Estrogenic and Antiestrogenic Properties of Resveratrol in Mammary Tumor Models[1]

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ABSTRACT

Trans-3,4′,5-trihydroxystilbene (resveratrol), a phytoalexin present in grapes and grape products such as wine, has been identified as a chemopreventive agent. Recent studies performed with MCF-7 human breast cancer cells have demonstrated superestrogenic effects with resveratrol. In contrast, studies performed using estrogen receptor-transfected cell lines have shown that resveratrol acts as a mixed agonist/antagonist. The major objective of this study was to characterize the estrogen-modulatory effects of resveratrol in a variety of *in vitro* and *in vivo* mammary models. Thus, the effect of resveratrol alone and in combination with 17β-estradiol (E_2) was assessed with MCF-7, T47D, LY2, and S30 mammary cancer cell lines. With cells transfected with reporter gene systems, the activation of estrogen response element-luciferase was studied, and using Western blot analysis, the expression of E_2-responsive progesterone receptor (PR) and presnelin 2 protein was monitored. Furthermore, the effect of resveratrol on formation of preneoplastic lesions (induced by 7,12-dimethylbenz(*a*)anthracene) and PR expression (with or without E_2) was evaluated with mammary glands of BALB/c mice placed in organ culture. Finally, the effect of p.o. administered resveratrol on *N*-methyl-*N*-nitrosourea-induced mammary tumors was studied in female Sprague Dawley rats. As a result, in transient transfection studies with MCF-7 cells, resveratrol showed a weak estrogenic response, but when resveratrol was combined with E_2 (1 nM), a clear dose-dependent antagonism was observed. Similar mixed estrogenic/antiestrogenic effects were noted with S30 cells, whereas resveratrol functioned as a pure estrogen antagonist with T47D and LY2 cells. Furthermore, in MCF-7 cells, resveratrol induced PR protein expression, but when resveratrol was combined with E_2, expression of PR was suppressed. With T47D cells, resveratrol significantly down-regulated steady-state and E_2-induced protein levels of PR. With LY2 and S30 cells, resveratrol down-regulated presnelin 2 protein expression. Using the mouse mammary organ culture model, resveratrol induced PR when administered alone, but expression was suppressed in the presence of E_2 (1 nM). Furthermore, resveratrol inhibited the formation of estrogen-dependent preneoplastic ductal lesions induced by 7,12-dimethylbenz(*a*)anthracene in these mammary glands ($IC_{50} = 3.2$ μM) and reduced *N*-methyl-*N*-nitrosourea-induced mammary tumorigenesis when administered to female Sprague Dawley rats by gavage. Therefore, in the absence of E_2, resveratrol exerts mixed estrogen agonist/antagonist activities in some mammary cancer cell lines, but in the presence of E_2, resveratrol functions as an antiestrogen. In rodent models, carcinogen-induced preneoplastic lesions and mammary tumors are inhibited. These data suggest that resveratrol may have beneficial effects if used as a chemopreventive agent for breast cancer.

INTRODUCTION

Despite years of intensive research, breast cancer remains a major cause of death among women in the United States (1). SERMs[3] such as tamoxifen, which are used clinically for the treatment of breast cancer, act as estrogen agonists in certain tissues but exhibit antiestrogenic effects in others (2). Data from adjuvant breast cancer trials have shown that tamoxifen not only suppresses the recurrence of breast cancer but also prevents the contralateral occurrence of second primary breast tumors (3). Additional beneficial effects include maintenance of bone density and decreased mortality from coronary heart disease (4, 5). Moreover, a double-blind clinical chemoprevention study with tamoxifen revealed a 49% reduction in the incidence of breast cancer among women with increased risk of developing this disease (6). However, tamoxifen may also promote the development of endometrial cancer. Raloxifene, a related SERM, exhibits antiestrogenic effects in the breast and uterus but estrogenic effects in the bone (7). As a result, a randomized double-blinded trial comparing the effectiveness of tamoxifen with that of raloxifene (STAR) is currently being conducted with postmenopausal women at increased risk for developing breast cancer (8).

To date, no plant-derived SERMs have been evaluated in clinical trials, although a number of herbal remedies such as extracts of black cohosh and red clover are touted for the relief of postmenopausal symptoms (9, 10). The precise constituents of these extracts that might be responsible for such pharmacological effects are not known; however, flavonoids and isoflavonoids such as quercetin and genistein have been reported to display both estrogenic and antiestrogenic effects (11). Genistein is about 1000-fold less potent than E_2 in its ability to bind to the ER, but its circulating concentrations among individuals consuming moderate amounts of soyfoods is nearly 1000-fold higher than peak levels of endogenous E_2 (12). Interestingly, Asian women who consume a soy-rich diet have about a 6-fold lower risk of developing breast cancer than their Western counterparts (13, 14), and population-based studies have suggested that consumption of a phytoestrogen-rich diet is protective against prostate and bowel cancer, as well as cardiovascular disease (15). Hence, it is possible that these phytoestrogens function as chemopreventive agents, and because human consumption of phytoestrogens is common, plant-derived agents are promising candidates for clinical trials.

Resveratrol, a stilbene found in the diet of humans, was identified as a potential chemopreventive agent (16). Subsequently, the compound was considered a phytoestrogen due to potent estrogenic and even superestrogenic (when combined with E_2) properties in MCF-7 mammary cancer cells (17), but additional studies conducted with these cells demonstrated antagonist activity in the presence of E_2 (18, 19). With ER-transfected cell lines, resveratrol has been reported to function as a mixed agonist/antagonist (20–22). For example, while

acting as an agonist with mammary tissues containing ER-α, estrogen antagonist activity was observed with ER-β (22). Using *in vivo* models, resveratrol has been shown to be an estrogen agonist in stroke-prone spontaneously hypertensive rats, in which it attenuated an increase in systolic blood pressure, enhanced endothelin-dependent vascular relaxation in response to acetylcholine, and prevented ovariectomy-induced decreases in femoral bone strength in a manner similar to E_2 (23). In contrast, however, using rat uterotrophic assays, it was shown that high doses of resveratrol antagonized the serum cholesterol-lowering activity of E_2 (24), mildly decreased uterine weight, and suppressed the expression of ER-α protein, similar to pure antiestrogens (25). Using a human endometrial cell line, we have recently demonstrated estrogen antagonistic responses with resveratrol (26).

Thus, the estrogen-modulatory potential of resveratrol in mammary tissues is not well delineated. In this study, a variety of cell lines were used to assess the potential of resveratrol to serve as a SERM. In addition, estrogen-dependent MMOC studies were performed, as well as full-term tumorigenesis studies in rats. The results obtained from both *in vitro* and *in vivo* studies suggest that resveratrol may serve as a cancer chemopreventive agent in the breast.

