Genistein: Does It Prevent or Promote Breast Cancer?

Kerrie B. Bouker and Leena Hilakivi-Clarke

Department of Oncology, Lombardi Cancer Center, Georgetown University, Washington, DC, USA

Diet is estimated to contribute to approximately 50% of all newly diagnosed breast cancers. As such, a search for dietary factors differentially consumed among populations with increased breast cancer risk (e.g., Caucasians) compared to those with low risk (e.g., Asians) has become a priority. One such dietary component, which is typical to the Asian but not the Caucasian diet, is soy. We review data relevant to attempts to determine whether soy, and more specifically genistein, is a dietary component that may help to explain the dramatic disparity in breast cancer risk among these populations. ***Key words*****: antiproliferative effects, breast cancer, estrogenic effects, genistein.** ***Environ Health Perspect*** **108:701–708 (2000). [Online 23 June 2000]**
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Epidemiologic data indicate a great disparity between breast cancer risks in Western and Eastern countries. Historically, the risk of American women developing breast cancer has been as high as 7 times that of Asian women (*1*). Today the disparity in risk is similarly significant, although the difference in incidence between Western and Eastern countries has narrowed slightly. For example, one in eight white women in the United States can expect to develop breast cancer in her lifetime; this risk is roughly 5-fold less in Japanese and Chinese women residing in Asia (*2*). However, extensive migration studies indicate that Asian women who immigrate to the United States and adopt a Western lifestyle develop risk comparable to Caucasian women within two generations (*3,4*). These studies provide strong evidence in support of other epidemiologic studies showing that 5–10% of breast cancer cases are estimated to be attributable to inherited factors, and thus > 90% of newly diagnosed breast cancers may be caused by unspecified factors probably related to lifestyle. Ziegler et al. (*5*) investigated the link between age of immigration to the United States and increased breast cancer risk in Chinese, Japanese, and Filipino women. The authors found a strong correlation between early age of immigration (< 35 years of age) and a marked increase in breast cancer risk (*5*). In fact, Asian women born in America, compared to their counterparts born in the East, had a 60% higher risk of breast cancer. Additionally, in all three ethnic groups, immigrants living in the United States for more than a decade had a significantly greater risk than more recent immigrants (*5*).

It is clear from both epidemiologic and clinical data that exposure to estrogens has significant influences on breast cancer development. Estrogens induce the proliferation of normal and malignant mammary cells, and are thus linked to breast cancer promotion and progression. Interestingly, a number of reports indicate that Asian women living in Asia have up to roughly 40% lower serum estrogen levels than Caucasian women living in the United States or Britain (*6,7*). Based on these data, it is increasingly clear that the protective effect seen in Asian countries does not correlate with genetic influences, but rather, with environmental and lifestyle factors. Thus, it has long been the goal of innumerable scientists to isolate those factors that may be responsible for the dramatic disparity in breast cancer risk between Caucasian and Asian women.

Diet is estimated to contribute to up to 50% of all newly diagnosed breast cancer cases (*8,9*). One particular class of dietary compounds that has received much attention, based on their high concentration in potentially protective foods and their reported antiproliferative effects, is phytoestrogens. Consumption of phytoestrogens, particularly soy products, as well as legumes, is higher in Asia than in the Western world (*10*). Soy-based diets are high in genistein (4,5,7-trihydroxyisoflavone), which has been widely studied for its potential anticancer properties. The exact mechanism by which genistein may exert its antitumorigenic effects is not clearly understood; however, it is a specific and potent inhibitor of both protein tyrosine kinases and topoisomerase II (*11,12*). Furthermore, genistein is able to inhibit angiogenesis and metastasis in some tumor models and to selectively reverse multidrug resistance protein-associated multidrug resistance in *in vitro* studies (*13–15*). Recently, genistein's ability to inhibit the cytochrome P450 enzyme CYP1A1 has been described (*16*). The inhibition of CYP1A1 may lead to a reduction in the production of DNA-damaging carcinogen metabolites and may be one mechanism by which genistein can protect against carcinogenesis (*16*). Given reports of its antiproliferative abilities, genistein appears to be a potentially powerful weapon in the breast cancer prevention and treatment arsenal. A recent review by Barnes (*17*) discussed the possible protective role of genistein in breast cancer, However, at closer look genistein may not be all it is touted to be.

Estrogenic Effects of Genistein

The phytoestrogen genistein is present naturally as several β-glucosides, which are metabolized by intestinal microflora to genistein (*15*). Genistein, a planar molecule with an aromatic A ring, has a chemical structure similar to steroidal estrogens, and its ability to behave as an estrogen in various tissues has been widely described. Observations of phytoestrogens' estrogenic properties date back to the 1950s, when it was discovered that the diadezan metabolite equol was the compound responsible for reduced reproductive capacity in sheep grazing on clover. Subsequently, countless studies have been conducted to characterize the hormonal effects of phytoestrogens including genistein's estrogenic and presumed antiestrogenic properties.

