DNA Repair Capacity, DNA-Strand Break Repair Gene Polymorphisms, and the Incidence of Hepatocellular Carcinoma in Southwestern Guangxi of China

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The associations between DNA repair capacity (DRC), DNA repair gene polymorphisms, and the incidence of hepatocellular carcinoma (HCC) have not been determined in high-risk areas. The aims of this study were to investigate whether DRC is related to the incidence of HCC and to determine whether polymorphisms in the DNA repair genes that regulate DRC are associated with the risk of HCC. First, a small case-control study was conducted to examine the association between DRC and the incidence of HCC and the environmental and genetic factors regulating DRC. Then, a large case-control study was conducted to determine whether those DNA repair gene polymorphisms shown to regulate DRC were related to the risk of HCC. The median DRC was significantly lower among the cases (0.80) than the controls (0.93). A multivariate linear regression analysis showed that the HBsAg status ($p < 0.01$), ethnicity ($p = 0.01$), and polymorphisms in the XRCC3-241 ($p = 0.01$) and APE1-148 ($p = 0.03$) gene loci may be impact factors for DRC. In the large case-control study, a stratified analysis showed that individuals with the APE1-148-combined genotype GT+TT likely had a significantly higher HCC risk compared with those with only the GG genotype (crude odds ratio=1.93, 95% confidence interval=1.17–3.17) among the Zhuang ethnicity. However, nonsignificant differences were observed between XRCC3-241 polymorphisms and the HCC risk. DRC may be related to the incidence of HCC as determined by environmental and genetic factors found in southwestern part of the Guangxi Province. Gene-environment interactions play an important role in the incidence and progression of HCC.

Introduction

HEPATOCELLULAR CARCINOMA (HCC) accounts for between 85% and 90% of all primary liver cancers, making it the fifth most common cancer and the third leading cause of cancer-related deaths worldwide (Parikh and Hyman, 2007). In China, HCC has been the second leading cause of cancer deaths since the 1990s, with an incidence rate of 26.26/100,000 in 2005 (Chen *et al.*, 2010). Many studies have shown that the major risk factors for HCC include chronic infection with hepatitis B (HBV) or hepatitis C (HCV) virus, exposure to dietary aflatoxin B1 (AFB1), and alcohol consumption. One of the major consequences of these risk factors is the DNA damage resulting from both endogenous and exogenous carcinogens, such as HBV DNA, peroxide generated by HCV protein or alcohol, DNA-adducts produced by AFB1 or peroxide, and others (Wang and Groop-

man, 1999; Kern *et al.*, 2002; Rusyn *et al.*, 2004). These carcinogens accumulate and can disrupt host DNA integrity, thus leading to carcinogenesis.

DNA repair systems are designed to protect the integrity of the genome, and any deficiency in this system likely leads to the development of cancers (Berwick and Vineis, 2000). Obviously, differences in these systems can lead to differences in DNA repair capacity (DRC), which is defined as the capacity of the DNA repair system to maintain genomic integrity and to repair DNA lesions and may reflect individual susceptibilities to cancer. Because DRC can be measured by a direct assay or indirectly by cultivation of peripheral blood lymphocytes (Berwick and Vineis, 2000), many studies have focused on the relationship between DRC and the cancer risk. Previous studies have reported that DRC deficiency likely increases the risk for several human cancers, including cancers of the lung, skin,

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prostate, and breast, as well as squamous cell carcinoma of the head and neck (Lockett *et al.*, 2005). Other studies have noted that this association may be modulated by other related risk factors, such as smoking status (Kosti *et al.*, 2010), caffeine intake (Natarajan *et al.*, 2006), and DNA repair gene polymorphisms (Shi *et al.*, 2004). However, limited data exist on the relationship between the HCC risk and DRC. Only Wu *et al.* (1998) have reported that increasing mutagen sensitivity (i.e., DRC deficiency) may be associated with the risk of HCC. However, Wu's study did not clearly illustrate the impact of HBV infection and other factors, such as alcohol consumption and family history, on DRC. Furthermore, as previously reported, DNA repair gene polymorphisms can regulate DRC (Qiao *et al.*, 2002; Voldicka *et al.*, 2004; Angelini *et al.*, 2008). Despite these results, the influence of DRC and DNA repair gene polymorphisms on the incidence of HCC in the high-risk HCC area of the Guangxi Province, China, where there is also a high prevalence of HBV infection and dietary AFB1 exposure (Yeh *et al.*, 1989), remains unclear.

