SCIENCE CHINA PRESS

SCIENCE CHINA Life Sciences
life.scichina.com link.springer.com
https://doi.org/10.1007/s11427-023-2512-6

RESEARCH PAPER

AP-1和SP1反式激活鸡AFB1生物激活中肝脏CYP1A1和CYP2A6的表达

AP-1 and SP1 *trans*-activate the expression of hepatic CYP1A1 and CYP2A6 in the bioactivation of AFB_1 in chicken

Jiang Deng[1†], Jia-Cheng Yang[1†], Yue Feng[1], Ze-Jing Xu[1], Kamil Kuča[2], Meng Liu[1*] & Lv-Hui Sun[1*]

[1]State Key Laboratory of Agricultural Microbiology, Hubei Hongshan Laboratory, Frontiers Science Center for Animal Breeding and Sustainable Production, College of Animal Sciences and Technology, Huazhong Agricultural University, Wuhan 430070, China;
[2]Department of Chemistry, Faculty of Science, University of Hradec Kralove, Hradec Kralove 50003, Czech Republic

†Contributed equally to this work
*Corresponding author (Lv-Hui Sun, email: lvhuisun@mail.hzau.edu.cn; Meng Liu, email: liumeng@mail.hzau.edu.cn)

Received 28 November 2023; Accepted 9 January 2024; Published online 18 April 2024

Dietary exposure to aflatoxin B_1 (AFB_1) is harmful to the health and performance of domestic animals. The hepatic cytochrome P450s (CYPs), CYP1A1 and CYP2A6, are the primary enzymes responsible for the bioactivation of AFB_1 to the highly toxic *exo*-AFB_1-8,9-epoxide (AFBO) in chicks. However, the transcriptional regulation mechanism of these CYP genes in the liver of chicks in AFB_1 metabolism remains unknown. Dual-luciferase reporter assay, bioinformatics and site-directed mutation results indicated that specificity protein 1 (SP1) and activator protein-1 (AP-1) motifs were located in the core region −1,063/−948, −606/−541 of the *CYP1A1* promoter as well as −636/−595, −503/−462, −147/−1 of the *CYP2A6* promoter. Furthermore, overexpression and decoy oligodeoxynucleotide technologies demonstrated that SP1 and AP-1 were pivotal transcriptional activators regulating the promoter activity of *CYP1A1* and *CYP2A6*. Moreover, bioactivation of AFB_1 to AFBO could be increased by upregulation of *CYP1A1* and *CYP2A6* expression, which was *trans*-activated owing to the upregulation of AP-1, rather than SP1, stimulated by AFB_1-induced reactive oxygen species. Additionally, nano-selenium could reduce ROS, downregulate AP-1 expression and then decrease the expression of *CYP1A1* and *CYP2A6*, thus alleviating the toxicity of AFB_1. In conclusion, AP-1 and SP1 played important roles in the transactivation of CYP1A1 and CYP2A6 expression and further bioactivated AFB_1 to AFBO in chicken liver, which could provide novel targets for the remediation of aflatoxicosis in chicks.

aflatoxin B_1 | CYP1A1 | CYP2A6 | SP1 | AP-1 | transcriptional activation

纳米硒可以减少ROS，下调AP-1表达，进而降低CYP1A1和CYP2A6的表达，从而减轻AFB1的毒性。
综上所述，AP-1和SP1在CYP1A1和CYP2A6表达的转录激活过程中发挥重要作用，并进一步将鸡肝中的 AFB1生物活化为AFBO，从而使AFB1的毒性降低。

INTRODUCTION

Aflatoxins are secondary metabolites mainly produced by the fungi *Aspergillus flavus* and *Aspergillus parasiticus* and ubiquitously contaminate groundnut- and corn-based foods, such as peanuts, wheat, maize and barley (Deng et al., 2018; Wei et al., 2019; Xue et al., 2016; Zhao et al., 2021a). Approximately 20 aflatoxins have been identified since they were first discovered in 1969, but only aflatoxin B_1 (AFB_1), B2 (AFB_2), G1 (AFG_1) and G2 (AFG_2) exist in nature (Liu et al., 2020; Rushing and Selim, 2019). AFB_1 is regarded as the most toxic among these aflatoxins due to its hepatoxicity, carcinogenicity, mutagenicity and immunogenicity (Liu et al., 2022a; Zhao et al., 2021b; Zhao et al., 2021c). Ingestion of foods or feeds exposed to AFB_1 has been associated with hepatocellular carcinoma and aflatoxicosis in chicks, as it is an important table food rich in protein and essential nutrients, consumption of chicken contaminated by AFB_1 leads to increasing safety and health concerns for humans (Benkerroum, 2020; Taranu et al., 2020; Wang et al., 2022a; Wu et al., 2016).

Following its absorption into the liver through the portal vein, AFB_1 can be bioactivated into highly toxic *exo*-AFB_1-8,9-epoxide (AFBO) and less toxic aflatoxin M_1, Q_1 and P_1 by liver microsomal cytochrome P450s (CYP450s) (Taranu et al., 2020; Yunus et al., 2011). AFBO, with extreme reactivity and electrophilicity, covalently binds to DNA and serum albumin lysine to form AFB_1-N^7-guanine and lysine adducts, resulting in DNA lesions and mutations, as well as cytotoxicity (Gan et al., 2018; Zhao et al., 2019; Zhao et al., 2021b). Meanwhile, AFB_1 induces the production of reactive oxygen species (ROS), leading to the oxidation of lipids and proteins (Benkerroum, 2020; Zhao et al., 2019). CYP450s, mostly located within the endoplasmic reticulum and mitochondria, play a significant role in the metabolism of AFB_1 in humans and animals (Deng et al., 2018). Previous studies have shown that there are 57 functional CYP450 genes in humans, compared with 108 in the mouse (Nelson et al., 2004; Zanger and Schwab, 2013). Numerous studies have demonstrated that various CYP450s produce different metabolites, but there are interindividual variations in the effectivity of bioactivation of AFB_1 across species (Dohnal et al., 2014). In humans, CYP1A2, CYP3A4, CYP3A5 and CYP3A7 catalyse 79%–95%, 4%–15%, 5%–7% and 1%–5% of AFBO formation in hepatic microsomes, whereas CYP2A13 is mainly expressed in the lung and transfers AFB_1 to AFBO (Deng et al., 2018; Dohnal et al., 2014; Zhang et al., 2014). In turkey liver, CYP1A5 is responsible for more than 98% generation of AFB_1 to AFBO, with a minority contribution by CYP1A2 and CYP2A6. In chicken, quail and ducklings, CYP1A1 and CYP2A6 play dominant roles in biotransformation of AFB_1 into AFBO in the liver; moreover, CYP1A2 and CYP3A4 catalyse the forma-

tion of AFBO from AFB_1 (Diaz et al., 2010a; Diaz et al., 2010b; Dohnal et al., 2014; Wang et al., 2018). Interestingly, *CYP1A1* and *CYP2A6* mRNA levels and/or enzyme activities significantly increased when broilers were exposed to AFB_1, while AFBO formation decreased by 80% after the activities of CYP1A1 and CYP2A6 were inhibited (Diaz et al., 2010b; Zhang et al., 2016). However, poultry, especially the young, are more susceptible than mammals to the toxicity of AFB_1, which might be associated with the low activity of glutathione *S*-transferase (GST) phase II detoxification enzymes in the conversion of AFBO to AFB_1-GSH in liver (Kim et al., 2011).

The toxicity and carcinogenicity of AFB_1 are closely related to the rate of activation as well as the capacity of detoxification in primary and secondary metabolism (Deng et al., 2018; Dohnal et al., 2014). Due to the low activities of GST in chicken, paying attention to the CYP450 phase I metabolizing enzyme and inhibiting its activity in the conversion of AFB_1 to AFBO would be of practical value. Considering the special interrelation between CYP1A1, CYP2A6 and AFB_1 bioactivation, the present study was designed to investigate the transcriptional regulation mechanism of crucial *CYP1A1* and *CYP2A6*, as well as their roles in AFB_1 bioactivation.

RESULTS

Chicken *CYP1A1* and *CYP2A6* gene structural analysis

The *CYP1A1* gene of *Gallus gallus* is located on chromosome 10 (NC_052541.1 2470514.2474266), has a full length of 3,753 bp, contains 7 exons and 6 introns and encodes 530 amino acids (Figure 1A). According to phylogenetic analysis, the putative *CYP2A6* gene of *Gallus gallus* is similar to *CYP2H1*. It is located on chromosome 6 (NC_052537.1 18351042.18358414), has a full length of 7,373 bp, contains 9 exons and 8 introns and encodes 491 amino acids (Figure 1B). Additionally, the promoter region of *CYP1A1* shows a high GC content with two CpG islands compared with *CYP2A6* (Figure 1C and D).

Promoter activity, sequence analysis and mutation trial

Thirteen recombinant plasmids of *CYP1A1* and 21 recombinant plasmids of *CYP2A6* were constructed. These plasmids were co-transfected with the phRL-TK normalizing vector into chicken Leghorn male hepatoma (LMH) cells to determine the promoter activity. The relative luciferase activities of p1A1-1816, p1A1-1214, p1A1-1161 and p1A1-1063 were significantly higher ($P<0.05$) than that of the negative control pGL3-Basic, while the activity of p1A1-948 exhibited no significant change ($P\geqslant0.05$) after deletion of promoter region −1,816/−948. However, p1A1-541 and p1A1-177 still showed high activities ($P<0.05$) after deletion of promoter region −1,816/−541 or −1816/−177 (Figure 2A). Subsequently, the potential transcription factor (TF) binding sites between −1,063/−948, −606/−541 and −177/−41 were analyzed with the ALGGEN-PROMO, and the results indicated that there were specificity protein 1 (SP1) binding sites in −1,063/−948, activator protein-1 (AP-1) and SP1 binding sites in −606/−541 and a TGGCA-binding protein binding sites in −177/−41 (Figure 2B). Similarly, the promoter region containing −636/1 possessed high relative luciferase activity ($P<0.05$). Compared with pGL3-Basic, p2A6-1941, p2A6-636, p2A6-530 and p2A6-147 possessed high activities ($P<0.05$), while the activity of p2A6-546 showed a sharp decrease ($P<0.05$) in comparison to p2A6-530 (Figure 2C). Sequence analysis predicted that there were SP1 and TGGCA-binding protein binding sites in −636/−595, TGGCA-binding protein and AP-1 binding sites in −530/−462 and −147/−1, but −546/−530 seemed to have no known TF binding sites predicted by ALGGEN-PROMO in chicken (Figure 2D). In view of the promoter activity, this study speculated that SP1, AP-1 and TGGCA-binding proteins could bind to the promoters of *CYP1A1* and *CTP2A6*.

