for Nationalities and Guangxi Medical University. All patients were the residents of Guangxi Zhuang Autonomous Region, a high AFB1 exposure area. A total of 585 HCC cases, including 458 patients previously studied^[25], were included for the present study. These cases included in present study represented for a significant portion of HCCs from Guangxi population. All cases were histopathologically confirmed and previously untreated with chemotherapy or radiotherapy. In this study, the response rate for the cases has been about 97%. Demographic information [including sex, age, ethnicity, hepatitis B virus (HBV) and hepatitis C virus (HCV) infection] were collected in the hospitals using a standard interviewer-administered questionnaire and/or medical records by a Youjiang Cancer Institution staff member. In this study, those having hepatitis B surface antigen (HBsAg) positive and anti-HCV positive in their peripheral serum were defined as groups infected with HBV and HCV. Additionally, after informed consent was obtained, surgically removed tumor samples and 4 mL of peripheral blood of all cases were collected for analyzing AFB1-DNA adduct levels, TP53M, and XRCC3 genotypes. The protocol of the study was approved by the Ethics Committees of the hospitals involved in the study. The study protocol was 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.

DNA extraction

Genomic DNA was extracted from HCC tumor tissues in a 1.5 mL microcentrifuge tube for deparaffinization and proteinase K digestion, as described by standard procedures (Protocol #BS474, Bio Basic, Inc., Ontario, Canada). For peripheral blood samples, Leukocytes 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.

AFB1 exposure data

In this study, AFB1 exposure levels were evaluated using the amounts of AFB1-DNA adducts in cancerous tissues. AFB1-DNA adducts levels of DNA samples from cancerous tissue were measured by competitive enzyme-linked immunosorbent assay (ELISA) with monoclonal antibody 6A10 and 50 ng of DNA as described by our previous report^[25]. The percent of inhibition was calculated by comparison with the non-modified heat-denatured calf thymus DNA control. DNA samples were assayed at 50 ng/well and quantitated relative to an imidazole ring-opened AFB1-DNA standard, which has a modification level of 4 adducts/10 nucleotides. Values below 20% inhibition, corresponding to 0.5 mmol/mol DNA, were considered not detectable. Each sample was measured in triplicate on the three different assay dates and had a variability of less than 10%.

XRCC3 Genotyping

XRCC3 codon 241 genotypes were analyzed using the DNA from

peripheral blood leukocytes by the TaqMan-PCR on iCycler iQ™ real-time PCR detection system (CFX Manager Version 2, Bio-Rad). Primer and probe sets are as followed: 5'-CCAGGGCCAGGCATCTG-3', 5'-CAGCACAGGGCTCTGGA-3', 5'-FAM-CAGCATGGCCCCCA-MGB-3', and 5'-VIC-CAGCGTGGCCCCCA-MGB-3'^[11]. PCR reactions were run in a 25 µL final volume containing 1×TaqMAN Universal Master Mix II (catalog # 4440041, ABI), 0.2 µM of each probe, 0.2 µM of each primer, and about 100 ng of genomic DNA. Cycling conditions were 95°C for 10 min, and 40 cycles of 95°C for 15 s and 60°C for 1 min. For quality control, controls were included in each run, and repeated genotyping and sequencing of a random 10% subset yielded 100% identical genotypes. Laboratory personnel were blinded to case and control status.

TP53M assay

TP53M in tumor tissues was tested by using aforementioned TaqMan-PCR technique. The primers and probes sets used for TaqMan-PCR assay were as previously published^[26,27].

Statistical analysis

All the analysis was performed by the statistical package for social science (SPSS) version 18.0 (SPSS Institute, Chicago, IL). The association between XRCC3 genotypes and the risk factors (including HBsAg, anti-HCV, and AFB1-DNA adducts), and TP53M was examined by the pair-wise comparisons of χ^2 test. Logistic regression with an adjustment for age, sex, ethnicity, HBsAg, anti-HCV, and AFB1-DNA adducts was used to estimate the odds ratio (OR) and the 95% confidence interval (CI) for the XRCC3 genotypes. The interactions between XRCC3 codon 241 genotypes and AFB1-DNA adducts on TP53M were also tested in the multiple logistic regression model. In this study, all statistical tests were based on 2-tailed probabilities, and a *P*-value of <0.05 was considered statistically significant.

RESULTS

Demographic features of cases

Table 1 showed the demographic data of the HCC cases. The HBV and HCV infective rates were 78.3% (458 of 585) and 20.0% (117 of 585), respectively. While AFB1-DNA adducts were detected in the 72.8% (426 of 585) of DNA samples from cancerous tissue of the patients with HCC.

