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Induction of apoptotic cell death by phytoestrogens by up-regulating the levels of phospho-p53 and p21 in normal and malignant estrogen receptor α–negative breast cells

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Abstract

In this study, we investigated the underlying mechanism by which phytoestrogens suppress the growth of normal (MCF-10A) and malignant (MDA-MB-231) estrogen receptor α (ERα)–negative breast cells. We hypothesized that phytoestrogen inhibits the proliferation of ERα-negative breast cancer cells. We found that all tested phytoestrogens (genistein, apigenin, and quercetin) suppressed the growth of both MCF-10A and MDA-MB-231 cells, as revealed by proliferation assays. These results were accompanied by an increase in the sub-G0/G1 apoptotic fractions as well as an increase in the cell population in the G2/M phase in both cell types, as revealed by cell cycle analysis. When we assessed the effect of phytoestrogens on the level of intracellular signaling molecules by Western blot analysis, we found that phytoestrogens increased the level of active p53 (phospho-p53) without changing the p53 level in both MCF-10A and MDA-MB-231 cells. Phytoestrogens also induced an increase in p21, a p53 target gene, and a decrease in either Bcl-xL or cyclin B1 in both cell types. In contrast, the protein levels of phosphatase and tensin homolog, cyclin D1, cell division control protein 2 homolog, phospho–cell division control protein 2 homolog, and p27 were not changed after phytoestrogen treatment. Our data indicate that phytoestrogens induce apoptotic cell death of ERα-negative breast cancer cells via p53-dependent pathway and suggest that phytoestrogens may be promising agents in the treatment and prevention of ERα-negative breast cancer.
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Keywords: Breast cancer; phytoestrogens; MCF-10A; MDA-MB-231; p53; p21

Abbreviations: ERα, estrogen receptor α; cdc2, cell division control protein 2 homolog; DMSO, dimethyl sulfoxide; CDK, cyclin-dependent kinase; PBS, phosphate-buffered saline; PTEN, phosphatase and tensin homolog; SDS, sodium dodecyl sulfate; TBST, Tris-Buffered Saline Tween-20.

1. Introduction

Breast cancer is the most common type of cancer and the leading cause of cancer deaths among women worldwide [1]. Significant improvements are being made in both prognosis and therapy of breast cancer. Drugs used to treat and prevent breast cancer include selective estrogen receptor modulators such as tamoxifen and raloxifene, as well as aromatase inhibitors. However, their effects are limited to estrogen receptor α (ERα)–positive breast cancers [2], and they therefore cannot be used to treat ERα-negative cancers. Estrogen receptor α–negative breast cancers are also more aggressive and have poorer clinical outcomes than ERα-positive cancers. Novel targeted therapies are urgently required for the treatment of ERα-negative breast cancer [2].

Phytoestrogens are phenolic compounds derived from soybean, tofu, vegetables, fruits, leaves, and grains that structurally mimic the principal mammalian estrogen 17β-

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estradiol, enabling them to bind to the ER [3-5]. There is also evidence that phytoestrogens have hormone-independent activities [5]. Nutrition of phytoestrogens is well studied and appreciated. The phytoestrogens' nutrition is known to reduce menopausal symptoms, risk of coronary heart disease, osteoporosis; help lower blood cholesterol and blood pressure levels; and protect against various cancers. Epidemiologic data have demonstrated that the consumption of phytoestrogens, especially from soy, decreases breast cancer incidence [5-7]. Several in vitro and in vivo animal studies have supported a potential role of phytoestrogens in the prevention of breast cancer development [6,8-10]. The anticancer activities of phytoestrogens may seem to involve tyrosine kinase inhibition, inhibition of angiogenesis, inhibition of tumor metastasis, inhibition of cell invasion, antioxidant activity, and DNA topoisomerase inhibition [5].

We previously reported that phytoestrogens (genistein and apigenin) stimulated the growth of ERα-positive breast cancer cells at lower concentrations (0.01-10 μmol/L), whereas they inhibited the growth of these cells at high concentrations (100 μmol/L) [11]. In contrast, phytoestrogens did not affect the growth of ERα-negative breast cancer cells at low concentrations, whereas they decreased the cell growth rate when used at high concentrations [11]. We hypothesized here that phytoestrogens inhibit the proliferation of ERα-negative normal and malignant breast cells. This hypothesis is supported by previous reports because it was stated that phytoestrogens (genistein, quercetin, or resveratrol) reduced proliferation of ERα-negative cell lines such as MDA-MB-468, MDA-MB-231, HCC-38, and HeLa cells even at low concentrations [4]. In an in vitro study, phytoestrogens seem to exert growth stimulatory activity via ERα-dependent manner but display the growth inhibitory activity via ERα-independent manner. These suggest that phytoestrogens may be either potential chemopreventive or potential chemotherapeutic compounds for ERα-negative breast cancer.

