Genistein stimulates growth of human breast cancer cells in a novel, postmenopausal animal model, with low plasma estradiol concentrations

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We have demonstrated that genistein (GEN) stimulates growth of estrogen-dependent breast tumors in vivo. In this study, we evaluated whether dietary GEN can act in an additive manner with low circulating levels of 17β-estradiol (E2). We developed an E2 delivery system using silastic implants that yield low circulating plasma E2 levels similar to those observed in postmenopausal women. We inserted various concentrations of E2 silastic implants (1:127, 1:63, 1:31, 1:15 and 1:7 = E2:cholesterol) and injected estrogen-dependent human breast cancer (MCF-7) cells into ovariectomized athymic mice. The E2 implants tested (1:127–1:7) generated 30.1–101.6 pM E2 in plasma, which is comparable to the E2 levels observed in postmenopausal women. The E2 implants stimulated MCF-7 tumor growth in a dose-dependent manner. We selected the 1:31 ratio of E2 implant to evaluate if dietary GEN acts in an additive manner with low E2 levels to influence the growth of MCF-7 tumors. Ovariectomized mice were divided into four groups: MCF-7 control, 500 ppm GEN, 1:31 E2, and 1:31 E2 + 500 ppm GEN. At week 17, the average tumor sizes were 7.6, 32.1, 67.4 and 106.8 mm² for these groups, respectively (P < 0.05), demonstrating that 500 ppm GEN additively stimulated MCF-7 tumor growth in the presence of low levels of E2.

In summary, we established a preclinical mouse model that results in E2 blood concentrations similar to those found in postmenopausal women. Further, we observed that these concentrations regulate the growth rate of MCF-7 breast tumors. Using this model, we demonstrated that dietary GEN in the presence of low levels of circulating E2 act in an additive manner to stimulate estrogen-dependent tumor growth in vivo. Results from this study suggest that consumption of products containing GEN may not be safe for postmenopausal women with estrogen-dependent breast cancer.

Abbreviations: AIN93G, American Institute of Nutrition 93 growth semi-purified diet; CD-BCS, charcoal-dextran stripped BCS; E2,17β-estradiol; ER, estrogen receptor; MCF-7: Michigan Cancer Foundation-7; qRT–PCR, quantitative reverse transcription polymerase chain reaction; RIA, radio-immunoassay; SEM, standard error of mean.

Introduction

In the United States, breast cancer is the second leading cause of cancer death (1) in women and has been increasing in men (2). Predictions indicate ~211,240 new cases and 40,410 deaths from breast cancer in American women in 2005 (3,4). Risk factors for breast cancer incidence include age, country, socioeconomic status, reproductive status, exogenous hormones [hormone replacement therapy (HRT) and contraceptives], lifestyle risk factors (alcohol, diet, obesity and physical activity), family history of breast cancer and mammary gland cellular density (5). Most breast cancer cases (~75%) occur in postmenopausal women, and most are estrogen-dependent (6). During the aging process, circulating E2 levels dramatically decrease upon the onset of menopause due to loss of ovarian function. Due to this decrease, women experience various symptoms associated with peri- and postmenopausal changes, including hot flashes, insomnia, migraines, and mood changes (7–9). To relieve these symptoms, many women have taken HRT (10). However, due to the warnings of side effects associated with HRT (such as increased risks of dementia, estrogen-regulated cancer, and diabetes) (11,12), many physicians have stopped prescribing HRT (10,11,13). In 2000, 87.3 million American women used an estrogen-progesterone HRT, but by 2003 the number of users dropped by 32% (10). As an alternative, many women, including patients with estrogen-dependent breast cancer who are suffering from postmenopausal symptoms as a result of both natural causes and side effects from anti-estrogen therapy (14), consume dietary supplements containing phytoestrogens such as GEN, without physician consent, believing that these ‘natural’ products are safe (15,16). In the last decade, phytoestrogen-containing products have increased in the market ~4-fold (17). However, most clinical trials examining dietary supplements have shown inconclusive results regarding their efficacy for relieving postmenopausal symptoms (18). Information about genistein (GEN) activity in the presence of E2 is limited. GEN has been described to be a weak estrogen, selective estrogen receptor modulator (SERM) (19) and/or to exhibit antiestrogenic properties (20). GEN antagonizes endogenous estrogen in sham-operated female mice as shown by inhibiting the uterine weight and by increasing the frequency of B lymphopoietic cells in bone marrow (21). We have demonstrated that dietary GEN stimulates estrogen-dependent tumor growth in mice at blood levels comparable to those observed in humans (22–27). In this report, we test if dietary GEN acts in an additive manner with low circulating E2 on the growth of MCF-7 tumors in order to evaluate if these women are at greater risk for development/exacerbation of breast cancer.

