Chapter 6

Molecular Mechanisms of Hepatocellular Carcinoma Related to Aflatoxins: An Update

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Abstract

Hepatocellular carcinoma (hepatocarcinoma) is a major type of primary liver cancer and one of the most frequent human malignant neoplasms. Aflatoxins are I-type chemical carcinogen for hepatocarcinoma. Increasing evidence has shown that hepatocarcinoma induced by aflatoxins is the result of interaction between aflatoxins and hereditary factor. Aflatoxins can induce DNA damage including DNA strand break, adducts formation, oxidative DNA damage, and gene mutation and determine which susceptible individuals feature cancer. Inheritance such as alterations may result in the activation of proto-oncogenes and the inactivation of tumor suppressor genes and determine individual susceptibility to cancer. Interaction between aflatoxins and genetic susceptible factors commonly involve in almost all pathologic sequence of hepatocarcinoma: chronic liver injury, cirrhosis, atypical hyperplastic nodules, and hepatocarcinoma of early stages. In this review, we discuss the biogenesis, toxification, and epidemiology of aflatoxins and signal pathways of aflatoxin-induced hepatocarcinoma. We also discuss the roles of some important genes related to cell apoptosis, DNA repair, drug metabolism, and tumor metastasis in hepatocarcinogenesis related to aflatoxins.

Keywords: hepatocellular carcinoma, molecular mechanism, aflatoxin

1. Introduction

Hepatocellular carcinoma (also called hepatocarcinoma or liver carcinoma) is a major type of primary liver cancer and one of the most frequent human malignant neoplasms. This malignancy has been proved to correlate with aflatoxins, especially aflatoxin B1 (AFB1) [1–3].

Increasing evidence has exhibited that several mechanisms, including the toxic production from metabolism, the accumulation of DNA damage and genic mutation–induced aflatoxins, the decreasing DNA repair capacity, and dysregulation of signal pathways may play a central role in the tumorigenesis of aflatoxin-induced hepatocarcinoma [4–6]. In this review, we discuss the biogenesis, metabolism, and genic toxification of aflatoxins. We also discuss the molecular mechanisms of aflatoxin-induced hepatocarcinoma, involving in aflatoxin toxification, abnormal change of tumor relative genes, the interaction of aflatoxins and genetic factors, and signal pathway for tumorigenesis. The roles of some important genes related to cell apoptosis, DNA repair, drug metabolism, and tumor metastasis in hepatocarcinogenesis related to aflatoxins are further emphasized.

2. Aflatoxin biosynthesis, metabolism, and toxification

2.1. Aflatoxin biosynthesis

The biosynthesis of aflatoxins has been fully summarized in several previous reviews [7, 8]. In brief, aflatoxins are an important type of mycotoxins, which were the most early identified in the *Aspergillus flavus* (*A. flavus*) and regarded as causative agents of "turkey X" disease in the late 1950s and early 1960s. Thus, these toxins were named as "aflatoxins (namely *A. flavus* toxins)" according to their origin fungus [9]. Until now, 17 related aflatoxin isoforms and aflatoxin metabolites have been identified, and 4 of them often contaminated a number of agricultural commodities [10]. According to the amounts and fluorescent reactions, four aflatoxins primarily identified in foodstuffs are named as AFB1, aflatoxin B2 (AFB2), aflatoxin G1 (AFG1), and aflatoxin G2 (AFG2). Among these four known aflatoxins, AFB1 and AFB2 are named as B-type aflatoxins because they are attached to a pentanone and can produce blue-color fluorescent under UV light, whereas AFG1 and AFG2 are termed as G-type aflatoxins because of their attachment to a 6-membered lactone and producing green fluorescent color feature. These aflatoxins are mainly produced by *A. flavus*, *Aspergillus parasiticus* (*A. parasiticus*), *Aspergillus nidulans* (*A. nidulans*), *Aspergillus pseudotamarii* (*A. pseudotamarii*), and *Aspergillus bombycis* (*A. bombycis*) [7, 8].

Toxigenic strains of *A. flavus* produce only B-type aflatoxins, but do not synthesize G-type aflatoxins due to the deletion of an unstable microsome enzyme and a-220 kDa cytosolic protein. The other aflatoxigenic species including *A. parasiticus*, *A. nidulans*, *A. pseudotamarii*, and *A. bombycis* can produce all four aflatoxins [8].

Numeral synthetical genes, such as aflatoxin regulatory protein gene (aflR), are required for aflatoxin biosynthesis and act as a huge neighbor gene cluster consisting of about 60–70 kb in original fungi (**Figure 1**) [8–10]. All corresponding gene-encoding enzymes and transcription factors produce aflatoxin production and regulate biosynthesis. Increasing evidence has proved that aflatoxin biosynthesis involves in at least 3 stages and 18 enzyme steps (**Figures 2–4**). The first stage, including the first (R01) to eighth reaction (R08) of biosynthesis, refers from acetyl CoA to hydroxyversicolorone. The primary product hydroxyversicolorone will be formed and regulated by transcription factors aflR and aflJ (**Figure 2**) [8, 10]. The second (biosynthesis

