Biochemical and Biophysical Research Communications xxx (2016) 1–7

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Exploration of Bcl-2 family and caspases-dependent apoptotic signaling pathway in Zearalenone-treated mouse endometrial stromal cells

Jin Hu, Minglong Xu, Yujian Dai, Xiaolin Ding, Cheng Xiao, Hongjun Ji, Yinxue Xu*

College of Animal Science and Technology, Nanjing Agricultural University, Nanjing 210095, PR China

ARTICLE INFO

Article history:
Received 14 May 2016
Accepted 29 May 2016
Available online xxx

Keywords:
ZEA
Cytotoxicity
Mouse
Endometrial stromal cells
Apoptosis
Signaling pathway

ABSTRACT

Zearalenone (ZEA) is a nonsteroidal estrogenic mycotoxin found in several food commodities worldwide. Although the toxicity of ZEA have been widely studied in a number of cell types, the mechanistic role of ZEA on apoptosis of endometrial stromal cells (ESCs) remains poorly understood. The objective of this study was to determine the effects of ZEA on apoptosis of mouse ESCs and explore the signaling pathway underlying the cytotoxicity of ZEA. The results showed that ZEA treatment caused obvious apoptosis in ESCs as determined by the flow cytometry and terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick end labeling (TUNEL) assay. Immunoblotting and real-time quantitative polymerase chain reaction (RT-qPCR) revealed that ZEA treatment increased the ratio of Bax/Bcl-2. The enzymatic activity assays revealed that caspases-3 and caspase-9 were activated by ZEA treatment in a dose-dependent manner. In addition, flow cytometry show that the apoptotic percentages of cells pretreated with Z-VAD-FMK and Z-LEHD-FMK were markedly reduced compared to the ZEA-treated cells. Overall, the results suggested that ZEA induced obvious apoptosis in ESCs via a Bcl-2 family and caspases-dependent signaling pathway.

1. Introduction

Zearalenone (ZEA), also known as the F-2 toxin, is a nonsteroidal mycotoxin produced by several species of *Fusarium*. It is a common fungal contaminant of cereal crops worldwide and commonly found in feed and foodstuff, such as maize, wheat, rye, and other cereals [1,2]. Due to its structural similarity to estrogen, ZEA competes with estradiol for binding to estrogen receptors and provokes estrogenic activities, which can cause several physiological alterations of the reproductive tract [3,4]. *In vitro* studies also indicated that ZEA can result in incidences of mycotoxicosis in farm animals [5,6] and dietary higher concentrations of ZEA cause abortion and reproductive failure [7]. And *in vitro* studies indicated that the cells with ZEA cytotoxicity suffer numerous changes which include alterations of some important metabolic processes, such as proliferation and cell differentiation, apoptosisand molecules synthesis *etc.* [8].

The endometrium of the uterus is lined by a columnar epithelium that is supported by a stromal cell foundation which consisting of a variety of cell types. The endometrial epithelial cells (EECs) and ESCs are the main two cell types as they have the absolute predominance in quantitative terms. The EECs actively take part in mucosal immune responses, including the antigen presentation, the transport of IgA, and the production of a variety of growth factors [9–12]. While the ESCs are known to produce numerous growth factors and cytokines that affect epithelial function. For example, epidermal growth factor, insulin-like growth factor-1, and hepatocyte growth factor are all produced in the ESCs and have been found to stimulate EECs mitogenesis and development [13–16]. Some studies revealed that the ESCs can promote EECs development or reprogramme EECs differentiation [17,18]. In addition, ESCs can be accompanied by extensive proliferation, differentiation, and endoreduplication (polyploidy) in the process of uterine decidualization at the site of embryo implantation, which is critical to the establishment of pregnancy in mice [19].

To the best of our knowledge, few studies were directly performed on exploring the mechanisms of ESCs damage caused by

* Corresponding author. Department of Animal Genetics, Breeding and Reproduction, College of Animal Science and Technology, Nanjing Agricultural University, Nanjing 210095, PR China.
E-mail address: xuyinxue@njau.edu.cn (Y. Xu).

http://dx.doi.org/10.1016/j.bbrc.2016.05.161
0006-291X/

Table 1
Primers used for quantitative real-time PCR.

Primer	Length (bp)	Primer sequence (5′–3′)	Tm(°C)
Caspase-9-F/R	109	TCCTGGTACATCGAGACCTTG/AAGTCCCTTTCGCAGAAACAG	61
Caspase-3-F/R	225	ACAGCACCTGGTTACTATTC/CAGTTCTTTCGTGAGCAT	60
Bax-F/R	139	CCAGGATGCGTCCACCAAGA/GGTGAGGACTCCAGCCACAA	58
Bcl-2-F/R	120	GTGGATGACTGAGTACCTGAACC/AGCCAGGAGAAATCAAACAGAG	58
β-actin-F/R	460	GCTGTCCCTGTATGCCTCT/GTCTTTACGGATGTCAACG	60

ZEA till now, though it has been shown that ZEA was toxic to female reproductive system intensified lately. To better understand the links of ZEA toxicity and ESCs, we investigated the effects of different ZEA concentrations on apoptosis in primary mouse ESCs. We also hypothesize that we can discover the apoptosis signaling pathway caused by the cytotoxicity of ZEA on mouse ESCs.

2. Materials and methods

2.1. Chemicals and reagents

Zearalenone (ZEA), Z-VAD-FMK, Z-LEHD-FMK, and Collagenasel were purchased from Sigma-Aldrich (St. Louis, USA). Antibodies to Bax, Bcl-2 and β-actin were purchased from Cell Signaling Technology (Boston, USA). Enhanced chemiluminescence (ECL) (TransGen, China). 0.25% Trypsin-EDTA, DMEM/F-12 medium and fetal bovine serum was obtained from GIBCO BRL (Grand Island, USA). Prime Script RT Master Mix, SYBR Green Master Mix and Annexin V-FITC Apoptosis Detection Kit (Vazyme, China), Caspase-3 and Caspase-9 Activity Assay Kit, DCFH-DA and JC-1 Assay Kit (Beyotime, China). All other chemicals and reagents were of the highest quality and obtained from standard commercial sources.

