We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,300

Open access books available

172,000

International authors and editors

190M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

The Blood AFB1-DNA Adduct Acting as a Biomarker for Predicting the Risk and Prognosis of Primary Hepatocellular Carcinoma

Qin-Qin Long, Xiao-Qin Wu and Jin-Guang Yao

Abstract

Aflatoxin B1 (AFB1) is an important carcinogen for primary hepatocellular carcinoma (PHCC). However, the values of blood AFB1-DNA adducts predicting HCC risk and prognosis have not still been clear. We conducted a hospital-based case-control study, consisting of 380 patients with pathologically diagnosed PHCC and 588 controls without any evidence of liver diseases, to elucidate the associations between the amount of AFB1-DNA adducts in the peripheral blood and the risk and outcome of HCC. All subjects had not the history of hepatitis B and C virus infection. AFB1-DNA adducts were tested using enzyme-linked immunosorbent assay. Cases with PHCC featured an increasing blood amount of AFB1-DNA adducts compared with controls (2.01 ± 0.71 vs. 0.98 ± 0.63 $\mu\text{mol/DNA}$). Increasing adduct amount significantly grew the risk of PHCC [risk values, 1.82 (1.34–2.48) and 3.82 (2.71–5.40) for medium and high adduct level, respectively]. Furthermore, compared with patients with low adduct level, these with medium or high adduct level faced a higher death and tumor-recurrence risk. These results suggest that the blood AFB1-DNA adducts may act as a potential biomarker for predicting the risk and prognosis of PHCC.

Keywords: AFB1, DNA adduct, primary hepatocellular carcinoma, biomarker, risk, prognosis

1. Introduction

Aflatoxin B1 (AFB1) is a knowledge I-type chemical carcinogen for primary hepatocellular carcinoma (PHCC) [1–3]. This carcinogen is mainly produced by *Aspergillus parasiticus* and *Aspergillus flavus* and often found in crops and food (including maize, nuts, and beans), which are raised in the areas with humid and hot environment [1, 4, 5]. Once these AFB1-contaminated crops and food are ingested by human bodies, AFB1 will be metabolized through two stage reactions consisting of detoxification stage (such as reduction, oxidation, and hydrolytic reaction) and covalent stage (such as binding reaction and

conjugating reaction) [2, 3]. During the process of AFB1's metabolism, AFB1-DNA adducts, including AFB1-formamidopyrimidine adduct (AFB1-FAPa) and AFB1's 8,9-dihydro-8-(N⁷-guanyl)-9-hydroxy-adduct (AFB1-GA), are frequently formed [2, 3]. Growing evidence has shown that AFB1-DNA adducts are usually tested in the tissue samples (such as liver and placenta tissues) of these individuals from high AFB1 exposure areas [6–10]. Recent studies have displayed that they are also found in the peripheral blood white cells of peoples who are from high AFB1 exposure areas and are associated with the time of AFB1 exposure [11–17]. However, the potential of blood AFB1-DNA adducts predicting PHCC risk and prognosis is not clear. Here, we specifically conducted a hospital-based case-control study to investigate whether blood AFB1-DNA adducts were related to the risk and outcome of PHCC.

2. Materials and methods

2.1 Study population

A total of 380 patients were recruited from the affiliated hospitals of Youjiang Medical University for Nationalities and Guangxi Medical University (two main medical universities in the AFB1-exposure areas in China) between 2011 and 2013. All cases were newly diagnosed as patients using histopathological method and they had no history of radiation or chemotherapy treatment before enrollment. A total of 588 controls, who were randomly recruited from a pool of healthy individuals in the same hospitals during the same time, were all volunteers without any evidence with liver diseases. To control the effects of confounder factors such as age, gender, and race, controls were individually matched with the cases on these factors. In this study, all cases and controls had no history of hepatitis B virus (HBV) and/ hepatitis C virus (HCV) infection, whereas these subjects with positive status of serum anti-HCV and/or hepatitis B surface antigen (HBsAg) were excluded. They all agreed to participate in this investigation and did not drop out. With informed consent, all clinicopathological data, including age, gender, race, hepatitis virus B and C infection information, survival follow-up information, were collected using healthy examination or medical records. Additionally, 10 ml of peripheral blood samples for all subjects were also collected for AFB1-DNA adduct analysis. In this study, the last following-up date was set on January 31, 2019. Overall survival (OS) and tumor recurrence-free survival (RFS) status were defined according to the previously described methods [11, 14, 18]. The study protocol was approved by the ethics committees of Youjiang Medical University for Nationalities and Guangxi Medical University.

