Chapter

X-Ray Repair Cross Complementing 4 (XRCC4) Genetic Single Nucleotide Polymorphisms and the Liver Toxicity of AFB1 in Hepatocellular Carcinoma

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Abstract

Our previous reports have shown that the genetic single-nucleotide polymorphisms (GSNPs) in the DNA repair gene X-ray repair cross complementing 4 (XRCC4) are involved in the carcinogenesis of hepatocellular carcinoma (HCC) induced by aflatoxin B1 (AFB1). However, the effects of GSNPs in the coding regions of XRCC4 on hepatic toxicity of AFB1 have been less investigated. We conducted a hospital-based clinic tissue samples with pathologically diagnosed HCC (n = 380) in a high AFB1 exposure area to explore the possible roles of GSNPs in the coding regions of XRCC4 in AFB1-induced HCC using liver toxicity assays. A total of 143 GSNPs were included in the present study and genotyped using the SNaPshot method, whereas the liver toxicity of AFB1 was evaluated using AFB1-DNA adducts in the tissues with HCC. In the clinicopathological samples with HCC, the average adduct amount is 2.27 ± 1.09 μmol/mol DNA. Among 143 GSNPs of XRCC4, only rs1237462915, rs28383151, rs762419679, rs766287987, and rs3734091 significantly increased the levels of AFB1-DNA adducts. Furthermore, XRCC4 GSNPs (including rs28383151, rs766287987, and rs3734091) also increased cumulative hazard for patients with HCC. These results suggest that the liver toxicity of AFB1 may be modified by XRCC4 GSNPs.

Keywords: AFB1, liver toxicity, XRCC4, genetic single-nucleotide polymorphism, hepatocellular carcinoma

1. Introduction

Aflatoxin B1 (AFB1) is an important type I chemical toxicant mainly produced by the toxigenic strains of *Aspergillus flavus* (*A. flavus*) and *Aspergillus parasiticus* (*A. parasiticus*) [1, 2]. This carcinogen is often taken into human body via contaminating human foods such as nuts and cereals and displays its toxic effects, especially hepatic toxicity [1–8]. AFB1-induced hepatic effects consist of acute toxic damages (such as severe DNA damage, severe liver degeneration and necrosis, and

the failure of hepatic function) and chronic cumulative damages (such as a series of cumulative DNA damage, slight hepatocellular degeneration and necrosis, chronic inflammation, liver cirrhosis, and liver cancer) [3–5]. Increasing evidence has shown that under the same exposure of AFB1, some individuals feature severe hepatic damage; others have no noticeable damage [9–14]. This suggests that different individuals have different responses to the toxic effects of AFB1 and genetic factors may play a central role in the AFB1-induced hepatic toxicity.

X-ray repair cross complementing 4 (XRCC4), an important DNA repair gene involved in nonhomologous end-joining (NHEJ) repair pathway, plays a scaffold function via stabilizing and localizing DNA repair enzymes LIG IV, Ku70/80 heterodimer, and the DNA-dependent protein kinase (DNA-PK) catalytic subunit (DNA-PKcs) in the ends of DNA double-stranded breaks (DSBs) during NHEJ [15, 16]. In the past decades, growing reports have exhibited that the abnormal structures and functions of XRCC4 may alter the capacity of DNA repair and ultimately result in human diseases [17–22]. Several recent studies have also shown that the genetic alterations in the coding regions of XRCC4 can modify hepatocellular carcinoma (HCC) risk and prognosis [23–27]. However, the effects of this genetic alteration on the hepatic toxicity of AFB1 is unclear. Here, we conducted a clinical sample study exposure to explore whether the genetic single-nucleotide polymorphisms (GSNPs, a type of genetic alterations) in the coding regions of XRCC4 modified the effects of AFB1 on hepatic damage.

2. Materials and methods

2.1 Study population

This was a hospital-based molecular epidemiological study conducted in high AFB1 exposure area, Guangxi Zhuang Region, China. All participants were newly diagnosed HCC cases and recruited from the Affiliated Hospitals of Youjiang Medical University for Nationalities (located at Bose region, a major AFB1 exposure area) between January 2010 and January 2013 inclusively. The inclusive criteria of cases consisted of (a) cases with ultimately histopathologically confirmed HCC; (b) cases without any evidence of hepatitis virus infection; (c) cases with the history of AFB1 exposure which was defined according to positive history of peripheral serum AFB1-albumin adducts [5, 24]; and (d) cases with available tumor tissue samples and clinicopathological data.

According to the criteria, a total of 380 cases with HCC were recruited in this study during the period. With informed consent, the tissue samples with HCC for all patients and clinicopathological data were collected. Additionally, survival follow-up information was also collected through cases themselves or their family contact. In this study, the last follow-up date was set on January 31, 2019. The protocol for clinical samples was approved by Youjiang Medical University for Nationalities Medical Ethics Committee.

2.2 The evaluation of AFB1-related hepatic toxicity

Hepatic toxicity of AFB1 was evaluated using AFB1-DNA adducts in the tissue samples with HCC, and the amounts of AFB1-DNA adducts were tested by the previously described enzyme-linked immunosorbent assay (ELISA).

2.3 GSNP selection

All GSNPs of XRCC4 gene were first screened from the SNPdatabase (http://asia.ensembl.org/Homo_sapiens/Gene/Variation_Gene/Table?db=core;g=ENSG00000152422;r=5:83077498-83353787). According to the data from SNPdatabase, a total of 143 GSNPs can result in missense mutations and the change of amino acids in XRCC4 protein, and thus they were ultimately selected for final analyses.

2.4 Genotypic analyses

Genomic DNA in all tumor tissue samples with HCC was standard phenol-chloroform extraction binding with proteinase K. The GSNPs of XRCC4 were genotyped using SNaPshot method (Applied Biosystems [ABI], Foster City, CA) as previously described [28]. For quality control, all laboratory personnel were blind to the status of every sample with hepatocarcinoma, and controls were also included in each analysis.

