INTRODUCTION

Hepatocellular carcinoma (HCC) is the most common type of primary liver cancer with cirrhosis and is the major cause of death worldwide. HCCs, even though represents the 7th common malignant tumour, it is the second principal source of cancer-related mortality in the world, with a 3%-5% overall 5-year survival rate. It is estimated that nearly 60 000 deaths happen annually worldwide.

Abstract

Aflatoxins are produced by Aspergillus flavus and Aspergillus parasiticus and are toxic carcinogens. These ‘fungal molds’ grow on corn, groundnuts, cereals and other grains. Of all the aflatoxins, Aflatoxin-B1 (AFB1) is considered the most toxic. Long-term exposure of AFB1 forms DNA adducts causing many genetic mutations and epigenetic alterations, ultimately leading to hepatocellular carcinoma (HCC). The liver is the major site of Aflatoxin detoxification; wherein cytochrome P-450 (CYP450) enzymes process the AFB1 into its epoxide AFB1-Exo-8,9-Epox (ABFO) and other less toxic metabolites. ABFO, in turn, reacts with DNA, RNA and protein molecules forming AFB adducts. The AFB1-DNA adducts in turn will induce various mutations, mainly mediated by G→T transversions. Aflatoxins are also known to cause HCC cell proliferation, growth, and invasion as well as angiogenesis by various epigenetic mechanisms including DNA methylation, histone post-translational modifications and non-coding RNA deregulation, etc. In this review, we will be emphasizing on epigenetic mechanisms by which aflatoxins induce hepatocarcinogenesis. In the last section, we will also discuss various methodologies to control aflatoxin contamination and detoxification of aflatoxin adducts using natural substances that are potentially anti-aflatoxins.

KEYWORDS

aflatoxin, cirrhosis, DNA methylation, hepatocellular carcinoma, histone modifications, non-coding RNA

1 | INTRODUCTION

Hepatocellular carcinoma (HCC) is the most common type of primary liver cancer with cirrhosis and is the major cause of death worldwide. HCCs, even though represents the 7th common malignant tumour, it is the second principal source of cancer-related mortality in the world, with a 3%-5% overall 5-year survival rate. It is estimated that nearly 60 000 deaths happen annually worldwide.
because of HCC. In the United States, there has been a fourfold increase in HCC incidence over the last four decades (1.6 per 100,000 population in 1975–1977 to 4.8 per 100,000 in 2005–2007, and 6.7 per 100,000 population by 2012). A similar rate in increase has also been reported in 2016. Incidence of HCC is also high in Asia and Sub-Saharan Africa because of endemic hepatitis B, compared to the United States and other developing countries. Mongolia has the highest reported incidence at 93.7 per 100,000, but China has the greatest number of cases, because of both an elevated rate (18.3 per 100,000) and the world’s largest population (1.4 billion persons). Males get affected with HCC than females. HCC is most likely linked with hepatitis B virus (HBV) and hepatitis C virus (HCV) infection, Aflatoxin exposure, or alcohol consumption. HCC, unlike other cancers, develops because of abnormal epigenetic alterations like DNA methylations, histone post-translational modifications, and unusual non-coding RNA (ncRNA) expression. Genetic alterations like mutations in genes such as p53, genomic instability as well as certain single nucleotide polymorphisms favour HCC development and progression. Up-regulation of proteins that play a key role in cell division, cell signalling pathways like p16 and p14ARF by Rb pathway, or down-regulation of mediators of FAS pathway, or up-regulation of proto-oncogenes, or JAK-STAT or WNT signalling pathways also contribute to HCC progression.

Mycotoxins are fungal metabolites that contaminate human food and animal feed. There are more than 20 different types of Aflatoxins that occur in nature, of which aflatoxin B1 (AFB1) is the most toxic carcinogen. AFB1 causes HCC by forming DNA adducts. Liver cells are largely affected as hepatocytes, since they produce cytochrome P-450 enzymes that metabolize aflatoxins into toxic substances along with other complex chemicals. Cytochrome 450 enzymes like CYP3A4 convert aflatoxin into epoxy-aflatoxin that reacts with amines such as guanine, which damages DNA, causing cancer. Globally 83% of HCC cases occurring in Asia and sub-Saharan Africa are because of aflatoxin exposure.

2 | RISK FACTORS

Hepatocellular carcinoma develops because of mutations and epigenetic alterations that avoid normal apoptosis as well as induce uncontrollable cell division. The most prominent factors associated with HCC include hepatitis B and hepatitis C infection, chronic alcohol consumption, aflatoxin-B1-contaminated food intake, gender differences as well as metabolic disorders like diabetes, etc.

Chronic infection with HBV and HCV accounts for nearly 80% of HCCs globally. In most cases, HBV or HCV infection leads to cirrhosis, which develops into chronic hepatitis. Nearly, in 2%-5% of the patients, cirrhosis subsequently transforms into HCC. It is important to note that in HBV patients, HCC can develop without cirrhosis. In Asia and Africa, the HBV infection is prevalent, and 30%-50% of patients develop HCC without cirrhosis, whereas in the USA, wherein the HBV is not endemic, >90% of patients with HBV-associated HCC have the cirrhotic liver disease.

Metabolic disorders contribute to a major segment (32%) of the population attributable fractions over time that result in HCC development. Metabolic disorders and genetic diseases associated with HCC include Porphyrias, α-1 antitrypsin disease, tyrosinemia, hemochromatosis, glycogen-storage disease types I and II, as well as Wilson’s disease. Individuals with hemochromatosis, non-alcoholic fatty liver disease (NAFLD), type 2 diabetes (T2DM) pose an increased risk of developing HCC. NAFLD is a major risk factor for HCC in developed countries like the USA and attributes to 10%-20% of HCCs. It was proposed that NAFLD is associated with a 2.6-fold increase in HCC risk, however, rigorous population studies are missing in this regard. On the other hand, alcoholic cirrhosis is the second most common risk factor for HCC development in the USA and Europe. Furthermore, in alcoholic cirrhosis patients, older age (>55 years) and thrombocytopenia (platelet count <125 000 per mm³ of blood) become independent HCC risk factors.

