Estrogen Receptor α Mediates the Proliferative but Not the Cytotoxic Dose-Dependent Effects of Two Major Phytoestrogens on Human Breast Cancer Cells

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ABSTRACT

Phytoestrogens are a chemically diverse group of compounds made by plants that can have estrogenic effects in animals. Both tumorigenic and antitumorigenic effects have been reported. Although estrogens stimulate the growth of many breast tumors, there is a negative correlation between the incidence of breast cancer and the phytoestrogen-rich diet of certain Asian populations. To begin to resolve this paradox, we have analyzed the estrogenic properties of genistein and quercetin, two flavonoid phytoestrogens particularly abundant in soybeans. Trans-activation experiments with a transfected reporter gene for nuclear estrogen receptors (ER) show strong activation of the endogenous ERα/H9251 by both phytoestrogens in two MCF7 human breast cancer cell lines. This is supported by the observation that the two phytoestrogens induce the downregulation of ERα mRNA and protein levels. Using chimeric proteins consisting of the hormone binding domains of ERα and ERβ fused to the Gal4 DNA binding domain, we have established that genistein and quercetin are full estrogenic agonists of both ER isoforms. Ligand binding experiments with purified ERα and ERβ confirm that the two phytoestrogens are ER ligands. At concentrations that are sufficient to obtain substantial transcriptional activity, they stimulate the proliferation of two ERα-dependent breast cancer cell lines. At high concentrations, such as those reached with a soy-rich diet, genistein and quercetin are strong cytotoxic agents that even kill ER-independent HeLa cells. Thus, the mode of action of phytoestrogens and the balance between being risk or chemopreventive factors for breast cancer may depend on the dietary load.

Breast cancer is the most frequent malignancy of women in North America, where every year about 200,000 new cases are diagnosed and 50,000 women die from the disease (Lopez-Otin and Diamandis, 1998). The age-adjusted death rates from breast tumors are 2- to 8-fold lower in Asian countries compared with the United States and Western Europe (Parker et al., 1996), which, together with migrant studies, suggests a primary role of dietary factors in reducing cancer risk in Asian women (Adlercreutz, 1995). Epidemiological and case-control studies have reported a negative correlation between breast cancer and the intake of soy products and the urinary excretion of phytoestrogens (Ingram et al., 1997; Kurzer and Xu, 1997). However, interpretations and conclusions have been contradictory (Messina et al., 1997; de Souza, 1998; Heaton and Lewis, 1998; Humfrey 1998; Mangtani and Silva, 1998; Tesarik and Mendoza, 1998).

The phytoestrogens genistein and quercetin (Fig. 1), abundantly present in soybeans, vegetables, and fruit (Price and Fenwick, 1985), have attracted research interest and have been considered natural chemopreventive agents (Larocca et al., 1990; Peterson and Barnes, 1991; Adlercreutz et al., 1995; Kuo, 1996). Many different mechanisms have been proposed to explain the antiproliferative effects exerted by these chemicals, including direct inhibition of tyrosine kinase activity (Akiyama et al., 1987, and references therein), DNA-topoisomerase II (Markovits et al., 1989), angiogenesis (Fotis et al., 1983), and synthesis of heat-shock proteins (Hansen et al., 1997). Flavonoids such as genistein and quercetin may prevent DNA damage as free radical scavengers (Wei et al., 1993); most importantly, however, they may act as partial estrogen agonists or antagonists (Kuiper et al., 1997; Barkhem et al., 1998).

