Original Article

Dietary Daidzein Enhances Antiapoptotic Effect of 17β-Estradiol (E_2) on Breast Cancer MCF-7 Cells

Xiao-fei Wu, Yi-jing Wang, Guo-liang Xia, Mei-jia Zhang*

State Key Laboratory for Agrobiotechnology, College of Biological Sciences, China Agricultural University, Beijing 100193, China

CLC number: R737.9 Document code: A Article ID: 1000-9604(2010)01-0010-07
DOI: 10.1007/s11670-010-0010-2

ABSTRACT

Objective: To investigate whether dietary daidzein interact with endogenous 17β-Estradiol (E_2) to give rise to additive or inhibitory effects on proliferation and apoptosis in breast cancer cells.

Methods: Cell cycle distribution and apoptosis induction were analyzed by using flow cytometry when breast cancer cell lines MCF-7 were cotreated with daidzein (1, 5 μmol/L) and E_2 (0.1−10 nmol/L) for 5 days. Whether daidzein could alter E_2-modulated mRNA expression of estrogen receptor alpha (ERα), estrogen receptor beta (ERβ) and ERβ-estrogen response element (ERE) dependent transcription was investigated by RT-PCR and luciferase induction assays. The effects of daidzein on E_2-modulated expression of proapoptotic *p53*, *bax* and antiapoptotic *bcl*-2 at both mRNA and protein levels were also investigated by RT-PCR and Western blot.

Results: Daidzein enhanced the antiapoptotic effect in an E_2 dose-dependent manner, but had no effect on E_2-induced proliferation. Daidzein antagonized E_2-induced ERβ mRNA expression and ERβ-ERE dependent transcription. In addition, daidzein only antagonized E_2-upregulated expression of *p53* and *bax*, but had no effect on E_2-upregulated expression of *bcl*-2.

Conclusion: Daidzein enhances the antiapoptotic effect of E_2 on breast cancer cells by inhibiting E_2-mediated *p53-bax* proapoptotic pathway. These results suggest that dietary daidzein may enhance deleterious effect of endogenous E_2 in hormone-dependent breast cancer.

Key words: Daidzein; E_2; Breast cancer; MCF-7 cells; Antiapoptotic effect; Estrogen receptor (ER)

INTRODUCTION

Daidzein, the second-most prominent insoflavone in soy products, has attracted attention because intake of it redounds to the protection against breast cancer [1, 2]. However, the anticancer effect of daidzein is only observed in vitro experiments at high concentrations (>10 μmol/L)[3]. In contrast, the dietary concentration of daidzein only reaches 1−5 μmol/L[4], which stimulates the growth of estrogen receptor (ER)-positive breast cancer cells in vitro, exhibiting estrogenic properties[5]. Estrogens are the most important risk factors for breast cancer, and endogenous E_2 present in breast tissue may achieve 0.1 to 10 nmol/L[6, 7]. The role of dietary daidzein in an endogenous estrogen environment still remains obscure.

Activation of ERα is known to promote cellular growth, but activation of ERβ has been proposed to inhibit proliferation and induce apoptosis in breast tumors[8−10]. ERβ mRNA was dose-dependently upregulated by high concentrations of E_2 (≥10 nmol/L) in breast cancer T47D cells[11]. E_2 may stimulate cellular growth via ERα, and synchronously induce cellular apoptosis via ERβ above 10 nmol/L in T47D cells. For example, the

Received 2009−08−12; **Accepted** 2009−10−23
This work was supported by the National Natural Science Foundation of China (No.30671508) and by State Key Laboratory for Agrobiotechnology of China (No.2009SKLAB07-5).
*Corresponding author.
E-mail: zmeijia@cau.edu.cn

proliferative rate rose from 0.1 to 10 nmol/L and dropped from 10 to 100 nmol/L[12]. Proapoptotic effect involves the *p53*-*bax* pathway. Proapoptotic protein *bax* can induce the mitochondrial pathway of apoptosis and tumor suppressor protein *p53* can induce *bax* expression[13]. *bcl*-2 is an antiapoptotic protein and its overexpression has been shown to inhibit apoptosis[14]. The *bcl*-2/*bax* protein ratio modulates cellular apoptosis[15].