In this study, we hypothesized that low DRC may increase the risk of HCC, which may be further modulated by other risk factors, such as HBV infection, or regulated by polymorphisms in DNA repair genes. Furthermore, we hypothesized that those DNA repair gene polymorphisms proven to regulate DRC may also be associated with the incidence of HCC. To test our hypotheses, we first designed a standardized microgel electrophoresis assay to measure DRC in 46 HCC cases and 46 cancer-free controls from the same hospital to evaluate the association between DRC and HCC and to examine the impact factors of DRC. Then, we conducted a large hospital-based case-control study to verify the relationship between DNA repair gene polymorphisms that regulate DRC and the HCC risk. In the assay, bleomycin (BLM) was used to induce DNA damage; BLM is a known carcinogenic simulator and inducer of single- and double-strand breaks in DNA (Wu *et al.*, 1998). Because such damage can be repaired by the base excision repair (BER) pathway, as well as homologous or nonhomologous recombination pathways, several single nucleotide polymorphism (SNP) loci (rs1799782, rs25489, rs25487, rs861539, rs3136820, and rs1052133) of the related genes *XRCC1*, *XRCC3*, *APE1*, and *hOGG1* (Wood *et al.*, 2001) were included in our study.

Materials and Methods

Study population

All HCC cases and cancer-free controls were recruited from two hospitals (First Affiliated Hospital of Guangxi Medical University and First Affiliated Hospital of Guangxi College of Traditional Chinese Medicine) from August 2007 to November 2008. Study subjects received a detailed description of the study protocol and signed the informed consent forms approved by the institutional review board for each medical center. The cases were all newly diagnosed with HCC, but had not been treated with radiation, chemotherapy, or surgical therapy before enrollment in the study. Controls were recruited from the Department of Orthopedics and Ophthalmology of the same hospitals and did not have cancer or other chronic diseases. For the two case-control studies, controls were frequency matched to the cases using age, gender, and ethnicity (Han or Zhuang). The study

protocol was approved by the institutional review board of the Guangxi Cancer Institute.

Data collection

After informed consent was obtained, a structured interview was conducted by trained interviewers. Data were collected from interviews and reviews of medical records regarding sociodemographic characteristics, recent and prior tobacco use, alcohol consumption, family history of cancer, and HBV virus markers. At the completion of the interview, blood was drawn into heparinized tubes for cytogenetic and molecular genetic analysis. All laboratory and questionnaire data were coded, entered by two investigators, and verified using EpiData 3.1 (www.epidata.dk/download.php). Neither the laboratory nor the data entry personnel had any knowledge of the subjects' case-control status.