Genistein has significant estrogenic properties in both *in vitro* and *in vivo* models (Table 1). Genistein binds to the estrogen receptor (ER), although its binding affinity is several-fold weaker than that of estradiol (*30*). Genistein can also activate a number of estrogen-responsive genes *in vitro*, including *pS2* and c-*fos* (*18,31*). Furthermore, when administered at low doses, genistein stimulates the growth of ER-positive (ER+) breast cancer cells (*18–20*). Findings in other tissue systems support the estrogenicity of genistein. For example, genistein is uterotrophic in a variety of species, resulting in impaired reproductive activity and increases in uterine wet weights (*21,25,26*). It is important to note that some studies have failed to see any effect of genistein on the uterus, including alterations in

Address correspondence to L. Hilakivi-Clarke, Lombardi Cancer Center, Research Building, Room W405, Georgetown University, 3970 Reservoir Rd., NW, Washington DC 20007 USA. Telephone: (202) 687-7237. Fax: (202) 687-7505. E-mail: clarkel@gunet.georgetown.edu

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Table 1. Estrogenic effects of genistein.

Observation	Reference
Inhibition of CYP1A1	(*16*)
Stimulation of ER+ human breast cancer cells *in vitro*	(*18–20*)
Stimulation of human ER+ breast cancer cells *in vivo*	(*18*)
Stimulation of rodent mammary gland	(*18,21,22*)
Stimulation of human breast	(*23,24*)
Stimulation of reproductive tissues	(*21,25,26*)
Estrogenic effects on bone, cardiovascular system, and lipid profiles	(*27–29*)

wet weight (*32,33*). Furthermore, findings with coumestrol, a more estrogenic phytoestrogen than genistein, indicate that although coumestrol increases uterine wet weights, it does not increase uterine DNA content or alter other indicators of more true estrogenic activity (*34*). Thus, an increase in uterine wet weight alone does not necessarily indicate that genistein has estrogenic properties.

In addition to directly binding to the ER, genistein may indirectly affect estrogenicity through inhibition of the cytochrome P450 enzyme CYP1A1. It has recently been shown that genistein is a noncompetitive inhibitor of the CYP1A1 enzyme, which apart from playing a role in the metabolism of carcinogens, is responsible for the metabolic degradation of 17β-estradiol. Thus, it is possible that genistein-mediated inhibition of estradiol degradation could result in higher levels of circulating estradiol and thus elevated ER activity (*16*).

Genistein has estrogenic effects on the hypothalamic/pituitary axis in ovariectomized Sprague-Dawley rats (*21*). Human studies indicate that high soy intake can disrupt the hypothalamic/pituitary/gonadal axis in premenopausal women similar to that seen in animal models (*23,35*). This perturbation is not seen in postmenopausal women (*36*), suggesting a differential effect of soy/genistein on pre- and postmenopausal women. Finally, genistein may exert beneficial effects on bone, cardiovasculature, and lipid profiles, all of which are effects characteristic of estrogen (*27–29*). Taken together, these studies indicate that genistein can behave as an estrogen and can mediate mitogenic effects via the ER.

Estrogens have long been identified as important mitogens in the breast and thus are associated with an increase in breast cancer risk. This is evidenced by the link between reproductive factors, including ages of first menarche, first pregnancy, and menopause, and breast cancer risk (*37*). This is further supported by studies showing that elevated concentrations of estrogens in serum and urine are associated with increased postmenopausal breast cancer risk (*38,39*). Additionally, estrogens induce mitogenic effects in both *in vitro* and *in vivo* models of breast cancer (*40*). The role of estrogen in this disease is supported by the fact that removal of ovarian estrogens by bilateral ovariectomy (*41*) or use of tamoxifen (which blocks ER in the mammary gland) (*42*) significantly reduces breast cancer risk. Because estrogen exposure presumably increases breast cancer risk, evidence showing that genistein acts in an estrogenic fashion is puzzling in light of the *in vitro* reports of genistein as an anticancer agent. To address studies that demonstrate the protective effects of genistein in *in vitro* and *in vivo* breast cancer models, it is important to determine whether genistein has antiestrogenic properties as well.

Possible Antiestrogenic Effects of Genistein

Genistein does not always induce proliferation of ER cells. For example, in some studies genistein exhibits an antiproliferative effect in mammary and uterine tissues (*19,43,44*). Thus, these data could be interpreted to indicate that genistein is acting as a classical antiestrogen; i.e., it competitively inhibits estrogen's binding to the ER and transactivation of estrogen-responsive genes. However, a more plausible explanation is that genistein inhibits estrogenicity in some other manner. For example, genistein may act in an antiestrogenic fashion through its inhibition of estrogen-metabolizing enzymes. *In vitro* studies in the human breast cancer cell line T47D show the ability of genistein to significantly inhibit the enzyme 17β-hydroxysteroid oxidoreductase type 1 (HSOR-1) (*45*). HSOR-1 belongs to the family of short-chain alcohol dehydrogenases, which are involved in the metabolism of steroids, antibiotics, and prostaglandins (*46*). HSOR-1 is necessary for estradiol secretion from the ovaries in premenopausal women. Additionally, it may be essential for the reduction of estrone to estradiol that occurs in the adipose and other tissues (*47*). Thus, inhibition of this enzyme could lead to decreased total estradiol. *In vitro* studies have also shown that isoflavones can inhibit the aromatase enzyme, which is responsible for the conversion of androgens to estrone in the peripheral (adipose) tissues, although this has not been shown with genistein specifically (*48*).

