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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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