DRC assessment

A standardized microgel electrophoresis assay was conducted to assess DRC as described previously (Schmezer *et al.*, 2001). Briefly, peripheral blood lymphocytes (PBLs) were isolated with a human lymphocyte separation kit (Chinese Academy of Medical Sciences and Peking Union Medical College Institute of Biomedical Engineering, 20080426). The cells were cultured for 20 h in 5% CO₂ at 37°C and subsequently treated with 20 µg/mL BLM. Before analysis by the microgel assay, the cells were incubated in RPMI 1640 at 37°C for 15 min and checked for viability using Trypan Blue. To perform the assay, fully frosted microscope slides were covered with a layer of normal melting point agarose followed by 20 µL of cells mixed with 100 µL of low melting point agarose at 37°C. The slides were then placed on ice for 10 min to allow solidification of the agarose. The cell layer was covered with a clean coverslip to make the agarose smooth and easy to observe under the microscope. The slides were carefully immersed in a lysis solution after removal of the coverslip and kept for 1 h at 4°C. After cell lysis, the slides were placed in a horizontal gel electrophoresis unit filled with an alkaline electrophoresis buffer at ambient temperature for 20 min to induce DNA unwinding. Electrophoresis was performed for 20 min at 25 V and 300 mA. The slides were then rinsed with a neutralization buffer (0.4 M Tris, pH 7.5), stained with 20 µg/mL ethidium bromide, and kept in a humidified box. To prevent additional DNA damage, the entire assay was conducted under red light conditions. The coded slides were evaluated under a fluorescence microscope coupled to an image analysis system (Nikon). Undamaged cells were defined as those with a tail moment of <2 as determined for 50 randomly selected cells per slide, usually with three parallel slides per data point. DRC was calculated after a fixed repair time of 15 min as the percentage of undamaged cells after BLM treatment divided by the percentage of undamaged cells in the control preparation.

Genotyping

Genomic DNA was extracted from peripheral blood by phenol-chloroform extraction and stored at -20°C. Genotyping of the polymorphisms was performed using high-flux TaqMan MGB, a fluorescent quantitative real-time

polymerase chain reaction (PCR) method. Each PCR reaction mixture (25 μ L) contained 1.00 μ L of DNA template, 10.25 μ L of H₂O, 12.50 μ L of 2 $\times$ TaqMan Universal PCR Mix, and 1.25 μ L of 20 $\times$ SNP Genotyping Assay Mix. The genomic DNA was amplified under the following conditions: 95°C for 10 min followed by 40 cycles of 92°C for 15 s and 60°C for 1 min. All genotyping reagents and analytical software were purchased from Applied Biosystems. Genotyping was performed according to the manufacturer's instructions.

Statistical analysis

All statistical analyses were performed using SPSS software, version 13.0 (SPSS Institute, Chicago, IL). The Wilcoxon rank-sum test was used to determine the statistical significance of the case-control comparisons of DRC (Schmezer *et al.*, 2001). The impact factors for DRC were evaluated by linear stepwise regression. Chi-squared analysis and unconditional logistic regression were used to analyze the risk of HCC by different genotypes. The interaction between environmental factors, such as HBV infection, and genomic variants was qualitatively analyzed by a stratified analysis and a logistic regression model. All *p*-values < 0.05 were considered statistically significant.

Smoking status and drinking status were categorized as dichotomized variables. Individuals who had smoked more than 100 cigarettes in his or her lifetime were defined as ever smokers; individuals who consumed three or more standard cups of alcohol per week for over 6 months were defined as ever drinkers. A family history of HCC was defined as having HCC in first- or second-degree biological relatives.

Results

As shown in Table 1, frequency matching using age, gender, and ethnicity was effective for the two separate studies, because no difference was observed between the cases and controls. However, the prevalence of smoking, drinking, and being HBsAg-positive was significantly higher among the cases than the controls (34.8% vs. 6.5%, 56.5% vs. 10.9%, and 84.9% vs. 13.0% for the DRC assay, respectively; 32.8% vs. 9.6%, 39.6% vs. 10.0%, and 76.7% vs. 11.6% for the large case-control study, respectively; *p*-values were all < 0.01). In the DRC assay, only 3 of the cases (and none of the controls) had a family history of HCC. However, in the large case-control study, the rate for family history of HCC in the cases was significantly higher than in the controls (9.5% vs. 0.6%, *p* < 0.01).

A standardized microgel electrophoresis assay was performed to assess DRC among the cases and controls (Fig. 1). We can find that DRC of the case was lower than the control for the first glance from the images. Since DRC was not normally distributed, we first compared the medians for DRC between the cases and controls using the Wilcoxon rank-sum test (Fig. 2). Overall, the median DRC was significantly lower among the cases (0.80) than the controls (0.93). The DRC medians were all consistently and significantly lower among the cases than the controls, regardless of whether they were stratified by smoking, drinking, or HBsAg status (Figs. 3–5).

