Chapter

The Carcinogenicity of Aflatoxin B1

Jie Li and Mengxi Liu

Abstract

Aflatoxins are a class of carcinogenic mycotoxins, products of *Aspergillus* fungi, which are known contaminants in a large portion of the world's food supply. Aflatoxin B1 (AFB1) is the most potent toxin, which has been strongly linked to the development of hepatocellular carcinoma (HCC), especially given coinfection with hepatitis B virus (HBV). AFB1 is catalyzed by cytochrome P450 (CYP450) into aflatoxin B1-8,9-exo-epoxide to form DNA adducts, which leads to carcinogenesis by disrupting DNA repair. AFB1-induced DNA damage is also caused by the production of excessive ROS, leading to the oxidation of DNA bases. The majority of AFB1-related to HCC carry G-to-T transversion of p53 gene. When the p53 gene is mutated, it shows a "gain of oncogenic function." In addition, epigenetic alterations may potentially be beneficial for the treatment of HCC, because the epigenetic changes are reversible. This chapter will provide important information on the carcinogenicity of AFB1, including DNA damage checkpoint response and epigenetic alteration.

Keywords: aflatoxins, hepatocellular carcinoma, DNA adducts, carcinogenicity

1. Introduction

Aflatoxins were reported to be potent liver carcinogens for laboratory animals since the 1960s [1]. The International Agency for Research on Cancer (IARC) has identified aflatoxins as one of the most harmful human carcinogens [2]. There are four main kinds of aflatoxins, B1, B2, G1, and G2, which are classified based on UV-induced fluorescence color and chromatography retention time [3].

Among these aflatoxins, aflatoxin B1 (AFB1), which is synthesized by *Aspergillus* fungi, is the most common carcinogenic and can be found in foods, such as corn, peanuts, cereals, rice, etc. [4, 5]. AFB1 is chemically stable and resistant to various thermal processes such as boiling, autoclaving, cooking, and fermentation [6]. AFB1 is catalyzed by cytochrome P450 into aflatoxin B1-8,9-exo-epoxide to form DNA adducts, which leads to carcinogenesis by disrupting DNA repair [7]. Although most countries introduce strict regulations for the maximum permitted concentrations of aflatoxin in food, excess of AFB1-DNA adducts has remained in normal and tumorous tissues of individuals with hepatocellular carcinoma (HCC) [8, 9]. HCC is ranked as the second leading cause of death from cancer globally, with 700,000 annual deaths recorded worldwide in 2012 [10, 11]. There are about 4.5 billion people in the world that are exposed to AFB1 and may develop HCC [12]. The risks of AFB1 to induce HCC are dependent on populations and areas, e.g., urban populations are generally exposed to lower levels of aflatoxins than rural populations. Moreover, AFB1 exposure is altered by strong seasonal variation that is correlated with increased food availability. In addition, hepatitis B virus (HBV) infection is associated with AFB1 exposure [12, 13].

Some studies reported that the detection of urinary AFB1-N7-guanine adduct, a good biomarker for AFB1 exposures and dietary AFB1 intake, has a significant association with HCC [13, 14]. The AFB1-DNA adduct is formed by the reactive intermediate of AFB1, AFB1-8,9-epoxide (AFB1-E), and binds to the DNA of hepatocytes. On the one hand, AFB1 is supposed to cause tumorigenesis by promoting the formation of DNA adducts, resulting in aberrant gene expression and genetic mutations, such as tumor suppressor gene p53, in targeted liver cells [15]. On the other hand, AFB1-developed HCC rat model presents various epigenetic (DNA methylation, histone modification, and noncoding RNA) alterations in hepatocytes [16, 17]. Therefore, this chapter focuses on the potential mechanisms of AFB1-induced development of HCC, including DNA damage checkpoint response and epigenetic alteration.