Obesity and/or diabetes are the major risk factors with the highest population-attributable fraction of over 37% in the United States. Similar to HBV, NAFLD-associated HCC can also occur in the absence of cirrhosis. Diabetes increases the chance of HCC by two to threefold. Diabetes primes insulin resistance and reactive oxygen production, which are in turn leading to hepatic inflammation, triggering carcinogenesis. Besides, hereditary Hemochromatosis increases HCC risk by 100- to 200-fold. Males are 2.4 times more affected by HCC than females, suggesting a crucial role of sex in HCC progression.

Chronic exposure to the fungal toxin, AFB1, is strongly associated with HCC development. Aflatoxins are poisonous, carcinogenic mycotoxins produced from molds that grow on decaying vegetation, grains, hay and soil. When improperly stored, they grow on edible commodities such as wheat, groundnuts, sorghum, chili peppers, cottonseed, millet, sesame seeds, peanuts, rice, cassava, corn, etc and release aflatoxins. Because of inapt post-harvest processing, aflatoxin exposure to humans is prominent in several west African countries, whereas in the western countries, Aflatoxin exposure is minimal.

3 | MECHANISMS OF AFLATOXIN-INDUCED HCC

Aflatoxins once ingested, interact with and affect a wide range of biomolecules, organs and tissues. Aflatoxins mainly interact with nucleic acids and other metabolic enzyme systems. They target DNA, RNA, as well as proteins and interfere with transcription, translation and other cellular pathways (Figure 1). In the liver, enzyme CYP3A4 metabolizes AFB1 into highly reactive epoxide; AFB0, which is later transformed to AFB1-8,9-diol that binds to the lysine present in albumin forming AFB-Lysine adduct. This AFB-Lysine adduct reacts with amines such as guanine, forming AFB1-DNA adduct, which damages DNA. The evidence supports the interpretation that the formation of AFB1-DNA adducts
Effects of aflatoxins and induction of hepatocellular carcinoma. Aflatoxins react with DNA, RNA, proteins and other compounds to form adducts. These Aflatoxin adducts cause many genetic mutations and epigenetic alterations leading to the deregulation of many cellular metabolic pathways affecting growth and normal functioning of cells in hepatocytes leads to a series of mutations, mainly G:C → T:A. More importantly, it was observed that 25% of all mutations were G → T in the CGC trinucleotide context, which leads to the substitution of Arginine to Serine (R249S) in p53 gene. In the regions of high aflatoxin-exposed areas. HCCs show tp53 R249S mutation as high as 50%-90%, whereas in regions such as the USA, where aflatoxin exposure is low, R249S mutation drops down to <6% of HCCs. In addition to p53 hot-spot mutation, mutational activation of proto-oncogenes such as H-RAS is also observed in HCC.

Rieswijk et al performed the whole-genome DNA methylation changes and the whole genome transcriptomic analysis in response to AFB1 exposure and found that TXNRD1, PCNA, CCNK, DIAPH3, RAB27A and HIST1H2BF are up-regulated because of promoter hypermethylation. Hypermethylation of RASSF1A promoter was also shown to be associated with AFB1 exposure. Zhu et al identified that AFB1 causes impairment in miRNA biogenesis. These authors also showed that AFB1 down-regulates Wnt/β-catenin signalling pathway by up-regulating miR-34a and is responsible for liver tumorigenesis. AFB1 also enhances HCC cell proliferation through an IGF-2-dependent signal axis. Finally, activation of oxidative stress and inflammatory factors accounts for histopathological progression of AFB1-induced hepatocarcinogenesis. Unlike HCV- and alcohol-induced hepatocarcinogenesis, there is no clear connection between AFB1 exposure and the development of cirrhosis, indicating that the mutational actions of this toxin might be the primary driver of HCC development. AFB1 exposure often coexists with HBV infection and such individuals possess a 5 to 10-fold increased risk of developing HCC compared with exposure to only one of these factors. Recently, AFB1 exposure was also shown to increase the risk of HCC associated with HCV infection or alcohol consumption. The mechanistic basis for this synergy is not yet established, it seems plausible that cooperation would derive from AFB1-induced mutagenesis and continuous hepatocyte turnover and regeneration during chronic infection. Aflatoxin-mediated DNA damage even affects the pro-apoptotic and cellular pathways such as c-Myc, p53, NF-kB, CDK, protein kinase A (PKA), pRb, Ras, protein kinase C (PKC), BCL2, Cyclins and CKI's, deregulating the cell cycle and causing HCC. AFB1 is also known to affect other metabolites, telomere length, oxidative phosphorylation and electron transport chain of carbohydrate metabolism.

4 | EPIGENETIC MECHANISMS IN AFLATOXIN-INDUCED HCC

Epigenetics is the study of heritable changes in gene expression without undergoing any alteration in its DNA sequence. Epigenetics is a Greek word, which means 'over and above the genome', a change in the phenotype is observed without any change in its genotype. Epigenetic modifications naturally occur in the developmental
process, during cellular differentiation, but they are largely influenced by several factors such as age, environmental and lifestyle changes leading to damaging effects such as cancer.\textsuperscript{53}

Epigenetic modifications induced by the aflatoxins include aberrant DNA methylation, histone post-translational modifications (methylation, acetylation, phosphorylation, ubiquitylation and sumoylation, etc) and irregular expression of ncRNA’s (Figure 2). These epigenetic modifications will, in turn, alter the gene expression profiles leading to HCC.\textsuperscript{54-56}

\subsection*{4.1 DNA methylation}

Methylation of DNA is a natural event that occurs in both prokaryotes and eukaryotes. In the eukaryotes, DNA methylation helps to regulate the gene expression.\textsuperscript{57,58} In cancer, aberrant DNA methylation is a widespread phenomenon and may be among the earliest changes to occur during the onset of oncogenesis.\textsuperscript{59-61} It also plays crucial roles in cell cycle regulation, genomic imprinting, X-chromosome inactivation and embryonic development.

