Research Article

SERMs Attenuate Estrogen-Induced Malignant Transformation of Human Mammary Epithelial Cells by Upregulating Detoxification of Oxidative Metabolites


Abstract

The risk of developing hormone-dependent cancers with long-term exposure to estrogens is attributed both to proliferative, hormonal actions at the estrogen receptor (ER) and to chemical carcinogenesis elicited by genotoxic, oxidative estrogen metabolites. Nontumorigenic MCF-10A human breast epithelial cells are classified as ER−/− and undergo estrogen-induced malignant transformation. Selective estrogen receptor modulators (SERM), in use for breast cancer chemoprevention and for postmenopausal osteoporosis, were observed to inhibit malignant transformation, as measured by anchorage-independent colony growth. This chemopreventive activity was observed to correlate with reduced levels of oxidative estrogen metabolites, cellular reactive oxygen species (ROS), and DNA oxidation. The ability of raloxifene, desmethylarzoxifene (DMA), and bazedoxifene to inhibit this chemical carcinogenesis pathway was not shared by 4-hydroxytamoxifen. Regulation of phase II rather than phase I metabolic enzymes was implicated mechanistically: raloxifene and DMA were observed to upregulate sulfotransferase (SULT 1E1) and glucuronidase (UGT 1A1). The results support upregulation of phase II metabolism in detoxification of catechol estrogen metabolites leading to attenuated ROS formation as a mechanism for inhibition of malignant transformation by a subset of clinically important SERMs.

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Introduction

Breast cancer is the leading cause of cancer death among women in Western countries. The association of hormone-dependent cancer with exposure to endogenous estrogens has been known for decades. Of the 2 major mechanisms of estrogen carcinogenesis, the hormonal pathway, mediated via the estrogen receptor (ER), has been extensively studied (1–4). Formation of highly reactive estrogen quinone metabolites, which can cause DNA damage, is believed to be a major contributor to chemical carcinogenesis (5–7).

In breast epithelial cells, the endogenous estrogens are metabolized to their 2-OH and 4-OH catechol metabolites, catalyzed by CYP450 1A1 and CYP450 1B1, respectively (Fig. 1). Further oxidation of estrogen catechols to quinones causes genotoxicity through electrophilic and oxidative DNA damage, including formation of 8-oxo-7,8-dehydro-2′-deoxyguanosine (8-oxo-dG; refs. 8–10). Formation of reactive oxygen species (ROS) from quinone redox cycling can amplify DNA damage (11, 12). Several lines of evidence strongly suggest that the estrogen catechols are the proximal carcinogens in chemical carcinogenesis (13–17). Prevention of estrogen-induced chemical carcinogenesis therefore can theoretically be achieved by ‘detoxification’ of estrogen catechols, via: (i) attenuated formation, (ii) enhanced conjugative metabolism and clearance, or (iii) trapping of quinones and ROS (ref. 18; Fig. 1).

Model systems for study of chemical carcinogenesis, a process envisioned to develop over many years of exposure to genotoxic insult, represent a challenge. MCF-10 cells are nontumorigenic human breast epithelial cells that undergo estrogen-induced malignant transformation. Owing to low ER levels and lack of proliferative response to estrogens, the cell line is of use in studying chemical carcinogenesis, in the absence of confounding hormonal proliferative signals (17, 19, 20).

Selective estrogen receptor modulators (SERM) are ER ligands that oppose the effects of endogenous estrogens in breast tissues. In the present study, the potential for prevention of estrogen-induced malignant transformation of MCF-10A cells was studied in response to raloxifene (Ral) and related SERMs. Ral and desmethylarzoxifene (DMA),

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the active metabolite of arzoxifene, were observed to inhibit malignant transformation.

The interconversion of estradiol (E2) with estrone (E1) is catalyzed by the enzyme 17β-hydroxysteroid dehydrogenase (17β-HSD; Fig. 1). In MCF-10A cells, as (i) the equilibrium lies strongly toward E1 and (ii) the stability of the methyl ether metabolites is superior to the catechol estrogen itself, MeOE1 represents a reliable, indirect measurement of estrogen oxidative metabolism (21). CYP450-1A1 (COMT) activity was not perturbed by SERMs. SERMs did not modulate expression of CYP450s in E2-treated cells.