2.5 Statistical analysis

The test for genotypic distribution of XRCC4 GSNPs among HCC cases featuring different AFB1-DNA adducts was accomplished using student *t*-test or one-way analysis of variance (ANOVA) test. Multiple tests were adjusted using a *Bonferroni* correction, and the threshold for GSNP screening was defined as $\alpha = 3.53 \times 10^{-4}$. Kaplan-Meier survival model with log-rank test and Cox regression model (the selection of significant varies based on forward-step method with likelihood ratio test) was used to analyze the association between XRCC4 GSNPs and HCC outcomes. Cumulative hazard value for the effects of XRCC4 GSNPs on the hepatic toxicity for AFB1 and corresponding 95% confidence interval (CI) were calculated using hazard ratio (HR) from significant multivariate Cox regression model (including all significant variates). All statistical analyses were performed with SPSS statistical package (Version 18, SPSS Institute, Chicago, IL, USA).

3. Results

3.1 The characteristics of subjects

All subjects suffered from hepatic carcinoma, and **Table 1** summarized their characteristics. The mean age of all participants was 50.74 ± 11.55 years, and more than 70% of them are male. For these cancer patients, 70.3% (267/380) and 26.3% (100/380) cases featured TNM II and III stages of tumor, and they also had an average AFB1 exposure value of 2.27 ± 1.09 μmol/mol DNA.

3.2 XRCC4 GSNPs increased AFB1-DNA adducts

A total of 143 GSNPs in the coding regions of XRCC4 gene were selected in our final analyses, and **Table 2** showed the genotypic distribution of all GSNPs. To evaluate the effects of these potential GSNPs on AFB1-DNA adducts, the role of each GSNP in the coding regions of XRCC4 gene was tested using Student *t*-test or ANOVA test with the adjustment of multiple test. Among these GSNPs, only rs1237462915 (cat#SNP016, at codon 38), rs28383151 (cat#SNP026, at codon 56), rs762419679 (cat#SNP069, at codon 127), rs766287987 (cat#SNP112, at codon 203),

	n	%
Total	380	100.0
Age (years)		
≤35	53	13.9
36–40	38	10.0
41–45	55	14.5
46–50	43	11.3
51–55	56	14.7
56–60	42	11.1
61–65	49	12.9
≥66	44	11.6
Gender		
Male	271	71.3
Female	109	28.7
Race		
Han	221	58.2
Zhuang	159	41.8
TNM stage		
I–II	13	3.4
III	267	70.3
IV	100	26.3

Table 1.
The characteristics of subjects.

and rs3734091 (cat#SNP138, at codon 247) significantly affected the levels of AFB1-DNA adducts in the tumor tissues with HCC. The adduct amounts of their wild genotypes (defined as XX genotype) were 2.15 ± 0.97 μmol/mol DNA, 2.07 ± 0.99 μmol/mol DNA, 2.12 ± 0.86 μmol/mol DNA, 2.11 ± 0.89 μmol/mol DNA, and 2.09 ± 0.97 μmol/mol DNA, respectively. For their mutant heterozygotic genotypes (defined as XY genotype), the amounts of AFB-DNA adduct were from 2.64 to 4.33 μmol/mol DNA, whereas the adduct levels were from 3.04 to 5.78 for the mutant homozygotic genotypes (defined as YY genotype) (**Table 2**).

Additionally, mutant genotypes of several other GSNPs, including rs761695470 (SNP008, at codon 18), rs758779099 (SNP018, at codon 40), rs144653114 (SNP054, at codon 103), rs1277864722 (SNP085, at codon 153), and rs777195630 (SNP100, at codon 180), also increased the amounts of AFB1-DNA adducts; however, they had no statistical significance according to screening threshold value.

3.3 XRCC4 GSNPs modified the AFB1-related HCC prognosis

Because the poor prognosis of patients with HCC has been associated with the toxicity of AFB1, we followed up the survival information of all patients and explored whether positive GSNPs of XRCC4 modified HCC outcomes, including overall survival (OS) and disease recurrence-free survival (RFS) (**Figures 1** and **2**). Results from Kaplan-Meier survival model (based on the cumulative risk models) and Cox regression model analyses showed that compared with their wild

No.	SNP ID	Chr: bp	Alleles (x/y)[a]	Genotypes (xx/xy/yy)[b]	Amino acid position	Amino acid change	$N_{xx/xy/yy}$[c]	Adducts (mean ± SD)	*P*[d]
SNP001	rs142575170	5:83104923	G/A	GG/GA/AA	2	E/K	323/54/3	2.28 ± 1.09/2.21 ± 1.08/ 2.36 ± 1.05	0.23
SNP002	rs1449631425	5:83104929	A/G	AA/AG/GG	4	K/E	377/3/0	2.27 ± 1.09/2.21 ± 0.54/–	0.99
SNP003	rs1166890864	5:83104930	A/G	AA/AG/GG	4	K/R	378/2/0	2.27 ± 1.09/1.57 ± 0.03/–	0.99
SNP004	rs1425642930	5:83104933	T/G	TT/TG/GG	5	I/R	376/4/0	2.27 ± 1.09/2.10 ± 0.61/–	0.96
SNP005	rs1252823908	5:83104946	C/A	CC/CA/AA	9	H/Q	376/4/0	2.27 ± 1.09/2.16 ± 0.56/–	0.94
SNP006	rs28383138	5:83104954	C/G	CC//CG/GG	12	S/C	319/47/14	2.26 ± 1.09/2.41 ± 1.06/ 2.00 ± 1.07	0.23
SNP007	rs774071130	5:83104966	T/C	TT/TC/CC	16	I/T	370/10/0	2.28 ± 1.09/2.05 ± 0.94/–	0.99
SNP008	rs761695470	5:83104971	C/T	CC/CT/TT	18	H/Y	371/6/3	2.24 ± 1.03/2.87 ± 2.00/ 4.42 ± 2.93	0.04
SNP009	rs755776572	5:83104983	G/A	GG/GA/AA	22	V/I	380/0/0	2.27 ± 1.09/–/–	—
SNP010	rs1271180927	5:83104986	T/A	TT/TA/AA	23	S/T	364/13/3	2.26 ± 1.09/2.52 ± 1.04/ 2.44 ± 0.77	0.89
SNP011	rs1288681786	5:83104992	G/A	GG/GA/AA	25	E/K	354/26/0	2.28 ± 1.10/2.16 ± 0.96/–	0.56
SNP012	rs550773308	5:83104999	C/T	CC/CT/TT	27	T/I	367/13/0	2.26 ± 1.06/2.51 ± 1.80/–	0.63
SNP013	rs1484250582	5:83105006	A/C	AA/AC/CC	29	E/D	376/4/0	2.27 ± 1.09/2.18 ± 1.42/–	0.87
SNP014	rs1305679408	5:83105013	T/A	TT/TA/AA	32	F/I	350/30/0	2.27 ± 1.08/2.26 ± 1.14/–	0.96
SNP015	rs1232077487	5:83105022	A/G	AA/AG/GG	35	T/A	380/0/0	2.27 ± 1.09/–/–	—
SNP016	rs1237462915	5:83105031	G/T	GG/GT/TT	38	D/Y	352/19/9	2.15 ± 0.97/3.62 ± 1.40/ 4.21 ± 0.93	2.96×10^{-5}
SNP017	rs748540743	5:83105032	A/T	AA/AT/TT	38	D/V	380/0/0	2.27 ± 1.09/–/–	—
SNP018	rs758779099	5:83105039	T/G	TT/TG/GG	40	H/Q	353/25/2	2.23 ± 1.03/2.78 ± 1.61/ 2.63 ± 0.01	0.04