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ABBREVIATIONS: ER, estrogen receptor; OHT, hydroxytamoxifen; OHF, hydroxyflutamide; ZK, ZK98299; HBD, hormone binding domain; PCR, polymerase chain reaction; wt, wild-type; FCS, fetal calf serum; DMEM, Dulbecco’s modified Eagle’s medium; CS, charcoal-stripped; RT, reverse transcription; AF, activation function.
Significant amounts of soy are “hidden” in normal processed food. In a recent study (Hargreaves et al., 1999), none of the subjects reported having knowingly consumed soy products despite displaying significant serum levels of phytoestrogens. Nanomolar amounts of phytoestrogens were measured in soy-supplemented premenopausal women and seemed to induce an estrogenic response in the breast. For postmenopausal women, it has also been reported that flavonoids may have some estrogenic activities, inducing vaginal cell maturation (Wilcox et al., 1990), reduction of hot flushes, and hepatic cholesterol synthesis (Anderson et al., 1995; Murkies et al., 1995).

The effects of 17β-estradiol (E2) and related compounds are mediated by two members of the nuclear receptor superfamily, the estrogen receptors (ER) α and β. Upon ligand binding, they undergo a conformational change allowing chromatin interaction and the regulation of transcription of target genes (Jensen, 1995). Estrogens stimulate the proliferation of many breast tumor cells, which has led to the use of such antiestrogens as hydroxytamoxifen for endocrine therapy. The presence of ERs in breast tumor biopsies has been recognized as a positive prognostic marker that correlates with higher survival rates and lower risk of relapse (Lopez-Otin and Diamandis, 1998).

Estrogenic compounds in the food, for example the natural flavonoids genistein and quercetin, might influence breast cancer progression in a dose-dependent fashion. To provide evidence for this hypothesis we have used the estrogen-dependent human breast cancer cell line MCF7, its hormone-independent variant MCF7SH (Kalkhoven et al., 1996), and the ER-negative HeLa cell line as model systems. We examined the ability of genistein and quercetin (1) to induce the transactivation function of ERα and ERβ, (2) to modulate ERα mRNA and protein levels, (3) to bind ERα and ERβ, and (4) to exert either growth stimulatory or antiproliferative effects at concentrations that are physiologically achievable through dietary uptake.

Materials and Methods

Reagents. E2, genistein, quercetin, and hydroxytamoxifen (OHT) were purchased from Sigma (St. Louis, MO). Hydroxyflutamide (OHF) and ZK98299 (ZK) were a gift from Schering (Berlin, Germany).

Plasmids. Firefly luciferase reporter plasmids used were XETL (Bunone et al., 1996) for the ERα and GK1 (Webb et al., 1998) for the Gal4 fusion proteins. The Renilla reniformis luciferase expression vector pRL-CMV (Promega, Madison, WI) was used as a transfection standard. Gal4 chimeras Gal-ERα, Gal-ERβ, Gal-ERβ, and Gal-ERβ were expressed from plasmids pCMVhERG4A (Gal93.ERΔF), pGal93.ERΔR (Gal93.ERΔR), pGal93.ERΔL, pGal93.ERΔ(L543/4AA), pGal93.ERΔF, and pGal93.ERΔβ, respectively. They were constructed by transferring the coding sequences for the hormone binding domain (HBD) of ERα (amino acids 282–595) from HEG0 (Tora et al., 1989), pCMVhERG521R (Ekena et al., 1996), pCMVhERL525A (Ekena et al., 1996), a PCR-mutagened intermediate with the point mutations M543A–L544A, and a PCR fragment lacking the coding sequences for the P domain and for the ERβ HBD (C-terminal 287 amino acids) from plasmid pCMV5-hERβ (a gift from J.-Å. Gustafsson) into the mammalian expression vector pSCTEVGal93 (Seipel et al., 1992).

Cell Culture. Wild-type human breast cancer MCF7 (MCF7wt) cells were a gift from E. Surmacz (Philadelphia, PA). MCF7wt and HeLa cells were maintained in DMEM without phenol red supplemented with 10% FCS. The variant cell line MCF7SH (Kalkhoven et al., 1996) was maintained in DMEM without phenol red supplemented with 5% charcoal-stripped (CS) FCS. MCF7wt and HeLa, and MCF7SH cells to be processed for immunoblot or RT-PCR assays were switched to DMEM supplemented with 5% CS-FCS and 1% CS-FCS, respectively, 4 days before treatments.

