Physiological Concentrations of Genistein and 17β-Estradiol Inhibit MDA-MB-231 Breast Cancer Cell Growth by Increasing BAX/BCL-2 and Reducing pERK1/2

TALITHA T. RAJAH, KEVIN J. PEINE, NGA DU, CHRISTINA A. SERRET and NEIL R. DREWS

Department of Biological Science, DePaul University, Chicago, IL, U.S.A.

Abstract. Aim: The aim of the present study was to identify the mechanism by which genistein and 17β-estradiol inhibit proliferation of MDA-MB-231 breast cancer cells. Materials and Methods: The expression of cell signaling proteins involved in cell apoptosis, proliferation, and survival (BCL-2 associated X protein, BAX; B-cell lymphoma 2, BCL-2; extracellular signal regulated kinase, pERK1/2; and protein kinase B, pAKT) were examined by western blotting, and tested whether these effects correlated with cell proliferation and apoptosis. Results: Compared to the control, 1 μM genistein plus 1 nM 17β-estradiol significantly increased apoptosis, and the BAX/BCL-2 ratio, with a concomitant decrease in ERK1/2 phosphorylation. High concentrations of genistein (100 μM) both in the presence and absence of 17β-estradiol also increased apoptosis; however, these changes were not correlated with the BAX/BCL-2 ratio or with phosphorylation of ERK1/2. Conclusion: These results suggest that different concentrations of genistein elicit cell responses through different signaling mechanisms. These results are especially relevant in premenopausal women with breast cancer who are on a soy diet.

Although the last two decades have seen an increase in the use of soy in the diet and as a safer alternative to hormone replacement therapy for the reduction of menopausal and cardiovascular symptoms, its efficacy for cardiovascular symptoms is debatable when tested in clinical trials (1-3). Phase II clinical trials have shown that genistein, a soy component, is able to inhibit levels of matrix metalloprotease-2 (a prostate cancer pro-metastatic protein) and reduce levels of serum prostate-specific antigen (a marker for prostate cancer) (4-5). According to the NIH Clinical Trials database, genistein is also in five currently ongoing phase II clinical trials for prevention and treatment of prostate, breast, and pancreatic cancers [clinicaltrials.gov]. Soy-based diets are high in isoflavonoids such as genistein, and have been credited with lowering the rate of breast cancer in Asian women (6-7).

Our previous studies in estrogen receptor (ER) β-positive and ERα-negative MDA-MB-231 breast cancer cells showed that physiological concentrations of genistein (1 nM to 1 μM) plus 17β-estradiol (1 nM) significantly inhibited cell proliferation when compared to controls (8). However, this effect was not seen in ERα- and ERβ-expressing T47D breast cancer cells, suggesting that the action of genistein plus 17β-estradiol in MDA-MB-231 cells is ERα independent. Genistein has a greater affinity for ERβ than for ERα and there is evidence that activation of ERβ in T47D cells with ERα:ERβ (9:1) can silence the growth-promoting effects of ERα (9). The purpose of this study was to identify the signaling mechanisms by which physiological concentrations of genistein and 17β-estradiol inhibit cell survival in ERβ-positive and ERα-negative MDA-MB-231 breast cancer cells.

With the recent trend towards increased human consumption of soy, either in the diet or as an alternative to estrogen replacement therapy, and ongoing clinical trials examining the potential chemopreventive effects of genistein, elucidating the cumulative or combined effects of signaling networks from genistein in the presence of 17β-estradiol on cell survival is highly relevant, especially for women with breast cancer of the ERβ-positive/ERα-negative type that accounts for 18% of all breast cancer cases (10).