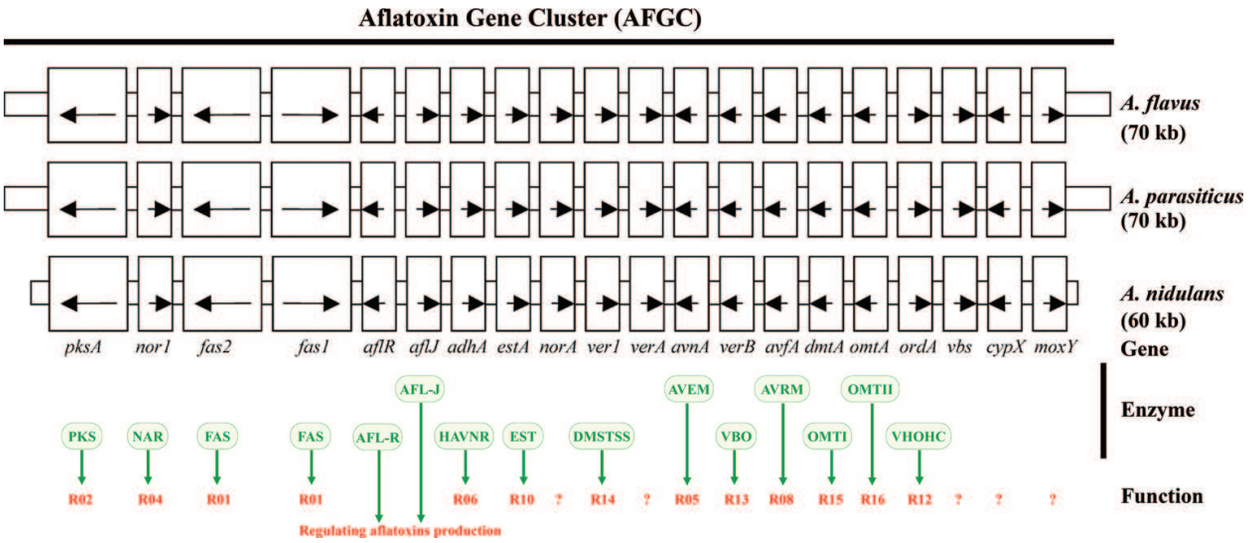

Figure 1. The aflatoxin gene cluster and their expression productions and functions. In the fungus-producing aflatoxins including *A. nidulans*, *A. parasiticus,* and *A. flavus*, genes encoding the enzymes and the transcription factors involving in aflatoxin biosynthesis commonly locate within a huge gene cluster of about 60–70 kb in the genomes. These genes, except for aflR and aflJ, involve in the 18 enzyme reaction steps (R01–R18) of aflatoxin biosynthesis, whereas aflR and aflJ expressing proteins are two important transcription factors and can regulate enzyme-related gene expression. "?" shows that the function of the corresponding gene is unknown (Note: adapted from Yabe and Nakajima [7]). *Abbreviations*. MCA, malonyl CoA; HAS, hexanoate synthase (also termed fatty acid synthase); PKS, polyketide synthase; NAS, Norsolorinic acid (NA) synthase; NAR, norsolorinic acid (NA) reductase; AVN, averantin; AVNM, averantin (AVN) monooxygenase; HAVN, 5′-hydroxyaverantin; HAVNR, 5′-hydroxyaverantin reductase; OVENC, 5′-oxoaverantin (OAVN) cyclase; AVRM, averufin (AVR) monooxygenase; VHAS, versiconal hemiacetal acetate (VHA) synthase; VHOHC, versiconal (VHOH) cyclase (also called versicolorin B synthase); VHAR, versiconal hemiacetal acetate (VHA) reductase; VBD, versicolorin B (VB) desaturase; DMSTSS, demethylsterigmatocystin (DMST) synthase system; OMTI, *O*-methyltransferase I; OMTII, *O*-methyltransferase II; OAE, OrdA enzyme.

reaction: R09–R12) (**Figure 3**) and third stages (biosynthesis reaction: R13–R18) (**Figure 4**) refer from hydroxyversicolorone to versicolorin B and from versicolorin B (VB) to the formation of ultimate products, respectively. These two stages involve in the formation of hydroxy- and non–hydroxy-versicolorone, and toxins. During the aflatoxin synthesis, more than 10 nicotinamide-adenine dinucleotide phosphate reduced form (NAPDH), one nicotinamide-adenine dinucleotide (NAD), and 2*S*-adenosylmethionine (SAM) are required. These cofactors may play a critical role in the control of aflatoxin biosynthesis [7–10].

2.2. The metabolism of aflatoxins in liver

Aflatoxins synthesized in the mycelia are finally excreted into such mediums as cereals (maize, wheat, sorghum, rice, and millet), nuts (peanuts, pistachios, walnuts, Brazil nut, and coconut), spices (chili, turmeric, paprika, black pepper, and ginger), and seeds. Epidemiological studies have exhibited that AFB1 is the most common in contaminated human foods [8, 10]. Once this aflatoxin in the mediums is taken into body, it is metabolized via two-stage reactions in the liver. The first-stage metabolisms include reduction reaction (ketoreduction to aflatoxicol), oxidative reaction (O-dealkylation to aflatoxin P1), and hydrolytic reactions (hydroxylation to aflatoxin M1, aflatoxin Q1, and aflatoxin B2). This stage reaction involves numerous enzymes such as cytochromes P450 (CYP450), monooxygenases, amino-oxidases,

alcohol dehydrogenases, epoxide-hydrolases, aldehyde-reductases, and ketone-reductases. The second-stage reaction mainly comprises covalent binding reaction (toxic produces) and conjugation reaction (excretion and detoxification). Through these metabolites, aflatoxins ultimately transform into nontoxic secretions and toxic products [10, 11].

2.3. The toxification of aflatoxins in liver

Toxification of aflatoxins in liver is mainly divided into acute and chronic toxic effects. Data from epidemiological, experimental, and clinical studies have shown that above 6000 mg exposure of aflatoxin through digestion will cause acute severe liver damage and subsequent