Transfections and Luciferase Assays. Cells were transferred into 24-well plates with 500 μl of regular growth medium/well the day before transfection. The medium was replaced with DMEM lacking phenol red as well as serum on the day of transfection, which was performed using the Fugene6 Reagent as recommended by the manufacturer (Roche Diagnostics, Mannheim, Germany) with a mixture containing 0.5 μg of reporter plasmid, 5 ng of pRL-CMV, and 0.1 μg of effecter plasmid where applicable. After 5 to 6 h the medium was added again with serum-free DMEM lacking phenol red, ligands were added at this point, and cells were incubated for 20 to 24 h. Luciferase activity was then measured with the Dual Luciferase Kit (Promega) according to the manufacturer’s recommendations. Firefly luciferase activity was normalized to the internal transfection control provided by the Renilla luciferase activity.

Ligand Binding Assay for ERs. The ability of genistein and quercetin to compete with [3H]E2 for binding to ERα and ERβ was...
evaluated and compared with that of E2. Two picomoles of purified recombinant human ERα and ERβ proteins (PanVera Corp., Madison, WI), each in 20 mM HEPES, pH 7.4, 1.5 mM EDTA, 0.5 mM dithiothreitol, and 10% (v/v) glycerol, were incubated with 1 nM [2,4,6,7-3H]E2 (72 Ci/mmol; Amersham Pharmacia Biotech, Little Chalfont, Buckinghamshire, UK) in the presence of serial dilutions of unlabeled E2, genistein, or quercetin for 20 to 22 h at 4°C. Bound and free radioligands were separated on Sephadex G-25 PD-10 columns. The amount of receptor-bound [3H]E2 was determined by liquid scintillation counting (Optiphase, HiSafe 3 and 1409; Wallac, Inc., Gaithersburg, MD). Relative counts per min were plotted against the concentration of the ligand, and data were evaluated with the use of a nonlinear, four-parameter logistic model to estimate the IC50 value (the concentration of competitor at half-maximal specific binding).

RT-PCR. The evaluation of gene expression was performed by semiquantitative RT-PCR as we have described previously (Maggiolini et al., 1999b). For ERα, pS2, and the internal control gene 36B4, the primers were: 5'-GTGTACAACCTCCCGAAGG3' (ER forward) and 5'-CAGATTCTATCAGCCGAACCGAGATG3' (ER reverse), 5'-TTCTATCCCTAATACCATCAGACG3' (pS2 forward) and 5'-TTTGAGTAGCTCAAGTGAGG3' (pS2 reverse), and 5'-CTCAACATCTCCCTTCCCT3' (36B4 forward) and 5'-CAAATCCCATATCCTCGCTCC3' (36B4 reverse) to yield products of 1172, 210, and 408 bp, respectively, with 20, 15, and 15 PCR cycles, respectively.

Immunoblotting. MCF7wt and MCF7SH cells were grown in 10-cm dishes and exposed to ligands for 24 h before lysis in 500 µl of 50 mM HEPES, pH 7.5, 150 mM NaCl, 1.5 mM MgCl2, 1 mM EGTA, 10% glycerol, 1% Triton X-100, a mixture of protease inhibitors (Aprotinin, PMSF), and Na-orthovanadate. Equal amounts of total protein were resolved on a 10% SDS-polyacrylamide gel. Proteins were transferred to a nitrocellulose membrane, probed with the antibodies F-10 against ERα and β-actin (Santa Cruz, Biotechnology, Santa Cruz, CA), and revealed using the enhanced chemiluminescence system (ECL, Amersham Pharmacia Biotech).