2. AFB1-DNA adduct formation

AFB1 is mainly absorbed in the small intestine after ingestion and transported from blood to the liver [18]. It passes through hepatocytes by nonionic diffusion, which is not dependent on metabolic energy status [19]. Although AFB1 cannot directly bind to DNA, it is metabolized into reactive epoxide named AFB1-E by cytochrome P450 (CYP450) in the liver [7]. AFB1-E can rapidly conjugate with N7 of guanine residues, resulting in the formation of highly mutagenic AFB1-E-deoxyguanosine or AFB1-formamidopyrimidine (AFB1-FaPy-dG) adducts [20]. In fact, AFB1-E can spontaneously and irreversibly conjugate with guanine residues to form 8,9-dihydro-8-(N7-guanyl)-9-hydroxyaflatoxin B1 (AFB1-E-N7-dG) adducts, leading to the occurrence of DNA damage and mutations [21, 22]. AFB1-E-N7-dG adduct may undergo imidazole ring opening or depurination spontaneously to yield AFB1-FaPy-dG, which has been found in many hepatocellular carcinoma cases [23]. Although both AFB1-FaPy and AFB1-E-N7-dG adducts have similar structures, they bind DNA in a different manner, which results in the differences in the lethality, mutagenicity, and repair capacity of these metabolites. In fact, enzymes involved in DNA repair pathways have higher affinity with AFB1-E-N7-dG than AFB1-FaPy analog. AFB1-FaPy-dG adduct block the replication more effectively than AFB1-E-N7-dG adduct, e.g., AFB1-FaPy-dG adducts induce at least six times more G-to-T transversion than AFB1-E-N7-dG [20]. Therefore, AFB1-FaPy-dG is recognized as the major adduct and the most lethal aflatoxin-induced replication block in vivo.

AFB1 is bioactivated by the predominant enzyme cytochrome P450 in human liver microsomes. Specifically, CYP1A2 is the major enzyme for AFB1-E-dG formation and DNA damage at the low dietary intake of AFB1, while CYP3A4 is the major enzyme at higher doses of exposure [24, 25]. In general, CYP1A2 contributes to 95% of AFB1-DNA adduct formation [26]. AFB1 exposure significantly affected the expression of almost 200 genes and exhibited a fivefold decrease in histone transcripts, which is revealed by microarray studies [27].

In addition, the formation of urinary AFB1-E-N7-guanine excretion and levels of AFB1-serum albumin adducts are highly associated to AFB1 intake [28]. Accordingly, AFB1-E-N7-dG adduct is closely related with the incidence of liver tumors [29, 30]. Then, AFB1-E-N7-dG adduct is rapidly removed from DNA and excreted solely in urine [30]. Actually, people who excreted AFB1-E-N7-dG adduct has 9.1 times more risk of developing HCC than the ones with no adducts [31]. These findings indicate that AFB1-DNA adducts have strong effects on HCC development [26, 27].

3. AFB1-induced oxidative stress

Besides the generation of AFB1-DNA adducts, AFB1-induced DNA damage is also caused by the production of excessive ROS, leading to the oxidation of DNA bases [32]. On the one hand, AFB1 increases ROS generation, superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT), and glutathione reductase (GR); on the other hand, AFB1 decreases glutathione (GSH) while increasing nitric oxide, fragmented DNA-conjugated dienes, caspase-3, superoxide anion radicals, lipid hydroperoxides, and malondialdehyde (MDA) [11, 33]. It suggests that AFB1-induced hepatocarcinogenesis is partially due to the increase of oxidative stress biomarkers and DNA damage [34].

In addition, the lipid peroxidation contributes to the progression of hepatocarcinogenesis by enhancing the susceptibility to mutation and provokes aldehyde-DNA adducts at p53 mutational hotspot codon 249 [35]. To prevent the further formation of ROS/RNS, p53 modulates the transcription of antioxidant genes; however, the extensive DNA damage promotes apoptosis by activating the prooxidant genes. The elevated oxidative stress is well correlated with the induction of AFB1-induced apoptosis of splenic lymphocytes [36]. Therefore, TP53 mutation is considered as a reliable biomarker for evaluating the extent of AFB1 exposure [37]. Vital antioxidant enzymes alleviate oxidative damage through removing free radicals, e.g., selenium, as a cofactor, and promote cell survival by activating the antioxidant system in response to AFB1-induced mitochondrial damage [38]. The dietary intake of AFB1 is associated with the amount of pathological lesions and apoptosis rate of hepatocytes [39]. Therefore, AFB1 causes instability between antioxidant system activity and ROS production, resulting in excessive reactive species that lead to the apoptosis of hepatocytes [11]. It has been reported that AFB1-treatment increases 8-hydroxy-2′-deoxyguanosine (8-OHdG), which was enormously found in tissues exposed to AFB1 [32]. The 8-OHdG adduct, which is generated from the binding between the hydroxyl radicals and guanine residues of DNA, is considered as a valuable biomarker for the measurement of oxidative DNA damage [40].

