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CBX4 Expression and AFB1-Related Liver Cancer Prognosis

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Abstract

Background: Previous studies have shown that chromobox 4 (CBX4) expression may involve in the progression of liver cancer, however, it is unclear whether it affects the prognosis of hepatocellular carcinoma (HCC) related to aflatoxin B1 (AFB1).

Methods: A retrospective study was conducted in the high AFB1 exposure areas and a total of 428 patients with HCC were included in the final survival analyses. AFB1 exposure levels and CBX4 expression in the tumor tissues were tested using enzyme-linked immunosorbent assay and immunohistochemistry, respectively. The effects of AFB1 and CBX4 on HCC outcome were elucidated by Kaplan–Meier survival method and Cox regression model.

Results: We found that the levels of AFB1 exposure and CBX4 expression in tumor tissues were significantly associated with some clinicopathological features such as microvessel density and tumor stage. Furthermore, both AFB1 and CBX4 significantly modified overall survival and tumor reoccurrence-free survival status of HCC. Additionally, some evidence of CBX4-AFB1 interaction affecting HCC prognosis was observed, with an interactive value of 1.98 for overall survival and 1.94 for tumor reoccurrence-free survival, respectively.

Conclusion: These results suggest that CBX4 expression might be a useful marker for AFB1-related HCC prognosis.

Keywords: CBX4, AFB1, HCC, prognosis

1. Introduction

Aflatoxin B1 (AFB1) is a type of secondary metabolite of *Aspergillus parasiticus* and *Aspergillus flavus*, and frequently contaminates a series of staple foods, such as ground nuts, and maize [1–3]. Once this type foods contaminated by AFB1 entered into human bodies, it is metabolized into its epoxides consisting of AFB1–8,9-exo-epoxide (AFBEX) and AFB1–8,9-endo-epoxide (AFBEN) by cytochrome P450 (CYP) metabolic system [3]. These products of AFB1, especially AFBEX, are characterized by high reaction, genic toxicity, and carcinogenicity [3]. Evidence from molecular epidemiology and animal models has shown that AFB1 is an important carcinogen inducing hepatocellular carcinoma (HCC) [4–10]. Mechanically, the carcinogenesis of AFB1-related HCC mainly involves in the formation of DNA damage (including AFB1-DNA adducts, DNA single-strand breaks, DNA double-strands breaks, and gene mutations), the inactivation of such tumor suppressor gene as TP53, and the activation of cancer genes such as Ras [3, 11–15]. Although some advance in the pathogenesis of AFB1-related HCC has obtained in the past decades [16–18], it is still far for us to elucidate more detailed mechanisms.

The chromobox 4 (Cbx4) (GenBank accession NO. 8535) consists of six exons and spans about 6.26 kb on chromosome 17q25.3. This gene encodes a 560-amino acid protein which is the important component of polycomb repressive complex 1 (PRC1) [19–22]. Functionally, CBX4 involves in PRC1-regulated transcription repression and post-translation modification [19–22]. Recently, increasing evidence has exhibited that the dysregulation of this gene may affect the carcinogenic process of some tumors such as HCC, colorectal cancer, breast cancer, and so on, and may be a significant prognostic biomarker [19, 21, 23–29]. However, it is not clear whether CBX4 modify the prognosis of AFB1-related HCC. Here, we conducted a hospital-based retrospective study to investigate whether the CBX4 expression in the cancerous tissues is associated with the outcome of HCC related to AFB1 expression in the Guangxi Region, a high AFB1 exposure area.