The idea of genistein leading to a decrease in estrogenicity through the inhibition of these estrogen-metabolizing enzymes is in contrast to the idea that genistein may increase estrogenicity by inhibiting CYP1A1. On one hand, the inhibition of HSOR-1 could lead to decreased production of estradiol in peripheral tissues and ovarian release of estradiol, thus resulting in a decrease in total estradiol, whereas inhibition of CYP1A1 could result in the opposite, leading to a buildup of total estradiol levels. It is not clear, therefore, what the net effect of genistein would be on overall estrogenicity (Figure 1). Furthermore, in addition to genistein's ability to inhibit the enzymatic activity of HSOR-1 and CYP1A1, genistein may affect these estrogen-metabolizing enzymes in another fashion. It is possible that the disruptive effects of genistein on the hypothalamic/pituitary/gonadal axis could lead to an upregulation of one or more of these enzymes, further complicating the issue of the overall estrogenic effect of genistein. However, depending on which, if any, of these enzymes would be upregulated in response to a perturbation of the hypothalamic/pituitary/gonadal axis, it is possible that the overall effect of genistein could be either an increase or decrease in estrogenicity. Thus, the overall estrogenic/antiestrogenic effects of genistein, as a result of inhibiting the activity of estrogen-metabolizing enzymes, are unclear and warrant further study.

In support of the possibility that genistein may have antiestrogenic effects by reducing circulating estrogen levels, there is some evidence that the consumption of high levels of soy products decreases plasma estradiol concentrations in premenopausal women (*49,50*). These women also had lengthened menstrual cycles (*23*). However, in some studies serum estradiol levels were not changed (*35,36,51,52*) or were increased in women supplemented with soy (*53*). Postmenopausal women did not show any of these effects. Perhaps the contrasting findings can be partially explained by the observation that genistein reduces gonadotrophin hormone-induced release of luteinizing hormone and follicular stimulating hormone (*54*). This in turn would initially lead to a reduction in estrogen production from the ovaries and eventually perhaps to an increase through a negative feedback mechanism acting on the hypothalamic/pituitary/gonadal axis (Figure 1). However, because genistein also interferes with HSOR-1, resulting in reduced estradiol production, and CYP1A1, resulting in increased estradiol levels, the net result may be either reduced or increased circulating estradiol levels, perhaps depending on the presence of additional simultaneous estrogenic stimuli (e.g., dietary fat).

Regardless of an effect on the concentration of circulating estradiol, genistein may be able to reduce the bioavailability of estradiol by increasing the synthesis of sex-hormone-binding globulin (SHBG) through stabilization of SHBG mRNA in a manner analogous to estradiol (*55*). SHBG binds and sequesters hormones, thereby reducing the concentration of bioavailable or free hormone.

However, in the presence of genistein, increased levels of SHBG may not necessarily lead to reduced estrogenicity. It is possible that when estradiol is bound to SHBG, genistein is free to bind to ER, resulting in transactivation of estrogen-responsive genes. Furthermore, given the structural similarities of genistein and endogenous estrogens, it is feasible that genistein could bind to SHBG and displace sequestered estrogens, thus effectively increasing the concentration of free estrogens in the body. However, studies by Baker et al. (*56*) and Martin et al. (*30*) show that genistein's binding affinities for rat alpha-fetoprotein (another member of the steroid-hormone-binding family of proteins) and human SHBG are significantly less than that of estradiol. This evidence suggests that genistein is probably not acting to displace endogenous estrogen from its binding proteins in humans and rodents (*56*). Thus, these data as a whole demonstrate that genistein may be a modulator of bioavailable estrogens. However, given its potential estrogenic activity, the cumulative effects of this reduction in endogenous estrogens, either by inhibiting metabolism or increasing sequestration, may not be significant.

Another possible antiestrogenic effect of genistein may be its ability to alter the kinetics of receptor translocation. Studies in the MCF-7 human breast cancer cell line indicate that genistein-bound ER may translocate to the nucleus more slowly than estrogen-bound ER, thus resulting in an antiestrogen-like effect of diminished signal transduction (*30*). However, this phenomenon has not been widely studied.

It is clear that *in vitro* genistein competes with estradiol for receptor binding (*57*). However, because genistein binds to both the classic ER-α and the novel ER-β receptor subtypes with relative binding affinities of roughly 20- and 5-fold less than that of estradiol, respectively (*58*), it is possible that genistein may not effectively compete with estradiol for ER binding *in vivo*. Furthermore, ER-α and ER-β, when bound to estradiol, appear to exert opposite effects on the AP1 transcription factor; i.e., estradiol leads to transcriptional activation when complexed with ER-α, whereas estradiol represses transcription when coupled with ER-β (*59*). There is some evidence to indicate that activation of ER-β inhibits cellular proliferation. For example, several antiestrogens behave as potent transcriptional activators when bound to ER-β at an AP1 site (*59*), and this may explain why an inhibition of cell growth occurs. Because genistein preferentially binds to ER-β, it may induce antiestrogenic effects through this receptor isoform.

In summary, genistein may have antiestrogenic effects through binding preferentially to ER-β, by inhibiting the activity of enzymes that participate in estrogen metabolism, or by affecting the hypothalamus in an estrogenic manner. The two latter effects can further lead to reduced circulating estrogen levels and increased menstrual cycle length. These possibilities are illustrated in Figure 1 and summarized in Table 2.