DNA methylation is generally considered as a heritable event and regulates gene expression by methylating cytosine at position-5. Methylation of critical regulatory regions such as promoters leads to the silencing of the gene expression, while the loss of methylation is associated with gene activation.\textsuperscript{62} DNA methylation occurs by a post-replication enzymatic modification and is carried by two enzymatic classes, namely maintenance methylation and de novo methylation.\textsuperscript{62} DNMT1 is said to be the maintenance methyltransferase, which during the DNA replication, copies methylation patterns to the newly synthesized daughter strand. DNMT3a and DNMT3b are the de novo methyltransferases that lead to DNA methylation, generally during the development. The methylation of cytosine occurs primarily at 5′-CpG-3′ sites in the genome.

The CpG sites or also called CG sites are the regions of DNA where a cytosine is followed by a guanine in the linear base sequence along its 5′→3′ direction. CpG islands are regions that consist of a high number of CpG sites. CpG island is the region in DNA with at least 200 bp, a GC percentage of more than 50%, and an observed to expected CpG ratio >60%. These CpG islands are frequently found in the upstream region of the gene. Many mammalian genes have CpG islands in their promoter regions (70%), which helps

\textbf{FIGURE 2} Epigenetic alterations in aflatoxin-induced HCC. Epigenetic alterations include changes in DNA methylation, histone modifications such as acetylation and methylation, and Non-coding RNAs that play a crucial role in gene regulation and expression. Aflatoxins alter these key regulatory mechanisms leading to HCC.
in gene annotation and prediction. In the human genome, it is estimated that there are about 28 million CpG islands.\textsuperscript{63,64} Methylation of multiple CpG sites in these islands of promoters leads to the stable gene silencing.\textsuperscript{65}

DNA methylation at CpG is associated with gene regulation and is known to importantly relate to many cancers, including HCC.\textsuperscript{66,67} This gene's functional regulation by methylation is done in two ways. Firstly, the DNA methylation may physically impede the transcriptional protein binding to the DNA and secondly, methyl-CpG-binding domain proteins (MBDs) may bind to these DNA methylated regions. These MBDs recruit histone deacetylases and other chromatin remodeling proteins, which modify the histones leading to the formation of heterochromatin.\textsuperscript{65}

Aflatoxins are shown to promote the methylation of the CpG islands near the promoters and bring changes in nucleosome occupancy.\textsuperscript{68} Methylation status of 92 cancer-associated genes was analysed by Zhu et al, in liver cancer patients, using the MSP method. In their study, they found that promoters of seven genes (MAGEA1, ASPH, OXCT, MTHFD2, SRP72, ENO3 and MDFI) had reduced methylation, while 25 other promoters (RASSF1A, GSPT1, SALL3, ASPH, OXCT, MTHFD2, SRP72, ENO3 and MDFI) had reduced methylation.\textsuperscript{65}

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Histone modifications play a key role in several biological processes, such as regulation of gene expression, spermatogenesis (meiosis), chromosome condensation (mitosis) as well as DNA repair.\textsuperscript{75} Of all the histone post-translational modifications, acetylation and methylation of lysine are caused because of AFB1 exposure that leads to HCC.\textsuperscript{76} Histone methylation and acetylation are crucial in gene expression and silencing. Histone methylation is done by histone methyltransferases, which can activate or repress the transcription.\textsuperscript{77} Histones are methylated on lysine (K) and arginine (R) residues only, but predominantly methylation occurs at lysine on H3 and H4 tails.\textsuperscript{78} Common histone modification sites for methylation for gene activation are H3K4, H3K48 and H3K79 and for gene inactivation are H3K9 and H3K27.\textsuperscript{79}

High exposure to Aflatoxins can alter the histone modification mechanisms causing HCC.\textsuperscript{35,44,80} Aflatoxin-induced HCC by the histone modification occurs, when Aflatoxin binds to the DNA at CpG sites, it employees proteins such as Mcp2, which on association recruits histone-modifying enzymes, such as histone deacetylases (HDAC) that condense the chromatin to block gene transcription.\textsuperscript{75,81} Histone acetylation and deacetylation also play a crucial role in gene expression initiation. When an acetyl group is added, histone DNA interaction is weakened leading to the activation of gene transcription. Acetylation is carried by a group of enzymes called histone acetyl transferases, and deacetylation is carried out by HDACs.\textsuperscript{82,83} HDACs are 12 in number (HDAC1 to HDAC11 along with Sirtuins) and are classified into four classes based on their location of action.\textsuperscript{76} Acetylation of amino acid tails induces a relaxed state of chromatin. Transcription factors (TF) can bind to the DNA promoting gene expression. Deacetylation tightly packs the nucleosomes, blocking transcriptional factors binding to DNA, suppressing the gene expression.\textsuperscript{84-86}

Several in vitro studies proved the HDAC6 role in HCC invasion and metastasis.\textsuperscript{28,87} Therefore, it can be a good biomarker for HCC progression. HDAC8 role in HCC was studied by a comparative study of HDAC8 expression levels in HCC cells and normal hepatocytes. HDAC8 repression causes inhibition of HCC cell proliferation, elevation in the expression and the acetylation of the p53 gene in lysine 382. HDAC8, when inhibited is also proved to increase the apoptotic rate in HCC cells, which can act as a good therapeutic target.\textsuperscript{88} Modifications such as histone H3 lysine9 methylation (H3K9me) or lysine27 methylation (H3K27me) are generally repressive marks, while the H3 lysine9 or lysine14 acetylation (H3K9Ac and H3K14Ac) and H3 lysine4 methylation (H3K4me) are generally considered as activation marks.\textsuperscript{89} Scientists have discovered that there is an increase in the levels of H3K27ac and H3K27me3 in HCC patients, when compared to normal individuals\textsuperscript{88} and hence can be used as markers in HCC diagnosis. AFB1 also activates AHR (Hydrocarbon receptor) in the liver that plays a major role in the high expression of HDAC8 leading to epigenetic deregulation and HCC.\textsuperscript{35,88}