Therefore, we examined whether dietary daidzein affected E_2-mediated proliferation and apoptosis of breast cancer cells, by altering E_2-modulated expression of ERα, ERβ, *p53*, *bax* and *bcl*-2.

MATERIALS AND METHODS

Reagents and Cell Culture

Daidzein, 17β-Estradiol (E_2) and Propidium iodide (PI) were purchased from Sigma and MPP dihydrochloride (highly selective ERα antagonist) from Tocris. Human breast cancer MCF-7 cells were obtained from ATCC. Cells were routinely maintained in DMEM medium (Gibco), supplemented with 10% FBS (Hyclone) and antibiotics (100 U/ml penicillin and 100 μg/ml streptomycin, Gibco) at 37°C in a humidified atmosphere containing 5% CO_2. Prior to each experiment, Media were changed into phenol red-free DMEM (Gibco) supplemented with 10% charcoal dextran-treated FBS (CDT-FBS, Hyclone) and cells were incubated for at least one week to deplete steroid hormones. Then, cells were incubated for five days in medium supplemented with 5% CDT-FBS containing various concentrations of daidzein or/and E_2.

Cell Cycle Analysis and Apoptosis Measurement

After treatment for 5 d, cells from two dishes in each group were put into a conical tube, and washed twice with PBS by centrifugating at 3000g for 5 min at room temperature. Cell cycle distribution and the rate of apoptosis were measured using flow cytometry as described before[16].

Values were expressed as $\bar{x}\pm s$ of two representative experiments, each performed in 4–6 replicates.

RNA Extraction and RT-PCR

After treatment for 72 h, Total RNA was isolated from cells by extraction using TRIZOL reagent. Lysates were extracted with chloroform and total RNA was precipitated with isopropanol. Verification of changed expression was done by semi-quantitative RT-PCR for ERα, *p53*, *bax* and *bcl*-2. Details of the primers for target genes and reaction conditions are listed in Table 1. GAPDH severed as the internal standard. The PCR products were separated by electrophoresis in a 1.5% agarose gel and visualized by ethidium bromide staining. Relative intensities were quantified using Gel-pro Analyzer 4.0. The ratio between the intensity of the bands was reported as the $\bar{x}\pm s$. For that the level of ERβ mRNA was low in MCF-7[17], real-time quantitative RT-PCR was performed to investigate the expression of ERβ as previously described[18]. Data represent the average of replicates with their standard errors.

Luciferase Induction Assays

The estrogen response element (ERE) reporter construct (pGL3-ERE-luciferase) and expression vectors for ERβ (psG5-hERβ) were kindly provided by Professor Dalong Ma (Chinese National Human Genome Center). Cells were transferred into 24-well plates (5×10^4 cells/well) with 500 μl phenol red-free DMEM (without antibiotics or fungicides) containing 3% CDT-FBS/well the day before transfection. One day later, 0.1 ml medium containing plasmid DNA (0.2 μg ERE reporter plasmid, 0.05 μg PRL-TK, and 0.5 μg ERβ expression vector) and FuGENE HD Transfection reagent (Roche) at a charge ratio of 1:3 was added, then cells were incubated for 14 h. The cells were incubated in medium containing 3% CDT-FBS plus test compounds for another 24 h. MPP dihydrochloride (100 nmol/L), an ERα-specific antagonist, was used with test compounds together to avoid the effect of ERα-ERE transactivation.
When ERβ is activated by the test ligands, ERβ forms a dimer that binds ERE, activating the transcription of luciferase reporter gene. Cell extracts were prepared for luciferase reporter assay (Dual-Luciferase Reporter Assay System, Promega). Transcriptional activity is represented as relative light units (RLUs) calculated as percentage of the maximal induction by E_2 (100 nmol/L) and standardized to the internal transfection control provided by *renilla* luciferase activity.