2. Materials and methods

2.1. Study population

Between January 2009 and December 2012, 428 consecutive patients with histopathologically confirmed hepatocarcinoma were recruited at the Divisions of Oncology and Pathology, the affiliated Hospitals of Guangxi Medical University and Youjiang Medical University for Nationalities. During the recruitment phase, only 5 cases refused to participate in the study (response rate 98.8%). All cases were from high AFB1 exposure areas, including Nanning, Bose, Tiandong, and Tianyang. After informed consent was obtained, surgically removed tumor samples were collected to analyze the amounts of AFB1-DNA adducts and CBX4 protein in the cancerous tissues. Additionally, all corresponding clinicopathological and survival following-up data were also collected in the hospitals as previously described methods [30-32]. In this study, the status of hepatitis B virus (HBV) and hepatitis C virus (HCV) infection was evaluated using serum hepatitis B surface antigen (HBsAg) and anti-HCV, respectively; whereas the grade and stage of tumor was elucidated using the Edmondson and Steiner (ES) grading system and the Barcelona Clinic Liver Cancer (BCLC) staging system, respectively. For survival analyses, the

last follow-up day was set on December 31, 2017. The study protocol was carried out according to the approved guidelines by the Institutional Ethics Committee from the Affiliated Hospitals of Youjiang Medical University for Nationalities and Guangxi Medical University.

2.2. Microvessel density (MVD) assay

MVD in the cancerous tissues was assessed using the immunohistochemistry staining of CD31 as our previously descripted [30]. In this study, positive status of MVD was defined as microvessel counts more than 50 per ×200 magnifications.

2.3. AFB1 exposure data

AFB1 exposure levels were evaluated using the amounts of AFB1-DNA adducts in the cancerous tissues as our previously described [31, 32]. The amounts of AFB1-DNA adduct were tested using the competitive enzyme-linked immunosorbent assay. In this study, a value than less 1.00 μmol/mol DNA was considered as negative status for AFB1 exposure.

2.4. CBX4 expression assays

The level of CBX4 protein expression in cancerous tissues was elucidated using our previously published immunohistochemistry method [33, 34]. Briefly, the amounts of CBX4 protein were tested using anti-CBX4 antibody and calculated using immunoreactive score system (IRS). In the present study, positive CBX4 protein in cancerous tissues was define as IRS > 4.

2.5. Statistical analysis

Logistic regression model with enter method for variables (including all known clinicopathological features) was used for statistical comparison between groups. The odd ratios (ODs) and corresponding 95% confidence intervals (CIs) were calculated in this model for evaluating the association between clinicopathological features of HCCs and either AFB1 exposure or CBX4 expression. Kaplan–Meier survival method with log-rank test was used for statistical comparisons between different levels of AFB1 expression and CBX4 expression. Multivariate Cox regression model (with retread method based on likelihood ratio test) analyses were performed to calculate the risk strength of independent variates and prognostic values. In this study, all analyses were finished using the SPSS soft version 18.0 (SPSS Inc. Chicago, IL), and a P-value less than 0.05 was defined as statistical significance.

3. Results

3.1. The clinicopathological and survival features of HCC cases

Table 1 gave the clinicopathological characteristics of all cases, and a total of 428 patients with HCC were included in the final analyses. All cases were followed-up more than 5 years to obtain median survival time. During the follow-up period, 261 patients with

Variables	n	%
Total	428	100.0
Age, years		
Mean ± SE	47.9 ± 10.1	—
Range	30–75	—
Sex		
Man	290	68.9
Female	138	32.8
Ethnicity		
Han	229	54.4
Zhuang	199	47.3
HBV status		
HBsAg (−)	113	26.8
HBsAg (+)	315	74.8
HCV status		
anti-HCV (−)	378	89.8
anti-HCV (+)	50	11.9
Smoking status		
No	315	74.8
Yes	113	26.8
Drinking status		
No	304	72.2
Yes	124	29.5
AFP (ng/mL)		
≤ 20	154	36.6
> 20	274	65.1
Liver cirrhosis		
No	104	24.7
Yes	324	77.0
BCLC stage		
A	167	39.7
B	121	28.7
C	140	33.3
Tumor size		
≤ 3 cm	211	50.1
> 3 cm	217	51.5
MVD		
Negative	192	45.6

Variables	n	%
Positive	236	56.1
ES grade		
Low	226	53.7
High	202	48.0

Abbreviations: AFP, α-fetoprotein; BCLC, the Barcelona Clinic Liver Cancer staging system; ES, Edmondson and Steiner grading system; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HCV, hepatitis C virus; MVD, microvessel density.

