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The epigenetic mechanisms in *Fusarium* mycotoxins induced toxicities

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ABSTRACT

Fusarium mycotoxins are the most economically important fungal toxins. Fumonisins, zearalenone and trichothecenes (T-2 toxin, HT-2 toxin, deoxynivalenol, nivalenol etc) are the major representatives and most studied of *Fusarium* mycotoxins. The *Fusarium* mycotoxins contaminate cereal grains, animal feeds and human food products, and cause huge economic losses and pose a threat to animal and human health globally. Depending on the type, the toxicity of *Fusarium* mycotoxins and the mechanisms have been well investigated. Epigenetic modifications (DNA methylation, histone modifications and regulation of non-coding RNA) have been implicated in various human diseases and the toxicities in animals caused by *Fusarium* mycotoxins, including carcinogenesis, genotoxicity and reproductive disorders. On the basis of recently documented data, this review discussed the relationship between the epigenetic modifications and *Fusarium* mycotoxins-induced toxicities.

1. Introduction of *Fusarium* mycotoxins

Approximately 25% of the global food and feed output is contaminated by mycotoxins according to a Food and Agriculture Organization study, of which *Fusarium* mycotoxins are the most economically important fungal toxins (Moretti et al., 2017). These mycotoxins are a large family of secondary metabolites produced by a host of *Fusarium* species, the most prevalent plant pathogens which could invade agriculturally important crops worldwide (Huang et al., 2017). Contamination of *Fusarium* mycotoxins in crops in the field or after harvesting has become a worldwide problem in common. *Fusarium* toxins contamination also occurs in prepared animal feeds and human food products (Moretti et al., 2017). The most toxicologically important classes of *Fusarium* mycotoxins with regard to human and animal health include fumonisins (FB), zearalenone (ZEA) and trichothecenes (Guo et al., 2018; Munkvold, 2017). The chemical structures of *Fusarium* mycotoxins are shown in Fig. 1.

Fusarium toxins have been reported to be associated with *Fusarium* head blight in plants. Also, these mycotoxins are able to induce both acute and chronic toxicities in animals. Typical clinical signs of acute mycotoxicoses in high doses for these toxins include alimentary toxic aleukia for T-2 toxin, abdominal distress, diarrhea and emesis in pigs for DON, pulmonary edema in pigs and equine leukoencephalomalacia in horses for fumonisins (Munkvold, 2017). Chronic exposure to low doses of *Fusarium* toxins which is more common in practice can damage the gastro-intestinal epithelial cell layer firstly. The damage is further worsen by the synergistic toxic effects due to the co-presence of *Fusarium* mycotoxins to animals, predisposing the animals more susceptible (Vejdovszky et al., 2017; Zhou et al., 2017a). These toxins can modulate the intestinal mucosal immunity, increase the permeability, and inhibit the viability and proliferation of intestinal epithelial cells (Akbari et al., 2017). After absorption, these toxins reach to the systemic compartment and exert their nephrotoxicity, hepatotoxicity, carcinogenicity, immunosuppressive activity and mutagenicity (Miller, 2016; Munkvold, 2017).

The mechanisms of *Fusarium* mycotoxins have been documented by various research studies depending on the type of toxin. For studying the mechanism of mycotoxins, a number of techniques like autophagy and epigenetic alterations have been used which now become a trend of toxicology studies. For example, some researches have been carried out to reveal the relationship of aflatoxin B1 (AFB1) exposure with the epigenetic modifications, particularly the DNA methylation in the