In polluted food and water, especially in the regions that are warm and have high humidity, AFB1 coexists with microcystin-LR (MC-LR), a hepatotoxic toxin produced by *Cyanobacteria*. DNA damage is enhanced when cells are exposed to both AFB1 and MC-LR, compared to the exposure to AFB1 or MC-LR, alone, because exposure to both AFB1 and MC-LR further increases the release of ROS. In this case, AFB1 induces genotoxicity through increasing oxidative stress and inhibiting the activity of DNA base excision repair genes [41]. Thus, induction of oxidative DNA damage is responsible for AFB1-induced hepatocarcinogenesis besides the elevated number of AFB1-DNA adducts.

4. AFB1-induced DNA damage checkpoint response

DNA damage checkpoint response is critical for the genomic integrity and the survival of the organism in response to AFB1-induced genotoxicity, especially in the DNA that is actively replicating [42]. Mechanically, AFB1 activates critical proteins in response to DNA damage, such as ataxia telangiectasia and Rad3-related (ATR), ataxia telangiectasia mutated (ATM), Chk2 (serine/threonine protein kinase Chk2 or checkpoint kinase 2), DNA repair enzymes, and p53 [43]. In this context, AFB1-induced DNA double-strand breaks trigger the activation of ATM, which is one of the earliest activated kinases in response to double-strand breaks [44, 45]. Notably, the ATR/Chk1 pathway is not activated in response to AFB1-induced DNA double-strand breaks [26].

Cell cycle-dependent regulation plays an important role in the modulation of DNA repair, such as checkpoint activation, which slows down the cell cycle progression to facilitate DNA repair [46]. In this way, temporary DNA lesions are restrained to become inheritable mutations [47]. AFB1 induces the mutation of ATM kinase, which results in the defectiveness of cell cycle checkpoints [48]. On the one hand, the damaged cells with activated checkpoints can be eliminated by apoptosis if the DNA lesions are severe; on the other hand, damaged cell can continue the cell cycle progression upon checkpoint termination if the DNA lesions have been repaired [49]. Actually, AFB1 decreased the rate of DNA double-strand break repair and apoptosis, resulting in elevated risk of cancer [50].

Since ATM plays a critical role in the activation of cell cycle checkpoints [51], the damaged DNA activates ATM kinase to trigger the DNA repair signaling pathways. One of the substrates of ATM kinase is the structural maintenance of chromosome 1 (SMC1) protein, which plays key roles in regulating DNA replication forks and DNA repair in response to the damage [52, 53]. The ATM signaling inhibits the cell cycle progression from G1 to S (the G1/S checkpoint) or G2 to mitosis (the G2/M checkpoint) by activating p53 and inhibiting cyclin-dependent kinases [45, 54]. Ultimately, ATM is involved in the regulation of G2/M checkpoint, which has a crucial role in the maintenance of genomic integrity [50, 55]. In addition, ATM activates Chk2 by promoting its phosphorylation in response to DNA damage [56]. Subsequently, phosphorylated Chk2 (pChk2) induces G2/M cell cycle checkpoint by activating p53 signal pathway [57, 58]. Indeed, AFB1 activates ATM-Chk2-p53 axis, leading to G2/M phase arrest via cdc25-cyclin B/cdc2 route [59]. In this signaling pathway, Mdm2 is an E3 ubiquitin ligase that facilitates the ubiquitination of p53 and proteasome-mediated degradation [60, 61]. Thus, ATM-Chk2-p53 axis plays a critical role in AFB1-induced DNA damage response.

5. AFB1 exposure and genetic mutation

More than 50% of HCC patients are from the regions where the daily food is contaminated by excess amount of AFB1 [62]. In these cases, the AFB1 exposure is associated partly with the genetic mutations [20]. For example, high frequent mutation of p53 gene at codon 249 with G-to-T transversion has been found in HCC patients who are exposed to excess dietary aflatoxin [30].