For ethical reasons, dietary supplements cannot be evaluated for stimulation of tumor growth in women. Therefore, there is a critical need to develop preclinical animal models that are
appropriate to evaluate the safety of these products in regard to estrogen-dependent tumor growth. Currently, progress in this research area has been hindered by the lack of an animal model that mimics the ovarian hormones and physiological state observed in postmenopausal women with estrogen-dependent breast cancer. Some animal models utilize ovariectomized female rats or mice. Other animal models, such as carcinogen-induced [either 7,12 dimethyl-benz[a]-anthracene (DMBA) or 1-methyl-1-nitrosourea (MNU)] mammary tumors in rodents, utilize young ovariectomized animals. Although the carcinogen-induced mammary tumor model is a well-established preclinical model (28), 80–90% of these tumors regress after ovariectomy (29), which indicates that it is not an appropriate preclinical model for postmenopausal breast cancer. The MCF-7 tumor implant model utilizes athymic mice that have been implanted with a high dose of E2 (1–2 mg), which results in very rapidly growing tumors that grow to near maximal rates (26,30). The implantation of pellets containing such a high level of E2 generates circulating E2 levels of 2–3 nM in athymic mice, which are similar to levels observed in premenopausal women (31–34). This is an excellent preclinical model for evaluation of current and new breast cancer therapies like the anti-estrogen, tamoxifen, which acts by blocking the agonist action of E2. Removal of the E2 pellet (1–2 mg) causes a decrease in plasma E2 levels to less than 25 pM, and in E2-stimulated MCF-7 tumor size. Using the unsupplemented ovariectomized athymic mice after initial stimulus by E2 we have demonstrated that dietary estrogens can provide the stimulus required for estrogen agonist-dependent tumor growth (26,35). The ovariectomized athymic models using either high E2 pellet (that generate greater than 1 nM of serum E2) or no supplementation (that generate less than 25 pM of serum E2) complicate the evaluation of additive effects of weak estrogen agonists because these tumors either grow too rapidly or regress, respectively. Here we report the development of an animal model in which we can modulate the circulating E2 level as well as tumor growth rate and, for the first time, demonstrate that dietary GEN, in the presence of circulating plasma levels of E2 that are typical for postmenopausal women, acts in an additive manner to more aggressively stimulate estrogen-dependent breast tumor growth.

Materials and methods

Materials

Minimal Essential Medium (MEM, without gentamicin, with glutamine) and phenol red-free MEM were purchased from the Media Facility at the University of Illinois at Urbana-Champaign. Bovine calf serum (BCS) was purchased from Hyclone (Logan, UT). Penicillin/streptomycin and trypsin/EDTA were purchased from Invitrogen (Carlsbad, CA). Laboratory animal diet and dietary components were purchased from Dyets (Bethlehem, PA). Reagents for qRT-PCR were purchased from PE Applied Biosystems (Foster City, CA). Synthegen (Houston, TX), and Invitrogen (Carlsbad, CA). E2 was purchased from Sigma (St Louis, MO). GEN was purchased from Indofine Chemical Company, Inc. (Hillsborough, NJ).

Athymic nude mice

Female Balb/c (nude) mice were purchased from Charles River Laboratories (Wilmington, MA). Mice were ovariectomized at 21-d of age by the vendor. After arrival, animals were acclimated for 1 week.

Diet formulation

American Institute of Nutrition 93 growth diet (AIN93G) semi-purified diet (Dyets, Bethlehem, PA) was selected as a base diet, as it has been established as meeting all of the nutritional requirements of mice (36). All the mice were fed AIN93G diet throughout the study. Soy oil was substituted with corn oil to remove additional soy components for Study 2.

Study 1: Effect of various dosages of E2 silastic implants on the growth of MCF-7 cells implanted into ovariectomized mice

Preparation of E2 silastic implants: Various ratios of E2 and cholesterol were mixed, using a mortar and pestle and packed into silastic tubes (0.1 cm inner diameter × 0.06 cm wall, 1.5 cm length). Both ends of the implant were sealed with silicon adhesive. Implants were sterilized with 70% ethanol and conditioned with phosphate buffered saline before implantation.