2.2. Cell isolation and culture

Mouse ESCs were isolated by using a previously described method [20] with some modifications. Briefly, the pieces of uterine horns were placed in PBS containing 0.25% trypsin for 30 min at 4 °C followed by 30 min at room temperature. After these digestion steps, the remaining tissues were washed twice in PBS and then placed in PBS containing 0.1% collagenase Type I for 1 h at 37 °C. The homogenate was added medium contain FBS and subsequently filtered through a BD Falcon cell strainers filter (nylon mesh size, 70 μm), and cells were collected for centrifugation at 1000 rpm for

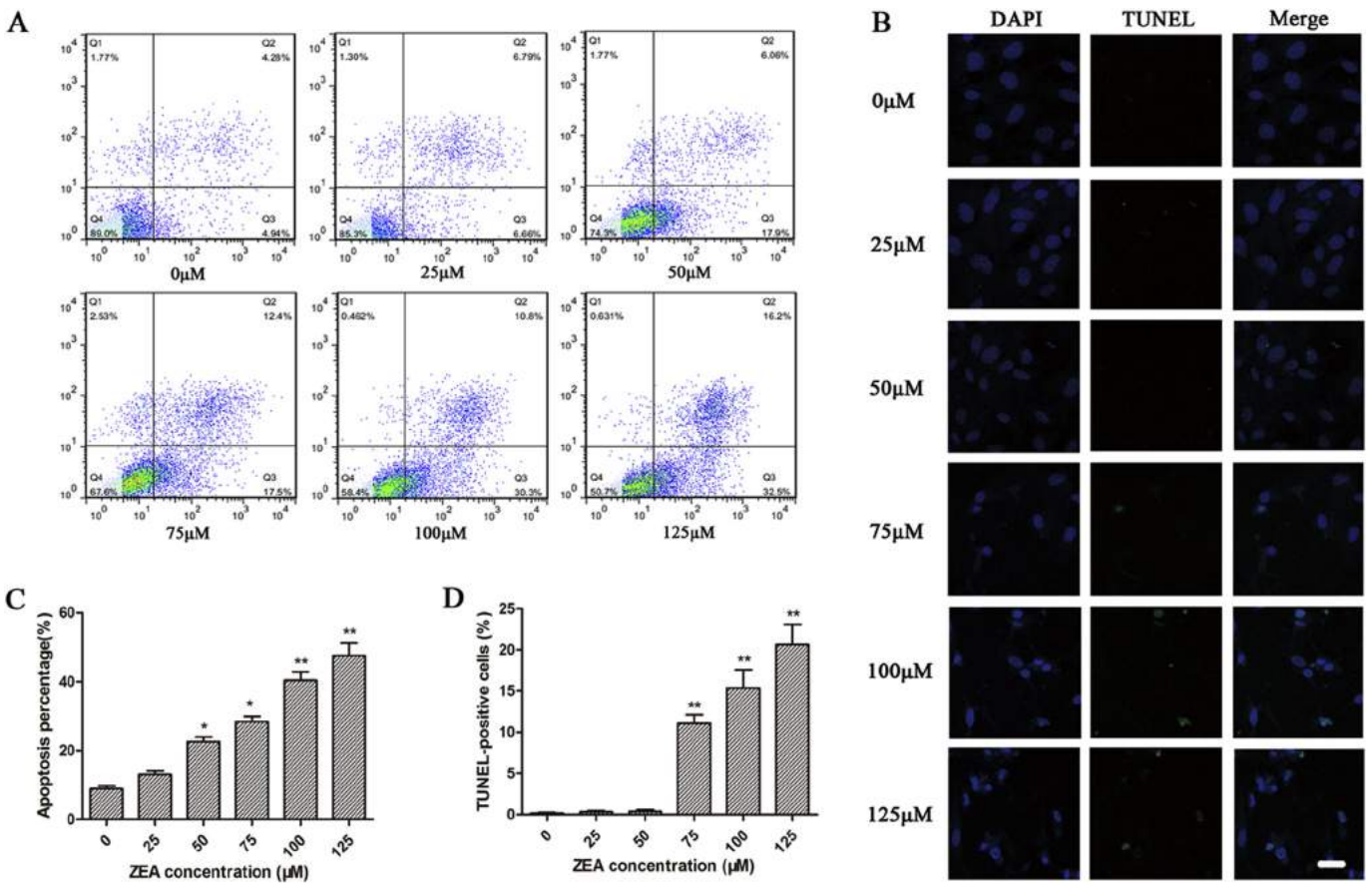

Fig. 1. Effect of ZEA treatment on apoptosis of mouse ESCs. A: Flow cytometry showed apoptosis percentage of mouse ESCs treated with various concentrations of ZEA treatment. B: TUNEL assays displayed the apoptosis in mouse ESCs by various concentrations of ZEA. C: Quantification of the apoptosis percentage in flow cytometry. D: Quantification of TUNEL-positive cells. The data are expressed as the mean ± SEM of three independent experiments. Statistically significant difference as compared with control group, "*"indicated significant difference ($P < 0.05$), "**" indicated extremely significant difference ($P < 0.01$).

Please cite this article in press as: J. Hu, et al., Exploration of Bcl-2 family and caspases-dependent apoptotic signaling pathway in Zearalenone-treated mouse endometrial stromal cells, Biochemical and Biophysical Research Communications (2016), http://dx.doi.org/10.1016/j.bbrc.2016.05.161

5 min. After washing for two times in PBS, the cells were resuspended with the DMEM-F12 medium with 10% FBS and seeded in relevant cultured bottle with cell suspension density of 3–6 × 10^5 cells/mL in a humidified atmosphere of 5% CO_2 at 37 °C.

2.3. Total RNA extraction and RT-qPCR

Total RNA was extracted from ESCs after exposure to ZEA (0, 25, 50, 75, and 100 μM) after 24 h by using the TRIzol reagent according to the manufacturer's instructions. The purity of RNA was determined by the quotient of OD at 260/280 nm (1.8–2.0). The Easyscript first-strand cDNA synthesis super mix kit was used for reverse transcription polymerase chain reaction (RT-PCR). The sequences of primers for the detected genes (shown in Table 1) which were designed by the Primer Premier 5 software. The reactions were performed in triplicate for each sample with 10 μl of AceQTM qPCR SYBR Green Master Mix, 0.8 μl of each primer, 0.4 μl of ROX Reference Dyel, 2 μL of cDNA (100 ng/μl) and RNase-free water to a final volume of 20 μl. PCR reaction was performed under the following conditions: Initial denaturation for 5 min at 95 °C, 40 cycles of denaturation for 10 s at 95 °C, annealing for 30 s and melting curve for 15 s at 95 °C, 60 °C, 15 s at 95 °C. The $2^{-\Delta\Delta Ct}$ method was used to identify differences in expression levels among the detected genes.