2.2 AFB1-DNA adducts data

The amount of AFB1-DNA adducts in the peripheral blood were tested using the previously published methods [8, 17]. Briefly, DNA samples were first extracted from the peripheral blood samples and adducts were next quantitated by the comparative enzyme-linked immunosorbent assay (ELISA). To investigate the association between different levels of AFB1-DNA adducts and the risk and prognosis of PHCC patients, the levels of AFB1-DNA adducts were divided into three subgroups according to the mean adduct amounts of cases and controls: low AFB1-DNA adduct level (LAL, <1.00 $\mu\text{mol/DNA}$), medium AFB1-DNA adduct level (MAL, 1.00–2.00 $\mu\text{mol/DNA}$), and high AFB1-DNA adduct level (HAL, >2.00 $\mu\text{mol/DNA}$).

2.3 Statistical analysis

All statistical analyses were accomplished with SPSS statistical package (Version 18, SPSS Institute, Chicago, IL, USA). Test for the distribution of age, gender, and race between patients with PHCC and controls was accomplished using chi-square test. The effects of blood AFB1-DNA adducts on PHCC risk were evaluated using odds ratio (OR) and 95% confidence interval (CI) in the conditional logistic regression model. For survival analyses, Kaplan-Meier survival model with *Log-Rank* test and Cox regression model (the selection of significant variates based on forward-step method with likelihood ratio test) was used to analyze the association between blood AFB1-DNA adducts and PHCC outcomes. Cumulative hazard value for the effects of adducts on the prognosis of patients with PHCC and corresponding 95% CI was calculated using hazard ratio (HR) from significant multivariate Cox regression model (including all significant variates). In this study, the *P* value <0.05 was defined as statistical significance.

3. Results

3.1 The features of study population

A total of 380 cases with PHCC and 588 controls were included in our final analyses. Baseline characteristics of all cases with PHCC and controls were summarized in **Table 1**, and results showed there were no significant distributions of age, gender, and race between cases and controls.

	Controls		PHCCs		<i>P</i>
	n	%	n	%	
Total	588	100.0	380	100.0	—
Gender					0.70
Male	426	72.4	271	71.3	
Female	162	27.6	109	28.7	
Age (years)					0.78
≤35	81	13.8	53	13.9	
36–40	62	10.5	38	10.0	
41–45	90	15.3	55	14.5	
46–50	83	14.1	43	11.3	
51–55	94	16.0	56	14.7	
56–60	56	9.5	42	11.1	
61–65	69	11.7	49	12.9	
≥66	53	9.0	44	11.6	
Race					0.88
Han	339	57.7	221	58.2	
Zhuang	249	42.3	159	41.8	

PHCCs, patients with primary hepatocellular carcinoma.

Table 1.
The characteristics of subjects.

AFB1-DNA adduct levels	Controls		PHCCs		OR (95% CI) ^a	P
	n	%	n	%		
Low	316	53.7	122	32.1	1	—
Medium	186	31.6	131	34.5	1.82 (1.34–2.48)	1.20 × 10 ^{−4}
High	86	14.6	127	33.4	3.82 (2.71–5.40)	2.35 × 10 ^{−14}

^aOR conditional on matched set.
AFB1, aflatoxin B1; PHCC, primary hepatocellular carcinoma.

Table 2.
Associations between AFB1-DNA adduct levels and PHCC risk.

3.2 Blood AFB1-DNA adducts correlating with PHCC risk

The amount of AFB1-DNA adducts in the peripheral white blood cells were calculated using ELISA technique. Compared to controls, patients with PHCC featured a higher level of blood AFB1-DNA adducts (0.98 ± 0.63 vs. 2.01 ± 0.71 $\mu\text{mol/DNA}$), suggesting blood AFB1-DNA adducts may play an important role in the PHCC carcinogenesis. To investigate possible correlation between AFB1-DNA adducts and PHCC risk, the levels of blood AFB1-DNA adducts were divided into three groups. Results from multivariable logistic regression analyses showed that these individuals with medium AFB1-DNA adduct level (MAL) had an increasing risk of PHCC compared to those with low AFB1-DNA adduct level (LAL) (OR = 1.82 and 95% CI = 1.34–2.48), whereas risk value for high AFB1-DNA adduct level (HAL) was 3.82 (2.71–5.40) (Table 2). Altogether, these data were indicative of important potential risk role of blood AFB1-DNA adducts in the carcinogenesis of PHCC.