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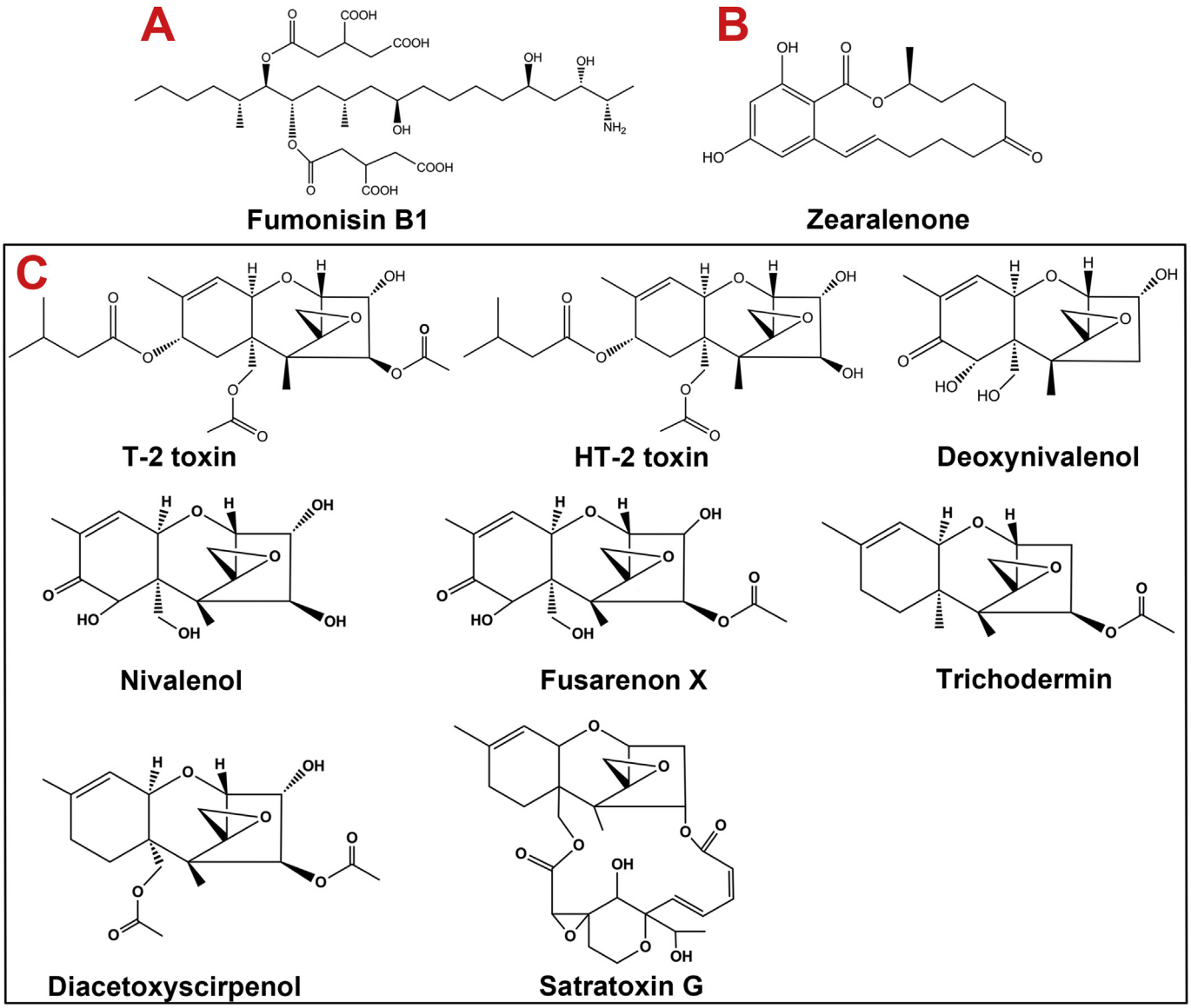

Fig. 1. The chemical structures of *Fusarium* mycotoxins. (A) Fumonisin B1. (B) Zearalenone. (C) Trichothecenes are sub-divided into types A, B, C and D. Type A trichothecenes include T-2 toxin, HT-2 toxin and diacetoxyscirpenol. Type B trichothecenes include fusarenon X, deoxynivalenol and nivalenol. Satratoxin G belong to Type D trichothecenes.

development of hepatocellular carcinoma in human (Rieswijk et al., 2016). Ochratoxin A (OTA) carcinogenicity involves a complex network of epigenetic mechanisms as well (Limbeck et al., 2018; Zhou et al., 2017b). Comprehensive knowledge regarding the epigenetic alterations induced by AFB1 and OTA has been summarized in excellent reviews (Dai et al., 2017; Zhu et al., 2017). A number of evidences indicated that epigenetic modifications play an important role in the toxicity of *Fusarium* mycotoxins, yet no efforts have been made to summarize these information. This review aims to gather the associated information and shape up the epigenetic regulating network of most important *Fusarium* mycotoxins.

2. Approach to literature review

For the purpose of this review, a systematic literature search using PubMed, Web of Science and Google Scholar was performed on 24 April 2018 and again on 10 August 2018 to gather the relevant studies related to the epigenetic alterations induced by the important *Fusarium* mycotoxins. In both title and abstract, this search used the following keywords: fusarium mycotoxins and epigenetic modifications (10 ones), fumonisin B1 and epigenetic (13 ones), fumonisin B1 and DNA methylation (14 ones), fumonisin B1 and histone modifications (6 ones), fumonisin B1 and microRNA or miRNA (2 ones), Fumonisin B1 and H2AX (one), Zearalenone and epigenetic (17 ones), Zearalenone and DNA methylation (17 ones), Zearalenone and histone modifications (7 ones), Zearalenone and microRNA or miRNA (2 ones), Zearalenone and H2AX (3 ones), trichothecenes and epigenetic (8 ones), trichothecenes and DNA methylation (6 ones), trichothecenes and histone modifications (6 ones), trichothecenes and microRNA or miRNA (one), trichothecenes and H2AX (3 ones). The articles searched in every keywords were summarized, and the duplicate articles were checked and removed. The articles on the epigenetic alterations caused by non-Fusarium mycotoxins were excluded. The empirical investigations (specific focus on the epigenetic modifications and Fusarium mycotoxins) which were peer-reviewed and published in a scientific journal were considered.

