Inhibition of Proliferation and Modulation of Estradiol Metabolism: Novel Mechanisms for Breast Cancer Prevention by the Phytochemical Indole-3-Carbinol

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Abstract. **Aberrant proliferation is an early-occurring intermediate event in carcinogenesis whose inhibition may represent preventive intervention. Indole-3-carbinol (I3C), a glucosinolate metabolite from cruciferous vegetables, inhibits organ site carcinogenesis in rodent models. Clinically relevant biochemical and cellular mechanisms for the anticarcinogenic effects of I3C, however, remain unclear. Experiments were conducted on reduction mammoplasty derived 184-B5 cells initiated with chemical carcinogen (184-B5/BP) or with oncogene (184-B5/HER), and on mammary-carcinoma–derived MDA-MD-231 cells to examine whether (i) I3C inhibits aberrant proliferation in initiated and transformed cells, and (ii) inhibition of aberrant proliferation is associated with altered cell-cycle progression, estradiol (E_2) metabolism, and apoptosis. Aberrant proliferation in 184-B5/BP, 184-B5/HER, and MDA-MB-231 cells was evident by a 55%–67% decrease in the ratio of quiescent (*Q* = G0) to proliferative (*P* = S + M) phase of the cell cycle, a 72%–90% decrease in apoptosis, and a 76%–106% increase in anchorage-dependent growth. These cells also exhibited a 88%–90% decrease in the ratio of C2 to C16α-hydroxylation products of E_2. Treatment of 184-B5/BP, 184-B5/HER, and MDA-MB-231 cells to cytostatic dose of 50 μ*M* I3C resulted in an 137%–210% increase in *Q/P* I3C ratio, a 4- to 18-fold increase in E_2 metabolite ratio, a 2-fold increase in cellular apoptosis, and a 54%–61% inhibition of growth. The preventive efficacy of I3C on human mammary carcinogenesis may be due in part to its ability to regulate cell-cycle progression, increase the formation of antiproliferative E_2 metabolite, and induce cellular apoptosis.** [P.S.E.B.M. 1997, Vol 216]

Experiments on animal models together with epidemiological investigations provide support to the concept that selected macronutrients, micronutrients, and non-nutritive phytochemicals present in vegetables, fruits, and grain products may lower the risk of organ site cancers in humans (1–5). The *in vivo* experiments have demonstrated that dietary intervention is associated with retardation of tumor growth and prolongation of the latent period of appearance of palpable tumors leading to inhibition of tumor incidence (1–4). These *in vivo* studies offer strong evidence for the ability of selected naturally occurring components of the diet to inhibit human tumor development. The clinical relevance of the molecular, biochemical, and cellular mechanisms responsible for tumor inhibitory effects remain equivocal.

In the multistep process of mammary carcinogenesis, early-occurring events of initiation and promotion of preneoplastic transformation represent intermediate steps that are detectable prior to tumorigenesis. Inhibition of preneoplastic transformation, therefore, may provide a marker for effective primary prevention preceding the appearance of overt cancer. Our previous studies utilizing *in vitro* models developed from noncancerous mammary tissue have iden-

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tified several biochemical and cellular surrogate end point biomarkers that are expressed in response to a carcinogenic insult *in vitro* prior to tumorigenesis *in vivo* (6–12). These surrogate end point biomarkers, therefore provide a spectrum of quantitative parameters to examine induction of preneoplastic transformation. Furthermore, exposure of initiated or tumorigenically transformed cells to naturally occurring and/or synthetic tumor inhibitory compounds results in downregulation of the perturbed surrogate end point biomarkers (6, 10–12). These observations provide support for the validity of biomarkers as quantitative end points for prevention of mammary carcinogenesis.

Cruciferous vegetables, a rich source of glucosinolates, have been reported as a protective dietary component against various organ site cancers (5, 13, 14). The hydrolysis products of glucosinolates such as indoles, brassinins, and isothiocyanates function as anticarcinogenic compounds both *in vivo* and *in vitro*, due in part to their antioxidant properties and/or their ability to induce specific Cyp450 Phase I and Phase II enzymes (14, 15). These observations, taken together, suggest that *in vivo* tumor inhibitory effect of glucosinolates may be a manifestation of biochemical interference in the initiational or postinitiational events in the multistep process of carcinogenesis. The postinitiational events are manifested as clonal expansion of the initiated phenotype leading to aberrant hyperproliferation. This cellular alteration has been observed in response to initiation by chemical carcinogens, oncogenes, or transforming retrovirus (7–9, 16, 17). Indole-3-carbinol (I3C), a glucosinolate metabolite, has been reported to inhibit carcinogen-induced and oncogene-induced aberrant hyperproliferation *in vitro* (10–12, 16, 17) and mammary tumorigenesis *in vivo* (18, 19).