Identification of the SP1 and AP-1 binding sites as transcriptional activators

According to the prediction of potential transcription factor binding sites in the essential promoter region of the chicken *CYP1A1* and *CYP2A6* genes, single or multiple base mutations of SP1, AP-1 and TGGCA binding sites in the promoter region of *CYP1A1* and *CYP2A6* were conducted. The relative luciferase activity decreased ($P<0.05$) by 45.51% or 22.52% after single mutation of SP1 binding site 1 or 2 located in −1,063/−948 compared with the wildtype p1A1-1063 (Figure 3A). Moreover, after mutation of AP-1 and SP1 in the −606/−541 interval, the luciferase activity of p1A1-606-AP-M increased ($P<0.05$) by 172.78%, while the activity of p1A1-606-SP1-M decreased ($P<0.05$) by 36.78% compared with the wildtype p1A1-606 (Figure 3B). Similarly, a single mutation of the TGGCA-binding protein, SP1 or AP-1 site resulted in a remarkable increase ($P<0.05$) in luciferase activity at −636/−595 (p2A6-636-SP1-M, 41.61%) and −485/−462 (p2A6-485-AP-M, 51.60%) and a decrease ($P<0.05$) at −530/−513 (p2A6-530-TG-M, 25.08%) and −513/−485 (p2A6-513-AP-M, 34.04%) (Figure 3C). Furthermore, multiple base mutation of those sites resulted in a dominant elevation ($P<0.05$) of luciferase activity in −636/−595 (p2A6-636-TG-M′, 399.57%; p2A6-636-SP1-M′, 264.09%), −530/−513 (p2A6-530-TG-M′, 62.33%), −513/−485 (p2A6-513-AP-M′, 101.93%) and −485/−462 (p2A6-485-AP-M′, 25.42%) (Figure 3D). These results indicated that SP1, AP-1 and TGGCA-binding protein motifs played important roles in the regulation of *CYP1A1* and *CYP2A6* as binding sites for these transcription factors.

Subsequently, this study constructed overexpression vectors utilizing pcDNA3.1(+) and overexpressed AP-1 and SP1 in LMH cells (Figure 4A). Then, AP-1 or SP1 overexpression plasmid was co-transferred with the luciferase vector containing the core promoter region of *CYP1A1* or *CYP2A6* and phRL-TK. The result displayed that AP-1 overexpression significantly raised ($P<0.05$) the luciferase activity of p1A1-606 but did not influence p2A6-147 ($P\geqslant0.05$) (Figure 4B and C). Moreover, overexpressed-SP1 decreased ($P<0.05$) the luciferase activity of p1A1-1063 and increased ($P<0.05$) that of p1A1-606, while p2A6-636 was not significantly changed ($P\geqslant0.05$) (Figure 4D–F).

Double-stranded oligodeoxynucleotides against AP-1 and SP1 binding sites were synthesized to block the TFs of the promoter region of *CYP1A1* and *CYP2A6*. Notably, AP-1 decoy oligodeoxynucleotides significantly decreased ($P<0.05$) the relative luciferase activity of p1A1-606 and p2A6-147 (Figure 5A and B). In addition, SP1 decoy oligodeoxynucleotides reduced ($P<0.05$) the activity of p1A1-1063, p1A1-606 and p2A6-147 (Figure 5C–E). These data demonstrated that AP-1 and SP1

https://doi.org/10.1007/s11427-023-2512-6

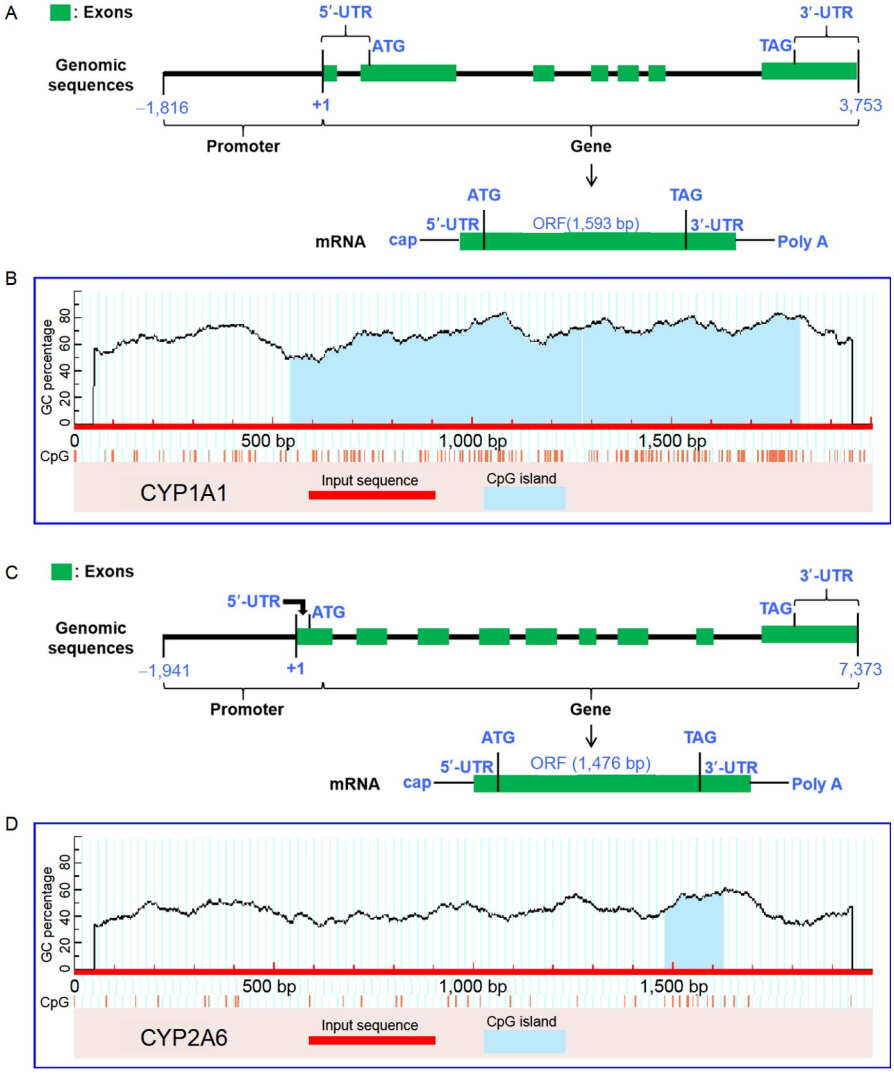

Figure 1. Structural characteristics of the chicken *CYP1A1* and *CYP2A6* genes. The structural characteristics, including the 5′/3′-untranslated region (5′/3′-UTR) and open reding frame (ORF) of the *CYP1A1* (A) and *CYP2A6* (B) genes. Schematic representation of the distribution of CpG islands in the *CYP1A1* (C) and *CYP2A6* (D) gene promoter regions. Dashed lines indicate the GC percentage, as represented on the *y*-axis, and the blue area represents CpG islands.

might work as an enhancer to bind to the corresponding *cis*-elements of the *CYP1A1* and *CYP2A6* promoter, thus promoting the transcription of *CYP1A1* and *CYP2A6*.

AFB_1 increased the transcriptional activation of *CYP1A1* and *CYP2A6* by upregulating the expression of AP-1

The results of this study showed that AFB_1 led to cell death and increased ($P<0.05$) the ROS level in chicken LMH cells (Figure 6A and B). At the same time, 40 μg L^{-1} AFB_1 elevated ($P<0.05$) the expression of AP-1, while it did not effected the expression of SP1, even at a dose of 500 μg L^{-1} ($P\geqslant0.05$) (Figure 6C–E). Moreover, AFB_1 increased ($P<0.05$) the mRNA expression of *CYP1A1* and *CYP2A6* (Figure 6F). Based on the previous results that AP-1 and SP1 performed as transcriptional enhancers, the above data suggested that AFB_1 induced an elevation of ROS, which stimulated the expression of AP-1, thus activating the transcriptional promoter activity of CYP1A1 and CYP2A6 and promoting their expression.

Nano-selenium mitigated the AFB_1-induced toxic effects through down-regulation of AP-1-mediated *CYP1A1* and *CYP2A6*

Several nutrients, present as trace elements, are important components of antioxidant enzymes and were selected for use in alleviating the toxicity of AFB_1 in LMH. The results showed that nano-selenium (nano-Se) and nano-zinc oxide (nano-ZnO), rather than copper amino acid complex and manganese amino acid complex, had superior mitigation effects on cell viability of LMH cell lines (Figure 7A; Figure S1A–C in Supporting Information). Moreover, only nano-Se decreased the ROS level induced by AFB_1 at concentrations of 0.50, 1.00 and 5.00 mg L^{-1}, and the concentration of 1.00 mg L^{-1} presented the best effects (Figure 7B; Figure S1D in Supporting Information). Furthermore, nano-Se decreased the expression of *CYP1A1*, *AP-1* and/or *CYP2A6* at concentrations of 1.00 and 5.00 mg L^{-1}, while only 1.00 mg L^{-1} nano-Se reduced the protein production of AP-1 (Figure 7C–E). These results showed

