Modulatory Effects of Phytoestrogens on the Expression of Fas Ligand and the Release of Cytochrome *C* in Normal and Cancerous Endometrial Cells

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Cytochrome c (CytC) released from mitochondria induces apoptosis in both normal and tumor cells. Expression of Fas ligand (FasL) helps maintain tumor cell survival by inducing apoptosis of Fas-bearing anti-tumor immune cells. A risk of endometrial cancer has been reported to associate with phytoestrogen consumption. Therefore the effects of phytoestrogens, genistein and daidzein, on FasL and CytC protein expression were examined in primary cultured porcine endometrial cells (PE) and human cancerous endometrial cells (RL95-2) by Western blot analysis. Both cells were cultured in standard medium (SM) and switched to estrogen-deprived medium (SF) with or without 17β-estradiol (E, 1 nM), genistein (10 μM) or daidzein (10 μM) for 48 h. FasL (25 kDa) which was found only in RL95-2 cells was upregulated in SF compared to SM. Treatment of RL95-2 cells with E, daidzein or genistein significantly increased the FasL expression by 7-10 folds. In the present study, low level of CytC was detected in both cells cultured in SM but markedly increased in SF by 1.5-2 folds. The SF-induced increase in CytC level was reversed by genistein or daidzein while E suppressed CytC in PE cells, but not in RL95-2 cells. The findings suggest that genistein and daidzein appear to act as a survival factor by inhibiting intracellular apoptogenic initiator in both normal and cancer endometrial cells. In addition, estrogen and phytoestrogens inducing the death signal FasL expressed by cancerous endometrial cells may cause the tumor progression. Thus, consuming phytoestrogen as a supplement should be awareness in patient with endometrial cancer.

Keywords: *Fas, FasL, Phytoestrogen, Cytochrome C, Estrogen*

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Apoptosis, programmed cell death, plays a critical role in the maintenance of tissue homeostasis and represents a physiological mechanism to eliminate excess or dysfunction cells in numerous tissues including endometrium. A well-characterized apoptotic pathway in reproductive tissues involves the death receptor pathway Fas/FasL and its cascade system via the cytosolic cytochrome c (CytC) produced by mitochondria[1]. Fas ligand (FasL), a 37 kDa protein belonging to the tumor necrosis factor (TNF) superfamily, is expressed mainly in immune-privileged organs including reproductive organs. Fas (CD95), a 45 kDa membrane protein also in the TNF family serves as the receptor for FasL. Upon binding of FasL to the Fas receptor, it signals the caspase cascades causing the release of CytC from mitochondrial membrane to cytosol[2,3]. Cytosolic CytC is a 13 kDa protein encoded by somatic *CYCS* gene and localized in the inner mitochondria memebrane[4]. The released CytC in response to apoptotic stimuli subsequently interacts with the specific enzyme to form an apoptosome to degrade the target molecules, leading to the apoptosis[5].

In endometrium, Fas/FasL apoptotic pathway interacts with immune system to promote the counterattack mechanism involved in reproductive functions particularly implantation[2,3]. Both FasL and Fas are expressed in endometrial stromal and glandular cells thoughout the menstrual cycle, suggesting the regulation by ovarian steroid estrogen and progesterone[1,6-8]. Upregulation of FasL in normal endometrial cells produced by local inflammation induces apoptosis of T-lymphocytes and causes a local immune tolerance, leading to development of ectopic implants and

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endometriosis[8]. On the other hand, tumor cells develop mechanism of upregulation of FasL (FasL/CD95L) expression during carcinogenesis in order to escape from immune evading. Progressive ovarian tumor cells to malignancy have been associated with the expression of membrane FasL or secretion of soluble FasL (MW = 25 kDa), leading to apoptosis of Fas-mediated T-cell and subsequently immune suppression[9].

Plant-derived phytoestrogens are used for the treatment and prevention of estrogen-related diseases including breast cancer[10]; however, a risk of endometrial cancer has been associated with phytoestrogen intake[11]. Genistein and daidzein are two major isoflavone phytoestrogens mostly present in soybean food[12]. However, modulatory effect of phytoestrogens on FasL expression in endometrial cell which is relevant to the pathogenesis of endometriosis and malignancy in normal and cancer endometrial cells has not been elucidated. On the other hand, the induction of CytC released to cytosol by phytoestrogens may lead to the apoptosis which could limit the growth of FasL-expressed endometrial cells. Therefore the present study aimed to examine the effects of phytoestrogens, genistein and daidzein, on regulation of FasL and CytC expression in primary cultured porcine endometrial epithelial cells and human endometrial carcinoma RL95-2. For an appropriate ethic, a primary culture of porcine endometrial cell representing a good normal model for human reproductive study was applied throughout the study due to its characteristic and function homologous to the human endometrium[13,14].