MATERIALS AND METHODS

Cell Culture. MCF-7 (ER$^+$/PR$^+$/pS2$^+$) and T47D (ER$^+$/PR$^+$/pS2$^-$) cells were obtained from American Type Culture Collection (Manassas, VA). LY2 cells (ER$^+$/PR$^-$/pS2$^+$) were provided by Dr. Marc E. Lippman (Georgetown University, Washington, D.C.), and S30 cells (ER$^+$/PR$^+$/pS2$^+$) were obtained from Dr. V. C. Jordan (Northwestern University, Chicago, IL). MCF-7, T47D, and LY2 cells were routinely maintained in MEME supplemented with 5% FBS, 100 μg/ml streptomycin, 100 units/ml penicillin, 10 μg/ml insulin, 2 mM L-glutamine, 1 mM sodium pyruvate, and nonessential amino acids at 37°C in a 5% CO_2 incubator. S30 cells were maintained in MEME supplemented with 5% DCC-FBS, 100 μg/ml streptomycin, 100 units/ml penicillin, 6 ng/ml insulin, 2 mM glutaMAX-1, 1 mM sodium pyruvate, and 500 μg/ml G418. All media components and reagents were purchased from Life Technologies, Inc. (Grand Island, NY), with the exception of FBS, which was purchased from Atlanta Biologicals (Atlanta, GA). One week before plating the cells (MCF-7, T47D, and LY2), the medium was changed to a phenol red-free formulation of MEME containing DCC-FBS.

Transfection and Luciferase Assays. The plasmid pUC 18 (provided by Dr. Peter J. Kushner; University of California at San Francisco) is a construct consisting of two copies of *Xenopus* vitellogenein A$_2$ ERE (GGTCACAGT-GACC) inserted upstream (-331 to -289) of a minimal thymidine kinase promoter (-109 to $+45$) linked to the luciferase gene. For transfection assays, cells were grown in estrogen-free media for 7 days and then plated in 12-well plates at a density of 10×10^4 cells/well. Experimental media containing various concentrations of resveratrol and E_2 were added 24 h later. Two h after changing to experimental media, the cells were transfected with 2 μg/well lipid-plasmid complex using FUGene 6 reagent (Roche Biochemicals, Indianapolis, IN). Luciferase activity was detected using a Luciferase Assay System (Promega, Madison, WI) approximately 24 h after transfection using a TD-20/20 luminometer (Turner Designs, Sunnyvale, CA). Luciferase induction responses for each treatment group were normalized to β-galactosidase activity and expressed as the means $\pm$ SD of three replicate experiments for each treatment group.

Western Blot Analysis for Protein Expression. Cells (50×10^4 cells/dish) were plated in 100-mm^2 Petri dishes, and compounds were added 24 h later. After 48 h of incubation, the cells were lysed using a $2\times$ electrophoresis sample buffer [250 mM Tris-HCl (pH 6.8), 4% SDS, 10% glycerol, 0.006% bromphenol blue, and 2% β-mercaptoethanol]. Twenty μg of protein lysate were fractionated using SDS-PAGE on 7.5% polyacrylamide gels. Anti-PR mouse monoclonal antibody was purchased from Lab Vision Corp. (Fremont, CA). Anti-pS2 goat monoclonal antibody was obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Anti-β-actin mouse polyclonal antibody was purchased from Sigma Chemical Co. (St. Louis, MO). The proteins were transferred onto nitrocellulose membranes by electroblotting and incubated with primary antibody (anti-PR, anti-pS2, and anti-β-actin) for 2 h. Secondary antibodies (antimouse or antirabbit) conjugated to horseradish peroxidase and streptavidin-horseradish peroxidase conjugate (Amersham Pharmacia Biotech, Piscataway, NJ) were diluted 1:2000 and incubated with the membrane for 30 min. Proteins were detected using an enhanced chemiluminescence plus detection system according to the manufacturer's instructions (Amersham Pharmacia Biotech) and quantified using Scion imaging software (Scion Corp., Frederick, MD).

Induction of Atypical Ductal Lesions in MMOC. BALB/c female mice (Charles River, Wilmington, MA), 3–4 weeks of age, were housed 5 mice/polycarbonate cage and maintained in accord with institutional guidelines. The animals were pretreated daily for 7 days with 1 μg of E_2 and 1 mg of progesterone by s.c. injections. The organ culture procedure has been described in detail elsewhere (27). Briefly, mice were killed by cervical dislocation, and the thoracic pair of mammary glands were dissected out on silk rafts and incubated for 10 days in Waymouth MB752/1 medium (5 glands/5 ml/dish; Life Technologies, Inc.) at 37°C in a 95% O_2 and 5% CO_2 environment. The medium was supplemented with 350 μg/ml glutamine, antibiotics (100 units/ml penicillin and 100 μg/ml streptomycin), and growth-promoting hormones (5 μg of insulin, 5 μg of prolactin, 1 ng of E_2, and 1 μg of progesterone per milliliter of medium; Sigma Chemical Co.). DMBA (2 μg/ml) was added to the medium between days 3 and 4, and control dishes contained DMSO as vehicle. On day 4, DMBA was removed from the medium by rinsing the glands with fresh medium, and the glands were transferred to new dishes containing carcinogen-free medium. Various concentrations of resveratrol (1–10 μM in DMSO) were included in the incubated media for the first 10 days of culture (10 glands/treatment). After 10 days of incubation, the glands were maintained for an additional 14 days in medium containing only insulin (5 μg/ml). At the end of the incubation period, glands were embedded in paraffin, and 5-μm longitudinal sections were cut. The sections were processed and stained with H&E, and ductal lesions were identified as hyperproliferative ductal epithelium with a $\geq$3-layer thickness of epithelial cells. Atypical ductal lesions were defined as ducts that were completely occluded with ductal epithelial cells that demonstrated nuclear pleomorphism and variability in size, form, staining intensity, chromatin composition, and nucleolar organization. The histological sections of the gland were divided into three regions, and each region was evaluated for the number of normal (single-layer thickness of epithelial cells), hyperplastic ($\geq$3-layers thick), or atypical (highly occluded, with no or very little lumen) areas. The percentage of multiplicity of lesions in control and treated glands was expressed as the mean $\pm$ SD, and the IC$_{50}$ of resveratrol was calculated.

Evaluation of PR Induction in MMOC. As described above, mouse mammary glands were incubated with growth-promoting hormones (insulin and prolactin) either alone or in the presence of resveratrol for 6 days. Glands were not exposed to DMBA, and the hormone withdrawal stage was omitted. The glands were fixed in buffered formalin, and 5-μm histological sections were prepared as described above. The sections were mounted on adhesive-coated slides (Superfrost; Fisher Scientific, Pittsburgh, PA), dried at 60°C overnight, deparaffinized in xylene, dehydrated in alcohol, and washed with PBS. To block nonspecific antibody reactions, sections were treated with 5% dried skim milk for 10 min and then incubated with a primary antibody solution against PR (2 μg/ml; Neomarkers, Fremont, CA) overnight at 0°C to 4°C. The tissues were rinsed in PBS and incubated with biotinylated antirabbit antimouse-linked antibody (Dako Corp., Carpinteria, CA) for 10 min, followed by a 10-min incubation with peroxidase-conjugated streptavidin (Dako Corp.) and AEC chromogen (BioGenex, San Ramon, CA) in H_2O_2 substrate for 5 min. The tissues were counterstained with Gill's hematoxylin (Fisher Scientific, Fairlawn, NJ), dehydrated through a graded series of alcohol and xylene, and mounted in Permount (Fisher Scientific). Slides were evaluated semiquantitatively for the expression of PR according to the proportion of positively stained cells (28). The cells were scored as follows: $-$, no staining; $+$, observable staining; $++$, marked staining; and $+++$, intense staining. The proportion of positively stained cells was calculated using the following formula: percentage of PR$+$ cells $= 100(n^+ + n^{++} + n^{+++})/(n^- + n^+ + n^{++} + n^{+++})$, where n is number of cells in each category of staining. The percentage of induction of PR in the control and treated glands were represented as the mean $\pm$ SD, and the IC$_{50}$ of resveratrol was calculated.