Materials and Methods

Chemicals and reagents

All chemicals, reagents, and enzymes were obtained from Sigma or Invitrogen unless stated otherwise. Antibodies were obtained from Santa Cruz Biotechnology, Cell Signal-
were extracted into dichloromethane and analyzed using MeOE1, respectively.

The ratio for the 4-OHE2–treated sample was taken as 100% for described (27). Anti-CYP450 1B1 (Sigma; AV51761), anti-sense and absence of SERMs (DMA, FDMA, Ral; 1 mL) and incubated overnight (16 hours) at 37°C. Samples were extracted into dichloromethane and analyzed using liquid chromatography/tandem mass spectrometry (LC/MS-MS) as previously described (27). Exemplar amounts of 2-MeOE1 and 4-MeOE1 are provided from standard curves for experiments in which cells were treated with E2 alone: 376 ± 22 and 319 ± 95 pmol/L, for 2-MeOE1 and 4-MeOE1, respectively.

ROS formation determined by CM-H2DCFDA

MCF-10A cells were grown (4 × 10^5 cells/ml) on each of 8 chambers on a sterile Nunc chambered coverglass and incubated overnight at 37°C with 5% CO2. Cells were treated with E2 (1 μmol/L) with and without SERMs (1 μmol/L) for 6 days. Treatments were renewed after 3 days. Formation of ROS was determined as previously described (31), using CM-H2DCFDA (10 μmol/L) and 0.2 μg/mL Hoechst stain for visualization of nuclei.

Detection and measurement of 8-oxo-dG formation

MCF-10A cells were plated in 15-cm diameter dishes at a density of 2 × 10^5 cells per dish in estrogen-free media. Cells were allowed to attach for 1 day and then were treated with 4-OHE2 (1 μmol/L) with and without SERMs (1 μmol/L; DMA, Ral, or FDMA) for 72 hours. 8-oxo-dG analysis was performed as described previously (17). The native dG was determined by HPLC (UV) scanning at 280 nm. 8-oxo-dG was detected by multiple reaction monitoring and collision-induced dissociation for the fragmentation pathway of m/z 284 → 168 and m/z 289 → 173 for (15N5)8-oxo-dG using positive ion electrospray. The amount of 8-oxo-dG formed per 10^9 of dG was plotted. Total 8-oxo-dG per 10^9 of dG ratio for the 4-OHE2–treated sample was taken as 100% for the purpose of calculation.

Anchorage-independent growth assay

Anchorage-independent colony formation cell transformation assay was performed as previously described (27). Spherical formation of more than 50 cells was taken as a colony. Number of colonies formed in each well were counted and represented as percentage colony efficiency ± SD. Percentage colony efficiency is calculated as the number of colonies formed per number of cells plated per well × 100.

Immunoblotting

MCF-10A cells were treated with E2 (1 μmol/L) in the presence and absence of SERMs (DMA, FDMA, Ral; 1 μmol/L). Protein expression of CYP450 1B1 and CYP450 1A1 was analyzed using Western blot experiments as previously described (27). Anti-CYP450 1B1 (Sigma; AV51761), anti-CYP450 1A1 (Santa Cruz; sc-20772), and anti-β-actin (Cell Signaling; #4967) antibodies were used as primary antibodies. Detoxification enzymes were also analyzed using anti-SULT1 (Santa Cruz; sc-32928), anti-SULT1A1 (Santa Cruz; sc-376009), anti-SULT1A1 (Santa Cruz; sc-130883), anti-GSTpi (Cell Signaling; #3369), anti-NQO1 (Santa Cruz; sc-32793), and anti COMT (Santa Cruz; sc-25844) as primary antibodies. Antibodies were diluted in blocking solution (5% non-fat milk in TBS with 0.1% Tween 20). Blots were incubated with primary antibody overnight at 4°C and with secondary antibody for 1 hour at room temperature. Blots were visualized using chemiluminescence substrate (Thermo Scientific). Imaging and analysis were done using FluoroChem software (Cell Biosciences). Each protein band density was normalized to the respective β-actin band density and was represented as the relative protein expression. Three independent experiments were performed and results were represented as average ± SD.