Maintenance of MCF-7 cells: MCF-7 cells are estrogen-dependent tumor cells isolated from a postmenopausal woman with metastatic infiltrating ductal carcinomas (37). MCF-7 cells were maintained in MEM supplemented with 5% BCS, 1% penicillin (100 U/ml)/streptomycin (100 μg/ml), and 1 mM E2. MCF-7 cells were maintained at 37°C in a humidified atmosphere of 5% CO2 in a 5% CO2/95% air atmosphere in plastic culture plates (100 mm diameter). One week before the injection of MCF-7 cells into athymic mice, the media was switched to phenol red-free MEM containing 5% charcoal-dextran stripped (CD)-BSC (26) and 1% pen/strep.

Analysis of tumor growth: Mice were divided into six treatment groups (10–11 mice/group): MCF-7, 1:127 E2, 1:63 E2, 1:31 E2, 1:15 E2 and 1:7 E2. E2 silastic tubes were implanted subcutaneously at 6.0 mg (5.0 × 10^6 cells per 40 μl of Matrigel® [Collaborative Biomedical Products, Bedford, MA]) were injected at 40 μl per site into four sites on the backs of the athymic mice (26–38,39). Tumor growth and body weight were measured weekly and cross-sectional area was determined using the formula (length/2 × width/2 × π) (27,40). Food intake was measured throughout the study. At the end of the study, tumors and blood samples were collected for analysis.

Analysis of plasma E2: Blood samples were collected by cardiac puncture and placed into EDTA-containing tubes and centrifuged at 500 g for 5 min. Plasma was stored at −20°C until analyzed using radioimmunoassay (RIA) (41). E2 levels in plasma were measured using an Ultra-sensitive Estradiol RIA kit according to the company’s protocol (Dynamics System Laboratory, Webster, TX). E2 in the plasma samples (50 μl) was extracted using toluene. The primary antibodies and 125I were used in a 1:4 dilution. Controls included plasma containing a low and a high concentration of E2, 0.1% gel-PBS control and a plasma blank plus a known amount of E2 (for recovery). The sensitivity of this RIA is 2.2 pg/ml (~8.1 × 10^-12 M) and the interassay coefficient variation (COV) was 2–4%.

RNA preparation from MCF-7 tumors and analysis of changes in pS2e expression using qRT-PCR: mRNA expression of an estrogen-response gene marker, pS2, was also analyzed using qRT-PCR (26). pS2 (also known as trefoil factor 1) is a peptide consisting of 60 amino acids and is the most abundant estrogen-induced mRNA in MCF-7 cells (42,43). Tumors with areas similar to the mean tumor surface area of each treatment group were used for mRNA analysis. Tumors (~200 mg) were prepared as described in Ju et al. (27,46). RNA was isolated using Trizol Reverse Transcription Reagents (PE Applied Biosystems, Foster City, CA). The pS2 primers and fluorescence (6-FAM)-labeled probes (pS2 forward: 5’-TCC-CCTGGTGCTCTACTATTCAA-3’, pS2 reverse: 5’-CGTCAGGATGCGAGCAGG-CAGT-3’, pS2 probe: 6-FAM-5’-ACCACGCTAGCCCTTCCACGAGA-GG-3’TARMA) were designed using Primer and Probe Design Express (PE Applied Biosystems) (27,38). The human GAPDH primers and a fluorescent (6-FAM/TARMA)-labeled probe (User Bulletin #2, PE Applied Biosystems) were used as a control. PCR and analysis of PCR products were performed using an ABI PRISM 7700 Sequence Detector (PE Applied Biosystems) (27,38). The human GAPDH primers and a fluorescent (6-FAM/TARMA)-labeled probe (User Bulletin #2, PE Applied Biosystems) were used as a control. PCR and analysis of PCR products were performed using an ABI PRISM 7700 Sequence Detector (PE Applied Biosystems). Data were analyzed using a comparative threshold cycle (Ct) method (User Bulletin, PE Applied Biosystems). One sample was run in triplicate in separate tubes to permit quantification of target genes normalized to a control, GAPDH.