2.4. Determination of apoptosis levels by flow cytometry and TUNEL

Apoptosis was determined by staining cells with Annexin V-FITC and Propidium Iodide (PI). ESCs exposed to various concentrations of ZEA (0, 25, 50, 75, and 100 μM), for 24 h, or pretreated with 20 μM of caspase inhibitor Z-VAD-FMK or 10 μM of caspase-9 inhibitor Ac-LEHD-FMK, for 2 h, and then treated with 100 μM ZEA for 24 h. The cells were stained with 5 μl of Annexin V-FITC and 5 μl of propidium iodide in 100 μl loading binding buffer for 15 min at room temperature in the dark. The fluorescent signal of the cells was measured with a flow cytometer (FACS Calibur; USA) according to the procedure as described previously [21].

The apoptosis rates were also determined by the TUNEL assay. The detailed procedure was performed according to the protocol of the In Situ Cell Death Detection Kit. Images were obtained using a laser scanning confocal microscopy (Zeiss 710, Germany).

2.5. Western blotting

Mouse ESCs were cultured in 6-well plates and treated with various concentrations of ZEA (0, 25, 50, 75 and 100 μM), for 24 h, cells were washed three times with cold PBS and lysed in 200 μl cold lysis buffer per 1 × 106 cells for 30 min at 4 °C. Samples were then clarified by centrifugation at 12,000 rpm for 10 min. Protein concentration was determined using the bicinchoninic acid (BCA)

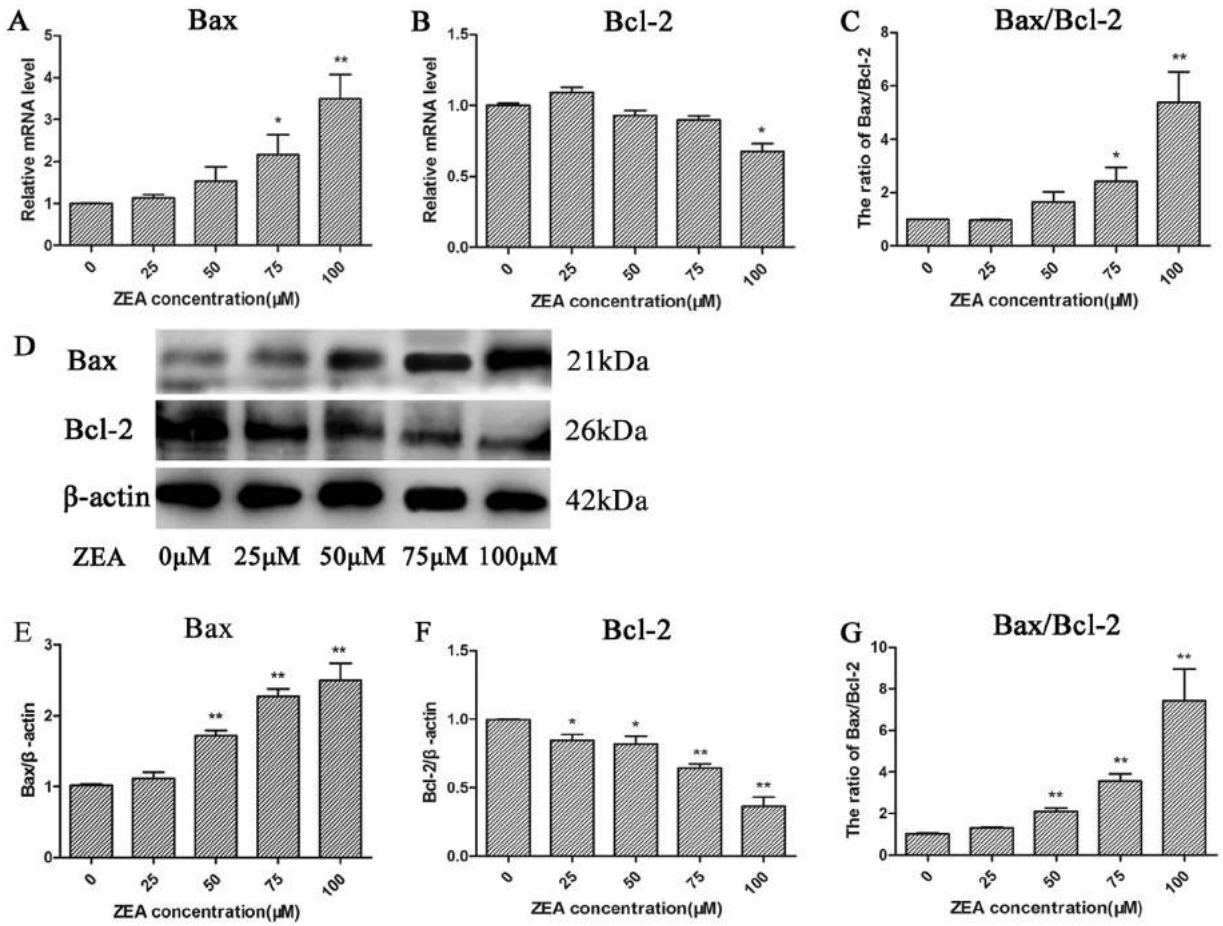

Fig. 2. Expression levels of Bax and Bcl-2 in mouse ESCs with various concentrations of ZEA treatment. A, B: The relative mRNA expression of Bax and Bcl-2; C: The ratio of Bax/Bcl-2 for mRNA expression; D: Western blotting showed the expression of Bax and Bcl-2, β-Actin was used as controls of loading proteins; E, F: The relative protein expression of Bax and Bcl-2; G: The ratio of Bax/Bcl-2 for protein expression. The data are expressed as the mean ± SEM of three independent experiments. Statistically significant difference as compared with control group, "*"indicated significant difference ($P < 0.05$), "**" indicated extremely significant difference ($P < 0.01$).