RNA isolation and quantification of metabolizing enzyme gene transcripts

MCF-10A cell were plated at a density of 2 × 10^5 cells per well in a 6-well plate and treated with E2 (1 μmol/L) with and without SERMs (1 μmol/L) for 24 hours. Total RNA was isolated from cells using QIAshredder columns and QIA-GEN RNeasy kit (Qiagen Inc.) according to the manufacturer’s protocol. Total RNA (1 μg) was used to synthesize cDNA using SuperScript III in a 20 μL reaction mixture according to manufacturer’s protocol. Quantitative PCR (qPCR) was done with respective primers. TaqMan FAM probes and primers (Applied Biosystems) were used for the gene analysis of SULT1A1, SULT1E1, and UGT1A1, whereas human β-actin gene amplification was used as the internal control. Expression of the gene of interest was normalized to the internal control and fold change in gene expression was calculated. Three independent experiments were performed in duplicates and the data were represented as an average ± SD.

Enzyme activity assays

Inhibition of CYP450 1B1 activity was analyzed using ethoxyresorufin O-dealkylase (EROD) assay as previously described (27). Inhibition of COMT was assayed by adaptation of a literature method (32). Recombinant COMT (10 μg/mL) was incubated in Tris (10 mmol/L, pH 7.4), MgCl2 (1 mmol/L), DTTP (1 mmol/L), S-(5’-adenosyl)-L-methionine (500 nmol/L) with or without Ral, Baze, or DMA (1 μmol/L) at 37°C for 5 minutes before initiation of reaction by addition of 6,7-dihydroxycoumarin (5 μmol/L). Reaction was monitored by fluorescence (λex = 355 nm, λem = 460 nm).

Statistical analysis

Three independent metabolism experiments were performed in triplicates and the data were represented as average ± SD. The statistical analysis of results consisted of t test or ANOVA using GraphPad Prism version 5 for Windows.
Results

DMA, Ral, and Baze, but not 4-OHTam, inhibit estrogen metabolism in MCF-10A cells

Analysis of E2 methoxy ethers is a useful indirect measurement of the formation of catechol metabolites in the presence of SERMs, as (i) in MCF-10A cells, catechol estrogens are largely metabolized to methoxyethers that cannot themselves be directly converted to quinones and (ii) SERMs do not inhibit COMT activity (Supplementary Fig. S1). After 6 days of E2 treatment, higher amounts of E1 relative to E2 metabolites and relatively higher amounts of the 2-MeOE1 isomer were observed in all treatments (Supplementary Fig. S2).

MCF-10A cells incubated with E2 (1 μmol/L) were treated with vehicle or SERMs (1 μmol/L) for 6 days and the formation of 4-MeOE1 and 2-MeOE1 was analyzed by LC/MS-MS, which provides a measure of catechol estrogen formation (Fig. 2; refs. 17, 27). Attenuation of MeOE1 formation with DMA and Ral reached significance for 4-MeOE1 (P < 0.05) whereas FDMA was without effect (Fig. 2A). For Ral and DMA, the reduction in catechol ether formation was found to be concentration-dependent (Figures 2B and C). The effects on estrogen metabolism of the clinical SERMs, Baze and Tam, were also studied. No significant effect on metabolite formation was observed with 4-OHTam, the active metabolite of Tam, whereas Baze showed significant inhibition of 4-MeOE1 formation (P < 0.05; Fig. 2D).

DMA, Ral, and Baze attenuate estrogen-induced ROS in MCF-10A cells

MCF-10A cells incubated with E2 for 6 days and treated with the reporter dye CM-H2DCFDA showed increased ROS levels compared with the dimethyl sulfoxide (DMSO) vehicle control (Fig. 3A). In ERα cells, localization of ER in the nucleus has been reported to produce nuclear ROS localization (31, 33); however, in MCF-10A cells, localization was not observed, compatible with the lack of function of ERα as a nuclear transcription factor in this cell line. E2-induced ROS formation was attenuated in cells cotreated with either DMA or Ral; however, there was no significant effect on the formation of ROS with FDMA cotreatment (Fig. 3A). Baze and 4-OHTam were also tested for their effect on E2-induced ROS formation in MCF-10A cells: no significant effect was observed on 4-OHTam treatment; however, Baze attenuated ROS formation (Fig. 3B).