Western Blot Analysis

Cells were lysed in ice-cold lysis buffer containing 50 mmol/L Tris-HCL, pH 7.5, 150

mmol/L NaCl, 10% (v/v) glycerol, 1% (v/v) Triton X-100, 1 mmol/L PMSF, 1 mmol/L NaF, 1% SDS (v/v), 2 mmol/L sodium orthovanadate, 0.2 mmol/L DTT, and complete TM protease inhibitor cocktail. The cellular debris was cleared by centrifugation (12,000×g, 10 min, 4°C). Protein content of the samples was determined by BCA procedure. Equal amounts of protein (80 μg/well) were separated by SDS-PAGE (15%) and electrotransferred onto nitrocellulose membranes (Bio-Rad, Hercules; Amersham Biosciences). The membranes were incubated overnight at 4°C with primary antibodies (Santa Cruz, CA, USA). Then, the membrane was incubated for 1 h with horseradish peroxidase-conjugated antibody at room temperature. Finally, ECL Plus reagent (Pierce Biotechnology, IL, USA) was used to detect the peroxidase activity and the signal was visualized by autoradiography.

Statistical Analysis

All experiments were repeated at least three times and the values are given as $\bar{x}\pm s$. All frequencies were subjected to arcsine transformation and analyzed by ANOVA followed by Duncan's multiple range tests. $P<0.05$ was considered statistically significant and groups with a common letter are not significantly different.

Table 1. Primer sequences and cycling conditions used for RT-PCR for ERα, ERβ, p53, bax and bcl-2 mRNA in MCF-7 cells

Genes	Primers PCR	Cycles	Annealing temperature (°C)	Product length (bp)
ERα	F: 5' GAAAGGTGGGATACGAAA 3'	32	55	478
	R: 5' AGAGCAAGTTAGGAGCAA 3'			
ERβ	F: 5' CGATGCTTTGGTTTGGGTG 3'	40	58	–
	R: 5' GTTAGCCAGGCGCATGGAT 3'			
p53	F: 5' TTGAGGTGCGTGTTTGTG 3'	30	56	335
	R: 5' TTTATGGCGGGAGGTAGA 3'			
bax	F: 5' TTCCCACCATTCTACCTGA 3'	34	57	536
	R: 5' AGATGAACTCCCTACTCCTTTT 3'			
bcl-2	F: 5' AGATGTCCAGCCAGCTGCAC 3'	30	59.5	331
	R: 5' GCCAAACTGAGCAGAGTCTTCA 3'			

RESULTS

Effects of Daidzein and E_2 on Cell Cycle Progression and Apoptosis in MCF-7 Cells

The concentrations of the compounds, the percentages of cells in G_0/G_1, S, G_2/M phases and sub-G_0/G_1 peak were presented in Table 2. The proliferation was indicated by the percentage of cells in the S phase of the cell cycle. Compared with control, Daidzein or E_2 significantly induced cellular proliferation ($P<0.05$, Figure 1). Neither additive nor antagonistic effects on proliferation could be observed with any of the daidzein/E_2 combinations (Figure 1). The rate of apoptosis was indicated by the percentage of sub-G_0/G_1 nuclei of all nuclei measured. In comparison with control, Daidzein significantly decreased the rate of apoptosis ($P<0.05$, Figure 1). However, E_2 produced a biphasic effect on cellular apoptosis (Figure 1), namely inhibiting apoptosis at low concentration (0.1 nmol/L, $P<0.05$) and inducing apoptosis at high concentration (10 nmol/L, $P<0.05$). E_2 exhibited (1 nmol/L) no significant effect on apoptosis. Compared with daidzein or E_2 alone treatment, the rate of apoptosis significantly decreased when cells were co-treated with daidzein and E_2 ($P<0.05$, Figure 1). In addition, daidzein enhanced the antiapoptotic effect in an E_2 dose-dependent manner.

Effects of Daidzein and E_2 on Gene Expression of ERα and ERβ

We first investigated whether daidzein could interfere with E_2-modulated expression of ERα and ERβ mRNA by RT-PCR after cells were treated for 72 h with various doses of daidzein or/and E_2. As shown in Figure 2A and B, E_2 dose-dependently decreased ERα mRNA and increased ERβ mRNA compared with the control. Daidzein could inhibit E_2-increased ERβ mRNA, but had no effect on ERα mRNA expression when cells were co-treated with daidzein and E_2.