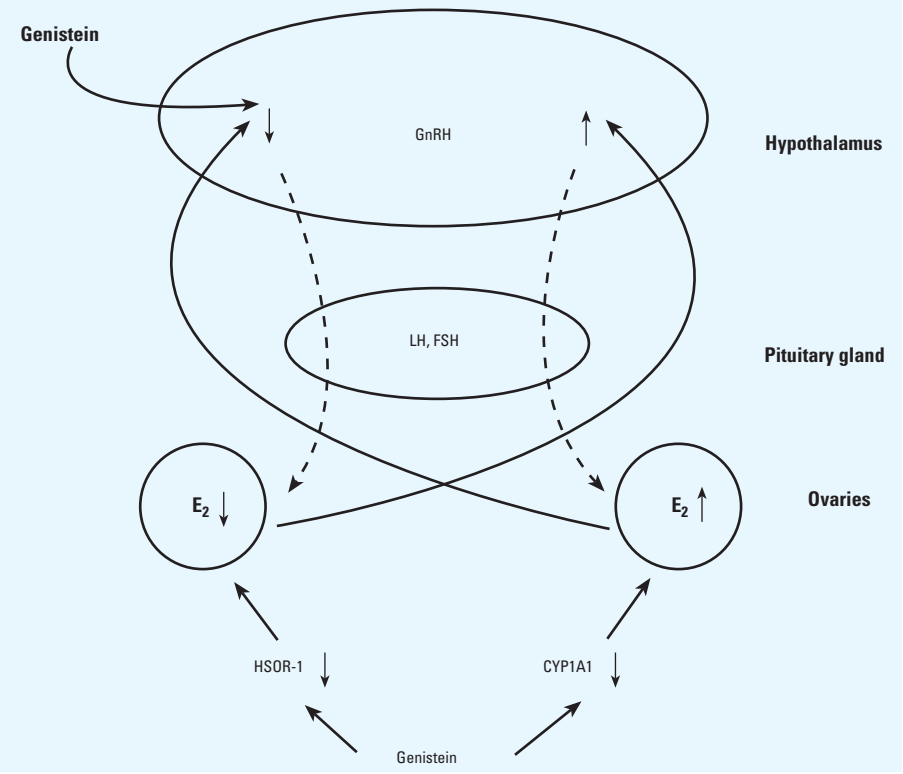

Figure 1. Potential pathways leading to estrogenic/antiestrogenic effects of genistein.

Genistein—Anticancer or Cancer-Promoting Compound?

Clearly, based on the findings of studies addressing the estrogenicity and antiestrogenicity of genistein, it cannot be concluded whether the net result of genistein consumption will be a proliferative or antiproliferative effect on breast cells. Many reports in the literature indicate that genistein is an inhibitor rather than a stimulator of mammary carcinogenesis. These reports consist of studies conducted in human breast cancer cells in which genistein inhibits cell growth (*20*), animal studies in which genistein inhibits initiation or promotion of carcinogen-induced mammary tumorigenesis (*44,60*), and epidemiologic studies in which high soy intake is linked to reduced breast cancer risk (*61,62*). However, the literature also contains many studies that have produced contrasting data.

In vitro *studies.* Studies conducted in human breast cancer cell lines indicate that genistein both inhibits and stimulates proliferation of these cells. For example, Hsieh et al. (*18*) observed a mitogenic effect of genistein at low doses (0.01–1 μM) and an antiproliferative effect at higher doses (> 10 μM). The results of this study are consistent with the data of Wang et al. (*20*), in which the growth of MCF-7 cells was stimulated and then inhibited by genistein in a dose-dependent manner. Thus, at doses of ≤ 1 μM, genistein appears to stimulate the growth of ER+ breast cancer cells (*18,20*). These doses correspond to the human (physiologic) exposure level because most Asians or Caucasians that consume a high soy diet have serum genistein levels of < 1 μM. Conversely, doses > 10 μM genistein inhibit the growth of both ER+ and ER-negative (ER–) breast cancer cells (*20*). This strongly implicates that the mechanisms of inhibition of cell proliferation by pharmacologic doses of genistein occur independently of the ER. It is also apparent that at physiologic exposure levels,

Table 2. Mechanisms through which genistein could reduce estrogenicity.

Observation	Reference
Inhibition of enzymes that metabolize estrogens	(*45,48*)
Reduction in circulating estradiol levels	(*49,50*)
Lengthened menstrual cycle	(*23*)
Stimulation of sex-hormone-binding protein	(*55*)
Preferential bindings to ER-β, leading to inhibition of AP-1 transcription	(*58*) (*59*)

Some of these findings are opposed or unconfirmed by other data.

it is more likely that breast cell growth is stimulated rather than inhibited by genistein.

Animal data. Most animal studies are supportive of the hypothesis that genistein or consumption of soy protein inhibits mammary tumor promotion (*63–66*). These findings were obtained from studies in intact rats and mice with functional ovaries that were exposed to carcinogens to initiate tumor formation. However, some studies show an ability of genistein to increase mammary tumorigenesis. Hsieh et al. (*18*) examined the effects of genistein exposure on ovariectomized athymic mice. Genistein, when administered through the diet at a dose of 750 ppm, enhanced epithelial proliferation in the mammary gland as well as the growth of MCF-7 cell (ER+) tumors *in vivo*. These *in vivo* results are particularly compelling because they were obtained in ovariectomized mice, which may represent a model for breast cancer development in postmenopausal women. Thus genistein, albeit a weak estrogen, could have mitogenic effects on mammary tissue when serum estrogen levels are low, such as in postmenopausal women.