Material and Method

Materials

17β-estradiol (E), genistein, daidzein, insulin, non-essential amino acid and high purity grade salts were purchased from Sigma Chemical Co., (St Louis, MO, USA). Dulbecco's modified Eagle's medium (DMEM), Dulbecco's phosphate buffer saline (DPBS), phenol red-free DMEM, fetal bovine serum (FBS), collagenase (type 1), 0.05% trypsin-0.53 mM ethylenediaminetetraacetic acid (EDTA), kanamycin, penicillin-streptomycin and fungizone were purchased from GIBCO BRL (Grand Island, NY). Charcoal-stripped FBS was purchased from Biowest Co., (Miami, FL).

Cell isolation and culture

Endometrial epithelial cells (PE cells) were isolated from immature pig uterus as described previously[14]. The isolated glands were seeded on 100-mm Costar® culture plate and allowed to grow in DMEM supplemented with 10% FBS, 10 mM *L*-glutamine, 1% non-essential amino acid, 100 U/ml penicillin and 100 mg/ml streptomycin (standard medium) at 37°C in a humidified atmosphere of 5% CO_2 in air. Culture medium was changed after 24 h and then every 2-3 days. The cells used in the experiment were within 2 weeks after isolation. For the human endometrial carcinoma cell line, RL95-2 purchased from the American Type Culture Collection (ATCC; Rockville, MD, USA), was cultured in same media and protocol as the PE cells.

Hormonal treatment

Prior to the hormonal treatment, both PE and RL95-2 cells were pre-conditioned for 3 days by substitution of the standard media with phenol red-free DMEM containing 2% charcoal-stripped FBS and other supplements as in the standard medium (estrogen-deprived medium, SF). This SF was used in order to minimize the estrogenic effect of the phenol red containing medium and ovarian steroids containing serum. After precondition, cells were trypsinized and then plated in the SF at the density of $2x10^4$ cells/well into 48-well culture plate (Costar®) and allowed to adhere for 18-24 h. E (1 nM), genistein (10 μM) or daidzein (10 μM) dissolved in dimethylsulfoxide (DMSO) were added and incubated in the SF for 48 h.

Western blot hybridization

After 48 h culturing of PE or RL95-2 cells in the SM or SF, cells were trypsinized and lysed in lysis buffer (50 mM tris HCl, 1% NP-40, 0.25% sodium deoxycholate, 150 mM NaCl, 1 mM EGTA, 1 mM phenylmethylsulfonylfluoride, 20 mg aprotinin and 1 mM NaF, pH 7.4). Protein concentrations were determined using a bicinchoninic acid (BCA) protein assay kit (Pierce Chemical Co., USA). To detect the protein expression, 60 mg of total protein per sample were separated on 10% sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE). Proteins were electrophoretically transferred to a polyvinylidene difluoride membrane (Pall Life Sciences, USA) in Tris-glycine transfer buffer. After blocking with 5% nonfat powdered milk in TBST (25 mM tris, 140 mM NaCl, 0.1% Tween 20) for 4 h at room temperature, the membranes were reacted with rabbit anti-human FasL antibody (1:400 dilution; Affinity Bioreagent, USA) or mouse anti-human cytochrome *c* antibody (1:100 dilution; Santa Cruz Biotechnology Inc., USA) at 4°C overnight. After several washings, the membranes were incubated for 2 h at room temperature with appropriate

secondary antibody conjugated with horseradish peroxidase (Zymed Laboratories Inc.). The immunoreactive proteins were visualized using the enhanced chemiluminesence (ECL) detection system (Santa Cruz Biotechnology Inc.) according to manufacturer instructions. Membranes were then exposed to film (Hyperfilm-ECL; Amersham Biosciences, USA) to visualize chemiluminescent bands. Reproceeded blot with anti β-actin antibody (Sigma) was used as an internal control. Differences in protein immunoreactivity between treatments were determined by scanning densitometry in proportion to β-actin immunoreactive bands (Scion Image; Scion Corporation, Frederick, MD). All experiments were repeated at least three times.