4.2 | Histone modifications

Histones are alkaline proteins (proteins with basic pH) found in eukaryotic cell nuclei and are associated with DNA because of their positive charge. They help in the packaging of the DNA into structural units called ‘nucleosomes’.\textsuperscript{73} The five major families of histones are H1/H5, H2A, H2B, H3 and H4. The ‘nucleosome core’ is formed of two H2A-H2B dimmers and an H3-H4 tetramer, forming a histone octamer.\textsuperscript{74} Histones undergo post-translational modifications, which alters their interaction with the DNA and other nuclear proteins. The long N-terminal tails of H3 and H4 histones protrude out from the core of the nucleosomes, and are covalently modified at several places. The various histone modifications include methylation, acetylation, phosphorylation, ubiquitination, SUMOylation, Citrullination and ADP-ribosylation, etc. The nucleosome core containing histones H2A and H2B can also be modified. These modifications and their combinations are thought to constitute what is called ‘the histone code’.\textsuperscript{50} Histone modifications play a key role in several biological processes, such as regulation of gene expression, spermatogenesis (meiosis), chromosome condensation (mitosis) as well as DNA repair.\textsuperscript{75} Of all the histone post-translational modifications, acetylation and methylation of lysine are caused because of AFB1 exposure that leads to HCC.\textsuperscript{76} Histone methylation and acetylation are crucial in gene expression and silencing. Histone methylation is done by histone methyltransferases, which can activate or repress the transcription.\textsuperscript{77} Histones are methylated on lysine (K) and arginine (R) residues only, but predominantly methylation occurs at lysine on H3 and H4 tails.\textsuperscript{78} Common histone modification sites for methylation for gene activation are H3K4, H3K48 and H3K79 and for gene inactivation are H3K9 and H3K27.\textsuperscript{79}

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ncRNA is usually the functional RNA that is transcribed from the DNA but is not translated and does not encode any protein. Most of the genomes of mammals and other organisms are transcribed into ncRNAs. ncRNAs include microRNAs, Linc RNA and snoRNAs, etc. 95 These ncRNAs comprise internal signals that control gene expression in physiology and development by playing critical roles in epigenetic memory and chromatin architecture, RNA splicing, translation, transcription and editing. 90 A limited number of transacting small ncRNAs in prokaryotes mainly regulate mRNA translation or stability. Eukaryotes have developed RNA processing and signalling systems, which are much complex and sophisticated than in prokaryotes to regulate the expression and activation of genes. 91-93 ncRNA’s also play an important role in chromosome maintenance and segregation 94 and implicated in the control of epigenetic memory and chromatin architecture. 95 Infrastructural ncRNAs have generally well-established functions. 96 They include rRNA, tRNA, snoRNA and snRNA. Both translation and splicing require core infrastructural RNAs not only to function as sequence-specific RNA substrates but also to carry out catalytic processes. 96-99 Most of the mammalian genome with estimated 5880 human transcription clusters from both sense and anti-sense strands are known to pair with anti-sense transcripts being ncRNA. 100,101 Evidence suggests that RNA signalling supports chromatin remodeling and epigenetic memory, although the mechanisms were not established. 102 Transcription itself is regulated by ncRNAs. 103 RNA polymerase II is also known to be regulated by ncRNA signalling. 104 ncRNAs also plays an important role in stress responses. Non-coding transcript, B2, is produced by RNA polymerase III from murine, short interspersed elements (SINEs) under heat shock and this B2 RNA binds to RNA polymerase II and represses the transcription after heat shock. 105 Hence, changes caused by the Aflatoxins in these ncRNA can lead to the up or down-regulation of genes. The silencing of genes by miRNA takes place when the duplex ribbon miRNA binds with the mRNA molecule in the 3’-UTR regions of the mRNA. 106 miR-34 directly represses the p53 gene, thus regulating its function. miR-1, miR-191, miR-124, miR-125b and miR-203 are found to be silenced in HCC tissues. 107 The miRNAs can be both oncogenes or can also act as tumour suppressors. 35,105,107 Other important miRNA that play a crucial role in Aflatoxin-induced HCC is miR-21, which modulates PTEN expression and PTEN-dependent pathways, essentially enhancing the AKT pathway. miR-21 is known to promote cell proliferation, avoid apoptosis and promote invasion as well as angiogenesis. 28,108,109 miR-221 overexpression causes inhibition of HDAC6, acts as a pro-apoptotic enzyme, and also a tumour suppressor gene. 109 miR-221, if overexpressed leads to repression of apoptosis inhibitor-S protein, which is needed in activation of caspase-2 to modulate the cellular apoptosis program. Aflatoxin-induced miR-221 overexpression correlates with cellular proliferation, metastasis, and invasion in HCC. 108-110 In a study on HCC patients, miR-7-2-3p, miR-4651, miR-1273p, miR-192-5p, miR-382-5p, miR-106-5p, miR-532-5p, miR-16-5p were all overexpressed of which miR-4651 showed highest correlation with HCC progression. 28,111 Other miRNAs involved with HCC are miR26a (regulates the expression of cyclin C2 and E2 and induces G1 arrest of human liver cancer cells. Its expression is found to be reduced in HCC. Systemic administration of miR-26a inhibits cancer cell proliferation and induces apoptosis, 110 while the miR-195, miR-17-5p expression leads to proliferation and migration. 112 miRNAs are epigenetically regulated and about 60% of human proteins are regulated by these miRNAs and about 50% of these miRNAs are known to be associated with CpG islands. When these CpG islands are repressed by methylation or any other epigenetic alterations such as histone modifications, miRNA expression is also affected causing HCC. 113 Aflatoxin exposure also modulates LncRNA expression. Lv et al showed that AFB1 upregulates the expression of LncRNA-H19, which in turn promotes the growth and invasion of Hepatocarcinoma cells in vitro. 114 Data from these authors convey that AFB1 induces E2F1-mediated transcriptional upregulation of H19 and either the overexpression of E2F1 or downregulation of H19 decreases the growth and invasion of HCC cells. 114 Recent transcriptomic analysis of chickens fed with AFB1 showed differential expression of ~164 LncRNA genes in their livers, through which AFB1 was shown to regulate hepatic fat deposition and hepatocyte apoptosis. 115 Shi et al observed an extensive alteration in LncRNA expression in rat liver cells exposed to AFB1. 116 These authors compared the LncRNA expression between the control and AFB1 exposed as well as AFB1 resistant samples and confirmed that AFB1 regulates the LncRNA expression differently between the carcinogenesis and resistance pathways. 116