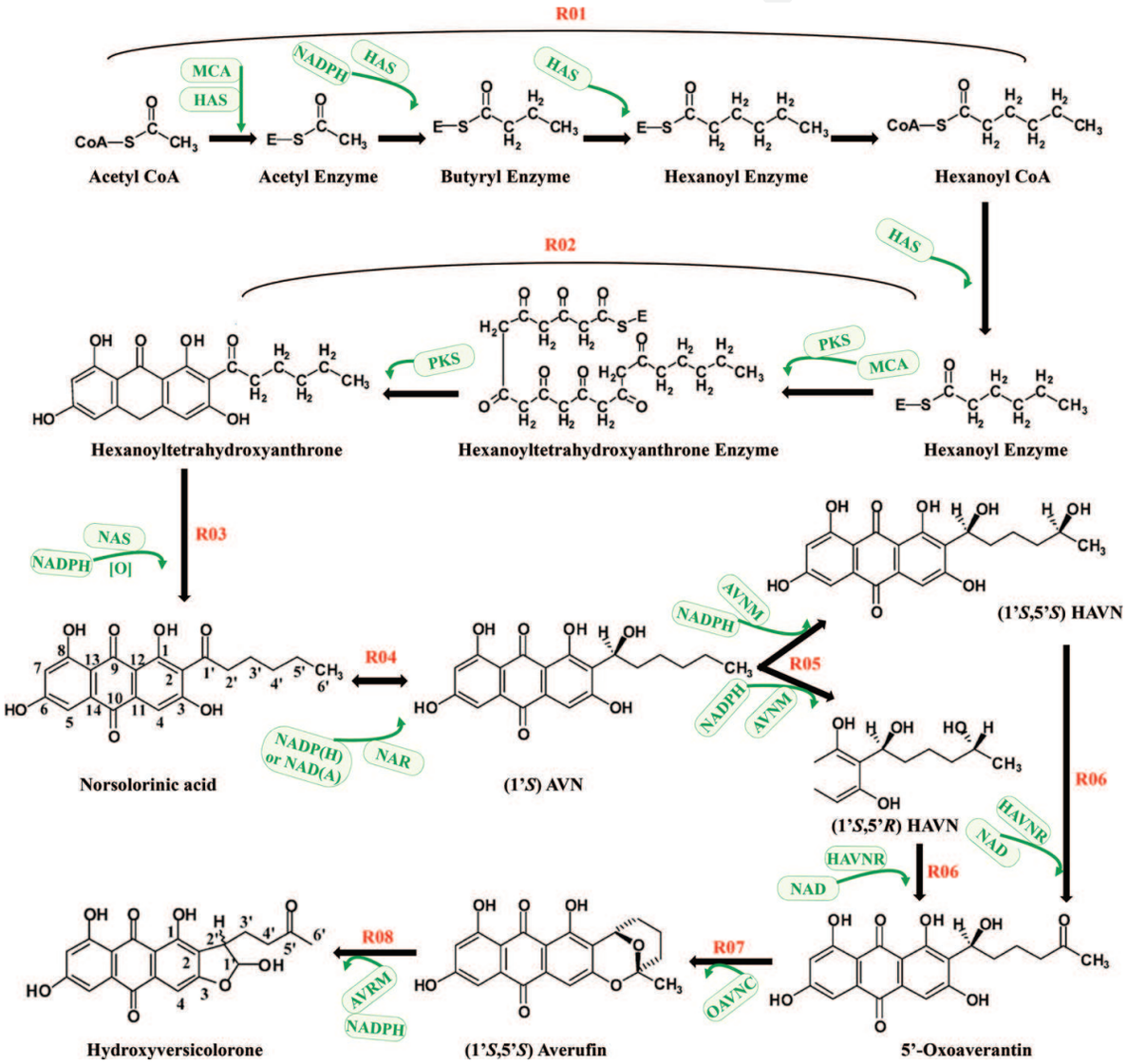

Figure 2. The first stage of aflatoxin biosynthesis. The first stage of aflatoxin biosynthesis, including the first (R01) to eighth reaction (R08) of biosynthesis, refers from acetyl CoA to hydroxyversicolorone. *Abbreviations*. MCA, malonyl CoA; HAS, hexanoate synthase (also termed fatty acid synthase); PKS, polyketide synthase; NAS, norsolorinic acid (NA) synthase; NAR, norsolorinic acid (NA) reductase; AVN, averantin; AVNM, averantin (AVN) monooxygenase; HAVN, 5′-hydroxyaverantin; HAVNR, 5′-hydroxyaverantin reductase; OVENC, 5′-oxoaverantin (OAVN) cyclase; AVRM, averufin (AVR) monooxygenase; NADP, nicotinamide adenine dinucleotide phosphate; NADPH, nicotinamide-adenine dinucleotide phosphate (reduced form); CoA, coenzyme A. *Noted:* adapted from Yabe and Nakajima [7].

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illness or death. This kind of acute effect is mainly associated with malfunction of the liver induced by toxic metabolic products. For chronic toxic effects, chronic exposure of aflatoxins can induce DNA damage and produce genotoxicity and carcinogenicity. In the past decades, increasing evidence has proved that AFB1 as aflatoxins often induce genic mutations such as TP53 and are among the most carcinogenic substances known and the major cancerous hepatocarcinoma risk factor.