Statistical analysis

All values are presented as mean ± standard error of mean (SEM). The differences between control and experimental means were analyzed using a Analysis of Variance (ANOVA). Difference among means following a significant ANOVA was identified by Dunnetts' comparison test (Prism™ 5.01, GraphPad Software, Inc., San Diego, CA). A value of $P<0.05$ was considered statistically significant.

Results

Glandular epithelial cells isolated from porcine uterus (PE) or human endometrial cancer cell lines (RL95-2) were tested with the effective concentration of estrogen (1 nM) or phytoestrogens (10 μM) as reported previously[11]. To determine whether the treatments of both cells with E, genistein or daidzein were related to apoptosis, the expressions of FasL apoptosis signal and apoptogenic enzyme CytC proteins were evaluated using semi-quantitative Western blot analysis following the 48-h treatment period.

Effects of 17β-estradiol and phytoestrogens on expression of FasL proteins

The effects of estrogen and phytoestrogens on FasL protein expression in the PE and RL95-2 cells were presented in Fig. 1A. The expression of FasL protein was not detected in the PE cells grown in both standard medium (SM) and estrogen-deprived medium (SF). However, the FasL antibody recognized the protein bands with an approximate molecular mass of 37 kDa in the RL95-2 cells. The FasL expression, as calculated by the ratio of FasL to β-actin density unit, of the RL95-2 cells grown in the SF was 0.79 ± 0.03 which was 6.5 fold higher than those in the SM (0.17 ± 0.08; $p < 0.01$, $n = 3$, Fig. 1B). Treatment with E, daidzein or genistein for 48 h significantly increased the FasL expression which was 1.02 ± 0.10, 1.24 ± 0.14 and 1.24 ± 0.14, respectively.

Effects of 17β-estradiol and phytoestrogens on apoptogenic cytosolic cytochrome c

To further investigate whether the mitochondria pathway was involved in the phytoestrogen-induced apoptosis, the level of CytC was determined in whole cell lysate of the PE or RL95-2 cells following the protocol of FasL expression study. As shown in

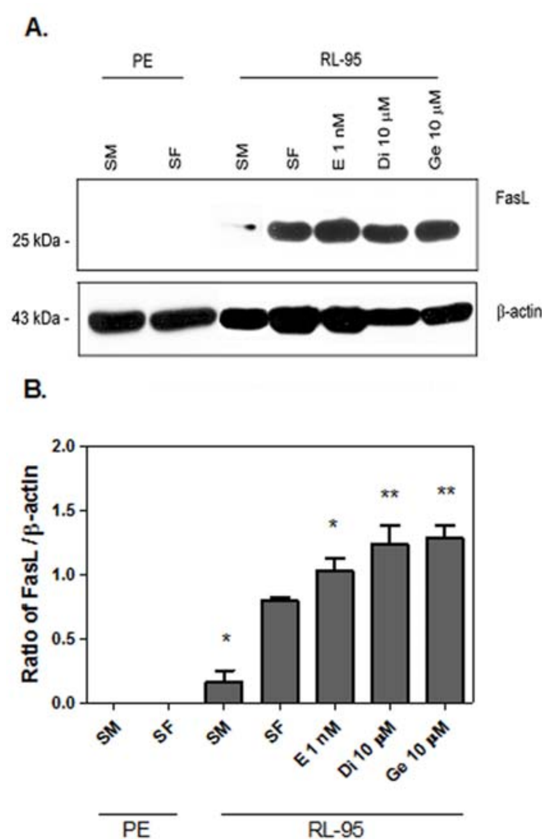

Fig. 1 (A) Western blot analysis of Fas ligand (FasL) protein and internal protein control (β-actin) in porcine endometrial epithelial cells (PE) and human endometrial cancer cells (RL95-2). The cells were grown in standard medium (SM), estrogen-deprived medium (SF) alone or in the presence of 17β-estradiol (E), daidzein (Di) or genistein (Ge) for 48 h. (B) Histograms represent mean ± SEM of the ratio of FasL/β-actin band density. $^{*}p < 0.05$ and $^{**}p < 0.01$ was considered significantly different versus SF control by One-Way ANOVA followed by Dunnett's test