In an effort to provide clinical relevance, we examined the effects of I3C on human mammary epithelial cells initiated for carcinogenesis by the chemical carcinogen benzo[*a*]pyrene (BP) or by the oncogene HER-2/neu, both of which are associated with human carcinogenesis (20,21). Present experiments utilizing the human reduction mammoplasty-derived, estrogen receptor–negative 184-B5 cells (22, 23) and human mammary carcinoma–derived, estrogen receptor–negative MDA-MB-231 cells (24) provide a system to examine the effects of I3C on BP- and/or HER-2/neu–initiated, as well as on fully transformed tumor cells phenotypes.

Materials and Methods

Cell Lines. The human reduction mammoplasty–derived cell line 184-B5 (22, 23) was maintained in a chemically defined, serum-free KBM-MEM medium (Clonetics Corp., Walkersville, MD) supplemented with 10 μg/ml insulin, 10 ng/ml epidermal growth factor (EGF), 10 μg/ml transferrin, 0.5 μg/ml hydrocortisone, and 5 μg/ml gentamicin. The HER-2/neu oncogene expressing 184-B5/HER cell line (23) was maintained in the supplemented KBM-MEM containing 200 μg/ml genetecin (G-418) to eliminate the expression of spontaneous revertants. The chemical carcinogen–initiated 184-B5/BP cell line was obtained after a single 24-hr exposure to 39 μ*M* benzo[*a*]pyrene (BP) and selective growth of the cells for 10 subsequent passages. The human carcinoma–derived MDA-MB-231 cell line was adapted to grow in serum-free DME-F12 medium supplemented with 2 m*M* L-glutamine, 100 IU/ml penicillin, 100 μg/ml streptomycin, 50 μg/ml gentamicin, and 10 μg/ml insulin. Routinely, the stock cultures were maintained at 37°C in an humidified atmosphere of 95% air–5% CO_2, fed every 48–96 hr, and subcultured at 1:10 split at 70% confluency.

Chemicals. The stock solution of I3C (Sigma Chemical Co., St. Louis, MO) was made up in dimethylsulfoxide (DMSO) at a concentration of 100 m*M*. This stock solution was appropriately diluted in the culture medium to obtain the final concentration of 50 μ*M*. The solvent controls were treated with 0.1% DMSO, a dose equivalent to that present in the highest final concentration of I3C tested. DMSO at 0.1% concentration did not influence the growth of the cell lines. The antibiotics, hormones, and growth factors used in the culture medium were obtained from Gibco-BRL (Grand Island, NY).

Cell-Cycle Analysis. Cultures of 184-B5/BP, 184-B5/HER, and MDA-MB-231 cells, after an initial attachment period of 24 hr, were treated with 0.1% DMSO or 50 μ*M* I3C for the following 24 hr. Cells were trypsinized and a propidium iodide (PI)–stained cell suspension ($\approx 1 \times 10^4$ cells) was analyzed by flow cytometry for relative distribution of cells in G0/G1, S and G2/M phases of the cell cycle (11, 25). The data was expressed as the ratio of cells in quiescent phase (Q = G0) over those in proliferative phase (P = S + M) of the cell cycle.

Estradiol Metabolism. Cultures of 184-B5/BP, 184-B5/HER, and MDA-MB-231 cells were treated with 0.1% DMSO or 50 μ*M* I3C were incubated for 48 hr with [C2-^{3}H] E_2 and [C16α-^{3}H] E_2. The culture medium was lyophilized, subliminated, and relative extent of ^3H_2O formation was determined by liquid scintillation counting (6, 7, 10, 11). The amounts of 2-hydroxyestrone (2-OHE_1) and 16α-hydroxyestrone (16α-OHE_1) formed *via* the C2 and C16α-hydroxylation pathways, respectively, were calculated from the specific activity of labeled E_2 based on its stoichiometric convertibility. The data expressed as the ratio of 2-OHE_1/16α-OHE_1 formation per 10^4 cells/48 hr.