Table 1. The clinic-pathological features of cases with hepatocellular carcinoma.

HCC featured cancer recurrences with 30.00 (22.20–37.80) months of median recurrence-free survival time (MRT), and 270 died with 45.00 (38.98–51.02) months of median overall survival time (MST).

3.2. The effects of AFB1 exposure on the clinicopathological features and the prognosis of HCC cases

In this study, the status of AFB1 exposure was elucidated using the amount of AFB1-DNA adducts in the cancerous tissues. Results from competitive ELISA exhibited the patients with HCC featured a 2.82 ± 1.60 μmol/mol DNA of AFB1 exposure level. To investigate the effects of AFB1 exposure on the clinicopathological features of HCC cases, we defined the amount of AFB1-DNA adducts ≤1.00 μmol/mol DNA as negative AFB1 exposure according to our previous published results [31, 32]. Our results showed that these patients with positive AFB1 status (AFB1-DNA adducts: > 1.00 μmol/mol DNA) had higher BCLC stage (adjusted OR = 2.09 and adjusted 95% CI = 1.04–4.24), bigger tumor size (adjusted OR = 69.06 and adjusted 95% CI = 33.62–141.86), and higher MVD (adjusted OR = 2.56 and adjusted 95% CI = 1.36–4.81) compared with those without positive AFB1 status (OR = 1) (**Table 2**). Additionally, we also found that the levels of AFB1 exposure were significantly associated with the age of patients with hepatocarcinoma (adjusted OR = 1.80, adjusted 95% CI = 1.22–2.66, and $P = 3.07 \times 10^{-3}$). However, AFB1 exposure was not correlated with other clinicopathological features of HCCs (**Table 2**).

Next, we investigated the effects of AFB1 exposure on the HCC prognosis using Kaplan–Meier survival model (**Figure 1A**). Results exhibited that HCC cases with negative AFB1 status (AFB1-DNA adducts: ≤ 1.00 μmol/mol DNA) featured longer median overall survival time (MST) [69.00 (55.41–82.59) months] and median tumor reoccurrence-free survival time (MRT) [70.00 (44.93–95.07) months] compared with those with positive AFB1 status [20.00 (13.04–26.96) months for MST and 13.00 (9.54–16.46) months for MRT, respectively].

3.3. The effects of CBX4 expression on the clinicopathological features and the prognosis of HCC cases

In the present, the levels of CBX4 protein in the cancerous tissues were amounted using immunohistochemistry technique with IRS counting system and the median IRS value was 5.58 for

Variables	AFB1 (−)		AFB1 (+)		OR (95% CI)	P_{trend}
	n	%	n	%		
Total	244	100.0	184	100.0	—	—
Age (years)						
≤ 48	148	60.7	86	46.7	Reference	
> 48	96	39.3	98	53.3	1.80 (1.22–2.66)	3.07×10^{-3}
Sex						
Man	160	65.6	130	70.7	Reference	
Female	84	34.4	54	29.3	1.13 (0.59–2.13)	0.72
Ethnicity						
Han	124	50.8	105	57.1	Reference	
Zhuang	120	49.2	79	42.9	0.99 (0.55–1.78)	0.98
HBsAg						
Negative	65	26.6	48	26.1	Reference	
Positive	179	73.4	136	73.9	1.19 (0.60–2.34)	0.61
anti-HCV						
Negative	217	88.9	161	87.5	Reference	
Positive	27	11.1	23	12.5	1.25 (0.51–3.09)	0.62
Smoking status						
No	181	74.2	134	72.8	Reference	
Yes	63	25.8	50	27.2	0.48 (0.12–1.85)	0.28
Drinking status						
No	174	71.3	130	70.7	Reference	
Yes	70	28.7	54	29.3	2.61 (0.69–9.89)	0.27
AFP (ng/mL)						
≤ 20	82	33.6	72	39.1	Reference	
> 20	162	66.4	112	60.9	1.00 (0.55–1.82)	0.99
Liver cirrhosis						
No	58	23.8	46	25.0	Reference	
Yes	186	76.2	138	75.0	0.84 (0.42–1.69)	0.63
BCLC stage						
A	113	46.3	54	29.3	Reference	
B	69	28.3	52	28.3	1.27 (0.61–2.61)	0.52
C	62	25.4	78	42.4	2.09 (1.04–4.24)	0.04
Tumor size						
≤ 3 cm	197	80.7	14	7.6	Reference	