Table 2. Effects of daidzein and E_2 on cell cycle and apoptosis in MCF-7 cells, $\bar{x}\pm s$, $n\geq 3$

Compounds	Cell cycle			Apoptosis
	G_0/G_1	S	G_2/M	Sub-G_0/G_1
Control	77.6%±1.6	15.8%±2.2	6.6%±3.1	27.8%±2.1
1 μmol/L daidzein	71.2%±2.2	21.2%±3.6	7.6%±3.5	20.8%±5.3
5 μmol/L daidzein	69.8%±4.4	22.8%±2.0	7.4%±2.6	22.3%±2.6
0.1 nmol/L E_2	70.9%±2.2	20.9%±4.4	8.2%±3.1	21.1%±3.8
1 nmol/L E_2	68.1%±2.5	25.6%±3.5	6.3%±2.8	25.6%±5.6
10 nmol/L E_2	65.9%±4.1	29.8%±4.5	4.3%±3.3	33.6%±4.4
1 μmol/L daidzein +0.1 nmol/L E_2	71.4%±2.1	21.5%±5.4	7.1%±3.2	15.2%±4.0
1 μmol/L daidzein +1 nmol/L E_2	70.6%±3.7	23.1%±3.4	6.3%±4.2	13.8%±3.5
1μmol/L daidzein +10 nmol/L E_2	68.1%±1.9	26.8%±4.2	5.1%±2.8	11.2%±5.5
5 μmol/L daidzein +0.1 nmol/L E_2	72.3%±3.2	22.9%±2.5	4.8%±2.0	17.1%±4.8
5 μmol/L daidzein +1 nmol/L E_2	67.8%±4.1	25.3%±4.5	6.9%±2.1	16.1%±3.2
5 μmol/L daidzein +10 nmol/L E_2	65.7%±4.5	28.6%±5.3	5.7%±3.7	12.5%±4.1

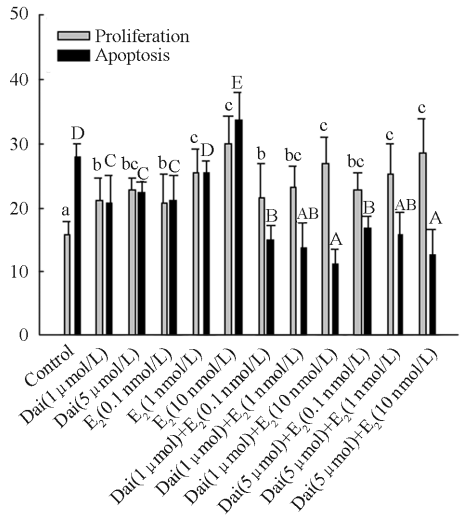

Figure 1. Effects of daidzein and E_2 on cell cycle progression and apoptosis in MCF-7 cells.

Luciferase Induction Assays

In order to investigate whether daidzein could inhibit E_2-mediated transcription via ERβ, we further performed luciferase induction assays in MCF-7 cells. Transcriptional activity is represented as relative light units (RLUs) calculated as percentage of the maximal induction by 100 nmol/L E_2. E_2 induced transactivation of the ERE reporter gene via ERβ in a concentration-dependent manner (Figure 3). Although daidzein induced ERβ-ERE transactivation, it could attenuate E_2-mediated ERE transactivation via ERβ when daidzein was used with 1 or 10 nmol/L E_2 together.

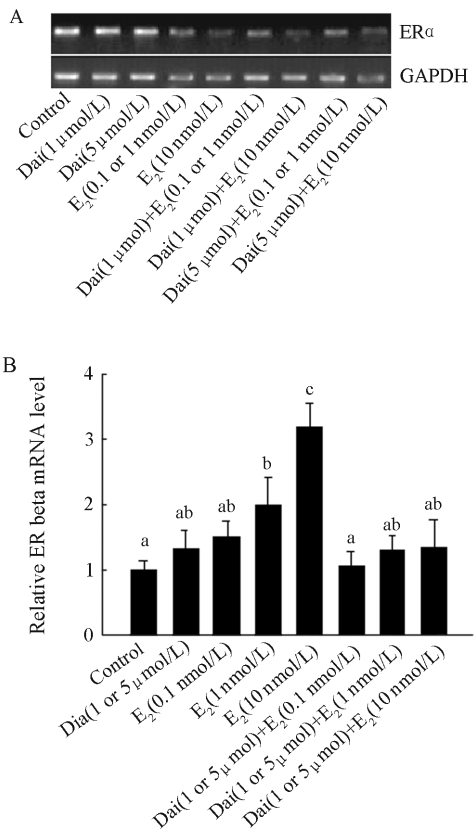

Figure 2. Effects of daidzein and E_2 on gene expression of ERα and ERβ. A: Representative gel showing ERα mRNA expression; B: ERβ mRNA expression values were expressed as fold changes compared with control (defined as 1).