No.	SNP ID	Chr: bp	Alleles (x/y)[a]	Genotypes (xx/xy/yy)[b]	Amino acid position	Amino acid change	$N_{xx/xy/yy}$[c]	Adducts (mean ± SD)	P[d]
SNP019	rs587779351	5:83105046	T/C	TT/TC/CC	43	W/R	380/0/0	2.27 ± 1.09/−/−	—
SNP020	rs771111816	5:83105050	C/T	CC/CT/TT	44	T/I	362/25/3	2.26 ± 1.08/2.33 ± 1.23/ 2.68 ± 0.07	0.78
SNP021	rs1460004120	5:83105053	G/C	GG/GC/CC	45	G/A	375/5/0	2.27 ± 1.09/2.21 ± 0.82/−	0.91
SNP022	rs1248835327	5:83111031	C/T	CC/CT/TT	48	S/F	370/10/0	2.27 ± 1.09/2.21 ± 1.01/−	0.87
SNP023	rs1326359694	5:83111033	G/A	GG/GA/AA	49	E/K	355/25/0	2.28 ± 1.11/2.16 ± 0.76/−	0.61
SNP024	rs868207005	5:83111039	G/A	GG/GA/AA	51	E/K	380/0/0	2.27 ± 1.09/−/−	—
SNP025	rs945155269	5:83111049	A/C	AA/AC/CC	54	Q/P	364/26/0	2.29 ± 1.07/2.02 ± 1.04/−	0.24
SNP026	rs28383151	5:83111054	G/A	GG/GA/AA	56	A/T	277/63/40	2.07 ± 0.99/2.64 ± 1.33/ 3.04 ± 0.78	2.83×10^{-55}
SNP027	rs1245127767	5:83111063	A/G	AA/AG/GG	59	M/V	354/25/1	2.27 ± 1.10/2.18 ± 0.92/2.78*	0.67*
SNP028	rs748124509	5:83111066	G/T	GG/GT/TT	60	A/S	354/23/3	2.25 ± 1.11/2.36 ± 0.74/2.78 ± 1.01	0.66
SNP029	rs760546308	5:83111070	T/C	TT/TC/CC	61	M/T	352/28/0	2.27 ± 1.04/2.25 ± 1.56/−	0.91
SNP030	rs1369565470	5:83111071	G/C	GG/GC/CC	61	M/I	380/0/0	2.27 ± 1.09/−/−	—
SNP031	rs867574505	5:83111072	G/A	GG/GA/AA	62	E/K	380/0/0	2.27 ± 1.09/−/−	—
SNP032	rs1285557699	5:83111073	A/G	AA/AG/GG	62	E/G	380/0/0	2.27 ± 1.09/−/−	—
SNP033	rs1248297453	5:83111085	A/G	AA/AG/GG	66	Y/C	351/29/0	2.26 ± 1.07/2.36 ± 1.25/−	0.63
SNP034	rs776362814	5:83111087	G/A	GG/GA/AA	67	V/I	350/30/0	2.26 ± 1.11/2.31 ± 0.84/−	0.83
SNP035	rs1423928075	5:83111090	G/A	GG/GA/AA	68	G/S	380/0/0	2.27 ± 1.09/−/−	—
SNP036	rs1478505961	5:83111093	G/A	GG/GA/AA	69	E/K	250/28/2	2.27 ± 1.07/2.24 ± 1.28/2.69 ± 1.16	0.86
SNP037	rs759064378	5:83111097	T/C	TT/TC/CC	70	L/P	354/26/0	2.28 ± 1.09/2.15 ± 1.01/−	0.58
SNP038	rs371307071	5:83111100	G/C	GG/GC/CC	71	R/T	380/0/0	2.27 ± 1.09/−/−	—