To determine whether the six SNPs associated with single- and double-strand breaks, in addition to other risk factors such as smoking, drinking, and HBsAg status, could predict lower DRC, we conducted a univariate analysis

TABLE 1. FREQUENCY DISTRIBUTION OF THE DEMOGRAPHIC CHARACTERISTICS BETWEEN CASES AND CONTROLS IN THE HOSPITAL-BASED STUDY IN SOUTHWESTERN GUANGXI, CHINA, 2008–2010

Factors	DRC assay			Large case-control study		
	Case n=46	Control n=46	p	Case n=497	Control n=500	p
Age (years)			0.13			0.68
<50	25	32		347	355	
≥50	21	14		150	145	
Gender			0.78			0.10
female	7	8		106	129	
male	39	38		391	371	
Nationality			0.24			0.14
Han	36	31		378	360	
Zhuang	10	15		119	140	
Smoking			<0.01			<0.01
never	30	43		334	452	
ever	16	3		163	48	
Drinking			<0.01			<0.01
never	20	41		300	450	
ever	26	5		197	50	
HBsAg status			<0.01			<0.01
negative	7	40		116	442	
positive	39	6		381	58	
Family history of cancer			0.24			<0.01
no	43	46		450	497	
yes	3	0		47	3	

Boldface indicates variables with statistical significant values (*p* < 0.05).

DRC, DNA repair capacity.

in both cases and controls (Table 2). Among controls, the HBsAg-positive status, XRCC3-241 wild-type CC genotypes, and APE1-148 GT+TT genotypes were found to be risk factors for low DRC, whereas similar risks among cases were found for drinking and XRCC3-241 CC genotypes. We also conducted a multivariate linear regression analysis by stepwise to screen the impact factors of DRC among controls (Table 3). We found that HBsAg status, ethnicity, and XRCC3-241 and APE1-148 polymorphisms may be impact factors for DRC.

Because the XRCC3-241 and APE1-148 polymorphisms were associated with DRC, we examined whether these SNPs were also associated with the HCC risk in a large hospital-based case-control study involving 497 HCC cases and 500 cancer-free controls. The distributions of the polymorphic genotypes for the two individual loci in the controls were both in Hardy-Weinberg equilibrium ($\chi^2=0.106$, *p*=0.745 for XRCC3-241 and $\chi^2=1.752$, *p*=0.185 for APE1-148). As shown in Table 4, for XRCC3-241, the risk for HCC was not significantly different for the CT heterozygote (adjusted odds ratio [OR]=1.06; 95% confidence interval [CI]=0.61–1.82) or the TT homozygote (adjusted OR=1.94; 95% CI=0.32–11.79) relative to the CC homozygote. Similarly, for APE1-148, the risk for HCC was not significantly different for the GT heterozygote (adjusted OR=0.73; 95% CI=0.50–1.07) or the TT homozygote (adjusted OR=1.19; 95% CI=0.68–2.07) relative to the GG homozygote. Moreover, a combination analysis was conducted and found to be nonsignificant (Table 4). Table 5

FIG. 1. Microgel electrophoresis assay for assessing DNA repair capacity (DRC) (comet cell with a typical tail is identified by arrow). Peripheral blood lymphocytes (PBLs) of a hepatocellular carcinoma (HCC) patient before bleomycin (BLM) exposure (A). PBLs of the same HCC patient after BLM exposure. More comet cells and typical tail indicate that the DRC is at a low level (B). PBLs of a patient in cancer-free control before BLM exposure (C). PBLs of the same patient in cancer-free control after BLM exposure. Less comet cells and nontypical tail indicate that the DRC is at a high level (D).

shows that the association between *APE1-148* variants and the HCC risk is modulated by ethnicity. The test of homogeneity on the ORs between the Han and Zhuang populations showed that they were not homogeneous ($p=0.006$). Among the Zhuang, individuals with the *APE1-148*-combined genotype *GT+TT* likely had a significantly increased HCC risk relative to those with the *GG* genotype (OR=1.93; 95% CI=1.17–3.17). However, after adjusting for age, gender, smoking, drinking, and HBsAg status, the results were not significant (OR=1.42; 95% CI=0.69–2.93). Differences between *XRCC3-241* polymorphisms and the HCC risk

stratified by ethnicity or HBsAg status were also not significant.

