Supplementary Meterials

Genetic Polymorphisms in DNA Repair Genes XRCC4 and XRCC5, and Aflatoxin B1-related Hepatocellular Carcinoma

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Table S1 Technical details of TaqMan-PCR analysis

Gene	Analysis	Primers	Probes
XRCC5	codon 247 genotype analysis	F[a] = TTGGCAAGGAAGATGGAAGTGT[b]	FAM-ACAGAGGAGAAGG-MGB[b]
		R[c] = TGGCCACCTAAGCGAAAGGT[b]	HEX-CAGAGGAGATGGC-MGB[b]
		Tm[d] = 60 ℃	
XRCC4	codon 247 genotype analysis	F = TGAGGAAAGTGAAAACCAAACTGATCT[b]	FAM-CCTGAAGACAACCC-MGB[b]
		R = GCCCAAATAAGATATTCAACAGAGGAGAT[b]	HEX-CCTGAAGCCAACCC-MGB[b]
		Tm = 60 ℃	
XRCC4	mRNA expression analysis	F = GCAATGGAAAAAGGGAAATATGTT[b]	FAM-TGAACTGAGAAAAGCATTG-MGB[b]
		R = GCTGGTCCTGCTCCTGACA[b]	
		Tm = 60 ℃	
UBC	Internal controls	F = GGGCACTGGTTTTCTTTCCA[b]	HEX-AGAGCGGAACAGGC-MGB[b]
		R = CGCCGAGAAGGGACTACTTTT[b]	
		Tm = 60 ℃	
TP53	TP53M analysis	F = TTGGCTCTGACTGTACCACCAT[e]	FAM-ACCGGAGTCCCATC-MGB[f]
		R = TGGAGTCTTCCAGTGTGATGATG[e]	VIC-AACCGGAGGCCCAT-MGB[f]
		*T*m = 60 ℃	

[a] Forward primer.

[b] To be synthesized by Shanghai GeneCore BioTechnologies Co., Ltd. (Shanghai, China).

[c] Reverse primer.

[d] Annealing temperature.

[e] To be synthesized by Introgen Bio., Ltd. (Shanghai, China).

[f] To be synthesized by Applied Biosystems.

Table S2 XRCC4P and HCC risk stratified by matching factors (HBV and HCV infection, age, gender, and race)

		Controls (n=2045)		HCCs (n=1499)		Adjusted OR	
		n	%	n	%	(95% CI)	P_{trend}
HBsAg[a]	XRCC4						
Negative	AA[b]	507	85.6	316	77.8	Reference	
	AS[b]	54	9.1	47	11.6	1.21(0.78-1.89)[c]	0.398
	SS[b]	31	5.2	43	10.6	2.03(1.21-3.40)[c]	0.007
	Ala	1068	90.2	679	83.6	Reference	
	Ser	116	9.8	133	16.4	1.80(1.38-2.36)	1.49×10^{-5}
Positive	AA	1223	84.2	834	76.3	Reference	
	AS	180	12.4	179	16.4	1.40(1.09-1.79)[c]	0.008
	SS	50	3.4	80	7.3	1.83(1.23-2.74)[c]	0.003
	Ala	2626	90.4	1847	84.5	Reference	
	Ser	280	9.6	339	15.5	1.72(1.45-2.04)	3.07×10^{-10}
Anti-HCV[d]	XRCC4						
Negative	AA	1417	84.4	940	76.9	Reference	
	AS	196	11.7	184	15.1	1.27(1.01-1.60)[c]	0.044
	SS	66	3.9	98	8.0	1.99(1.41-2.82)[c]	1.01×10^{-4}
	Ala	3030	90.2	2064	84.5	Reference	
	Ser	328	9.8	380	15.5	1.70(1.45-1.99)	4.41×10^{-11}
Positive	AA	313	85.5	210	75.8	Reference	
	AS	38	10.4	42	15.2	1.46(1.08-2.20)[c]	0.026
	SS	15	4.1	25	9.0	2.28(1.05-4.97)[c]	0.038
	Ala	664	90.7	462	83.4	Reference	
	Ser	68	9.3	92	16.6	1.94(1.39-2.72)	1.01×10^{-4}
Age (yr)[e]	XRCC4						
≤49	AA	923	55.0	593	48.5	Reference	
	AS	140	8.3	120	9.8	1.30(0.97-1.75)[c]	0.085
	SS	43	2.6	63	5.2	2.04(1.28-3.24)[c]	0.003
	Ala	1986	89.8	1306	84.1	Reference	
	Ser	226	10.2	246	15.9	1.66(1.36-2.01)	3.37×10^{-7}
>49	AA	807	20.5	557	20.1	Reference	
	AS	94	25.7	106	38.3	1.40(1.02-1.91)[c]	0.039
	SS	38	10.4	60	21.7	2.16(1.39-3.36)[c]	0.001
	Ala	1708	90.9	1220	84.4	Reference	
	Ser	170	9.1	226	15.6	1.86(1.51-2.30)	9.46×10^{-9}
Gender[f]	XRCC4						
Female	AA	421	85.1	287	23.5	Reference	
	AS	59	11.9	58	4.7	1.43(0.89-2.28)[c]	0.138
	SS	15	3.0	27	2.2	2.80(1.32-5.93)[c]	0.007
	Ala	901	91.0	632	84.9	Reference	
	Ser	89	9.0	112	15.1	1.79(1.33-2.41)	1.11×10^{-4}
Male	AA	1309	84.5	863	31.6	Reference	