Induction of Mammary Tumors in Rats. Virgin female Sprague Dawley rats were received from Harlan Sprague Dawley (Indianapolis, IN) at 35 days

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Fig. 1. Effects of resveratrol on ERE-luciferase activity with transiently transfected breast cancer cells. Panels represent data obtained with MCF-7, S30, T47D, and LY2 cells. Cells were treated with the indicated concentrations of resveratrol alone (■) or in combination with 1 nM E_2 (▦) as described in "Materials and Methods." Two h later, cells were transfected with ERE-luciferase plasmid, and luciferase activity was measured after 24 h. Induction values are averaged from triplicate experiments. C*olumns*, average of triplicate samples; *bars*, SE. Values with an *asterisk* are significantly different from the respective controls. *, $P < 0.05$; **, $P < 0.005$ (Student's *t* test).
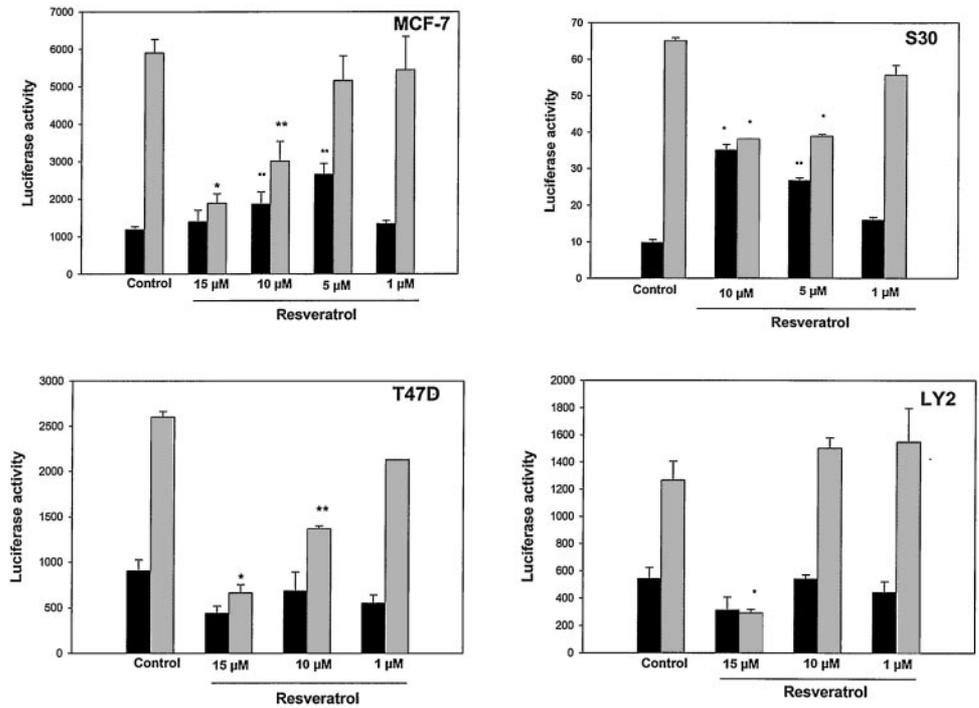
of age, placed on a diet of Teklad 4% rat/mouse chow (Harlan Teklad, Madison, WI), and maintained in accord with institutional guidelines. After 1 week, at 42 days of age, animals were randomized by weight into six groups of 20 animals each and treated with resveratrol (dissolved in 6.5% ethanol and 93.5% neobee oil) at 10 and 100 mg/kg body weight i.g. (5 days a week). Two groups served as controls for each concentration of resveratrol to monitor potential toxicity (without NMU). One group received only NMU (50 mg/kg) in saline, and one group received only vehicle. Resveratrol administration began 1 week before NMU injection (day 49, i.v.). During the experimental period, all animals were weighed weekly. Palpation for mammary tumors began 3 weeks after the animals received NMU and continued until termination of the study (120 days after administration of NMU). The date of appearance and location of all tumors were recorded. Animals were observed twice daily to assess general health. For histopathological studies, tumors were removed, fixed in 10% neutral formalin, and embedded in paraffin. Four-μm sections were stained with H&E for light microscopy.

Statistical Methods. For the transient transfection experiments, significant induction responses compared to control experiments were determined using Student's *t* test. For the induction of ductal hyperplasia or PR protein expression in the mammary organ culture model, differences between means (control *versus* treated) were analyzed with *t* tests for independent samples. *P*s of ≤0.05 were used to denote statistical significance. The NMU tumor incidence was evaluated with Fisher's exact test, and the log-rank test was used for tumor incidence rate. Tumor multiplicity was calculated using Armitage's test for trends in proportions, and latency was evaluated by unpaired *t* test. The percentage survival at the end of the study was analyzed by log-rank analysis.

RESULTS

Modulation of ERE-Luciferase Activity in Mammary Tumor Cell Lines. In the first series of studies, we compared the effect of resveratrol on ERE-luciferase activity with four different cell lines: MCF-7, T47D, LY2, and S30. In these models, ligand-activated ER binds to the ERE, and its functional activation is monitored using a luciferase reporter gene linked to its enhancer (ERE) driven by the minimal thymidine kinase promoter. Relative to DMSO controls, E_2 (1 nM) caused a 3–10-fold increase in luciferase activity (Fig. 1). With MCF-7 and S30 cells, resveratrol (10 μM) caused a significant increase in luciferase activity when administered alone ($P < 0.05$), but

when combined with E_2 (1 nM), resveratrol antagonized luciferase induction with each of the cell lines tested (Fig. 1). No statistically significant induction was observed at a concentration of 1 μM, and, unlike previous reports (17), no additive or superagonist activities were seen. In T47D and LY2 cells, resveratrol behaved as a pure antiestrogen, with no statistically significant induction of luciferase observed at the three concentrations tested (Fig. 1). When resveratrol was coadministered with E_2, potent antagonism was observed in a dose-dependent manner with approximately 50% inhibition at 10 μM ($P < 0.005$) in T47D cells. In tamoxifen-resistant LY2 cells, resveratrol also responded as an antagonist in the presence of E_2 at a concentration of 15 μM ($P < 0.005$).

Modulation of Estrogen-inducible Protein Expression in Mammary Cancer Cell Lines. We compared the expression of PR and pS2 in the four different mammary cancer cell lines. MCF-7 cells expressed both PR and pS2, T47D cells expressed PR but not pS2, and S30 and LY2 cells expressed pS2 but not PR (Fig. 2). Cells were treated with resveratrol in the presence or absence of E_2 for 48 h and analyzed for protein expression. Treatment of MCF-7 cells with E_2 caused a significant increase in PR expression. Resveratrol caused similar induction of PR expression at concentrations of 10 and 15 μM, but no effect was seen at 1 μM. When combined with E_2, resveratrol suppressed PR expression in a dose-dependent manner. At a concentration of 15 μM, resveratrol blocked the induction of PR by ~90%.

With T47D cells, even after incubation with phenol red-free media containing DCC-FBS for 1 week, basal levels of PR expression were not minimized, and the expression was not increased by the addition of E_2. However, resveratrol significantly suppressed basal levels of PR expression and down-regulated PR levels in the presence of E_2 in a dose-dependent fashion (Fig. 2).