Fig. 2A, the protein band with an approximate molecular weight of 25 kDa of CytC protein was detected in the PE cells. Removal of estrogen from the medium (SF) significantly increased ratio of CytC to β-actin to 1.23 $\pm$ 0.14 as compared to 0.37 $\pm$ 0.17 in the SM ($p < 0.01$, n = 5, Fig 2B). The presence of E or daidzein in the SF decreased the cytosolic CytC level to the same level as in the SM (0.40 $\pm$ 0.09 for E and 0.34 $\pm$ 0.08 for daidzein) whereas genistein markedly suppressed the normalized CytC to 0.03 $\pm$ 0.001.

In the RL95-2 cells, however, a molecular weight of 32 kDa of CytC protein was detected in cells cultured in any medium condition (Fig 3A). Like the PE cells, switching from the SM to the SF produced twofold increases in the ratio of CytC to β-actin level from 0.33 $\pm$ 0.03 to 0.83 $\pm$ 0.09 ($p < 0.05$, n = 3, Fig 3B). Treatment of the RL95-2 cells with E did not affect the level of CytC released to cytosol (0.79 $\pm$ 0.12) whereas daidzein decreased this protein to the same level as in the SM (0.44 $\pm$ 0.01). Similar to daidzein, genistein markedly decreased the amount of CytC protein (0.12 $\pm$ 0.37) as observed with the PE cells.

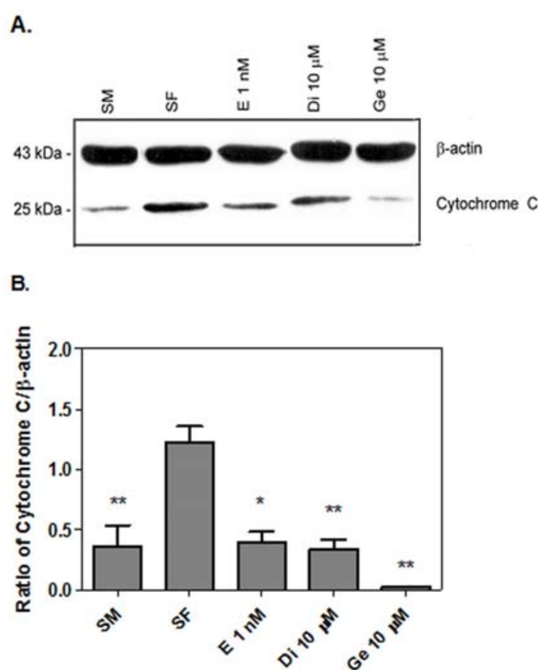

Fig. 2 (A) Western blot analysis of cytochrome c protein and internal protein control (β-actin) in whole-cell lysates of porcine endometrial epithelial cells. The cells were grown in standard medium (SM) or estrogen-deprived medium (SF) alone or in the presence of 17β-estradiol (E), daidzein (Di) or genistein (Ge) for 48 h. (B) Histograms represent mean $\pm$ SEM of the ratio of cytochrome c/β-actin band density. *$p < 0.05$ and **$p < 0.01$ was considered significantly different versus SF control by One-Way ANOVA followed by Dunnett's test

Discussion

Apoptosis has been implicated for several normal processes in the reproductive organs particularly the secretory phase of menstrual cycle and implantation of fetus during pregnancy[15]. Two major apoptotic pathways in mammalian cells, the death receptor pathway and the mitochondrial pathway, have been suggested[16]. The death receptor pathway induces the apoptosis via FasL, the extracellular signal which may exist as either membrane bound form (37 kDa) or soluble form (26 kDa) presented by the nearby