Please cite this article in press as: J. Hu, et al., Exploration of Bcl-2 family and caspases-dependent apoptotic signaling pathway in Zearalenone-treated mouse endometrial stromal cells, Biochemical and Biophysical Research Communications (2016), http://dx.doi.org/10.1016/j.bbrc.2016.05.161

assay kit. Equal amounts of protein were subjected to SDS-PAGE then transferred to nitrocellulose membranes according to the methods as described previously [22]. After transfer, the membranes were blocked with Trisbuffered saline (TBS) containing 0.1% Tween-20 (TBST) and 5% BSA for 2 h at room temperature. The membranes were then incubated with the chosen antibodies, including antibody to Bax, Bcl-2, and β-actin, at a dilution of 1:500 in TBST at 4 °C overnight. After three washes with TBST, the membranes were incubated with horseradish peroxidase-conjugated secondary antibody at room temperature for 2 h.Then visualized with the ECL-plus Western Blotting Detection System (LAS-4000 imager, Fujifilm, Tokyo, Japan) using Cool Saver AE-6955 (Pierce, Rockford, IL, USA). Densitometry analyses were performed using Image-J 1.42q software, and the values for target proteins were normalized to β-actin as the endogenous control.

2.6. Caspase activity assay

The activity of caspase family was detected using caspase colorimetric protease assay kit [23]. Caspase-3 can catalyze acetyl-Asp-Glu-Val-Asp *p*-nitroanilide (Ac-DEVD-*p*NA) to *p*-nitroaniline (*p*NA) and Caspase-9 can catalyze acetyl-Leu-Glu-His-Asp *p*-nitroanilide (Ac-LEHD-*p*NA) to *p*NA. An increase in absorbance at 405 nm was used to quantify the activation of caspases activities. Endometrial stromal cells exposed to various concentrations of ZEA (0, 25, 50, 75, and 100 μM), for 24 h, pretreated with 20 μM of caspases inhibitor Z-VAD-FMK or 10 μM of caspase-9 inhibitor Ac-LEHD-FMK, for 2 h, and then treated with 100 μM ZEA for 24 h. Subsequently, cells from 6-wells were collected and rinsed with cold PBS, and then lysed by lysis buffer for 15 min on ice. Cell lysates were centrifuged at 18,000 × g for 10 min at 4 °C. Caspase-3 and caspase-9 activities in the supernatant were assayed using the kit. The caspase activities were expressed as percentage of enzyme activity compared to control.

2.7. Statistical analysis

At least three replicates were performed for each treatment and results were analyzed using SPSS version 18.0 (SPSS Inc., USA) and given as means ± SEM. Statistical comparisons were made by analysis of variance (ANOVA), followed by Duncan's multiple comparisons test. P values < 0.05 were accepted as significant, P values < 0.01 were accepted as extremely significant. Fluorescence intensity was quantified by Image J software (NIH, Bethesda, MD). All experiments were repeated at least three times.

3. Results

3.1. Effect of ZEA on apoptosis of mouse ESCs

Flow cytometry was used to measure the apoptosis percentage of ESCs treated with various concentrations of ZEA (0, 25, 50, 75, 100 and 125 μM) for 24 h (Fig. 1A). It is feasible to identify and quantitate apoptotic cells on a single-cell basis using flow cytometry by conjugating FITC to Annexin V. The result of flow cytometry displayed that apoptotic percentage of mouse ESCs were significantly increased by ZEA treatment (Fig. 1C). TUNEL assay revealed that ZEA treatment induced a remarkable increase in the TUNEL-positive cells of mouse ESCs in a dose-dependent manner compared to the control (Fig. 1B and D). These results indicated that