Circulating cell-free DNA (ccfDNA) is a fragmented form of DNA found in the blood circulation. ccfDNA can be detectable in both healthy individuals as well as patients with or without cancer. During the normal turnover of lymphoid and myeloid cells, they shed their DNA into circulation, which mainly responsible for the presence of ccfDNA in healthy individuals. 117 Whereas in the cancer patients, the DNA that sheds from the apoptotic and/or necrotic tumour cells or from the dying macrophages that had phagocytosed the necrotic tumour cells, constitutes the major amount of ccfDNA. 118 ccfDNA can be used effectively as a marker of tumour prognosis as well as to analyse the effectiveness of therapy. 118 At the population level, the Aflatoxin role in HCC development is estimated through TP53 - R249S mutation. HCC patients show a significant increase in cirrhosis of liver due to AFB1 exposure. 119,120 This hotspot mutation in p53 leads to abnormal cell proliferation. Zhang et al had recently identified mutations in ADGRB1, an orphan G-protein-coupled receptor in Aflatoxin-induced HCC. 121 By identifying mutations in the circulating DNA, it is possible to segregate the high-risk population that is exposed to Aflatoxin and can develop HCC.
and eliminate Aspergillus fungi contamination in food is essential to reduce human exposure. To minimize Aflatoxin contamination in food, many physical and chemical methods are used. These methods focus on inhibiting the sporulation and mycelia formation of fungus, inactivation of Aflatoxins by transforming them into non-toxic compounds. The most common methods include the use of synthetic fungicides, X-ray radiation, control of environmental factors during harvest and storage as well as a healthy cooking process. But the usage of synthetic chemicals is of great risk and may alter the physical properties of the food. Hence natural ways of Aflatoxin inhibition and control are being developed. The possible ways of controlling are by preventing Aflatoxin contamination, by using compounds that inhibit Aflatoxin biosynthesis and detoxification of Aflatoxins from contaminated food. Some of the bacteria and other fungi are naturally resistant to Aflatoxins, hence, the natural sources that are potentially anti-Aflatoxins that are produced by these bacteria and fungi can be isolated and used for controlling Aflatoxin growth and contamination. Many substances that can inhibit the growth and biosynthesis of Aflatoxins are isolated.

Detoxification of Aflatoxin-contaminated food can be possible by (a) removing the Aflatoxin through surface adsorption; (b) transforming Aflatoxin into the non-toxic compound; (c) inhibiting the absorption from gastrointestinal tract, and by 4) the metabolism of Aflatoxin into non-toxic compounds. Removal of AFB1-DNA adducts is possible by dietary compounds such as cruciferous vegetables such as broccoli sprouts, cabbage, which contain several compounds like di-thiol-ethiones (DTTs) that inhibit carcinogen metabolism. Glucoraphanin present in broccoli has been shown to help in detoxification of aflatoxins. Phenols such as butylated hydroxytoluene (BHT) and ellagic acid (EA) reduces the AFB1-DNA adduct levels, inhibiting mutagenesis. Other substances like indomethacin, selenium, coumarin, cafestol and kahewool, terpenes, vitamin such as β-carotene, β-apo-8’carotenal, astaxanthin and canthaxanthin, 3 methyl-cholanthrene (3-MC) and anti-cancer herbs such as oldenlandia and scutellaria effectively reduces the AFB1-DNA adducts, thus essentially decreasing the potential HCC burden.

6 | CONCLUSIONS

Hepatocellular carcinoma incidence is increasing every year and it is the 5th most common cancer in the world. Although HCC occurrence is because of multiple factors, a continuous and higher level of aflatoxin exposure leads to cancer progression. In children, it has been observed that aflatoxin exposure causes stunted growth. Of all the types of Aflatoxins in nature, AFB1 is considered most toxic. AFB1, AFB3, and other metabolites interact with various cellular components such as DNA, RNA, and they affect several cellular metabolic pathways such as protein synthesis, carbohydrate metabolism, cell signalling pathways, and electron transport chain involved in ATP production. Aflatoxin exposure mainly causes HCC as the aflatoxins are metabolized by cytochrome- P450 enzymes such as CYP 1A2/ CYP 3A4 converted into AFB Exo-8, 9-epoxide (AFBO). This AFBO epoxide is highly reactive especially towards amines and hence it readily forms adducts with various cellular components such as DNA, RNA, and proteins causing transversion of G:C to T:A on the 3rd base in codon 249 of TP53. This leads to mutations in the p53 gene. Along with these genetic mutations, aflatoxins also induce various epigenetic alterations such as DNA methylation, histone modifications and irregular expression of ncRNA. Aflatoxins induce frequent methylation of gene promoters causing activation of oncogenes and inactivation of tumour suppressor genes. Aflatoxin-induced HCC by histone modifications occurs when Aflatoxin binds to DNA at CpG sites and recruits proteins such as MeCP2, which on association recruit’s histone-modifying enzymes such as HDAC that condense chromatin and blocks the process of gene transcription. When aflatoxins induce chromatin condensation in tumour suppressor genes, those genes get post-transcriptionally repressed causing HCC. miRNAs also act as a post-transcriptional gene repressor by binding to mRNA transcript. These miRNA’s regulate many genes including the p53 gene. miRNAs are non-coding RNAs whose expression levels are also modified by Aflatoxins in HCC. Epigenetic alterations can act as both biomarkers and for systemic therapies in treating Aflatoxin-induced HCC. New biomarkers should be designed to detect early Aflatoxin exposure that can help in the prevention of HCC. Crops and grains should be devoid of Aflatoxin exposure and strict governance should be adopted. New genetically modified crops with better Aflatoxin resistance should be produced. New anti-fungal, anti-Aflatoxin pesticides should be used for crop improvement. Awareness of nutritional role in detoxifying Aflatoxins post-ingestion should be carried out intensively and new drug formulations should be carried out for controlling Aflatoxin-induced HCC deaths.