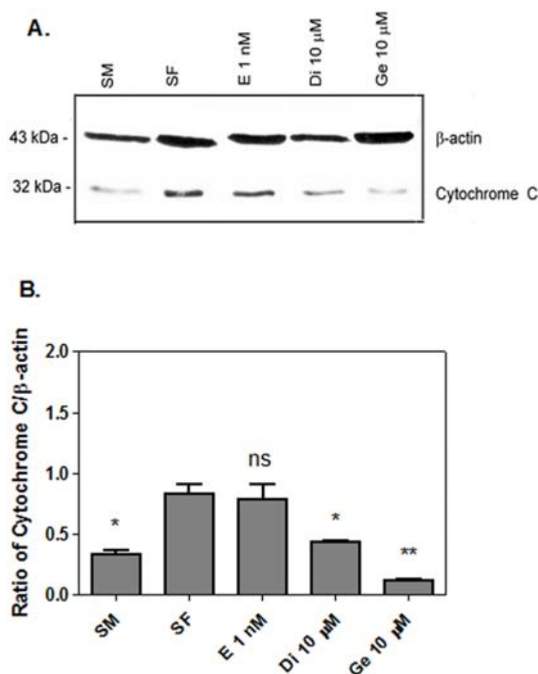

Fig. 3 (A) Western blot analysis of cytochrome *c* protein and internal protein control (β-actin) in whole-cell lysates of human endometrial cancer cells (RL95-2). The cells were grown in standard medium (SM) or estrogen-deprived medium (SF) alone or in the presence of 17β-estradiol (E), daidzein (Di) or genistein (Ge) for 48 h. (B) Histograms represent mean $\pm$ SEM of the ratio of cytochrome *c*/β-actin band density. *$p < 0.05$ and **$p < 0.01$ was considered significantly difference versus SF control by One-Way ANOVA followed by Dunnett's test. NS was not considered as significantly different ($p > 0.05$) from SF control

cells. The soluble form can be released from cells upon cleavage by metalloproteinases. Binding of FasL to its receptor, Fas, leads to oligomerization of the receptor and triggers apoptotic cell death through interaction with other proteins i.e. caspases to cascade the CytC release from mitochondrial membrane[2,3]. FasL normally expressed in the activated T-lymphocytes, natural killer (NK) cells and the tissues of immune-privilege sites, particularly the epithelial lining of visceral organs plays role in the cell-mediated immunity to eliminate differentiated, senescent, or dysfunctional cells[2-4]. Acquired resistance to apoptosis is a hallmark of most cancer cells[17]. It is known that carcinogenesis involves selective failure of apoptosis and consequently prolongs the survival of cells carrying mutagenesis DNA damage[18,19]. It is possible that carcinoma cells or normal cells expressing high level of death signal FasL affected by some factors may induce the death of immune cells and embryo. Some tumor cell types express FasL and allow cell survival through the apoptosis of Fas-positive lymphocytes[20]. During the menstrual cycle, FasL expressed on the endometrial stromal cells is stimulated by macrophage derived growth factors leading to cell adhesion to extracellular matrix, and this suggests a role of FasL in the pathogenesis of endometriosis[21]. Therefore, in the present study, the authors examined the expression of FasL, rather than Fas receptor, on the normal and cancer endometrial cells.

In the present study, the expression of FasL was not detected in normal endometrial cells grown in any conditioned medium. Although the primary culture of porcine endometrial cell was used as a model for non-cancer cell, the possibility of not being cross reaction of the polyclonal rabbit anti-human FasL antibody with pig tissues was unlikely to occur since the human FasL nucleotides and protein are homology to those of porcine about 89-90%[22,23]. However, our results were not in accordance with those of Yamashita and co-workers[7] which demonstrated the presence of Fas and FasL in normal human endometrium using electron microscopy and immunohistochemistry technique. FasL mRNA and protein expression is cycle-dependent regulated by ovarian steroid hormones *i.e.* its expression starts at the proliferative phase and peaks at the secretory phase of menstrual cycle[8]. Other study additionally indicates that the regulation of endometrial apoptosis is important for the onset of menstruation and the regulation of immune tolerance to fetoplacental reception[15]. In the present study, the PE cells were cultured in the condition without sex steroids in order to demonstrate the regulatory effect of phytoestrogens on FasL expression. Treatment with genistein or daidzein failed to induce the FasL expression in normal endometrial cells (data not shown). However, modulation of FasL expression by phytoestrogens in the presence of ovarian steroids following the ovarian cycle should be further investigated.