Discussion

To the best of our knowledge, this study is the first to demonstrate that reduced DRC was associated with the occurrence of HCC. HBsAg status, ethnicity, and *XRCC3-241* and *APE1-148* polymorphisms may be determining factors of

FIG. 2. Box-plot for distributions of DRC in the cases and controls. (p -values were analyzed by the Wilcoxon rank-sum test). The box for each group represents the interquartile range (25–75th percentile), and the line within this box is the median value; bottom and top bars of the whisker indicate the minimum and maximum values excluded the outlier value.

FIG. 3. Box-plot for distributions of DRC in the cases and controls stratified by smoking (p -values were analyzed by the Wilcoxon rank-sum test; the outlier value is identified by "○" with the sample number).

FIG. 4. Box-plot for distributions of DRC in the cases and controls stratified by drinking (p -values were analyzed by the Wilcoxon rank-sum test).

DRC in high-risk HCC regions with a high prevalence of HBV infection and AFB1 exposure. However, we found that the polymorphisms of these two loci may not associate with the HCC risk. The findings support our hypothesis that low DRC may increase the risk of HCC as modulated by environmental and genetic factors.

FIG. 5. Box-plot for distributions of DRC in the cases and controls stratified by the HBsAg status (p -values were analyzed by the Wilcoxon rank-sum test; the outlier value is identified by "O" with the sample number).

TABLE 2. SINGLE-FACTOR ANALYSIS FOR DNA REPAIR CAPACITY IN THE CASES AND CONTROLS FOR THE DNA REPAIR CAPACITY ASSAY USING THE WILCOXON RANK-SUM TEST

		Case (n=46)			Control (n=46)		
Factors		n	Median of DRC	p	n	Median of DRC	p
Smoking	never	30	0.82	0.58	43	0.95	0.10
	ever	16	0.78		3	0.97	
Drinking	never	20	0.86	0.05	41	0.96	0.80
	ever	26	0.78		5	0.93	
HBsAg status							
	negative	7	0.79	0.76	40	0.96	<0.01
	positive	39	0.80		6	0.80	
XRCC1 rs1799782	CC	23	0.80	0.72	26	0.96	0.42
	CT+TT	23	0.80		20	0.93	
rs25489	GG	39	0.83	0.22	35	0.95	0.99
	AG+AA	7	0.76		11	0.96	
rs25487	GG	33	0.80	0.71	25	0.93	0.55
	AG+AA	13	0.83		21	0.96	
XRCC3 rs861539	CC	44	0.80	0.04	38	0.93	0.03
	CT+TT	2	0.91		8	0.96	
APE1 rs3136820	GG	20	0.80	0.88	12	0.96	<0.01
	GT+TT	26	0.81		34	0.93	
hOGG1 rs1052133	CC	16	0.81	0.70	15	0.95	0.71
	CG+GG	30	0.80		31	0.96	

Boldface indicates variables with statistical significant values ($p < 0.05$).

In the past two decades, increasing epidemiological and genetic evidence has shown that higher mutagen sensitivity is a risk factor for cancer development (Wu *et al.*, 2007). Lower DRC is associated with higher mutagen sensitivity (Schmezer *et al.*, 2001). For these reasons, DRC has been recommended as a molecular epidemiological marker for cancer research. Our results indicate that DRC was significantly lower in the cases than the controls, and that this result was independent of smoking, drinking, or HBsAg status. This result agrees with the previous study by Wu *et al.*, which found that higher mutagen sensitivity may increase the HCC risk (Wu *et al.*, 1998). Additionally, studies

TABLE 3. MULTIVARIATE ANALYSIS FOR DNA REPAIR CAPACITY BY LINEAR REGRESSION IN THE CONTROLS

Factors ^a	B	Std. error	Beta	t	p
HBsAg status	-0.06	0.01	-0.56	-5.09	<0.01
APE1 SNP	-0.02	0.01	-0.31	-2.89	0.01
XRCC3 SNP	0.03	0.01	0.33	2.98	0.01
Nationality	-0.02	0.01	-0.23	-2.27	0.03
Constant	0.98	0.02		51.31	<0.01

Boldface indicates variables with statistical significant values ($p < 0.05$).