Effects of Daidzein and E_2 on the Expression of *p53*, *bax* and *bcl*-2 at both mRNA and Protein Levels

After cells were treated for 72 h, the effects of daidzein on E_2-modulated the expression of *p53*, *bax* and *bcl*-2 at both mRNA and protein levels were investigated by RT-PCR and Western blot. In comparison with the control, 1 or 5 μmol/L daidzein significantly decreasesed *bax* mRNA (P<0.05), and E_2 dose-dependently increased *p53*, *bax* and *bcl*-2 mRNA (Figure 4A, B). When cells were co-treated with daidzein and E_2, daidzein only antagonized E_2-upregulated mRNA expression of *p53* and *bax* (Figure 4A, B). Additional Western blot assays were also examined and the results were shown in Figure 4C. Daidzein did not alter the protein levels of *p53* and *bcl*-2, but markedly decreased the level of *bax* protein. However, E_2 dose-dependently increased the protein levels of *p53* and *bax*, and upregulated *bcl*-2 protein expression to the same level (Figure 4C). When cells were co-treated with daidzein and E_2, daidzein antagonized E_2-induced expression of *p53* and *bax* proteins, but had no effect on E_2-upregulated expression of *bcl*-2 protein and *bcl*-2/*bax* protein ratio was higher than daidzein or E_2 treatment alone (Figure 4C). Hence, daidzein antagonized E_2-induced expression of *p53* and *bax* at both mRNA and protein levels.

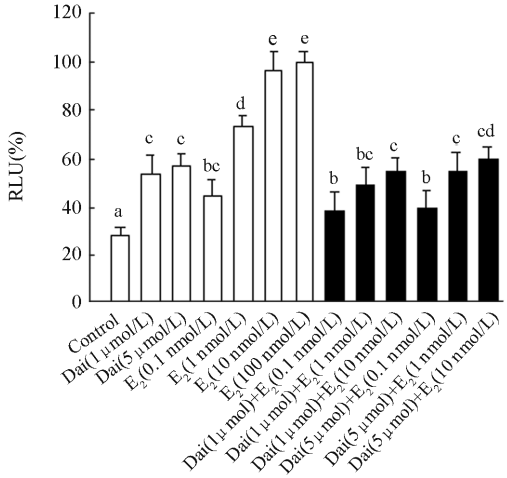

Figure 3. Daidzein attenuated E_2-mediated transactivation via ERβ.