DMA and Ral significantly attenuate 4-OHE2–induced 8-oxo-dG formation

Measurement of 8-oxo-dG is routinely used to determine the level of oxidative DNA damage in cells and in vivo (34). After 3 days treatment of MCF-10A cells with E2, formation of 8-oxo-dG did not reach significance relative to DMSO control (data not shown); therefore, MCF-10A cells were treated directly with the catechol estrogen metabolite, 4-OHE2 (1 μmol/L), for 3 days revealing a significant increase in 8-oxo-dG relative to DMSO control (P < 0.001). Cotreatment with either DMA or Ral significantly reduced (P < 0.05) 8-oxo-dG levels induced by 4-OHE2. Coadministration of FDMA was again without effect (Fig. 3C).

DMA and Ral do not decrease CYP450 expression or activity

CYP450 enzymes mediate estrogen-induced chemical carcinogenesis by catalyzing catechol estrogen formation (Fig. 1). CYP450 expression was analyzed by immunoblotting after treatment of MCF-10A cells with E2 in the presence or absence of SERMs, showing no effect of SERM cotreatment on CYP450 levels (Supplementary Fig. S3). Measurement of CYP450 1B1 activity using the EROD assay revealed the expected inhibition by SERMs at high concentrations, but not at the 1 μmol/L concentration applied to cells (Supplementary Fig. S4).

DMA and Ral detoxify estrogen metabolites via action on phase II enzymes

Sulfation and glucuronidation play key roles in conjugative detoxification; therefore, levels of estrogen metabolites in cell media were measured in the presence of a sulfatase/β-glucuronidase cocktail that causes enzymatic hydrolysis of conjugates. The attenuation of 4-MeOE1 and 2-MeOE1 formation by SERMs was completely lost under these conditions (Fig. 4), leading to the conclusion that conjugative metabolism is responsible for the attenuation of catechol estrogen metabolite formation caused by SERM cotreatment.

Extending this observation, cotreatment of MCF-10A cells with E2 and either DMA or Ral significantly elevated immunoreactivity of SULT1 family enzymes (Fig. 5A). Further analysis indicated that expression of SULT1E1 was induced by cotreatment with DMA or Ral (Supplementary Fig. S5) but that SULT1A1 expression was not significantly changed (Supplementary Fig. S5). E2 itself did not modulate SULT1 expression and once again FDMA was unable to mimic the effects of Ral and DMA (Fig. 5A). Reduction of quinones by NQO1 is able to maintain a reducing cellular environment, unless this activity contributes to redox cycling (11, 22) (Fig. 1). The reduction in NQO1 expression after treatment of MCF-10A cells with E2 was negated or reversed by cotreatment with SERMs (Supplementary Fig. S4). Similar analyses of COMT and GST-P1 expression showed no effect from cotreatment with SERMs (Supplementary Fig. S5). The lack of sensitivity of COMT to E2 and drug treatments further supports measurements of MeOE1 as reflective of catechol estrogen formation.

Because immunoblotting showed induction of SULT1 family proteins after cotreatment with DMA or Ral, qPCR experiments were conducted to examine the effect of SERMs on SULT1E1 and SULT1A1. A significant increase in gene transcription of SULT1E1 was observed with cotreatment of DMA and Ral (P < 0.05) whereas the effect of FDMA was not significant (Fig. 5B). There was an induction of SULT1A1 gene transcription in E2 incubations, which was not
significantly perturbed by SERM cotreatment (Supplementary Fig. S5). Induction of \( \text{UGT1A1} \) \((P < 0.05)\) was observed with both DMA and Ral cotreatment, whereas the effect of FDMA was not significant (Fig. 5C). Transcription of \( \text{SULT1E1} \), in response to SERM cotreatment, mirrored the observations on protein expression.

**DMA and Ral significantly inhibit \( E_2 \)-induced anchorage-independent colony formation**

Upon exposure to chemical carcinogens, MCF-10 cells can be transformed into a malignant phenotype reflected by formation of anchorage-independent colonies (35, 36). MCF-10A cells treated with \( E_2 \) (1 \( \mu \text{mol/L} \)) for 3 weeks...
underwent malignant transformation as shown by formation of colonies in soft agar (27). E2-induced colony formation was significantly inhibited by cotreatment with DMA and Ral (P < 0.05) whereas FDMA was without effect (Fig. 5D).