AFB1 preferentially induces G-to-T transversion in exon 7 of p53 tumor suppressor gene, so as to the frequency of G-to-T transversion of p53 significantly associated with AFB1-DNA adducts [63]. Thus, HCC patients who have AFB1-DNA adducts also have AGG-to-AGT mutation at codon 249 of p53 gene [64]. In addition, AFB1 also promotes G-to-T transversion of ras genes, which can enhance the malignant transformation of normal cells. AFB1-FaPy-dG adduct that is a highly persistent DNA lesion in the liver is associated with the lethality and mutagenicity potential [65]. Indeed, the metabolically activated AFB1-E and its cationic metabolite, AFB1-E-N7-dG, are highly related with the genotoxicity of AFB1 [66]. Additionally, there are at least 80% of HCC patients who have chronic infection with hepatitis B virus (HBV) or hepatitis C virus (HCV) [67]. Although AFB1 exposure slows down the replication of HBV, AFB1-mediated DNA damage and p53 induction are not associated with virus infection [68].

6. AFB1 and epigenetic alteration

Epigenetic alterations are heritable phenotype changes that do not include alteration of the DNA sequences [17]. AFB1 exposure induces various kinds of

epigenetic alterations, such as DNA methylation and histone modifications, leading to the development of HCC.

DNA methylation is an enzymatic process that a methyl group is conjugated into the CpG site of DNA, where a cytosine (C) is found next to a guanine (G) separated by a single phosphate (p) [69]. AFB1-induced change of DNA methylation is considered as one of the major mechanisms that are associated with AFB1-induced hepatocarcinogenesis [70]. The global hypomethylation of DNA has been identified as one of the most common biomarkers in human cancer [71]. It can lead to the silencing of critical tumor suppressor genes, such as p53 [72]. AFB1 exposure interferes DNA methylation patterns, resulting in an interruption of the methylation machinery associated with HCC development.

A nucleosome is composed of a segment of DNA, which is wrapped around an octamer of histone protein cores composed of two copies of each of the protein histones H2A, H2B, H3, and H4 [73, 74]. Thousands of nucleosome units highly compact into a structure called chromatin [17, 75]. Several translational modifications occur at the N-terminal domains of the histones, e.g., acetylation, methylation, phosphorylation, and ubiquitination, which regulate important cellular processes, including DNA repair, chromatin structure, gene expression, and DNA replication [74]. AFB1 exposure can alter the pattern of modifications in histones, resulting in an increase or repression of genes, facilitating the development of cancer, such as HCC [76].

The most common modifications in histones regulated by AFB1 are methylation and acetylation [74]. Methylation is catalyzed by enzymes called histone methyltransferases, which add the methyl group into the lysine of the histones H3 and H4, occurring as three different forms, including mono-, di-, or trimethylation. Additionally, histone demethylase can remove the methyl group. AFB1-induced histone methylation causes conformational changes of chromatin, leading to gene silencing [77, 78]. In contrast to methylation, acetylation leads to a relaxation of chromatin, resulting in the activation of the gene transcription. Histone acetyltransferases, which catalyze the addition of the acetyl group, and the histone deacetylase (HDAC) enzymes, which remove the acetyl group, are the major enzymes involved in this dynamic process [73, 79]. The increased expression of HDACs causes the hypoacetylation of histone H3, leading to the repression of the silencing of specific tumor suppressor genes, such as p21, inducing the differentiation and proliferation of liver tumor cells [74, 80, 81].

7. Conclusion

Aflatoxin B1 (AFB1), which is synthesized by *Aspergillus* fungi, is the most common carcinogenic type of aflatoxins and can be found in human diet, such as corn, peanuts, cereals, rice, etc. Although AFB1 cannot directly conjugate with DNA, its metabolite, AFB1-E, can form carcinogenic AFB1-E-N7-dG and AFB1-FaPy-dG adducts spontaneously and irreversibly. Besides the generation of AFB1-DNA adducts, AFB1-induced DNA damage is also caused by the production of excessive ROS, leading to the oxidation of DNA bases. The majority of AFB1-related HCC carry G-to-T transversion of p53 gene. When the p53 protein is mutated, it shows a "gain of oncogenic function." In addition, epigenetic alterations may potentially be beneficial for the treatment of HCC, because the epigenetic changes are reversible.