	AS	175	11.3	168	60.6	1.30(1.02-1.65) [c]	0.036
	SS	66	4.3	96	34.7	1.93(1.36-2.73) [c]	2.12×10^{-4}
	Ala	2793	90.1	1894	84.0	Reference	
	Ser	307	9.9	360	16.0	1.73(1.47-2.04)	4.60×10^{-11}
Race[g]	XRCC4						
Han	AA	754	44.9	515	42.1	Reference	
	AS	115	6.8	122	10.0	1.25(0.92-1.70) [c]	0.149
	SS	43	2.6	69	5.6	1.97(1.28-3.02) [c]	0.002
	Ala	1623	89.0	1152	81.6	Reference	
	Ser	201	11.0	260	18.4	1.82(1.49-2.22)	3.38×10^{-9}
Zhuang	AA	976	26.7	635	29.2	Reference	
	AS	119	32.5	104	37.5	1.38(1.02-1.86) [c]	0.036
	SS	38	10.4	54	19.5	2.08(1.30-3.33) [c]	0.002
	Ala	2071	91.4	1374	86.6	Reference	
	Ser	195	8.605	212	13.4	1.64(1.33-2.01)	2.64×10^{-6}

[a] Likelihood ration test for interaction of the stratified variable (HBsAg-negative and positive) and XRCC4 genotype was calculated as test for the heterogeneity of ORs across strata ($P_{interaction} = 0.970$).

[b] AA, AS and SS represented the homozygotes of XRCC4 codon 247 Ala alleles, the heterozygotes of XRCC4 codon 247 Ala and Ser allele, and the homozygotes of XRCC4 codon 247 Ser alleles, respectively.

[c] OR conditional on matched set.

[d] Likelihood ration test for interaction of the stratified variable (anti-HCV-negative and positive) and XRCC4 genotype was calculated as test for the heterogeneity of ORs across strata ($P_{interaction} = 0.820$).

[e] Likelihood ration test for interaction of the stratified variable (Age: ≤49 yrs and > 49 yrs) and XRCC4 genotype was calculated as test for the heterogeneity of ORs across strata ($P_{interaction} = 0.615$).

[f] Likelihood ration test for interaction of the stratified variable (female and male) and XRCC4 genotype was calculated as test for the heterogeneity of ORs across strata ($P_{interaction} = 0.947$).

[g] Likelihood ration test for interaction of the stratified variable (Han and Zhuang) and XRCC4 genotype was calculated as test for the heterogeneity of ORs across strata ($P_{interaction} = 0.759$).