**Modulation of estrogen metabolism in MCF-10A cells is not mediated by classical ER**

MCF-10A cells are formally considered as ER−/C0, as estrogen does not induce proliferation; however, the presence of ER protein and mRNA has been determined in MCF-10A cells (37–41). As the primary biologic target of SERMs is ER, it was essential to determine whether classical ER signaling via these proximal receptors was causal in modulation of oxidative metabolism to produce catechol estrogens. We therefore chose to study the effects on E2 metabolism of analogues of DMA (BTC, HP-BTC, AcBTC, TolBTC) with varied activity at ERα and ERβ (42). The formation of 4-MeOE1 was measured in E2-treated MCF-10A cells cotreated with DMA analogues: TolBTC was without effect, whereas BTC, HP-BTC, and AcBTC significantly reduced oxidative metabolite formation (Fig. 6A). Using ERE luciferase reporters, full concentration–response curves were obtained for DMA analogues in MCF-7 and in MDA-MB-231:β41 cells to determine EC50 for classical ERα and ERβ signaling, respectively (Supplementary Fig. S6). The relative luciferase activity for the DMA analogues illustrates that BTC is an ERβ selective agonist, TolBTC is an ERα selective agonist, Ac-BTC is a nonselective agonist, and HP-BTC was observed to be an antagonist (Fig. 6B). Ral, DMA, FDMA, 4-OHTam, and Baze have been extensively profiled by ourselves and others as classical ERα antagonists in mammary epithelial cell lines.
preclinical SERMs, formation of MeOE₁ catechol estrogen metabolites was significantly attenuated. The attenuated metabolism correlated with the effect of Ral and DMA in preventing E₂-induced malignant transformation of MCF-10A cells (Fig. 5D), representing the first evidence that modulation by SERMs of estrogen metabolism in mammary cells attenuates malignant transformation. In contrast to DMA and Ral, FDMA, an analogue of DMA, with similar affinity and potency at ER to Ral and DMA (46, 47), caused no significant attenuation of estrogen metabolism and did not inhibit malignant transformation. The clinical SERM, Baze, was also observed to inhibit formation of oxidative estrogen metabolites; whereas Tam had no effect on metabolism (Fig. 2D). Therefore, attenuation of estrogen oxidative metabolism is not a feature common to the entire SERM drug class; however, where studied, attenuated metabolism correlated with inhibition of malignant transformation.

Both estrogen-induced ROS formation and formation of 8-oxo-dG are indicators of oxidative stress and possible genotoxicity leading to carcinogenesis (34, 48, 49). Exposure of breast epithelial cells to catechol estrogen metabolites is associated with ROS formation (12, 50). In the present study, we observed that exposure to E₂ for 6 days significantly increased ROS in MCF-10A cells (Fig. 3A). There was a clear correlation between ROS formation and metabolism to catechol estrogens with all clinical and preclinical SERMs tested. Treatment of MCF-10A cells directly with the catechol estrogen metabolite, 4-OH₂, gave a significant increase in 8-oxo-dG after 3 days, compatible with induction of oxidative DNA damage by this carcinogenic metabolite (48, 51). Cotreatment of cells with DMA and Ral, but not FDMA, led to inhibition of both estrogen-induced ROS and 8-oxo-dG formation (Fig. 3).

The MCF-10A cell line represents a model system to evaluate estrogen metabolism and malignant transformation in vitro (21). Previous studies have shown that both MCF-10A and MCF-10F cells can be transformed into a malignant phenotype upon exposure to E₂ and 4-OH₂ (27, 52) leading to the formation of anchorage-independent colonies in semi-solid media. It has been previously reported that a botanical extract of hops (Humulus lupulus) could significantly reduce estrogen-induced malignant transformation in MCF-10A cells via attenuation of oxidative estrogen metabolism (27). Malignant transformation of MCF-10F cells, induced by a combination of estrogen and TCDD, was also reported to be inhibited by attenuation of estrogen metabolism, on resveratrol cotreatment (19, 53).