^aAge, gender, nationality, smoking status, drinking status, HBsAg status, and polymorphisms of APE1-148 and XRCC3-241 were included.

TABLE 4. POLYMORPHISMS OF XRCC3-241 AND APE1-148 AND HEPATOCELLULAR CARCINOMA RISK IN THE LARGE CASE-CONTROL STUDY

Gene	Case (n=497)	Control (n=500)	Crude OR	Adjusted OR ^a
XRCC3-241				
CC	440	432	Reference	Reference
CT	50	65	0.75(0.51–1.12)	1.06(0.61–1.82)
TT	7	3	2.29(0.59–8.91)	1.94(0.32–11.79)
CT+TT	57	68	0.82(0.57–1.20)	1.11(0.66–1.87)
APE1-148				
GG	233	241	Reference	Reference
GT	198	203	1.01(0.77–1.32)	0.73(0.50–1.07)
TT	66	56	1.22(0.82–1.82)	1.19(0.68–2.07)
GT+TT	264	259	1.05(0.82–1.35)	0.85(0.60–1.20)

^aOR, odds ratio, adjusted for nationality, age, gender, family history of tumor, smoking, alcohol drinking, and HBsAg status.

on other solid tumors, such as cancers of the lung (Wu *et al.*, 1997; Spitz *et al.*, 2001), breast (Kosti *et al.*, 2010), and prostate (Hu *et al.*, 2004), have also elucidated the same association between DRC and the cancer risk, although different DRC-measuring methods were used.

In the present study, DRC was significantly influenced by two environmental factors, HBsAg status and ethnicity, and two DNA repair gene SNPs, XRCC3-241 and APE1-148. The results indicate that DRC may be jointly determined by both genes and the environment. Similar conclusions have been reached before. Qiao and his colleagues have reported that DRC was likely modulated by polymorphisms in several DNA repair genes, including XRCC1, XPA, and XPD (Qiao *et al.*, 2002; Wang *et al.*, 2003; Wu *et al.*, 2003; Shi *et al.*, 2004),

whereas Vodicka *et al.* (2004) and Angelini *et al.* (2008) have consistently noted that polymorphisms in XRCC1 may regulate DRC. Shen *et al.* (2006) have found that XPA, XPC, and XPG, and smoking all modestly predicted DRC after adjusting for age. Wu *et al.* (2006) have also demonstrated that a gene-smoking interaction can modulate DRC. These seemingly variable results are likely the result of two factors. First, induction methods using simulated carcinogens include the use of BLM, benzo[a]pyrene diol epoxide (BPDE), and irradiation. The damage induced by these agents, however, involves different repair pathways. BLM is known to induce single- and double-strand breaks in DNA, and our results indicate that the capacity to repair this damage may be determined by polymorphisms in the genes involved in single- and double-strand break repair. Second, the geographical regions encompassed by these studies vary from Asia (for the present study) to America to Europe, all of which have different backgrounds of environmental carcinogens. The Guangxi Province is the principle homeland for the Zhuang Chinese, a group of 15.18 million people who account for 38.5% of the total population of this autonomous region. Guangxi is also a high-risk area for HCC, as well as having a high prevalence of HBV infection and high levels of AFB1 exposure (Yeh *et al.*, 1989; Long *et al.*, 2008). The Zhuang live predominantly in the southwestern part of Guangxi where the major food sources, such as rice, corn, and peanut, are easily contaminated by AFB1. We hypothesize that the high levels of environmental carcinogens present in Southwestern Guangxi may influence DRC, and that DRC may be useful in predicting background levels of environmental carcinogens and the HCC risk.

