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

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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

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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

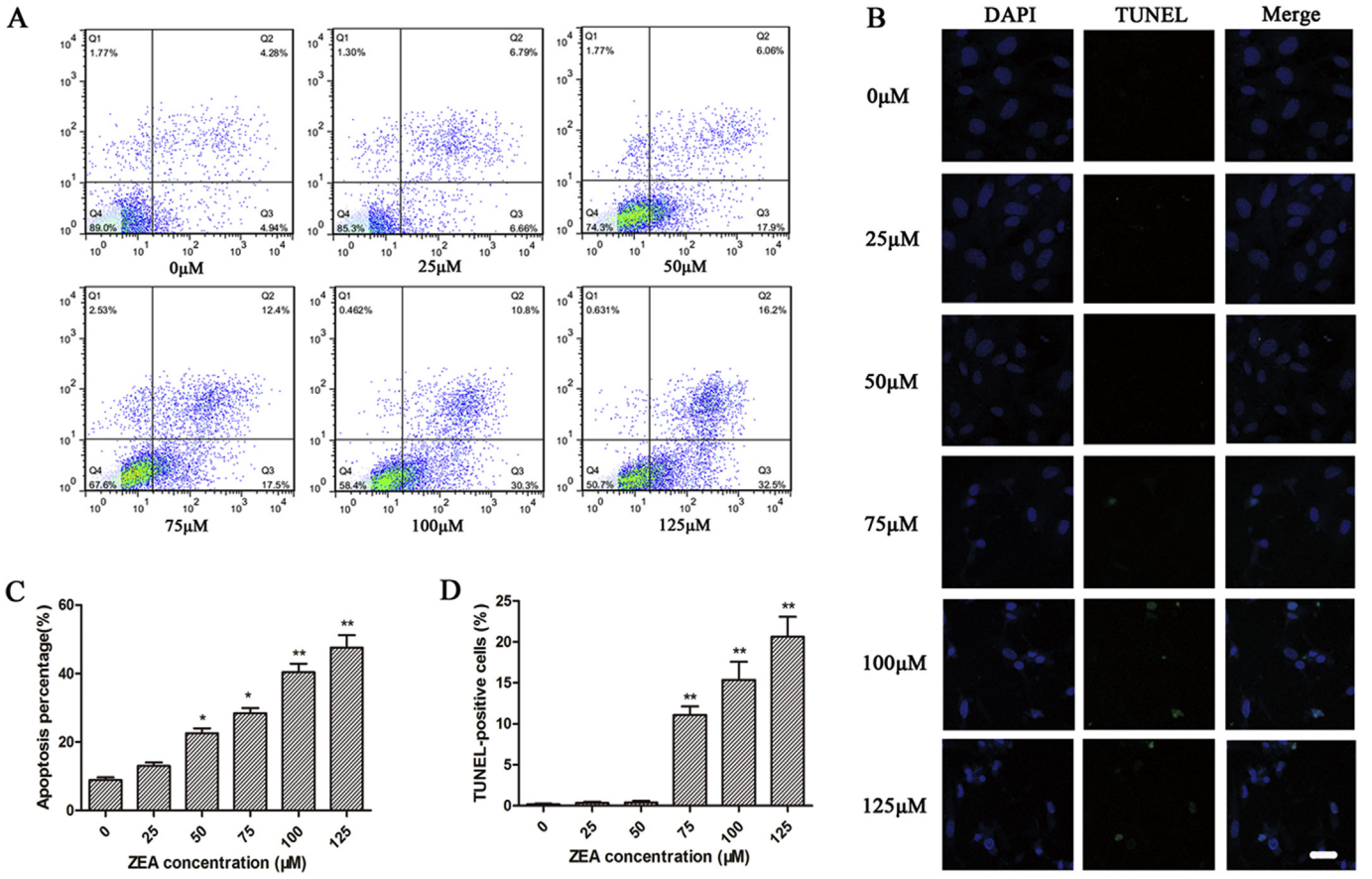

Fig. 1. Effect of ZEA treatment on apoptosis of mouse ESCs. A: Flow cytometry showed apoptosis percentage of mouse ESCs treated with various concentrations of ZEA treatment. B: TUNEL assays displayed the apoptosis in mouse ESCs by various concentrations of ZEA. C: Quantification of the apoptosis percentage 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 ($P < 0.01$).