Human data. The estrogenic effects of genistein/soy are not only seen in *in vitro* studies or in animal models. Petrakis et al. (*23*) found that 5 months of daily intake of 38 g soy protein isolate containing 38 mg genistein increased the yield of nipple aspirate fluid (NAF) and the appearance of hyperplastic epithelial cells in premenopausal women. There were no significant changes observed in postmenopausal women (*23*). Previous studies showed a reduced total volume, a lighter color, and less atypical epithelial cells in the NAF obtained from women with low breast cancer risk (i.e., Chinese and Japanese women) compared to women with high breast cancer risk (*67–69*). These studies suggest a correlation between volume, color, and cytology of NAF with breast cancer risk. In another study, 14-day daily intake of 60 g soy supplement containing 45 mg isoflavones significantly increased the proliferative rate of breast lobular epithelium in premenopausal women (*24*). Both of these studies indicate that soy/genistein has an estrogenic effect on the human breast. Furthermore, the results obtained in the soy/NAF/breast proliferation studies would seem to suggest that the genistein in soy is not acting in a protective manner in premenopausal women and may have no effect in postmenopausal women.

Caution must be exercised when interpreting the data of these two studies that apparently indicate an estrogenic effect of soy on the human breast. In particular, the Petrakis et al. (*23*) study contains several potential confounding factors. First, menstrual cycling was not controlled for in this study, and thus NAFs were not obtained at the same phase of the menstrual cycle. However, this may not have affected the results because menstrual cycle has not been found to affect either the hormonal or cytologic content of NAF (*70*). Second, some women in the study were exposed either to oral contraceptives or hormone replacement therapy, and this could have interacted with the effects of soy on NAF. Third, all women previously yielded NAF, and because yielders are at a higher risk to develop breast cancer than nonyielders (*67,68*), study subjects may have been particularly sensitive to the estrogenic effects of genistein in soy. Finally, it is possible that other components of a Western diet may have interacted with soy administration, producing a different outcome than if study subjects had been Asian women consuming an Asian diet.

The epidemiologic evidence supporting the idea that high soy consumption protects against breast cancer is also inconsistent (*61*). Three studies suggest that soy intake is associated with lower breast cancer risk. A study in Singaporean women found that high soy intake is associated with a lower breast cancer risk among premenopausal but not postmenopausal women (*62*). A study in Asian-American women living in the West indicated that breast cancer risk decreases with increasing frequency of tofu (bean curd) intake in both pre- and postmenopausal women (*71*). Finally, a study that measured urinary excretion levels of phytoestrogens reported that a high excretion of isoflavones (genistein was not included) was associated with a substantial reduction in breast cancer risk (*72*). A similar but more recent study did not find significant differences in soy protein intake or urinary excretion levels of daidzein or genistein between breast cancer cases and their controls in Shanghai; however, total isoflavonoid levels in urine were lower in the breast cancer cases (*73*). Four other studies also suggest that soy consumption is not associated with a reduced risk of breast cancer (*74–76*). These significant inconsistencies may reflect differences in the end points used for soy/genistein intake (consumption of tofu or miso, or serum isoflavone concentrations). They may also suggest that high intake of genistein is not the key factor behind low breast cancer incidence in Asian countries. We recently performed a meta-analysis of all the epidemiologic studies currently available. The results indicate that high soy intake might reduce the risk of developing premenopausal breast cancer, but has no effect on postmenopausal breast cancer risk (*77*).

Conclusions from* in vitro *and* in vivo *data. There appears to be a great disparity among the findings of both animal and human studies. Is genistein an anticancer or a cancer-promoting agent? The answer may very well be both. One of the most convincing explanations for the duality of this compound lies in the experimental design—specifically dosage. It is well documented that the classical antiestrogen tamoxifen has agonist properties when administered at low doses and antagonistic properties when administered at higher doses (*78*). Thus studies showing antiproliferative effects using genistein at high doses (> 1 μM) could show similar antiestrogenic actions such as those with tamoxifen. Indeed, many *in vitro* studies conducted using human breast cancer cell lines indicate a biphasic effect of genistein. Interestingly, a study by Anderson et al. (*27*) showed that at low doses, genistein exerted estrogen-like beneficial effects on bone tissue in ovariectomized rats, whereas at high concentrations these estrogenic effects were not seen. Given the potentially biphasic effects of genistein, it is important to determine what the relevance of these concentrations is to women on soy-supplemented diets. Reports in women consuming large amounts of soy products indicate concentrations of up to 0.25 mg/kg genistein in the plasma and urine (*10*). It appears from *in vitro* and *in vivo* animal studies that pharmacologic doses may be required for the breast cancer preventative effects seen with genistein. It is possible that in humans a diet containing foods high in genistein will never reach the levels that are able to effectively inhibit mammary tumorigenesis *in vitro* and in animal models. However, this conclusion is contradicted by our meta-analysis, which showed a protective effect of high soy intake among premenopausal women (*77*), and in animal studies in which rats were given a physiologic dose of genistein prepubertally, resulting in reduced mammary tumor incidence (*79*).

Genistein and Nonestrogenic Pathways of Action

The antiproliferative effects seen with pharmacologic doses of genistein are unlikely to be mediated by the ER. Instead, they are probably due to other biological effects of genistein. For example, genistein is a specific inhibitor of protein tyrosine kinase (PTK) (*11*). Genistein induces a reversible G_2M cell cycle arrest, which may be related to its ability to inhibit PTK (*80,81*). Other important mechanisms by which genistein may exert its antiproliferative effects are through its ability to inhibit both topoisomerase II and angiogenesis (*12,13*). Additionally, it has recently been suggested that the growth inhibitory effects of genistein may be due to modulations in transforming growth factor-β signal transduction (*82*).