Cellular Apoptosis. The relative extent of apoptosis in 184-B5/BP, 184-B5/HER, and MDA-MD-231 cells treated for 24 hr with 0.1% DMSO or 50 μ*M* I3C was determined from the extent of Sub G0 peak detected in flow cytometric profile of propidium iodide-stained cell suspensions. In addition, the presence of apoptotic cells was confirmed by epifluorescence in cells double labeled using PI and fluorescein isothiocyanate (FITC) labeled apoptag (Oncor, Gaithersburg, MD).

Anchorage-Dependent Growth. The long-term growth inhibitory effect of I3C was determined using the

Table I. Characteristics of Human Mammary Epithelial Cells

Cell line	Surrogate end point biomarker[a]			
	Q/P ratio[b]	E_2 metabolism[c] (2-/16α-OHE_1)	Apoptosis[d] (%)	AD-CFE[e] (%)
184-B5	1.8 ± 0.3	4.2 ± 0.3	18.9 ± 2.6	10.4 ± 0.6
184-B5/BP	0.8 ± 0.1	0.5 ± 0.2	1.8 ± 0.5	18.3 ± 1.0
184-B5/HER	0.8 ± 0.1	0.5 ± 0.1	5.2 ± 0.6	20.1 ± 0.7
MDA-MB-231	0.6 ± 0.1	0.4 ± 0.1	3.8 ± 0.5	21.4 ± 1.6

[a] Values are mean ± SD, n = 4/cell line.
[b] *Q/P*, ratio of cells in quiescent (G0) and proliferative (S + M) phases of cell cycle.
[c] Ratio of 2-OHE_1/16α-OHE_1 formation.
[d] Sub-G_0 (apoptotic) phase.
[e] AD-CFE, anchorage-dependent colony-forming efficiency.

anchorage-dependent colony forming assay. 184-B5/BP, 184-B5/HER, and MDA-MB-231 cells were plated at a seeding density of 200 cells/well in six-well cluster plates. After a 24-hr attachment period, the cultures were continually exposed to 0.1% DMSO or 50 μ*M* I3C for 21 days. The cultures were fixed in 10% buffered formalin and stained with 0.5% Giemsa. Number of adherent colonies formed of more than 100 cells were counted. The data was expressed as the percentage anchorage-dependent colony-forming efficiency (AD-CFE).

Statistical Analysis. The statistical significance of differences between the treatment groups was estimated by the two-tailed *t* test using the Statview 4.01 statistical software.

Results

Aberrant Proliferation and Altered E_2 Metabolism in Human Mammary Epithelial Cells. The growth kinetics of 184-B5/BP, 184-B5/HER, and MDA-MB-231 cell lines were evaluated by determining the relative extent of *Q/P* ratio, apoptosis, and AD-CFE. Alteration in the metabolism of estradiol was evaluated by determining the 2-OHE_1/16α-OHE_1 ratio (Table 1). Relative to the 184-B5/BP cell line, cells initiated with BP or with HER-2/neu exhibited a 55.6% decrease in *Q/P* ratio, a 90.5% and 72.5% inhibition in apoptosis, and 75.9% and 93.3% inhibition in anchorage-dependent growth respectively. The tumor-derived MDA-MB-231 used as the positive control also showed a comparable change in the same growth parameters. At the biochemical level, cellular metabolism of E_2 was altered as seen by a 88%–90% decrease in 2-OHE_1/16α-OHE_1 ratio in the initiated as well as transformed cells relative to that observed in nontumorigenic 184-B5 cells. The observed changes in the quantitative parameters of growth suggest that aberrant proliferation is induced in response to treatment with BP or overexpression of HER-2/neu oncogene. A similar perturbation in transformed MDA-MB-231 cells provides evidence that aberrant proliferation may represent a relevant quantitative marker for tumorigenic transformation.

Effect of I3C on Aberrant Proliferation. The experiment presented in Table II was designed to examine whether I3C treatment affects aberrant proliferation by regulating the status of cell cycle progression. A 24-hr exposure to 50 μ*M* I3C resulted in increased G0 phase and a concomitant decrease in S + M phases of the cell cycle. G0 represents the quiescent phase, while S + M represent the proliferative phase in the cell cycle. Substantial increases in *Q/P* ratio in I3C-treated cultures relative to that in DMSO-treated solvent controls indicated that I3C downregulates the extent of aberrant proliferation in 184-B5/BP, 184-B5/HER, and MDA-MB-231 cells.