3. Epigenetic modifications

"Epigenetic", literally refers to "outside conventional genetics",

which means the stable alterations in gene expression that do not result from an alteration in the DNA sequence itself (Vrtacnik et al., 2014). Epigenetic modifications are essential for the normal development and maintenance of tissue-specific gene expression patterns in mammals. While aberrant epigenetic modifications tend to render the genome more vulnerable to environment, and may activate various gene expression, including the oncogenes, or silence expression of particularly the tumor suppressor genes (TSGs) (Dai et al., 2017). Epigenetic modifications have been associated with human diseases, including cancers, cardiovascular diseases, neurological disorders, diabetes and asthma, and has also been considered an underlying mechanism in toxic chemicals and mycotoxins mediated toxicity and carcinogenesis (Ostry et al., 2017; Portela and Esteller, 2010). Besides, epigenetic alterations often precede disease pathology and can be detected in stool, urine, blood and diseased tissues, which makes them valuable diagnostic indicators in early detection of diseases or prognostic indicators for disease progression (Kelly et al., 2010; Yokoi et al., 2017). Epigenetic modifications mainly include DNA methylation, histone modifications, nucleosome positioning and expression of non-coding RNA such as microRNAs (miRNAs) and long intergenic non-coding RNAs (lincRNA). These modifications to DNA and histones and nucleosome positioning can determine accessibility of the activators and transcription factors to their site on DNA, thereby affecting the gene transcription. miRNAs mainly act to inhibit protein translation of the target mRNA by binding to the 3' -untranslated region of mRNA with a base pairing mechanism and degrade it.

4. Fumonisin B1

Fumonisins (FB) is a group of mycotoxins mainly produced by *Fusarium* verticillioides, with Fumonisins B1 (FB1) being the most toxic one. Existing evidence has suggested that FB1 promoted the carcinogenesis in the liver and kidney of rats and mice, which was linked to human oesophageal and liver cancer in China and South Africa (Sun et al., 2007). In animals, FB1 also causes an acute pulmonary edema in pigs and equine leucoence phalomalacia. Fumonisins toxicity is believed to derive from its ability to inhibit ceramide synthase, resulting in disruption of sphingolipid biosynthesis (Singh and Kang, 2017). However, this may not fully explain the toxicity of FB1, especially the carcinogenic properties of this mycotoxin. In recent years, the epigenetic mechanisms of FB1 have been investigated that epigenetic modifications are widely involved in the carcinogenesis and this may explain the carcinogenic potency of FB1 (Bayoglu et al., 2017).

4.1. DNA methylation and Fumonisin B1 toxicity

DNA methylation is one of the most common epigenetic events occurring in cytosine and adenine of mammalian DNA, while adenine methylation received considerably less attention (Dai et al., 2017). DNA methylation that occurs in CpG islands of gene promoter typically acts to repress gene transcription. The CpG islands are regions with (1) a length greater than 200 bp, (2) a GC content greater than 50%, (3) a ratio of observed/expected CpG greater than 0.6, according to a typical definition (Gardinergarden and Frommer, 1987). DNA methylation is brought about by a series of enzymes known as DNA methyltransferases (DNMTs), which catalyze the process that a methyl group is selectively added to the fifth carbon of the cytosine in 5′-CG-3′ dinucleotide, forming the 5-methylcytosine (5 mC) (see Fig. 2). The main active DNMTs include DNMT1, DNMT3a and 3b. DNMT1 possess de novo (when CpG dinucleotides on both DNA strands are unmethylated) as well as maintenance (when CpG dinucleotides on one strand are methylated) methyltransferase activity, while DNMT3a and 3b are de novo methyltransferases. On the other hand, a mammalian methyl DNA binding protein MBD2, as well as 5-methylcytosine glycosylase is shown to act as demethylases to activate transcription.