Proliferation Assays. For quantitative proliferation assays 1 x 104 MCF7wt, MCF7SH, or HeLa cells were seeded in 24-well plates in regular growth medium. Cells were washed extensively once they had attached and further incubated in medium without serum for 24 h. On the second day, the medium of MCF7wt and HeLa cells was changed and supplemented with 5% CS-FCS, and the medium of MCF7SH cells was supplemented with 1% CS-FCS. Ligands were added at this point; thereafter, medium was changed every day (with ligands). On day six, cells were trypsinized and counted in a hemocytometer.

Results

Genistein and Quercetin Activate Endogenous ERα. We began by examining whether a transiently transfected ER reporter gene is able to respond to the flavonoids genistein and quercetin (Fig. 1). The reporter plasmid XETL carries firefly luciferase sequences under the control of an estrogen response element upstream of the thymidine kinase promoter. As an internal transfection control, we cotransfected a plasmid that expresses Renilla luciferase, which is enzymatically distinguishable from firefly luciferase, from the strong cytomegalovirus enhancer/promoter. Figure 2 shows the results obtained with two related human breast cancer cell lines that exclusively express ERα and no ERβ as judged by RT-PCR (data not shown): MCF7wt and its variant MCF7SH. The latter expresses 10-fold elevated levels of ERα, which might contribute to its unique properties. Although MCF7SH cells can proliferate in a seemingly estrogen-independent fashion, they are still dependent on ERα and blocked by antiestrogens (Kalkhoven et al., 1996). More-
over, MCF7SH cells support E2-induced reporter gene expression with exquisite sensitivity and efficacy (“fold induction”) (Fig. 2A; see also Maggiolini et al., 1999a).

In MCF7SH cells, E2 induces luciferase expression from the transfected reporter plasmid XETL about 60-fold with a half-maximal stimulation at a concentration that is consistent with the quoted hormone binding affinity (Kd = 0.2 nM) of ERα from these cells. Both phytoestrogens are strong inducers of XETL expression, albeit at higher ligand concentrations and with somewhat reduced efficacies. Although maximal stimulation by genistein and quercetin is reached only at 1 μM, 10 nM is sufficient to achieve a substantial induction (20- and 14-fold, respectively). The same transfection experiments with MCF7wt cells (Fig. 2B) confirmed the ability of both phytoestrogens to activate ER reporter gene expression, albeit with reduced maximal efficacy.

These transfection experiments suggested that the two phytoestrogens signal through the endogenous ERα rather than another steroid receptor. This was verified with anti-hormones in parallel experiments. The antiestrogen OHT abolishes the activation by E2 and by both phytoestrogens (Fig. 2C) whereas the antiandrogen OHF, and the anti-progesterin and antiglucocorticoid ZK 98’299 have no effect (Fig. 2C). Thus, the two phytoestrogens seem to be able to activate ERα directly.

Transcriptional Activation of ERα and ERβ by Genistein and Quercetin in a Heterologous System. To provide further evidence that genistein and quercetin transactivate ERα directly, and to examine the response of the other ER isoform, ERβ, we turned to a completely heterologous system. Nuclear receptors such as ERα contain two main transcription activation functions (AF): the N-terminal AF1 and the C-terminal, HBD-associated AF2. The former is constitutively active and not further stimulated by phytoestrogens (data not shown), whereas the latter is known to be dependent on a full agonist (Kumar et al., 1987). Chimeric proteins consisting of the heterologous DNA binding domain of the yeast transcription factor Gal4 and the ERα or ERβ HBDs respond to both E2 and phytoestrogens in a transient expression assay in HeLa cells (Fig. 3A). These results demonstrate that the HBD of each ER isoform is sufficient for the response and that genistein and quercetin are AF2 agonists.

We also assessed the response of ERα HBD mutants with the use of Gal4 fusion proteins (Fig. 3B). The two point mutants L525A and G521R that require considerably higher E2 concentrations for activation (Ekena et al., 1996) fail to respond to 1 μM genistein or quercetin. The AF2 mutant M543/L544A does not respond to either E2 or the two phytoestrogens. The mutant ΔF, which lacks the F-domain located C-terminal to the HBD core, displays a robust response to both E2 and the phytoestrogens. These data argue that the transcriptional response to phytoestrogens depends on an intact hormone binding pocket and that it is mediated by AF2 (and presumably also AF1 in the context of the full-length receptor).