Table II. Effect of Indole-3-Carbinol on Cell-Cycle Regulation in Human Mammary Epithelial Cells

Cell line	Treatment	% distribution of cells[a,b]		
		Quiescent phase (G0)	Proliferative phase (S + M)	*Q/P* ratio[c]
184-B5/BP	DMSO	43.8 ± 2.1	56.2 ± 2.1	0.78 ± 0.70
	I3C	70.2 ± 2.9	29.8 ± 2.9	2.38 ± 0.33
184-B5/HER	DMSO	45.6 ± 2.3	54.4 ± 3.3	0.84 ± 0.11
	I3C	65.8 ± 3.3	34.2 ± 6.3	1.99 ± 0.56
MDA-MB-231	DMSO	36.1 ± 1.6	63.9 ± 1.6	0.59 ± 0.05
	I3C	64.6 ± 2.1	35.4 ± 2.1	1.83 ± 0.20

[a] Determined from flow cytometry of propidium iodide-stained cell suspension.
[b] Values are mean ± SD, n = 4/treatment group.
[c] 184-B5/BP, P = 0.001; 184-B5/HER, P = 0.004; MBA-MD-231, P = 0.001.

Effect of I3C on Estradiol Metabolism. The ability of I3C to alter the relative extent of estradiol metabolism *via* the C2- and C16α-hydroxylation pathways was determined by radiometrically measuring the formation of 2-OHE_1 and 16α-OHE_1 respectively Table III). The two metabolites represent the intermediate products of the two principal hydroxylation pathways. In the three cell lines, a 48-hr exposure to 50 μ*M* I3C resulted in a substantial increase in the amount of 2-OHE_1 and a concomitant decrease in the amount of 16α-OHE_1 formed. Thus ratio of 2-OHE_1/16α-OHE_1 was found consistently to be increased in the presence of I3C.

Effect of I3C on Cellular Apoptosis. The experiment designed to examine whether the downregulation of aberrant proliferation induced by I3C is associated with apoptosis, utilized flow cytometric measurement of sub-G0 peak (Table IV). A single 24-hr exposure to cytostatic dose of 50 μ*M* I3C induced apoptosis in 184-B5/BP, 184-B5/HER, and MDA-MD-231 cells. The presence of apoptosis was confirmed by increased immunoreactivity to FITC-labeled apoptag, a specific antibody to apoptotic DNA (data not shown).

Effect of I3C on Anchorage-Dependent Growth. The long-term effect of I3C on growth inhibition was evaluated after a continuous 21-day exposure to 184-B5/BP, 184-B5/HER and MDA-MB-231 cells (Table V). This experiment revealed a 54% to 61% inhibition in anchorage-dependent colony-forming efficiency by I3C in the three cell lines tested.

Discussion

The experiments in this study on human mammary epithelial cells initiated for carcinogenic transformation by chemical carcinogen or oncogene were designed to examine whether the naturally occurring dietary component of I3C inhibits aberrant proliferation and to identify possible mechanism(s) responsible for such growth inhibition.

Aberrant proliferation represents one of the early-occurring events in the multistep process of carcinogenesis. This cellular alteration is detectable *in vitro* in response to mutagenic DNA damage or overexpression of oncogenes prior to tumorigenicity *in vivo* (6–10, 26, 27). Aberrant proliferation leads to disrupted growth control, and impaired cellular homeostasis in part due to an imbalance between positive and negative regulators of growth such as oncogenes and tumor suppressor genes, alteration in hormone responsiveness, and disrupted signaling associated with cellular apoptosis (28–31). Furthermore, loss of apoptosis and deregulated expression of antiapoptotic *Bcl-2* gene has been correlated with lymph node metastasis of breast cancer (32). Consistent with these observations, 184-B5/BP and 184-B5/HER cells, initiated for carcinogenesis by BP and HER-2/neu, respectively, exhibited decreased *Q/P* ratio, decreased apoptosis and increased anchorage-dependent growth relative to that observed in non-carcinogenic 184-B5 cells. Thus, upregulation of proliferation and downregulation of apoptosis observed in BP- and HER-2/neu–initiated cells, in a manner similar to that seen