By contrast, the endometrial cancer cell RL95-2 expressed the FasL in both standard and estrogen-deprived media even though the expression was very low in the standard medium. Depletion of estrogen in the medium significantly induced the FasL protein expression. In addition, supplementation with estrogen or phytoestrogens to SF enhanced FasL expression. The stimulatory effect of estrogen in cancer cells seemed to correlate with the increased FasL expression in response to estrogen or during the late proliferative to secretory phase of menstrual cycle[8,15]. However, the exact mechanism and modulation of FasL expression by sex steroids or phytoestrogens in cancer cells remains unknown. Overexpression of FasL on the cancer cells is taken into considerations when using phytoestrogens in endometrial cancer patients because it may induce the death of Fas-bearing cells, activated T-lymphocytes, and natural killer (NK) cells[24]. Our findings also support the precaution of using phytoestrogens in breast cancer therapy which is likely to enhance endometrial cancer[25]. However, we speculate that the concomitant increases in FasL together with Fas expression in carcinoma cells by genistein or daidzein could be good prognosis because it may induce apoptosis via Fas/FasL pathway in nearby cancer cell. Alternatively, finding the chemicals or drugs to decrease FasL expression in endometrial stromal cells and endometrial carcinoma should be examined for successful pregnancy or cancer treatment, respectively.

The present study also demonstrated that the cytosolic CytC protein was found in both normal and cancerous endometrial cells while FasL was expressed only in the cancer cells. As expected, the CytC was apparent in the absence or low estrogen medium bathing the PE or RL95-2 cells. This evidence was in agreement with the findings of hormonal withdrawal giving rise to endometrial apoptosis[26]. Supplementation of normal cells with physiological concentration of E (1 nM) or highest safety concentration of soybean phytoestrogens genistein or daidzein (10 µM) decreased the CytC, suggesting that estrogen and phytoestrogens may act as the survival factor by inhibiting the default apoptotic pathway. It should be

noted that absence or decrease of cytosolic CytC reflects less apoptotic cells unless the mitochondria does not function. However, detection of CytC activity in an isolated subcellular fraction of intact and functional mitochondria was not performed in the present study.

Basically, CytC is released by mitochondrial membrane triggered by intracellular signals including Fas/FasL signaling system[27]. Each step of the CytC release is controlled by many intracellular factors including estrogen through ER-α or ER-β[28]. Estrogen treatment has been shown to increase mitochondrial CytC protein and mRNA, leading to apoptosis in both normal and cancer cells[28]. However, a recent study has reported that estrogen binding to mitochondrial ER inhibits UV radiation-induced CytC release through the formation of mitochondrial-generated ROS (mROS). The mROS induces translocation of the apoptotic Bax protein to mitochondria which subsequently increases mitochondria membrane permeability, and thereby CytC release[29]. Therefore, in the present study, the increased cytosolic CytC upon depletion of estrogen may be a result of mROS production, and the treatment of estrogen or phytoestrogens may defend yet unidentified mitochondrial event of cell death induced by mROS. However, estrogen treatment in the RL95-2 cells could not suppress the CytC release which may be explained by the direct effect of estrogen on mitochondrial membrane permeability mediated by different subtypes of estrogen receptors or signaling mechanism in cancer cells.

In the present results, the CytC protein revealed molecular weight of 25 kDa and 32 kDa for PE and RL95-2 cells, respectively, which were different from cytosolic CytC (MW= 12.7 kDa) as has been reported for RL95-2 in other study[30]. CytC are highly conserved among the mammal species including pig. After being released from mitochondria to cytosol, CytC can form multimeric complex with other macromolecules (i.e. Apaf-1) in term of apoptosome, thus heterogeneity of cytosolic CytC protein bands with high molecular weight could be seen[31].

In conclusion, the present findings showed that estrogen or soybean phytoestrogens, genistein and daidzein, has modulatory effects on the expression of apoptogenic signal FasL presented in endometrial cancer cells; even though, this effect was not indicated in normal endometrial cells. In addition, decreasing effects of genistein or daidzein on the mitochondrial cytochrome *c* released to cytosol may serve as anti-apoptogenic activity for both normal and cancer endometrial cells. As an anti-apoptogenic activity of phytoestrogen, it may benefit for protection of cell death. Unfortunately, the increased death signal FasL produced by genistein and daidzein may enhance the progressive of mutant or cancer cells. Thus, careful consideration must be taken concerning the use of estrogen and phytoestrogens in patient with endometrial cancer cells.

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Potential conflicts of interest

None.