Study 2: Effect of GEN in the presence of low levels of E2 levels

Analysis of tumor growth: Ovariectomized mice were divided into four groups: MCF-7, E2 (1:31), 500 ppm GEN and E2 (1:31) + 500 ppm GEN. E2 (1:31 = E2:cholesterol) silastic tubes were implanted into mice in the E2 and E2 + GEN groups, and MCF-7 cells (1 × 10^5 cells per 40 μl) were prepared and injected into all mice as described in Study 1. Then dietary GEN treatment was initiated. Mice in the MCF-7 and E2 control groups were fed AIN93G diet. Mice in the GEN and E2 + GEN groups were fed AIN93G diet containing 500 ppm GEN (500 mg/kg diet). GEN (500 ppm) will generate 2–3 μM total serum GEN in mice, levels that are relevant to human soy product consumption (44,45). Soy oil was substituted with corn oil as a fat source in order to prevent any additional components of soy from being added to the diets. During the study, tumor growth and body weight were monitored weekly and food intake was measured throughout the study. Blood E2 levels were measured using RIA (25,27).
Statistics: Data from plasma E2 analysis, expression of pS2, the tumor growth study, and food intake measurements were analyzed by one-way or repeated-measures analysis of variance according to the characteristics of the dataset using the SAS program (SAS, Cary, NC). If the overall treatment F-ratio was significant \((P < 0.05)\), the differences between treatment group means were tested with Fisher’s Least Significant Difference (LSD) test.

Results

Study 1: Effects of low E2-containing silastic implants on MCF-7 tumor growth

As the tumor cross-sectional area of each group reached approximately 100 mm\(^2\), the animals in that group were killed in order to monitor the tumor growth curve generated by each dosage of E2 implant and tissues and blood were collected. Tumors grew in an E2 dose-dependent manner. At 16 weeks the 1:7 E2 implant group reached an average tumor area of 108.3 mm\(^2\) (40 tumors from 10 mice); at 22 weeks the tumors in the 1:15 E2 implant group reached 102.5 ± 13.7 mm\(^2\) (28 tumors from 7 mice) and at 31 weeks the 1:31 E2 implant group reached 102.5 ± 13.0 mm\(^2\) (36 tumors from 9 mice). The remaining groups, 1:63 E2, 1:127 E2, and the MCF-7 control group, were killed at 44 weeks because this was the maximum time of release of E2 from the implant. At 44 weeks the average tumor sizes were 64.8 ± 13.4 mm\(^2\) for the 1:63 E2 group (40 tumors from 10 mice), 33.5 ± 9.2 mm\(^2\) for the 1:127 E2 group (34 tumors from 9 mice) and 8.6 ± 1.3 mm\(^2\) for the MCF-7 control group (22 tumors from 9 mice) (Figure 1).

Body weights were measured weekly and food intake was measured throughout the study. No significant difference in body weight or food intake was observed among treatment groups (data not shown).

E2 levels in blood: Mice in the MCF-7 control, 1:127 E2 and 1:63 E2 groups were killed at week 44, at which time E2 levels in plasma were 30.4 ± 1.7, 30.6 ± 1.9 and 32.4 ± 1.8 pm, respectively. The 1:31 E2 group was killed at week 31 and E2 levels in plasma were 36.3 ± 1.7 pm. The 1:15 E2 group was killed at week 22 and E2 levels in plasma were 49.9 ± 2.6 pm. The 1:7 E2 group was killed at week 16 and E2 levels in plasma were 101.6 ± 13.8 pm. The 1:31 E2 (\(P = 0.48\)), 1:63 E2 (\(P = 0.81\)), 1:127 E2 (\(P = 0.98\)) and the MCF-7 control groups were not statistically different. The 1:31 E2 and 1:15 E2 groups were not statistically different from one another (\(P = 0.11\)), but the 1:15 group was significantly higher than the MCF-7 control, 1:127 E2, and 1:63 E2 groups (\(P < 0.05\)). The 1:7 E2 group was significantly higher than all other groups (\(P < 0.05\)) (Figure 2).

pS2 mRNA expression in tumors: pS2 expression is presented as relative pS2 mRNA expression level. Fold increases in pS2 expression over the MCF-7 control group were observed to be: 1.3 (± 0.5) for the 1:127 E2 group, 1.8 (± 0.4) for the 1:63 E2 group, 1.8 (± 0.4) for the 1:31 E2 group, 1.7 (± 0.4) for the 1:15 E2 group and 2.7 (± 0.5) for the 1:7 E2 group. Expression of pS2 was not statistically different in the 1:127 E2 (\(P = 0.69\)), 1:63 E2 (\(P = 0.25\)), 1:31 E2 (\(P = 0.31\)) and 1:15 E2 (\(P = 0.35\)) implant groups. The 1:7 E2 implant group significantly increased pS2 expression (\(P < 0.05\)) (Figure 3).