Estrogen influences multiple signal transduction pathways related to cell proliferation and survival, including the mitogen-activated protein kinase (MAPK), G-protein-coupled receptor, and protein kinase B (AKT) pathways (11-12). The receptor(s) through which estrogen activates these pathways is not clear. The signaling molecules of the MAPK pathway that are rapidly activated by estrogen include the
extracellular signal regulated protein kinases 1 and 2 (ERK-1 and ERK-2) (11), which influence cell growth, differentiation, and development. In addition, estrogen stimulates nitric oxide synthase to generate nitric oxide, thereby activating phosphatidylinositol-3-kinase (PI3K), a member of the AKT signaling pathway (11). The AKT signaling pathway is antiapoptotic and is involved in regulating cell survival, cell cycle, and metabolism. The PI3K pathway, activated by estrogen, leads to the activation of AKT, an important player in cell survival. Activated AKT inhibits the proapoptotic BCL-2 family protein BCL-2 antagonist of cell death (BAD), directly inhibits caspase-9, influences cell cycle proteins p21 and mouse double minute 2 (MDM2), and inhibits additional apoptotic pathways linked to Forkhead homologue in rhabdomyosarcoma (FKHR) and Glycogen synthase kinase 3 (GSK-3) (13). The BCL-2 family protects the integrity of mitochondria, preventing cytochrome c release and subsequent activation of caspase-9. The end result of these cascades includes execution of complex cellular processes such as proliferation, differentiation, metabolism, and apoptosis.

Genistein is a known tyrosine kinase inhibitor that has been shown by various studies to influence signaling proteins, such as AKT, Nuclear factor kappa B (NFkB), and ERK-1/2 that regulate cell proliferation and survival. In addition, genistein influences various apoptotic protein regulators such as BAX, BCL-2, the caspas, and BAD (14-15).

This suggests that the actions of both estrogen and genistein are pleiotropic, involving a network of genes that regulate multiple signaling pathways to modulate the execution of complex cellular programs such as proliferation, differentiation, and apoptosis, key events in carcinogenesis and cancer progression. The integration of diverse mechanisms through which these agents modulate the resulting cellular behavior is not known. Clarke et al. proposed that biological events regulated by antiestrogens reflect the existence of a complex and coordinated network of genes that function interactively and/or interdependently in a dose-, tissue-, and time-dependent manner (16, 17). The key biological outcome is determined by the manner in which the sum of the signals affects the cell’s choice to proliferate, differentiate, or die.

Most previous studies with genistein have been carried out on single signaling pathways and an overview of literature has shown that genistein is pleiotropic in action, acting on several different signaling pathways. Thus, the combined effect of these pathways on carcinogenic cellular behavior is not known. Our hypothesis is that the cumulative or combined signaling actions of genistein plus 17β-estradiol from different signaling pathways is likely to direct the cell’s choice either to proliferate or enter the apoptotic pathway. The combined effects from multiple signaling pathways induced by physiological concentrations of genistein and 17β-estradiol on ERβ-positive and ERα-negative breast cancer cells has yet to be studied.

In this study, the effects of physiological concentrations of genistein and 17β-estradiol were determined on key signaling proteins from different pathways involved in carcinogenic cellular behaviors (ERK-1/2 for cell proliferation, AKT for cell survival, and proapoptotic BAX and antiapoptotic BCL-2), and the results were correlated with cellular outcomes of cell proliferation and apoptosis. Specifically, we exposed MDA-MB-231 cells to physiological concentrations of genistein and 17β-estradiol for 24 h to examine changes in the levels of signaling proteins and we correlated these changes with cell proliferation and apoptosis at 24 and 48 h.

Materials and Methods

Chemicals and reagents. Genistein, 17β-estradiol, acridine orange, ethidium bromide, bovine serum albumin (BSA), 3-[4,5-dimethyl-2-thiazolyl]-2,5-diphenyl-2H-tetrazolium bromide (MTT) dye, and RPMI-1640 media were purchased from Sigma-Aldrich (St Louis, MO, USA). Genistein was dissolved in dimethyl sulfoxide (DMSO; Sigma-Aldrich) and was added to cell cultures at the desired dilutions in culture medium without serum supplementation. Control cultures received 0.1% of DMSO alone, which has been demonstrated to have no effect on cell proliferation. 17β-Estradiol (water-soluble) was dissolved in sterile deionized water and added to cell cultures at a final concentration of 1 nM. Control cultures received an equal volume of sterilized deionized water.

Cell culture. MDA-MB-231 breast cancer cells were obtained from the ATCC (Manassas, VA, USA. Cells were grown as a monolayer culture in RPMI-1640 medium (without phenol red) supplemented with L-glutamine (2 mM), gentamicin (50 μg/ml), penicillin (100 U/ml), streptomycin (100 μg/ml), and fetal bovine serum (5%).