Genistein and Quercetin Down-Regulate ERα mRNA and Protein. E2 is known to down-regulate the levels of ERα in breast cancer cells lines through an increased turnover of the E2-activated ERα protein and a reduced transcription rate of its own gene (Santagati et al., 1997). This down-regulation represents an additional hallmark of activation of ERα by an agonist. This prompted us to investigate whether ERα mRNA and protein levels are sensitive to phytoestrogens in MCF7wt cells. ERα mRNA levels were compared by semiquantitative RT-PCR and standardized on the mRNA levels of the house-keeping gene 36B4 (Fig. 4A and B). A treatment for 24 h with 10 μM genistein or quercetin down-regulates the levels of ERα mRNA. As in the trans-activation assays (Fig. 2B), the rank order of efficacy is E2 > genistein > quercetin. Using the same treatments, we also observed a dose-dependent down-regulation of ERα protein content (Fig. 4C and D) that is independent of new protein synthesis (Fig. 4E). At 100 nM, the down-regulation is again most obvious with E2 and genistein, whereas higher concentrations are required for quercetin.

Genistein but Not Quercetin Up-Regulates pS2 mRNA. Having determined that both phytoestrogens down-regulate ERα mRNA and protein, we examined the induction of pS2, a well-known endogenous ER target gene, by RT-
PCR. Of the two phytoestrogens, only genistein is able to induce an up-regulation of pS2 mRNA (Fig. 4, A and B). Thus, structurally and functionally similar phytoestrogens may have overlapping and yet distinct effects on endogenous targets.

Genistein and Quercetin Are Ligands for Both ERα and ERβ. The aforementioned results strongly suggested that the two phytoestrogens are ER ligands. This issue was examined directly by a binding competition experiment with purified recombinant human ERα and ERβ proteins. We found that genistein and quercetin compete with the radiolabeled E2 tracer for binding to the ERs in a concentration-dependent manner (Fig. 5). Half-maximal competition for ERα and ERβ occurs at 756 and 1015 nM and 22 and 113 nM with genistein and quercetin, respectively. Whereas our data demonstrate that both phytoestrogens are direct ligands for both ERα and ERβ, quercetin is clearly a poorer binder of ERα, because competition is barely more than 60% even at the highest concentration (10 μM). We found the apparent affinities of ERβ compared with ERα to be ~34- and ~9-fold higher for genistein and quercetin, respectively. This is largely consistent with previous reports on ER isoform-specific preferences in phytoestrogen binding (Kuiper et al., 1997; Barkhem et al., 1998) (see under Discussion).

Both Genistein and Quercetin Display a Biphasic Effect on Proliferation of Breast Cancer Cells. Having established that genistein and quercetin are ER ligands and activators, we wanted to evaluate a more complex physiological response. We analyzed the effects of the phytoestrogens on the proliferation of estrogen-dependent MCF7wt and estrogen-stimulated MCF7SH cells. Cells were treated for 5 days with the different ligands, counted, and compared with untreated cells. At concentrations up to 1 μM, both phytoestrogens stimulate the proliferation of MCF7wt cells (Fig. 6A); this can be inhibited by the antiestrogen hydroxytamoxifen (Fig. 6B), confirming that the effect is ERα-mediated. At 10 μM and more, genistein and quercetin addition leads to a severe drop in cell numbers indicative of massive cell death (Fig. 6A), an inhibitory effect that cannot be reversed by the addition of E2 (Fig. 6B). The results were qualitatively similar to those of MCF7SH cells, except that only the highest dose of genistein (100 μM) interferes with proliferation (Fig. 6, A and B), whereas high levels of E2 (up to 100 μM) have no cytotoxic effect (data not shown). Further tests with intermediate concentrations indicate that there might be a rather sharp transition from growth stimulatory to cytotoxic doses (data not shown).