No.	SNP ID	Chr: bp	Alleles (x/y)[a]	Genotypes (xx/xy/yy)[b]	Amino acid position	Amino acid change	$N_{xx/xy/yy}$[c]	Adducts (mean ± SD)	*P*[d]
SNP039	rs61762970	5:83111112	T/C	TT/TC/CC	75	L/S	356/24/0	2.29 ± 1.10/1.99 ± 0.89/–	0.20
SNP040	rs763501478	5:83111117	G/A	GG/GA/AA	77	G/R	361/29/0	2.27 ± 1.09/–/–	—
SNP041	rs764572419	5:83111120	G/T	GG/GT/TT	78	A/S	349/31/0	2.26 ± 1.09/2.39 ± 1.09/–	0.52
SNP042	rs528259464	5:83111121	C/T	CC/CT/TT	78	A/V	376/4/0	2.26 ± 1.09/2.77 ± 0.44/–	0.36
SNP043	rs757644947	5:83111126	C/T	CC/CT/TT	80	P/S	369/11/0	2.26 ± 1.07/2.51 ± 1.51/–	0.46
SNP044	rs572613361	5:83111138	T/C	TT/TC/CC	84	Y/H	351/27/2	2.27 ± /2.24 ± 1.02/3.09 ± 0.47	0.57
SNP045	rs1398927737	5:83111141	A/C	AA/AC/CC	85	T/P	380/0/0	2.27 ± 1.09/–/–	—
SNP046	rs756247552	5:83111142	C/T	CC/CT/TT	85	T/M	374/6/0	2.26 ± 1.08/2.56 ± 1.35/–	0.50
SNP047	rs149355996	5:83111147	A/T	AA/AT/TT	87	N/Y	371/9/0	2.27 ± 1.10/2.36 ± 0.70/–	0.81
SNP048	rs1049631686	5:83111162	T/A	TT/TA/AA	92	S/T	364/26/0	2.27 ± 1.09/–/–	—
SNP049	rs772190420	5:83111169	A/G	AA/AG/GG	94	Y/C	354/26/0	2.28 ± 1.08/2.08 ± 1.23/–	0.36
SNP050	rs1239084198	5:83111171	T/G	TT/TG/GG	95	F/V	356/24/0	2.26 ± 1.07/2.36 ± 1.34/–	0.68
SNP051	rs1184763400	5:83111174	T/A	TT/TA/AA	96	F/I	380/0/0	2.27 ± 1.09/–/–	—
SNP052	rs1236326383	5:83111177	T/C	TT/TC/CC	97	F/L	366/14/0	2.28 ± 1.09/1.92 ± 0.92/–	0.24
SNP053	rs1472843465	5:83111180	G/A	GG/GA/AA	98	E/K	377/3/0	2.27 ± 1.09/2.58 ± 0.84/–	0.63
SNP054	rs144653114	5:83111195	G/A	GG/GA/AA	103	N/D	240/127/13	2.17 ± 1.09/2.48 ± 1.08/2.11 ± 0.84	0.03
SNP055	rs79561451	5:83195782	T/C	TT/TC/CC	110	S/P	352/25/3	2.28 ± 1.11/2.20 ± 0.84/1.82 ± 0.37	0.74
SNP056	rs757113391	5:83195787	C/G	CC//CG/GG	111	F/L	380/0/0	2.27 ± 1.09/–/–	—
SNP057	rs1378785946	5:83195794	G/C	GG/GC/CC	114	E/Q	360/20/0	2.277 ± 1.10/2.227 ± 0.84/–	0.83
SNP058	rs1240771489	5:83195795	A/G	AA/AG/GG	114	E/G	281/80/19	2.297 ± 1.09/2.197 ± 1.06/–	0.75

No.	SNP ID	Chr: bp	Alleles (x/y)[a]	Genotypes (xx/xy/yy)[b]	Amino acid position	Amino acid change	$N_{xx/xy/yy}$[c]	Adducts (mean ± SD)	P[d]
SNP059	rs769556494	5:83195797	A/G	AA/AG/GG	115	K/E	340/40/0	2.277 ± 1.09/2.277 ± 1.04/–	0.99
SNP060	rs1056939125	5:83195798	A/C	AA/AC/CC	115	K/T	380/0/0	2.27 ± 1.09/–/–	—
SNP061	rs1301137729	5:83195800	G/C	GG/GC/CC	116	V/L	359/21/0	2.27 ± 1.09/2.19 ± 0.99/–	0.74
SNP062	rs550178738	5:83195807	A/T	AA/AT/TT	118	N/I	341/39/0	2.27 ± 1.10/2.28 ± 1.00/–	0.93
SNP063	rs375157105	5:83195809	C/A	CC/CA/AA	119	P/T	352/28/0	2.29 ± 1.102.06 ± 0.83/–	0.28
SNP064	rs768175717	5:83195810	C/T	CC/CT/TT	119	P/L	380/0/0	2.27 ± 1.09/–/–	—
SNP065	rs369641536	5:83195816	A/G	AA/AG/GG	121	E/G	380/0/0	2.27 ± 1.09/–/–	—
SNP066	rs1395194011	5:83195819	T/A	TT/TA/AA	122	V/D	289/81/10	2.24 ± 1.07/2.62 ± 1.22/–	0.05
SNP067	rs1198491910	5:83195821	A/G	AA/AG/GG	123	I/V	348/32/0	2.27 ± 1.09/2.25 ± 1.13/–	0.95
SNP068	rs1159852376	5:83195824	A/G	AA/AG/GG	124	R/G	369/11/0	2.26 ± 1.10/2.40 ± 0.88/–	0.64
SNP069	rs762419679	5:83195834	T/C	TT/TC/CC	127	I/T	359/15/6	2.12 ± 0.86/4.33 ± 1.99/5.99 ± 1.12	1.17×10^{-43}
SNP070	rs1412506484	5:83195840	A/G	AA/AG/GG	129	Y/C	349/31/0	2.27 ± 1.10/2.26 ± 1.32/–	0.96
SNP071	rs1178870682	5:83195848	G/C	GG/GC/CC	132	D/H	365/15/0	2.27 ± 1.10/2.26 ± 0.78/–	0.98
SNP072	rs1484047716	5:83195852	C/G	CC//CG/GG	133	T/S	374/6/0	2.26 ± 1.07/2.71 ± 1.90/–	0.32
SNP073	rs28360135	5:83195855	T/C	TT/TC/CC	134	I/T	304/64/12	2.31 ± 1.13/2.12 ± 0.90/ 2.12 ± 0.69	0.39
SNP074	rs1335801774	5:83195856	T/G	TT/TG/GG	134	I/M	380/0/0	2.27 ± 1.09/–/–	—
SNP075	rs372774793	5:83195857	G/C	GG/GC/CC	135	A/P	358/22/0	2.27 ± 1.06/2.25 ± 1.46/–	0.92
SNP076	rs1384832919	5:83195863	A/G	AA/AG/GG	137	N/D	368/12/0	2.27 ± 1.10/2.00 ± 0.59/–	0.38
SNP077	rs56334522	5:83195865	T/G	TT/TG/GG	137	N/K	366/13/1	2.26 ± 1.10/2.53 ± 0.80/1.34b	0.89
SNP078	rs370037164	5:83195869	G/A	GG/GA/AA	139	A/T	292/84/4	2.28 ± 1.06/2.23 ± 1.18/ 2.49 ± 0.83	0.89