In the present study, DMA and Ral significantly inhibited E₂-induced malignant transformation by attenuation of catechol estrogen metabolite formation and concomitant reduction in levels of ROS and 8-oxo-dG. Transformation of normal breast epithelial cells into a malignant phenotype, measured by formation of anchorage-independent colonies in semi-solid media, is dependent upon oxidative hydroxylation of E₂ to a catechol metabolite. Estrogen metabolism can be modulated either via: (i) downregulating or inhibiting CYP450 enzymes and thereby reducing the formation of catechols and quinones.

Discussion

SERMs are used in the treatment and prevention of postmenopausal osteoporosis (44) and also in primary and secondary prevention of ER⁺ breast cancer, with STAR (Study of Tamoxifen and Ral) and IBIS2 (International Breast cancer Intervention Study 2) reporting data on primary chemoprevention (45). In light of the clinical use of SERMs and both the current and potential use in breast cancer chemoprevention, the present study was designed to determine the effect of SERMs on estrogen-induced chemical carcinogenesis, a pathway that is independent of the formal ER status of cells and tissues. We hypothesized that modulation of oxidative estrogen metabolism in mammary epithelial cells by SERMs would influence the estrogen-induced malignant transformation of these cells and be of relevance to chemical carcinogenesis.

When MCF-10A nontumorigenic breast epithelial cells, in the presence of E₂, were cotreated with selected clinical or...
The expression of SULT and UGT was assayed with and without SERM cotreatment in E2-treated MCF-10A cells: SULT 1E1 expression was induced by DMA and Ral. UGT 1A1 was also induced by DMA and Ral cotreatment; however, the expression of UGT 1A1 was low in MCF-10A cells when tested by immunoblotting. The inhibitory effects of DMA and Ral on MeOE1 formation were lost when supernatants were treated with sulfatase/glucuronidase (Fig. 4). Future studies of the role of glucuronidation in detoxification will interrogate formation of the hydrophilic glucuronate and sulfate metabolites. However, the combined observations support induction of phase II metabolism, and particularly SULT 1E1 expression, as a mechanism of detoxification of carcinogenic estrogen metabolites by selected SERMs in breast epithelial cells.

whereas FDMA had no effect compared with E2 treatment alone. C, gene transcription of UGT 1A1 was significantly induced on cotreatment with DMA or Ral as measured by qPCR after isolating RNA from 24 hours treated MCF-10A cells. Each data point represents an average of 3 independent experiments ± SD. *, *P < 0.05; **, *P < 0.01. D, cotreatment with DMA or Ral significantly inhibited E2-induced anchorage-independent colony growth of MCF-10A cells in soft agar, whereas FDMA cotreatment had little effect. Cells were treated twice a week, in the presence and absence of SERMs, over the course of 3 weeks. DMSO (0.01%) was used as the vehicle control in the experiments in the absence of E2 treatment. Cells were plated on soft agar and maintained for 3 weeks. Relative colony efficiency is calculated by dividing the number of colonies counted in a well by the number of cells plated in each well, normalized to DMSO vehicle. Data show mean from 3 independent experiments ± SD. **, *P < 0.005.

Figure 5. A, a significant induction in SULT1 enzyme expression was observed by immunoblotting on cotreatment of MCF-10A cells with DMA or Ral. Relative protein amounts were determined by densitometric analysis of SULT1 protein after Western blotting, loading with 30 μg of total protein. Each treatment was normalized to the loading and transferring control, β-actin. Each data point represents an average of 3 independent experiments in duplicate ± SD. *, *P < 0.05. B, gene transcription of SULT 1E1 was significantly induced by DMA and Ral, or (ii) induction of phase II enzymes that “detoxify” these catechol metabolites by conjugation and elimination. Hops extract and other agents have been reported to inhibit the expression of CYP450 1B1 in human breast epithelial cells (27, 53, 54); however, no evidence for regulation or inhibition of CYP450 1A1 or CYP450 1B1 by Ral or DMA was observed at the concentrations used in MCF-10A cell cultures.