Cell proliferation assay. The effects of genistein on cell proliferation were assessed using the MTT assay as described (8). The MTT assay is commonly used to measure cell proliferation and viability by measuring the reduction of the yellow MTT dye by mitochondrial dehydrogenases in viable cells to yield purple formazan crystals, which can be detected colorimetrically at 590 nm. Briefly, breast cancer cells were seeded in 24-well plates at approximately 3×10⁵ cells/well and allowed to attach overnight. They were then estrogen starved for 24 h by replacing the medium with phenol red free RPMI-1640 with 0.2% BSA, which is completely devoid of estrogen. Following estrogen starvation, cells were exposed to genistein (1 nM to 100 μM) alone and in combination with 17β-estradiol (1 and 10 nM) for 24 and 48 h. Finally, cells were incubated for 2 h in the presence of 10% MTT (5 mg/ml) and the resulting formazan crystals were dissolved in MTT solubilization solution (10% Triton X-100, 0.1 N HCl in isopropanol). The absorbance of the resulting solution was measured at 590 nm, with background absorbance measured at 690 nm. The background was subtracted from the Δ Abs measurement, and cell survival was calculated as a percentage that of the control.

Apoptosis assay. Apoptosis was assayed by examining cytoplasmic condensation and nuclear fragmentation, two hallmarks of apoptotic cells (18), using differential uptake of the fluorescent
Differentiated from necrotic cells, which have a structurally normal condensed and fragmented orange chromatin. These are nucleus. Late apoptotic cells take up both dyes and display a condensed or fragmented acridine orange, but are identified by a condensed or fragmented membrane integrity and stains the nucleus red. Healthy cells take up acridine orange but not ethidium bromide and are stained green with a normal nuclear morphology. Early apoptotic cells also take up acridine orange but not ethidium bromide and are stained green.

DNA-binding dyes ethidium bromide and acridine orange. Acridine orange permeates all cells and stains the nucleus green, whereas ethidium bromide is taken up by cells with compromised cell membrane integrity and stains the nucleus red. Healthy cells take up acridine orange but not ethidium bromide and are stained green with a normal nuclear morphology. Early apoptotic cells also take up acridine orange but are identified by a condensed or fragmented nucleus. Late apoptotic cells take up both dyes and display a condensed and fragmented orange chromatin. These are differentiated from necrotic cells, which have a structurally normal but orange nucleus.

The ethidium bromide/acridine orange apoptotic assay was conducted as described (19-20) with a few modifications. MDA-MB-231 cells were seeded in 100-mm Petri dishes and allowed to attach overnight. They were estrogen starved in RPMI with 0.2% BSA for 24 h and then exposed to genistein (1 and 100 μM) in the presence, or absence of 17β-estradiol (1 nM) for 48 h. Cells were trypsinized and a dye mix containing a 1:25 ratio of acridine orange (100 μg/ml) and ethidium bromide (100 μg/ml) was added to the cell suspension. Cells were placed in the dark for 10-15 min and were examined using a Nikon Eclipse E600 fluorescence microscope at ×40 magnification with a 480/30-nm excitation filter, 505-nm LP dichromatic mirror cut-on, and 535/40-nm barrier filter (Nikon, Melville, NY, USA). Each experiment was carried out in triplicate and repeated three times. A total of approximately 250 cells in 10 to 15 fields per treatment were counted for analysis. The percentage of apoptosis was calculated as the total number of apoptotic cells divided by the number of total cells counted multiplied by 100.