Table 1 Characteristics of the patients with HCC.

Characteristics	
Age, yr	
Mean ± SE	48.47 ± 11.92
Range	19-86
Sex	
Man, <i>n</i> (%)	498 (85.1)
Female, <i>n</i> (%)	87 (14.9)
Ethnicity	
Han, <i>n</i> (%)	315 (53.8)
Zhuang, <i>n</i> (%)	270 (46.2)
HBV infection	
HBsAg(-), <i>n</i> (%)	127 (21.1)
HBsAg(+), <i>n</i> (%)	458 (78.3)
HCV infection	
anti-HCV (-), <i>n</i> (%)	468 (80.0)
anti-HCV (+), <i>n</i> (%)	117 (20.0)
AFB1-DNA adducts	
Nondetected, <i>n</i> (%)	157 (27.2)
Detected, <i>n</i> (%)	426 (72.8)
Total, <i>n</i> (%)	585 (100)

AFB1 exposure levels increased TP53M risk

Cancerous tissue samples from the 585 HCC patients were examined for TP53M by TaqMan-PCR (Table 2). We found that three hundred fifty (59.8%) of the 585 HCCs showed TP53M. Individuals with AFB1-DNA adducts in the cancerous tissue had a significantly increasing risk of TP53M than those without AFB1-DNA adducts in their cancerous tissue (OR=3.30, 95% CI=2.26-4.82, $P=3.386 \times 10^{-10}$). However, the frequency of hotspot mutation was not related with HBV- or HCV- infection status ($P>0.05$, Table 2).

Table 2 The association of hotspot mutation in codon 249 of the p53 gene (TP53M) and risk factors.

Risk factor	TP53M (-) (n=235)		TP53M (+) (n=350)		OR (95% CI)
	n	%	n	%	
HBsAg ¹					
Negative	43	18.3	84	24.0	Reference
Positive	192	81.7	266	76.0	0.68 (0.44-1.04) ²
anti-HCV ³					
Negative	191	81.3	277	79.1	Reference
Positive	44	18.7	73	20.9	1.20 (0.77-1.85) ⁴
AFB1-DNA adducts ⁵					
Nondetected	97	41.3	62	17.7	Reference
Detected	138	58.7	288	82.3	3.30 (2.26-4.82) ⁶

¹ Two-sided $\chi^2 = 2.690$, $P = 0.101$;
² Adjusted for age, sex, ethnicity, anti-HCV, and AFB1-DNA adducts;
³ Two-sided $\chi^2 = 0.400$, $P = 0.527$;
⁴ Adjusted for age, sex, ethnicity, HBsAg, and AFB1-DNA adducts;
⁵ Two-sided $\chi^2 = 39.439$, $P = 3.386 \times 10^{-10}$;
⁶ Adjusted for age, sex, ethnicity, HBsAg, and anti-HCV.

XRCC3 codon 241 polymorphism modified TP53M risk

The association between the polymorphism of XRCC3 codon 241 and TP53M among the HCC patients was shown in table 3. Individuals with the heterozygotes of XRCC3 codon 241 (XRCC3-TM) or the variant homozygotes of XRCC3 codon 241 (XRCC3-MM) were more probably to have hotspot mutation in codon 249 of the p53 gene than those with the wild-type homozygote of XRCC3 codon 241 (XRCC3-TT). Its adjusted ORs (95% CIs) were 4.57 (3.10-6.73) and 6.26 (3.54-11.06) for XRCC3-TM and XRCC3-MM, respectively. These results suggest that the risk of high TP53M risk was related with the number of codon 241 Met alleles.

Table 3 XRCC3 polymorphism and TP53M risk.

XRCC3 genotypes ¹	TP53M (-) (n=235)		TP53M (+) (n=350)		OR (95% CI) ²
	n	%	n	%	
TT	155	66.0	97	27.7	Reference
TM	61	26.0	197	56.3	4.57 (3.10-6.73)
MM	19	8.1	74	21.1	6.26 (3.54-11.06)
TM/MM	80	34.0	271	77.4	4.96 (3.46-7.11)

¹ Two-sided $\chi^2 = 84.553$, $P = 4.360 \times 10^{-19}$;
² Adjusted for age, sex, ethnicity, HBsAg, anti-HCV, and AFB1-DNA adduct levels.

Because the different status of HBV and HCV infection is different in HCC carcinogenesis and might modify the effects of XRCC3 polymorphism on TP53M risk^[1-3,28-30], we investigated the TP53M risk associated with genetic polymorphism of XRCC3 codon 241 separately among different stratified variables including HBsAg (negative and positive) and anti-HCV (negative and positive) (Table 4). Higher risk value of TP53M was observed among either negative HBsAg or negative anti-HCV status. For example, these HBsAg-positive cases having XRCC3-TM/MM featured 5.78 times of TP53M risk, and this risk role would increase to 12.75 times under the conditions of HBsAg-negative status.