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References

[1] Stoloff L. Aflatoxin is not a probably human carcinogen: The published evidence is sufficient. Regulatory Toxicology and Pharmacology. 1989;**10**(3):272-283

[2] Ostry V, Malir F, Toman J, Grosse Y. Mycotoxins as human carcinogens—The IARC monographs classification. Mycotoxin Research. 2017;**33**(1):65-73

[3] Bennett JW, Klich M. Mycotoxins. Clinical Microbiology Reviews. 2003;**16**(3):497-516

[4] Kumar P, Mahato DK, Kamle M, Mohanta TK, Kang SG. Aflatoxins: A global concern for food safety, human health and their management. Frontiers in Microbiology. 2016;**7**:2170

[5] Gross-Steinmeyer K, Eaton DL. Dietary modulation of the biotransformation and genotoxicity of aflatoxin B(1). Toxicology. 2012;**299**(2-3):69-79

[6] Wang Y et al. Effective biodegradation of aflatoxin B1 using the *Bacillus licheniformis* (BL010) strain. Toxins (Basel). 2018;**10**(12):497

[7] Rushing BR, Selim MI. Aflatoxin B1: A review on metabolism, toxicity, occurrence in food, occupational exposure, and detoxification methods. Food and Chemical Toxicology. 2019;**124**:81-100

[8] Harrison JC, Carvajal M, Garner RC. Does aflatoxin exposure in the United Kingdom constitute a cancer risk? Environmental Health Perspectives. 1993;**99**:99-105

[9] Turner PC, Flannery B, Isitt C, Ali M, Pestka J. The role of biomarkers in evaluating human health concerns from fungal contaminants in food. Nutrition Research Reviews. 2012;**25**(1):162-179

[10] Ferlay J et al. Cancer incidence and mortality worldwide: Sources, methods and major patterns in GLOBOCAN 2012. International Journal of Cancer. 2015;**136**(5):E359-E386

[11] Mughal MJ, Xi P, Yi Z, Jing F. Aflatoxin B1 invokes apoptosis via death receptor pathway in hepatocytes. Oncotarget. 2017;**8**(5):8239-8249

[12] Liu Y, Wu F. Global burden of aflatoxin-induced hepatocellular carcinoma: A risk assessment. Environmental Health Perspectives. 2010;**118**(6):818-824

[13] Asim M, Sarma MP, Thayumanavan L, Kar P. Role of aflatoxin B1 as a risk for primary liver cancer in North Indian population. Clinical Biochemistry. 2011;**44**(14-15):1235-1240

[14] Yu MW, Chiang YC, Lien JP, Chen CJ. Plasma antioxidant vitamins, chronic hepatitis B virus infection and urinary aflatoxin B1-DNA adducts in healthy males. Carcinogenesis. 1997;**18**(6):1189-1194

[15] Hamid AS, Tesfamariam IG, Zhang Y, Zhang ZG. Aflatoxin B1-induced hepatocellular carcinoma in developing countries: Geographical distribution, mechanism of action and prevention. Oncology Letters. 2013;**5**(4):1087-1092

[16] Kew MC. Aflatoxins as a cause of hepatocellular carcinoma. Journal of Gastrointestinal and Liver Diseases. 2013;**22**(3):305-310

[17] Dai Y, Huang K, Zhang B, Zhu L, Xu W. Aflatoxin B1-induced epigenetic alterations: An overview. Food and Chemical Toxicology. 2017;**109**(Pt 1): 683-689

[18] Kumagai S. Intestinal absorption and excretion of aflatoxin in rats.

Toxicology and Applied Pharmacology. 1989;**97**(1):88-97

[19] Muller N, Petzinger E. Hepatocellular uptake of aflatoxin B1 by non-ionic diffusion. Inhibition of bile acid transport by interference with membrane lipids. Biochimica et Biophysica Acta. 1988;**938**(3):334-344

[20] Smela ME et al. The aflatoxin B(1) formamidopyrimidine adduct plays a major role in causing the types of mutations observed in human hepatocellular carcinoma. Proceedings of the National Academy of Sciences of the United States of America. 2002;**99**(10):6655-6660