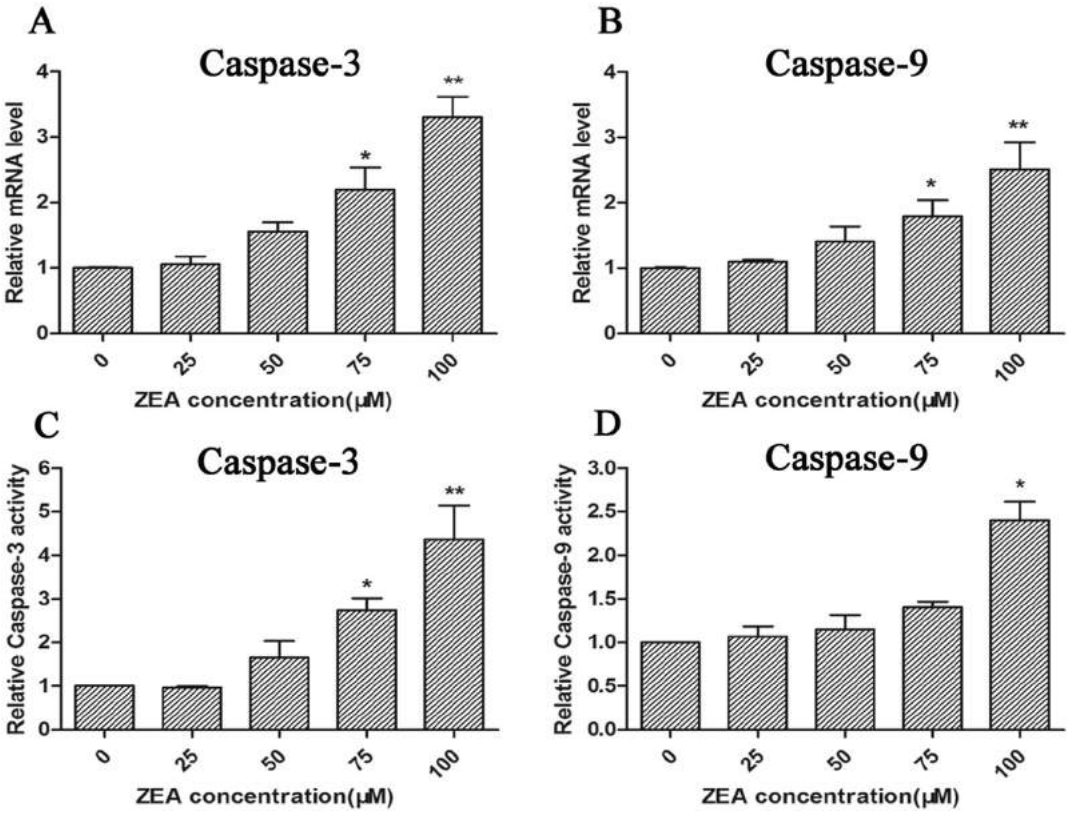

Fig. 3. Expression of caspase-3 and caspase-9 in mouse ESCs with various concentrations of ZEA treatment. A: The relative mRNA expression of caspase-3; B: The relative mRNA expression of caspase-9; C: The relative activity of caspase-3; D: The relative activity of caspase-9. The data are expressed as the mean ± SEM of three independent experiments. Statistically significant difference as compared with control group, "*"indicated significant difference ($P < 0.05$), "**" indicated extremely significant difference ($P < 0.01$).

Please cite this article in press as: J. Hu, et al., Exploration of Bcl-2 family and caspases-dependent apoptotic signaling pathway in Zearalenone-treated mouse endometrial stromal cells, Biochemical and Biophysical Research Communications (2016), http://dx.doi.org/10.1016/j.bbrc.2016.05.161

exposure of ZEA to mouse ESCs results in an obvious apoptosis in a dose-dependent manner.

3.2. ZEA activates caspase-9, caspase-3, and regulates Bcl-2 family members to induce apoptosis of mouse ESCs

To gain a novel insight into molecular mechanism that regulates the apoptosis of mouse ESCs induced by ZEA treatment. Firstly, we evaluated the expression levels of Bax and Bcl-2 by RT-qPCR and Western-Blotting (Fig. 2). The results showed that the expression of Bax gradually increased in a dose-dependent manner of the ESCs after treating with various ZEA concentrations for 24 h. However, the expression of Bcl-2 showed an opposite pattern with the expression of Bax. Thus, the ratio of Bax/Bcl-2 increased. Next, the expression and activity of caspase-9 and caspase-3 in mouse ESCs were examined after treating with various ZEA concentrations for 24 h (Fig. 3). Both RT-qPCR and enzymatic activity assays showed that the expression of caspase-9 and caspase-3 gradually increased in a dose-dependent manner.

3.3. Inhibitors of Z-VAD-FMK and Z-LEHD-FMK prevent ZEA-induced apoptosis

Given the above finding, we probed whether the mitochondrial pathway is involved in ZEA-induced apoptosis, then we hypothesized that inhibitor of the caspase could prevent the apoptosis of mouse ESCs from ZEA treatment. Therefore, mouse ESCs we pretreated with caspase inhibitor Z-VAD-FMK (a broad caspase inhibitor) and caspase-9 inhibitor Z-LEHD-FMK, respectively for 2 h, then treated with or without 100 μM ZEA for 24 h. Firstly, morphological changes in mouse ESCs were observed under a light microscope. There were more viable cells and cell morphological