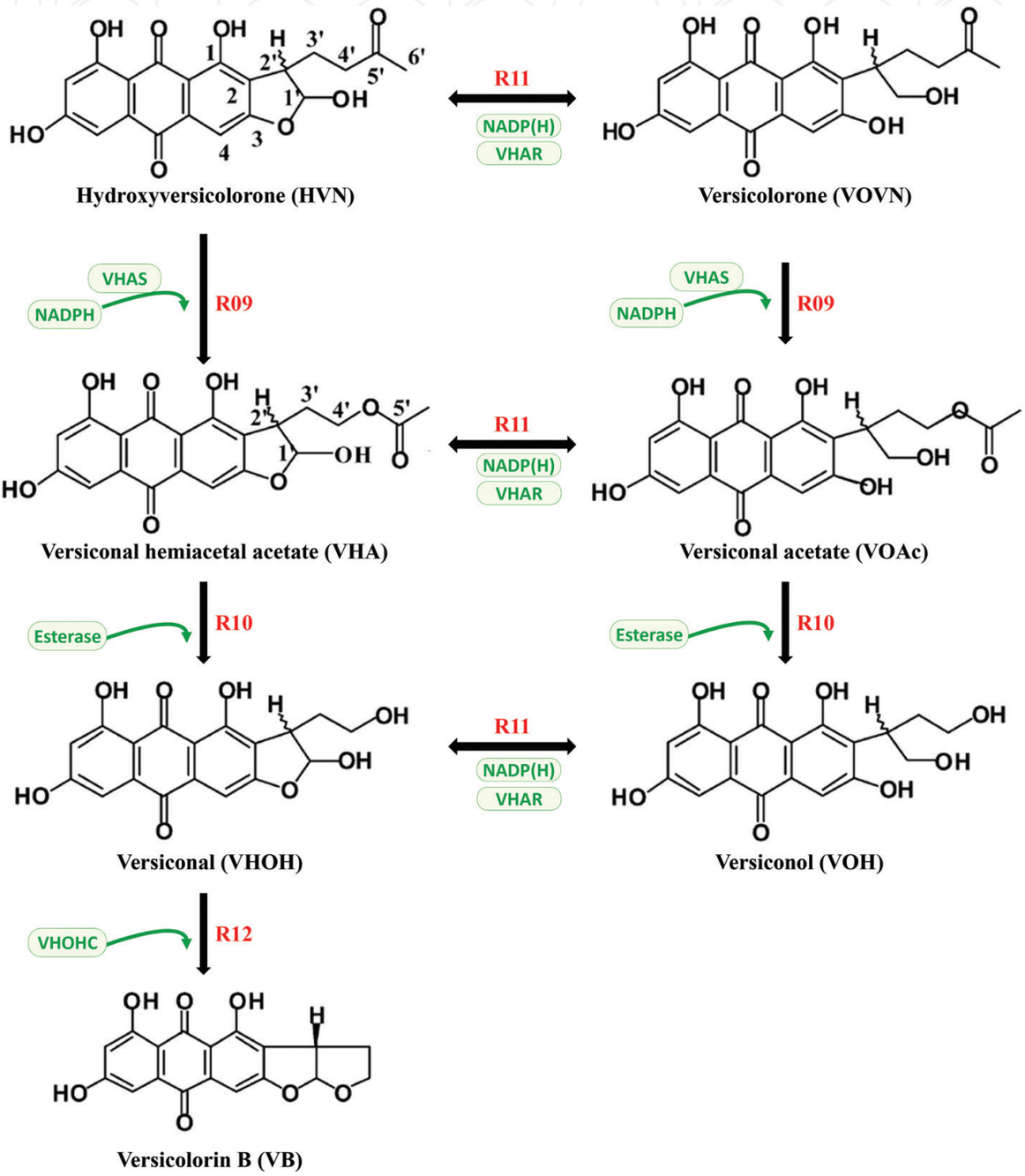

Figure 3. The second stage of aflatoxin biosynthesis. The second stage of aflatoxin biosynthesis, including the ninth (R09) to twelfth reaction (R12) of biosynthesis, refers from hydroxyversicolorone to versicolorin B (VB). *Abbreviations*. VHAS, versiconal hemiacetal acetate (VHA) synthase; VHOHC, versiconal (VHOH) cyclase (also called versicolorin B synthase); VHAR, versiconal hemiacetal acetate (VHA) reductase; NADP, nicotinamide adenine dinucleotide phosphate; NADPH, nicotinamide-adenine dinucleotide phosphate (reduced form). *Noted:* adapted from Yabe and Nakajima [7].

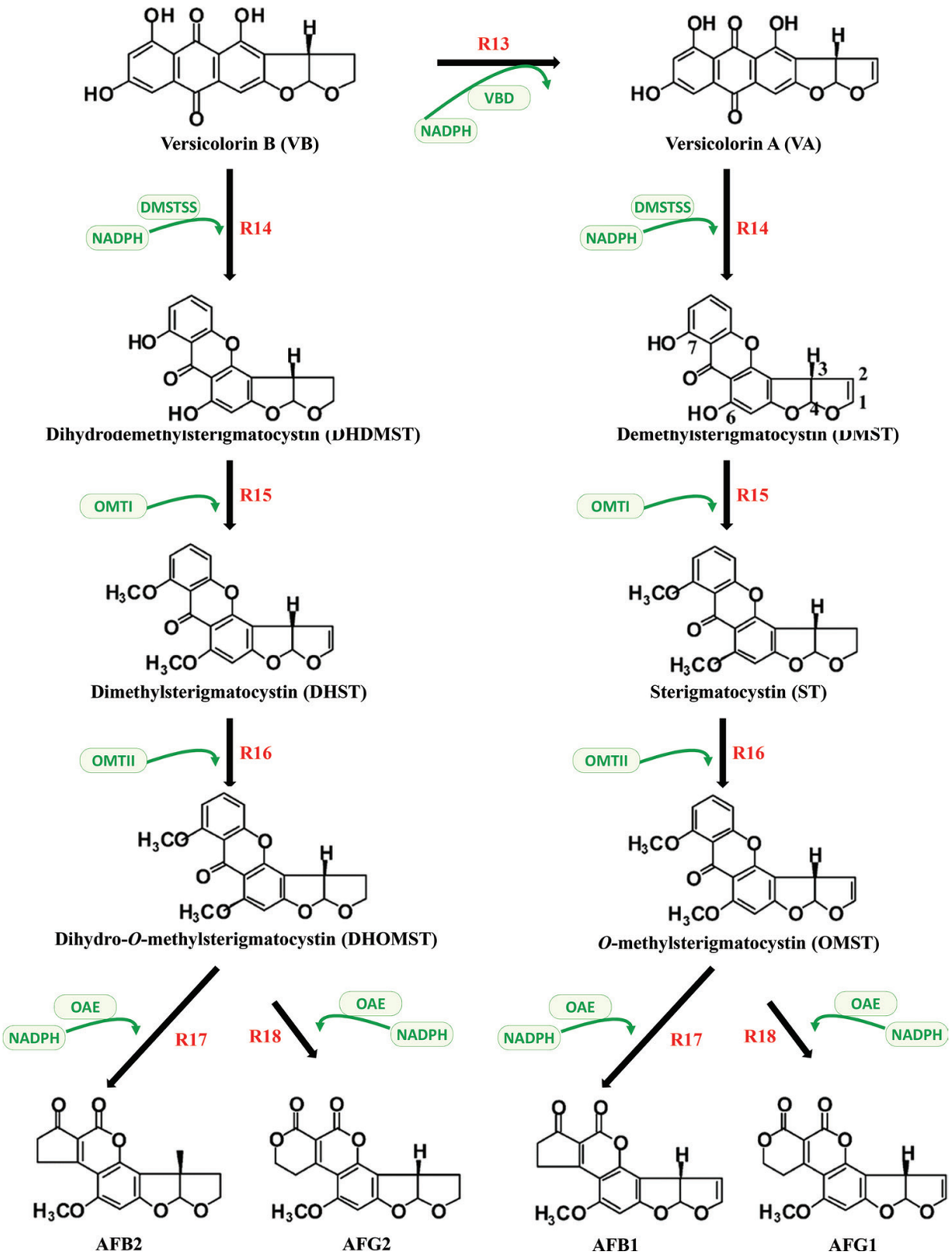

Figure 4. The third stage of aflatoxin biosynthesis. The third stage of aflatoxin biosynthesis, including the 13th (R13) to 18th reaction (R18) of biosynthesis, refers from versicolorin B (VB) to the formation of aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1), and aflatoxin G2 (AFG2). *Abbreviations*. VBD, versicolorin B (VB) desaturase; DMSTSS, demethylsterigmatocystin (DMST) synthase system; OMTI, *O*-methyltransferase I; OMTII, *O*-methyltransferase II; OAE, OrdA enzyme; NADP, nicotinamide adenine dinucleotide phosphate; NADPH, nicotinamide-adenine dinucleotide phosphate (reduced form). *Noted:* adapted from Yabe and Nakajima [7].