Phase II conjugative enzyme activity has been reported to correlate with malignant transformation in vitro, tumorigenesis in vivo, and breast cancer risk in human subjects. SULTs play a major role in hepatic and extrahepatic detoxification of xenobiotics and other toxic metabolites. It has been reported that SULT 1E1 and SULT 2B1 are responsible for detoxification of estrogene catechols via sulfation (40). In MCF-10A cells, one article reported that both SULTs were equally expressed at the mRNA level and more highly so than in breast cancer cells such as T47D, SKBR3, and MDA-MB-231 (40). Another group reported expression of SULT 1E1 mRNA alone in MCF-10A cells and observed both epigenetic regulation of SULT 1E1 mRNA and repression in transformed MCF-10A–derived cells (39). There is some evidence to suggest that SULT 1E1 gene transcription is mediated via the aryl hydrocarbon receptor in MCF-10A cells (55). In addition, there may be an association between breast cancer and genetic polymorphisms in human UGT1A1, another key mediator of conjugative metabolism (56).

The expression of SULT and UGT was assayed with and without SERM cotreatment in E2-treated MCF-10A cells: SULT 1E1 expression was induced by DMA and Ral. UGT 1A1 was also induced by DMA and Ral cotreatment; however, the expression of UGT 1A1 was low in MCF-10A cells when tested by immunoblotting. The inhibitory effects of DMA and Ral on MeOE1 formation were lost when supernatants were treated with sulfatase/glucuronidase (Fig. 4). Future studies of the role of glucuronidation in detoxification will interrogate formation of the hydrophilic glucuronate and sulfate metabolites. However, the combined observations support induction of phase II metabolism, and particularly SULT 1E1 expression, as a mechanism of detoxification of carcinogenic estrogen metabolites by selected SERMs in breast epithelial cells.
FDMA, proved to be an excellent probe of mechanism, because of the SERMs studied, FDMA alone did not attenuate metabolite levels, nor upregulate SULT 1E1, nor inhibit both ROS and 8-oxo-dG formation. Clear trends were observed in reduction of the 2-MeOE1 metabolite by DMA and Ral, although significance was not reached. Several lines of evidence strongly suggest that the 4-OH catechol estrogen is the proximal carcinogen in estrogen chemical carcinogenesis (13–17). It is possible that in the model system under study, malignant transformation can be elicited by both isomeric catechol estrogens; however, more detailed study would be needed.

It was important to confirm that the observed expression of SULT 1E1, attenuation of estrogen metabolism, and inhibition of malignant transformation were not mediated via ligand binding to ER and resulting classical, ERE-mediated transcription. The lack of effect observed for FDMA and 4HO-Tam, both high-affinity ER ligands, supports this assertion. Furthermore, estrogen metabolism was measured in the presence of DMA analogues that manifest varied agonist/antagonist activity at ER; no correlation was observed between attenuation of estrogen metabolism and ER/ERE signaling.

Interestingly, the phytoestrogen genistein has been the subject of 2 recent studies in MCF-10A cells, implicating independently upregulation of detoxification enzymes (57) and of PTEN (58) in mediating chemoprevention. The cause was speculatively attributed to ligand binding to ER or the G-protein–coupled receptor, GPR30 (GPER; ref. 59). GPR30 mediates many nonclassical, extranuclear actions of estrogens and anti-estrogens, including the actions of Ral, DMA, and DMA analogues (42). However, several SERMs are able to act as phenolic antioxidants and to activate stress response via Nrf2 and the antioxidant response element (ARE; refs. 60–63); therefore, ER-independent pathways are known that might regulate function in MCF-10A cells by DMA, Ral, and Baze.

The present study demonstrates that clinical SERMs can attenuate estrogen chemical carcinogenesis by modulating oxidative estrogen metabolism. Treatment of human breast epithelial cells with the SERMs, Ral, DMA, and Baze, but not 4-OHTam, led to inhibition of oxidative estrogen metabolism. Attenuated oxidative metabolism and lower levels of ROS were correlated with inhibition of E2-induced malignant transformation. The mechanism of inhibition by Ral and DMA was shown to be detoxification of genotoxic estrogen metabolite accumulation mediated via upregulation of SULT 1E1. Further studies are underway to identify the proximal receptor for these SERMs and to extend studies to animal models.

### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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SERMs Upregulate Estrogen Detoxification


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SERMs Attenuate Estrogen-Induced Malignant Transformation of Human Mammary Epithelial Cells by Upregulating Detoxification of Oxidative Metabolites


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