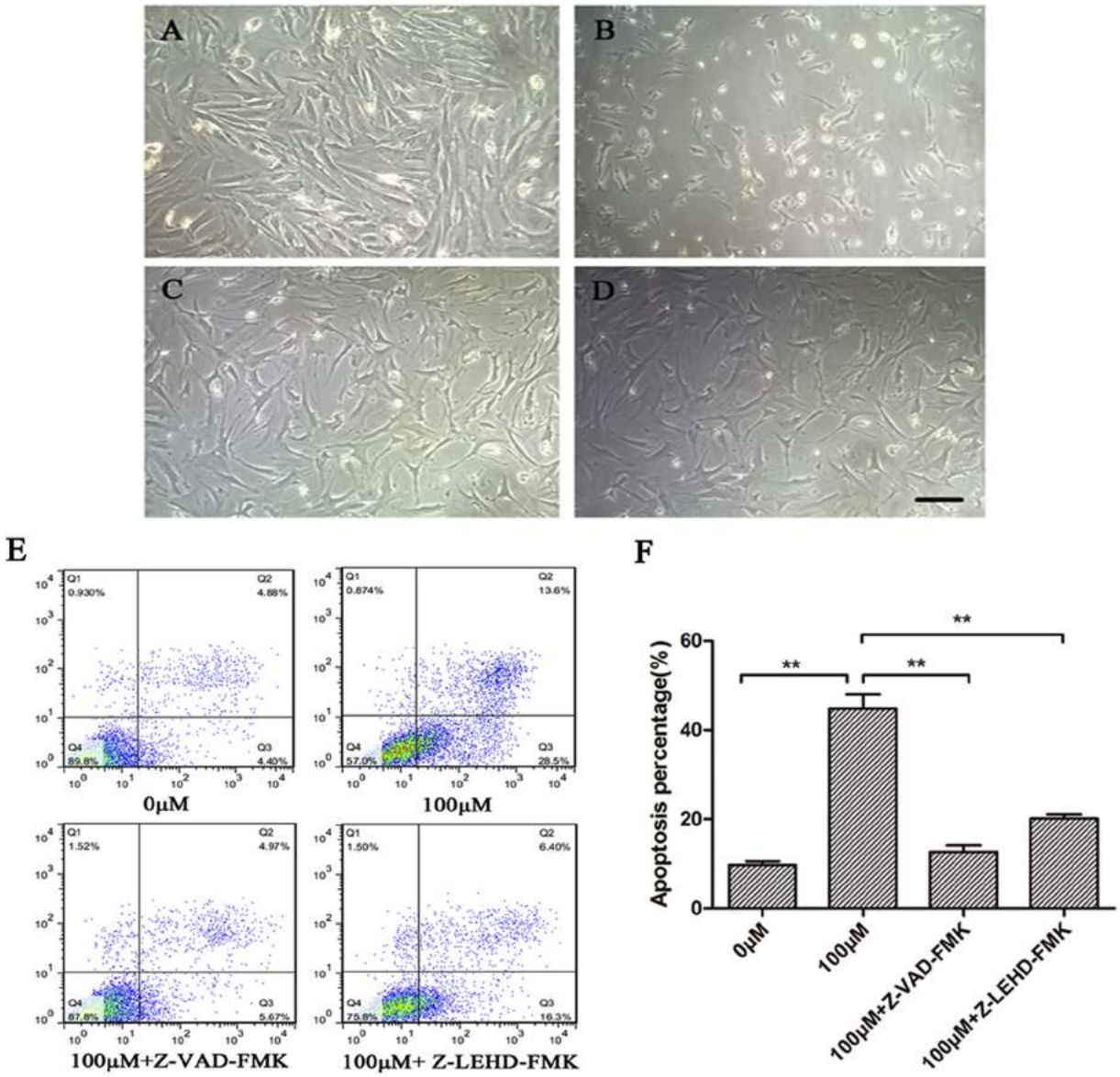

Fig. 4. Inhibitors of Z-VAD-FMK and Z-LEHD-FMK prevent ZEA-induced apoptosis. A–D: The cells were pretreated with or without Z-VAD-FMK and Z-LEHD-FMK followed by phase imaging (A: ZEA-untreated cells; B: 100 μM ZEA treated cells; C: Z-VAD-FMK and 100 μM ZEA treated cells; D: Z-LEHD-FMK and 100 μM ZEA treated cells); E: Flow cytometry showed apoptosis percentage of ESCs treated with or without Z-VAD-FMK and Z-LEHD-FMK; F: Quantification of the apoptosis percentage in flow cytometry. The data are expressed as the mean ± SEM of three independent experiments. Statistically significant difference as compared with control group, "*"indicated significant difference ($P < 0.05$), "**" indicated extremely significant difference ($P < 0.01$).

Please cite this article in press as: J. Hu, et al., Exploration of Bcl-2 family and caspases-dependent apoptotic signaling pathway in Zearalenone-treated mouse endometrial stromal cells, Biochemical and Biophysical Research Communications (2016), http://dx.doi.org/10.1016/j.bbrc.2016.05.161

integrity in pretreated with Z-VAD-FMK and Z-LEHD-FMK cells compared to treated with ZEA alone group (Fig. 4A–D). To investigate further, the results of flow cytometry showed that the cell apoptotic percentages treated with Z-VAD-FMK and Z-LEHD-FMK were markedly reduced compared to the ZEA-treated cells (Fig. 4E and F). These results further indicated that ZEA treatment caused an apoptosis in mouse ESCs via the caspase-9 and caspase-3-dependent mitochondrial signaling pathway.

4. Discussion

Previous studies have proved that ZEA is toxic for human and animal health, and the main studies about the toxic of ZEA in female reproduction were related to the granulosa cells [24–27]. ESCs play a key role in the process of uterine decidualization at the site of embryo implantation, which is critical to the establishment of pregnancy in female reproduction [19]. However, the detailed molecular and cellular mechanisms of ZEA toxicity on mouse ESCs have not yet been explained. The main focus of this study was to explore the effects on the apoptosis in ZEA-treated primary mouse ESCs and to evaluate the effects of the two common caspase inhibitors (Z-VAD-FMK and Z-LEHD-FMK) in primary mouse ESCs.

Apoptosis is a programmed cell death and it plays an important role in cellular homeostasis, embryogenesis and metamorphosis. We discovered that ZEA treatment caused an obvious apoptosis of mouse ESCs in a dose-dependent manner using flow cytometry and TUNEL assay. In mammalian cells, the death receptor-dependent, endoplasmic reticulum (ER) stress-dependent and mitochondrial-mediated pathways are the three major apoptotic pathways. In the death receptor-dependent pathway, the death receptors can activate caspase-8 which further activates downstream effectors caspases such as caspase-3 with committing the cells to apoptosis [28]. In the ER stress-dependent pathway, the ER stress response up-regulates GRP78 and subsequently initiates UPR signal transduction pathways in an attempt to restore homeostasis in the ER and promote cell survival [29]. In the mitochondrial pathway, when the cells are subjected to stimulus, the ratio of pro-apoptotic factors/anti-apoptotic factors was increased which can activate caspase-9 and further activate downstream effectors caspases such as caspase-3 with committing the cells to apoptosis.

Caspases are a family of cysteine-aspartic proteases or cysteine-dependent aspartate-directed proteases that play essential roles in apoptosis [30]. According to their activities, caspases are classified into two families; three well-known effector caspases (caspase-3, caspase-6, and caspase-7) and four initiator caspases (caspase-2, caspase-8, caspase-9, and caspase-10). Previous studies have proved the activation of the caspase cascade triggers apoptosis in some ZEA-treated cells [24,31,32].

Bcl-2 family proteins are known to modulate apoptosis through the regulation of mitochondrial apoptosis pathway [33]. Bad, Bax, Bak, Bik and HRK are promoters of apoptosis [34], while Bcl-2 functions as a major inhibitor of cell death, therefore guarding against apoptosis [34–36]. The correct balance of pro-apoptotic and anti-apoptotic proteins can determine whether apoptosis occurs or not [37]. In viable cells, a substantial portion of Bax is monomeric and found either in the cytosol or loosely attached to membranes. When the cells are subjected to stimulus, monomeric Bax translocate from the cytosol to the mitochondrial where it becomes an integral membrane protein and cross-linkable as a homodimer and accelerates the opening of the mitochondrial voltage-dependent anion channel, which is one of the components of the permeability transition and allows cytochrome *c* to pass through the anion channel out of mitochondria [38,39]. During this process, the anti-apoptotic molecule Bcl-2 can inhibit the activation of Bax following a death signal, which can inhibit the cytochrome *c* releasing from mitochondria into cytoplasm; thereby the down-regulation of Bcl-2 promoting intrinsic mitochondria-mediated apoptosis [40,41]. When the ratio of pro-apoptotic factors/anti-apoptotic factors (Bax/Bcl-2) increased, which led to the disruption of mitochondrial membrane, which in turn would cause cytochrome *c* release from intra-mitochondria into the cytosol, and then cytochrome *c* forms a complex with both apoptotic protease-activating factor-1 (Apaf-1) and pro-caspase-9 in the presence of dATP or ATP. This complex then activated caspase-9, an "initiator caspase" that could in turn activate "effector caspase" as caspase-3 which then cleaved the death substrates, leading to apoptosis [39,42].