3.3 Blood AFB1-DNA adducts correlating with PHCC outcome

To explore the effects of blood AFB1-DNA adducts on the prognosis of patients with PHCC, we accomplished two survival model analyses. Kaplan-Meier’s

Figure 1.
The aflatoxin B1 (AFB1)-DNA adducts in peripheral blood white cells significantly correlating with the overall survival (OS) and tumor recurrence-free survival (RFS) of primary hepatocellular carcinoma (PHCC). Cumulative hazard function was plotted by Kaplan-Meier’s 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. LAL, low AFB1-DNA adduct level; MAL, medium low AFB1-DNA adduct level; HAL, high low AFB1-DNA adduct level.

survival analyses first tested the association between blood AFB₁-DNA adducts and patients' OS and results displayed that increasing level of adducts significantly shorten the OS time of patients ($P = 1.33 \times 10^{-5}$) (**Figure 1**, left). Similar effects were also found in the RFS analyses ($P = 2.88 \times 10^{-7}$) (**Figure 1**, right). Results from multivariate Cox's regression models further exhibited that these cases with an increasing level of blood AFB₁-DNA adducts faced an increasing risk of death [HRs (95% CIs) = 1.44 (1.11–1.86) for MAL and 1.93 (1.47–2.54) for HAL, respectively] (**Figure 1**, left). For RFS, the corresponding tumor-recurrence risk was 1.49 (1.18–1.89) for MAL and 2.98 (1.93–4.60) for HAL, respectively (**Figure 1**, right).

4. Discussion

In this study, we explored the relationship between the blood AFB₁-DNA adducts and the risk and prognosis of PPHCC. We found that individuals with an increasing level of AFB₁-DNA adducts in peripheral blood white cells would feature higher PHCC risk (OR = 1.82 for MAL and 3.82 for HAL, respectively). Furthermore, the blood AFB₁-DNA adduct levels were significantly associated with poor OS and RFS of patients with PHCC.

AFB₁ acts as a major cause of PHCC in the southeast areas of China and is taken into human bodies through its contaminating staple foods [2]. AFB₁ is transferred into AFB₁-DNA adducts and displays its genic toxicity and hepato carcinogenicity [3, 19]. Mechanically, PHCC induced by AFB₁ is mainly concerned with DNA damage (including DNA single-/double-strand breaks, base damage, adduct formation, genic mutation), the dysregulation of DNA repair, the activation of cancer genes (such as ras and myc), the inactivation of cancer suppressor genes (such as TP53, BP1, H2AX, bcl2, p21, and p27), inheritance alterations, and/or abnormal immunoreaction [1, 20–25]. Among these knowledge mechanisms and pathways, AFB₁-DNA adducts and mutations at codon 249 of TP53 gene (also termed as hot-spot mutation induced by AFB₁) have been especially concerned in the past decades [26–29]. This is mainly because AFB₁-DNA adducts are the key central forms in the metabolism of AFB₁ in human bodies [19, 26, 30], whereas spot mutations at codon 249 of TP53 gene are highly frequent in HCC patients with AFB₁ exposure [31–36]. Evidence from clinical epidemiology and experimental animal models has exhibited that they are constantly tested in biopsy samples, such as liver tissues, tumor tissues, placenta tissues, and blood cells, of individuals from AFB₁ exposure areas [6, 8, 11, 12, 15–17].

For example, Hsieh and Hsieh [8] examined the amounts of AFB₁-DNA adducts in the 120 placenta tissue samples from women in Taipei, a high AFB₁ exposure area, and observed that 57.5% (69/120) of samples were positive AFB₁-DNA adducts with the range of 0.6 and 6.3 $\mu\text{mol/mol}$ DNA. Furthermore, they found higher amount of AFB₁-DNA adducts in samples collected in the summer than in the winter. Shirabe et al. [37] investigated the association between AFB₁-DNA adducts in hepatocyte nuclei and TP53 mutation in PHCC among Japanese population. They found that 6% (118/279) patients with PHCC and 16% (13/83) patients with HBV- and HCV-negative PHCC were positive for AFB₁-DNA adducts. Higher hot-spot mutations in the TP53 gene were also found in these with positive AFB₁-DNA adduct status [37]. A relatively large-size sample clinical study, including 501 PHCC cases with different AFB₁ exposure, also shows that positive status of AFB₁-DNA adducts in the tumor tissues significantly increases the risk of TP53 mutations (OR = 3.38 and 95% CI = 2.23–5.11) [7].