We then analyzed the expression of pS2, another estrogen-inducible protein, in S30 cells. Based on the analysis of pS2 protein expression by Western blots, high basal levels were present, and E_2 did not cause any increase. However, resveratrol down-regulated pS2 expression at all concentrations tested as a single agent, and in the presence of E_2, suppression was observed in a dose-dependent manner (Fig. 2).
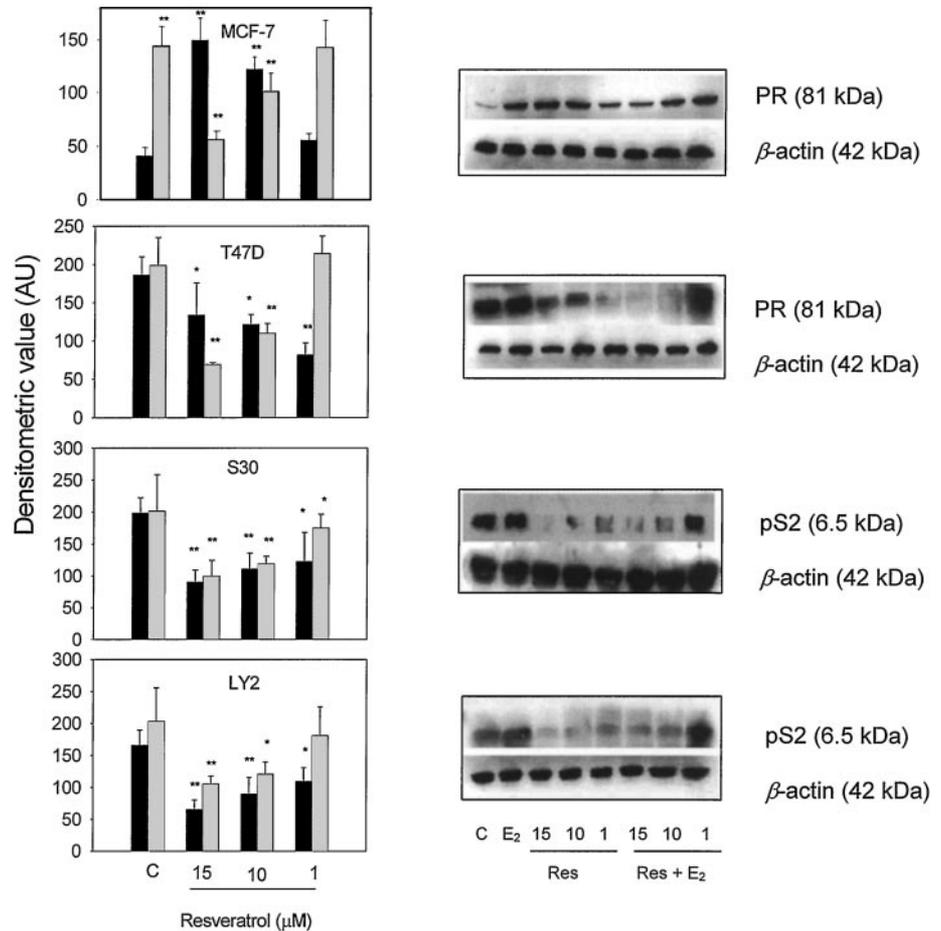
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Fig. 2. Effect of resveratrol on PR and pS2 protein expression in breast cancer cells. Panels represent densitomeric graphs (*left panels*) of immunoreactive bands (*right panels*) from MCF-7, T47D, S30, and LY2 cells. Cells were treated with various concentrations of resveratrol with (▨) or without E_2 (■), and protein expression was analyzed using Western blotting. Immunoreactive bands for PR and pS2 proteins were identified using enhanced chemiluminescence and quantitated as described in "Materials and Methods." Densitometric values are averaged from triplicate experiments. C*olumns*, average of triplicate samples; *bars*, SE. Values with an *asterisk* are significantly different from the respective controls. ∗, $P < 0.05$; ∗∗, $P < 0.005$ (Student's *t* test). *Res*, resveratrol; *AU*, arbitrary units.

LY2 cells responded to E_2 treatment by a slight increase of pS2 expression relative to controls. However, production of pS2 protein was down-regulated by resveratrol in the presence or absence of E_2 in a dose-dependent manner (Fig. 2).

Atypical Ductal Lesions in MMOC. Experiments were carried out to evaluate the effect of resveratrol on estrogen-dependent DMBA-induced ductal lesions in MMOC. DMSO-treated control glands exhibited normal morphology with branching ducts and alveolar structures, whereas alveoli in glands treated with DMBA in the presence of E_2 and progesterone were sparse (data not shown). On histological examination, it was found that DMSO-treated glands showed a single layer of epithelium in the lumen (Fig. 3*A*). When treated with DMBA, however, 4–5 layers of epithelial cells resulting in thickened ducts exhibiting a hyperplastic nature (Fig. 3*B*) were observed. In some ductal structures, the intraductal outgrowths were aggressive and resulted in a complete occlusion of the ducts (Fig. 3*C*). The percentage of incidence of hyperplastic and aggressive ductal lesions induced by DMBA ($63.0 \pm 8.1\%$; $P < 0.005$) was reduced by resveratrol in a dose-dependent manner (Fig. 3*D*). The percentage of areas with abnormal histology (hyperplastic and atypical ductal lesions) in control and resveratrol groups is shown in Table 1. Based on these data, the IC_{50} of resveratrol was approximately 3 μM.

Modulation of PR Expression in MMOC. To evaluate a functional aspect of estrogen action in MMOC, induction of PR was examined. Mammary glands were treated for 6 days with estrogen in insulin- and prolactin-containing medium, sections were prepared, and expression of PR was assessed and quantified by immunohistochemistry. With control glands (in the absence of E_2), basal levels of PR were expressed with a proportion of positively stained cells equal

to $22.8 \pm 4.7\%$ (Fig. 4*A*). In the presence of insulin, prolactin, and E_2, both the intensity (data not shown) and the proportion of positively stained cells for PR expression ($85 \pm 14.8\%$; control *versus* estrogen-induced PR expression, $P < 0.005$) were increased (Fig. 4*B*). When glands were treated in a similar manner without E_2 in the presence of resveratrol, expression of PR was induced to a proportion of $58.3 \pm 10.5\%$ (control *versus* resveratrol-induced PR expression, $P < 0.005$) at 10 μM (Fig. 4*C*). Lower concentrations of resveratrol did not cause statistically significant induction of PR. Nonetheless, when E_2 (1 nM) was combined with resveratrol, estrogen-induced PR expression was suppressed to $23.9 \pm 7\%$ (estrogen-induced PR expression *versus* resveratrol + estrogen-induced PR expression, $P < 0.005$; Fig. 4*D*) at 10 μM, and the effect was dose-dependent (Fig. 4*E*). Resveratrol also decreased the intensity of PR staining in estrogen-induced tissues (data not shown). When tested at a concentration of 20 μM, diffuse cellular structures indicative of toxicity were observed in the glands. Based on these data, the IC_{50} was approximately 4.0 μM.