Curiously, although HBV infection and ethnicity were found to modulate DRC, only ethnicity was found to significantly

TABLE 5. STRATIFIED ANALYSIS FOR POLYMORPHISMS OF XRCC3-241 AND APE1-148 AND HEPATOCELLULAR CARCINOMA RISK

Gene	Factor	Genotype	Case (n=497)	Control (n=500)	OR ^a	p ^b	OR ^c
XRCC3-241	Nationality					0.29	
	Han	CC	334	305			
		CT+TT	44	55	0.73(0.48–1.12)		0.90(0.50–1.62)
	Zhuang	CC	106	127			
		CT+TT	13	13	1.20(0.55–2.70)		1.93(0.62–6.01)
	HBsAg status					0.91	
APE1-148	negative	CC	100	380			
		CT+TT	16	62	0.98(0.54–1.77)		1.06(0.56–2.01)
	positive	CC	340	52			
		CT+TT	41	6	1.05(0.42–2.58)		1.13(0.45–2.83)
	Nationality					0.01	
	Han	GG	186	163			
		GT+TT	192	197	0.85(0.64–1.14)		0.72(0.48–1.07)
	Zhuang	GG	47	78			
		GT+TT	72	62	1.93(1.17–3.17)		1.42(0.69–2.93)
	HBsAg status					0.31	
	negative	GG	64	214			
		GT+TT	52	228	0.76(0.51–1.15)		1.28(0.80–2.04)
	positive	GG	169	27			
		GT+TT	212	31	1.09(0.63–1.90)		1.50(0.85–2.64)

Boldface indicates variables with statistical significant values ($p < 0.05$).

^aOR, odds ratio, not adjusted.

^bp-value for Breslow-Day tests of homogeneity of the odds ratios.

^cOR, odds ratio, adjusted for nationality, age, gender, smoking, alcohol drinking, and HBsAg status.

affect the association between *APE1-148* polymorphisms and the risk of HCC. This result may indicate that a gene proven to influence DRC may not necessarily predict the HCC risk by itself, but may instead function through gene–environment interactions. Previously, Long *et al.* (2008) reported that individuals with the *XRCC3-241 T* allele had an increased risk of HCC compared with those with the CC homozygote, which is inconsistent with the present study. The main reason for this inconsistency may be due to the difference between Long's study sample and ours; Long *et al.* selected more than 50% of the minority (mainly Zhuang) living in Southwestern Guangxi, while we included only 26.5% of the minority (the rest of our sample was Han). Recently, Long *et al.* reported that *XPC* and *XPB* polymorphisms may be associated with AFB1-related HCC (Long *et al.*, 2009, 2010). These results showed that even if the genes do not modulate DRC, they can predict the HCC risk in Southwestern Guangxi, thus supporting observations concerning the high levels of environmental carcinogens in this region. All data indicate that AFB1 contamination interacting with DNA repair gene polymorphisms may play an important role in the incidence and progression of HCC in Southwestern Guangxi.

In summary, as a comprehensive molecular marker, DRC may be determined by HCC risk factors such as HBV infection, AFB1 exposure, and specific DNA repair genes in Guangxi. Genes related to DRC may not predict the risk of HCC, but gene–environment interactions may play an important role in HCC development in high-risk regions. HCC, with its high degree of malignancy and poor prognosis, is one of the most important disease burdens in Guangxi. DRC, which partly reflects the background of environmental carcinogens, may be used as a biomarker for estimating the risk of HCC for the purpose of early prevention, which is essential for controlling the HCC prevalence. Although our results are only preliminary, we collected meaningful data on the association between DNA-strand repair systems and HCC. Because the DNA repair system is so comprehensive, such that the analysis of only a single pathway may be limiting, future research should focus on studies involving more pathways.

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Disclosure Statement

The authors declare no conflicts of interest.

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