Variables	AFB1 (−)		AFB1 (+)		OR (95% CI)	P_{trend}
	n	%	n	%		
> 3 cm	47	19.3	170	92.4	69.06 (33.62–141.86)	9.36×10^{-31}
MVD						
Negative	118	48.4	74	40.2	Reference	
Positive	126	51.6	110	59.8	2.56 (1.36–4.81)	3.46×10^{-3}
ES grade						
Low	129	52.9	97	52.7	Reference	
High	115	47.1	87	47.3	1.52 (0.64–2.07)	0.64

Abbreviations: AFP, α-fetoprotein; BCLC, the Barcelona Clinic Liver Cancer staging system; ES, Edmondson and Steiner grading system; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HCV, hepatitis C virus; MVD, microvessel density.

Table 2. The association between AFB1 exposure and clinic-pathological features of hepatocellular carcinoma cases.

all cases with hepatocarcinoma. According to the results from the CBX4 expression in cancerous tissues based on a large sample, IRS > 4 was regarded as positive CBX4 status. **Table 3** summarized the association between CBX4 expression in the cancerous tissues and the clinicopathological features, and results from multivariable logistic regression models proved that the levels of CBX4 expression were significantly related to increasing risk of liver cirrhosis (OR = 1.75 and 95% CI = 1.07–2.88), higher tumor stage (OR = 2.02 and 95% CI = 1.23–3.33), and increasing MVD (OR = 2.66 and 95% CI = 1.74–4.07). However, CBX4 expression levels did not affect other clinicopathological features such as tumor size, grade, AFP, and so on.

Results from Kaplan–Meier survival analyses further displayed that HCC patients with positive status of CBX4 protein expression had short MST [22.00 (18.00–26.00) months] and MRT [16.00 (10.88–21.12) months] compared with those with negative-status CBX4 protein [69.00 (52.75–85.25) months for MST and 48.00 (23.69–72.31) months for MRT, respectively] (**Figure 1B**). Taken together, CBX4 expression in the cancerous might be an important biomarker for HCC prognosis.