Interestingly, ER-negative and estrogen-independent HeLa cells show only the inhibitory effects of high concentrations of genistein and quercetin (Fig. 6A). These data suggest very strongly that the cytotoxic effects of these two phytoestrogens are not mediated by an ER-dependent mechanism.

Discussion

Our results provide mechanistic insights into the mode of action of two very abundant dietary phytoestrogens. At relatively low concentrations, genistein and quercetin are full agonists for ERα and ERβ as well as for the proliferation of ER-dependent breast cancer cells. In the same cells, they are cytotoxic in an ER-independent fashion at concentrations that are reached only in humans with a soy-rich diet. In combination, these observations suggest an explanation for the confusing state of the literature on the potential role of dietary phytoestrogens in breast cancer (Ingram et al., 1997; Kuiper et al., 1997; Barkhem et al., 1998) (see under Discussion).

We have presented several lines of evidence that the flavonoids genistein and quercetin are full agonists and ligands of both ER isoforms: 1) They potently activate endogenous ERα in two different breast cancer cell lines, 2) they autoregulate ERα mRNA and protein levels, 3) they activate the agonist-dependent AF2 trans-activation functions of ERα and ERβ in the context of chimeric proteins with the Gal4 DNA binding domain, and this agonist effect depends on an intact hormone binding pocket and AF2, 4) they compete with E2 for binding to purified ERα and ERβ. In all these assays, they qualitatively behave like the physiological estrogenic ligand E2. Quantitatively similar effects are typically achieved only at concentrations that are 2 to 3 orders of magnitude higher but can be reached physiologically with a phytoestrogen-rich diet (see below).

It has previously been reported that structurally distinct phytoestrogens, including the flavonoids genistein and quercetin, exert their estrogenic effects through direct binding and activation of the ERs (Kuiper et al., 1997; Barkhem et al., 1998). Genistein has been recognized as an ERβ-selective ligand because of its higher apparent affinity for this isoform (Kuiper et al., 1997, see also Fig. 5). Moreover, the structure of a genistein-ERβ HBD cocrystal has been solved, establishing unambiguously that this flavonoid is an ERβ ligand that is bound in the same cavity as E2 (Pike et al., 1999). Our data, although qualitatively consistent with these reports, extend them in several important points. They demonstrate that these two phytoestrogens are full and strong ER ago-

Fig. 5. Genistein and quercetin compete with [3H]E2 for binding to purified recombinant human ERα (A) and ERβ (B) protein. Each data point represents the mean of triplicate samples of three separate experiments.

Fig. 6. Genistein and quercetin have opposite effects on proliferation of human breast cancer cells at different concentrations. A, phytoestrogens stimulate the proliferation of MCF7wt and MCF7SH cells at low and intermediate concentrations and are cytotoxic at high concentrations, even for HeLa cells. Low numbers of cells were seeded in 24-multwell plates, treated with 10 nM E2 or increasing concentrations (logarithmic scale) of phytoestrogens, and counted on day 6 as described in under Materials and Methods. Cell numbers are expressed as percentage of numbers of cells treated with vehicle alone. B, proliferative but not cytotoxic effects are ERα-mediated. Proliferation of MCF7wt and MCF7SH cells was assayed as above except that cells were treated with 100 μM phytoestrogens plus 100 nM E2 (G+E and Q+E) or 100 nM phytoestrogens plus 1 μM OHT (G+OHT and Q+OHT). Each data point is the average of several independent experiments.
nists. In our trans-activation assays that monitor activation of endogenous ERα in breast cancer cells, both genistein and quercetin are strong activators both for their potencies (“dose dependence of response”) and their efficacies (“fold induction”). Differences in experimental approaches (endogenous versus transfected ERs) and cell type may explain why other authors (Kuiper et al., 1997) have missed the strong agonist activity of quercetin. Its only slightly lower binding affinities for ERs compared with genistein (Fig. 5) may have been overlooked in previous surveys (Kuiper et al., 1997), perhaps because it had not been tested in hormone binding assays with soluble receptor. Based on our results with the Gal4 chimeras, it is clear that genistein and quercetin are full ER agonists. Unlike partial agonists such as the anti-estrogen OHT, they are able to activate AF2, which is known to be associated with a characteristic repositioning of helix 12 of the ligand-binding domain. Unexpectedly, the ERβ-genistein crystal structure was found to resemble that of an antagonist-bound HBD (Pike et al., 1999). Our results lend further support to the speculation that a crystallization artifact may have affected this structure.