Following epidemiological studies on based clinical samples further prove that the amount of AFB1-DNA adducts is higher in the tumor tissues than in the peritumor tissues [6]. This increasing tissular AFB1-DNA adducts are significantly associated with poor OS and RFS of patients with PHCC [6].

In this study, we designed and finished a hospital-based case-control study in the southwestern of Guangxi, a knowledge-high AFB1 exposure area. Our data exhibited that increasing the amount of AFB1-DNA adducts in peripheral white blood cells not only increased PHCC risk, but also modified the OS and RFS of patients with PHCC. Supporting our findings through several studies from high AFB1 exposure areas, the amount of blood AFB1-DNA adducts can reflect the levels of AFB1 exposure information and may be related to PHCC risk and prognosis [11, 12, 14, 15, 17, 38]. Taken together, these results suggest that AFB1-DNA adducts in the blood as well as in the tumor tissues may be potential biomarkers for PHCC risk and outcome.

This study has several strengthens. We accomplished the predictive value analyses using these individuals only with AFB1 exposure but without HBV or HCV. This is done mainly because both HBV and HCV infection will alter effects of AFB1-DNA adducts predicting the risk and outcome of PHCC. Additionally, to control potential confounders such as age, gender, and race, the individually matched design was finished in this study. Therefore, our study may represent a relatively more actual predictive role of blood AFB1-DNA adducts.

To conclude, this study explored the association between blood AFB1-DNA adducts and the risk and prognosis of PHCC using a retrospective clinic-sample research approach and displayed that blood AFB1-DNA adduct may be a potential biomarker for HCC risk and outcome. Several limitations should be focused for our study. First, relatively small-size samples may underestimate the effects of blood AFB1-DNA adducts on PHCC risk and outcome. Second, selective bias may happen because of this hospital-based retrospective investigative design. Finally, the mechanical analyses for AFB1-DNA adducts predicting PHCC risk and prognosis were not finished. Thus, the blood AFB1-DNA adducts may be valuable biomarkers for predicting the risk and prognosis of PHCC once the present findings were proved by larger clinic samples and functional analyses.

Acknowledgements

We thank Dr Yuan-Feng Zhou for sample collection and management.

Conflict of interests and source of funding

The authors declare no competing financial interests. This study was supported in part by the National Natural Science Foundation of China (Nos. 81860489, 81760502, 81572353, and 81660495), the Natural Science Foundation of Guangxi (Nos. 2018GXNSFAA281043, 2017GXNSFAA198002, and 2017GXNSFGA198002), Research Program of Guangxi “Zhouyue Scholar” (No. 2017-38), Research Program of Guangxi Specially invited Expert (No. 2017-6th), the “12th Five” Planning Program of Guangxi Education Science (No. 2015C397), the Innovative Program of Guangxi Graduate Education (No. JGY2015139), Research Program of Guangxi Clinic Research Center of Hepatobiliary Diseases (No. AD17129025), and Open Research Program from Molecular Immunity Study Room Involving in Acute and Severe Diseases in Guangxi Colleges and Universities (Nos. kfkt20160062 and kfkt20160063).

Abbreviations

AFB1	Aflatoxin B1
AFB1-FAPa	AFB1-formamidopyrimidine adduct
AFB1-GA	AFB1's 8,9-dihydro-8-(N ⁷ -guanyl)-9-hydroxy-adduct
HAL	high AFB1-DNA adduct level
HBV	hepatitis B virus
HBsAg	hepatitis B surface antigen
HCV	hepatitis C virus
CI	confidence interval
HR	hazard ratio
LAL	low AFB1-DNA adduct level
MAL	medium AFB1-DNA adduct level
OR	odds ratio
PHCC	primary hepatocellular carcinoma
OS	overall survival
RFS	tumor recurrence-free survival

Author details

Qin-Qin Long[†], Xiao-Qin Wu[†] and Jin-Guang Yao*
Department of Pathology, The Affiliated Hospital of Youjiang Medical University
for Nationalities, Baise, China

*Address all correspondence to: yyyaojg001@163.com

[†] These authors contributed equally to this work.