3.4. The joint effects of AFB1 exposure and CBX4 expression on HCC prognosis

Given that both AFB1 exposure and CBX4 expression modified HCC outcome, we questioned whether CBX4 expression interacted with AFB1 expression, and whether this interaction affected the prognosis of hepatocarcinoma. First, we analyzed the joint effects of AFB1 exposure and CBX4 expression on the prognosis of patients with HCC using Kaplan–Meier survival model (**Figure 2**). In this model, the combination of AFB1 exposure and CBX4 expression was divided into four groups: cases with negative-AFB1 and negative-CBX4 status (AC-1), cases with negative-AFB1 and positive-CBX4 status (AC-2), cases with positive-AFB1 and negative-CBX4 status (AC-3), and cases with positive-AFB1 and positive-CBX4 status (AC-4). We found MST and MRT gradually decreased from AC-1 to AC-4 (89.00–11.00 months for MST and more than 125.00–7.00 months for MRT, respectively) (**Figure 2A** and **B**).
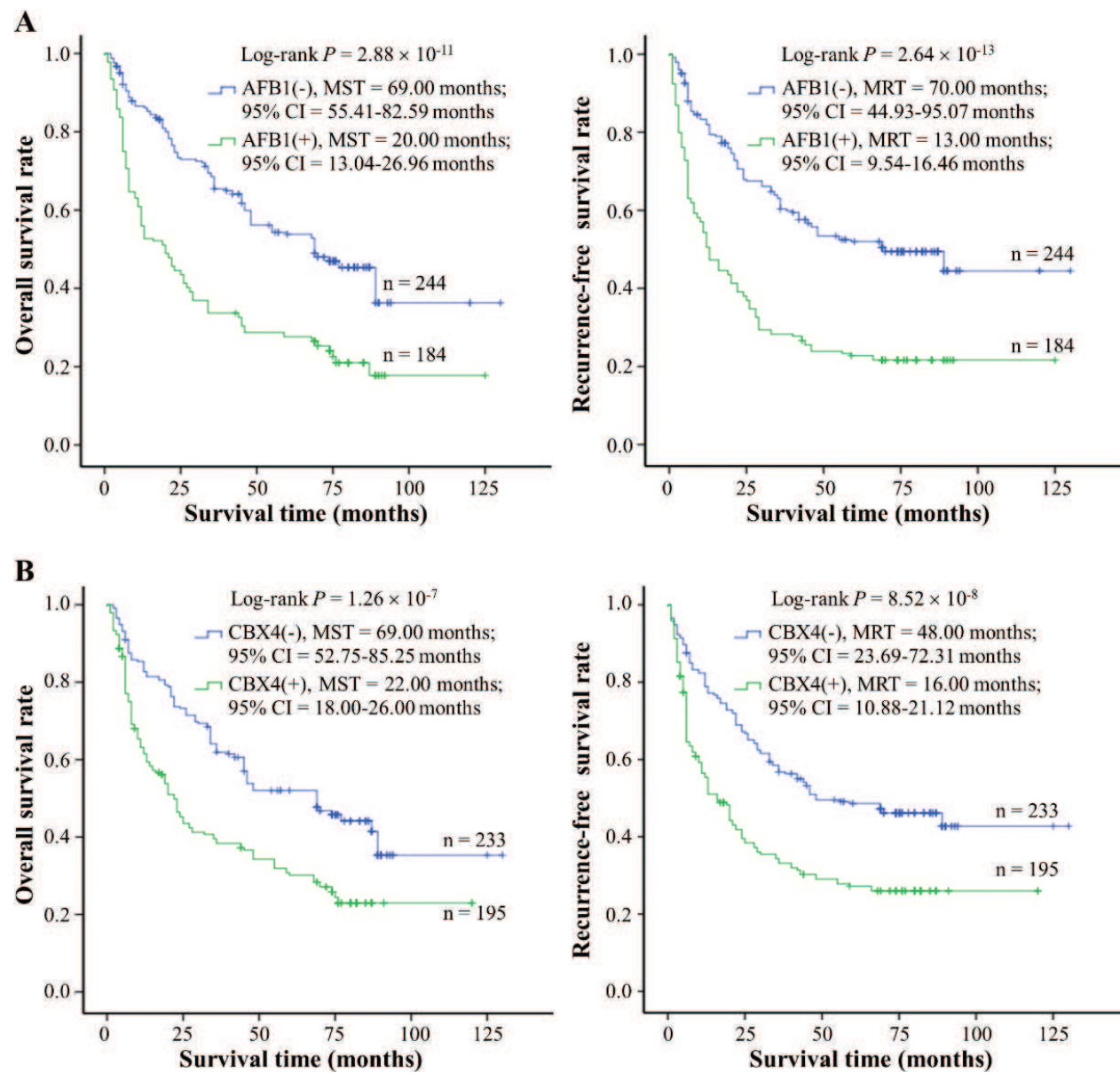
Figure 1. Both AFB1 exposure and CBX4 expression significantly correlating with hepatocellular carcinoma. AFB1 exposure levels were elucidated using the amount of AFB1-DNA adducts in the cancerous tissues. The CBX4 expression in cancerous tissues from 428 patients with hepatocellular carcinoma was tested using immunohistochemistry technique based on immunoreactive score system (IRS). To analyze, the levels of CBX4 expression were divided into two groups: Negative group (IRS ≤ 4) and positive group (IRS > 4). AFB1 exposure (A) and CBX4 expression (B) are associated with overall survival (left) and tumor recurrence-free survival (right) of hepatocellular carcinoma. Cumulative hazard function was plotted by Kaplan–Meier's methodology, and P value was calculated with two-sided log-rank tests. *Abbreviations:* CBX4, chromobox 4; MST, median overall survival time; MRT, median tumor recurrence-free survival time; CI, confidence interval.