Western blot analysis. The expression of signaling proteins was carried out by western blot analysis as described (21). Briefly, MDA-MB-231 breast cancer cells were seeded into 100-mm tissue culture dishes, allowed to reach 60 to 70% confluence, estrogen starved in RPMI containing 0.2% BSA for 24 h, and exposed to genistein (1 and 100 μM) in the presence or absence of 17β-estradiol (1 nM) for 24 h. Cells were washed in ice-cold phosphate buffered saline (PBS) and the total protein was extracted using the Whole Cell Extraction Kit (Chemicon/Millipore, Danvers, MA, USA) according to the manufacturer’s instructions. Equal amounts of protein were run on a 4-20% acrylamide gradient gel (Pierce/Thermo Fisher Scientific, Rockford, IL, USA) and were transferred to polyvinylidene fluoride membranes. Membranes were blocked for 1 h at room temperature in Tris-buffered saline (1% Tween 20 (TBS-T) plus 5% milk, then rinsed three times in TBS-T and incubated overnight with primary antibodies to BAX, BCL-2, β-actin, ERK1/2, pERK1/2, AKT, and pAKT (Cell Signaling Technologies, Danvers, MA, USA). Membranes were washed three times in TBS-T, incubated with horseradish peroxidase conjugated secondary antibodies for 1 h at room temperature, and washed again. Protein bands were visualized using Super Signal West Pico Chemiluminescent Substrate (Thermo Fisher Scientific). Chemiluminescent images were captured using a Chemi-Imager (Alpha Innotech, Santa Clara, CA, USA), and the optical density of bands was quantified using AlphaView software, version 3.0 (Alpha Innotech). Expression of BAX and BCL-2 (in optical density units) was normalized to the expression of β-actin, whereas phosphorylated ERK1/2 and AKT were normalized to their corresponding non-phosphorylated proteins. β-Actin was used as a loading control.

Statistical analysis. Data are expressed as the means±SEM of at least three independent experiments. Statistical differences were analyzed using the two-tailed unpaired Student’s t-test. A value of p<0.05 was considered statistically significant.

Results

Effect of genistein and 17-β estradiol on cell proliferation. MDA-MB-231 cells display highly aggressive metastatic characteristics and have the ERβ receptor but are ERα negative (22). To determine the effects of genistein and 17β-estradiol on cell proliferation, the cells were exposed to a range of genistein concentrations (1 nM to 100 μM) in the presence and absence of 17β-estradiol (1 nM) and cell proliferation was determined by the MTT assay at 24 and 48 h. When used alone, genistein at the concentration range of 1 nM to 10 μM showed no appreciable effect on cell proliferation when compared to the control. At the higher concentration range (25 to 100 μM), however, genistein significantly reduced cell proliferation (20-40%, p<0.05) compared to the control (Figure 1A). 17β-Estradiol alone did not have any appreciable effect on the percentage cell proliferation at either concentration tested (1 and 10 nM; Figure 1B). However, in the presence of physiological concentrations of 17β-estradiol (1 nM), low concentrations of genistein (1 nM to 10 μM) led to a significant 30-40% decrease in cell proliferation (p<0.01) compared to the control, and higher concentrations (25 to 100 μM) of genistein led to a 40% decrease in cell proliferation (p<0.01; Figure 1B). These differences were observed both at 24 and 48 h.

Effect of genistein and 17-β estradiol on apoptosis. To determine if these decreases in cell proliferation were due to apoptosis, an apoptosis assay was carried out at 48 h after treatment with 1 and 100 μM genistein. At 1 μM, genistein alone significantly increased apoptosis by 1.6-fold compared to the control (Figure 2). In the presence of 1 nM 17β-estradiol, 1 μM genistein led to a 2.1-fold increase in apoptosis compared to the control. At these concentrations, genistein plus 17β-estradiol significantly increased apoptosis by 1.35-fold over that with genistein alone. A high concentration of genistein (100 μM) significantly increased apoptosis by 3-fold compared to the control, both in the presence and absence of 17β-estradiol. At 100 μM genistein, the difference in apoptosis between genistein and genistein plus estradiol was not statistically significant.