[21] Eaton DL, Gallagher EP. Mechanisms of aflatoxin carcinogenesis. Annual Review of Pharmacology and Toxicology. 1994;**34**:135-172

[22] Shimada T, Guengerich FP. Evidence for cytochrome P-450NF, the nifedipine oxidase, being the principal enzyme involved in the bioactivation of aflatoxins in human liver. Proceedings of the National Academy of Sciences of the United States of America. 1989;**86**(2):462-465

[23] Lin YC et al. DNA polymerase zeta limits chromosomal damage and promotes cell survival following aflatoxin exposure. Proceedings of the National Academy of Sciences of the United States of America. 2016;**113**(48):13774-13779

[24] Gallagher EP, Kunze KL, Stapleton PL, Eaton DL. The kinetics of aflatoxin B1 oxidation by human cDNA-expressed and human liver microsomal cytochromes P450 1A2 and 3A4. Toxicology and Applied Pharmacology. 1996;**141**(2):595-606

[25] Kamdem LK, Meineke I, Godtel-Armbrust U, Brockmoller J, Wojnowski L. Dominant contribution of P450 3A4 to the hepatic carcinogenic activation of aflatoxin B1. Chemical Research in Toxicology. 2006;**19**(4):577-586

[26] 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**(4):561-571

[27] Guo Y et al. Analysis of cellular responses to aflatoxin B(1) in yeast expressing human cytochrome P450 1A2 using cDNA microarrays. Mutation Research. 2006;**593**(1-2):121-142

[28] Groopman JD, DeMatos P, Egner PA, Love-Hunt A, Kensler TW. Molecular dosimetry of urinary aflatoxin-N7-guanine and serum aflatoxin-albumin adducts predicts chemoprotection by 1,2-dithiole-3-thione in rats. Carcinogenesis. 1992;**13**(1):101-106

[29] Lin YC et al. Error-prone replication bypass of the primary aflatoxin B1 DNA adduct, AFB1-N7-Gua. The Journal of Biological Chemistry. 2014;**289**(26):18497-18506

[30] Wang JS, Groopman JD. DNA damage by mycotoxins. Mutation Research. 1999;**424**(1-2):167-181

[31] Poirier MC. DNA adducts as exposure biomarkers and indicators of cancer risk. Environmental Health Perspectives. 1997;**105**(Suppl 4):907-912

[32] Guindon KA, Bedard LL, Massey TE. Elevation of 8-hydroxydeoxyguanosine in DNA from isolated mouse lung cells following in vivo treatment with aflatoxin B(1). Toxicological Sciences. 2007;**98**(1):57-62

[33] Ajiboye TO, Yakubu MT, Oladiji AT. Lophirones B and C prevent aflatoxin B1-induced oxidative stress and DNA fragmentation in rat

hepatocytes. Pharmaceutical Biology. 2016;**54**(10):1962-1970

[34] Ajiboye TO, Yakubu MT, Oladiji AT. Lophirones B and C extenuate AFB1-mediated oxidative onslaught on cellular proteins, lipids, and DNA through Nrf-2 expression. Journal of Biochemical and Molecular Toxicology. 2014;**28**(12):558-567

[35] Weng MW et al. AFB1 hepatocarcinogenesis is via lipid peroxidation that inhibits DNA repair, sensitizes mutation susceptibility and induces aldehyde-DNA adducts at p53 mutational hotspot codon 249. Oncotarget. 2017;**8**(11):18213-18226

[36] Chen J et al. Effects of aflatoxin B1 on oxidative stress markers and apoptosis of spleens in broilers. Toxicology and Industrial Health. 2016;**32**(2):278-284

[37] Hussain SP, Schwank J, Staib F, Wang XW, Harris CC. TP53 mutations and hepatocellular carcinoma: Insights into the etiology and pathogenesis of liver cancer. Oncogene. 2007;**26**(15):2166-2176

[38] Shi D et al. Protective effects of selenium on aflatoxin B1-induced mitochondrial permeability transition, DNA damage, and histological alterations in duckling liver. Biological Trace Element Research. 2015;**163**(1-2):162-168

[39] Yang J et al. Effects of feeding corn naturally contaminated with aflatoxin B1 and B2 on hepatic functions of broilers. Poultry Science. 2012;**91**(11):2792-2801