IntechOpen

© 2019 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/3.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

References

- [1] Long XD, Deng Y, Huang XY, Yao JG, Su QY, Wu XM, et al. Molecular mechanisms of hepatocellular carcinoma related to aflatoxins: An update. In: Rodrigo L, editor. *Liver Research and Clinical Management*. Vol. 1.1. Rijeka: InTech; 2018. pp. 113-136. DOI: 10.5772/intechopen.72883
- [2] Kew MC. Aflatoxins as a cause of hepatocellular carcinoma. *Journal of Gastrointestinal and Liver Diseases*. 2013;**22**:305-310. DOI: PMID.24078988
- [3] Kensler TW, Roebuck BD, Wogan GN, Groopman JD. Aflatoxin: A 50-year odyssey of mechanistic and translational toxicology. *Toxicological Sciences*. 2011;**120**(Suppl 1):S28-S48. DOI: 10.1093/toxsci/kfq283
- [4] Wu XM, Xi ZF, Lu J, Wang XZ, Zhang TQ, Huang XY, et al. Genetic single nucleotide polymorphisms (GSNPs) in the DNA repair genes and hepatocellular carcinoma related to aflatoxin B1 among Guangxi population. In: Parine NR, editor. *Genetic Polymorphisms*. Vol. 1. Rijeka: InTech; 2017. pp. 97-119. DOI: 10.5772/intechopen.69530
- [5] Long XD, Yao JD, Yang Q, Huang CH, Liao P, Nong LG, et al. Polymorphisms of DNA repair genes and toxicological effects of aflatoxin B1 exposure. In: Faulkner AG, editor. *Aflatoxins: Food Sources, Occurrence and Toxicological Effects*. 1st ed. Nova Science Publishers: New York; 2014. pp. 125-156. DOI: 978-1-63117-298-4
- [6] Liu YX, Long XD, Xi ZF, Ma Y, Huang XY, Yao JG, et al. MicroRNA-24 modulates aflatoxin B1-related hepatocellular carcinoma prognosis and tumorigenesis. *BioMed Research International*. 2014;**2014**:482926. DOI: 10.1155/2014/482926
- [7] Long XD, Ma Y, Huang HD, Yao JG, Qu de Y, Lu YL. Polymorphism of XRCC1 and the frequency of mutation in codon 249 of the p53 gene in hepatocellular carcinoma among Guangxi population, China. *Molecular Carcinogenesis*. 2008;**47**:295-300. DOI: 10.1002/mc.20384
- [8] Hsieh LL, Hsieh TT. Detection of aflatoxin B1-DNA adducts in human placenta and cord blood. *Cancer Research*. 1993;**53**:1278-1280. DOI: PMID.8383006
- [9] Tulayakul P, Dong KS, Li JY, Manabe N, Kumagai S. The effect of feeding piglets with the diet containing green tea extracts or coumarin on in vitro metabolism of aflatoxin B1 by their tissues. *Toxicon*. 2007;**50**:339-348. DOI: 10.1016/j.toxicon.2007.04.005
- [10] Zhang YJ, Chen S, Tsai WY, Ahsan H, Lunn RM, Wang L, et al. Expression of cytochrome P450 1A1/2 and 3A4 in liver tissues of hepatocellular carcinoma cases and controls from Taiwan and their relationship to hepatitis B virus and aflatoxin B1-and 4-aminobiphenyl-DNA adducts. *Biomarkers*. 2000;**5**:295-306. DOI: 10.1080/135475000413845
- [11] Long XD, Zhao D, Wang C, Huang XY, Yao JG, Ma Y, et al. Genetic polymorphisms in DNA repair genes XRCC4 and XRCC5 and aflatoxin B1-related hepatocellular carcinoma. *Epidemiology*. 2013;**24**:671-681. DOI: 10.1097/EDE.0b013e31829d2744
- [12] Long XD, Yao JG, Huang YZ, Huang XY, Ban FZ, Yao LM, et al. DNA repair gene XRCC7 polymorphisms (rs#7003908 and rs#10109984) and hepatocellular carcinoma related to AFB1 exposure among Guangxi population, China. *Hepatology Research*. 2011;**41**:1085-1093. DOI: 10.1111/j.1872-034X.2011.00866.x
- [13] Wu XM, Ma Y, Deng ZL, Long XD. The polymorphism at codon