Study 2: Evaluation of the additive effect of dietary GEN with low E2 on in vivo estrogen-dependent tumor growth

MCF-7 tumor growth: In Study 1, all the dosages of E2 were able to generate serum E2 levels similar to those observed in postmenopausal women. We selected one dosage of E2 (1:31) to evaluate the effects of an interaction of dietary GEN and low circulating E2 on estrogen-dependent breast tumor growth. This dosage was selected based on our reasoning that higher...
dosages of E2 implants (1:7 or 1:15) would reach the study termination point (~10% body wt = ~120–130 mm² of average tumor size) too fast and would not give enough time to monitor dietary effects of GEN, whereas a lower concentration of E2 implant (1:63 and 1:127) would take too long to show an estrogen-induced effect. At 17 weeks after dietary treatment was initiated, the 1:31 E2 + 500 ppm GEN group reached an average tumor area of 106.8 ± 10.6 mm² (50 tumors from 16 mice) and the study was terminated. The average tumor surface areas were 7.6 ± 0.4 mm² (24 tumors from 15 mice) for the MCF-7 control group, 32.1 ± 7.0 mm² (68 tumors from 20 mice) for the 500 ppm GEN group and 67.4 ± 7.7 mm² for the 1:31 E2 group (80 tumors from 20 mice) (Figure 4). All the treatments were statistically different from each other (P < 0.05). No significant differences in body weight or food intake were observed among treatment groups (data not shown).

**Discussion**

Dietary supplement usage has increased dramatically in the US over the past decade (46,47). Also of note is the increasing trend for these products to include non-nutritional ingredients, such as herbal and botanical compounds. In fact, some of the most widely used dietary supplements by postmenopausal

![Relative pS2 mRNA Expression](image)

**Fig. 3.** Relative pS2 mRNA expression in tumors. Six tumors (≤200 mg each) from each treatment group were used for qRT-PCR analysis. Bars with different letters are significantly different, P < 0.05.

![Effect of the interaction of GEN with E2 (1:31)](image)

**Fig. 4.** Effect of the interaction of GEN with E2 (1:31) on growth of estrogen-dependent human breast cancer (MCF-7) cells implanted into ovariectomized athymic mice. Groups included: MCF-7 control, 500 ppm GEN, 1:31 E2, and 1:31 E2 + 500 ppm GEN. Data are expressed as weekly average cross-sectional tumor areas (mm² ± SEM) for all tumors in each treatment. Bars with different letters at week 17 are significantly different, P < 0.05.
women are herbal products that are enriched with bioactive components to the point that they become compounds with potent estrogenic activity. The use of these supplements by women has been driven in part because phytoestrogens are natural products, and the assumption is made that these products are a safe alternative to HRT for relieving menopausal symptoms. This assumption may be incorrect.

Another source of phytoestrogens is the increased use of food products containing high soy protein isolates, soy germ flour and isoflavone-containing soy extracts as generally recognized as safe (GRAS)-listed ingredients. With the increasing popularity and availability of phytoestrogen-containing dietary supplements and food ingredients, postmenopausal women with estrogen-dependent cancer should be cautious about consuming these products because of the potential risk. Isoflavone components found within dietary supplements are biochanin A, genistin, GEN, formononetin, daidzin and daidzein (48–53). Isoflavone levels in dietary supplements vary, with some products containing over 100 mg per serving and/or dosage (54). In these products, GEN is typically the isoflavone in the highest concentration. GEN binds to both ERs, though with higher affinity to ERβ (55–57), and behaves either as an estrogen agonist or antagonist to one or both ERs depending on dosage and timing of exposure (58–60). The isoflavone content in dietary supplements or isoflavone-enriched foods generate total GEN levels (i.e. inactive conjugated forms plus the active aglycone) in human plasma up to 5 μM. Less than 5% of total GEN is present as aglycone (26,27). It has been determined that this concentration range produces estrogen agonist effects on the growth of breast cancer cells and tumors (22,26). Yet, an important unanswered question is whether dietary GEN can act in an additive manner in the presence of low circulating levels of E2 as observed in postmenopausal women. We have addressed this critical issue using silastic implants containing various amounts of E2 as a delivery system that produces low circulating E2 levels similar to those in postmenopausal women. These levels of E2 were able to stimulate estrogen-dependent tumor growth, making it an excellent preclinical model of breast cancer growth in postmenopausal women.