NMU-induced Mammary Carcinogenesis. To determine the effect of resveratrol on mammary tumorigenesis, the NMU model with rats was used. Resveratrol was administered i.g. (5 days/week) for the entire period of study, starting 7 days before NMU administration. Doses of 10 and 100 mg/kg body weight were selected on the basis of previous studies (16). As shown in Fig. 5*A*, under these experimental conditions, resveratrol had no effect on body weight. Tumors were palpable in the control group by day 21 after NMU treatment (5.3%), and the percentage of incidence reached 100% on day 111 (Fig. 5*B*). The high dose of resveratrol delayed tumorigenesis. Notably, on day 40, 0% incidence was observed in the resveratrol-treated group,
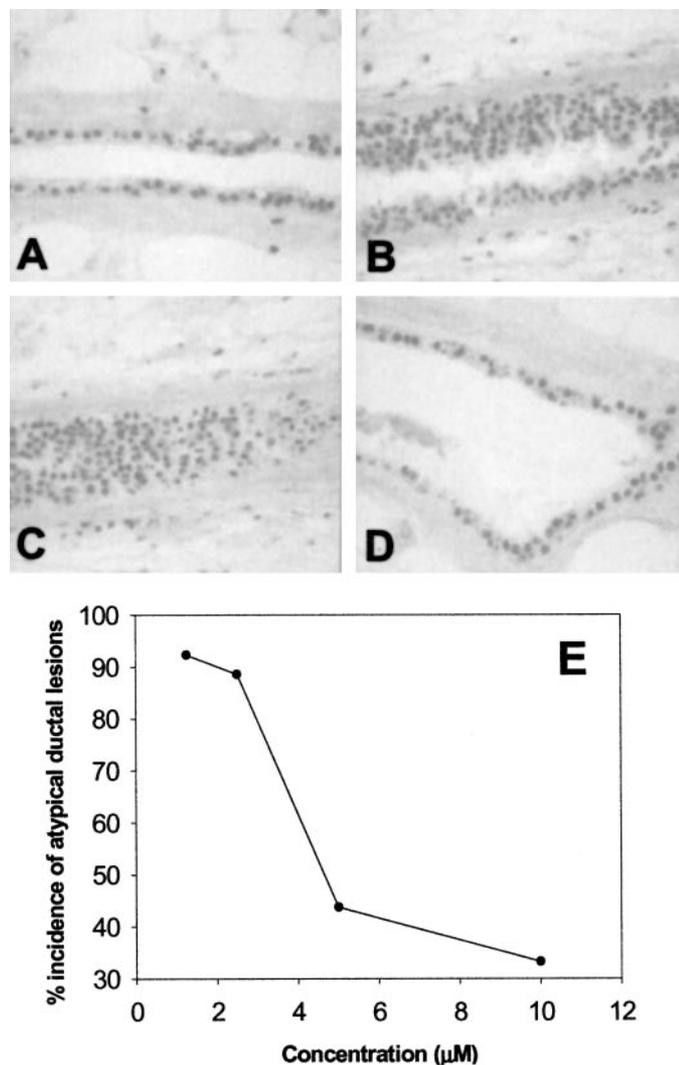
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Fig. 3. Effect of resveratrol on DMBA-induced atypical ductal lesions in MMOC. Mouse mammary glands in culture were incubated with insulin, prolactin, E_2, and progesterone, with or without resveratrol, for the first 10 days in culture, followed by withdrawal of all hormones (and resveratrol) except insulin and incubation for an additional 14 days. Ductal lesions were induced by addition of DMBA (2 μg/ml) on day 3 for 24 h. Glands were then fixed in formalin, processed, and stained with H&E for histopathological evaluation. *A,* control glands receiving only DMSO (without DMBA) for the first 10 days show a single layer of epithelial cells lining the ducts. Glands treated with DMBA exhibit (*B*) hyperplastic lesions or (*C*) completely occluded duct with atypical epithelial cells. *D*, glands treated with 10 μM resveratrol for the first 10 days of culture demonstrate normal mammary ductal structures comparable to control. *E*, dose-response curve for the inhibition of DMBA-induced atypical ductal (hyperplastic and aggressive) lesions by resveratrol. Mouse mammary glands were treated with the indicated concentrations of resveratrol, as described above. The histological sections of the glands were divided into three regions, and each region was evaluated for the number of normal (single-layer thickness of epithelial cells), hyperplastic ($\geq$3 layers of thickness), or aggressive (highly occluded, with no or very little lumen) areas. Data points indicate the mean percentage of incidence of atypical lesions in resveratrol-treated glands. Original magnification for *A–D*, ×40.

whereas 42% incidence was found in the control group. Overall, the median time for appearance of the first tumor was 79.5 days in the high-dose resveratrol group compared with 51.5 days in the control group. Thus, resveratrol caused a 28-day increase in tumor latency ($P = 0.0326$, unpaired two-tailed t test). In addition, at the termination of the study, resveratrol treatment (100 mg/kg) reduced the multiplicity of tumors from 6.0 in the control group to 3.9 in the treatment groups ($P = 0.0015$, Armitage test for trends in proportions, one-tailed test; Fig. 5*C*), and there was a decrease in the total number of tumors (Fig. 5*D*). In the low-dose resveratrol group, no significant differences in percentage incidence, tumor latency, or multiplicity were observed (Fig. 5, *A–D*).

Tumor Morphology. Histomorphology of 15 mammary tumors derived from rats treated with high-dose resveratrol was compared with control tumors. Control tumors were composed mainly of alveolar or papillary structures (Fig. 6*A*); in the majority of the tumors (11 of 15 tumors), central necrosis was observed. In animals treated with resveratrol, an overall increase in differentiated alveolar structures among tumor parenchyma was observed. In addition, resveratrol facilitated focal reduction of cell layers within alveolar structures, with simultaneous formation of numerous luminal openings in these areas as a result of tissue disintegration (Fig. 6*B*). Necrosis was seen in small areas of some (5 of 15) tumors from resveratrol-treated animals, as were apoptotic cells with typical shrinkage of the cytoplasm and condensation of chromatin (Fig. 6*C*). Apoptotic cells in tumors from resveratrol-treated animals were noted predominantly in the peripheral areas, unlike tumors from control animals, in which apoptotic cells predominated in the central areas and close to areas of necrosis. Thus, resveratrol treatment increased alveolar and adipocyte differentiation of mammary tumor cells (Fig. 6*D*).

DISCUSSION

Although a number of previous studies have been conducted, the potential of resveratrol to function as a SERM remains unclear. For example, whereas it was shown with MCF-7 cells in culture that resveratrol is a superagonist when combined with E_2 (17), resveratrol was also shown to be antiestrogenic with the same cell line (18, 19). Similarly, the ability of resveratrol to interact with ER is not conclusive. Whereas some researchers have shown that resveratrol binds to ER from MCF-7 cells ($IC_{50} = \sim 10$ μM) and rat uterine cytosolic extracts ($IC_{50} = \sim 100$ μM; Refs. 17 and 29), other studies have shown that resveratrol does not bind to ER in cell extracts from PR1 pituitary cells or partially purified mouse uterus, even at concentrations as high as 100 μM (21, 30). More recently, it was shown that resveratrol acts as a mixed agonist/antagonist in cells transiently transfected with ER and mediates higher transcriptional activity when bound to ER-β than to ER-α (22). Moreover, resveratrol showed antagonist activity with ER-α, but not with ER-β (22). In accord with these data, we have recently observed that resveratrol mediates antiestrogenic effects in endometrial cancer (Ishikawa) cells by a novel mechanism that involves selective down-regulation of ER-α but not ER-β manifested as suppression of estrogen-dependent alkaline phos-

Table 1 *Effect of resveratrol on percentage of incidence of atypical ductal lesions in mouse mammary organ culture[a]*