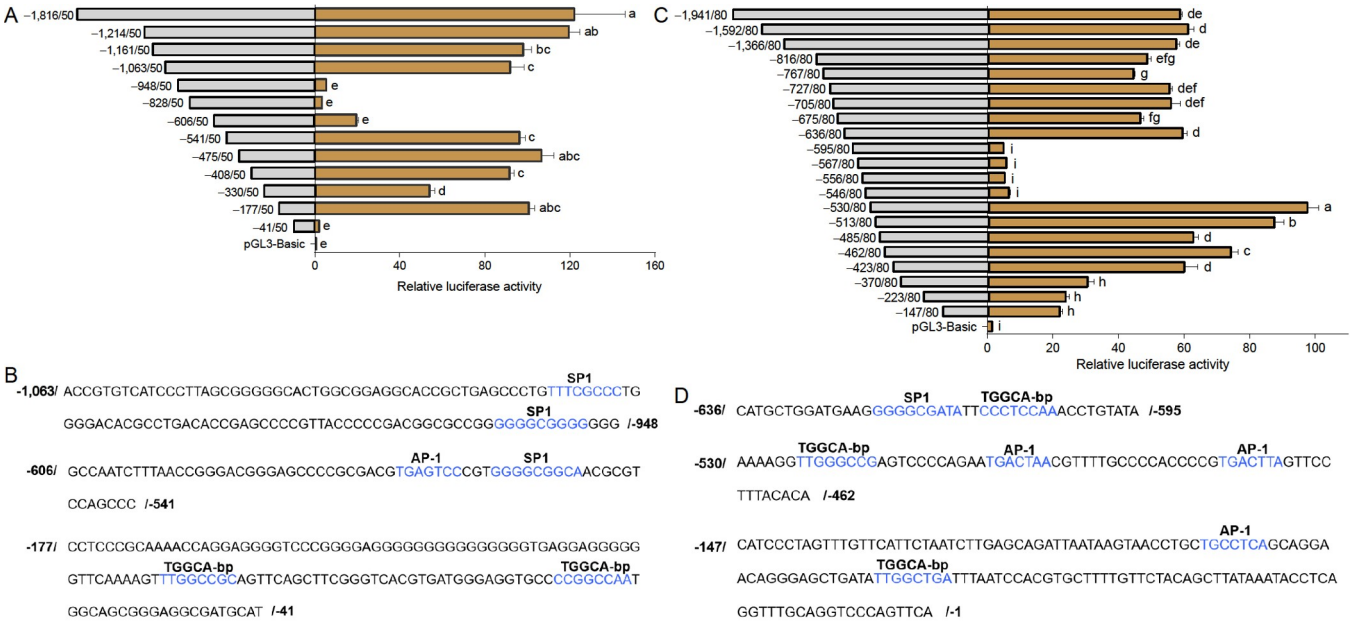

Figure 2. (Color online) Luciferase activities of the chicken *CYP1A1* and *CYP2A6* promoter regions in the LMH cell line. A and C, A series of plasmids containing 5′ unidirectional deletions of the promoter region of the *CYP1A1* gene (A, p1A1-1816, -1214, -1161, -1063, -948, -828, -606, -541, -475, -408, -330, -177, -41 and pGL3-basic) and *CYP2A6* gene (C, p2A6-1941, -1592, -1366, -816, -767, -727, -705, -675, -636, -595, -567, -556, -546, -530, -513, -485, -462, -423, -370, -223, -147 and pGL3-basic) fused in frame to the luciferase gene were transfected into the LMH cell line. Values are means±SE based on the firefly luciferase activity normalized against the Renilla luciferase activity, n=6–8. Different letters between groups represent significant differences, P<0.05. B and D, Analysis of the *cis*-acting elements within the core promoter region of the *CYP1A1* (B) and *CYP2A6* (D) genes using online software. The short sequences marked in blue are the putative transcription factor binding sites, and the transcription factor names are SP1, AP-1 and TGGCA-binding protein (TGGCA-bp).

that nano-Se could increase cell viability and significantly decrease the ROS level through down-regulation of *CYP1A1* and *CYP2A6*, mediated by AP-1.

DISCUSSION

CYP450 phase I metabolizing enzymes play crucial roles in the metabolism of 92%–96% of xenobiotics and drugs in the liver (Deng et al., 2018); however, they can also bioactivate procarcinogens or prodrugs to electrophilic metabolites, thus inducing cytotoxicity, DNA lesions and cell death (Manikandan and Nagini, 2018). CYP1A1 and CYP2A6 are the isozymes most responsible for the biotransformation of AFB_1 into AFBO in chicken liver (Diaz et al., 2010b; Muhammad et al., 2017; Zhang et al., 2016). The chicken *CYP1A1* gene sequence was reported first by Gilday et al. (1996), while the *CYP2A6* gene sequence was still unknown, since it was identified as a main enzyme involved in the bioactivation of AFB_1 into AFBO, until Muhammad et al. computationally identified that the nucleotide sequence of *CYP2A6* showed a similarity to *CYP2H1* (Diaz et al., 2010b; Muhammad et al., 2017). Then, this study analysed and cloned the promoter sequence of the *CYP1A1* and *CYP2A6* genes according to previous studies and conducted luciferase activity determination.

Generally, the transcription start site (TSS) is defined as "+1" in the promoter sequence of genes, and upstream of the TSS is represented as "–". In the present study, dual luciferase activity assay indicated that the core promoter regions of *CYP1A1* were −1,063/−948, −606/−541 and −177/−41, while the core promoter regions of *CYP2A6* were −636/−595, −546/−530 and −147/1. SP1, AP-1 and TGGCA-binding protein potential binding sites were predicted in these core regions. SP1 recognized and bound GC-rich sites at the promoter via 3-carboxyterminal Cys2His2 zinc-finger motifs, thus regulating the transcription of target genes (Chuang et al., 2011; Jiang et al., 2018; Song et al., 2022). Notably, the typical sequence of the SP1 binding site was 5′-(G/T)GGGCGG(G/A)(G/A)-3′ in the promoter region (Jiang et al., 2018). The sequence analysis showed that there were two CpG islands in the promoter sequence of the *CYP1A1* gene, and the sequences of the putative SP1 binding sites, which were 5′-TTTCGCCC-3′, 5′-GGGGCGGGG-3′, 5′-GGGGCGGCA-3′ and 5′-GGGGCGA TA-3′ in this study, partially corresponded to the canonical sequence. SP1 could interact with itself when bound to distant sites in *cis*-acting elements (Li et al., 1991; Su et al., 1991), which suggested that SP1 might establish interactions between promoters and distant regulatory elements *in vivo* through a loop formation (Ptashne, 1986). In the present study, the luciferase activity was higher for p1A1-1063, whereas it was lower for p1A1-606, which might imply that potential SP1 sites located in the −1,063/−948 and −606/−541 regions of the *CYP1A1* promoter interacted with each other, thus blocking other potential TFs to maintain the high luciferase activity of the whole promoter region. AP-1 was composed of Fos family proteins dimerized with Jun family proteins, and it could be induced in response to extracellular signals, which always bound to 12-O-tetradecanoylphorbol-13-acetate response elements (5′-TGAG/CTCA-3′), cAMP response elements (5′-TGACG/TCA-3′), and variants of these sequences (Bejjani et al., 2019; Koo et al., 2020; Lee et al., 2013). TGGCA-binding proteins have been shown to be functionally equivalent to nuclear factor I, which was ubiquitous among higher eukaryotes (Miksicek et al., 1987; Rupp et al., 1990). In this study, single or multiple base mutations of SP1, AP-1 and TGGCA-binding protein binding elements located in the core promoter region cause a notable

https://doi.org/10.1007/s11427-023-2512-6

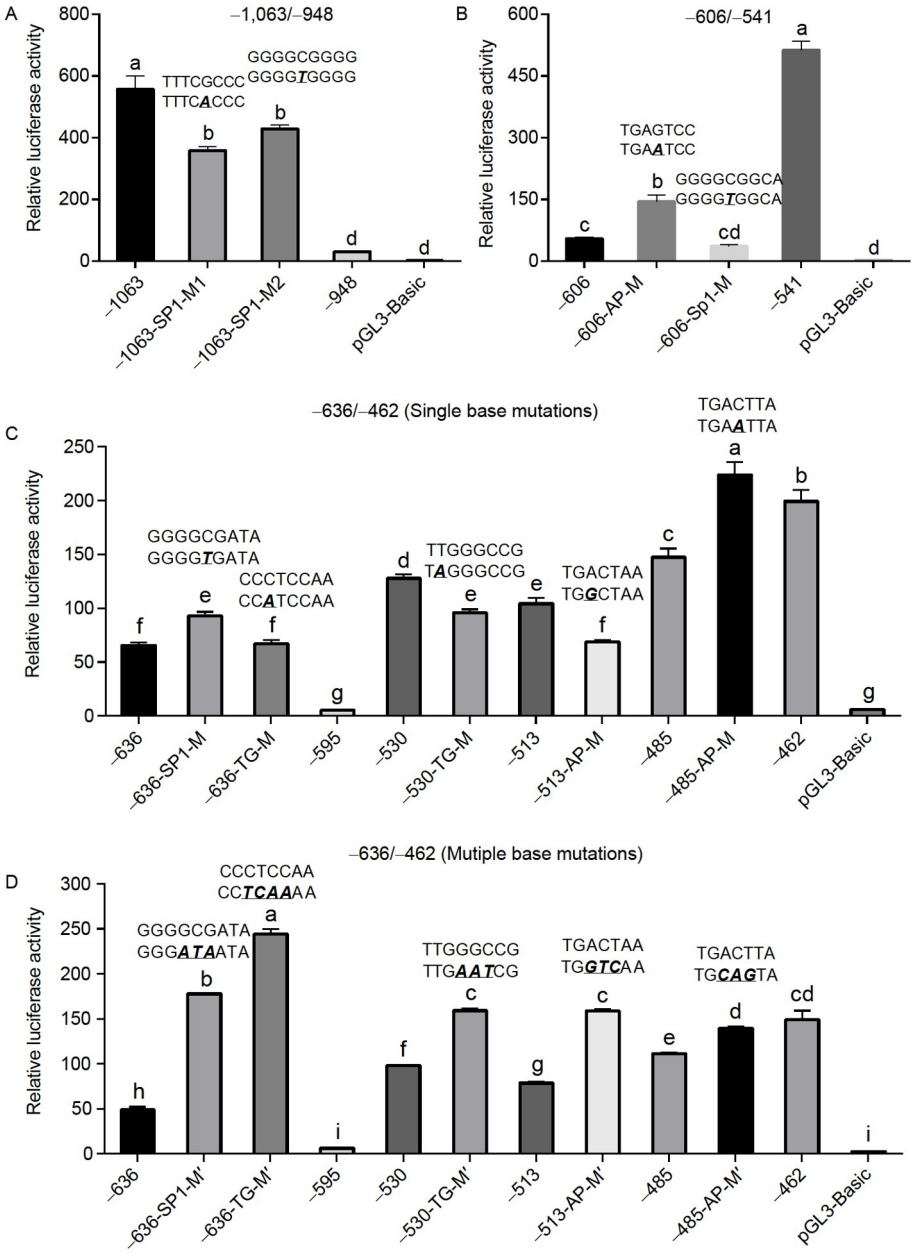

Figure 3. Analysis of potential SP1, AP-1 and TGGCA-binding protein motifs by site-directed mutagenesis. Site-directed mutagenesis was conducted in the construct vectors p1A1-1063 and p1A1-606 of the *CYP1A1* gene (A and B) with single base mutations, and p2A6-636, p2A6-530, p2A6-513 and p2A6-485 of the *CYP2A6* gene with single (C) or multiple (D) base mutations. The wildtype sequence and mutated sequence of each potential transcription factor binding sites were shown, and mutated base were marked in bold, italic and underlined in the corresponding group. Values are means±SE based on the firefly luciferase activity normalized against the Renilla luciferase activity, n=6–8. Different letters between groups represent significant differences, P<0.05.

decrease or increase of the relative luciferase activity compared with the wild type. These results suggested that potential binding sites for SP1, AP-1 and TGGCA-binding protein might played key roles in the transcriptional regulation of *CYP1A1* and *CYP2A6*.