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CONFLICT OF INTEREST

No potential conflict of interest was disclosed by the author.

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REFERENCES

1. Forner A, Llovet JM, Bruix J. Hepatocellular carcinoma. Lancet. 2012;379(9822):1245-1255.
43. Chu YJ, Yang HI, Wu HC, et al. Aflatoxin B1 exposure increases the risk of hepatocellular carcinoma associated with hepatitis C virus infection or alcohol consumption. Eur J Cancer. 2018;94:37-46.

44. Dai Y, Huang K, Zhang B, Zhu L, Xu W. Aflatoxin B1-induced epigenetic alterations: an overview. Food Chem Toxicol. 2017;109(Pt 1):683-689.

45. Ueno Y, Li Y, Liang J, Hou P. Hypermethylation in gastric cancer. Crit Rev Toxicol. 1985;14(2):99-132.

46. Betina V. Structure-activity relationships among mycotoxins. Chem Biol Interact. 1989;71(2-3):105-146.

47. Stark AA. Threat assessment of mycotoxins as weapons: molecular mechanisms of acute toxicity. J Food Prot. 2005;68(6):1285-1293.

48. Shephard GS, Van Der Westhuizen L, Sewram V. Biomarkers of exposure to fumonisin mycotoxins: a review. Food Addit Contam. 2007;24(10):1196-1201.

49. Paterson RR, Lima N. Toxicology of mycotoxins. Exs. 2010;100:31-63.

50. Allis CD, Jenuwein T. The molecular hallmarks of epigenetic control. Nat Rev Genet. 2016;17(8):487-500.

51. Soshnev AA, Josepowicz SZ, Allis CD. Greater than the sum of parts: complexity of the dynamic epigenome. Mol Cell. 2016;62(5):681-694.

52. Meccariello R, Santoro A, D’Angelo S, et al. The epigenetics of the endocannabinoid system. Int J Mol Sci. 2020;21(3):1113-https://doi.org/10.3390/ijms21031113

53. Berger SL, Kouzarides T, Shiekhattar R, Shilatifard A. An operational definition of epigenetics. Genes Dev. 2009;23(7):781-783.

54. Ostojic S, Perez N, Kapovic M. A current genetic and epigenetic view on human aging mechanisms. Colloquium Antropologicum. 2009;33(2):687-699.

55. Li Y, Daniel M, Tollefsbol TO. Epigenetic regulation of cancer. Nat Rev Genet. 2016;17(8):487-500.

56. Morgan AE, Davies TJ, Mc Auley MT. The role of DNA methylation in ageing and cancer. Proc Nutr Soc. 2018;77(4):412-422.

57. Borchelli M, Ummarino S, Di Ruscio A. The bright and dark side of DNA methylation: a matter of balance. Cells. 2019;8(10):1243.

58. Culbrett M, Aschner M. Methylmercury epigenetics. Toxics. 2019;7:4.

59. Stirzaker C, Millar DS, Paul CL, et al. Extensive DNA methylation spanning the Rho promoter in retinoblastoma tumors. Cancer Res. 1999;57:2229-2237.

60. Li Y, Liang J, Hou P. Hypermethylation in gastric cancer. Clin Chim Acta. 2015;448:124–132.

61. Bouras E, Karakioulaki M, Bougioukas KI, Aivaliotis M, Tzimagiorgis G, Chourdakis M. Gene promoter methylation and cancer: an umbrella review. Gene. 2019;710:333-340.

62. Kulis M, Esteller M. DNA methylation and cancer. Adv Genet. 2010;70:27-56.

63. Sridhar GR. Impact of human genome project on medical practice. J Assoc Physicians India. 2001;49:995-998.

64. Mattick JS. The human genome and the future of medicine. Med J Australia. 2003;179(4):212-216.

65. Deaton AM, Bird A. CpG islands and the regulation of transcription. Genes Dev. 2011;25(10):1010-1022.

66. Herath NI, Leggett BA, MacDonald GA. Review of genetic and epigenetic alterations in hepatocarcinogenesis. J Gastroenterol Hepatol. 2006;21(1 Pt 1):15-21.

67. Sceusi EL, Loose DS, Wray CJ. Clinical implications of DNA methylation in hepatocellular carcinoma. HPB (Oxford). 2011;13(6):369-376.

68. You JS, Jones PA. Cancer genetics and epigenetics: two sides of the same coin? Cancer Cell. 2012;22(2):9-20.

69. Zhu JD. The altered DNA methylation pattern and its implications in liver cancer. Cell Res. 2005;15(4):272-280.

70. Toft J. DNA methylation: an introduction to the biology and the disease-associated changes of a promising biomarker. Mol Biotechnol. 2010;44(1):71-81.

71. Wang S, He Z, Li D, et al. Aberrant methylation of RUNX3 is present in aflatoxin B1-induced transformation of the L02R cell line. Toxicology. 2017;385:1-9.