Group	Normal regions	Hyperplastic lesions	Atypical lesions
DMBA	37.0 ± 8.1	23.4 ± 6.8	39.6 ± 11.1
DMBA + resveratrol (10 μM)	79.2 ± 15.4 (<0.001)	15.3 ± 1.5 (<0.01)	5.5 ± 0.2 (<0.001)
DMBA + resveratrol (5 μM)	79.4 ± 23.6 (<0.001)	16.2 ± 5.1 (<0.01)	4.9 ± 1.0 (<0.001)
DMBA + resveratrol (2.5 μM)	43.5 ± 12.3[b]	18.4 ± 2.5[b]	38.1 ± 7.8[b]
DMBA + resveratrol (1 μM)	31.5 ± 12.1[b]	26.8 ± 4.5[b]	41.7 ± 12.7[b]

[a] Values represent the mean percentage of each region in total glands that were classified as normal, hyperlastic, and aggressive, as defined in "Materials and Methods" ± SEs ($n = 10$). Numbers shown in parentheses are Ps of a two-tailed t test for independent samples.
[b] Not significantly different from the control group that received only DMBA.
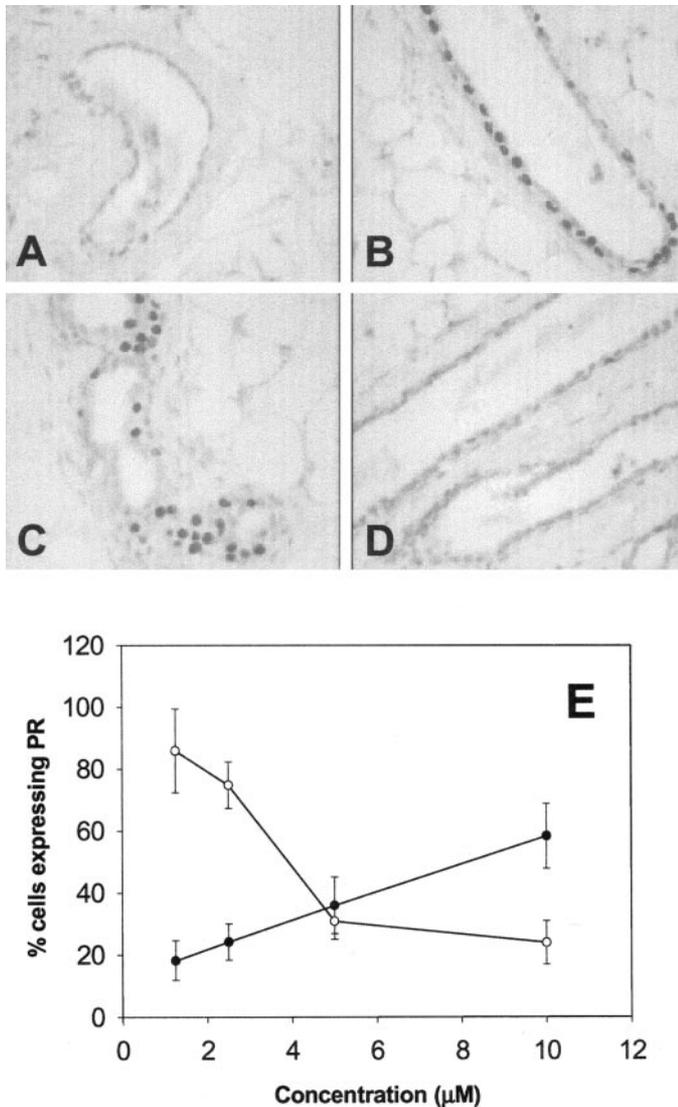
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Fig. 4. Immunohistochemistry of PR expression in MMOC. Mouse mammary glands in culture were incubated with insulin and prolactin for 6 days. At termination, histological preparations were made, and slides were subjected to PR immunohistochemical analysis as described in "Materials and Methods." *A,* control glands show very few cells expressing PR in the epithelial cells. *B,* glands treated with E_2 (1 nM) show induced PR expression. *C,* glands treated with resveratrol (10 μM) also show induction of PR expression. *D,* glands treated with E_2 (1 nM) plus resveratrol (10 μM) show minimal expression of PR. *E,* dose-response curve for PR expression by resveratrol in MMOC. Mouse mammary glands were treated with the indicated concentrations of resveratrol with ($\bigcirc$) or without ($\bullet$) E_2 as described above. The proportion of cells expressing PR was calculated as described in "Materials and Methods," and the percentage of induction of PR in the glands was represented as mean $\pm$ SD. Original magnification for *A–D,* $\times$40.

phatase and ERE-luciferase activities, as well as PR and α1 integrin expression (26). To further clarify the estrogen-modulatory effects of resveratrol, we examined its effect with *in vitro* and *in vivo* mammary models.

In the first set of experiments, we examined the effect of resveratrol on ERE-luciferase activity in various transiently transfected mammary cancer cell lines. Consistent with previous reports, resveratrol, when administered alone, stimulated luciferase activity at concentrations ranging from 5–15 μM in MCF-7 cells. However, luciferase activity induced by 1 nM E_2 was antagonized by resveratrol in the same concentration range, and, in contrast to previous work (17), no superagonist activity was observed under any experimental conditions. Because previous studies (17) had used ethanol as the solvent, and ethanol has the potential to induce ER-α mammary cancer cells

(31, 32), it may be speculated that synergistic effects of resveratrol and ethanol could explain the reported superagonist activity of resveratrol. Also, because it is known that resveratrol is UV light and pH sensitive in solution (33), we speculated that improper storage could lead to degraded products capable of mediating superagonism. However, in studies conducted with MCF-7 cells and resveratrol that was intentionally degraded, superagonist activity was not observed (34). In the current report, a profile similar to that of MCF-7 cells (estrogenic alone and antiestrogenic when combined with E_2) was observed when S30 cells were treated with resveratrol, whereas with T47D and LY2 cells, resveratrol acted as a pure antagonist.

Induction of breast cancer cell growth, up-regulation of estrogen-inducible genes, and tissue-, species-, and promoter-specific suppression of these parameters in the presence of E_2 are some of the classic effects of partial estrogens such as 4-hydroxytamoxifen (35–37). However, in the case of resveratrol, the differential activities observed with various cell lines may be due to the relative expression levels of ER-α and ER-β. As demonstrated by Pettersson *et al.* (38), heterodimerization of ER-α and ER-β has a major influence on cellular responses to agonists and antagonists; ER-β has the ability to dominantly regulate the activity of the α/β heterodimer positively (genistein) and negatively (tamoxifen), probably by modifying the activity of ER-α AF-1. Although a comprehensive study to establish the relative levels of these receptors in mammary cancer cell lines remains to be done, some reports have indicated that ER-β is virtually absent in MCF-7 cells but is expressed highly in T47D cells (39–41). S30 cells have been stably transfected with ER-α and contain low levels of ER-β (42), and data are not available with respect to the relative levels of the ER subtypes in LY2 cells. Nonetheless, based on the response profile currently reported (Fig. 1), it is reasonable to suggest that resveratrol exerts partial agonist activity in tissues that express predominantly ER-α (*e.g.,* MCF-7 and S30) but exerts antagonist activity when ER-β is coexpressed (*e.g.,* T47D). Interestingly, both T47D and LY2, in which resveratrol acted as a pure antagonist, are antiestrogen-resistant cell lines (43). Therefore, it would be reasonable to evaluate resveratrol in combination with an agent such as tamoxifen.