Numerous studies have reported that SP1 and AP-1 are important transcription factors in a variety of physiological and pathological processes, including cell proliferation, cell cycle progression, differentiation, apoptosis and cancer progression (Vizcaíno et al., 2015; Young et al., 2022). Furthermore, previous studies have reported that SP1 expression is associated with the expression of CYPs, such as the decrease in *CYP1A1* and *CYP1B1* induced by SP1 downregulation in breast cancer cells (Do et al., 2014) and the downregulation of *CYP1A1* induced by knockdown of SP1 (Xie et al., 2018). Moreover, AP-1 could upregulate the expression of *CYP2A8* in Syrian hamsters, *CYP4A2* in rat hepatocytes and *CYP1A1* in HepG2 cell (Fiala-Beer et al., 2007; Tohkin et al., 1996; Ung et al., 2021). Therefore, this study paid more attention to SP1 and AP-1, and their overexpression activated the promoter activity of p1A1-606. Subsequently, double-stranded linear DNA was synthesized as decoy oligodeoxynucleotides, which could competitively bind to specific TFs, thus blocking the subsequent binding of TFs to the promoter of target genes (Dzau, 2002; Kume et al., 2002; Remes et al., 2021). In the present study, transfection of decoy oligodeoxynucleotides against SP1 markedly decreased the promoter activities of p1A1-1063, p1A1-606 and p2A6-636;

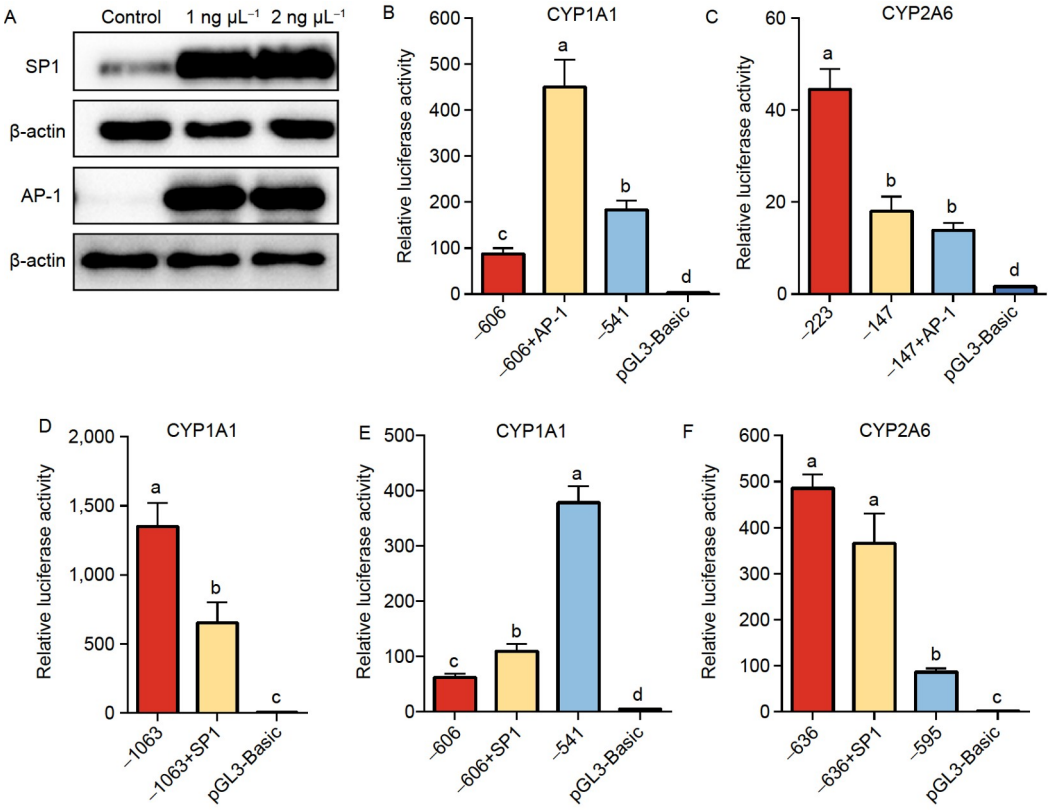

Figure 4. Luciferase activities of core region-containing vectors co-transfected with overexpressed AP-1 and SP1 in chicken LMH cells. A, Overexpression of AP-1 and SP1 in LMH cells, n=4. B–F, Luciferase activities of p1A1-606 (B) and p2A6-147 (C) co-transfected with overexpressed AP-1, p1A1-1063 (D), p1A1-606 (E) and p2A6-147 (F) co-transfected with overexpressed SP1, n=6–8. Values are means±SE based on the firefly luciferase activity normalized against the Renilla luciferase activity. Different letters between groups represent significant differences, P<0.05.

moreover, decoy oligodeoxynucleotides against AP-1 led to the noticeable reduction of the promoter activities of p1A1-606 and p2A6-147. These results implied that SP1 and AP-1 might functioned as enhancers to promote the transcription of *CYP1A1* and *CYP2A6*.

AP-1, a master integrator of a myriad of extracellular signals, could be activated immediately or early by external stimulation, such as inflammation, oxidative stress or growth factors (Beisaw et al., 2020; Bejjani et al., 2019; Zanconato et al., 2015). Moreover, a "respiratory burst" occurred at sites of inflammatory damage due to the increased uptake of oxygen by mast cells and leukocytes, thus leading to increased release and accumulation of ROS, which induced changes in the expression of AP-1 and SP1 (Cheng et al., 1999; Coussens and Werb, 2002; Reuter et al., 2010). Previous studies showed that certain chemicals, such as uric acid and anlotinib, could increase ROS production and then activate c-Jun N-terminal kinase (JNK), which phosphorylated the AP-1 subunit c-jun, resulting in increased transcriptional activity (Luo et al., 2022; Xie et al., 2021). Consistent with previous studies, this study found that AFB_1 led to the accumulation of ROS and cell death (Chen et al., 2019; Mo et al., 2023) and was the first to discover that AFB_1-induced ROS upregulated the expression of AP-1 in LMH cells (Luo et al., 2022; Xie et al., 2021). Meanwhile, AFB_1 upregulated the expression of *CYP1A1* and *CYP2A6*, which were consistent with previous studies (Ates and Ortatatli, 2021; Sang et al., 2023). However, the expression of SP1 was not affected by AFB_1-induced ROS in this study, which was inconclusive according to previous studies (Gao et al., 2021; Yang et al., 2022). This discrepancy could be due to differences in the experimental durations and doses of AFB_1. The above results suggested that AFB_1-induced ROS elevation activated and promoted the expression of AP-1 and then enhanced the promoter activities of *CYP1A1* and *CYP2A6* genes and increased their expression.

Trace elements such as selenium, zinc, copper and manganese are important nutrients for biological processes and they are crucial cofactors of various enzymes, such as antioxidant enzymes (Deng et al., 2024; Liu et al., 2022b; Ma et al., 2023; Schwarz et al., 2019; Wan and Yin, 2023). This study selected four chemical reagents, nano-Se, nano-ZnO, copper amino acid complex and manganese amino acid complex, to explore whether they could alleviate the toxicity of AFB_1 in LMH cells. The results showed that Nano-Se and nano-ZnO exerted protective effects on cell viability when exposed to AFB_1, which were in agreement with previous studies that Nano-Se and nano-ZnO had anti-apoptotic activity, antioxidant capacity and antagonistic effects against heavy metals (Bi et al., 2022; Kang et al., 2022; Shetty et al., 2015; Wang et al., 2022b; Wang et al., 2022c; Zhao et al., 2023). Copper amino acid complex and manganese amino acid complex showed few protective effects, which might be due to these trace elements displaying better effectiveness *in vivo* than *in vitro* model (Medeiros-Ventura et al., 2020; Studer et al., 2021). In addition, nano-Se decreased the ROS level in LMH cells exposed to AFB_1 in this study, which was consistent with a previous study and a summary by Jin et al. (Jin et al., 2023; Yan et al., 2024). Meanwhile, this study also found that nano-Se down-regulated

https://doi.org/10.1007/s11427-023-2512-6

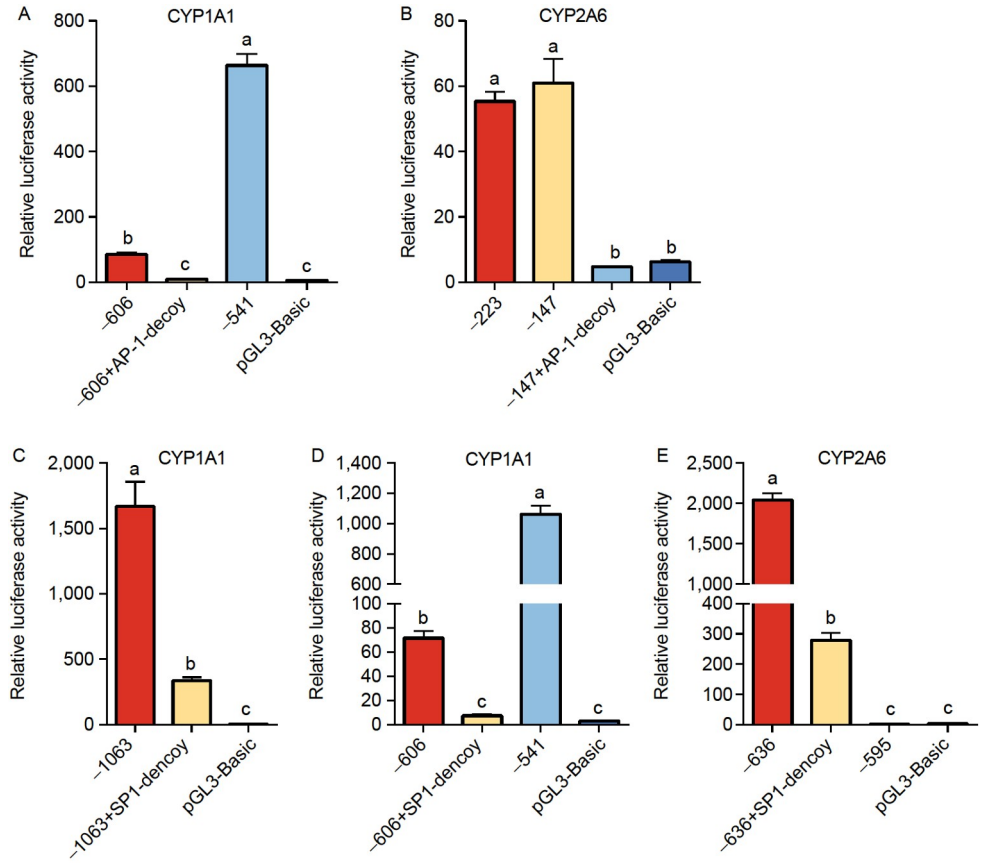

Figure 5. Luciferase activities of core region-containing vectors co-transfected with decoy oligodeoxynucleotides against AP-1 and SP1 in chicken LMH cells. The luciferase activities of p1A1-606 (A) and p2A6-147 (B) co-transfected with AP-1 decoy oligodeoxynucleotides, p1A1-1063 (C), p1A1-606 (D) and p2A6-147 (E) co-transfected with SP1 decoy oligodeoxynucleotides. Values are means±SE based on the firefly luciferase activity normalized against the Renilla luciferase activity, n=6–8. Different letters between groups represent significant differences, P<0.05.

the expression of AP-1, *CYP1A1* and *CYP2A6*, which were crucial to the bioactivation of AFB_1 to AFBO (Ates and Ortatatli, 2021; Sang et al., 2023). These results indicated that nano-Se could alleviate the cell toxicity induced by AFB_1. However, nano-ZnO did not reduce ROS generation at the applied concentration in this study, and this result seemed inconsistent with previous studies, which might be due to the different cell lines and the concentration of nano-ZnO applied (Zhang et al., 2023).