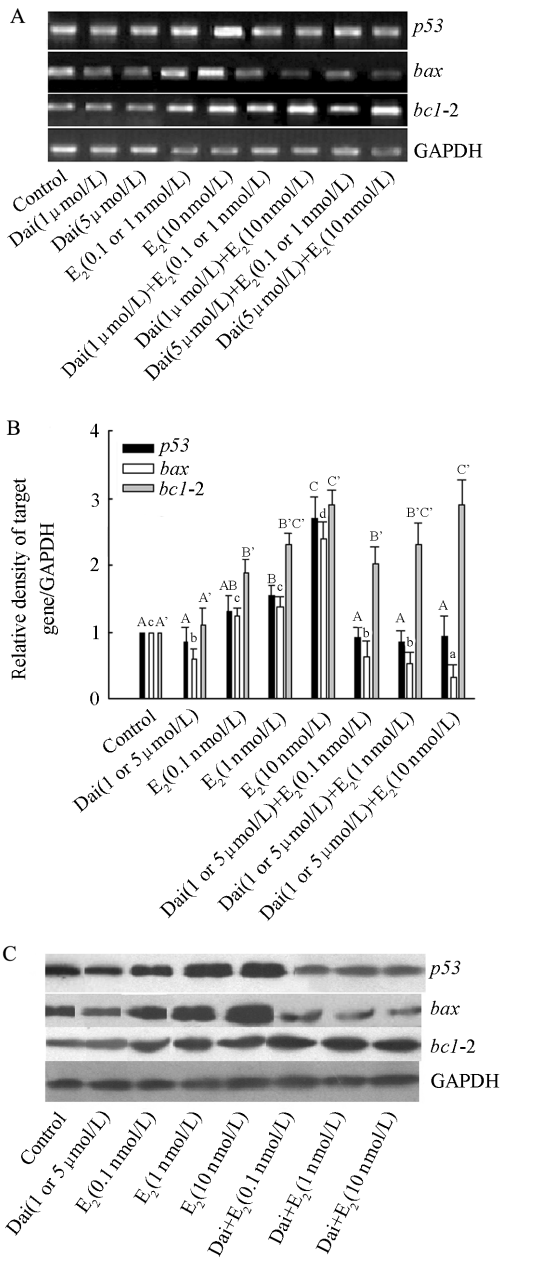

Figure 4. Effects of daidzein and E_2 on expression of *p53*, *bax* and *bcl*-2 at both mRNA and protein levels were investigated by RT-PCR and Western blot. A: Representative gel showing gene expression of *p53*, *bax* and *bcl*-2; B: Summary of the relative densities of *p53*, *bax* and *bcl*-2 genes; C: Representative blot showing protein expression of *p53*, *bax* and *bcl*-2.

DISCUSSION

In the present study, we investigated the combinatory effect of dietary daidzein and endogenous E_2 (0.1–10 nmol/L) on proliferation and apoptosis of breast cancer cells. Daidzein enhanced the antiapoptotic effect in an E_2 dose-dependent manner, but had no effect on E_2-induced proliferation when MCF-7 cells were cotreated with daidzein and E_2. The result is similar to previous report that anticancer effect of genistein

was abolished by cotreatment with higher concentration of E_2 (25 nmol/L or 50 nmol/L)[19]. Our results suggest that dietary daidzein may increase the risk of estrogen-dependent breast cancer especially for postmenopausal women, in whose breast tissue E_2 is transnormal.

In addition, we further examined the mechanism by which daidzein enhances the antiapoptotic effect of E_2 on breast cancer cells. Daidzein antagonized E_2-induced ERβ mRNA expression and ERβ-ERE dependent transactivation, but had no effect on E_2-modulated expression of ERα and *bcl*-2 mRNA. Daidzein antagonized E_2-upregulated expression of *p53* and *bax* at both mRNA and protein levels, inducing a marked increase in *bcl*-2/*bax* protein ratio when cells were co-treated with daidzein and E_2. Thus, the molecular mechanism is that daidzein do not interfere with the antiapoptotic effect by E_2, but inhibits E_2-induced proapoptotic effect through *p53*-*bax* pathway.

Daidzein had no antagonistic effect on E_2-induced cellular proliferation in our and other study[20]. It has been reported that genistein is more effective than daidzein in competing with E_2 for binding to ERα[20]. Its strong affinity for ERα generally correlates with the antagonistic effect of genistein on E_2-induced proliferation, possibly by downregulating ERα expression[8, 20]. Daidzein had no effect on E_2-downregulated expression of ERα mRNA in our study. All these studies show that daidzein has no effect on E_2-induced proliferation due to its weak affinity for ERα.

Activation of ERβ may inhibit cellular growth in breast cancer cells[9, 10]. For example, Pyranocoumarin compound induced apoptosis, accompanied by an increased expression of ERβ[21]. The antiproliferative effect of apigenin was effectively abrogated by ERβ siRNA to downregulate ERβ[22]. Our results showed that daidzein could antagonize E_2-upregulated ERβ mRNA expression and attenuate E_2-mediated transactivation via ERβ. Generally, the ability for binding of a phytoestrogen to a specific ER subtype correlates with its ability to transactivate gene expression through that receptor. So, daidzein attenuates E_2-mediated transactivation via ERβ by competing with E_2 for binding to ERβ. This is likely associated with that daidzein has a stronger affinity for ERβ than ERα, and E_2 binds to ERα and ERβ with equal affinity[23, 24]. Our result is inconsistent with previous investigation that 1 μmol/L daidzein could not interfere with 0.5 nmol/L E_2-mediated transactivation via ERβ[24]. The differences result from that endogenous ERα might interfere with the activity of the exogenously introduced ERβ in their system, however ERα-specific antagonist was used to avoid the effect of ERα-ERE transactivation in ours.

