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Short communication

Aflatoxin B_1 induced upregulation of protein arginine methyltransferase 5 in human cell lines

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ABSTRACT

The exposure of naturally occurring mycotoxins affects human health and play a vital role in cancer initiation and progression. Aflatoxin B_1 is a difuranocoumarin mycotoxin, classified as a group I carcinogen. The present study was conducted to assess the effect of aflatoxin B_1 on epigenetic regulatory proteins. The protein arginine methyltransferase 5 expression was induced upon aflatoxin B_1 treatment in a dose and time dependent manner. Further global arginine methylation was also increased in the same manner. This is the first report showing the induction of epigenetic regulatory protein, protein arginine methyltransferase 5 upon aflatoxin B_1 treatment. Further study is required to establish the detailed pathway of PRMT5 induction.

Dietary exposure of aflatoxin from contaminated food is a world-wide problem. These aflatoxins are more abundant in nature and not detoxified completely through metabolic processes, can accumulate in the tissues which may adversely affect human. Aflatoxins are non-volatile, relatively low-molecular weight products produced by *Aspergillus flavus* and *Aspergillus parasiticus* (Bräse et al., 2009). It belongs to a group of difuranocoumarins mainly divided into four major types, B_1, B_2, G_1, G_2, and are known to be carcinogenic for human and animals. Among all these toxins, aflatoxin B_1 (AFB_1) is the most prevalent, and potent carcinogen (IARC, 2002). According to US Food and Drug Administration, AFB_1 is considered as an unavoidable contaminant of food but its amount can be minimized (Hamid et al., 2013).

There are two major routes where human and animals can get the exposure of this toxin, first by direct intake of aflatoxin contaminated food and second by the inhalation of conidial spores (Agag, 2004). The AFB_1 exposure can predominantly cause damage to liver, and it is considered as a potent hepatotoxic, hepatocarcinogenic and a main contributor of HCC (Groopman et al., 2005; Lewis et al., 2005). It can also cause acute damage to respiratory system, heart, kidneys and skin (Massey et al., 2000; Richard and Payne, 2003; Boonen et al., 2012).

Other than cancer, AFB_1 can cause numerous health effects such as suppression of immune system, reduction in growth rate and reproductivity. The aflatoxin act as genotoxic agent, where it cause genetic alterations by forming adduct with DNA, leading to DNA strand breakage, and this oxidative DNA damage ultimately leads to cancer (Hamid et al., 2013). The p53, a tumor suppressor gene, is mutated at third base of codon 249 of TP53 in AFB_1-related HCC patients and even in lung cancers (Hamid et al., 2013; Massey et al., 2000). In liver AFB_1 and its metabolites form adduct with DNA, this damage if not repaired before DNA replication leads to mutation. AFB_1 up regulate the expression of an onco-fetal gene H19 by activating the E2F1 transcription factor and promote cell growth and invasion in hepatocellular carcinoma (Lv et al., 2014). Even-though liver is a primary target, lungs and other organs are also affected by this mycotoxin. The epidemiological and experimental evidences supports a vital role of AFB_1 in the initiation of human lung tumorigenesis. Lung cells also biotransform the AFB_1 into DNA binding adduct (Massey et al., 2000). In lung cells, oncogene K-ras was activated due to the point mutation generated by AFB_1 and its metabolite has an integral role in tumorigenesis (Massey et al.,

Abbreviation: AFB_1, aflatoxin B_1; HCC, hepatocellular carcinoma; PRMT5, protein arginine methyltransferase 5; SDMA, symmetric dimethyl arginine; HaCaT, immortalized human keratinocytes; HepG2, human hepatoblastoma cells; HEK, human embryonic kidney cells; L-132, human embryonic pulmonary epithelial cells; E2F1, E2F transcription factor 1; CAR, constitutive androstane receptor; PXR, pregnane X receptor; AhR, aryl hydrocarbon receptor.