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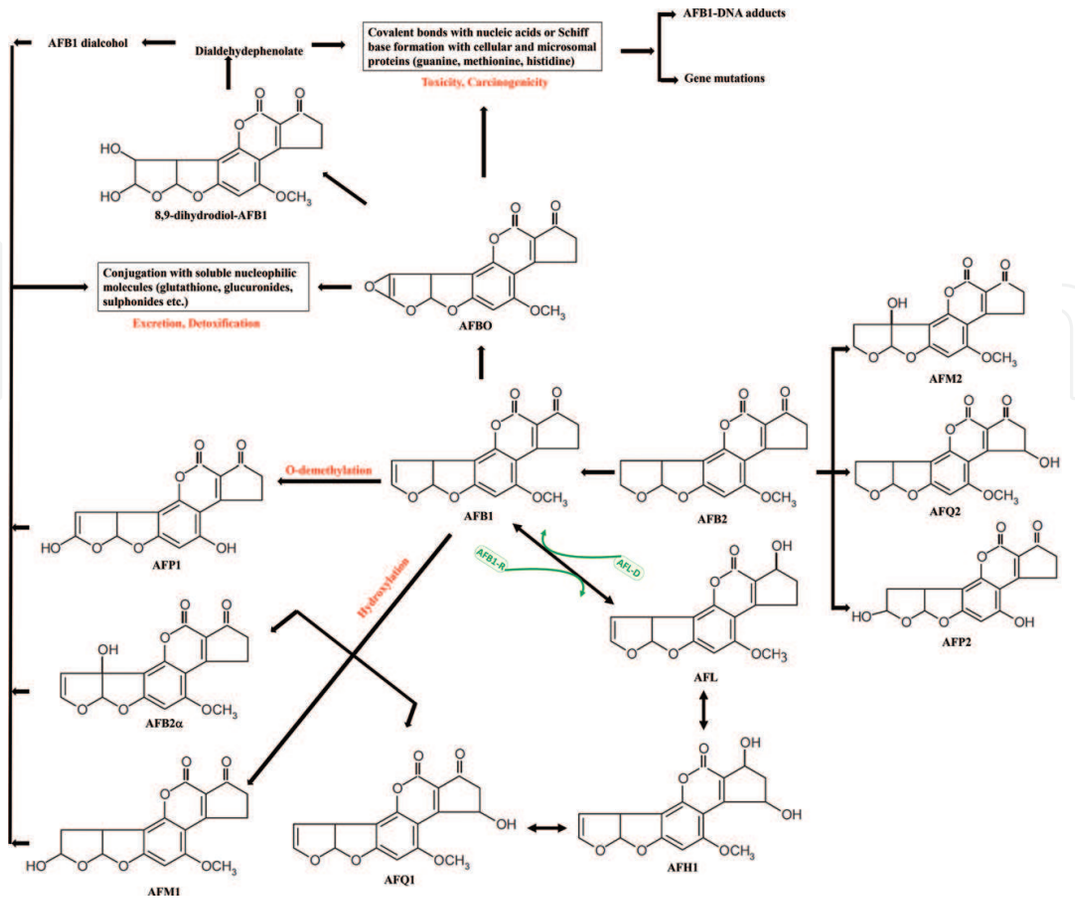

Figure 5. The metabolite of aflatoxins in the liver. Aflatoxins are metabolized via four metabolic pathways: O-dealkylation to aflatoxin P1 (AFP1), ketoreduction to aflatoxicol (AFL), epoxidation to AFB1-8,9-epoxide (AFBO, highly toxic, mutagenic, and carcinogenic), and hydroxylation to aflatoxin M1 (AFM1, highly toxic), AFP1, aflatoxin Q1 (AFQ1), or aflatoxin B2a (AFB2a). *Abbreviations*. AFM2, aflatoxin M2; AFP2, aflatoxin P2; AFQ2, aflatoxin Q2; AFL-D, aflatoxicol dehydrogenase; AFB1-R, aflatoxin B1 reductase. *Noted:* adapted from Wu and Jezkova [10].

3. The molecular mechanisms of aflatoxin-induced hepatocarcinoma

As described earlier, the main chronic toxification of aflatoxins is chronic liver damage and induced tumorigenesis of hepatocarcinoma. AFB1 has been proved as an I-type chemical carcinogen. Mechanisms of AFB1-induced hepatocarcinoma mainly involve in DNA damage and repair, the inactivation of tumor suppressor genes and the activation of oncogenes from genic mutations, abnormal immunoreaction, and inheritance alterations.

3.1. Aflatoxin-induced DNA damage

Increasing evidence has shown that the carcinogenicity of aflatoxins results from aflatoxin-induced DNA damage, including the formation of DNA adducts, DNA single strand breaks (SSBs) or double strand breaks (DSBs), chromosomal aberration damage (CAD), unscheduled DNA synthesis (USDS), abnormal chromatid exchange (ACE), the formation of micronuclei and macronuclei, and oxidation DNA damage. Of these DNA damages, AFB1-DNA adducts

are the most common damage types and consist of 8,9-dihydro-8-(N^7-guanyl)-9-hydroxy–AFB1 adduct (AFB1-GA) and ring-opened formamidopyrimidine AFB1 adduct (AFB1-FAPYA). The formation of AFB1-GA begins from AFB1 covalent binding to DNA and its product 8,9-epoxide-AFB1 (AFBE) by CYP450 [12, 13]. This adduct can automatically not only give rise to AFB1-FAYPA, which is accumulated using a time-dependence and nonenzyme pathway, but also be transferred into AFP1, AFM1, AFQ1, and other products by metabolic enzymes.