939 of xeroderma pigmentosum C gene and hepatocellular carcinoma among Guangxi population. *Zhonghua Xiaohua Neijing Zazhi*. 2010;**30**:846-848. DOI: 10.3760/cma.j.issn.0254-1432.2010.11.018

[14] Long XD, Ma Y, Zhou YF, Ma AM, Fu GH. Polymorphism in xeroderma pigmentosum complementation group C codon 939 and aflatoxin B₁-related hepatocellular carcinoma in the Guangxi population. *Hepatology*. 2010;**52**:1301-1309. DOI: 10.1002/hep.23807

[15] Long XD, Ma Y, Zhou YF, Yao JG, Ban FZ, Huang YZ, et al. XPD codon 312 and 751 polymorphisms, and AFB₁ exposure, and hepatocellular carcinoma risk. *BMC Cancer*. 2009;**9**:400. DOI: 10.1186/1471-2407-9-400

[16] Long XD, Ma Y, Deng ZL. GSTM1 and XRCC3 polymorphisms: Effects on levels of aflatoxin B₁-DNA adducts. *Chinese Journal of Cancer Research*. 2009;**21**:177-184. DOI: 10.1007/s11670-009-0177-6

[17] Long XD, Ma Y, Qu de Y, Liu YG, Huang ZQ, Huang YZ, et al. The polymorphism of XRCC3 codon 241 and AFB₁-related hepatocellular carcinoma in Guangxi population, China. *Annals of Epidemiology*. 2008;**18**:572-578. DOI: 10.1016/j.annepidem.2008.03.003

[18] Long XD, Yao JG, Zeng Z, Ma Y, Huang XY, Wei ZH, et al. Polymorphisms in the coding region of X-ray repair complementing group 4 and aflatoxin B₁-related hepatocellular carcinoma. *Hepatology*. 2013;**58**:171-181. DOI: 10.1002/hep.26311

[19] Wang JS, Groopman JD. DNA damage by mycotoxins. *Mutation Research*. 1999;**424**:167-181. DOI: 10.1016/S0027-5107(99)00017-2

[20] Zuberi Z, Eeza MNH, Matysik J, Berry JP, Alia A. NMR-based metabolic profiles of intact Zebrafish embryos

exposed to aflatoxin B₁ recapitulates hepatotoxicity and supports possible neurotoxicity. *Toxins (Basel)*. 2019;**11**:258. DOI: 10.3390/toxins11050258

[21] Zhou Y, Jin Y, Yu H, Shan A, Shen J, Zhou C, et al. Resveratrol inhibits aflatoxin B₁-induced oxidative stress and apoptosis in bovine mammary epithelial cells and is involved the Nrf2 signaling pathway. *Toxicon*. 2019;**164**:10-15. DOI: 10.1016/j.toxicon.2019.03.022

[22] Zhou X, Gan F, Hou L, Liu Z, Su J, Lin Z, et al. Aflatoxin B₁ induces immunotoxicity through the DNA methyltransferase-mediated JAK2/STAT3 pathway in 3D4/21 cells. *Journal of Agricultural and Food Chemistry*. 2019;**67**:3772-3780. DOI: 10.1021/acs.jafc.8b07309

[23] Zhou J, Tang L, Wang JS. Assessment of the adverse impacts of aflatoxin B₁ on gut-microbiota dependent metabolism in F344 rats. *Chemosphere*. 2019;**217**:618-628. DOI: 10.1016/j.chemosphere.2018.11.044

[24] Zhao L, Feng Y, Deng J, Zhang NY, Zhang WP, Liu XL, et al. Selenium deficiency aggravates aflatoxin B₁-induced immunotoxicity in chick spleen by regulating 6 selenoprotein genes and redox/inflammation/apoptotic signaling. *The Journal of Nutrition*. 2019;**149**:894-901. DOI: 10.1093/jn/nxz019

[25] Zhao F, Tian Y, Shen Q, Liu R, Shi R, Wang H, et al. A novel nanobody and mimotope based immunoassay for rapid analysis of aflatoxin B₁. *Talanta*. 2019;**195**:55-61. DOI: 10.1016/j.talanta.2018.11.013

[26] Li S, Muhammad I, Yu H, Sun X, Zhang X. Detection of aflatoxin adducts as potential markers and the role of curcumin in alleviating AFB₁-induced liver damage in chickens.