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2000). Dermal absorption of this aflatoxin in particular environmental condition can lead to major health risks for agricultural workers who are exposed to a mycotoxin contaminated solution (Boonen et al., 2012). Recent reports are focused on epigenetic regulation of AFB_1 and its metabolite AFB_1-exo-8,9-epoxide. The epigenetic events like DNA methylation, histone modifications, chromatin condensation, and micro RNA expression can lead to altered gene expression, cellular differentiation and growth upon induction by toxicants. Epigenetic role of aflatoxin in the development of tumorigenesis, angiogenesis, invasion and metastasis in hepatocellular carcinoma has recently been reported (Bbosa et al., 2013). The reduced oocyte development of mouse upon mycotoxin-containing diet suggested the possible mechanism causing epigenetic modifications (Zhu et al., 2014). In the present study, we have also reported the AFB_1 induced effect on an epigenetic regulatory protein.

Protein arginine methyltransferase 5 (PRMT5), is a major type II methyltransferase that symmetrically dimethylates arginine residues of proteins thereby alter the target protein function (Bedford and Clarke, 2009). PRMT5 regulates a host of cellular events and cooperates with the cellular proteins including chromatin remodelers and co-repressors which results in the transcriptional repression of critical genes involved in cell cycle regulation, tumor cell invasion and metastasis. It also has an essential role in embryogenesis (Xu et al., 2010; Karkhanis et al., 2011). Increased level of PRMT5 has been reported in leukemia, lymphoma, glioma, ovarian, breast, prostate, and lung cancer (Powers et al., 2011; Ibrahim et al., 2014; Gu et al., 2012; Nicholas et al., 2013; Yan et al., 2014). Therefore, this enzyme has generated the increased interest as a therapeutic target.

The objective of this study was to assess the effect of AFB_1 on epigenetic regulatory protein expression using different cell lines. We have used the hepatocarcinoma cells (HepG2), human lung epithelial cells (L-132), immortalized keratinocytes (HaCaT) and human embryonic kidney cells (HEK 293) for study. The cytotoxic effect of AFB_1 was assessed by MTT assay and by counting the viable cells after trypan blue exclusion staining (Fig. 1) at varying concentration of AFB_1 ranging from 1 nM to 100 μM for 24 h (Supplimentary Data). At low concentration of toxin, cell viability was increased significantly, in case of L-132, HaCaT and HepG2 cells up to 1 μM but at higher concentration of 10 μM and above, it was cytotoxic with reduced number of viable cells (Fig. 1A–C, and E). However in HEK 293 cells, viability significantly increased up to 100 nM but at higher concentration (1–100 μM) cell death was observed (Fig. 1D). The calculated IC_{50} value are 37, 29, 19, and 27 μM for L-132, HaCaT, HepG2, and HEK 293, respectively. In addition, the counting of viable cells by trypan blue exclusion assay, show that the cell number increased significantly up to 1 μM concentration (Fig. 1E). This clearly demonstrated that very low concentration of AFB_1 induced the proliferation (Al-Terehi et al., 2013) in a dose and time-dependent manner (Baptista et al., 2008). The IC_{50} value for HepG2 cells reported recently by Liu et al. (2014) is similar with our value. The studies on the cytotoxic effect of AFB_1 has been reported in different cells such as in cultured Vero cells, from African green monkey's kidney (IC_{50} value of 30 μM) (Golli-Bennour et al., 2010). For further studies we have used AFB_1 concentration well below the IC_{50} value.