The joint effects of AFB1 exposure levels and XRCC3 codon 241 polymorphism on TP53M risk

To study the relationship between XRCC3 codon 241 polymorphism and AFB1 exposure levels in TP53M, the joint analysis was carried out (Table 5). In this analysis model, the combination of AFB1-DNA adduct levels and XRCC3 genotypes was divided into six strata and the lowest risk group, namely those who had XRCC3-TT and low AFB1-exposure levels, was used as a reference (OR=1). The results demonstrated that: compared to subjects with the XRCC3-TT and AFB1-DNA adducts non-detected in their cancerous tissue, those with the XRCC3-MM and AFB1-DNA adducts faced a greater risk of TP53M (OR was 2.20 to 28.38, $P<0.01$). According to the previously published formula ($OR_{reg} < OR_{reg}^1 \times OR_{reg}^2$)^[31], some significant evidence of multiplicatively interactive effects of risk genotypes and AFB1 exposure on TP53M risk were observed (28.38 > 1.98×2.20).

Table 4 XRCC3 polymorphism and TP53M risk stratified by HBsAg and anti-HCV status.

	XRCC3 genotypes	TP53M (-) (n=235)		TP53M (+) (n=350)		OR (95% CI)
		n	%	n	%	
HBsAg						
Negative	TT	31	72.1	20	23.8	Reference
	TM	11	25.6	46	54.8	10.28(3.61-29.27) ¹
	MM	1	2.3	18	21.4	31.24(3.43-284.42) ¹
	TM/MM	12	27.9	64	76.2	12.75(4.72-34.41) ¹
Positive	TT	124	64.6	77	28.9	Reference
	TM	50	26.0	133	50.0	5.44(3.36-8.79) ¹
	MM	18	9.4	56	21.1	6.78(3.48-13.19) ¹
	TM/MM	68	35.4	189	71.1	5.78(3.70-9.02) ¹
anti-HCV						
Negative	TT	129	67.5	76	27.4	Reference
	TM	47	24.6	140	50.5	6.84(4.18-11.18) ²
	MM	15	7.9	61	22.0	8.79(4.40-17.54) ²
	TM/MM	62	32.5	201	72.6	7.33(4.64-11.56) ²
Positive	TT	26	59.1	21	28.8	Reference
	TM	14	31.8	39	53.4	4.41(1.72-11.30) ²
	MM	4	9.1	13	17.8	4.60(1.12-18.92) ²
	TM/MM	18	40.9	52	71.2	4.45(1.87-10.63) ²

¹ Adjusted for age, sex, ethnicity, anti-HCV, and AFB1-DNA adduct levels;
² Adjusted for age, sex, ethnicity, HBsAg, and AFB1-DNA adduct levels.

Table 5 Joint effects of XRCC3 genotypes and AFB1-DNA adducts on TP53M risk.

AFB1-DNA adducts ¹	XRCC3 genotypes	TP53M (-) (n=235)		TP53M (+) (n=350)		OR (95% CI) ²
		n	%	n	%	
Nondetected	TT	45	19.1	17	4.9	Reference
	TM	39	16.6	35	10.0	2.34 (1.13-4.86)
	MM	13	5.5	10	2.9	2.20 (0.80-6.04)
	TM/MM	52	22.1	45	12.9	2.31 (1.15-4.62)
Detected	TT	110	46.8	80	22.9	1.98 (1.05-3.74)
	TM	22	9.4	144	41.1	17.33 (8.44-35.62)
	MM	6	2.6	64	18.3	28.38 (10.34-77.93)
	TM/MM	28	11.9	208	59.4	19.70 (9.90-39.18)

¹ Two-sided; $\chi^2=138.453$, $P = 3.815 \times 10^{-28}$; ² Adjusted for age, sex, ethnicity, HBsAg, and anti-HCV.

DISCUSSION

To the best of our knowledge, few studies have investigated the role of DNA-repair gene XRCC3 codon 241 polymorphism in TP53M from AFB1- exposure areas. In this study, we analyzed the association between XRCC3 codon 241 polymorphism and TP53M risk among Guangxi HCCs, and found that this polymorphism was significantly associated with the increasing TP53M risk (OR was 4.96, 95% CI was 3.46-7.11). Additionally, a gene-levels of AFB1-DNA adducts effect was also observed for those patients who had the risk genotypes of XRCC3 and AFB1-DNA adducts in their cancerous tissue (OR was 19.70, 95% CI was 9.90-39.18). These imply that there should be interaction among the genotypes of XRCC3 and the levels of AFB1-DNA adducts, and that this polymorphism may have functional significance in the detoxification of AFB1 and repair of AFB1-induced p53 mutations in codon 249.