In summary, the present study was the first to report that the transcription factors SP1 and AP-1 were in the core region of the *CYP1A1* and *CYP2A6* promoter sequence. These factors played pivotal roles as activators influencing the transcriptional activity of both *CYP1A1* and *CYP2A6*. AFB_1 led to an increase in the expression of *CYP1A1* and *CYP2A6*, which were associated with the upregulation of AP-1 induced by the elevation of ROS (Figure 8). The high expression of *CYP1A1* and *CYP2A6* proved responsible for the bioactivation of AFB_1 to AFBO, thus leading to DNA lesions and cytotoxicity. Therefore, the findings of this study provided AP-1 as a novel and promising target for potential nutritional strategies to prevent aflatoxicosis in chicks.

MATERIALS AND METHODS

Materials and cell culture

The pGL3-Basic vector and Renilla luciferase expression vector phRL-TK were provided by the Animal Physiology Laboratory, Huazhong Agricultural University. AFB_1 and DMSO were purchased from Sigma-Aldrich (USA). Nano-selenium, copper and manganese amino acid complexes were provided by Beijing Deyuanshun Biotechnology Co., Ltd. (Beijing, China), and nano-zinc oxide was purchased from Aladdin (Shanghai, China). The chicken hepatocellular carcinoma cell line (LMH) was obtained from the ATCC (USA). Cells were grown in DMEM/F12 supplemented with 10% fetal bovine serum and 100 μg mL^{-1} penicillin/streptomycin/gentamicin (Invitrogen, Gibco, USA) under conditions of 95% air and 5% CO_2 in a humidified atmosphere at 37°C (Liu et al., 2023a).

Sequence analysis and plasmid construction

Following genomic DNA extraction from chicken liver using the Tissue Genome DNA Kit (Omega Bio-Tek, USA), pairs of primers containing the Hind III restriction enzyme sites were designed using the Primer 5.0 program to amplify the promoter sequences (http://genome.UCSC.edu/) of *CYP1A1* and *CYP2A6* (the translational start site was designated as +1). The potential TF binding sites and CpG islands were analysed using ALGGEN (http://ALGGEN.LSI.UPC.ES) and MethPrimer (http://www.urogene.org/methprimer/), respectively. Then, luciferase vectors were constructed by homologous recombination. In detail, each purified PCR product was combined with the linearized pGL3-

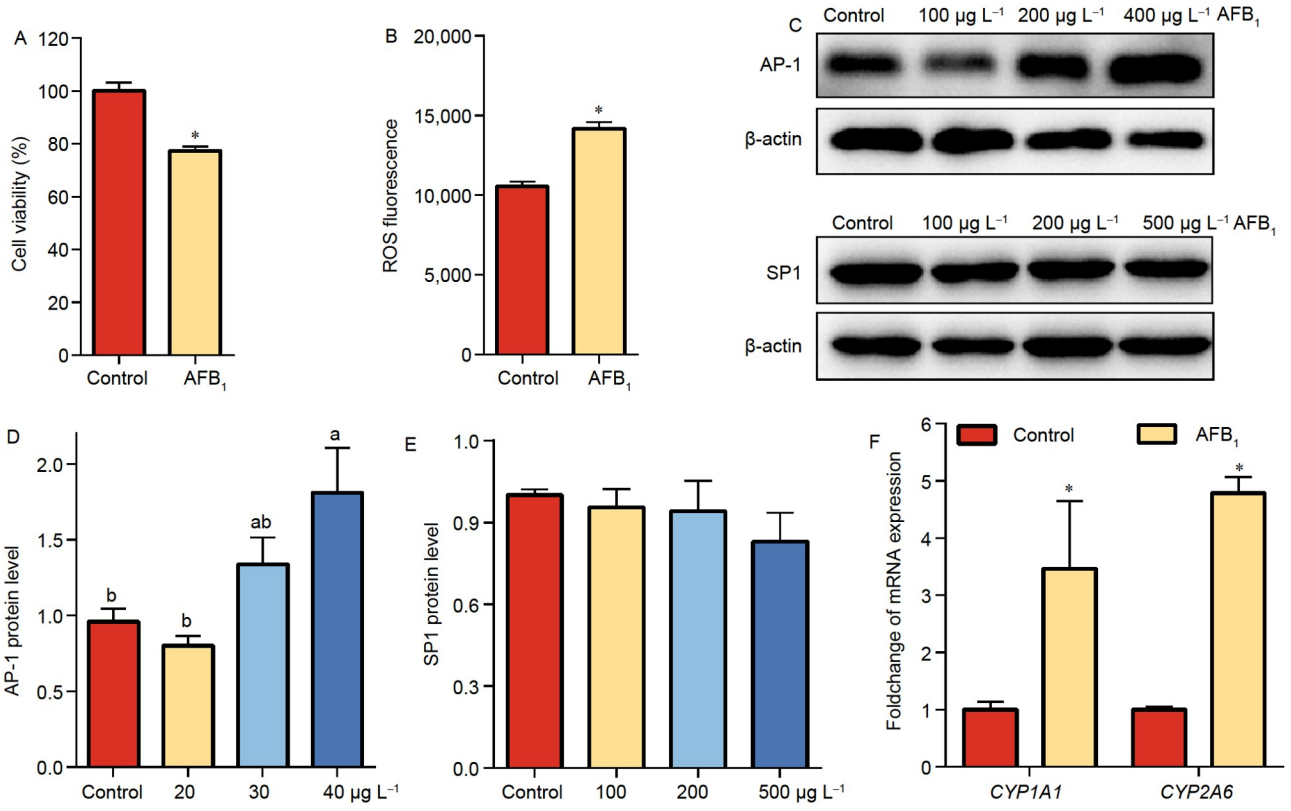

Figure 6. AFB_1 increased the transcriptional activation of *CYP2A6* by increasing the expression of AP-1 in chicken LMH cells. A and B, The cytotoxicity of AFB_1 on LMH cells (A) and the ROS level induced by AFB_1 (B), n=8–10. C–E, The effects of different concentrations of AFB_1 on the protein expression of AP-1 and SP1, n=3–4. F, The effects of AFB_1 on the mRNA levels of *CYP1A1* and *CYP2A6*, n=6. Values are means±SE; different letters between groups represent significant differences, P<0.05.

Basic plasmid, and the ligation catalysed by Etnas II (Vazyme, Nanjing, China) according to the manufacturer's instructions. A series of truncated luciferase vectors and their primers (Tsingke Biotechnology, Wuhan, China) are listed in Table S1 in Supporting Information.

Additionally, primers (listed in Table S1 in Supporting Information) were designed to amplify the genes SP1 and AP-1 using liver cDNA as the template. PCR products were confirmed by sequencing, purified and mixed with linearized pcDNA3.1(+) plasmid to obtain overexpression vectors through homologous recombination. These plasmids were extracted using an Endotoxin-free Plasmid Mini kit (Omega Bio-Tek), and their concentrations were determined using a Nanodrop 2000 (Thermo Fisher Scientific, USA).

Transient transfection and detection of dual luciferase activity

Cells were grown in 48-well plates until their density reached 70%–80%. A total of 200 ng of luciferase vector was co-transfected with 10 ng phRL-TK normalizing vector using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions. At 24 h post-transfection, cells were lysed and collected to determine the relative transcriptional activity of each fragment with the Dual-Luciferase Reporter Assay System (Yeasen, Shanghai, China) by using a Fluoroskan Ascent FL (Thermo Fisher Scientific).

Synthesis of decoy oligodeoxynucleotides

The synthesis of oligodeoxynucleotides was conducted by Tsingke Biotechnology, and the sequences of double-stranded oligodeoxynucleotides against AP-1 and SP1 binding sites were as follows: AP-1 decoy ODN, 5′-AGCTTGTGAGTCAGAAGCT-3′, 3′-TCGAACACTCAGTCTT CGA-5′ (Kume et al., 2002); SP1 decoy ODN, 5′-TTGATCGGGGCGGGGCGAGC TTTGC-3′, 3′-AACTAGCCCCGCCCCGCTCGAAACG-5′. Annealing was completed in a thermocycler (Applied Biosystems, USA). Briefly, sense and antisense oligonucleotides were mixed at a 1:1 mole ratio and then annealed in an annealing buffer (Solarbio, Beijing, China). The thermal program was set to decrease the temperature by 1°C in 90 s per cycle until 70 cycles were completed. Additionally, a purified decoy ODN was co-transfected with 200 ng of luciferase vector and 10 ng phRL-TK normalizing vector for determination of dual luciferase activity.