Effect of genistein and 17-β estradiol on BAX and BCL-2 protein expression. To determine the mechanism by which low concentrations of genistein plus 17β-estradiol inhibit MDA-MB-231 proliferation, we examined the expression of the proapoptotic protein BAX and the antiapoptotic protein BCL-2. Representative western blots for BAX and BCL-2 are shown in Figure 3. No significant differences were observed in the expression of BAX and BCL-2 upon treatment of cells with
Figure 1. Effect of genistein and 17β-estradiol on proliferation of MDA-MB 231 human breast cancer cells. Cells were seeded in 24-well plates and allowed to attach overnight. Following estrogen starvation, cells were treated for 24 and 48 h with genistein (1 nM to 100 μM) in the absence (A) or presence (B) of 17β-estradiol (1 and 10 nM). Cell survival was determined by the MTT assay and calculated as a percentage that of the vehicle-treated control. Data are presented as the mean from at least three experiments±SEM. *p<0.05 and **p<0.01 compared to control.
1 nM 17β-estradiol alone. Only marginal decreases of both BAX and BCL-2 were seen in cells treated with 1 μM genistein alone. However, in cells treated with 1 μM genistein plus 1 nM 17β-estradiol, there was a significant increase in the expression of proapoptotic protein BAX and a significant decrease in the expression of the antiapoptotic protein BCL-2. Cells treated with 100 μM genistein alone showed a significant increase in BAX but no significant change in BCL-2 compared to the control. In the presence of 1 nM 17β-estradiol, 100 μM genistein did not affect BAX or BCL-2 expression compared to the control.

Effect of genistein and 17-β estradiol on phosphorylation of ERK1/2 and AKT. To further determine the mechanism by which low concentrations of genistein plus 17β-estradiol inhibit MDA-MB-231 cell proliferation, we examined the phosphorylation of the cell proliferation protein ERK1/2 and the cell survival protein AKT. Representative western blots for ERK1/2, pERK1/2, AKT, and pAKT are shown in Figure 4. 17β-Estradiol alone (1 nM) caused a significant decrease in phosphorylated ERK1/2 and a significant increase in phosphorylated AKT. At 1 μM genistein, there was a significant decrease in phosphorylated ERK1/2 in both the presence and the absence of 17β-estradiol. Cells in this treatment group also exhibited increases in the phosphorylation of AKT, although the changes were not statistically significant. Cells treated with 100 μM genistein exhibited significant decreases in phosphorylation of AKT both in the presence and absence of 17β-estradiol and significant increase in phosphorylation of ERK1/2 in the absence of 17β-estradiol.

Combined effects on expression of signaling proteins. The balance between proapoptotic BAX and antiapoptotic BCL-2 is important for inducing apoptosis and an increase in the BAX/BCL-2 ratio is considered a key determinant for mitochondrial contribution to apoptosis (23). Cells treated with 1 μM genistein plus 1 nM 17β-estradiol had the highest BAX/BCL-2 ratio (2.6-fold), along with reduced phosphorylation of ERK1/2 but no appreciable difference in phosphorylation of pAKT (Figure 5). When used alone, 1 μM genistein led to only a marginal increase in the BAX/BCL-2 ratio.
ratio and significantly reduced the ratio of pERK1/2 to ERK1/2 relative to the control. Cells treated with 100 μM genistein in the absence of 17β-estradiol had an increased BAX/BCL-2 ratio and increased phosphorylation of ERK1/2. In contrast, no appreciable differences in the expression of BAX or BCL-2 or the phosphorylation of ERK1/2 were seen in the presence of 17β-estradiol. Although a statistically significant decrease in the phosphorylation of ERK1/2 was observed in this treatment group, its biological relevance is questionable. Under both treatment conditions (100 μM genistein with/without 17β-estradiol), the phosphorylation of AKT was significantly inhibited.
Discussion

Previous reports suggest that genistein is involved in regulation of signaling proteins from several pathways. It is likely that the networking of these pathways drives a cell's choice to proliferate, differentiate, or enter apoptosis. The concept of a network differs from that of a signal transduction pathway in that it requires the integration of different pathways, de-emphasizes the role of well-established single signal transduction pathways, and acknowledges the likelihood that few cellular behaviors are likely to be driven by a single gene or pathway (16, 17). Our previous studies have shown that at physiological concentrations, genistein in the presence of 17β-estradiol reduces cell proliferation of MDA-MB-231 cells that are ERα negative and ERβ positive (8). However, the
mechanism by which this reduction in proliferation is brought about remains unknown. In the present study, the effect of multiple signaling pathways induced by genistein plus 17β-estradiol was evaluated by examining expression of BAX and BCL-2, phosphorylation of ERK1/2 and AKT, and two key carcinogenic cellular behaviors, cell proliferation and apoptosis.