PTKs regulate a number of growth factor receptor signal transduction pathways, which can become oncogenic when altered. Therefore, a potent inhibitor of PTK could counteract this oncogenic activity. One widely studied PTK-associated growth-promoting pathway is the epidermal growth factor pathway. Increased expression of the epidermal growth factor receptor (EGFR) in breast cancer cells has been associated with accelerated growth and metastasis and is an indicator of poor prognosis (*83*). EGFR contains a PTK domain and upon activation and dimerization of the receptor, phosphorylation of the PTK domain occurs. This results in signaling events to downstream effector molecules, ultimately leading to an inhibition of apoptosis. It has been postulated that genistein's ability to inhibit the PTK of EGFR (or other potentially oncogenic proteins) may be an important mechanism mediating its observed antiproliferative effects in breast cancer. In support of this, a number of studies have demonstrated genistein's ability to inhibit both PTK and the proliferation of a number of ER+ and ER– breast cancer cell lines (*84,85*). An indication that genistein-induced inhibition of PTK may be independent of ER-mediated functions was shown by Schultze-Mosgau et al. (*85*). The authors demonstrated that pharmacologic doses of genistein inhibit the PTK-dependent transcription of c-*fos* and subsequent cellular proliferation in an ER- human breast cancer cell line. Thus, the antiproliferative effects appear to be due to an inhibition of PTK rather than an inhibition of ER signaling. Additionally, a study by Uckun et al. (*86*) showed that targeting nanomolar concentrations of genistein to the EGFR–PTK complex by means of an EGF–genistein conjugate resulted in a rapid apoptotic effect and inhibition of *in vitro* clonogenicity in human breast cancer cells. Similar results were seen when targeting the Src family of PTK with an anti-CD19 antibody–genistein conjugate (*87*). As with the EGF–genistein conjugate, this was effective at increasing apoptosis and decreasing cell growth at nanomolar concentrations.

Genistein's ability to inhibit tyrosine phosphorylation not only allows for an inhibition of a proliferation of cancer cells, but it may also lead to an inhibition of metastasis. It has been suggested that tyrosine phosphorylation of membrane proteins plays a critical role in the mediation of degradation of the extracellular matrix, thus allowing for cellular invasion (*88*). In a recent study, Connolly et al. (*89*) attempted to reverse the metastasis promoting effects of *n*-6 polyunsaturated fatty acids (PUFAs) in intact nude mice transplanted with ER– human breast cancer cells (MDA-MB-435) by feeding the animals soy protein. They found that soy increased, rather than decreased, the size of both primary tumors and lung micrometastases in the high *n*-6 PUFA group. However, the number of macrometastases was significantly reduced by soy. These results suggest that genistein/soy may promote mammary tumor growth in the nude mouse model both in ER+ (*18*) and ER– tumor cells (*89*) but has both inhibitory and stimulatory effects on various aspects of metastasis (*89*). In addition, Li et al. (*90*) demonstrated that genistein inhibits the secretion of matrix metalloproteinase (proteinases which have been implicated in invasion and metastasis) in MDA-MB-435 breast cancer cells.

It is important to note that pharmacologic doses are required for all of these non-ER-mediated effects of genistein [concentration that inhibits cell growth by 50% (IC_{50}) > 1 μM], with the exception of the genistein conjugates used by Uckun et al. (*86,87*). Interestingly, Peterson et al. (*84*) demonstrated that although genistein can induce growth inhibition of a number of breast cancer cell lines at pharmacologic doses (IC_{50} 2.3–20 μg/mL), these doses do not correlate with PTK inhibition. Doses of 50 μg/mL genistein were required to significantly inhibit EGFR tyrosine phosphorylation. Schultze-Mosgau et al. (*85*) showed similar findings for EGFR in MDA-MB-468 breast cancer cells. Genistein was able to inhibit the growth of these cells at an IC_{50} of < 10 μM, whereas PTK was inhibited at doses of 60 μM (*86*). Additionally, Peterson and Barnes (*84*) showed that doses up to 20 μg/mL genistein were insufficient to inhibit the tyrosine phosphorylation of other PTKs such as phospholipase Cγ and Raf, whereas doses up to 50 μg/mL did not inhibit tyrosine phosphorylation of MAPK or PI3K. This is further supported by Koroma et al. (*80*), who reported that in bovine aortic endothelial cells, genistein doses of > 300 μM were required to inhibit PTK, whereas growth was inhibited at concentrations of < 30 μM. Thus, these data strongly suggest that the antiproliferative effects of genistein are not mediated through inhibition of PTK.

However, although genistein does not appear to be inhibiting EGFR (or other PTKs that have been studied) at physiologic doses, or even at pharmacologic doses associated with growth inhibition, this does not preclude the possibility that genistein mediates its growth-inhibitory effects through the inhibition of other PTK-dependent pathways. It is possible that at lower pharmacologic doses (those associated with genistein's growth inhibitory effects) genistein targets an as yet unidentified kinase or a kinase which has not been examined within this context. Additionally, kinase inhibitors specific for a particular kinase can inhibit other kinases when administered at extremely high doses. This may be due to the similarities in structure of these inhibitors, given that many are designed to target the adenosine triphosphate (ATP) binding site of PTKs. The possibility of genistein mediating its growth-suppressive effects through the inhibition of an unknown kinase at lower pharmacologic doses and nonspecifically inhibiting other kinases at high doses could explain the observed lack of correlation between growth inhibition at lower doses and PTK inhibition of EGFR by higher dose genistein.