In the last decade, some studies reported that FB1 could induce DNA methylation in various cells. In human intestinal Caco-2 cells, FB1 (10, 20, 40 μM for 24 h) was shown to significantly increase the DNA methylation by raising the percentage of 5 mC in genomic DNA from 4.5% to 9% (Kouadio et al., 2007). In rat C6 glioma cells, FB1 induced significant DNA hypermethylation after 24 h of exposure time at the doses of 9 and 18 μM (Mobio et al., 2000). However, in rat liver cells Clone 9 and kidney epithelial cells (NRK-52E), FB1 (1–50 μM) exposure for 24 h did not reveal significant dose-related effects on global DNA methylation compared with controls (Demirel et al., 2015). CpG promoter DNA methylation of specific TSGs (*p15*, *p16*, *VHL*, *c-Myc*, *e-cadherin*) were also evaluated in this study, and the results revealed that *VHL* gene was methylated in its CpG promoter region in both cells, while *c-Myc* and *p16* gene were methylated in Clone 9 or NRK-52E alone, respectively (Demirel et al., 2015). In contrary, in human hepatocellular carcinoma HepG2 cells, FB1 (200 μM) treatment for 24 h significantly induced global DNA hypomethylation and histone demethylation which could cause chromatin instability and liver tumourigenesis. FB1 significantly decreased the expression and activities of DNMT1, DNMT3a and DNMT3b, but increased the expression and activity of demethylases MBD2. Furthermore, FB1 induced a significant up-regulation of histone demethylase KDM5B and KDM5C (Chuturgoon et al., 2014a). These discrepancies may result from different reasons. It has been known that DNA methylation can control gene expression in a cell type- and tissue-specific manner during differentiation, leading to different phenotypes (Hodjat et al., 2017). The heterogeneity in the different type of cells chosen in the above studies may primarily lead to the discrepancy results. In addition, the doses of the FB1 may be another causative factor, because in the previous studies of Kouadio et al. (2007), Mobio et al. (2000) and Demirel et al. (2015), the exposure of the cells to low doses FB1 (< 50 μM) resulted in DNA hypermethylation or methylation of specific TSGs, while relatively high dose FB1 (> 200 μM) exposure led to DNA hypomethylation. The global DNA hypermethylation in FB1-induced toxicity remains unclear so far, and only detecting the global DNA hypermethylation seems not sufficient to explain the specific toxic effects of FB1. Furthermore, the above discrepancy reports suggest that more detailed studies in DNA hypermethylation in other various cell types are dire need of the day. However, to study the aberrant promoter hypermethylation of TSGs as well as genome-wide hypomethylation in FB1-treated cells will be of significance, since this can lead to the inactivation of TSGs and affect the genome stability, which both increase the chance to develop cancers. Gene-specific hypermethylation at the CpG islands of certain promoters can interfere with the gene expression involved in main cellular pathways like DNA repair (*Hmlh1, MGMT, WRN, BRCA1*), Ras signaling (*RASSF1A, NOREIA*), cell cycle control ($p16^{INK4a}$, $p15^{INK4b}$, *RB*), p53 network (*p53,* $p14^{ARF}$, *p73, HIC-1*) and apoptosis (*TMS1, DAPK1, WIF-1, SFRP1*) (Patra, 2008). Meanwhile, a massive global loss of DNA methylation has been a hallmark in cancer cells. The DNA methylation in TSGs and global hypomethylation seem to explain the promoting effect of carcinogenesis induced by FB1.

4.2. Histone modifications and Fumonisin B1 toxicity

Histone modifications are covalent post-translational modification (PTM) that mediates changes in chromatin structure and gene expression. Two copies of each of the core histone H2A, H2B, H3 and H4 form the core structure of nucleosomes, which are the fundamental repeating units of eukaryotic chromatin (Portela and Esteller, 2010). All of the histones have a globular C-terminal and unstructured N-terminal tails that protrude from the nucleosome. Histone modification that occurs within the N-terminal tail include methylation of arginine (R), methylation, acetylation, ubiquitination and sumoylation of lysines (K), and phosphorylation of serine (S), threonine (T) and tyrosines (Y) (Hodjat et al., 2017). Among the histone modifications implicated in gene transcription, the best characterized ones belong to histone acetylation (ac) and methylation of histone H3 and H4 at lysine residues. The histone methylation includes mono-methylation (me), di-methylation