Additionally, AFB1 also induces oxidation DNA damage such as 8-oxodeoxyguanosine (8-_{oxyG}). These damages induced by aflatoxins, if not timely repaired, can cause subsequent repair-resistant adducts and depurination or lead to error-prone DNA repair resulting in DSBs, SSBs, USDs, CAD, ACE, and frame shift mutations. Interestingly, the accumulation of DNA damages is positively associated with the time and the levels of aflatoxin exposure and modifies the risk of hepatocarcinoma through regulating the expression of some genes such as a disintegrin and metalloproteinase with thrombospondin motifs 5 (ADAMTS5) [14], X-ray repair complementing 4 (XRCC4) [15], microRNA-4651 [16], and so on (**Table 1**). For example, Huang et al. [14] investigated the association between AFB1-DNA adducts via a hospital-based case control study and found increasing AFB1-DNA adducts negatively correlated with ADAMTS5 expression. It is known that ADAMTS5 may act as a tumor suppressor gene via decreasing vascular endothelial growth factor (VEGF) expression and inhibiting tumor angiogenesis and metastasis [17]. The downregulation of XRCC4 by increasing AFB1-DNA adducts decreases repair capacity for SSBs and DSBs and increases risk of tumor suppressor gene TP53 mutation and tumors [15, 18–22]. These genes progress the tumorigenesis and progression of hepatocarcinoma via regulating DNA repair capacity and angiogenesis. Although AFB1-DNA adducts are mainly produced in liver cells, they are also found in the immune cells and may regulate the immune function. Thus, DNA damage may be an important molecular event and may play a crucial role in the carcinogenesis of hepatocarcinoma caused by aflatoxins.

3.2. The mutagenesis of aflatoxins

Aflatoxin-induced DNA adducts can produce depurination, DSBs, the substitution of DNA bases, and frame shift mutations. In the past decades, the *in vivo* and *in vitro* studies have shown that the mutagenesis of aflatoxins can induce the mutation from GC to TA. As previously shown, mispairing of the aflatoxin-DNA adducts can cause both transition and transversion mutations [25–27]. In an *in vitro* non-sense analysis, Foster et al. found that the action form of AFB1 (namely AFBE) can induce more than 90% of GC to TA mutation [28]. This

Gene	Expression change	Role of change in the hepatocarcinoma carcinogenesis	Ref
ADAMTS5	Down	Angiogenesis, metastasis, prognosis	[14]
XRCC4	Down	Low DNA repair capacity, gene mutation	[15]
MicroRNA-4651	Up	Angiogenesis, metastasis, prognosis	[16]
MicroRNA-24	Up	Angiogenesis, metastasis, prognosis	[23]
MicroRNA-429	Up	Angiogenesis, metastasis, prognosis	[24]

Table 1. The change of gene expression related to DNA damage induced by aflatoxins.

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mutation was further proved to locate in the GC-rich regions via the plasmid system identifying mutational target enzyme and named as hot-spot regions for aflatoxin-induced mutations [29–31]. Results from quantitative analyses based on the *in vitro* cell model, which was transfected by pS189 (a shuttle vector having mutative targets), also showed that more than 90% of mutative spectra caused by aflatoxins was GC to TA (about 50% of mutations) and GG to TC transversion (about 30% of mutations) [32]. It has been proved that the accumulation of these transversions will result in the mutations of some important genes such as TP53 and Ras and promote hepatocarcinogenesis [31, 33].

3.3. The abnormality of tumor suppressor genes induced by aflatoxins

Studies *in vivo* and *in vitro* have examined the abnormality of tumor suppressor genes by aflatoxin exposure (**Table 2**). Among these known genes, the abnormality of TP53 induced by aflatoxins has been proved to be an important molecule change [34, 35]. In high aflatoxin-exposure areas, the mutations of TP53 gene, especially hot-spot mutation at codon 249, are present among more than 40% of patients with AFB1-related hepatocarcinoma, whereas this kind of mutation is very rare among cases with null or low AFB1 exposure [14, 36, 37]. Therefore, the mutation at codon 249 of TP53 gene has been defined as a molecular symbol for hepatocarcinoma caused by AFB1 exposure. Results from clinical sample and experimental studies further display that consistent exposure of aflatoxins may result in the accumulation of TP53 mutant protein and abnormal DNA damage repair, apoptosis, and immunoreaction [38]. Other genes such as bcl2, p27, p16, and p21 are found to produce different expression or abnormal structural change under the conditions of aflatoxin expression (**Table 2**). Taken together, inactivation of tumor suppressor genes from mutation and increasing mutant expression may be a crucial step of malignant transformation for liver cells.

3.4. The abnormality of oncogenes induced by aflatoxins

In the past decades, the abnormality of oncogenes induced by aflatoxins has mainly been focused on c-myc and ras genes, involving in the activation, expression, and mutation of proto-oncogenes (**Table 3**). For example, Tashiro et al. investigated the effects of AFB1 exposure on oncogenes based on rat model with AFB1-induced hepatomas and found that the expression of both c-myc and c-Ha-ras was upregulated in all the tumors [65]. They also observed c-Ha-ras amplification and rearrangement [65]. In Fischer rat models with AFB1- and AFG1-induced liver tumors, Sinha et al. observed that aflatoxins can induce activation of N-ras and spot mutation of G to A at codon 12 of Ki-ras [66]. This type of activation and mutation will increase in the tissues with liver cancer than those with noncancers [66–69]. Results from *in vitro* studies have further proved that aflatoxins can induce gene mutations of oncogenes [70]. Together, these data suggest that aflatoxins may activate proto-oncogenes by inducing gene mutations and promote the carcinogenesis of hepatocarcinoma.

3.5. The interaction of aflatoxins and hepatitis B virus promoting hepatocarcinogenesis

The interaction of aflatoxins and hepatitis B virus (HBV) has been proved in the carcinogenesis of hepatocarcinoma by molecular epidemiological and clinicopathological studies and sys-