The Uckun studies (*86,87*) targeting conjugated genistein into direct association with PTK raise an important possibility. In these studies conjugated genistein was able to inhibit PTK at nanomolar concentrations; in the same experiments, genistein alone was unable to achieve this even at micromolar concentrations (> 10 μM for EGFR inhibition). However, when directed to PTK genistein is effective at physiologic concentrations in the nanomolar range. Proposed explanations for the efficacy of these genistein conjugates at nanomolar concentrations are increased delivery of genistein, direct contact with PTK, and localization in close proximity to ATP binding domains of PTK, thus possibly increasing the binding constant. Nevertheless, despite a lack of understanding of the exact mechanism of genistein's antiproliferative action, it is clear that at pharmacologic doses genistein may be an important inhibitor of breast cancer cell growth and may alter metastatic properties. Additionally, it is possible that even at physiologic concentrations genistein has the potential to exert anticancer properties through inhibition of PTK, but lacks the ability to effectively reach such target molecules.

Timing of Genistein Exposure and Mammary Tumorigenesis

In addition to dose, timing of administration may explain the apparent dual effects of genistein. Throughout the life span, estrogens increase mammary cell proliferation, but depending on the overall hormonal environment, estrogens also activate expression of other factors that could induce differentiation or affect mammary growth by other means. Thus, estrogens can have a different impact on the breast if the exposure occurs *in utero*; during childhood, puberty, or pregnancy; premenopausally; or during postmenopause (*91*). There is evidence that genistein also has different effects on the breast depending on the timing of exposure.

We examined the effects of exposing pregnant rats to genistein (at doses ranging from 20 to 300 μg) on mammary gland development and tumorigenesis among the

offspring (*22,92*). The rationale for this study was based on the fact that mice and rats exposed to estradiol or other estrogenic compounds *in utero* only, i.e., time of exposure was restricted to the *in utero* period, exhibit an increase in mammary tumorigenesis (*93,94*). Furthermore, epidemiologic data suggest that high estrogen levels during pregnancy increase breast cancer risk among daughters (*95–97*). Our results indicate that *in utero* exposure to genistein through a pregnant mother increases the risk to develop 7,12-dimethylbenz[*a*]anthracene (DMBA)-induced mammary tumorigenesis in rats but does not increase the number of tumors per rat (multiplicity) (*92*). Thus *in utero* genistein may act as an estrogen in the mammary gland. Interestingly, a recent study found that the free fraction of estradiol in fetal cord serum is 0.05% of that in adult human serum (*98*). Nagel et al. (*98*) therefore postulated that there may be an undefined mechanism limiting the uptake of endogenous estrogens. They go on to suggest that phytoestrogens may not be limited by this uptake mechanism, which could result in increased exposure to these exogenous estrogens during prenatal life. This is speculative, however, and needs to be further substantiated.

The mechanism mediating the effects of *in utero* estrogen/genistein exposure on mammary tumorigenesis may be related to changes in mammary gland morphology. In the mammary gland, terminal end buds (TEBs) are the least mature ductal structures, containing multipotent stem cells, and are the most susceptible to carcinogens. With maturation, TEBs either differentiate into alveolar buds (which comprise type I and II lobules) or regress to terminal ducts. During pregnancy, lobules I and II further mature into type III lobules. In rats, and perhaps also in humans, more differentiated lobular structures give rise only to benign tumors (*99*). Our data indicate that in animals exposed to estrogens or genistein *in utero*, the number of TEBs in the mammary gland is increased and remains increased, and the number of terminal ducts and differentiated alveolar buds is reduced, when compared to animals exposed to vehicle control *in utero* (*22*).

The findings indicating that *in utero* exposure to genistein increases mammary tumorigenesis in rats are in sharp contrast to findings in Asian women. These women consume high levels of soy products, including during pregnancy, and their newborns have high plasma levels of phytoestrogens at birth similar to their mothers' levels (*100*). However, breast cancer risk is low among Asian women. One explanation may be that Asian women are exposed to high levels of phytoestrogens throughout their lives, whereas in our study rats received genistein only *in utero*. Another explanation could be that soy has many other components in addition to genistein, and these components may antagonize the estrogenic effects of genistein *in utero*. This interpretation is supported by our unpublished study (*101*) in which no changes in DMBA-induced mammary tumorigenesis were noted in offspring of rat dams who consumed varying levels of soy protein during pregnancy.