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ผลของสารไฟโตเอสโตรเจนต่อการแสดงออกของฟาสไลแกนด์ และการปลดปล่อยไซโตโครมซีในเซลล์ปกติและเซลล์มะเร็งของเยื่อบุมดลูก

สุทธาสินี ปุญญโชติ, ฉัตรศรี เดชะปัญญา

การปล่อยไซโตโครมซีจากไมโตคอนเดรียสู่ไซโตพลาสซึม ทำให้เกิดการตายแบบอะพอพโทซิสทั้งในเซลล์ปกติและเซลล์มะเร็ง การปรากฏของฟาสไลแกนด์ (FasL) บนผิวเซลล์ช่วยให้เซลล์มะเร็งมีชีวิตรอดโดยเหนี่ยวนำให้เซลล์ภูมิคุ้มกันที่ต่อต้านมะเร็งและมีตัวรับของฟาสไลแกนด์ (Fas) เกิดการตายแบบอะพอพโทซิส มีรายงานว่าการบริโภคสารไฟโตเอสโตรเจนมีความเสี่ยงต่อการเกิดมะเร็งเยื่อบุมดลูก การศึกษาครั้งนี้จึงมีวัตถุประสงค์เพื่อศึกษาผลของสารไฟโตเอสโตรเจน ได้แก่ เจนีสทีอีน และไดแอดซีนต่อการแสดงออกของฟาสไลแกนด์และไซโตโครมซีในเซลล์ปฐมภูมิของเยื่อบุมดลูกที่แยกได้จากสุกร (PE) และเซลล์มะเร็งเยื่อบุมดลูกคน (RL95-2) ด้วยเทคนิค Western blot โดยนำเซลล์ทั้งสองชนิดที่เลี้ยงในน้ำเลี้ยงเซลล์ปกติ (SM) มาเลี้ยงต่อในน้ำเลี้ยงเซลล์ที่พร่องสารออกฤทธิ์เหมือนเอสโตรเจน (SF) และมีการเติมเอสโตรเจน (E; 1 นาโนโมลาร์) เจนีสทีอีน (10 ไมโครโมลาร์) หรือไดแอดซีน (10 ไมโครโมลาร์) เป็นเวลา 48 ชั่วโมง ผลการทดลองไม่พบการปรากฏของฟาสไลแกนด์ (25 กิโลดาลตัน) ในเซลล์ PE แต่พบฟาสไลแกนด์ในเซลล์ RL95-2 ที่เลี้ยงใน SM และเพิ่มมากขึ้นเมื่อนำไปเลี้ยงใน SF การเติมเอสโตรเจน เจนีสทีอีน หรือ ไดแอดซีน เพิ่มการปรากฏของฟาสไลแกนด์ 7-10 เท่า ในการศึกษาครั้งนี้พบปริมาณโปรตีนไซโตโครมซี ในระดับต่ำทั้งในเซลล์ PE และ RL95-2 ที่เลี้ยงใน SM แต่กลับเพิ่มขึ้น1.5-2 เท่า ใน SF การเพิ่มขึ้นของไซโตโครม ซีที่เหนี่ยวนำโดย SF ของทั้งสองเซลล์กลับคืนสู่ค่าปกติเมื่อมีการเติมเจนีสทีอีนและไดแอดซีน การให้เอสโตรเจนมีผลทำให้ระดับไซโตโครมซีต่ำในเซลล์ PE ลดลงแต่กลับไม่มีผลต่อระดับไซโตโครมซีที่เหนี่ยวนำโดย SF ในเซลล์ RL95-2 ผลดังกล่าวแสดงให้เห็นว่าเจนีสทีอีนและไดแอดซีน มีฤทธิ์เสมือนปัจจัยช่วยชีวิต โดยยับยั้งการปลดปล่อยไซโตโครมซีซึ่งเป็นสัญญาณเริ่มต้นที่ทำให้เกิดกระบวนการอะพอพโทซิสภายในเซลล์ปกติและเซลล์มะเร็งของเยื่อบุมดลูก นอกจากนี้ เอสโตรเจน และไฟโตเอสโตรเจนยังเพิ่มการแสดงออกของฟาสไลแกนด์บนผิวเซลล์มะเร็งที่เป็นสัญญาณทำให้เกิดการตายต่อเซลล์อื่นซึ่งอาจช่วยทำให้เกิดการเจริญของเซลล์มะเร็งต่อไป การศึกษาครั้งนี้จึงสรุปได้ว่าควรมีความระมัดระวังในการบริโภคอาหารที่มีไฟโตเอสโตรเจนในผู้ป่วยมะเร็งเยื่อบุมดลูก