For this study, based on results from the cell proliferation assay, 1 μM genistein was chosen as being representative of physiological concentrations, and 100 μM genistein was considered as a high concentration. For comparison, the serum concentration of genistein after intake of a soy-rich meal or low-dose isoflavones ranges between 1 and 5 μM (24).

In MDA-MB-231 cells exposed to 1 μM genistein plus 1 nM 17β-estradiol, we observed an increase in BAX expression, a decrease in BCL-2 expression, an increase in the BAX/BCL-2 ratio, a decrease in phosphorylation of ERK1/2, and no change in phosphorylation of AKT. Whereas BCL-2 is an antiapoptotic protein and is overexpressed in about 40-70% of breast carcinomas (25), BAX is a proapoptotic protein for which an increase in expression induces apoptosis. BAX has homologous sequences with BCL-2 and forms heterodimers with BCL-2, thereby inhibiting the antiapoptotic activity of the latter. Hence, the ratio of BAX/BCL-2, rather than the absolute expression of each protein singly, has been suggested to be an important determinant for apoptosis (23). Cells exposed to genistein in the presence of 17β-estradiol had a significantly increased BAX/BCL-2 ratio and a corresponding significant increase in apoptosis at 48 h. The increased BAX/BCL-2 ratio observed in cells treated with 1 μM genistein in the presence of 1 nM 17β-estradiol was higher than for any other treatment condition tested. An increase in the BAX/BCL-2 ratio stimulates the release of cytochrome c from the mitochondria into the cytosol, which then leads to the activation of caspase-3, one of the executioners of apoptosis (26).

In cells treated with 1 μM genistein and 1 nM 17β-estradiol, we also observed an inhibition in the phosphorylation status of ERK1/2 but no difference in the phosphorylation of AKT. In pancreatic cells, activation of ERK signaling causes overexpression of BCL-2 (27). However, these effects appear to be dependent on the cell lines studied, as Chang et al. observed no effect on BCL-2 levels upon inhibition of ERK in lung cancer cells (28). Sakamoto et al. suggest that genistein alone does not activate ERK1/2 or AKT and that the induction of apoptosis via BCL-2 may not involve these signaling molecules (29). Although the role of phosphorylated ERK1/2 and AKT in apoptosis induction via
BCL-2 remains to be elucidated, our results suggest that in conjunction with an increased BAX/BCL-2 ratio and suppression of cell proliferative signals (activation of ERK1/2), genistein plus 17β-estradiol shifts the balance of cellular behavior towards apoptosis by increasing proapoptotic signals and concurrently reducing cell proliferative signals.

Physiological concentrations of genistein plus 17β-estradiol significantly inhibited cell proliferation at both 24 and 48 h when compared to the control and genistein alone. To determine if this decrease was due to apoptosis, apoptosis was measured by microscopic analysis. Our results show that genistein increased apoptosis at both concentrations and in both the presence and the absence of 17β-estradiol. At 1 μM genistein, apoptosis observed in the genistein plus 17β-estradiol group was significantly higher than that seen with genistein alone. The decrease in cell proliferation seen at the low concentration range of genistein plus 17β-estradiol could be due to apoptosis. Previous studies have shown that up to 1 μM genistein in the presence of 17β-estradiol inhibits apoptosis in ERα-positive MCF7 breast cancer cells (30, 31). However, to our knowledge, this is the first report of the apoptotic effect of low genistein concentrations in the presence of 17β-estradiol in ERα-negative, ERβ-positive MDA-MB-231 breast cancer cells.