[40] Shen HM, Ong CN, Lee BL, Shi CY. Aflatoxin B1-induced 8-hydroxydeoxyguanosine formation in rat hepatic DNA. Carcinogenesis. 1995;**16**(2):419-422

[41] Liu W et al. Microcystin-LR increases genotoxicity induced by aflatoxin B1 through oxidative stress and DNA base excision repair genes in human hepatic cell lines. Environmental Pollution. 2018;**233**:455-463

[42] Fasullo M, Sun M, Egner P. Stimulation of sister chromatid exchanges and mutation by aflatoxin B1-DNA adducts in Saccharomyces cerevisiae requires MEC1 (ATR), RAD53, and DUN1. Molecular Carcinogenesis. 2008;**47**(8):608-615

[43] Yang X et al. Cytochrome P450 2A13 enhances the sensitivity of human bronchial epithelial cells to aflatoxin B1-induced DNA damage. Toxicology and Applied Pharmacology. 2013;**270**(2):114-121

[44] Burma S, Chen BP, Murphy M, Kurimasa A, Chen DJ. ATM phosphorylates histone H2AX in response to DNA double-strand breaks. The Journal of Biological Chemistry. 2001;**276**(45):42462-42467

[45] Sancar A, Lindsey-Boltz LA, Unsal-Kacmaz K, Linn S. Molecular mechanisms of mammalian DNA repair and the DNA damage checkpoints. Annual Review of Biochemistry. 2004;**73**:39-85

[46] Warmerdam DO, Kanaar R. Dealing with DNA damage: Relationships between checkpoint and repair pathways. Mutation Research. 2010;**704**(1-3):2-11

[47] Yang XH, Zou L. Checkpoint and coordinated cellular responses to DNA damage. Results and Problems in Cell Differentiation. 2006;**42**:65-92

[48] Branzei D, Foiani M. Regulation of DNA repair throughout the cell cycle. Nature Reviews. Molecular Cell Biology. 2008;**9**(4):297-308

[49] Bartek J, Lukas J. DNA damage checkpoints: From initiation to recovery or adaptation. Current Opinion in Cell Biology. 2007;**19**(2):238-245

[50] Lavin MF, Kozlov S. ATM activation and DNA damage response. Cell Cycle. 2007;**6**(8):931-942

[51] Kurz EU, Lees-Miller SP. DNA damage-induced activation of ATM and ATM-dependent signaling pathways. DNA Repair (Amst). 2004;**3**(8-9):889-900

[52] Kitagawa R, Kastan MB. The ATM-dependent DNA damage signaling pathway. Cold Spring Harbor Symposia on Quantitative Biology. 2005;**70**:99-109

[53] Lee JH, Paull TT. Activation and regulation of ATM kinase activity in response to DNA double-strand breaks. Oncogene. 2007;**26**(56):7741-7748

[54] Niida H, Nakanishi M. DNA damage checkpoints in mammals. Mutagenesis. 2006;**21**(1):3-9

[55] Kudoh A et al. *Epstein-Barr* virus lytic replication elicits ATM checkpoint signal transduction while providing an S-phase-like cellular environment. The Journal of Biological Chemistry. 2005;**280**(9):8156-8163

[56] Wilson KA, Stern DF. NFBD1/MDC1, 53BP1 and BRCA1 have both redundant and unique roles in the ATM pathway. Cell Cycle. 2008;**7**(22):3584-3594

[57] Hirao A et al. DNA damage-induced activation of p53 by the checkpoint kinase Chk2. Science. 2000;**287**(5459):1824-1827

[58] Yu Q et al. UCN-01 inhibits p53 up-regulation and abrogates gamma-radiation-induced G(2)-M checkpoint independently of p53 by targeting both of the checkpoint kinases, Chk2 and Chk1. Cancer Research. 2002;**62**(20):5743-5748

[59] Yin H et al. The molecular mechanism of G2M cell cycle arrest induced by AFB1 in the jejunum. Oncotarget. 2016;**7**(24):35592-35606

[60] Chen X et al. MDM2 promotes invasion and metastasis in invasive ductal breast carcinoma by inducing matrix metalloproteinase-9. PLoS ONE. 2013;**8**(11):e78794