Previous results have demonstrated that pharmacological concentration of daidzein induced cellular apoptosis of MCF-7 cells[3], followed by upregulation of the expression of ERβ, *p53* and *bax* mRNA[25]. In contrast, the expression of *p53* and *bax* mRNA were downregulated by daidzein in MCF10a devoid of ERβ[25]. Proapoptotic effect may be relevant to the activation of ERβ-*p53*-*bax* pathway[25, 26]. Thus, daidzein antagonized E_2-activated *p53*-*bax* pathway possibly through attenuating E_2-upregulated ERβ mRNA expression and ERβ-ERE dependent transactivation. Proapototic *p53* protein induces apoptosis by transcriptional activation of proapoptotic gene *bax*[13]. However, *bcl*-2 protein can dimerize with *bax* to silence its apoptotic functions[14]. The ratio of *bcl*-2/*bax* protein modulates apoptosis[15]. In this study, E_2 dose-dependently increased the levels of proapoptotic *p53* and *bax* proteins in MCF-7 cells. These increases coincided with that E_2 induces cellular apoptosis at high concentration. Daidzein only antagonized E_2-upregulated expression of *p53* and *bax* proteins. E_2 can induce *bcl*-2 expression via ERα-ERE in MCF-7 cells[27]. As mentioned above, daidzein hardly competes with E_2 for ERα binding and interferes with its transactivation via ERα[20, 24]. That daidzein has no effect on E_2-upregulated expression of *bcl*-2 may result from these. So, *bcl*-2/*bax* protein ratio markedly increased when cells were co-treated with daidzein and E_2. It can be seen that daidzein attenuates the proapoptotic effect of E_2, and do not inhibit the antiapoptotic effect of E_2.

In summary, dietary daidzein enhances the antiapoptotic effect in an E_2 dose-dependent manner by inhibiting E_2-induced proapoptotic *p53*-*bax* pathway. Thus, we suggest that dietary daidzein may potentiate deleterious effect of endogenous estrogen in hormone-dependent breast cancer.

Acknowledgements

We thank Professor Da-long Ma (Chinese National Human Genome Center, P.R. China) for donating the plasmids that were used in this study.

REFERENCES

[1] Adlercreutz H. Phyto-estrogens and cancer [J]. Lancet Oncol 2002; 3:364–73.