Gene	Study design	Change	Significance	Ref
TP53	Mice model with HNP	Expression ↑	DNA damage ↑	[39]
bcl2	Mice model with HNP	Expression ↓	DNA damage ↑	[39]
p27	Hepatocytes *in vitro*	Expression ↓	DNA damage ↑	[40]
p21	Hepatocytes *in vitro*	Expression ↓	DNA damage ↑	[40]
TP53	HCCs (n = 223)	Expression ↑, multiplot mutation	Carcinogenesis	[41]
TP53	HCCs (n = 124)	Mutation at codon 249: 60%	Carcinogenesis	[42]
H2AX	HCC cells *in vitro*	Phosphorylation	Carcinogenesis	[43]
BP1	HCC cells *in vitro*	Phosphorylation	Carcinogenesis	[43]
TP53	HCCs (n = 52)	Mutation at codon 249: 50%	Carcinogenesis	[44]
p16	HCCs (n = 40)	Methylation	Carcinogenesis	[45]
p53	HCCs (n = 40)	Multiplot mutation	Carcinogenesis	[45]
p53	AFB1-induced mutation *in vitro*	Multiplot mutation at CpG	Carcinogenesis	[46]
TP53	HCCs (n = 64) plus a meta-analysis	Mutation at codon 249: 36%, protein accumulation: 50%	Carcinogenesis	[47]
TP53	Mice model with HNP	Multiplot mutation	Carcinogenesis	[48]
TP53	HCC cells *in vitro*	AFB1-induced mutation at codon 249 promoting IGF-II expression	Carcinogenesis	[49]
TP53	Atcc-Ccl13 *in vitro*	Mutation at codon 249	Carcinogenesis	[50]
TP53	HCCs (n = 36)	Mutation at codon 249	Carcinogenesis	[51]
TP53	Mice model	Mutation at codon 249 and 346, mutant protein increasing	Carcinogenesis	[52–57]
TP53	HCCs (n = 60)	Mutation at codon 249: 69%	Carcinogenesis	[58, 59]
TP53	Hepatocytes *in vitro*	Multiplot mutation	Carcinogenesis	[60]
TP53	HCCs (n = 110)	Mutation at codon 249: 69%	DNA damage, carcinogenesis	[61]
TP53	HCCs (n = 15)	Mutation at codon 249 and 254	Carcinogenesis	[62]
TP53	HCC cells *in vitro*	AFB1-induced Mutation at codon 249	Carcinogenesis	[63]
TP53	HCCs (n = 18)	Mutation at codon 249: 53%	Carcinogenesis	[64]

Abbreviations. HNP, hepatic neoplasms; HCC, hepatocarcinoma.

Table 2. The change information of tumor suppressor genes induced by aflatoxins in hepatic cells and hepatocarcinoma cells.

Gene	Study design	Change	Significance	Ref
N-ras	HCCs (n = 36)	Mutation at codon 61	Carcinogenesis	[51]
c-myc	Mice model with HNP	Expression ↑, amplification, rearrangement	Carcinogenesis	[65]
c-Ha-ras	Mice model with HNP	Expression ↑, amplification, rearrangement	Carcinogenesis	[65]
Ki-ras	Mice model with HNP	Activation	Carcinogenesis	[69]
N-ras	Mice model with HNP	Activation	Carcinogenesis	[66]
Ki-ras	Mice model with HNP	Mutation at codon 12	Carcinogenesis	[66]
N-ras	Mice model with HCC	Activation	Carcinogenesis	[67]
Ki-ras	Mice model with HCC	Activation	Carcinogenesis	[67]
c-Ha-ras	Mice model with HNP	Mutation at codon 61: 40–60%	Carcinogenesis	[71, 72]

Abbreviations. HNP, hepatic neoplasms; HCC, hepatocarcinoma.

Table 3. The change information of oncogenes induced by aflatoxins.

tematically reviewed by several studies [73–75]. In brief, the first clinicopathological evidence of aflatoxins interacting with HBV was provided by Yeh et al. [76]. Through a case-control study design conducted in Guangxi Area, they found that these HBV-positive individuals with high AFB1 exposure consumption featured 10-times the mortality rate compared with those with low exposure consumption. Results from multivariable interactive analyses have further convinced that AFB1 multiplicatively interacted with HBV status for promoting hepatocarcinoma risk [77–80]. For example, Williams et al. reported that the risk of developing hepatocarcinoma was 6.37 for aflatoxin exposure, 11.3 for HBV infection, and 73.0 for the combination of aflatoxin and HBV [77]. The following several molecular epidemiological studies with large-size samples from areas with high aflatoxin exposure and high HBV infection in China showed remarkably multiplicative effect for hepatocarcinoma risk (multiplicative interaction: $63.2_{\text{(both positive)}} > 1.9_{\text{(AFB1 positive)}} \times 9.5_{\text{(HBV positive)}}$ [78–80].

This interaction of two hepatocarcinogenic causes has been proved in the transgenic mice models with overexpressing HBV large envelope polypeptide [81]. Results from this study exhibited that animals will produce more rapid and extensive hepatic dysplasia and hepatocarcinoma under the conditions with aflatoxin consumption [81]. Similar findings have also shown in the studies based on woodchuck and duck models [82–84].

The aflatoxins interacting with HBV infection promoting hepatocarcinoma development mechanically involve in the following aspects. First, HBV infection directly or indirectly increases the sensitivity of hepatocytes on the toxification of aflatoxins. Evidence from observation studies have displayed that HBV-positive carriers have more amount of aflatoxin adducts than those with negative HBV status, although they are from the same high aflatoxin exposure area [85, 86]. The active product of aflatoxin AFBE is found to significantly increase the risk of viral DNA integrating into damaged DNA strand [87]. This promotes malignant transformation of damaged hepatocytes by aflatoxins. Second, HBV

infection increases the mutation frequency at codon 249 of TP53 gene and coordinates with aflatoxins for abrogating the normal functions of TP53 (such as the control of cell cycle, DNA damage repair, and cell apoptosis), which contributes to multisteps of hepatic carcinogenesis [64, 88]. Third, the HBV X gene–expressing protein inhibits base excision repair potential and results in an increasing accumulation of aflatoxin-DNA adducts [89]. Finally, HBV infections can cause hepatocytic necrosis, inflammatory proliferation, and oxygen/nitrogen active products, which may increase the likelihood of aflatoxin-induced mutations and the cellular clonal expansion containing mutations [90–92].

3.6. The interaction of aflatoxins and inheritance alterations promoting hepatocarcinogenesis

Increasing evidence has exhibited that the genetic alterations in DNA repair genes increase the amount of AFB1-DNA adducts and the frequency of hot-spot mutation at codon 249 of TP53 gene and may promote hepatic toxification of aflatoxins [1, 19, 20, 22, 37, 93–98]. Joint analyses based on meta-analyses further showed this kind of toxic effects (**Table 4**) [1, 22]. The genetic variants in other genes, such as CYP450, glutathione *S*-transferase T1 (GSTT1), glutathione *S*-transferase M1 (GSTM1), and microsomal epoxide hydrolase (HEHY), also display similar modificative effects on aflatoxin-induced hepatocarcinoma [98–101]. Interestingly, the multiplicatively interactive effects between aflatoxins and genetic alterations in these genes have been identified in the risk elucidation of hepatocarcinoma related to aflatoxins [22]. Taken together, genetic deficiency in the DNA repair and detoxification capacity may play a vital role in the carcinogenetic process of aflatoxin-induced hepatocarcinoma.