AFB1 is an important mycotoxin produced by the moulds *Aspergillus parasiticus* and *Aspergillus flavus* and mainly occurs in poorly stored corns and peanuts^[7,8,32]. This mycotoxin is mainly metabolized by CYP into the genotoxic metabolic AFB1-epoxide. This metabolite can bind to DNA and cause DNA damage (including AFB1-DNA adduct, base damage, and DSBs, etc.) that can be repaired by ways of excision repair and DSBs repair (DSBR)^[1-3,7,8,32]. If these DNA damages are not repaired duly and efficiently, however, they may lead to gene mutations such as p53 gene^[6,9,10,33]. Clinic and experimental studies have shown that AFB1 mainly induces the point mutation in codon 249 of the p53 gene (namely: G:C to T:A transversion at the third base position, also called TP53M)^[9,34-36]. Therefore, the DNA repair capacity (limited to DNA repair genes) might modify the frequency of this mutation.

While the protein encoded by XRCC3 gene is one of the 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 for HRR. XRCC3-deficient hamster cells showed high frequency of multiple centrosomes and abnormal spindle formation^[11-13]. Previous studies have shown that a common polymorphism (rs861539) at codon 241 of XRCC3 gene modifies the function of this gene, because this polymorphism changes the amino acids from one with a neutral hydrophilic hydroxyl group (Thr) to a hydrophobic one with a methyl sulfur group (Met)^[20,22,37]. The XRCC3 241 Met variation is evolutionarily conserved, which may be associated with reduced deficiency of DNA repair, subsequently resulting to gene mutations^[20,22,37]. Recently, we reported that those polymorphism is significantly associated with increasing the levels of AFB1-DNA adducts and risk of HCC induced by AFB1^[23,24]. Moreover, there is interaction of risk genotypes and levels of AFB1 exposure, which suggests that there may be relationship between this polymorphism and AFB1 exposure in the

process of TP53M for HCC patients^[32,33]. To explore this possible modified role and the molecular carcinogenesis of AFB1 resulting in HCC, we studied the correlation between the XRCC3 codon 241 polymorphism and TP53M risk. As a result, we found individuals, especially those featuring AFB1-DNA adducts detected in their tissular DNA, with XRCC3 codon 241 Met alleles were probably to have higher risk of TP53M. Interestingly, some genotypes-AFB1 exposure interaction evidence was also observed in the joint analysis, possibly because the decreasing DNA repair capacity resulting from XRCC3 codon 241 polymorphism is contributed to the toxicological effects of AFB1 and the amounts of TP53M is easily accumulated^[11].

Because a previous study has shown that hepatitis viruses and AFB1 have different carcinogenesis and that this difference might affect TP53M risk^[1,29,38], we also investigated the possible modifying effects of HBV and HCV infection on TP53M risk correlated with genetic polymorphism of XRCC3 and observed an increasing risk value of TP53M induced by AFB1 for negative hepatitis virus status. These different TP53M risk value suggests that AFB1-induced HCC should differ from HCC to hepatitis virus (including HBV and HCV) infection.

In conclusion, this is the first report to investigate association between XRCC3 codon 241 polymorphism and the higher frequency of TP53M in HCCs for Guangxi population from an high AFB1 exposure area. We find evidence to suggest that the XRCC3 codon 241 Mets are related to increasing TP53M risk that may be because of reducing DNA repair function. Given that TP53M is an important molecular marker of AFB1-induced liver cancer and liver cancer is a highly fatal tumor^[4-6], the finding of a genetic susceptibility (if confirmed) may have implications for screening and prevention. However, there were several limitations to our study. Despite risk role of XRCC3 codon 241 polymorphism on TP53M analyzed, we did not examine the functions of TP53M risk associated with XRCC3 polymorphism in the HCC carcinogenesis. Also, we did not analyzed the polymorphisms of other DNA repair genes involved in AFB1-induced DNA damage repair, such as XPC, XRCC4, and so on^[2,26,27,31]. Because this study based on hospitals, the selective bias might happen. Additionally, the power to elucidate gene-environment interactions was limited because of the small magnitudes of the overall associations and the relatively low frequency of high-risk genotypes. Future studies are need to characterize the roles of XRCC3 codon 241 Met alleles in detoxification and DNA repair and to test whether it affects the amounts of other biomarkers of gene mutations.

CONFLICTS OF INTEREST AND SOURCE OF FUNDING

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