At 1 μM, genistein alone caused statistically significant but biologically modest decreases in BAX and BCL-2, a modest increase in the BAX/BCL-2 ratio, a statistically significant decrease in phosphorylated ERK1/2, and no change in phosphorylation of AKT. Although the BAX/BCL-2 ratio for genistein plus 17β-estradiol was about 2.6-fold higher than that of the control, genistein alone resulted in similar BAX/BCL-2 ratios as compared to the control. The levels of phosphorylated ERK1/2 were comparable for genistein in the presence and absence of 17β-estradiol. These results suggest that the higher cell death in MDA-MB-231 cells treated with genistein plus 17β-estradiol are due to an increase in the BAX/BCL-2 ratio and a concomitant decrease in ERK1/2 phosphorylation, whereas the lower cell death with genistein alone could be due to a decrease in phosphorylated ERK1/2 alone. Recent findings have shown that genistein alone at 5-30 μM up-regulates BAX and down-regulates BCL-2 in MDA-MB-231 cells (32, 33) by down-regulation of NFκB via the mitogen-activated protein ERK5 pathway (33). However, the cells in these studies were not serum starved but rather grown in the presence of fetal calf serum, which contains physiological levels of 17β-estradiol, suggesting that the observed increases in the BAX/BCL-2 ratio could be due to the presence of genistein plus 17β-estradiol and not to genistein alone.

Our results show that 17β-estradiol alone did not have any effect on the BAX/BCL-2 ratio in MDA-MB-231 cells. Reports on the effects of 17β-estradiol on BAX and BCL-2 expression are controversial. 17β-Estradiol increases BAX in the anterior pituitary glands from rats in the proestrus phase, and in testicular cells, which is correlated to apoptosis (34-35). Furthermore, treatment with 17β-estradiol of ovariectomized rats increased the BAX/BCL-2 ratio and induced apoptosis (33). Contrary to these reports, a recent study has shown that 17β-estradiol increases the levels of the antiapoptotic protein BCL-2 in the ER-positive breast cancer cell line KPL (36). Whether ERα and or β play any role in the expression of BAX and BCL-2 is not known.

MDA-MB-231 cells treated with 100 μM genistein in the presence and absence of 17β-estradiol exhibited slight increases in BAX/BCL-2 and phosphorylation of ERK1/2, but also a significant inhibition of the phosphorylation of AKT. Xu and Loo have shown that the BAX/BCL-2 ratio in MDA-MB-231 cells is inhibited by exposure to 50 μM genistein for 18 h to 6 days (37). The discrepancy between these results could be due to differences in genistein concentrations and exposure times. High concentrations of genistein have been found to inhibit the phosphorylation of AKT at Ser473 without affecting total AKT levels in MDA-MB-231 cells (38). AKT inhibits cell death through inactivation of the proapoptotic proteins BAD, caspases, and FKHR, thereby inhibiting apoptosis and causing cell cycle arrest.

In the higher concentration range (10 to 100 μM), genistein reduced cell proliferation and increased apoptosis in both the presence and absence of 17β-estradiol. The effect of genistein at higher concentrations has been attributed to general cytotoxic effects rather than specific estrogen-mediated effects. Some of the mechanisms that have been attributed to effects at higher concentrations of genistein include inhibition of tyrosine kinase and DNA topoisomerase activity (15). Furthermore, decreased survival of MDA-MB-231 cells at high concentrations of genistein is due to cell cycle arrest at the G2/M phase (39), or apoptosis through a caspase-3-dependent (33), ERα- and p53-independent (32) pathway. In addition, high concentrations of genistein mediate apoptosis through the down-regulation of AKT and NFκB (33, 38). The relevance of in vitro studies using high concentrations of genistein to physiological conditions is in question because the physiological levels of genistein achieved by the diet ranges only from 1 to 5 μM (24).

In conclusion, our results suggest that physiological concentrations of genistein and 17β-estradiol induce apoptosis of MDA-MB-231 breast cancer cells by regulating multiple signaling molecules from different pathways, and this effect appears to be both concentration- and estrogen-dependent. Physiological concentrations of genistein and 17β-estradiol inhibit the growth of MDA-MB-231 cells by increasing the BAX/BCL-2 ratio and reducing the phosphorylation of ERK1/2. The balance between the activities of these signaling molecules appears to drive cellular fate. These results suggest that genistein may be useful as a chemopreventive agent against breast cancer cells of the ERβ-positive/ERα-negative type, which account for approximately 18% of all breast carcinomas (10).
Acknowledgements

The Authors are grateful to Dr. Jingjing Kipp for critical review of the manuscript. This study was supported by grants from the University Research Council and College of Liberal Arts and Sciences, DePaul University.

References


Received February 3, 2012
Revised February 28, 2012
Accepted February 29, 2012