We next finished multivariable Cox regression analyses based on the retread method with likelihood ratio test (including significant variables and all kinds of possible interactive variables) (**Table 4**), and found both AFB1 exposure and CBX4 expression in the cancerous tissues were independent prognostic factors. Furthermore, we also observed that AFB1 exposure significantly and multiplicatively interacted with CBX4 protein expression (interactive values, 1.98 for overall survival and 1.94 for tumor reoccurrence-free survival, respectively).

	CBX4 (−)		CBX4 (+)			
Variables	n	%	n	%	OR (95% CI)	P_{trend}
Total	233	100.0	195	100.0	—	—
Age (years)						
≤ 48	136	58.4	98	50.3	Reference	
> 48	97	41.6	97	49.7	1.35 (0.89–2.05)	0.67
Sex						
Man	160	68.7	130	66.7	Reference	
Female	73	31.3	65	33.3	1.14 (0.73–1.78)	0.57
Ethnicity						
Han	121	51.9	108	55.4	Reference	
Zhuang	112	48.1	87	44.6	0.96 (0.63–1.46)	0.85
HBsAg						
Negative	65	27.9	48	24.6	Reference	
Positive	168	72.1	148	75.9	1.17 (0.73–1.89)	0.51
anti-HCV						
Negative	209	89.7	169	86.7	Reference	
Positive	24	10.3	26	13.3	1.43 (0.74–2.75)	0.29
Smoking status						
No	176	75.5	139	71.3	Reference	
Yes	57	24.5	56	28.7	1.04 (0.37–2.93)	0.94
Drinking status						
No	172	73.8	132	67.7	Reference	
Yes	61	26.2	63	32.3	1.40 (0.51–3.83)	0.51
AFP (ng/mL)						
≤ 20	84	36.1	70	35.9	Reference	
> 20	149	63.9	125	64.1	1.11 (0.72–1.70)	0.64
Liver cirrhosis						
No	69	29.6	35	17.9	Reference	
Yes	164	70.4	160	82.1	1.75 (1.07–2.88)	0.03
BCLC stage						
A	112	48.1	55	28.2	Reference	
B	58	24.9	63	32.3	1.94 (1.16–3.24)	0.01
C	63	27.0	77	39.5	2.02 (1.23–3.33)	5.79×10^{-3}
Tumor size						
≤ 3 cm	121	51.9	90	46.2	Reference	
> 3 cm	112	48.1	105	53.8	1.24 (0.81–1.88)	0.33

	CBX4 (–)		CBX4 (+)			
MVD						
Negative	131	56.2	61	31.3	Reference	
Positive	102	43.8	134	68.7	2.66 (1.74–4.07)	6.65×10^{-6}
ES grade						
Low	132	56.7	94	48.2	Reference	
High	101	43.3	101	51.8	1.39 (0.92–2.11)	0.12

Abbreviations: AFP, α-fetoprotein; BCLC, the Barcelona Clinic Liver Cancer staging system; CBX4, chromobox 4; ES, Edmondson and Steiner grading system; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HCV, hepatitis C virus; MVD, microvessel density.