No.	SNP ID	Chr: bp	Alleles (x/y)[a]	Genotypes (xx/xy/yy)[b]	Amino acid position	Amino acid change	$N_{xx/xy/yy}$[c]	Adducts (mean ± SD)	*P*[d]
SNP079	rs374892515	5:83195876	A/G	AA/AG/GG	141	N/S	380/0/0	2.27 ± 1.09/−/−	—
SNP080	rs28360136	5:83195878	G/C	GG/GC/CC	142	E/Q	254/111/15	2.33 ± 1.10/2.12 ± 1.02/2.35 ± 1.26	0.21
SNP081	rs553111266	5:83195883	C/A	CC/CA/AA	143	H/Q	373/7/0	2.26 ± 1.09/2.75 ± 1.07/−	0.24
SNP082	rs1022211508	5:83195897	A/G	AA/AG/GG	148	N/S	342/38/0	2.30 ± 1.11/2.03 ± 0.79/−	0.15
SNP083	rs757278630	5:83195903	G/A	GG/GA/AA	150	R/K	378/12/0	2.27 ± 1.09/1.99 ± 1.01/−	0.36
SNP084	rs1375107569	5:83195905	C/T	CC/CT/TT	151	L/F	380/0/0	2.27 ± 1.09/−/−	—
SNP085	rs1277864722	5:83195912	G/A	GG/GA/AA	153	R/K	355/25/0	2.29 ± 1.01/1.93 ± 0.72/−	0.02
SNP086	rs745751715	5:83195922	T/G	TT/TG/GG	156	N/K	289/89/2	2.28 ± 1.04/2.25 ± 1.23/2.12 ± 1.83	0.97
SNP087	rs1201811742	5:83195924	A/C	AA/AC/CC	157	D/A	353/26/1	2.24 ± 1.07/2.67 ± 1.22/2.72[e]	0.13
SNP088	rs369499884	5:83195930	A/G	AA/AG/GG	159	Q/R	367/13/0	2.28 ± 1.09/2.03 ± 1.08/−	0.42
SNP089	rs372493882	5:83195933	G/T	GG/GT/TT	160	G/V	250/30/0	2.29 ± 1.10/2.07 ± 0.97/−	0.30
SNP090	rs1156689163	5:83203553	T/G	TT/TG/GG	162	F/V	380/0/0	2.27 ± 1.09/−/−	—
SNP091	rs1325151692	5:83203554	T/A	TT/TA/AA	162	F/Y	345/95/0	2.26 ± 1.04/2.39 ± 1.46/−	0.49
SNP092	rs375731584	5:83203566	T/G	TT/TG/GG	166	V/G	355/25/0	2.25 ± 1.09/2.48 ± 0.97/−	0.32
SNP093	rs1359488275	5:83203568	A/T	AA/AT/TT	167	S/C	380/0/0	2.27 ± 1.09/−/−	—
SNP094	rs753605351	5:83203575	A/C	AA/AC/CC	169	K/T	385/15/0	2.26 ± 1.10/2.40 ± 0.89/−	0.64
SNP095	rs1376295451	5:83203587	A/T	AA/AT/TT	173	E/V	340/40/0	2.28 ± 1.10/2.18 ± 0.98/−	0.58
SNP096	rs778422015	5:83203595	C/T	CC/CT/TT	176	L/F	380/0/0	2.27 ± 1.09/−/−	—
SNP097	rs777300742	5:83203599	A/G	AA/AG/GG	177	Y/C	354/26/0	2.26 ± 1.08/2.39 ± 1.24/−	0.57
SNP098	rs140143447	5:83203604	C/T	CC/CT/TT	179	R/W	372/8/0	2.28 ± 1.09/1.69 ± 0.56/−	0.13
SNP099	rs771544881	5:83203605	G/A	GG/GA/AA	179	R/Q	380/0/0	2.27 ± 1.09/−/−	—

No.	SNP ID	Chr: bp	Alleles (x/y)[a]	Genotypes (xx/xy/yy)[b]	Amino acid position	Amino acid change	$N_{xx/xy/yy}$[c]	Adducts (mean ± SD)	*P*[d]
SNP100	rs777195630	5:83203607	T/C	TT/TC/CC	180	F/L	354/26/0	2.22 ± 1.04/2.89 ± 1.51/–	0.04
SNP101	rs1379408635	5:83203610	A/T	AA/AT/TT	181	I/F	338/41/1	2.26 ± 1.07/2.37 ± 1.22/2.98[e]	0.67
SNP102	rs1199884817	5:83203611	T/C	TT/TC/CC	181	I/T	345/35/–	2.27 ± 1.08/2.26 ± 1.20/–	0.96
SNP103	rs1018879495	5:83203623	A/T	AA/AT/TT	185	N/I	366/14/0	2.27 ± 1.09/2.18 ± 0.94/–	0.76
SNP104	rs1482288279	5:83203635	C/T	CC/CT/TT	189	T/I	380/0/0	2.27 ± 1.09/–/–	—
SNP105	rs770217916	5:83203641	T/A	TT/TA/AA	191	I/N	341/38/1	2.28 ± 1.10/2.10 ± 0.90/4.31b	0.11
SNP106	rs775587299	5:83203644	G/T	GG/GT/TT	192	R/I	351/24/5	2.28 ± 1.09/2.24 ± 1.16/ 1.94 ± 0.64	0.79
SNP107	rs763186148	5:83203652	C/T	CC/CT/TT	195	H/Y	328/50/2	2.28 ± 1.11/2.23 ± 0.97/2.18 ± 0.47	0.95
SNP108	rs1173748737	5:83203653	A/G	AA/AG/GG	195	H/R	380/0/0	2.27 ± 1.09/–/–	—
SNP109	rs764109844	5:83203654	T/G	TT/TG/GG	195	H/Q	380/0/0	2.27 ± 1.09/–/–	—
SNP110	rs1458486332	5:83203659	A/G	AA/AG/GG	197	K/R	380/0/0	2.27 ± 1.09/–/–	—
SNP111	rs1263079073	5:83203676	C/G	CC//CG/GG	203	Q/E	355/25/–	2.28 ± 1.09/2.18 ± 1.09/–	0.69
SNP112	rs766287987	5:83203678	A/T	AA/AT/TT	203	Q/H	357/16/7	2.11 ± 0.89/4.15 ± 0.77/5.78 ± 1.17	1.05×10^{-59}
SNP113	rs778723397	5:83203683	G/A	GG/GA/AA	205	R/Q	365/15/0	2.29 ± 1.09/1.87 ± 0.97/–	0.15
SNP114	rs777199609	5:83203691	G/C	GG/GC/CC	208	D/H	292/86/2	2.22 ± 1.05/2.43 ± 1.18/ 2.86 ± 1.40	0.20
SNP115	rs1224705261	5:83203694	A/G	AA/AG/GG	209	I/V	380/0/0	2.27 ± 1.09/–/–	—
SNP116	rs201604424	5:83203698	A/G	AA/AG/GG	210	K/R	300/72/8	2.29 ± 1.11/2.15 ± 0.97/2.77 ± 1.03	0.26
SNP117	rs1276157833	5:83203701	A/G	AA/AG/GG	211	Q/R	371/9/0	2.28 ± 1.08/1.82 ± 1.17/–	0.22
SNP118	rs1298401873	5:83203706	G/A	GG/GA/AA	213	G/R	359/21/0	2.29 ± 1.10/1.93 ± 0.87/–	0.14
SNP119	rs746407658	5:83204816	G/A	GG/GA/AA	214	E/K	291/79/10	2.30 ± 1.10/2.22 ± 1.09/1.72 ± 072	0.23