Table S3 Joint effects of AFB1 exposure and XRCC4P on HCC risk

AFB1 exposure	XRCC4	Controls		HCCs		OR(95% CI)[a]	P_{trend}
		n	%	n	%		
Years	Allele						
Short	Ala	1949	38.5	565	13.5	Reference	
	Ser	205	4.1	81	1.9	1.36(1.04-1.79)	0.027
Medium	Ala	980	19.4	783	18.8	2.76(2.41-3.15)	9.96×10^{-51}
	Ser	148	2.9	165	4.0	3.85(3.02-4.89)	5.86×10^{-28}
Long	Ala	765	15.1	1178	28.2	5.31(4.66-6.05)	1.22×10^{-138}
	Ser	43	0.9	226	5.4	18.13(12.91-25.45)	6.77×10^{-63}
Levels	Allele						
Low	Ala	1944	38.5	621	15.0	Reference	
	Ser	226	4.5	87	2.1	1.21(0.93-1.57)	0.165
Medium	Ala	1138	22.5	732	17.7	2.01(1.77-2.29)	3.37×10^{-26}
	Ser	92	1.8	112	2.7	3.81(2.85-5.09)	1.63×10^{-19}
High	Ala	612	12.1	1173	28.3	6.00(5.25-6.85)	1.99×10^{-153}
	Ser	78	1.5	273	6.6	10.96(8.39-14.32)	6.02×10^{-69}

[a] Calculated by non-conditional logistic regression.

Table S4 XRCC4P and the expression levels of XRCC4 protein[a]

	XRCC4-AA		XRCC4-AS		XRCC4-SS	
Expression levels	n	%	n	%	n	%
Low	127	11.0	64	28.3	57	46.3
Medium	378	32.9	94	41.6	47	38.2
High	645	56.1	68	30.1	19	15.4
Total	1150	100	226	100	123	100

[a] Spearman r test, $r = -0.314$, $P = 1.15 \times 10^{-69}$.

Table S5 Genotype distributions of XRCC4 codon 247 among HCC cases with curative treatment

Characters	AA		AS/SS[a]			Two-sided
	n	%	n	%	χ^2	*P*
Total	859	100.0	233	100.0		
Sex					0.415	0.520
Femal	58	6.8	13	5.6		
Male	801	93.2	220	94.4		
Age (years)					0.837	0.360
≤49	454	52.9	131	56.2		
>49	405	47.1	102	43.8		
Ethnicity					0.003	0.954
Han	400	46.6	108	46.4		
Minority	459	53.4	125	53.6		
HBV infection					0.63×10^{-5}	0.993
HBsAg (－)	232	27.0	63	27.0		
HBsAg (＋)	627	73.0	170	73.0		
HCV infection					4.250	0.039
Anti-HCV (－)	703	81.8	204	87.6		
Anti-HCV (＋)	156	18.2	29	12.4		
AFB1-exposure years					2.815	0.245
Short	196	22.8	45	19.3		
Median	274	31.9	87	37.3		
Long	389	45.3	101	43.3		
AFB1-exposure levels					12.232	0.002
Low	219	25.5	40	17.2		
Median	245	28.5	57	24.5		
High	395	46.0	136	58.4		
Tumor size					20.482	6.02×10^{-6}
≤5 cm	191	22.2	21	9.0		
>5 cm	668	77.8	212	91.0		
Liver cirrhosis					0.117	0.732
No	230	26.6	65	27.9		
Yes	629	73.2	168	72.1		
TNM stage					0.900	0.343
I	92	10.7	20	8.6		
II	767	89.3	213	91.4		
Recurrence					135.73	2.29×10^{-31}
No	506	58.9	37	15.9		
Yes	353	41.1	196	84.1		

[a] AS/SS represented the genotypes with XRCC4 codon 247 Ser alleles.

Table S6 Cox proportional hazard model analysis for multivariate analysis of potential predictor factors for recurrence-free survival of HCC cases with curative treatment

Variable	HR (95% CI)	*P*
Tumor size		
≤5 cm	Reference	
>5 cm	2.06(1.52-2.79)	3.18×10^{-6}
TNM stage		
I	Reference	
II	3.17(2.13-4.71)	1.13×10^{-8}
AFB1-exposure years		
Short	Reference	
Median	2.90 (2.22-3.80)	3.78×10^{-15}
Long	5.21 (4.00-6.78)	1.36×10^{-34}
AFB1-exposure levels		
Low	Reference	
Median	1.52(1.11-2.08)	0.010
High	4.06(3.40-5.32)	3.11×10^{-24}
XRCC4 codon 247 genotypes		
AA	Reference	
AS	2.64(2.13-3.26)	3.18×10^{-19}
SS	5.05(3.88-6.56)	1.42×10^{-33}

Table S7 Cox proportional hazard model analysis for multivariate analysis of potential predictor factors for overall survival of HCC cases with curative treatment