As PRMT5 is overexpressed in may cancers, we wanted to explore whether AFB_1 modulate the PRMT5 function. For that, first we treated different cell lines with AFB_1 at varying concentrations and assessed the PRMT5 gene expression level. The gene expression experiment was shown only in L-132 cells with different doses of AFB_1 (10 nM, 100 nM, 1 μM) for a period of 12 and 24 h. We observed a dose- and time-dependent overexpression in PRMT5 mRNA level with significant increase at 12 and 24 h at 1 μM dose of AFB_1 (4 and 8 fold respectively, Fig. 1F, and G). Increased level of PRMT5 is reported in lung cancer cell lines as well as in tumor samples and found that its activity is essential for the proliferation of lung cancer cells (Massey et al., 2000; Gu et al., 2012). Further we analysed the PRMT5 protein level in L-132, HaCaT, HepG2, and HEK 293 cells using anti-PRMT5 antibody. Our results demonstrated that PRMT5 protein increased in a dose dependent manner as shown in Fig. 2. The significant increase in the expression of PRMT5 protein was observed at 100 nM and 1000 nM in L-132, HaCaT, and HepG2 cells (Fig. 2A, C, and D), whereas in HEK 293 cells, significant expression ($p < 0.001$) of PRMT5 was observed at 100 nM dose (Fig. 2E). A fixed dose 1000 nM of AFB_1 exposure significantly increased PRMT5 level in a time dependent manner in L-132 cells, and maximum protein expression ($p < 0.001$) was observed at 24 h (Fig. 2B).

Symmetric dimethylation of arginine (SDMA), catalysed by PRMT5 occurs at glycine- and arginine-rich (GAR) motifs on target proteins. We analysed the PRMT5 activity by checking the global arginine methylation using anti-SDMA antibody (Supplimentary Data). Cell lysates derived from AFB_1 treated cells were prepared for anti-SDMA immunoblot (Supplimentary Data). As shown in Fig. 2, a significant increase in global SDMA intensity in AFB_1 treated human cell lines was observed in a dose dependent manner with maximum intensity at 1000 nM exposure of AFB_1 in L-132, HaCaT, HepG2 cells (Fig. 2A, C and D). In case of HEK 293 cells, average SDMA intensity was increased ($p < 0.001$) at 100 nM concentration (Fig. 2E). Similarly, treatment with a 1000 nM concentration of AFB_1, significantly increased the formation of SDMA in L-132 cells, and maximum average SDMA intensity ($p < 0.001$) seen at 24 h of treatment (Fig. 2B). The identification of candidate protein is important to establish the function.

Carcinogenesis may be induced either by genotoxic or non-genotoxic mechanisms; but both cause prominent epigenetic changes (Pogribny and Rusyn, 2013). Our results suggest that AFB_1 mediate the overexpression of emerging oncoprotein PRMT5, which might play an important role in AFB_1 induced tumorigenesis. The mechanism of upregulation of PRMT5 in cancer is yet to be discovered. The studies report that PKC regulates PRMT5 expression in different cells (Kanade and Eckert, 2012; Zhang et al., 2014). The activation of PRMT5 by AFB_1 may be different levels, the AFB_1 and its metabolite could be transcriptionally activate PRMT5 gene and/or post transcriptionally modulate PRMT5 activity, but further studies are essential to establish the mechanism of up regulation of this epigenetic regulatory protein. In summary taking an advantage of cell lines our result suggest the AFB_1 mediated induction of PRMT5. It is difficult to speculate the nature of receptor that recognize AFB_1 and activation of associated signaling mechanism. However it is reported that AFB_1 associated with receptors like PXR, CAR, and AhR (Ayed-Boussema et al., 2012; Yang and Wang, 2014) in different cells. These receptors may also have role in induction of PRMT5, because it is reported that PRMT5 associated with protein complex, which is activated by CAR (Inouye, 2016) but it needs to be established by new experimental evidences. The accumulating research publications on the versatile role of PRMT5 in cancer tumorigenesis and as a marker of clinical outcome making this protein as a potential oncogene (Nicole et al., 2015; Mundade et al., 2014). Whatever it could be, mechanism of activation of AFB_1 on PRMT5 induction remains to be demonstrated throughly in different models. In short our results showing that AFB_1 modulate the epigenetic process, however detailed studies are required to establish the signaling pathway involved in this process to understand the mechanism.