Table III. Modulation of Estradiol Metabolism by Indole-3-Carbinol in Human Mammary Epithelial Cells

Cell line	Treatment	Estradiol metabolism[a,b] (pmole/10^4 cells/48 hr)		
		2-OHE_1	16α-OHE_1	2/16α ratio[c]
184-B5/BP	DMSO	1.3 ± 0.2	2.2 ± 0.2	0.6 ± 0.2
	I3C	12.7 ± 0.2	1.4 ± 0.2	9.2 ± 1.4
184-B5/HER	DMSO	1.4 ± 0.2	2.7 ± 0.1	0.5 ± 0.2
	I3C	11.5 ± 0.7	1.2 ± 0.3	9.7 ± 2.1
MDA-MB-231	DMSO	1.5 ± 0.2	3.8 ± 0.7	0.4 ± 0.1
	I3C	3.6 ± 0.6	1.9 ± 0.2	2.1 ± 0.3

[a] Determined by the radiometric assay measuring ^3H_2O formation after a 48-hr incubation with [C2-^{3}H] E_2 or [C16α-^{3}H] E_2 in the presence of 0.1% DMSO or 50 μ*M* I3C.
[b] Metabolite concentrations calculated from stoichiometric convertibility of specifically labeled E_2. Values are mean ± SD, *n* = 18/treatment group.
[c] 184-B5/BP, *P* = 0.001; 184-B5/HER, *P* = 0.004; MBA-MD-231, *P* = 0.001.

Table IV. Induction of Apoptosis in Human Mammary Epithelial Cells by Indole-3-Carbinol

Cell line	Treatment	% apoptosis[a,b]	*P*
184-B5/BP	DMSO	1.8 ± 0.5	—
	I3C	5.6 ± 0.6	0.04
184-B5/HER	DMSO	5.3 ± 0.6	—
	I3C	16.1 ± 2.5	0.01
MDA-MB-231	DMSO	2.9 ± 0.4	—
	I3C	6.9 ± 0.5	0.01

[a] Determined from the extent of sub-G0 (apoptotic) phase using flow cytometry of propidium iodide-stained cell suspension.
[b] Values are mean ± SD, *n* = 4/treatment group.

Table V. Inhibition of Anchorage-Dependent Growth of Human Mammary Epithelial Cells by Indole-3-Carbinol

Cell line	Treatment[a]	Anchorage-dependent colony-forming efficiency (AD-CFE)[b,c]
184-B5/BP	DMSO	19.3 ± 1.6
	I3C	8.9 ± 2.1
184-B5/HER	DMSO	20.5 ± 1.4
	I3C	9.2 ± 3.3
MDA-MB-231	DMSO	23.1 ± 2.2
	I3C	9.0 ± 1.2

[a] Cells exposed to 0.1% DMSO or 50 μ*M* I3C for 21 days, and number of anchorage-dependent (adherent) colonies determined.
[b] AD-CFE:

$$\frac{\text{Number of Colonies}}{\text{Initial Seeding Density}} \times 100.$$

[c] Values are mean ± SD, *n* = 18/treatment group. DMSO versus I3C, *P* = 0.01.

in the fully transformed MDA-MD-231 tumor cell phenotype, provides evidence for the relevance of these surrogate end point biomarkers to the process of mammary carcinogenesis.

Cellular metabolism of estradiol has been reported to alter in response to carcinogenic insult and is related to the risk of developing breast cancer (6, 7, 17, 26, 27, 33, 34). The Cyp450-dependent, competitive metabolic pathways lead to the formation of antiproliferative 2-OHE_1 and proliferative 16α-OHE_1, each of which possess specific modulatory effects on the initiational and the post initiational events in mammary carcinogenesis (30, 35). The ratio of 2-OHE_1/16α-OHE_1 therefore provides comparative evidence for alteration in overall metabolism of estradiol relevant to carcinogenesis. In this context, it is noteworthy that, in 184-B5/BP as well as in 184-B5/HER cells, the ratio of 2-OHE_1/16α-OHE_1 was substantially decreased relative to that in 184-B5 cells, and that the observed decrease in initiated cells was comparable to that seen in fully transformed, tumor-derived MDA-MB-231 cells. Thus, the decrease in the E_2 metabolite ratio appears to correspond with the progression of tumorigenic transformation.