[61] Noon AP et al. p53 and MDM2 in renal cell carcinoma: Biomarkers for disease progression and future therapeutic targets? Cancer. 2010;**116**(4):780-790

[62] Shen HM, Ong CN. Mutations of the p53 tumor suppressor gene and ras oncogenes in aflatoxin hepatocarcinogenesis. Mutation Research. 1996;**366**(1):23-44

[63] Aguilar F, Hussain SP, Cerutti P. Aflatoxin B1 induces the transversion of G-->T in codon 249 of the p53 tumor suppressor gene in human hepatocytes. Proceedings of the National Academy of Sciences of the United States of America. 1993;**90**(18):8586-8590

[64] Shirabe K 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**(9):1366-1372

[65] Croy RG, Wogan GN. Quantitative comparison of covalent aflatoxin-DNA adducts formed in rat and mouse livers and kidneys. Journal of the National Cancer Institute. 1981;**66**(4):761-768

[66] Brown KL et al. Unraveling the aflatoxin-FAPY conundrum: Structural basis for differential replicative processing of isomeric forms of the formamidopyrimidine-type DNA adduct of aflatoxin B1. Journal of the American Chemical Society. 2006;**128**(47):15188-15199

[67] McGlynn KA, London WT. The global epidemiology of hepatocellular

carcinoma: Present and future. Clinics in Liver Disease. 2011;**15**(2):223-243, vii-x

[68] Lereau M et al. Interactions between hepatitis B virus and aflatoxin B(1): Effects on p53 induction in HepaRG cells. The Journal of General Virology. 2012;**93**(Pt 3):640-650

[69] Goel N, Karir P, Garg VK. Role of DNA methylation in human age prediction. Mechanisms of Ageing and Development. 2017;**166**:33-41

[70] Udali S, Guarini P, Moruzzi S, Choi SW, Friso S. Cardiovascular epigenetics: From DNA methylation to microRNAs. Molecular Aspects of Medicine. 2013;**34**(4):883-901

[71] Udali S et al. Hepcidin and DNA promoter methylation in hepatocellular carcinoma. European Journal of Clinical Investigation. 2018;**48**(2):e12870

[72] You JS, Jones PA. Cancer genetics and epigenetics: Two sides of the same coin? Cancer Cell. 2012;**22**(1):9-20

[73] Ma L, Chua MS, Andrisani O, So S. Epigenetics in hepatocellular carcinoma: An update and future therapy perspectives. World Journal of Gastroenterology. 2014;**20**(2):333-345

[74] Chrun ES, Modolo F, Daniel FI. Histone modifications: A review about the presence of this epigenetic phenomenon in carcinogenesis. Pathology, Research and Practice. 2017;**213**(11):1329-1339

[75] Anestopoulos I et al. Epigenetic therapy as a novel approach in hepatocellular carcinoma. Pharmacology & Therapeutics. 2015;**145**:103-119

[76] Chappell G, Pogribny IP, Guyton KZ, Rusyn I. Epigenetic alterations induced by genotoxic occupational and environmental human chemical carcinogens: A systematic literature review. Mutation Research, Reviews in Mutation Research. 2016;**768**:27-45

[77] Rieswijk L et al. Aflatoxin B1 induces persistent epigenomic effects in primary human hepatocytes associated with hepatocellular carcinoma. Toxicology. 2016;**350-352**:31-39

[78] Valinluck V et al. Oxidative damage to methyl-CpG sequences inhibits the binding of the methyl-CpG binding domain (MBD) of methyl-CpG binding protein 2 (MeCP2). Nucleic Acids Research. 2004;**32**(14):4100-4108

[79] Zhao N et al. Expression of microRNA-195 is transactivated by Sp1 but inhibited by histone deacetylase 3 in hepatocellular carcinoma cells. Biochimica et Biophysica Acta. 2016;**1859**(7):933-942

[80] Buurman R, Sandbothe M, Schlegelberger B, Skawran B. HDAC inhibition activates the apoptosome via Apaf1 upregulation in hepatocellular carcinoma. European Journal of Medical Research. 2016;**21**(1):26

[81] Lai YC, Cheng CC, Lai YS, Liu YH. Cytokeratin 18-associated histone 3 modulation in hepatocellular carcinoma: A mini review. Cancer Genomics Proteomics. 2017;**14**(4):219-223