We then studied the expression of two estrogen-responsive proteins, PR and pS2. MCF-7 and T47D cells were compared for expression of PR. With MCF-7 cells, consistent with the transfection experiments, PR was induced by treatment with resveratrol as a single agent and suppressed when resveratrol was given in combination with E_2. With T47D cells, even after long-term replacement with DCC-FBS, basal levels of PR were not reduced. Nonetheless, PR protein levels were down-regulated on treatment with resveratrol. In LY2 and S30 cells, resveratrol suppressed pS2 expression irrespective of the presence of E_2. Although these data do not correlate directly with the transient transfection studies performed with these cells, it is possible that the natural, imperfect ERE sequences for PR and pS2, which differ slightly from the consensus sequence, may account for the differential effects. For example, the ERE from the human pS2 gene, which varies from the consensus ERE by one base change in the right arm, binds to ER with 3-fold lower affinity than the vitellogenin A_2 ERE (44).

Recently, we established a model in which DMBA can induce estrogen- and progesterone-dependent mammary ductal lesions with high frequency in organ culture (45). Several estrogen modulators, including tamoxifen, ICI182780, toremefine, and aminoglutethimide, selectively suppress proliferation of estrogen- and progesterone-induced end-bud proliferation and development of hyperplastic ducts, indicating the general utility of the model (45). In a similar manner, resveratrol suppressed the formation of these lesions in a dose-dependent manner. Although resveratrol can inhibit ovarian hormone-independent, DMBA-induced alveolar lesions in MMOC (16), this novel model that selectively induces the formation of ductal hyperplasia in the presence of ovarian hormones (E_2 + progesterone) indicates that
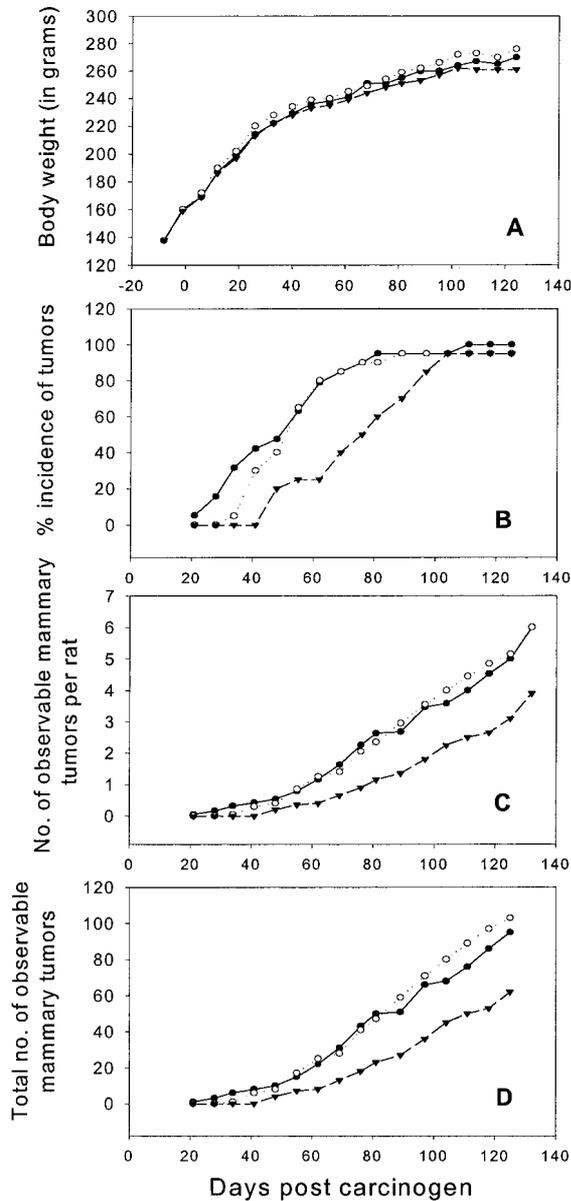
Fig. 5. Effect of resveratrol on (*A*) body weight, (*B*) percentage incidence of mammary tumors, (*C*) number of observable mammary tumors/rat, and (*D*) total number of observable mammary tumors in female Sprague Dawley rats given a single i.v. dose of NMU. As described in "Materials and Methods," the rat treatment groups were as follows: ○, NMU in sesame oil; ●, NMU and 10 mg/kg body weight resveratrol administered i.g; and ▼, NMU and 100 mg/kg body weight resveratrol administered i.g.

prevention studies (49, 50). Resveratrol caused a significant suppression of tumor multiplicity and increased the latency period by 27 days. Tumor incidence in resveratrol-treated animals was reduced by ~50% for 69 days, after which it gradually increased until the end of the experiment, when it reached that of control animals (95%). Histopathological examination revealed response to resveratrol treatment, as evidenced by increased alveolar and adipocyte differentiation and the occurrence of apoptotic cells within peripheral tumor areas, which are rare in these areas. It is, of course, difficult to affirm whether the chemopreventive response mediated by resveratrol is due exclusively to antiestrogenic effects. However, our observation that resveratrol delays the occurrence of mammary tumors and suppresses incidence at the early time points (until day 69) suggests that the agent may be more efficacious in early stages, when hyperplastic and premalignant lesions predominate in the mammary gland. These lesions are also small in size and composed entirely of ER^+ cells (51, 52), and their response to ER agonists or antagonists should be more profound than the response of large mammary tumors, in which a selection of ER^- tumor cells may also occur and thus nullify antiestrogenic effects. Studying the effect of resveratrol in rats in which E_2 administration reactivates NMU tumor growth after an ovariectomy-induced tumor regression would be significant in this regard. Also, it may be of interest to investigate whether higher does of resveratrol (>100 mg/kg) can cause further reduction of the incidence and multiplicity of tumors. Although relatively high doses of a chemopreventive agent may be scrutinized for physiological relevance, justification for using such doses is provided by the necessity of inducing tumors in model systems by treatment with high doses of chemical carcinogens. In addition, the effect of metabolism needs to be investigated because studies have shown that resveratrol, when administered p.o. in rats, is sulfated, and hepatic and duodenal sulfation might limit the bioavailability of this compound (53). Alternatively, metabolites themselves may mediate biological responses, as in the case of tamoxifen.

In conclusion, the data presented in this study emphasize the mixed estrogenic/antiestrogenic activity of resveratrol in mammary tissues. In the absence of E_2, resveratrol weakly induced ER-dependent transcriptional events in some mammary tumor cell lines, but down-regulation was invariably observed when the agent was coadministered with the

resveratrol functions as an antiestrogen. Furthermore, one of the initial effects of E_2 in mammary cell differentiation is the induction of estrogen-inducible genes including PR (46, 47). In the current report, we have shown that expression of PR is localized in the epithelial lining of glands incubated with insulin, prolactin, and E_2 and that treatment with resveratrol for 6 days reduced induction of PR. These data indirectly suggest a regulatory role of progesterone and PR in carcinogenesis and are consistent with a recent report in which PR knockout mice developed fewer DMBA-induced tumors as compared with the isogenic wild-type mice (48). It would be of interest to evaluate resveratrol in this model.