Our results showed that the Bax was increased in a dose-dependent manner by RT-qPCR and western-blotting analysis. In contrast, the expression level of Bcl-2 was reduced in a dose-dependent manner. Thus, ZEA treatment increased the ratio of pro-apoptotic factors/anti-apoptotic factors (Bax/Bcl-2) which led to apoptosis. Moreover, enzymatic activity assays analysis showed that caspase-3 and caspase-9 are activated by ZEA treatment in mouse ESCs. As we hypothesize that pretreated with caspases inhibitor (Z-VAD-FMK) and caspase-9 inhibitor (Z-LEHD-FMK) could decrease the apoptotic after ZEA treatment. In summary, the results demonstrated that ZEA caused an obvious apoptosis of mouse ESCs in a dose-dependent manner. Both the ratio of Bax/Bcl-2 and the activities of caspase-9 and caspase-3 were increased with ZEA treatment. While the effect of ZEA treatment on apoptotic significantly decreased after pretreated with Z-VAD-FMK and Z-LEHD-FMK. Overall, we concluded that ZEA treatment induced apoptosis in mouse ESCs via a Bcl-2 family and caspases-dependent signaling pathway.

Conflicts of interest statement

The authors declare that there are no conflicts of interest.

Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.bbrc.2016.05.161.

References

[1] A. Zinedine, J.M. Soriano, J.C. Moltó, J. Mañes, Review on the toxicity, occurrence, metabolism, detoxification, regulations and intake of zearalenone: an oestrogenic mycotoxin, Food & Chem. Toxicol. 45 (2007) 1–18.
[2] K.-G. T, S. PM, W. H, Risk assessment of the mycotoxin zearalenone, Regul. Toxicol. Pharmacol. 7 (1987) 253–306.
[3] P.H. Hidy, R.S. Baldwin, R.L. Greasham, C.L. Keith, M. JR, Zearalenone and some derivatives: production and biological activities, Adv. Appl. Microbiol. 22 (1977) 59–82.
[4] M. Gajęcka, L. Rybarczyk, E. Jakimiuk, K. Zielonka, Obremski, W. Zwierzchowski, G.C. M, The effect of experimental long-term exposure to low-dose zearalenone on uterine histology in sexually immature gilts, Exp. Toxicol. Pathol. 64 (2012) 537–542.
[5] W. Zwierzchowski, M. Przybyłowicz, K. Obremski, L. Zielonka, E. Skorska-Wyszyńska, M. Gajecka, M. Polak, E. Jakimiuk, B. Jana, L. Rybarczyk, Level of zearalenone in blood serum and lesions in ovarian follicles of sexually immature gilts in the course of zearalenone micotoxicosis, Pol. J. Veterinary Sci. 8 (2005) 209–218.
[6] M.L. Gonzalez Pereyra, C.M. Pereyra, M.L. Ramirez, C.A.R. Rosa, A.M. Dalcero, L.R. Cavaglieri, Determination of mycobiota and mycotoxins in pig feed in central Argentina, Lett. Appl. Microbiol. 46 (2008) 555–561 (557).
[7] H. Alm, K.P. Brüssow, H. Torner, J. Vanselow, W. Tomek, S. Dänicke, U. Tiemann, Influence of Fusarium-toxin contaminated feed on initial quality and meiotic competence of gilt oocytes, Reprod. Toxicol. 22 (2006) 44–50.
[8] D.E. Marin, I. Taranu, R. Burlacu, D.S. Tudor, Effects of zearalenone and its derivatives on the innate immune response of swine, Toxicon 56 (2010) 956–963.
[9] S.A. Robertson, G. Mayrhofer, R.F. Seamark, Uterine epithelial-cells synthesize granulocyte-macrophage colony-stimulating factor and interleukin-6 in pregnant and nonpregnant mice, Biol. Reprod. 46 (1992) 1069–1079.
[10] D.A. Sullivan, W. CR, Hormonal regulation of immunoglobulins in the rat

Please cite this article in press as: J. Hu, et al., Exploration of Bcl-2 family and caspases-dependent apoptotic signaling pathway in Zearalenone-treated mouse endometrial stromal cells, Biochemical and Biophysical Research Communications (2016), http://dx.doi.org/10.1016/j.bbrc.2016.05.161