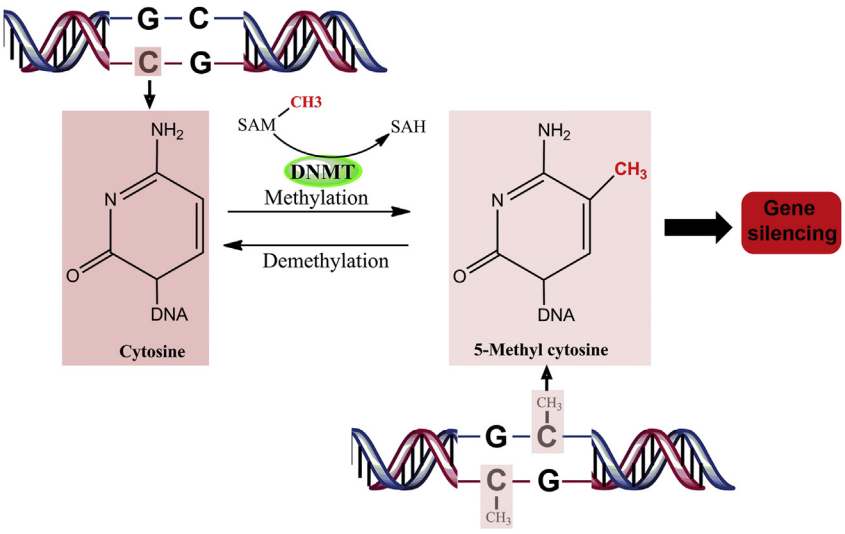

Fig. 2. The DNA methylation process catalyzed by DNMTs. DNA methylation is a process by which a methyl group is selectively added to the fifth carbon of the cytosine in 5′-CG-3′ dinucleotide, forming the 5-methylcytosine. DNMTs can catalyze the transfer of a methyl group from S-adenosyl-methionine (SAM) to DNA, which induce hypermethylation and silencing of genes (Smith and Meissner, 2013).

(me2) and tri-methylation (me3). Generally, methylation of histone H3/H4 on lysine 4, 36, 79 and 20 (H3K4, H3K36, H3K79 and H4K20) and high levels of acetylation are associated with an "open" euchromatin structure and transcription activation, whereas H3K9, H3K20 and H3K27 methylations and low levels of acetylation are associated with a "closed" heterochromatin structure and gene silencing (Ren et al., 2011). Histone phosphorylation, as another type PTM of histones, has been shown to modify the chromatin structure by changing the protein/DNA or protein/protein interactions. Histone phosphorylation is a transient histone modification and can be induced by extracellular signals, DNA damage or entry into mitosis (Sawicka and Seiser, 2014). All the core histones H2A, H2B, H3 and H4 and the linker histone H1 can be phosphorylated, while the most-studied histone phosphorylation is that of H2AX (Rossetto et al., 2012). The histone H2AX phosphorylation to γ-H2AX, performed mainly by the ataxia telangiectasia mutated (ATM), is a sensitive marker of DNA double strand breaks (DSB) and a predictor of cancer risk (Matthaios et al., 2013).

FB1 has been reported to change the histone modification profiles. Previous study have shown that FB1 (25, 50 and 100 μM) induced a significant increase in the level of global H3K9me2 and H3K9me3, and a decrease in the level of H4K20me3 in rat kidney epithelial cells. Furthermore, FB1 significantly decreased the acetylation level of H3K9 (Sancak and Ozden, 2015). Another study also reported that a combination of a methyl-deficient diet and FB1 exposure in pregnant rats increased the H3K9me3 level and decreased the H4K20me3 level (Pellanda et al., 2012). All these effects pointed to a probability that FB1 could lead to a condensed heterochromatin structure, which resulted in inactive gene expression of particularly TSGs. However, the study of Chuturgoon et al. (2014b) indicated that FB1 significantly increased the expression of two histone demethylase genes *KDM5B* and *KDM5C*, which may result in increased H3K4me3/me2 demethylation. But this is not observed when rat kidney epithelial cells were treated with FB1 (5–100 μM) for 24 h in the study of Sancak and Ozden (2015).

Histone phosphorylation, as a unique epigenetic form, also contributes to the toxicity of FB1. The study of Chuturgoon et al. (2015) showed that 200 μM FB1 treatment in HepG2 cells caused a significant decrease in phosphorylated γ-H2AX and cleaved poly ADP-ribose polymerase (PARP) levels. In addition, FB1 inhibited the apoptosis of the cells. The decrease in phosphorylated γ-H2AX levels would make the cells lack sufficient ability to maintain genomic stability and to activate appropriate DNA damage responses, leading to gene mutation or tumorigenesis (Matthaios et al., 2013). The decreased cleaved PARP (a DNA repair enzyme involved in apoptosis) levels also demonstrated that FB1 treated cells lack the ability to repair the damaged DNA, which can lead to uncontrolled cell proliferation, malignant transformation and eventually to liver tumorigenesis.

4.3. MiRNAs and Fumonisin B1 toxicity

Few studies were conducted to reveal the role of non-coding RNA in the toxicity of FB1. The only study, as far as we know, reported that in HepG2 cells, FB1 significantly down-regulated the expression of miR-27b, and up-regulated the expression of CYP1B1 that catalyzes the metabolic activation of many pro-carcinogens. Furthermore, miR-27b post-transcriptionally regulated CYP1B1 expression in HepG2 cells (Chuturgoon et al., 2014b), indicating that modulation of miR-27b by FB1 may be a contributor in hepatic neoplastic transformation.