No.	SNP ID	Chr: bp	Alleles (x/y)[a]	Genotypes (xx/xy/yy)[b]	Amino acid position	Amino acid change	$N_{xx/xy/yy}$[c]	Adducts (mean ± SD)	*P*[d]
SNP120	rs969467594	5:83204820	C/G	CC//CG/GG	215	T/S	380/0/0	2.27 ± 1.09/−/−	—
SNP121	rs780330653	5:83204823	C/A	CC/CA/AA	216	A/E	307/61/12	2.24 ± 1.08/2.46 ± 1.19/ 2.06 ± 0.72	0.28
SNP122	rs1348464342	5:83204825	A/G	AA/AG/GG	217	I/V	375/5/0	2.27 ± 1.09/2.46 ± 0.94/−	0.69
SNP123	rs749647860	5:83204828	T/G	TT/TG/GG	218	C/G	358/22/0	2.27 ± 1.08/2.22 ± 1.23/−	0.84
SNP124	rs908326126	5:83204840	A/G	AA/AG/GG	222	T/A	380/0/0	2.27 ± 1.09/−/−	—
SNP125	rs1347664669	5:83204847	A/G	AA/AG/GG	224	D/G	354/26/0	2.26 ± 1.09/2.38 ± 1.12/−	0.58
SNP126	rs866477694	5:83204848	C/A	CC/CA/AA	224	D/E	380/0/0	2.27 ± 1.09/−/−	—
SNP127	rs774555675	5:83204850	G/A	GG/GA/AA	225	R/Q	348/32/0	2.28 ± 1.10/2.11 ± 0.97/−	0.39
SNP128	rs748307585	5:83204852	G/A	GG/GA/AA	226	D/N	285/86/9	2.29 ± 1.09/2.20 ± 1.09/2.37 ± 1.12	0.77
SNP129	rs368106955	5:83204867	G/A	GG/GA/AA	231	E/K	380/0/0	2.27 ± 1.09/−/−	—
SNP130	rs140579916	5:83204874	C/A	CC/CA/AA	233	T/N	342/38/0	2.26 ± 1.07/2.39 ± 1.23/2.27 ± 1.09	0.46
SNP131	rs762812825	5:83204882	G/A	GG/GA/AA	236	E/K	357/21/2	2.26 ± 1.08/2.43 ± 1.33/ 2.23 ± 0.26	0.78
SNP132	rs574436773	5:83204897	A/T	AA/AT/TT	241	T/S	376/4/0	2.27 ± 1.09/1.96 ± 1.19/−	0.57
SNP133	rs542187236	5:83204901	A/G	AA/AG/GG	242	D/G	380/0/0	2.27 ± 1.09/−/−	—
SNP134	rs1013137284	5:83204904	T/A	TT/TA/AA	243	L/H	374/6/0	2.26 ± 1.09/3.00 ± 0.56/−	0.10
SNP135	rs1261641487	5:83204906	T/C	TT/TC/CC	244	S/P	380/0/0	2.27 ± 1.09/−/−	—
SNP136	rs767176080	5:83204907	C/T	CC/CT/TT	244	S/F	376/6/0	2.27 ± 1.09/1.71 ± 0.34/−	0.31
SNP137	rs371824973	5:83204910	G/A	GG/GA/AA	245	G/E	380/0/0	2.27 ± 1.09/−/−	—
SNP138	rs3734091	5:83204915	G/T	GG/GT/TT	247	A/S	296/46/38	2.09 ± 0.97/2.79 ± 1.49/ 3.08 ± 0.77	1.20×10^{-70}
SNP139	rs141122119	5:83258556	A/G	AA/AG/GG	258	I/V	363/17/0	2.28 ± 1.10/2.04 ± 0.82/−	0.37

No.	SNP ID	Chr: bp	Alleles (x/y)[a]	Genotypes (xx/xy/yy)[b]	Amino acid position	Amino acid change	$N_{xx/xy/yy}$[c]	Adducts (mean ± SD)	*P*[d]
SNP140	rs138837678	5:83258665	A/C	AA/AC/CC	294	Q/P	372/8/0	2.28 ± 1.09/1.98 ± 0.79/−	0.45
SNP141	rs61749611	5:83353207	A/C	AA/AC/CC	324	N/H	348/30/2	2.27 ± 1.09/2.21 ± 1.14/2.53 ± 0.16	0.90
SNP142	rs148273490	5:83353231	G/C	GG/GC/CC	332	D/H	356/24/0	2.29 ± 1.10/2.02 ± 0.86/−	0.25
SNP143	rs141304949	5:83353238	T/C	TT/TC/CC	334	I/T	344/35/1	2.26 ± 1.07/2.39 ± 1.26/1.37[e]	0.58

[a] *x/y represents wild-type allele/ variant type allele.*
[b] *xx/xy/yy represents wild-type homozygote/heterozygote/variant-type homozygote.*
[c] *$N_{xx/xy/yy}$ represents the number of subjects with xx genotype, the number of subjects with xy genotype, and the number of subjects with yy genotype.*
[d] *P values are calculated using t-test or one-way analysis of variance.*
[e] *SD is not determined.*
** SD for genotype yy is not determined and P-value is used for genotypes xx and xy.*

Table 2.
The association between SNPs in the coding region of XRCC4 and AFB1-DNA adducts in tissues with hepatocellular carcinoma.