- Ecotoxicology and Environmental Safety. 2019;**176**:137-145. DOI: 10.1016/j.ecoenv.2019.03.089
- [27] Engin AB, Engin A. DNA damage checkpoint response to aflatoxin B1. *Environmental Toxicology and Pharmacology*. 2019;**65**:90-96. DOI: 10.1016/j.etap.2018.12.006
- [28] Coskun E, Jaruga P, Vartanian V, Erdem O, Egner PA, Groopman JD, et al. Aflatoxin-guanine DNA adducts and oxidatively induced DNA damage in aflatoxin-treated mice in vivo as measured by liquid chromatography-tandem mass spectrometry with isotope dilution. *Chemical Research in Toxicology*. 2019;**32**:80-89. DOI: 10.1021/acs.chemrestox.8b00202
- [29] Liang TJ. p53 proteins and aflatoxin B1: The good, the bad, and the ugly. *Hepatology*. 1995;**22**:1330-1332. PMID: 7557889
- [30] Poapolathep S, Imsilp K, Machii K, Kumagai S, Poapolathep A. The effects of curcumin on aflatoxin B1-induced toxicity in rats. *Biocontrol Science*. 2015;**20**:171-177. DOI: 10.4265/bio.20.171
- [31] Bayram S, Rencuzogullari E, Almas AM, Genc A. Effect of p53 Arg72Polymorphism on the induction of micronucleus by aflatoxin B1 in in vitro in human blood lymphocytes. *Drug and Chemical Toxicology*. 2016;**39**:331-337. DOI: 10.3109/01480545.2015.1121275
- [32] Qi LN, Bai T, Chen ZS, Wu FX, Chen YY, De Xiang B, et al. The p53 mutation spectrum in hepatocellular carcinoma from Guangxi, China: Role of chronic hepatitis B virus infection and aflatoxin B1 exposure. *Liver International*. 2015;**35**:999-1009. DOI: 10.1111/liv.12460
- [33] Chittmittrapap S, Chieochansin T, Chaiteerakij R, Treeprasertsuk S, Klaikaew N, Tangkijvanich P, et al. Prevalence of aflatoxin induced p53 mutation at codon 249 (R249s) in hepatocellular carcinoma patients with and without hepatitis B surface antigen (HBsAg). *Asian Pacific Journal of Cancer Prevention*. 2013;**14**:7675-7679. DOI: PMID24460352
- [34] Gursoy-Yuzugullu O, Yuzugullu H, Yilmaz M, Ozturk M. Aflatoxin genotoxicity is associated with a defective DNA damage response bypassing p53 activation. *Liver International*. 2011;**31**:561-571. DOI: 10.1111/j.1478-3231.2011.02474.x
- [35] Van Vleet TR, Watterson TL, Klein PJ, Coulombe RA Jr. Aflatoxin B1 alters the expression of p53 in cytochrome P450-expressing human lung cells. *Toxicological Sciences*. 2006;**89**:399-407. DOI: 10.1093/toxsci/kfj039
- [36] Chan KT, Hsieh DP, Lung ML. In vitro aflatoxin B1-induced p53 mutations. *Cancer Letters*. 2003;**199**:1-7. DOI: 10.1016/S0304-3835(03)00337-9
- [37] Shirabe K, Toshima T, Taketomi A, Taguchi K, Yoshizumi T, Uchiyama H, et al. Hepatic aflatoxin B1-DNA adducts and TP53 mutations in patients with hepatocellular carcinoma despite low exposure to aflatoxin B1 in southern Japan. *Liver International*. 2011;**31**:1366-1372. DOI: 10.1111/j.1478-3231.2011.02572.x
- [38] Long XD, Yao JG, Zeng Z, Huang CH, Huang ZS, Huang YZ, et al. DNA repair capacity-related to genetic polymorphisms of DNA repair genes and aflatoxin B1-related hepatocellular carcinoma among Chinese population. In: Kruman I, editor. *DNA Repair*. Rijeka: InTech; 2011. pp. 505-524. DOI: 10.5772/20792