5. Zearalenone

Zearalenone (ZEA) is a non-steroidal estrogen-like mycotoxin produced by *Fusarium* graminearum, F. culmorum, F. crookwellense, F. equiseti and F. semitectum. The major and well-documented toxicity of ZEA is the reproductive disorders in several mammalian species like pigs, which is due to that ZEA can completely bind to estrogen receptors (ERs) ER-α and ER-β (Zinedine et al., 2007). A previous study has reported that ZEA and its metabolites α-zearalenol and β-zearalenol could increase the production of progesterone, estradiol, testosterone and cortisol hormones in human adrenocortical carcinoma cells (Frizzell et al., 2011). ZEN treatment in mice during early pregnancy led to obstruction in establishing and maintaining pregnancy, and activation of luteal function in a manner similar with 17β-estradiol. ZEA also induced delayed implantation, loss of conceptuses and retarded growth of the fetuses (Kunishige et al., 2017).

5.1. DNA methylation, histone modifications and zearalenone toxicity

The previous studies have indicated that the changes in chromatin structure mediated by epigenetic modifications could affect the reprogramming of gene expression and were related to the acquisition of meiotic and developmental competence during oocyte maturation (Akiyama et al., 2004; De La Fuente, 2006). The estrogen-like effect of ZEA is probably related to the epigenetic modifications. In porcine oocytes, ZEA (30 μM) treatment disrupted the oocyte maturation and early embryonic development. It also significantly increased the global 5 mC level and the gene expression of DNMT3a and DNMT3b. The global levels of H3K4me2, H3K9me3, H3K27me3 and gene levels of their histone methyltransferase *ASH2*, *SUV39H2*, *EZH2* and *EED* were all significantly increased (Han et al., 2015). Zhang et al. (2017a)

investigated the mechanism of ZEA on mammalian ovarian folliculogenesis. It showed that ZEA (10 and 30 μM) exposure to newborn mouse ovaries led to remarkably higher level of DNA methylation in the CpG site of the *LHX8* gene and accordingly decreased its expression. LHX8 protein is specifically expressed in oocytes and is a transcription factor connected with early stages of ovarian follicle formation (Ren et al., 2015). The DNA methylation of *LHX8* gene would impair the primordial follicle formation, and also indicated that estrogenic compounds could affect epigenetic genomic changes in oocytes and contributed to the down-regulation of specific gene mRNA abundance (Ren et al., 2015). Another study investigated the effect of ZEA on mouse egg developmental competence, and the results showed that ZEA (10 and 50 μM) increased the 5 mC level, and decreased the levels of H3K4me2, H3K9me3 and H4K20me1, me2, me3 (Zhu et al., 2014).

ZEA has been suggested to be an airborne mycotoxin. In Dalian, China, the daily inhaled ZEA by a worker in a poultry house could reached 17.432–20.512 ng (Wang et al., 2008). Thus, a study has been carried out to investigate the epigenetic mechanisms of ZEA in human bronchial epithelial BEAS-2B cells. It was shown that 40 μM ZEA significantly decreased the global DNA methylation to 53.09% relative to controls, and also down-regulated the expression of HDAC1 and 2. Besides, ZEA induced the DNA damage and inhibited the expression of genes *RAD51 and BLM* that is related to DNA repair in the BEAS-2B cells (So et al., 2014). Previous report also showed that in the lung of patients with chronic obstructive pulmonary disease, the activity and expression of particularly of HDAC1 and 2 is reduced, which resulted in increased expression of multiple inflammatory genes (Barnes, 2006). The decrease in global DNA methylation and the down-regulation of HDAC1 and 2 suggested that ZEA could induce transcription activation and genome stability, which may activate the expression of some oncogenes. The DNA damage and inhibition of DNA repair by ZEA in BEAS-2B cells can cause mutations in critical TSGs which promote the transformation of normal cells into cancerous cells and cause lung carcinogenesis.

The reproductive toxicity of ZEA is probably related to the histone phosphorylation. In the study of Zhang et al. (2017a), the treatment of murine newborn ovaries with 10 and 30 μM ZEA in vitro decreased the numbers of germ cells and PARP1 positive cells, and increased the numbers of apoptotic and γ-H2AX positive cells within mouse ovaries. In porcine granulosa cells, 10 and 30 μM ZEA treatment also induced apoptosis, increased the percentage of γ-H2AX positive cells, and increased the expression of genes, *BRCA1, RAD51 and PRKDC* that are related to DNA damage and repair (Liu et al., 2018).