uterus: uterine response to multiple estradiol treatments, Endocrinology 114 (1984) 650–658.
[11] R.H. Prabhala, C.R. Wira, Sex hormone and IL-6 regulation of antigen presentation in the female reproductive tract mucosal tissues, J. Immunol. 155 (1995) 5566–5573.
[12] C.R. Wira, R. RM, Antigen-presenting cells in the female reproductive tract: influence of the estrous cycle on antigen presentation by uterine epithelial and stromal cells, Endocrinology 136 (1995) 4526–4534.
[13] A.A. Donjacour, G.R. Cunha, Stromal Regulation of Epithelial Function, Springer, US, 1991.
[14] P.S. Cooke, D.L. Buchanan, P. Young, T. Setiawan, J. Brody, K.S. Korach, J. Taylor, D.B. Lubahn, G.R. Cunha, Stromal estrogen receptors mediate mitogenic effects of estradiol on uterine65epithelium, Proc. Natl. Acad. Sci. 94 (1997) 6535–6540.
[15] L.J. Murphy, G. A, Uterine insulin-like growth factor-1: regulation of expression and its role in estrogen-induced uterine proliferation, Endocr. Rev. 11 (1990).
[16] Y.K. Hom, P. Young, J.F. Wiesen, P.J. Miettinen, R. Derynck, Z. Werb, G.R. Cunha, Uterine and vaginal organ growth requires epidermal growth factor receptor signaling from stroma, Endocrinology 139 (1998) 913–921.
[17] P.S. Cooke, G.R. Cunha, Restoration of normal morphology and estrogen responsiveness in cultured vaginal and uterine epithelia transplanted with stroma, Proc. Natl. Acad. Sci. U. S. A. 83 (1986) 2109–2113.
[18] R.M. Bigsby, C. GR, Estrogen stimulation of deoxyribonucleic acid synthesis in uterine epithelial cells which lack estrogen receptors, Endocrinology 119 (1986) 390–396.
[19] E.Y. Adashi, J.A. Rock, Z. Rosenwaks, Reproductive Endocrinology, Surgery, and Technology, Lippincott-Ravan, 1996.
[20] Y. Tan, M. Li, S. Cox, M.K. Davis, O. Tawfik, B.C. Paria, S.K. Das, HB-EGF directs stromal cell polyploidy and decidualization via cyclin D3 during implantation, Dev. Biol. 265 (2004) 181–195.
[21] M. Zhang, Z. He, L. Wen, J. Wu, L. Yuan, Y. Lu, C. Guo, L. Zhu, S. Deng, H. Yuan, Cadmium suppresses the proliferation of piglet Sertoli cells and causes their DNA damage, cell apoptosis and aberrant ultrastructure, Reprod. Biol. Endocrinol. 8 (2010), 10.1186.
[22] Z. He, J. Jiang, M. Kokkinaki, M. Dym, Nodal signaling via an autocrine pathway promotes proliferation of mouse spermatogonial stem/progenitor cells through Smad2/3 and Oct-4 activation, Stem Cells 27 (2009) 2580–2590.
[23] C. Liu, K. Yu, X. Shi, J. Wang, P.K. Lam, R.S. Wu, B. Zhou, Induction of oxidative stress and apoptosis by PFOS and PFOA in primary cultured hepatocytes of freshwater tilapia (Oreochromis niloticus), Aquat. Toxicol. 82 (2007) 135–143.
[24] L. Zhu, H. Yuan, C. Guo, Y. Lu, S. Deng, Y. Yang, Q. Wei, L. Wen, Z. He, Zearalenone induces apoptosis and necrosis in porcine granulosa cells via a caspase-3-and caspase-9-dependent mitochondrial signaling pathway, J. Cell. Physiol. 227 (2012) 1814–1820.
[25] U. Tiemann, W. Tomek, F. Schneider, J. Vanselow, Effects of the mycotoxins α- and β-zearalenol on regulation of progesterone synthesis in cultured granulosa cells from porcine ovaries, Reprod. Toxicol. 17 (2003) 673–681.
[26] F. Minervini, A. Giannoccaro, F. Fornelli, M.E. Dell'Aquila, P. Minoia, A. Visconti, Influence of mycotoxin zearalenone and its derivatives (alpha and beta zearalenol) on apoptosis and proliferation of cultured granulosa cells from equine ovaries, Reprod. Biol. Endocrinol. 4 (2006) 62.
[27] G. Ranzenigo, F. Caloni, F. Cremonesi, P.Y. Aad, L.J. Spicer, Effects of Fusarium mycotoxins on steroid production by porcine granulosa cells, Animal Reprod. Sci. 107 (2008) 115–130.
[28] A. Ashkenazi, V.M. Dixit, Death receptors: signaling and modulation, Science 281 (1998) 1305–1308.
[29] S.S. Cao, R.J. Kaufman, Unfolded protein response, Curr. Biol. 22 (2012) R622–R626.
[30] N.A. Thornberry, Y. Lazebnik, Caspases: enemies within, Science 281 (1998) 1312–1316.
[31] I. Ayed-Boussema, C. Bouaziz, K. Rjiba, K. Valenti, F. Laporte, H. Bacha, W. Hassen, The mycotoxin Zearalenone induces apoptosis in human hepatocytes (HepG2) via p53-dependent mitochondrial signaling pathway, Toxicol. Vitro 22 (2008) 1671–1680.
[32] C. Bouaziz, O.S.E. Dein, E.E. Golli, C. Brenner, C. Lemaire, H. Bacha, S. Abid-Essefi, Different apoptotic pathways induced by zearalenone, T-2 toxin and ochratoxin A in human hepatoma cells, Toxicology 254 (2008) 19–28.
[33] S. Cory, D.C. Huang, J.M. Adams, The Bcl-2 family: roles in cell survival and oncogenesis, Oncogene 22 (2003) 8590–8607.
[34] M. Story, R. Kodym, Signal transduction during apoptosis; implications for cancer therapy, Front. Biosci. 3 (1998) d365–375.
[35] S.C. Dixon, B.J. Soriano, R.M. Lush, M.M. Borner, W.D. Figg, Apoptosis: its role in the development of malignancies and its potential as a novel therapeutic target, Ann. Pharmacother. 31 (1997) 76–82.
[36] S. Cory, J.M. Adams, The Bcl2 family: regulators of the cellular life-or-death switch, Nat. Rev. Cancer 2 (2002) 647–656.
[37] X.W. Wang, Q. Zhan, J.D. Coursen, M.A. Khan, H.U. Kontny, L. Yu, M.C. Hollander, P.M. O'Connor, A.J. Fornace, C.C. Harris, GADD45 induction of a G2/M cell cycle checkpoint, Proc. Natl. Acad. Sci. 96 (1999) 3706–3711.
[38] H. Kashkar, K. Wiegmann, B. Yazdanpanah, D. Haubert, M. Krönke, Acid sphingomyelinase is indispensable for UV light-induced Bax conformational change at the mitochondrial membrane, J. Biol. Chem. 280 (2005) 20804–20813.
[39] S. Shimizu, M. Narita, Y. Tsujimoto, Bcl-2 family proteins regulate the release of apoptogenic cytochrome c by the mitochondrial channel VDAC, Nature 399 (1999) 483–487.
[40] P. Costantini, E. Jacotot, D. Decaudin, G. Kroemer, Mitochondrion as a novel target of anticancer chemotherapy, J. Natl. Cancer Inst. 92 (2000) 1042–1053.
[41] M.O. Hengartner, The biochemistry of apoptosis, Nature 407 (2000) 770–776.
[42] M. Chen, A.D. Guerrero, L. Huang, Z. Shabier, M. Pan, T.-H. Tan, J. Wang, Caspase-9-induced mitochondrial disruption through cleavage of anti-apoptotic BCL-2 family members, J. Biol. Chem. 282 (2007) 33888–33895.

Please cite this article in press as: J. Hu, et al., Exploration of Bcl-2 family and caspases-dependent apoptotic signaling pathway in Zearalenone-treated mouse endometrial stromal cells, Biochemical and Biophysical Research Communications (2016), http://dx.doi.org/10.1016/j.bbrc.2016.05.161