In this study, we investigated the underlying mechanisms by which phytoestrogens (genistein, apigenin, and quercetin) suppressed the growth of normal (MCF-10A) and malignant (MDA-MB-231) ERα-negative breast cells. At high concentrations, we found that all tested phytoestrogens strongly inhibited the growth of both MCF-10A and MDA-MB-231 cells. This growth inhibition was associated with an increase in apoptosis and an accumulation of the cell population in the G2/M phase of the cell cycle. Phytoestrogens also had a tendency to increase the level of proapoptotic proteins (phospho-p53 [p-p53] and p21) and to decrease the level of antiapoptotic proteins (Bcl-xL or cyclin B1). This suggests that phytoestrogens induce apoptotic cell death of ERα-negative breast cancer cells via p53-p21–dependent manner. Because we report here that genistein, apigenin, and quercetin may suppress the growth of ERα-negative breast cancer, the outcome of this study may help to advance human health.

2. Methods and materials

2.1. Compounds and antibodies

Genistein (4′,5,7-trihydroxyisoflavone), apigenin (4′,5,7-trihydroxyflavone), and quercetin (3,3′,4′,5,7-pentahydroxyflavone) were purchased from Sigma Chemical Co (St Louis, Mo). These compounds were dissolved in dimethyl sulfoxide (DMSO), and the final concentration of DMSO in the controls and each sample did not exceed 0.2%. We found that 0.2% DMSO did not affect the cell growth rate as compared with 0% DMSO (no treatment) in both MCF-10A and MDA-MB-231 cells (data not shown).

Antibodies to ββ-tubulin, p21, p27, p53, cyclin D1, and PTEN were purchased from Santa Cruz Biotechnology, Inc (Santa Cruz, Calif). Anti–cell division control protein 2 homolog (cdc2) antibody was obtained from Upstate (Billerica, Mass), whereas phospho-cdc2 (p-cdc2) (Thr161), p-p53 (Ser15), and Bcl-xL antibodies were purchased from Cell Signaling Technology (Danvers, Mass). Cyclin B1 antibody was obtained from Abcam (Cambridge, Mass).

2.2. Culture materials

The MCF-10A normal mammary epithelial cell line was cultured in Dulbecco modified Eagle medium/F12 (1:1) media (Invitrogen, Carlsbad, CA) containing 5% horse serum (Invitrogen), 20 ng/mL epidermal growth factor (Peprotech, Seoul, Korea), 0.5 mg/mL hydrocortisone (Sigma), 100 ng/mL cholera toxin (Sigma), 10 μg/mL insulin (Sigma), 100 U/mL penicillin, and 100 mg/mL streptomycin (Welgene, Daegu, Korea). The MDA-MB-231 cell line was cultured in Dulbecco modified Eagle medium (Invitrogen) containing 10% fetal bovine serum (Thermo Fisher Scientific, Inc, Waltham, Mass), 100 U/mL penicillin, and 100 mg/mL streptomycin. All cell lines were incubated at 37°C with 5% CO_2 in a humidified atmosphere.

2.3. Proliferation assays

MCF-10A and MDA-MB-231 cells were seeded in 12-well culture plates at a density of 5×10^4 cells/well. The next day, cells were treated with DMSO or phytoestrogens for 3 days. Cells were then trypsinized and counted in triplicate with a hemocytometer.

2.4. Cell cycle analyses by flow cytometry

Cells were harvested with 0.25% trypsin and washed once with phosphate-buffered saline (PBS). After centrifugation, cells were fixed in 100% ice-cold methanol overnight at −20°C. Cells were then incubated in 50 μg/mL of propidium iodide in PBS and 1 mg/mL of ribonuclease in PBS for 30 minutes. Cell cycle analyses were performed on a FACScalibur (Becton & Dickinson Biosciences, Mountain View, Calif), and the data were analyzed using Cell Quest software.

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