5.2. MiRNAs and zearalenone toxicity

The miRNAs have been suggested to influence the synthesis and secretion of pituitary hormones, and also involved in the reproductive disorder in animals (Cao et al., 2018; Das and Kumar, 2018). A recent study reported that miR-7 could be overexpressed in the pig pituitary after administration of ZEA at a dose of 7.5 mg/kg body weight in vivo, and in cultured porcine pituitary primary cells in vitro at a dose of 1 μM of ZEA. ZEA regulated the miR-7 expression through protein kinase C (PKC) and p38 signal pathway. The overexpression of miR-7 further mediated the inhibition of follicle-stimulating hormone (FSH) synthesis and secretion by ZEA, and the FOS gene (FBJ murine osteosarcoma viral oncogene homolog) acted as a direct target of miR-7 to regulate this process (He et al., 2018). The miRNAs expression in liver and colon (including ascending and descending colon) of sexually immature gilts treated with ZEA (40 μg/kg/d) was analyzed. In the liver, ZEA treatment for 35 days resulted in a significant increase in the expression of miR-15a, miR-21 and miR-192. In ascending colon, ZEA treatment for 42 days increased the expression of miR-15a, miR-34a and miR-192. However, in descending colon, only the miR-15a was significantly increased after ZEA treatment for 35 days (Brzuzan et al., 2015). The miR-15a and miR-34a primarily function as tumor suppressors and are often down-regulated in multiple types of tumor cells. Overexpression of them can result in growth arrest, apoptosis and inhibition of migration and invasion of cancer cells (Jin et al., 2018). The miR-192 is also a tumor suppressor that has a significant effect on cell cycle control and cell proliferation with a p53-dependent manner (Wang et al., 2016). The miR-21 is an oncogenic miRNA that is highly overexpressed in multiple types of tumors. Inhibition of miR-21 can lead to reduced cell growth and apoptosis by promoting the expression of tumor suppressors, phosphatase and tensin homolog (PTEN) and protein programmed cell death 4 (PDCD4) (Zhao et al., 2018).

6. Trichothecenes

Trichothecenes are a family of toxic mycotoxins commonly found in wheat, barley, oats and maize. The producing genera of trichothecenes include *Fusarium, Stachybotrys, Mycothecium, Cephalosporium, Verticimonosporium, Trichoderma* and *Trichothecium* (Wu et al., 2017). Of all *Fusarium* mycotoxins identified, trichothecenes are the most economically important mycotoxins, and have most often been associated with the acute and chronic toxicoses in human and livestock. These mycotoxins can cause growth retardation, reproductive disorders, immune system suppression, anorexia, vomiting, hemorrhages, diarrhea, and even death in experimental and farm animals (Wang et al., 2015). The most representative compounds of trichothecenes include T-2 toxin, HT-2 toxin, trichodermin, deoxynivalenol (or vomitoxin, DON), fusarenon X, nivalenol, diacetoxyscirpenol and satratoxin G.

The canonical mechanism of trichothecenes has been attributed to inhibit the eukaryotic ribosomal protein synthesis, which may be a reason for their inhibition effects on DNA and RNA synthesis (Deyu et al., 2018). Trichothecenes also induced the cell cycle arrest and apoptosis (Fatima et al., 2018). New evidence suggested that trichothecenes could decrease the mitochondrial protein synthesis by inhibiting the mitochondrial translation and induce mitochondrial dysfunction (Bin-Umer et al., 2011). However, there are still few studies regarding the trichothecene-induced epigenetic alterations which may be a mechanism of the extensive gene expression induced by the trichothecenes.

6.1. DNA methylation, histone modifications and trichothecenes toxicity

Trichothecenes have been shown to affect the oocyte developmental potential in domestic animals (Han et al., 2016; Zhu et al., 2016). The possible mechanism of HT-2 toxin-induced disruption of mouse oocyte maturation is previously investigated. The results showed that HT-2 toxin could disrupt the oocyte maturation and meiotic spindle morphology, reduced the actin expression, and induced the apoptosis and autophagy in mouse oocyte cells. Furthermore, HT-2 toxin increased the global 5 mC level, and decreased the H3K9me2 and H3K27me3 levels (Zhu et al., 2016). Also, in porcine oocyte cells, T-2 toxin and HT-2 toxin significantly increased the global 5 mC level and H3K4me2 and H3K9me2 levels, which may be a cause for the disruption of porcine oocyte maturation and embryo development (Zhang et al., 2017b). In porcine oocyte cells, DON significantly inhibited the porcine oocyte maturation, disrupted the meiotic spindle formation and induced the apoptosis and autophagy. DON also induced DNA hypermethylation by increasing the DNMT3a expression, and increased the H3K4me2 and H3K27me3 levels and the expressions their corresponding methyltransferases of EZH2, SETDB1 and SUV39H2 (Han et al., 2016). Similarly, DON inhibited mouse oocyte maturation, caused early embryo cleavage, and disrupted the meiotic spindle formation and kinetochore-microtubule attachment which might be through decreasing the acetylated tubulin level. Moreover, DON increased the global 5 mC level and decreased the H3K9me2 level (Lan et al., 2018). By summarizing all these facts it is concluded that trichothecenes decreased the oocyte developmental potential which is possible related to the alterations in the epigenetic modifications.