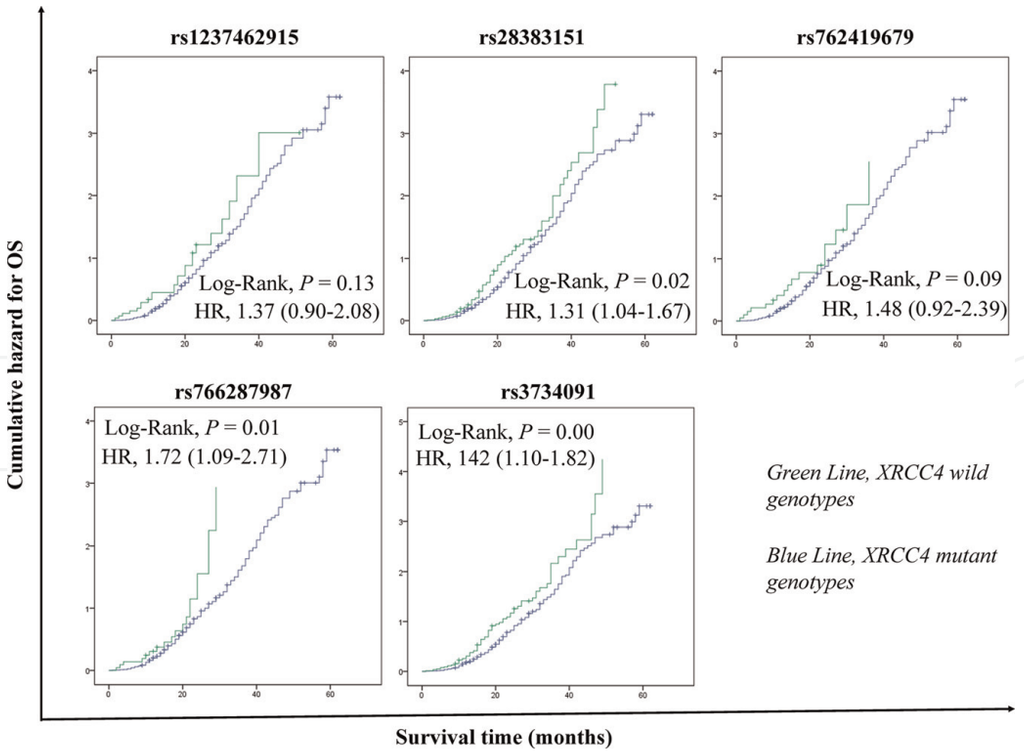

Figure 1.
XRCC4 GSNPs significantly correlating with the overall survival (OS) of hepatocellular carcinoma (HCC). Cumulative hazard function was plotted by Kaplan-Meier methodology, and P *value was calculated with two-sided log-rank tests. The relative hazard ratio (HR) values for genotypes were calculated using multivariable Cox regression models (with all significant variables) based on forward-step method with likelihood ratio test.*

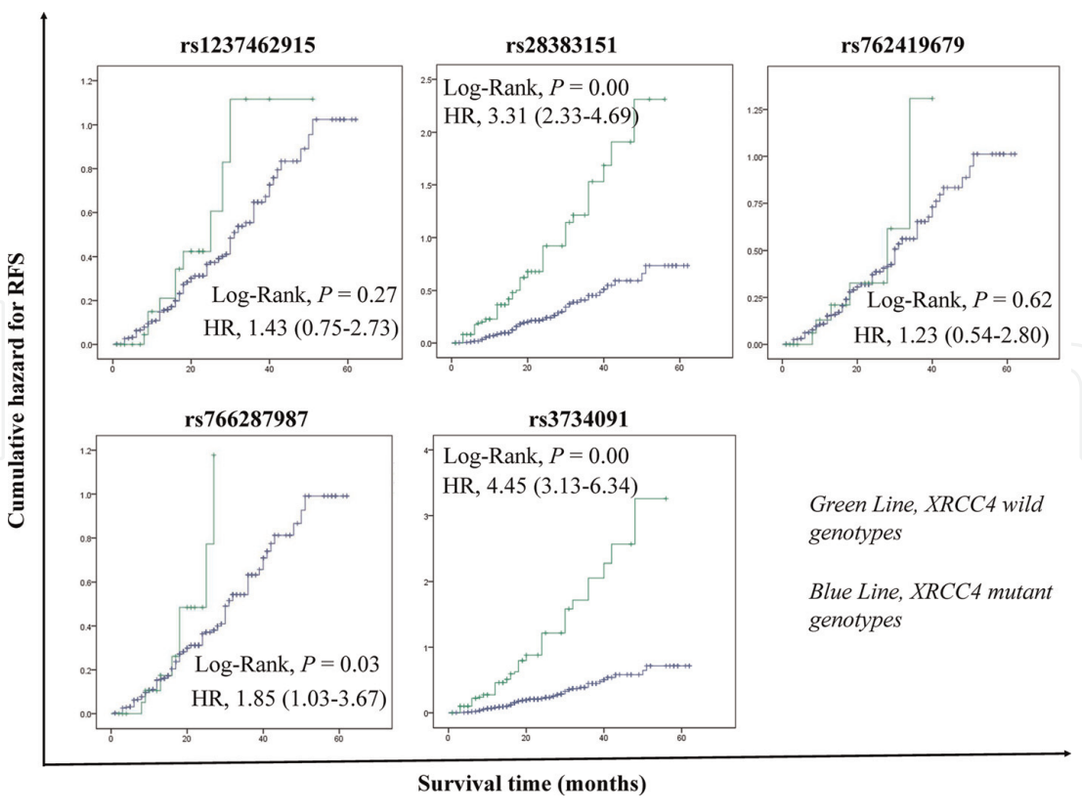

Figure 2.
XRCC4 GSNPs significantly correlating with the disease recurrence-free survival (RFS) of hepatocellular carcinoma (HCC). Cumulative hazard function was plotted by Kaplan-Meier methodology, and P *value was calculated with two-sided log-rank tests. The relative hazard ratio (HR) values for genotypes were calculated using multivariable Cox regression models (with all significant variables) based on forward-step method with likelihood ratio test.*

genotypes (XX genotypes), the mutant genotypes (including XY and YY genotypes) of rs28383151, rs766287987, and rs3734091 polymorphisms increased cumulative hazard for OS [HR = 1.31 (1.04–1.67), 1.72 (1.09–2.71), and 1.42 (1.10–1.82), respectively] (**Figure 1**). For RFS, the corresponding hazard values were 3.31 (2.33–4.69) for rs28383151, 1.85 (1.03–3.67) for rs766287987, and 4.45 (3.13–6.34) for rs3734091, respectively (**Figure 2**).