Variable	HR (95% CI)	*P*
Tumor size		
≤5 cm	Reference	
>5 cm	2.33 (1.84-2.95)	2.59×10^{-12}
TNM stage		
I	Reference	
II	3.89 (2.85-5.29)	7.73×10^{-18}
AFB1-exposure years		
Short	Reference	
Median	3.34 (2.46-4.53)	1.03×10^{-14}
Long	7.06 (5.22-9.55)	6.77×10^{-37}
AFB1-exposure levels		
Low	Reference	
Median	1.06 (0.88-1.27)	0.537
High	1.35 (1.15-1.60)	2.94×10^{-4}
XRCC4 codon 247 genotypes		
AA	Reference	
AS	1.19 (0.98-1.46)	0.086
SS	1.82 (1.38-2.39)	1.91×10^{-5}
Interaction of XRCC4 and AFB1 exposure years[a]		
SS × long exposure years	2.13 (1.05-4.31)	0.035

[a] The likelihood ratio test was used to test the interaction of XRCC4 genotypes and AFB1 exposure years.

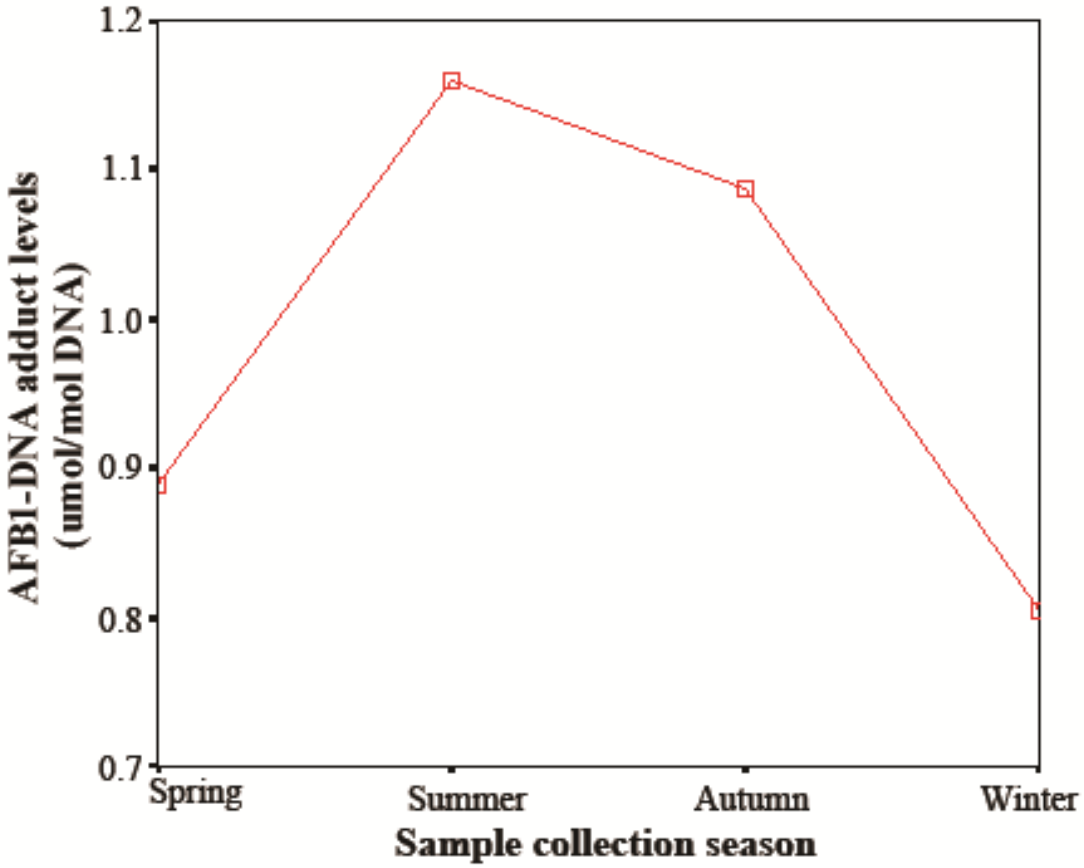

Figure S1 The episodic features of AFB1-DNA adduct levels of peripheral blood leukocytes. Peripheral blood samples were collected from 91 controls in different time spot (spring, summer, autumn, and winter), and AFB1-DNA adduct levels were tested using comparative enzyme-linked immunosorbent assay. Data were analyzed using the one-way analysis of variance test and are shown as means.