3.7. The aflatoxin-caused immunosuppression promoting hepatocarcinogenesis

Increasing evidence from *in vitro* and *in vivo* studies has proved that the immunosuppression induced by aflatoxins plays an important role in the carcinogenesis of hepatocarcinoma. Several known mechanisms may involve in this progression step. First, aflatoxins can significantly suppress the functions of macrophages via affecting the expression and secretions of cytokines such as tumor necrosis factor (TNF)-α, interleukin (IL)-1, IL-2, IL-3, IL-6, and reactive intermediates (including nitric oxide, hydrogen peroxide, and superoxide anion) [102, 103]. The suppression of macrophages by aflatoxins may be also correlated with the arrest in the G1/G0 phase [104] and altered expression of CD14 (a cell surface protein functionally regulating immunoreaction) [105]. This suppression may result in the dysregulation of the immune response and homeostasis, which contributes to the accumulation of abnormal cells with DNA damage and altered genome induced by aflatoxins, and ultimately progresses tumorigenesis. Second, aflatoxin exposure can decrease the secretion of antibody such as IgA [106]. For example, Turner et al. investigated effects of aflatoxin exposure on antibody production based on a large molecular epidemiological study [106]. In their study, they tested the levels of saliva secretory IgA (sIgA) in Gambian children (n = 472) with different degree exposure of aflatoxins and found that these individuals with high aflatoxin exposure featured lower level of sIgA in their saliva compared to those without high exposure (50.4 vs. 70.2 μg/mg protein). Finally, aflatoxins may alter T-cell functions (including decreased T-cell populations and suppressed CD4+ T-cell function) and increase individuals' susceptibility to other carcinogens [77, 107].

Gene	RS#	Genotype	TP53M			DNA adducts	
			%	Risk	*P*	Mean	*P*
XRCC1	rs25487	CC	46.51	Reference		3.276	
		CT	45.25	2.419	3.371×10^{-11}	3.264	0.899
		TT	8.24	5.028	6.651×10^{-6}	3.640	0.026
XRCC3	rs861539	GG	32.17	Reference		2.990	
		GA	43.55	1.380	0.018	3.216	0.025
		AA	24.28	1.524	0.011	3.897	4.962×10^{-14}
XRCC7	rs7003908	AA	21.24	Reference		2.879	
		AC	46.06	1.883	1.372×10^{-5}	3.347	1.663×10^{-5}
		CC	32.71	2.089	4.368×10^{-6}	3.550	1.751×10^{-8}
XRCC4	rs28383151	GG	67.03	Reference		3.308	
		GA	21.68	1.688	0.001	3.405	0.069
		AA	11.29	3.829	7.387×10^{-6}	3.721	2.867×10^{-4}
XRCC4	rs3734091	GG	72.31	Reference		3.229	
		GT	17.56	2.799	9.191×10^{-7}	3.439	0.095
		TT	10.13	5.104	3.826×10^{-6}	3.654	0.005
XPD	rs13181	TT	34.41	Reference		2.926	
		TG	41.85	1.458	0.005	3.253	0.011
		GG	23.75	1.744	0.001	4.062	4.265×10^{-6}
XPC	rs2228001	TT	34.05	Reference		3.083	
		TG	48.30	1.500	0.002	3.332	0.001
		GG	17.65	1.818	0.001	3.666	3.404×10^{-22}

Noted: Adapted from Refs. [13] and [84]. *Abbreviations*. TP53M, hot-spot mutation at codon 249 of TP53 gene; RS#, the number of polymorphism.

Table 4. Polymorphisms in DNA repair genes and HCC risk.

Altogether, the data available to date make it clear that aflatoxins can exert an immunosuppressive effect via different pathways. However, more detailed mechanisms by which this effect is mediated remain unknown.

4. Limitation and further direction

In the past decades, the advance in pathological mechanisms of aflatoxin-related hepatocarcinoma held great promise. However, we are still far from a comprehensive view of this kind of potentials. First, the detailed metabolic step and corresponding enzymes, especially the first-stage

reaction and toxicity mechanisms, have not been elucidated. Second, although the activation of aflatoxins is found to act as a crucial step, it is unclear how the tumorigenesis of hepatocarcinoma is triggered by aflatoxins. Third, the vast literature for aflatoxin-induced hepatocarcinoma mainly focuses on the studies on AFB1, and some important information may have been lost. Fourth, in spite of some evidence of AFB1 inducing abnormal immunoreaction and interacting with hepatitis virus and genetic factors, they are at the primary stage and still far from elucidation. Therefore, the detailed toxicity mechanisms of aflatoxins and corresponding carcinogenesis mechanism will greatly benefit our understanding of aflatoxin-related hepatocarcinoma.

5. Summary

It has been shown that increasing exposure of aflatoxins may promote the carcinogenesis of hepatocarcinoma. Molecular mechanisms of aflatoxin-induced hepatocarcinoma involve in DNA damage, gene mutations, the inactivation of such tumor suppressor gene as TP53, the activation of proto-oncogenes, abnormal immunoreaction, and the interaction between aflatoxins and other carcinogens such as HBV. However, an understanding of aflatoxin-induced hepatocarcinoma is far from complete, and further research in this field is looked forward to elucidating more detailed mechanisms responsible for hepatocarcinoma related to aflatoxins in the future.

Conflicts of interest and source of funding

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Abbreviations

AFB1	aflatoxin B1
AFB2	aflatoxin B2
AFG1	aflatoxin G1

AFG2	aflatoxin G2
AFP	α-fetoprotein
A. flavus	*Aspergillus flavus*
A. parasiticus	*Aspergillus parasiticus*
A. nidulans	*Aspergillus nidulans*
A. pseudotamarii	*Aspergillus pseudotamarii*
A. bombycis	*Aspergillus bombycis*
HBV	hepatitis virus B
HCV	hepatitis virus C
Hepatocarcinoma	hepatocellular carcinoma
NAPDH	nicotinamide-adenine dinucleotide phosphate reduced form
NAD	one nicotinamide-adenine dinucleotide
SAM	*S*-adenosylmethionine
CYP450	cytochromes P450

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