Most importantly, we show that both genistein and quercetin are full ERα agonists in a more complex biological system. At concentrations that are sufficient to elicit substantial transcriptional activity in transfection experiments (≤1 μM), they both stimulate the proliferation of two breast cancer cell lines that are dependent on ERα. This confirms previous reports on genistein (Hsieh et al., 1998) and extends them to quercetin. The potencies and efficacies of genistein and quercetin are particularly striking with the MCF7 variant cell line MCF7SH. It is hypersensitive for E2, but responds very efficaciously to all ER agonists (see also Maggini et al., 1999a). Genistein or quercetin (10 nM) already induce reporter gene expression 10- to 20-fold and stimulate proliferation 4-fold. Because long-term estrogen-deprived breast tumor cells, for which MCF7SH cells may be a model, can be extremely sensitive to E2 (Kalkhoven et al., 1996; Shim et al., 2000), these findings illustrate that even very low levels of phytoestrogens may be able to stimulate the growth of certain breast tumors.

The proliferative responses of our breast cancer cell lines to the two phytoestrogens are biphasic. At concentrations ≥10 μM, both phytoestrogens become cytotoxic. Cell death becomes apparent by about 72 h (data not shown) whereas exposure to 10 μM for only 24 h is tolerated, resulting, for example, in maximal transcriptional stimulation in a trans-activation assay. Because this treatment also kills ER-negative and independent HeLa cells, this probably involves non-specific inhibitory effects such as the well known inhibition of tyrosine kinases (Akiyama et al., 1987, and references there-in).

On the other hand, the consumption of soy products has been associated with low rates of hormone-dependent and hormone-independent cancers, and genistein has been shown to inhibit the growth of a wide variety of tumor cell types in culture (Peterson, 1995, and references therein).

Our work offers a framework for explaining the negative correlation between a soy-rich diet and low incidence of breast cancer in some Asian countries. Indeed, phytoestrogen concentrations can be above 5 μM in adults with a typical Japanese diet (Morton et al., 1994) or in infants fed exclusively with soy-based formula (Setchell et al., 1997). The maximal concentration that can be reached physiologically has been reported to be 18.5 μM (Barnes, 1995). At these concentrations, one would expect phytoestrogens to be primarily antitumorigenic. They may act directly by being cyto-toxic through inhibition of tyrosine kinases and topoisomerase II (Akiyama et al., 1987; Markovits et al., 1989) and, in the case of quercetin, may also act as phosphoinositide 3-kinase inhibitors (Walker et al., 2000), and/or indirectly through their antiangiogenic and antioxidant effects (Fotsis et al., 1993; Wei et al., 1993; Hansen et al., 1997). In contrast, exposure to phytoestrogen levels below 1 μM would be expected to have estrogenic effects (Hsieh et al., 1998). Upon long-term exposure to phytoestrogen levels remaining below 1 μM, potentially they may promote breast cancer development and stimulate the progression in breast cancer patients with estrogen-dependent tumors. One may have to conclude that one should either eat a lot of phytoestrogen-rich food or as little as possible. Advocating phytoestrogen-rich food or even phytoestrogen pills without medical follow-up for post-menopausal women should be considered critically. Moreover, it also follows that the diet of breast cancer patients should be matched carefully with their particular tumor types.

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References


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