[2] Magee PJ, Rowland IR. Phyto-oestrogens, their mechanism of action: current evidence for a role in breast and prostate cancer [J]. Br J Nutr 2004; 91:513–31.
[3] Choi EJ, Kim GH. Daidzein causes cell cycle arrest at the G1 and G2/M phases in human breast cancer MCF-7 and MDA-MB-453 cells [J]. Phytomedicine 2008; 15:683–90.
[4] Setchell KD. Phytoestrogens: the biochemistry, physiology, and implications for human health of soy isoflavones [J]. Am J Clin Nutr 1998; 68: 1333s–46s.
[5] Murata M, Midorikawa K, Koh M, et al. Genistein and daidzein induce cell proliferation and their metabolites cause oxidative DNA damage in relation to isoflavone-induced cancer of estrogen-sensitive organs [J]. Biochemistry 2004; 43:2569–77.
[6] Pasqualini JR, Chetrite GS. Recent insight on the control of enzymes involved in estrogen formation and transformation in human breast cancer [J]. J Steroid Biochem Mol Biol 2005; 93:221–36.
[7] van Landeghem AA, Poortman J, Nabuurs M, et al. Endogenous concentration and subcellular distribution of estrogens in normal and malignant human breast tissue [J]. Cancer Res 1985; 45: 2900–6.
[8] Seo HS, DeNardo DG, Jacquot Y, et al. Stimulatory effect of genistein and apigenin on the growth of breast cancer cells correlates with their ability to activate ER alpha [J]. Breast Cancer Res Treat 2006; 99: 121–34.
[9] Rice S, Whitehead SA. Phytoestogens and breast cancer--promoters or protectors? [J] Endocr Relat Cancer 2006; 13:995–1015.
[10] Matthews J, Gustafsson JA. Estrogen signaling: a subtle balance between ERalpha and ERbeta [J]. Mol Interv 2003; 3:281–92.
[11] Cappelletti V, Miodini P, Giovanni DF, et al. Modulation of estrogen receptor-beta isoforms by phytoestrogens in breast cancer cells [J]. Int J Oncol 2006; 28: 1185–91.
[12] Hirsch K, Atzmon A, Danilenko M, et al. Lycopene and other carotenoids inhibit estrogenic activity of 17beta-estradiol and genistein in cancer cells [J]. Breast Cancer Res Treat 2007; 104:221–30.
[13] Schuler M, Green DR. Mechanisms of *p53*-dependent apoptosis [J]. Biochem Soc Trans 2001; 29:684–8.
[14] Oltvai ZN, Milliman CL, Korsmeyer SJ. Bcl-2 heterodimerizes in vivo with a conserved homolog, *Bax*, that accelerates programmed cell death [J]. Cell 1993; 74:609–19.
[15] Williams GT, Smith CA. Molecular regulation of apoptosis: genetic controls on cell death [J]. Cell 1993; 74:777–9.
[16] Diel P, Olff S, Schmidt S, et al. Effects of the environmental estrogens bisphenol A, o, p'-DDT, p-tert-octylphenol and coumestrol on apoptosis induction, cell proliferation and the expression of estrogen sensitive molecular parameters in the human breast cancer cell line MCF-7 [J]. J Steroid Biochem Mol Biol 2002; 80:61–70.
[17] Dotzlaw H, Leygue E, Watson PH, et al. Expression of estrogen receptor-beta in human breast tumors [J]. J Clin Endocrinol Metab 1997; 82:2371–4.
[18] Le Corre L, Fustier P, Chalabi N, et al. Effects of resveratrol on the expression of a panel of genes interacting with the BRCA1 oncosuppressor in human breast cell lines [J]. Clin Chim Acta 2004; 344:115–21.
[19] Park OJ, Shin JI. Proapoptotic potentials of Genistein under growth stimulation by estrogen [J]. Ann N Y Acad Sci 2004; 1030:410–8.
[20] Matsumura A, Ghosh A, Pope GS, et al. Comparative study of oestrogenic properties of eight phytoestrogens in MCF-7 human breast cancer cells [J]. J Steroid Biochem Mol Biol 2005; 94:431–43.
[21] Jiang C, Guo J, Wang Z, et al. Decursin and decursinol angelate inhibit estrogen-stimulated and estrogen-independent growth and survival of breast cancer cells [J]. Breast Cancer Res 2007; 9:R77.
[22] Mar P, Leung YK, Tang WY, et al. Apigenin suppresses cancer cell growth through ERbeta [J]. Neoplasia 2006; 8:896–904.
[23] Kuiper GG, Lemmen JG, Carlsson B, et al. Interaction of estrogenic chemicals and phytoestrogens with oestrogen receptor beta [J]. Endocrinology 1998; 139:4252–63.
[24] Harris DM, Besselink E, Henning SM, et al. Phytoestrogens induce differential estrogen receptor alpha- or beta-mediated responses in transfected breast cancer cells [J]. Exp Biol Med 2005; 230:558–68.
[25] Caëtano B, Le Corre L, Chalabi N, et al. Soya phytonutrients act on a panel of genes implicated with BRCA1 and BRCA2 oncosuppressors in human breast cell lines [J]. Br J Nutr 2006; 95: 406–13.
[26] Zhang H, Somasundaram K, Peng Y, et al. BRCA1 physically associates with *p53* and stimulates its transcriptional activity [J]. Oncogene 1998; 16: 1713–21.
[27] Perillo B, Sasso A, Abbondanza C, et al. 17beta-estradiol inhibits apoptosis in MCF-7 cells, inducing bcl-2 expression via two estrogen-responsive elements present in the coding sequence [J]. Mol Cell Biol 2000; 20: 2890–901.