The period when the breast is particularly vulnerable to the effects of carcinogens is between puberty and a first full-term pregnancy. During this time, there are a high percentage of TEBs and many actively proliferating cells in the breast. We (*79*) and others (*60,102,103*) have studied the effects of prepubertal genistein exposure on mammary tumorigenesis induced by DMBA. Studies by Lamartiniere et al. (*60*), Murrill et al. (*102*), and Brown et al. (*103*) demonstrated that postpartum (days 2, 4, and 6) or prepubertal (days 16, 18, and 20) treatment of rats with 5 mg genistein resulted in increased latency and reduced the incidence and multiplicity of breast tumors. We (*79*) recently replicated these findings by administering 20 μg genistein between postnatal days 7 and 21; this dose is closer to the human exposure range. At day 21, the genistein-exposed animals had a higher percentage of TEBs and increased cellular proliferation compared to control animals, but by day 50 the TEBs had differentiated to lobular structures, which were not susceptible to malignant growth, and the glands exhibited less cellular proliferation (*60,102*). Thus, genistein administered after birth but before the onset of puberty may, in the long term, have a differentiating effect on mammary gland ductal structures and may be chemopreventive (*60*). It is also important to point out that prepubertal genistein administration did not alter puberty onset, although estradiol exposure during the same time period effectively advances puberty. The results of studies in which genistein was administered *in utero* or before puberty indicate that these exposures can have significant but opposing effects on the normal development of TEBs in the mammary gland and influence its susceptibility to carcinogenesis. Fritz et al. (*104*) recently conducted a study in which rats were exposed via diet to genistein from conception (and throughout fetal life) to postpartum day 21. This perinatal exposure significantly reduced the multiplicity (number of tumors per animal) of DMBA-induced mammary tumors but did not affect the proportion of animals per group who developed tumors (tumor incidence). Thus, it appears that some of the adverse effects of *in utero* genistein exposure on mammary tumorigenesis can be partially reversed by prepubertal exposure to the same phytoestrogen.

It is difficult to conceptualize why genistein would have different effects on breast cancer risk based on the timing of exposure. Do these findings suggest that genistein is estrogenic *in utero* and antiestrogenic during adolescence? In addition, is genistein also antiestrogenic during the reproductive years and estrogenic again postmenopausally? It is more likely that genistein is always acting as an estrogen if the level of exposure is maintained at a level low enough as to stimulate only the ER. The differentiating effect on the mammary gland with prepubertal genistein exposure also occurs after prepubertal estradiol exposure. For example, animal studies indicate that neonatal and postpubertal exposure to estrogens reduces subsequent mammary tumorigenesis (*105,106*). Furthermore, in human studies, high fat intake or high body mass index at puberty, both of which increase availability of nongonadal estrogens, are linked to reduced (not increased) breast cancer risk (*107,108*). These data suggest that high prepubertal estrogen levels may effectively protect the breast from malignant transformation, perhaps by inducing early breast differentiation.

During the reproductive years, genistein increases mammary gland proliferation, as is evident in two human studies (*23,24*). Animal studies also show that genistein induces proliferation of the mammary epithelial structures (*18*). However, there is no evidence that genistein increases breast cancer risk in premenopausal women; it may modestly reduce it (*77*). Animal data suggest that genistein may promote breast cancer growth in ovariectomized mice (*18*); i.e., in a postmenopausal breast cancer model. The apparent difference in the effects of genistein on breast cancer risk premenopausally and postmenopausally may be explained by the fact that the breasts of older women are more likely to contain malignant cells as compared to the breasts of younger women. Although the proliferative effects of genistein on postmenopausal women have not been extensively studied, it is possible that genistein induces proliferation of mammary cells in both pre- and postmenopausal women. However, in postmenopausal women, who are more likely to have accumulated malignancies than their younger counterparts, genistein may stimulate the proliferation of these malignant cells leading to the formation of breast cancer. Therefore, genistein may not be antiestrogenic premenopausally, but rather, the mammary gland of younger women may be less fertile ground for the development of cancer.

In conclusion, the differential effects of genistein on breast cancer risk throughout

life from *in utero* to the postmenopausal period appear similar to those of endogenous estrogens, further supporting the role of genistein as an estrogenic compound.

Conclusion

Based on data in the literature, it appears that genistein can act as both an estrogen and an antiproliferative agent. These effects may be both dose and tissue dependent. This is in agreement with data from studies with other estrogenic compounds such as tamoxifen (a partial estrogen receptor agonist) and diethylstilbestrol (a potent ER agonist). Additionally, the timing of exposure may be critical in determining the carcinogenic/anticarcinogenic potential of genistein. Gonadal and placental estrogen production varies dramatically during a woman's life span, as does the production of other factors that regulate the breast. It is plausible that genistein has different effects on the breast in the presence of high estrogen levels (such as during pregnancy), moderate levels (such as during premenopausal life), and low levels (such as during childhood and postmenopause).

It is clear from epidemiologic data that Asian women living in Asia (where a diet high in soy is consumed) have a decreased risk for breast cancer. One possible explanation for the apparent lack of tumor-promoting effects of genistein in Asian populations is that a lifetime exposure to genistein may have a protective effect. It is also possible that soy contains other factors besides genistein that oppose the estrogenic effects of genistein and actually reduce breast cancer risk. Additionally, other environmental or lifestyle factors may be more related to the low breast cancer risk seen in this population than soy/genistein consumption.

Although the evidence of the range of genistein's effects is far from conclusive, it is tempting for some in the scientific community to tout genistein as a potential chemopreventive agent or alternative to hormone replacement therapy. However, studies indicating a potential cancer-promoting effect of genistein should not be taken lightly. Further studies must be done before the true scope of genistein's actions can be understood. Given the recent U.S. Food and Drug Administration approval of an over-the-counter soy supplement and recent media reports touting this as an alternative to hormone replacement therapy, the need for a clearer understanding of the potential cancer-promoting effects of genistein is paramount. Fortunately, genistein continues to be an active area of research interest, and therefore an explanation for the dual nature of genistein may not be too far away.

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