4. Discussion

In this study, we investigated the association between the GSNPs in the coding regions of XRCC4 gene and the toxic effects of AFB1 on the liver. We found that five XRCC4 GSNPs, including rs1237462915 (at codon 38), rs28383151 (at codon 56), rs762419679 (at codon 127), rs766287987 (at codon 203), and rs3734091 (at codon 247), significantly increased the amount of AFB1-DNA adducts in tissues with HCC (2.07–2.15 μmol/mol DNA for XY genotypes and 2.64–4.33 μmol/mol DNA for YY genotypes, respectively) and progressed the cumulative hazard of AFB1 hepatic toxicity.

AFB1 acts as a type of human chemical toxicant, and the toxic effects of this toxicant are characterized by organophilism (mainly causing hepatic damage), genic toxicity (mainly inducing DNA damages such as hotspot mutation at codon 249 of TP53 gene, AFB1-DNA adduct formation, and so on), and carcinogenicity (mainly resulting in HCC) [6–8]. Among the hepatic toxicity of AFB1, the formation of AFB1-DNA adducts in hepatic cells is a key step during the metabolism of this toxicant [9–14]. Evidence from molecular epidemiological studies and clinical studies has proved that the levels of AFB1-DNA adducts in the hepatic tissues are positively associated with the levels and time of AFB1 exposure [3, 24, 26–44]. This is indicative of AFB1-DNA adduct acting as the biomarker for AFB1's toxic capacity in the liver. In this study, AFB1-DNA adduct in the tumor tissues with HCC was used to evaluate hepatic toxicity related to AFB1, mainly because normal liver tissue samples cannot be obtained. Our results exhibited HCC tumor samples from high AFB1 exposure areas have an average adduct amount of 2.27 ± 1.09 μmol/mol DNA. Supporting our findings, several studies from high AFB1 exposure areas Nanning and Tiandong, China, have also shown the similar level of DNA adducts [4, 5, 26, 27, 37, 39, 45]. Taken together, the amount of AFB1-DNA adducts should be able to reflect the hepatic toxic potential of AFB1.

XRCC4, a key gene in the V(D)J recombination repair pathway, is located at 5q14.2 and consists of 13 exons (PubMed). Normally, XRCC4 is mainly expressed in genital meatus, alimentary tract, and lymphoid tissue; however, its expression will noticeably increase in other tissues such as the skin and liver under the condition of in vitro and in vivo injuries. This gene's encoding protein plays a vital role in both NHEJ and the completion of V(D)J recombination via acting as a scaffold protein for DNA ligase IV and DNA-PK in the repair of DNA DSBs [15, 19]. Mutations in XRCC4, including GSNPs and other non-GSNPs variants, can cause endocrine dysfunction, microcephaly, short stature, and diseases [16, 21]. With the development of human Geno projects, more than 1000 GSNPs are identified. Among these GSNPs, we focused on genetic alterations in the coding regions of XRCC4, mainly because they will result in missense mutations and ultimately cause the structure damage and function deficiency of XRCC4 protein. Molecular epidemiological studies have displayed that the GSNPs in the XRCC4 genes can increase DNA repair capacity and increase the risk of some tumors such as lung cancer, colon cancer, HCC, and so on [21, 46–51]. Evidence from in vitro and in vivo studies has also proved that XRCC4 GSNPs increase the amount of DNA damage and induce more

gene mutations [23, 24, 26, 27]. In our study, we tested the genotypic distributions of all known GSNPs in the coding region of XRCC4 in liver tumor tissues. Five positive GSNPs were identified, and they result in the change of amino acid D to Y at codon 38 for rs1237462915, A to T at codon 56 for rs28383151, I to T at codon 127 for rs762419679, Q to H at codon 203 for rs766287987, and A to S at codon 247 for rs3734091, respectively. Although evidence that several other GSNPs, including rs761695470, rs758779099, rs144653114, rs1277864722, and rs777195630, increased the amounts of AFB1-DNA adducts was not statistically significant according to our defined threshold value, their effects should not be neglected because small-size samples may underestimate values.

Because the toxic effects of AFB1 also modify the prognosis of patients with HCC [26, 27, 33, 52, 53], we accomplished patients' survival analyses on the basis of the cumulative risk models and found only rs28383151, rs766287987, and rs3734091 polymorphisms shortened HCC cases' OS and RFS. Supporting our findings, several previous reports have proved that XRCC4 GSNPs can alter the levels of XRCC4 mRNA and protein expression and dysregulation of XRCC4 expression increasing the amount of AFB1-DNA adducts and mutative risk of TP53 gene [23, 24, 26, 27].

To conclude, this study is the first report investigating the modified function of XRCC4 GSNPs on AFB1's hepatic toxicity. Our findings suggest that the GSNPs in the coding regions of XRCC4 gene, like rs1237462915, rs28383151, rs762419679, rs766287987, and rs3734091, may alter the DNA repair capacity of DNA damage induced by AFB1. If these individuals with mutant genotypes of these GSNPs decrease their exposure to AFB1, they will be free from toxic effects of AFB1 on hepatic damage. Several limitations should be focused for our study. First, relatively small-size samples may underestimate the effects of XRCC4 GSNPs on AFB1 hepatic toxicity. Second, the hospital-based design may result in selective bias. Third, we only accomplished the cumulative risk analyses but not the cumulative survival analyses. Finally, we did not finish functional and mechanical analyses. Thus, XRCC4 GSNPs may be valuable biomarkers for predicting the toxic effects of AFB1 on the liver once the present findings were proved by larger samples and toxic function analyses.

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Conflicts of interest and source of funding

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Abbreviations

AFB1	aflatoxin B1
CI	confidence interval
GSNPs	the genetic single-nucleotide polymorphisms
HCC	hepatocellular carcinoma
HR	hazard ratio
OS	overall survival
RFS	disease recurrence-free survival
XRCC4	X-ray repair cross complementing 4

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