Table 3. The correlation between CBX4 expression and clinical pathological features of hepatocellular carcinoma.
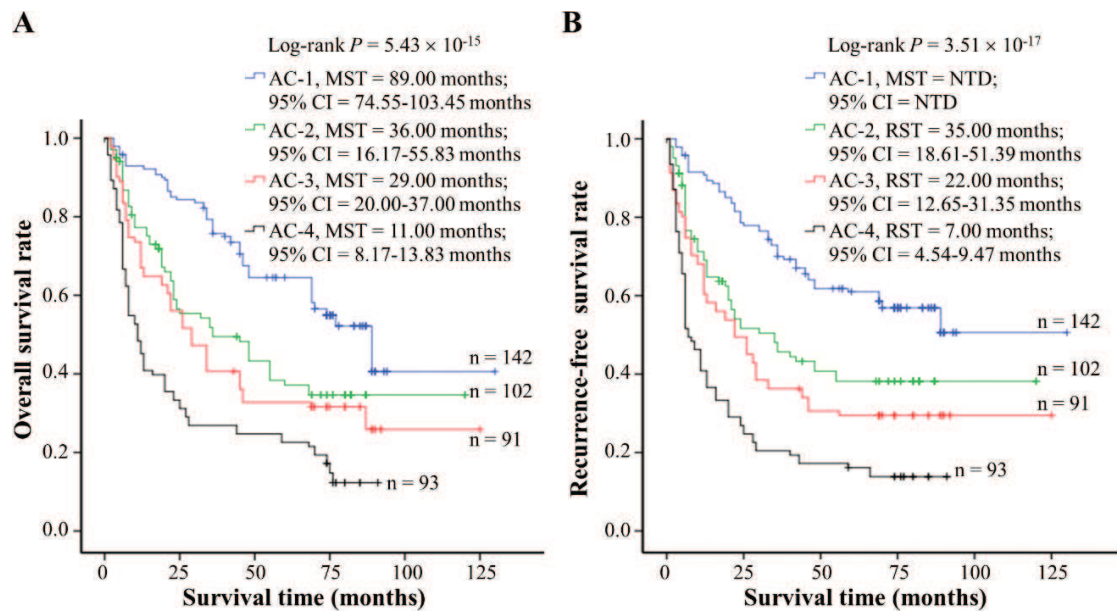
Figure 2. Survival analysis of CBX4 expression binding AFB1 exposure levels. The combination of CBX4 expression and AFB1 exposure was divided into 4 strata: Cases with negative-AFB1 and negative-CBX4 status (AC-1), cases with negative-AFB1 and positive-CBX4 status (AC-2), cases with positive-AFB1 and negative-CBX4 status (AC-3), and cases with positive-AFB1 and positive-CBX4 status (AC-4). This kind of joint analyses showed that interactive effects on the overall survival (A) and tumor recurrence-free survival (B) of patients with hepatocarcinoma. Cumulative hazard function was plotted by Kaplan–Meier's methodology, and *P* value was calculated with two-sided log-rank tests. *Abbreviations:* CBX4, chromobox 4; MST, median overall survival time; MRT, median tumor recurrence-free survival time; CI, confidence interval.

4. Discussion

In Guangxi Zhuang Autonomous Region, HCC is the most malignant disease. In the past decades, the annual incidence and death rate (AIR and ADR) of hepatocarcinoma in this area has been reported to remarkably increase (up to about 100–200 per 10,000 for AIR about 50 per 10,000 for ADR) [1]. Lots of epidemiological studies have shown that AFB1 exposure is

	OS		RFS	
Variables	HR (95%CI)	Ptrend	HR (95%CI)	Ptrend
AFB1	2.09 (1.64–2.65)	2.34×10^{-9}	2.29 (1.79–2.93)	3.82×10^{-11}
CBX4	1.76 (1.38–2.24)	4.66×10^{-6}	1.80 (1.41–2.30)	3.12×10^{-6}
AFB1 × CBX4	1.98 (1.61–2.59)	9.43×10^{-7}	1.94 (1.58–2.54)	8.17×10^{-5}

HR and corresponding 95% CI was calculated using multivariable Cox regression model (with retread method based on likelihood ratio test). *Abbreviations:* AFB1, aflatoxin B1; CBX4, chromobox 4; OS, overall survival; RFS, tumor reoccurrence-free survival; HR, hazard ratio; CI, confidence interval.