72. Zhang YJ, Chen Y, Ahsan H, et al. Silencing of glutathione S-transferase P1 by promoter hypermethylation and its relationship to environmental chemical carcinogens in hepatocellular carcinoma. Cancer Lett. 2005;221(2):135-143.

73. Luger K, Mader AW, Richmond RK, Sargent DF, Richmond TJ. Crystal structure of the nucleosome core particle at 2.8 Å resolution. Nature. 1997;389(6648):251-260.

74. Strahl BD, Allis CD. The language of covalent histone modifications. Nature. 2000;403(6765):41:45.

75. Audia JE, Campbell RM. Histone modifications and cancer. Cold Spring Harbor Perspect Biol. 2016;8(4):a019521.

76. Chrun ES, Modolo F, Daniel FI. Histone modifications: A review about the presence of this epigenetic phenomenon in carcinogenesis. Pathol Res Pract. 2017;213(11):1329-1339.

77. Greer EL, Shi Y. Histone methylation: a dynamic mark in health, disease and inheritance. Nat Rev Genet. 2012;13(5):343-357.

78. Wang Y, Jia S. Degrees make all the difference: the multifunctionality of histone H4 lysine 20 methylation. Epigenetics. 2009;4(5):273-276.

79. Nicolas D, Phillips NE, Naef F. What shapes eukaryotic transcriptional bursting? Mol BioSyst. 2017;13(7):1280–1290.

80. Chappell G, Pogribny IP, Guyton KZ, Rusyn I. Epigenetic alterations induced by genotoxic occupational and environmental human chemical carcinogens: a systematic literature review. Mutat Res Rev Mutat Res. 2016;768:27-45.

81. Valinluck V, Tsai HH, Rogstad DK, Burdzy A, Bird A, Sowers LC. 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 Res. 2004;32(14):4100-4108.

82. Ma L, Chua MS, Andrisani O, So S. Epigenetics in hepatocellular carcinoma: an update and future therapy perspectives. World J Gastroenterol. 2014;20(2):333-345.

83. Zhao N, Li S, Wang R, et al. Expression of microRNA-195 is transcriptionally induced by Sp1 but inhibited by histone deacetylase 3 in hepatocellular carcinoma cells. Biochem Biophys Acta. 2016;1859(7):933-942.

84. Struhl K. Histone acetylation and transcriptional regulatory mechanisms. Genes Dev. 1999;12(5):599-606.

85. de Ruiter AJ, van Gennip AH, Caron HN, Kemp S, van Kuiilenburg AB. Histone deacetylases (HDACs): characterization of the classical HDAC family. Biochem J. 2003;370(PT 3):737-749.

86. Gallinari P, Di Marco S, Jones P, Pallaoaro M, Steinkühler C. HDACs, histone deacetylation and gene transcription: from molecular biology to cancer therapeutics. Cell Res. 2007;17(3):195-211.

87. Kanno K, Kanno S, Nitta H, et al. Overexpression of histone deacetylase 6 contributes to accelerated migration and invasion activity of hepatocellular carcinoma cells. Oncol Rep. 2012;28(3):867-873.

88. Hayashi A, Yamauchi N, Shibahara J, et al. Concurrent activation of acetylation and tri-methylation of H3K27 in a subset of hepatocellular carcinoma with aggressive behavior. PLoS One. 2014;9(3):e91330.

89. Latham JA, Dent SYR. Cross-regulation of histone modifications. Nat Struct Mol Biol. 2007;14(11):1017–1024.

90. Mattick JS, Makunin IV. Non-coding RNA. Hum Mol Genet. 2006;15(suppl_1):R17-R29.

91. Mattick JS, Gagen MJ. The evolution of controlled multitasked gene networks: the role of introns and other noncoding RNAs in the development of complex organisms. Mol Biol Evol. 2001;18(9):1611-1630.
92. Mattick JS. Challenging the dogma: the hidden layer of non-protein-coding RNAs in complex organisms. BioEssays. 2003;25(10):930-939.

93. Mattick JS, Makunin IV. Small regulatory RNAs in mammals. Hum Mol Genet. 2005;14(suppl_1):R121-R132.

94. Bernstein E, Allis CD. RNA meets chromatin. Genes Dev. 2005;19(14):1655-1665.

95. Morison IM, Ramsay JP, Spencer HG. A census of mammalian im.

96. Steitz TA, Moore PB. RNA, the first macromolecular catalyst: the ribosome is a ribozyme. Trends Biochem Sci. 2003;28(8):411-418.

97. Nilsen TW. The spliceosome: the most complex macromolecular machine in the cell. BioEssays. 2003;25(12):1147-1149.

98. Butcher SE, Brow DA. Towards understanding the catalytic core structure of the spliceosome. Biochem Soc Trans. 2005;33(Pt 3):447-449.

99. Noller HF. RNA structure: reading the ribosome. Science. 2005;309(5740):1508-1514.

100. Chen J, Sun M, Kent WJ, et al. Over 20% of human transcripts might form sense-antisense pairs. Nucleic Acids Res. 2004;32(16):4812-4820.

101. Dahary D, Elroy-Stein O, Sorek R. Naturally occurring antisense: transcriptional leakage or real overlap? Genome Res. 2005;15(3):364-368.

102. Morey C, Avner P. Employment opportunities for non-coding RNAs. FEBS Lett. 2004;567(1):27-34.

103. Corey D. Regulating mammalian transcription with RNA. Trends Biochem Sci. 2006;30(12):655-658.

104. Espinoza CA, Allen TA, Hieb AR, Kugel JF, Goodrich JA. B2 RNA binds directly to RNA polymerase II to repress transcript synthesis. Nat Struct Mol Biol. 2004;11(9):822-829.

105. Valgardsson R, Chiiodi I, Giordano M, Cobianchi F, Riva S, Biamonti G. Structural and functional characterization of noncoding repetitive RNAs transcribed in stressed human cells. Mol Biol Cell. 2005;16(6):2597-2604.