Cell viability assay

The cytotoxic effect of AFB_1 on LMH cells was evaluated using the cell counting kit-8 (CCK-8) assay as previously described (Wang et al., 2023). In brief, cells were seeded in a 96-well plate and treated with AFB_1 for 48 h. Then, 10 μL CCK-8 solution (Dojindo, Japan) was added according to the manufacturer's instructions. At the indicated time, the absorbance at 450 nm was determined by using a microplate reader (LabSerV K3, Thermo Fisher Scientific).

https://doi.org/10.1007/s11427-023-2512-6

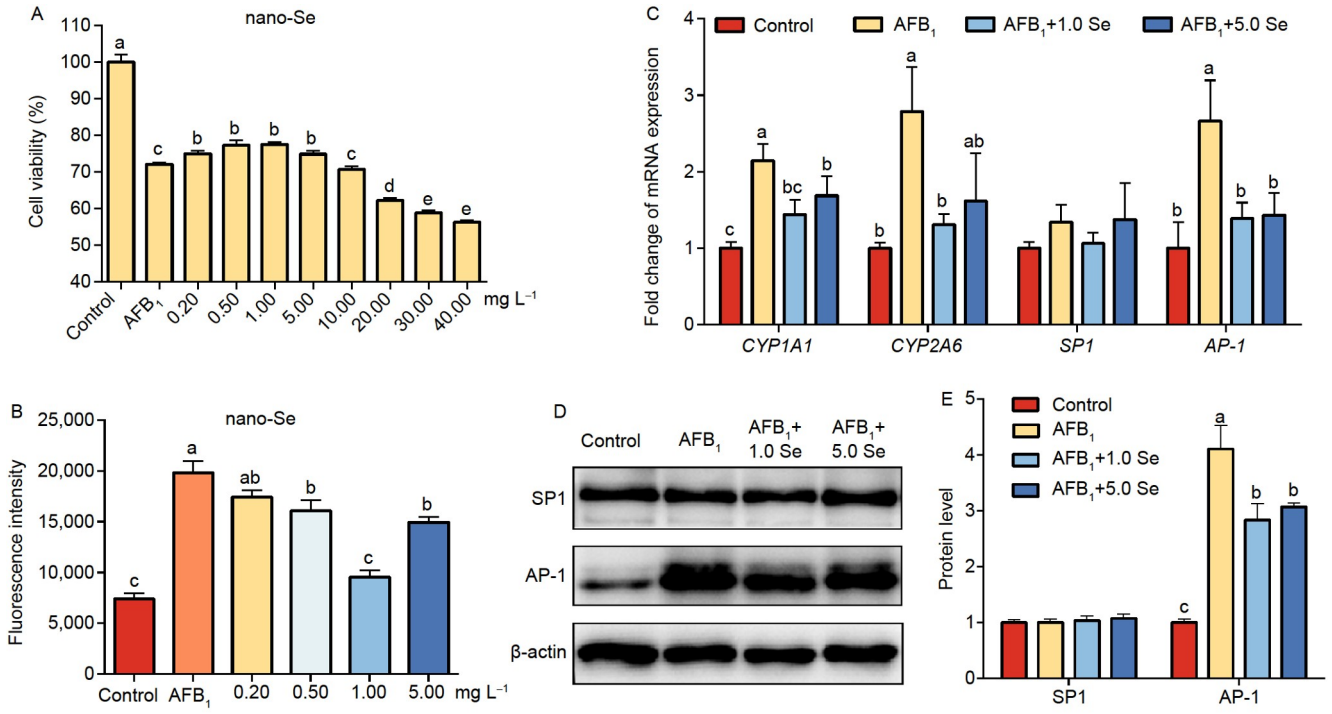

Figure 7. Nano-selenium mitigates the cytotoxicity of AFB_1 on LMH cells via down-regulation of AP-1. A and B, The mitigating effects of nano-Se on cell viability (A) and ROS levels (B) induced by AFB_1 on LMH cells, horizontal axis represented different doses of nano-Se, n=8–10. C, The effects of nano-Se on the expression of *CYP1A1*, *CYP2A6*, *SP1* and *AP-1*, n=6. D and E, The effects of nano-Se on the protein level of SP1 and AP-1, n=4. Values are means±SE; different letters between groups represent significant differences, P<0.05. Control, cells treated without AFB_1 and nano-Se; AFB_1, cells treated with AFB_1 at the IC_{30}; AFB_1+1.0 Se, cells with AFB_1 and 1.00 mg L^{-1} nano-Se; AFB_1+5.0 Se, cells treated with AFB_1 and 5.00 mg L^{-1} nano-Se.

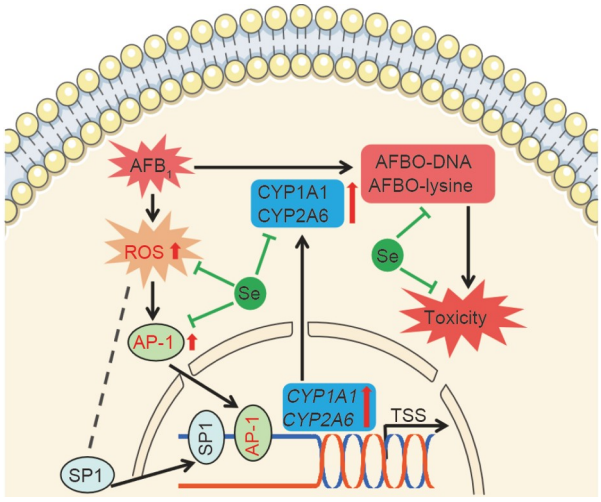

Figure 8. Illustration of AFB_1 bioactivation via upregulation of *CYP1A1* and *CYP2A6* through AP-1 and SP1 *trans*-activation. The "red arrow" means upregulation, the "dashed line" means there was no influence and the "green line" means inhibition.

Determination of reactive oxygen species

The concentration of ROS was determined with specific assay kits (Beyotime Biotechnology, Shanghai, China). Equivalent numbers of cells were seeded in a 12-well plate and treated with AFB_1 for 48 h. Cells were incubated with DCFH-DA at a concentration of 10 μmol L^{-1} for 30 min at 37°C. After three washes with free-serum media, cells were collected and counted. Then, fluorescence values of cells were measured with a fluorescence microplate reader (Thermo Fisher Scientific) with excitation and emission wavelengths at 488 and 525 nm.

Real-time qPCR and Western blotting analysis

Total RNA was isolated from LMH cells, and the relative mRNA abundance was quantified as described previously (Dong et al., 2023; Du et al., 2023; Luo et al., 2019). The target genes and their primers are listed in Table S1 in Supporting Information. Relative mRNA expression was calculated by using the $2^{-\Delta\Delta Ct}$ method with β-actin as a reference gene, and the relative abundance was normalized to that of the control (set as 1) (Deng et al., 2023). Total proteins were extracted from cells using radioimmunoprecipitation assay buffer with 1.0% phenylmethylsulphonyl fluoride (Beyotime Biotechnology), and Western blot analysis of the cell was conducted as previously described (Liu et al., 2023b). The primary antibodies of SP1 and AP-1 in this study were purchased from (ABclonal Technology, Wuhan, China). All protein levels were normalised to that of the housekeeping protein β-actin, and densitometric quantification of the Western blotting bands was performed using ImageJ 1.51j8 software.

Data availability

The data that support the findings of this study are available from the corresponding author on reasonable request.

Compliance and ethics

The author(s) declare that they have no conflict of interest.

Acknowledgement

This work was partly supported by the Chinese Natural Science Foundation Projects (31772636, 32072775, 32272915), the National Key Research and Development Program of China (2023YFD1301003), the Fundamental Research Funds for the Central Universities (2662023DKPY002) and the Top-notch Young Talent Supporting Program to LHS, a gift from Beijing Deyuanshun Biotechnology Co., Ltd.

Supporting information

The supporting information is available online at https://doi.org/10.1007/s11427-023-2512-6. The supporting materials are published as submitted, without typesetting or editing. The responsibility for scientific accuracy and content remains entirely with the authors.

References

Ates, M.B., and Ortatatli, M. (2021). Phase-1 bioactivation mechanisms of aflatoxin through AhR, CAR and PXR nuclear receptors and the interactions with Nigella sativa seeds and thymoquinone in broilers. Ecotoxicol Environ Saf 208, 111774.

Benkerroum, N. (2020). Chronic and acute toxicities of aflatoxins: mechanisms of action. Int J Environ Res Public Health 17, 423.

Bejjani, F., Evanno, E., Zibara, K., Piechaczyk, M., and Jariel-Encontre, I. (2019). The AP-1 transcriptional complex: local switch or remote command? Biochim Biophys Acta Rev Cancer 1872, 11–23.

Beisaw, A., Kuenne, C., Guenther, S., Dallmann, J., Wu, C.C., Bentsen, M., Looso, M., and Stainier, D.Y.R. (2020). AP-1 contributes to chromatin accessibility to promote sarcomere disassembly and cardiomyocyte protrusion during zebrafish heart regeneration. Circ Res 126, 1760–1778.

Bi, S.S., Talukder, M., Jin, H.T., Lv, M.W., Ge, J., Zhang, C., and Li, J.L. (2022). Nano-selenium alleviates cadmium-induced cerebellar injury by activating metal regulatory transcription factor 1 mediated metal response. anim Nutr 11, 402–412.

Chen, X., Li, C., Chen, Y., Ni, C., Chen, X., Zhang, L., Xu, X., Chen, M., Ma, X., Zhan, H., et al. (2019). Aflatoxin B1 impairs leydig cells through inhibiting AMPK/mTOR-mediated autophagy flux pathway. Chemosphere 233, 261–272.

Cheng, T.H., Shih, N.L., Chen, S.Y., Wang, D.L., and Chen, J.J. (1999). Reactive oxygen species modulate endothelin-I-induced c-*fos* gene expression in cardiomyocytes. Cardiovasc Res 41, 654–662.

Chuang, J.Y., Chang, W.C., and Hung, J.J. (2011). Hydrogen peroxide induces Sp1 methylation and thereby suppresses cyclin B_1 via recruitment of Suv39H1 and HDAC1 in cancer cells. Free Radic Biol Med 51, 2309–2318.

Coussens, L.M., and Werb, Z. (2002). Inflammation and cancer. Nature 420, 860–867.

Deng, J., Zhao, L., Zhang, N.Y., Karrow, N.A., Krumm, C.S., Qi, D.S., and Sun, L.H. (2018). Aflatoxin B_1 metabolism: regulation by phase I and II metabolizing enzymes and chemoprotective agents. Mutat Res Rev Mutat Res 778, 79–89.

Deng, Z.C., Yang, J.C., Huang, Y.X., Zhao, L., Zheng, J., Xu, Q.B., Guan, L., and Sun, L.H. (2023). Translocation of gut microbes to epididymal white adipose tissue drives lipid metabolism disorder under heat stress. Sci China Life Sci 66, 2877–2895.

Deng, Z.C., Wang, J., Wang, J., Yan, Y.Q., Huang, Y.X., Chen, C.Q., Sun, L.H., and Liu, M. (2024). Tannic acid extracted from gallnut improves intestinal health with regulation of redox homeostasis and gut microbiota of weaned piglets. Anim Res One Health 2, 16–27.

Diaz, G.J., Murcia, H.W., Cepeda, S.M., and Boermans, H.J. (2010a). The role of selected cytochrome P450 enzymes on the bioactivation of aflatoxin B_1 by duck liver microsomes. Avian Pathol 39, 279–285.