The xenograft model has been used as a preclinical model to understand breast tumor growth and to evaluate the potential efficacy of many of the current breast cancer therapies (39,61–63) and will continue to be the predominant model for preclinical studies. Clinically active anti-estrogens and selective ER modulators (SERMs) have been developed for treatment of estrogen-dependent breast cancer using E2 implantation models (64,65). Previously reported E2 pellet implantation models allowed researchers to evaluate potential estrogen antagonistic effects of test drugs such as tamoxifen in a high E2 environment. However, the E2 pellet generates high plasma E2 levels and rapid near maximal estrogen-dependent tumor growth rate and cannot be effectively utilized to evaluate the additive effect of weak dietary phytoestrogens such as GEN.

We have developed a model using various concentrations of E2 in a silastic implant similar to the approach used for birth control implants (66) and numerous other studies for steroid hormone replacement (67,68). It has been shown that such silastic implants release E2 at a slow and constant rate for extended periods of time (27,69–74). We have also demonstrated that E2 (1:47) and tamoxifen implants lasted up to 32 weeks (27). This method of low dosage E2 delivery in vivo results in conditions that are very relevant to the human postmenopausal stage. During menopause the ovaries stop producing female hormones such as estrogen. Circulating E2 levels in postmenopausal women range from 20–150 pM (75,76), which is much lower than the E2 levels observed in premenopausal women (700–3500 pM at pre-ovulation) (31–34). We evaluated the effects of silastic capsules containing different ratios of E2 and cholesterol on estrogen-dependent breast tumor growth and circulating E2 levels. The E2 blood levels in the ovariectomized nude mice with E2 implants were comparable to those of postmenopausal women. We observed that E2 silastic implants at concentrations between 1:127 and 1:7 (E2:cholesterol) were able to generate 30.6–101.6 pM E2 in the blood (Figure 2), but no linear co-relationship between dosages of E2 implant and circulating concentrations of E2 was observed. This is probably due to the differential distribution of E2 in various tissues (77). For example, E2 levels are higher in lipid containing tissues (78,79). Additionally, E2 concentration in breast tumors is higher than that observed in the blood (80,81). A recent study using dogs as the animal model showed that mammary tumor E2 levels were higher than that in the normal mammary tissues (by 3.5-fold), tumor E2 levels were higher than serum E2 levels (by 1.3-fold), and E2 levels in invasive and ulcerative tumors were higher than those in non-invasive tumors (by 1.3–1.4 –fold) (82).

Using this model, we were able to address this critical issue of whether dietary GEN can act in an additive or inhibitory manner in the presence of low plasma E2 levels to stimulate growth of estrogen-dependent breast cancer tumors above that observed in the presence of low levels of E2. We evaluated the effect of GEN in the diet at 500 ppm in the presence of low levels of circulating E2 (1:31 = E2:cholesterol). This dosage was selected because our previous investigations indicated that it produces blood concentrations in the same
range as typical human levels (1–3 μM) of total GEN in plasma (26,27). We have demonstrated that dietary GEN produced additive stimulation of MCF-7 tumor growth beyond that produced by low E2 levels (Figure 4). In our published (27) and unpublished studies, we observed that dietary GEN (500–1000 ppm) stimulated MCF-7 tumor growth, uterine proliferation, and mammary gland differentiation in ovariec-tomized athymic mice, while lowering circulating E2 levels. Previously, we detected 2–3 μM of total plasma GEN from ovariec-tomized (OVX) or OVX + E2 implant mice fed with 500 ppm GEN. We also observed that dietary GEN treatment lowered circulating E2 levels in OVX + E2 implant mice (27). Also, a uterotrophic effect was observed from GEN alone (Figure 5).

In summary, we developed an estrogen-dependent tumor model with slow-growing tumors in ovariec-tomized athymic mice using silastic implants to deliver E2 at levels similar to those observed in postmenopausal women. This feature is a significant advancement over previous models used in the evaluation of breast cancer anti-estrogen therapy (the high E2 implant) or the unsupplemented tumor growth models. Although we specifically evaluated the effects of E2 implants on estrogen-dependent breast cancer, this low E2 implantation model may be applicable to other chronic diseases and health conditions, such as coronary heart disease, osteoporosis and postmenopausal symptoms.

We have consistently observed that dietary GEN in various forms (glycosides, soy protein isolates, and soy extracts) stimulates estrogen-dependent breast cancer in a non-E2 supplemented tumor growth model (23,24,26,27,35,83). Here we have demonstrated using a new preclinical model of postmenopausal breast cancer that dietary GEN acts in an additive manner with low circulating E2 levels to stimulate estrogen-dependent tumor growth.

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References

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