106. Eichhorn SW, Guo H, McGarvey SE, et al. mRNA destabilization is the dominant effect of mammalian microRNAs by the time substantial repression ensues. Mol Cell. 2014;56(1):104-115.

107. Shen J, Wang S, Zhang YJ, et al. Genome-wide aberrant DNA methylation of microRNA host genes in hepatocellular carcinoma. Epigenetics. 2012;7(11):1230-1237.

108. Ma D, Tao X, Gao F, Fan C, Wu D. miR-224 functions as an oncogenic RNA in heterogeneous primary hepatocellular carcinoma cells by activating AKT signaling. Oncol Lett. 2014;8(3):483-488.

109. Bae HJ, Jung KH, Eun JW, et al. MicroRNA-211 governs tumor suppressor HDAC6 to potentiate malignant progression of liver cancer. J Hepatol. 2015;63(2):408-419.

110. Imre G, Berthelet J, Heering J, et al. Apoptosis inhibitor 5 is an endogenous inhibitor of caspase-2. EMBO Rep. 2017;18(5):733-744.

111. Wu XM, Xi ZF, Liao P, et al. Diagnostic and prognostic potential of serum microRNA-4651 for patients with hepatocellular carcinoma related to aflatoxin B1. Oncotarget. 2017;8(46):81235-81249.

112. Bao L, Yan Y, Xu C, et al. MicroRNA-21 suppresses PTEN and hSulf-1 expression and promotes hepatocellular carcinoma progression through AKT/ERK pathways. Cancer Lett. 2013;337(2):226-236.

113. Bbosa G, Kitya D, Onda J, Ogwal-Okeny J. Aflatoxin metabolism, effects on epigenetic mechanisms and their role in carcinogenesis. Health. 2013;5:14-34.

114. Lv J, Yu YQ, Li SQ, Luo L, Wang Q. Aflatoxin B1 promotes cell growth and invasion in hepatocellular carcinoma HepG2 cells through H19 and E2F1. Asian Pac J Cancer Prev. 2014;15(6):2565-2570.

115. Liu X, Kumar Mishra S, Wang T, et al. AF81 induced transcriptional regulation related to apoptosis and lipid metabolism in liver of chicken. Toxins. 2020;12(5):290.

116. Shi J, He J, Lin J, et al. Distinct response of the hepatic transcriptome to Aflatoxin B1 induced hepatocellular carcinogenesis and resistance in rats. Sci Rep. 2016;22(6):31898.

117. Snyder MW, Kircher M, Hill AJ, Daza RM, Shendure J. Cell-free DNA comprises an in vivo nucleosome footprint that informs its tissues-of-origin. Cell. 2016;164(1-2):57-68.

118. Li X, Wang H, Li T, et al. Circulating tumor DNA/circulating tumor cells and the applicability in different causes induced hepatocellular carcinoma. Curr Probl Cancer. 2020;44(2):100516.

119. Jiao J, Niu W, Wang Y, et al. Prevalence of aflatoxin-associated TP53R249S mutation in hepatocellular carcinoma in hispanics in South Texas. Cancer Prev Res (Phila). 2018;11(2):103-112.

120. Marchio A, Amougou Atsama M, Béré A, et al. Droplet digital PCR detects high rate of TP53 R249S mutants in cell-free DNA of middle African patients with hepatocellular carcinoma. Clin Exp Med. 2018;18(3):421-431.

121. Zhang W, He H, Zang M, et al. Genetic features of aflatoxin-associated hepatocellular carcinoma. Gastroenterology. 2017;153(1):249-262.e2.

122. Holmes RA, Boston RS, Payne GA. Diverse inhibitors of aflatoxin biosynthesis. Appl Microbiol Biotechnol. 2008;78(4):559-572.

123. Yazdani D, Mior Ahmad ZA, Yee How T, Jaganath IB, Shahnazi S. Inhibition of aflatoxin biosynthesis in Aspergillus flavus by phenolic compounds extracted of Piper betle L. Iranian J Microb. 2013;5(4):428-433.

124. Yan S, Liang Y, Zhang J, Chen Z, Liu CM. Autoxidized linolenic acid inhibits aflatoxin biosynthesis in Aspergillus flavus via ooxylipin species. Fungal Genet Biol. 2015;81:229-237.

125. Ross RP, Morgan S, Hill C. Preservation and fermentation: past, present and future. Int J Food Microbiol. 2002;79(1-2):3-16.

126. Tian F, Chun H. Natural products for preventing and controlling aflatoxin contamination of food. 2017. https://doi.org/10.5772/intechopen.68413.

127. Wei YK, Zhao XM, Li MM, et al. Detoxification of aflatoxins on prospective approach: effect on structural, mechanical, and optical properties under pressures. Interdiscip Sci Computat Life Sci. 2018;10(2):311-319.

128. Benson AB 3rd. Oltipraz: a laboratory and clinical review. J Cell Biochem Suppl. 1993;17F:282-829.

129. Kessler TW. Chemoprevention by inducers of carcinogen de-toxication enzymes. Environ Health Perspect. 1997;105(Suppl 4):955-970.

130. Mandal S, Ahuja A, Shivarupkar NM, Cheng SJ, Groopman JD, Stoner GD. Inhibition of aflatoxin B1 mutagenesis in Salmonella typhimurium and DNA damage in cultured rat and human tracheobronchial tissues by eflagic acid. Carcinogenesis. 1987;8(11):1651-1656.

131. Allameh A. Comparison of the effect of low- and high-dose dietary butylated hydroxytoluene on microsome-mediated aflatoxin B1-DNA binding. Cancer Lett. 1997;114(1-2):217-220.

132. Carvajal-Moreno M. Metabolic changes of aflatoxin B1 to become an active carcinogen and the control of this toxin. Immunome Res. 2015;11(3): https://doi.org/10.4172/1745-7580.10000104

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