Diaz, G.J., Murcia, H.W., and Cepeda, S.M. (2010b). Cytochrome P450 enzymes involved in the metabolism of aflatoxin B_1 in chickens and quail. Poult Sci 89, 2461–2469.

Do, M.T., Kim, H.G., Tran, T.T.P., Khanal, T., Choi, J.H., Chung, Y.C., Jeong, T.C., and Jeong, H.G. (2014). Metformin suppresses CYP1A1 and CYP1B1 expression in breast cancer cells by down-regulating aryl hydrocarbon receptor expression. Toxicol Appl Pharmacol 280, 138–148.

Dohnal, V., Wu, Q., and Kuča, K. (2014). Metabolism of aflatoxins: key enzymes and interindividual as well as interspecies differences. Arch Toxicol 88, 1635–1644.

Dong, Z., Liu, S., Deng, Q., Li, G., Tang, Y., Wu, X., Wan, D., and Yin, Y. (2023). Role of iron in host-microbiota interaction and its effects on intestinal mucosal growth and immune plasticity in a piglet model. Sci China Life Sci 66, 2086–2098.

Du, S., Zeng, S., Song, L., Ma, H., Chen, R., Luo, J., Wang, X., Ma, T., Xu, X., Sun, H., et al. (2023). Functional characterization of novel NPRL3 mutations identified in three families with focal epilepsy. Sci China Life Sci 66, 2152–2166.

Dzau, V.J. (2002). Transcription factor decoy. Circ Res 90, 1234–1236.

Fiala-Beer, E., Lee, A.C., and Murray, M. (2007). Regulation of the rat CYP4A2 gene promoter by c-Jun and octamer binding protein-1. Int J Biochem Cell Biol 39, 1235–1247.

Gan, F., Yang, Y., Chen, Y., Che, C., Pan, C., and Huang, K. (2018). Bush sophora root polysaccharide could help prevent aflatoxin B_1-induced hepatotoxicity in the primary chicken hepatocytes. Toxicon 150, 180–187.

Gao, X., Zhang, C., Zheng, P., Dan, Q., Luo, H., Ma, X., and Lu, C. (2021). Arsenic suppresses GDF1 expression via ROS-dependent downregulation of specificity protein 1. Environ Pollut 271, 116302.

Gilday, D., Gannon, M., Yutzey, K., Bader, D., and Rifkind, A.B. (1996). Molecular cloning and expression of two novel avian cytochrome P450 1A enzymes induced by 2,3,7,8-tetrachlorodibenzo-p-dioxin. J Biol Chem 271, 33054–33059.

Jiang, D., He, Z., Wang, C., Zhou, Y., Li, F., Pu, W., Zhang, X., Feng, X., Zhang, M., Yecheng, X., et al. (2018). Epigenetic silencing of ZNF132 mediated by methylation-sensitive Sp1 binding promotes cancer progression in esophageal squamous cell carcinoma. Cell Death Dis 10, 1.

Jin, S., He, L., Yang, C., He, X., Chen, H., Feng, Y., Tang, W., Li, J., Liu, D., and Li, T. (2023). Crosstalk between trace elements and T-cell immunity during early-life health in pigs. Sci China Life Sci 66, 1994–2005.

Kang, L., Wu, Y., Zhang, J., An, Q., Zhou, C., Li, D., and Pan, C. (2022). Nano-selenium enhances the antioxidant capacity, organic acids and cucurbitacin B in melon (*Cucumis melo* L.) plants. Ecotoxicol Environ Saf 241, 113777.

Kim, J.E., Bunderson, B.R., Croasdell, A., and Coulombe Jr, R.A. (2011). Functional characterization of alpha-class glutathione *S*-transferases from the turkey (*Meleagris Gallopavo*). Toxicol Sci 124, 45–53.

Koo, J.H., Plouffe, S.W., Meng, Z., Lee, D.H., Yang, D., Lim, D.S., Wang, C.Y., and Guan, K.L. (2020). Induction of AP-1 by YAP/TAZ contributes to cell proliferation and organ growth. Genes Dev 34, 72–86.

Kume, M., Komori, K., Matsumoto, T., Onohara, T., Takeuchi, K., Yonemitsu, Y., and Sugimachi, K. (2002). Administration of a decoy against the activator protein-1 binding site suppresses neointimal thickening in rabbit balloon-injured arteries. Circulation 105, 1226–1232.

Lee, J.K.H., Pearson, J.D., Maser, B.E., and Ingham, R.J. (2013). Cleavage of the JunB transcription factor by caspases generates a carboxyl-terminal fragment that inhibits activator protein-1 transcriptional activity. J Biol Chem 288, 21482–21495.

Li, R., Knight, J.D., Jackson, S.P., Tjian, R., and Botchan, M.R. (1991). Direct interaction between Sp1 and the BPV enhancer E2 protein mediates synergistic activation of transcription. Cell 65, 493–505.

Liu, X., Kumar Mishra, S., Wang, T., Xu, Z., Zhao, X., Wang, Y., Yin, H., Fan, X., Zeng, B., Yang, M., et al. (2020). AFB_1 induced transcriptional regulation related to apoptosis and lipid metabolism in liver of chicken. Toxins 12, 290.

Liu, S., Kang, W., Mao, X., Ge, L., Du, H., Li, J., Hou, L., Liu, D., Yin, Y., Liu, Y., et al. (2022a). Melatonin mitigates aflatoxin B1-induced liver injury via modulation of gut microbiota/intestinal FXR/liver TLR4 signaling axis in mice. J Pineal Res 73, e12812.

Liu, M., Sun, X., Chen, B., Dai, R., Xi, Z., and Xu, H. (2022b). Insights into manganese superoxide dismutase and human diseases. Int J Mol Sci 23, 15893.

Liu, M., Zhang, L., Mo, Y., Li, J., Yang, J., Wang, J., Karrow, N.A., Wu, H., and Sun, L. (2023a). Ferroptosis is involved in deoxynivalenol-induced intestinal damage in pigs. J Anim Sci Biotechnol 14, 29.

Liu, S., Dong, Z., Tang, W., Zhou, J., Guo, L., Gong, C., Liu, G., Wan, D., and Yin, Y. (2023b). Dietary iron regulates intestinal goblet cell function and alleviates Salmonella typhimurium invasion in mice. Sci China Life Sci 66, 2006–2019.

Luo, J.J., Zhang, Y., Sun, H., Wei, J.T., Khalil, M.M., Wang, Y.W., Dai, J.F., Zhang, N.Y., Qi, D.S., and Sun, L.H. (2019). The response of glandular gastric transcriptome to T-2 toxin in chicks. Food Chem Toxicol 132, 110658.

Luo, B., Zhang, S., Tan, D., Yu, X., Lin, J., and Wang, M. (2022). Anlotinib benefits the αPDL1 immunotherapy by activating ROS/JNK/AP-1 pathway to upregulate PDL1 expression in colorectal cancer. Oxid Med Cell Longev 2022, 8965903.

Ma, Y., Fei, Y., Ding, S., Jiang, H., Fang, J., and Liu, G. (2023). Trace metal elements: a bridge between host and intestinal microorganisms. Sci China Life Sci 66, 1976–1993.

Manikandan, P., and Nagini, S. (2018). Cytochrome P450 structure, function and clinical significance: a review. Curr Drug Targets 19, 38–54.

Medeiros-Ventura, W.R.L., Rabello, C.B.V., Barros, M.R., Silva Junior, R.V., Oliveira, H.B., Faria, A.G., Silva, A.F., Soares, P.C., Pereira, C.G., Santos, M.J.B., et al. (2020). Zinc, manganese, and copper amino acid complexes improve performance and bone characteristics of layer-type chicks under thermoneutral and cold stress conditions. Poult Sci 99, 5718–5727.

Miksicek, R., Borgmeyer, U., and Nowock, J. (1987). Interaction of the TGGCA-binding protein with upstream sequences is required for efficient transcription of mouse mammary tumor virus. EMBO J 6, 1355–1360.

Mo, Y.X., Ruan, M.L., Wang, J., Liu, Y., Wu, Y.Y., Wang, G.L., Han, Y.M., Wan, H.F., Lamesgen, D., Kuča, K., et al. (2023). Mitigating the adverse effects of Aflatoxin B1 in LMH, IPEC-J2 and 3D4/21 cells by a novel integrated agent. Food Chem Toxicol 178, 113907.

Muhammad, I., Sun, X., Wang, H., Li, W., Wang, X., Cheng, P., Li, S., Zhang, X., and Hamid, S. (2017). Curcumin successfully inhibited the computationally identified