The anticarcinogenic effect of dietary glucosinolates such as indoles and isothiocyanates is believed to be due in part to their antioxidant effects and induction of xenobiotic metabolizing enzyme systems (13, 14, 36–40). These properties may provide protection against metabolic activation of chemical carcinogens and thereby inhibit events related to DNA damage and mutagenic perturbation. Several *in vitro* and *in vivo* investigations have demonstrated that the naturally occurring glucosinolate metabolite I3C functions as a potent inducer of C2-hydroxylation pathway of estradiol metabolism in rodent models (6, 10, 11, 17–19, 26, 27). Additionally, administration of I3C or dietary supplementation with cruciferous vegetables has been reported to induce 2-hydroxylation of estradiol in humans (14, 41, 42). The inhibition of mammary tumorigenesis by I3C may thus be due in part to increased formation of the antiproliferative 2-OHE_1 (10, 11, 17, 18).

Treatment of aberrantly proliferative cultures of 184-B5/BP, 184-B5/HER, and MDA-MD-231 with I3C exhibited substantial accumulation of cells in G0 phase and decreased number of cells in S + M phases of the cell cycle. This alteration resulted in increased *Q/P* ratio, suggesting that antiproliferative effect of I3C may be cell-cycle dependent. Similar to the previous studies on murine systems (10, 11, 17, 18, 26), I3C in this human mammary-cell culture system, induced a substantial increase in 2-OHE_1 formation and a decrease in 16α-OHE_1 formation. The estradiol metabolite 16α-OHE_1 has been demonstrated to initiate aberrant proliferation in noncancerous cells and promote proliferation in carcinogen-initiated cells (10, 17, 26, 27, 33). The ability of I3C to alter the 2-OHE_1/16α-OHE_1 ratio in favor of 2-OHE_1 formation, therefore, provides evidence for a possible mechanism for the inhibitory effect of this phytochemical on human mammary carcinogenesis. It is now of considerable interest to examine whether treatment of 184-B5/BP, 184-B5/HER, and MDA-MB-231 cells with 2-OHE_1 and 16α-OHE_1 modulates the surrogate end point biomarkers for carcinogenesis in a manner similar to that reported in other cell systems (17, 26, 27, 33).

Cellular apoptosis, in concert with proliferation and cytodifferentiation, plays an important role in homeostasis. During carcinogenesis, aberrant proliferation disrupts growth control leading to downregulation of apoptosis (28, 29). Aberrant hyperproliferation in 184-B5/BP and 184-B5/HER cells was accompanied by decreased apoptosis. The ability of I3C to increase the extent of apoptosis suggests an additional mechanism responsible for growth regulatory effect of this compound.

Positive regulation of *p53* and negative regulation of *Bcl-2* play important roles in mediating apoptosis (43–47). Thus, inverse correlation of the two gene products, together with upregulated cell-cycle progression, may lead to aberrant hyperproliferation and carcinogenesis. It is noteworthy that an inverse correlation has been observed between *p53* and *Bcl-2* expression in advanced breast cancers (32), raising the possibility that the two genes may participate in a common pathway for the control of cell survival. In an independent study, exposure of 184-B5/HER cells to a naturally occurring tea polyphenol has been shown to inhibit immunoreactivity to proliferation specific regulatory proteins, downregulate cell-cycle progression, induce cellular apoptosis, and inhibit immunoreactivity to *Bcl-2* (48). The data generated from the present experiments showing regulation of cell-cycle progression and induction of apoptosis by I3C in 184-B5/BP, 184-B5/HER, and MDA-MB-231 cells therefore emphasizes the need for future experiments to focus on understanding the role of *p53* and/or *Bcl-2* in the mechanisms for induction of apoptosis by phytochemicals. The status of immunoreactivity to cell-cycle regulatory, and apoptosis specific gene products, together with analysis of gene expression at RNA and protein levels, should elucidate the responsible mechanisms.

In conclusion, the data generated from the present study demonstrates the validity of a clinically relevant model for evaluating preventive and/or therapeutic efficacy of naturally occurring dietary phytochemicals that are documented tumor inhibitors in animal models (3, 4, 18, 19, 49–51). This preclinical approach utilizing a human tissue-derived cell culture model should identify promising compounds for clinical trials in high risk (asymptomatic) individuals as well as breast cancer patients.

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