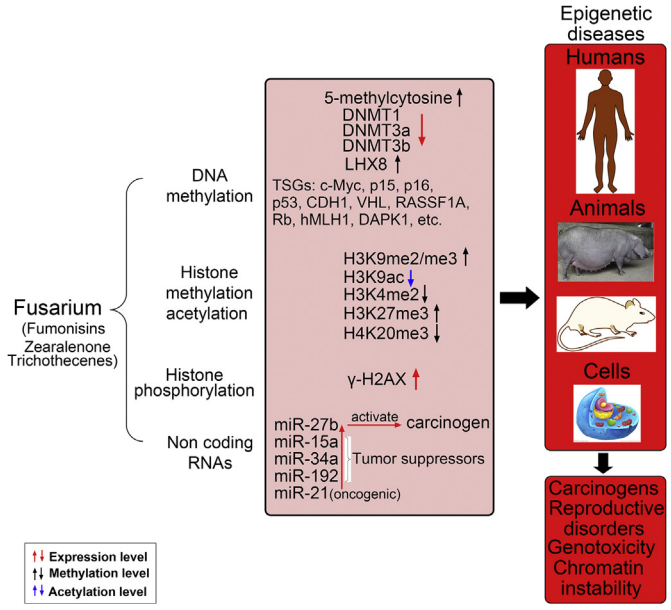

Fig. 3. Epigenetic mechanisms of *Fusarium* mycotoxins-induced toxicity. *Fusarium* mycotoxins change the expression of DNMTs and tumor suppressor genes (TSGs) and global 5-methylcytosine level, change the methylation or acetylation states of H3K4, H3K9, H3K20, and H3K27, increase the γ-H2AX level, and activate or inhibit the tumor suppressors or oncogenic miRNAs. These epigenetic alterations could cause carcinogenesis, reproductive disorders and genotoxicity in humans and animals.

The DNA damage response indicated by H2AX phosphorylation caused by trichothecenes has been reported. For example, in human monocytic THP-1 cells, T-2 toxin and satratoxin G induced oxidative DNA damage, activated the ATM kinase that then rapidly phosphorylate the H2AX to form γ-H2AX as early as within 3 h (Rakkestad et al., 2010). The DON has been characterized as a skin tumor promoter because it can induce edema, hyperplasia, DNA damage and activation of MAPKs pathway in mouse skin (Mishra et al., 2016). Further mechanism study in human HaCaT keratinocytes revealed that DON significantly increased the level of γ-H2AX and 8-hydroxy-2′-deoxyguanosine (8-OHdG), a widely used biomarker for oxidative stress and carcinogenesis (Mishra et al., 2016).

6.2. MiRNAs and trichothecenes toxicity

The analysis of miRNA expression profiles in the liver, ascending and descending colon of gilts which are exposed to DON (12 μg/kg/d) showed that DON treatment for 21 days significantly decreased the miR-15a level in the liver, while DON treatment for 7 days resulted in a remarkable increase of 50-folds in the expression of miR-21in the ascending colon (Brzuzan et al., 2015).

7. Conclusion

By analyzing all the previous facts and figures it is concluded that epigenetic modifications are involved in the toxicity of *Fusarium* mycotoxins, as shown in Fig. 3. It is further noted that the epigenetic modifications are implicated in the etiology of many human diseases and pathological conditions in various cells in vitro. On the basis of these findings it is suggested to extend the epigenetic researches to the epigenotoxic impacts of not only *Fusarium* mycotoxins, but also for other types of mycotoxins and toxic chemicals. However, the epigenetic studies about *Fusarium* mycotoxins are still sporadically reported, but the current evidences showed that the *Fusarium* mycotoxins indeed can result in toxicities such as reproductive disorders through the epigenetic mechanisms. In general, the current epigenotoxic studies of *Fusarium* mycotoxins are not specific and thus, more attempts are needed to elucidate the gene-specific epigenetic modifications and their relationship with downstream gene expression and particular toxicological phenotype.

Conflicts of interest

The authors declare no conflicts of interest.

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