CYP2A6 enzyme-mediated bioactivation of aflatoxin B_1 in Arbor Acres broiler. Front Pharmacol 8.
Nelson, D.R., Zeldin, D.C., Hoffman, S.M., Maltais, L.J., Wain, H.M., and Nebert, D.W. (2004). Comparison of cytochrome P450 (*CYP*) genes from the mouse and human genomes, including nomenclature recommendations for genes, pseudogenes and alternative-splice variants. Pharmacogenetics 14, 1–18.
Ptashne, M. (1986). Gene regulation by proteins acting nearby and at a distance. Nature 322, 697–701.
Reuter, S., Gupta, S.C., Chaturvedi, M.M., and Aggarwal, B.B. (2010). Oxidative stress, inflammation, and cancer: how are they linked? Free Radic Biol Med 49, 1603–1616.
Remes, A., Arif, R., Franz, M., Jungmann, A., Zaradzki, M., Puehler, T., Heckmann, M. B., Frey, N., Karck, M., Kallenbach, K., et al. (2021). AAV-mediated AP-1 decoy oligonucleotide expression inhibits aortic elastolysis in a mouse model of Marfan syndrome. Cardiovasc Res 117, 2459–2473.
Rupp, R.A.W., Kruse, U., Multhaup, G., Göbel, U., Beyreuther, K., and Sippel, A.E. (1990). Chicken NFI/TGGCA proteins are encoded by at least three independent genes: NFI-A, NFI-B and NFI-C with homologues in mammalian genomes. Nucl Acids Res 18, 2607–2616.
Rushing, B.R., and Selim, M.I. (2019). Aflatoxin B_1: a review on metabolism, toxicity, occurrence in food, occupational exposure, and detoxification methods. Food Chem Toxicol 124, 81–100.
Sang, R., Ge, B., Li, H., Zhou, H., Yan, K., Wang, W., Cui, Q., and Zhang, X. (2023). Taraxasterol alleviates aflatoxin B_1-induced liver damage in broiler chickens via regulation of oxidative stress, apoptosis and autophagy. Ecotoxicol Environ Saf 251, 114546.
Schwarz, M., Lossow, K., Kopp, J.F., Schwerdtle, T., and Kipp, A.P. (2019). Crosstalk of Nrf2 with the trace elements selenium, iron, zinc, and copper. Nutrients 11, 2112.
Shetty, P.K., Venuvanka, V., Jagani, H.V., Chethan, G.H., Ligade, V.S., Musmade, P.B., Nayak, U.Y., Reddy, M.S., Kalthur, G., Udupa, N., et al. (2015). Development and evaluation of sunscreen creams containing morin-encapsulated nanoparticles for enhanced UV radiation protection and antioxidant activity. Int J Nanomedicine 10, 6477–6491.
Song, J., Nabeel-Shah, S., Pu, S., Lee, H., Braunschweig, U., Ni, Z., Ahmed, N., Marcon, E., Zhong, G., Ray, D., et al. (2022). Regulation of alternative polyadenylation by the C2H2-zinc-finger protein Sp1. Mol Cell 82, 3135–3150.e9.
Studer, J.M., Kiefer, Z.E., Goetz, B.M., Keating, A.F., Baumgard, L.H., Rambo, Z.J., Schweer, W.P., Wilson, M.E., Rapp, C., and Ross, J.W. (2021). Evaluation of the molecular response of corpora lutea to manganese-amino acid complex supplementation in gilts. J Anim Sci 99, skab245.
Su, W., Jackson, S., Tjian, R., and Echols, H. (1991). DNA looping between sites for transcriptional activation: self-association of DNA-bound Sp1. Genes Dev 5, 820–826.
Taranu, I., Hermenean, A., Bulgaru, C., Pistol, G.C., Ciceu, A., Grosu, I.A., and Marin, D.E. (2020). Diet containing grape seed meal by-product counteracts AFB_1 toxicity in liver of pig after weaning. Ecotoxicol Environ Saf 203, 110899.
Tohkin, M., Kurose, K., and Fukuhara, M. (1996). Okadaic acid potentiates 3-methylcholanthrene-induced CYP2A8 gene expression in primary cultures of Syrian hamster hepatocytes: possible involvement of activator protein-1. Mol Pharmacol 50, 556–564.
Ung, T.T., Nguyen, T.T., Li, S., Han, J.Y., and Jung, Y.D. (2021). Nicotine stimulates CYP1A1 expression in human hepatocellular carcinoma cells via AP-1, NF-κB, and AhR. Toxicol Lett 349, 155–164.
Vizcaíno, C., Mansilla, S., and Portugal, J. (2015). Sp1 transcription factor: a long-standing target in cancer chemotherapy. Pharmacol Ther 152, 111–124.
Wan, D., and Yin, Y. (2023). Trace elements in nutrition and health: a deep dive into essentiality and mechanism of their biological roles. Sci China Life Sci 66, 1949–1951.
Wang, H., Li, W., Muhammad, I., Sun, X., Cui, X., Cheng, P., Qayum, A., and Zhang, X. (2018). Biochemical basis for the age-related sensitivity of broilers to aflatoxin B_1. Toxicol Mech Methods 28, 361–368.
Wang, R., Bai, Z., Chang, J., Li, Q., Hristov, A.N., Smith, P., Yin, Y., Tan, Z., and Wang, M. (2022a). China's low-emission pathways toward climate-neutral livestock production for animal-derived foods. Innovation 3, 100220.
Wang, C., Gu, Z., Gu, X., Tan, X., Wang, S., Zhang, R., Li, R., Sun, M., Gui, C., Li, S., et al. (2022b). Nano-selenium attenuates mitochondrial-associated apoptosis via the PI3K/AKT pathway in nickel-induced hepatotoxicity *in vivo* and *in vitro*. Environ Toxicol 37, 101–119.
Wang, X., Yang, F., Na, L., Jia, M., Ishfaq, M., Zhang, Y., Liu, M., and Wu, C. (2022c). Ferulic acid alleviates AFB_1-induced duodenal barrier damage in rats via up-regulating tight junction proteins, down-regulating ROCK, competing CYP450 enzyme and activating GST. Ecotoxicol Environ Saf 241, 113805.
Wang, D., Kuang, Y., Lv, Q., Xie, W., Xu, X., Zhu, H., Zhang, Y., Cong, X., Cheng, S., and Liu, Y. (2023). Selenium-enriched cardamine violifolia protects against sepsis-induced intestinal injury by regulating mitochondrial fusion in weaned pigs. Sci China Life Sci 66, 2099–2111.
Wei, T., Ren, P., Huang, L., Ouyang, Z., Wang, Z., Kong, X., Li, T., Yin, Y., Wu, Y., and He, Q. (2019). Simultaneous detection of aflatoxin B1, ochratoxin A, zearalenone and deoxynivalenol in corn and wheat using surface plasmon resonance. Food Chem 300, 125176.
Xie, D., Zhao, H., Lu, J., He, F., Liu, W., Yu, W., Wang, Q., Hisatome, I., Yamamoto, T., Koyama, H., et al. (2018). High uric acid induces liver fat accumulation via ROS/JNK/AP-1 signaling. Am J Physiol-Endocrinol Metab 320, E1032–E1043.
Xie, Z., Yang, X., Duan, Y., Han, J., and Liao, C. (2021). Small-molecule kinase inhibitors for the treatment of nononcologic diseases. J Med Chem 64, 1283–1345.
Wu, L., Li, J., Li, Y., Li, T., He, Q., Tang, Y., Liu, H., Su, Y., Yin, Y., and Liao, P. (2016). Aflatoxin B1, zearalenone and deoxynivalenol in feed ingredients and complete feed from different Province in China. J Anim Sci Biotechnol 7, 63.
Xue, K.S., Cai, W., Tang, L., and Wang, J.S. (2016). Aflatoxin B_1-lysine adduct in dried blood spot samples of animals and humans. Food Chem Toxicol 98, 210–219.
Yan, Y.Q., Liu, M., Xu, Z.J., Xu Z.J., Huang, Y.X., Li, X.M., Chen, C.J., Zuo, G., Yang, J. C., Lei, X.G., et al. (2024). Optimum doses and forms of selenium maintaining reproductive health via regulating homeostasis of gut microbiota and testicular redox, inflammation, cell proliferation, and apoptosis in roosters. J Nutr 154, 369–380.
Yang, C.C., Hsiao, L.D., Shih, Y.F., Chang, C.I., and Yang, C.M. (2022). Induction of heme oxygenase-1 by 15d-prostaglandin J2 mediated via a ROS-dependent Sp1 and AP-1 cascade suppresses lipopolysaccharide-triggered interleukin-6 expression in mouse brain microvascular endothelial cells. Antioxidants 11, 719.
Young, M.J., Chen, Y.C., Wang, S.A., Chang, H.P., Yang, W.B., Lee, C.C., Liu, C.Y., Tseng, Y.L., Wang, Y.C., Sun, H.S., et al. (2022). Estradiol-mediated inhibition of Sp1 decreases miR-3194-5p expression to enhance CD44 expression during lung cancer progression. J Biomed Sci 29, 3.
Yunus, A.W., Razzazi-Fazeli, E., and Bohm, J. (2011). Aflatoxin B1 in affecting broiler's performance, immunity, and gastrointestinal tract: a review of history and contemporary issues. Toxins 3, 566–590.
Zanconato, F., Forcato, M., Battilana, G., Azzolin, L., Quaranta, E., Bodega, B., Rosato, A., Bicciato, S., Cordenonsi, M., and Piccolo, S. (2015). Genome-wide association between YAP/TAZ/TEAD and AP-1 at enhancers drives oncogenic growth. Nat Cell Biol 17, 1218–1227.
Zanger, U.M., and Schwab, M. (2013). Cytochrome P450 enzymes in drug metabolism: regulation of gene expression, enzyme activities, and impact of genetic variation. Pharmacol Ther 138, 103–141.
Zhao, L., Feng, Y., Deng, J., Zhang, N.Y., Zhang, W.P., Liu, X.L., Rajput, S.A., Qi, D.S., and Sun, L.H. (2019). Selenium deficiency aggravates aflatoxin B1-induced immunotoxicity in chick spleen by regulating 6 selenoprotein genes and redox/inflammation/apoptotic signaling. J Nutr 149, 894–901.
Zhao, L., Deng, J., Ma, L.B., Zhang, W.P., Khalil, M.M., Karrow, N.A., Qi, D.S., and Sun, L.H. (2021a). Dietary Se deficiency dysregulates metabolic and cell death signaling in aggravating the AFB_1 hepatotoxicity of chicks. Food Chem Toxicol 149, 111938.
Zhao, L., Feng, Y., Xu, Z.J., Zhang, N.Y., Zhang, W.P., Zuo, G., Khalil, M.M., and Sun, L.H. (2021b). Selenium mitigated aflatoxin B_1-induced cardiotoxicity with potential regulation of 4 selenoproteins and ferroptosis signaling in chicks. Food Chem Toxicol 154, 112320.
Zhao, L., Feng, Y., Wei, J.T., Zhu, M.X., Zhang, L., Zhang, J.C., Karrow, N.A., Han, Y. M., Wu, Y.Y., Guo, Y.M., et al. (2021c). Mitigation effects of bentonite and yeast cell wall binders on AFB_1, DON, and OTA induced changes in laying hen performance, egg quality, and health. Toxins 13, 156.
Zhao, L., Liu, M., Sun, H., Yang, J.C., Huang, Y.X., Huang, J.Q., Lei, X., and Sun, L.H. (2023). Selenium deficiency-induced multiple tissue damage with dysregulation of immune and redox homeostasis in broiler chicks under heat stress. Sci China Life Sci 66, 2056–2069.
Zhang, Z., Lu, H., Huan, F., Meghan, C., Yang, X., Wang, Y., Wang, X., Wang, X., and Wang, S. (2014). Cytochrome P450 2A13 mediates the neoplastic transformation of human bronchial epithelial cells at a low concentration of aflatoxin B_1. Intl J Cancer 134, 1539–1548.
Zhang, N.Y., Qi, M., Gao, X., Zhao, L., Liu, J., Gu, C.Q., Song, W.J., Krumm, C.S., Sun, L.H., and Qi, D.S. (2016). Response of the hepatic transcriptome to aflatoxin B_1 in ducklings. Toxicon 111, 69–76.
Zhang, B., Li, M., Zhou, G., Gu, X., Xie, L., Zhao, M., Xu, Q., Tan, G., and Zhang, N. (2023). ZnO-NPs alleviate aflatoxin B1-induced hepatoxicity in ducklings by promoting hepatic metallothionein expression. Ecotoxicol Environ Saf 256, 114826.