Table 4. The effects of AFB1 and CBX4 expression on the prognosis of cases with hepatocellular carcinoma.

the most important cause for this high AIR and ADR [1]. AFB1 is a known I-type chemical carcinogen produced by *Aspergillus parasiticus* and *Aspergillus flavus*, and has been proved to involve in the carcinogenesis and progression of HCC [4–10]. This carcinogenicity of AFB1 mainly results from its metabolic product binding to DNA and inducing DNA damage. Among DNA damage types induced by AFB1, AFB1-DNA adducts are very important, because of its non-enzymatic, time-dependent, and apparent persistent characteristics in the genomic DNA strands [3, 35]. Our previous studies have exhibited that AFB1-DNA adducts, especially from liver tissues, are highly associated not only with increasing HCC risk, but with the poor prognosis of HCC [2, 31, 36–39]. Here, our data displayed that increasing levels of AFB1 exposure significantly correlated with higher tumor stage, increasing tumor size, and higher MVD; furthermore, AFB1 was also poor prognostic marker for HCC. Taken together, these data suggest that AFB1 may involve in the startup and progression of HCC.

Because several previous studies have exhibited that CBX4 can progress tumorigenesis via several signal pathways, including CBX4/HIF-1α/VEGF pathway [20, 25, 26, 34], CBX4/ HDAC3/Runx2 pathway [21], CBX4/P63 pathway [22], CBX4/miR-195 pathway [40], CBX4/ CtIP pathway [41], and CBX4/P53 pathway [42, 43], here we investigated the effects of CXB4 expression on HCC outcome. We not only found that increasing CBX4 expression in the cancerous tissues is a poor prognostic biomarker for HCC, but this increasing expression is associated with clinicopathological features such as tumor size, tumor stage, and angiogenesis. Supporting our findings, several recent reports further prove that CBX4 can govern the several biofunctions of HCC, including proliferation, invasion and metastasis, angiogenesis, and metastasis [20, 25, 26, 34, 40, 44].

Noticeably, some evidence of the joint effects of CBX4 and AFB1 on HCC outcome was observed in the prognostic analyses based on the gene-environmental joint effects. Our results showed that CBX4 expression significantly and multiplicatively interacted with AFB1 exposure levels, and that this multiplicative interaction remarkably increased the death risk and tumor reoccurrence risk of patients with HCC. Recently, two studies from high AFB1 exposure areas have also reported that the dysregulation of CBX4 in the cancerous tissues from patients with hepatocarcinoma increases MVD, promotes angiogenesis, and increases sensitivity of HCC cells on anti-cancer drugs [33, 34]. Altogether, these results are indicative of the angiogenesis induced by CBX4 involving in the progression of AFB1-related HCC.

In summary, our present study proposes that CBX4 expression in the cancerous tissues can act as a valuable biomarker for AFB1-related HCC. However, several limitations confine the value of this study. First, because of the hospital-based retrospective design, selective bias may take place. Second, because liver damage itself affects AFB1 metabolite and may increase the amount of AFB1-DNA adducts, the prognostic and interactive values of AFB1 and CBX4 may be underestimated. Finally, we did not do functional and mechanical analyses. Therefore, detailed functional analyses deserve further evaluation on the basis of the foresighted design and the combination of AFB1 and CBX4.

Conflicts of interest and source of funding

Abbreviations

AFB1	aflatoxin B1
AFBEX	AFB1–8,9-exo-epoxide
AFBEN	AFB1–8,9-endo-epoxide
BCLC	The Barcelona Clinic Liver Cancer
CBX4	chromobox 4
CI	confidence interval
CYP	cytochrome P450
ES	The Edmondson and Steiner
HBV	hepatitis B virus
HCC	hepatocellular carcinoma
HCV	hepatitis C virus

HBsAg	hepatitis B surface antigen
IRS	immunoreactive score system
MRT	median tumor reoccurrence-free survival time
MST	median overall survival time
MVD	microvessel density
OD	odd ratio.

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