In the current study, we did examine the effect of resveratrol on the formation of NMU-induced mammary tumors in Sprague Dawley rats. The etiology of this model is generally considered relevant to human breast cancer, and it has been widely used in various chemo-
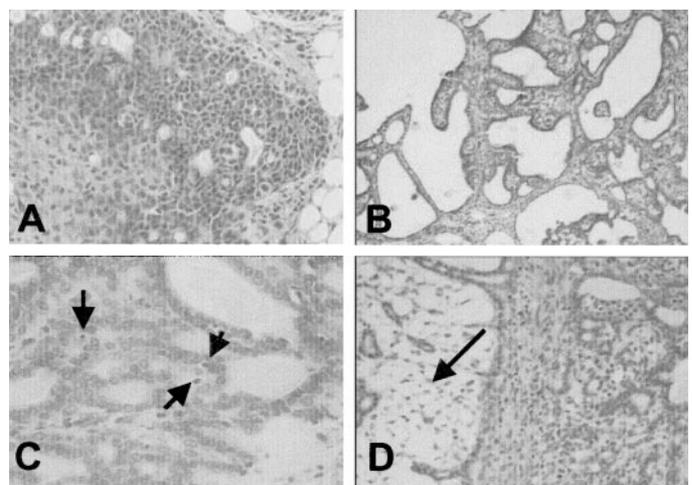

Fig. 6. Histomorphology of mammary adenocarcinoma in rats treated with NMU. At the end of the study (120 days), animals were sacrificed, and mammary tumors were removed, fixed in formalin, processed, and stained with H&E for histopathological evaluation. *A*, control mammary tumor composed of alveolar and solid cellular structures. *B*, loss of cellularity and formation of cystic structures within parenchyma in a tumor from a rat treated with resveratrol (100 mg/kg body weight, i.g.). *C*, apoptotic cells identified with condensed chromatin (*arrows*) in a tumor from a resveratrol-treated rat. *D*, adipocyte differentiation (*arrow*) in parenchyma of a tumor from a resveratrol-treated rat. Original magnification: *A*, *B*, and *D*, ×20; *C*, ×40.

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hormone. In mouse mammary glands grown in culture, resveratrol inhibited the formation of DMBA-induced, E_2-promoted, atypical ductal hyperplasia. Furthermore, resveratrol inhibited the early stages of NMU-induced mammary carcinogenesis when administered to female Sprague Dawley rats. In conjunction with previous work reported in the literature, these studies support the suggestion that resveratrol is a novel SERM that may be useful for the chemoprevention of breast cancer.

ACKNOWLEDGMENTS

REFERENCES

1. Landis, S. H., Murray, T., Bolden, S., and Wingo, P. A. Cancer statistics. CA Cancer J. Clin., *49:* 8–31, 1999.
2. Rosenbaum Smith, S. M., and Osborne, M. P. Breast cancer chemoprevention. Am. J. Surg., *180:* 249–251, 2000.
3. Dunn, B. K., Kramer, B. S., and Ford, L. G. Phase III, large-scale chemoprevention trials. Approach to chemoprevention clinical trials and Phase III clinical trial of tamoxifen as a chemopreventive for breast cancer: the US National Cancer Institute experience. Hematol. Oncol. Clin. N. Am., *12:* 1019–1036, 1998.
4. Love, R. R., Mazess, R. B., Barden, H. S., Epstein, S., Newcomb, P. A., Jordan, V. C., Carbone, P. P., and DeMets, D. L. Effects of tamoxifen on bone mineral density in postmenopausal women with breast cancer. N. Engl. J. Med., *326:* 852–856, 1992.
5. MacGregor, J. I., and Jordan, V. C. Basic guide to the mechanisms of antiestrogen action. Pharmacol. Rev., *50:* 151–196, 1998.
6. Fisher, B., Costantino, J. P., Wickerham, D. L., Redmond, C. K., Kavanah, M., Cronin, W. M., Vogel, V., Robidoux, A., Dimitrov, N., Atkins, J., Daly, M., Wieand, S., Tan-Chiu, E., Ford, L., and Wolmark, N. Tamoxifen for prevention of breast cancer: report of the National Surgical Adjuvant Breast and Bowel Project P-1 Study. J. Natl. Cancer Inst. (Bethesda), *90:* 1371–1388, 1998.
7. Gustafsson, J-A. Raloxifene. Magic bullet for the heart and bone? Nat. Med., *4:* 152–153, 1998.
8. Rhodes, D. J., Hartmann, L. C., and Perez, E. A. Breast cancer prevention trials. Curr. Oncol. Rep., *2:* 558–565, 2000.
9. Wade, C., Kronenberg, F., Kelly, A., and Murphy, P. A. Hormone-modulating herbs: implications for women's health. J. Am. Med. Women's Assoc., *54:* 181–183, 1999.
10. Zava, D. T., Dollbaum, C. M., and Blen, M. Estrogen and progestin bioactivity of foods, herbs, and spices. Proc. Soc. Exp. Biol. Med., *217:* 369–378, 1998.
11. Davis, S. R., Dalais, F. S., Simpson, E. R., and Murkies, A. L. Phytoestrogens in health and disease. Recent Prog. Horm. Res., *54:* 185–210, 1999.
12. Adlercreutz, C. H., Goldin, B. R., Gorbach, S. L., Hockerstedt, K. A., Watanabe, S., Hamalainen, E. K., Markkanen, M. H. Makela, T. H., Wahala, K. T., and Adlercreutz, T. Soybean phytoestrogen intake and cancer risk. J. Nutr., *125:* 757S–770S, 1995.
13. Key, T. J., Chen, J., Wang, D. Y., Pike, M. C., and Boreham, J. Sex hormones in women in rural China and in Britain. Br. J. Cancer, *62:* 631–636, 1990.
14. Rose, D. P. Dietary fiber, phytoestrogens, and breast cancer. Nutrition, *8:* 47–54, 1992.
15. Wiseman, H. The therapeutic potential of phytoestrogens. Expert Opin. Investig. Drugs, *9:* 1829–1840, 2000.
16. Jang, M., Cai, L., Udeani, G. O., Slowing, K. V., Thomas, C. F., Beecher, C. W. W., Fong, H. H., Farnsworth, N. R., Kinghorn, A. D., Mehta, R. G., Moon, R. C., and Pezzuto, J. M. Cancer chemopreventive activity of resveratrol, a natural product derived from grapes. Science (Wash. DC), *275:* 218–220, 1997.
17. Gehm, B. D., McAndrews, J. M., Chien, P. Y., and Jameson, J. L. Resveratrol, a polyphenolic compound found in grapes and wine, is an agonist for the estrogen receptor. Proc. Natl. Acad. Sci. USA, *94:* 14138–14143, 1997.
18. Lu, R., and Serrero, G. Resveratrol, a natural product derived from grape, exhibits antiestrogenic activity and inhibits the growth of human breast cancer cells. J. Cell. Physiol., *179:* 297–304, 1999.
19. Mgbonyebi, O. P., Russo, J., and Russo, I. H. Antiproliferative effect of synthetic resveratrol on human breast epithelial cells. Int. J. Oncol., *12:* 865–869, 1998.
20. Mizutani, K., Ikeda, K., Kawai, Y., and Yamori, Y. Resveratrol stimulates the proliferation and differentiation of osteoblastic MC3T3–E1 cells. Biochem. Biophys. Res. Commun., *253:* 859–863, 1998.
21. Yoon, K., Pellaroni, L., Ramamoorthy, K., Gaido, K., and Safe, S. Ligand structure-dependent differences in activation of estrogen receptor α in human HepG2 liver and U2 osteogenic cancer cell lines. Mol. Cell. Endocrinol., *162:* 211–220, 2000.
22. Bowers, J. L., Tyulmenkov, V. V., Jernigan, S. C., and Klinge, C. M. Resveratrol acts as a mixed agonist/antagonist for estrogen receptors α and β. Endocrinology, *141:* 3657–3667, 2000.
23. Mizutani, K., Ikeda, K., Kawai, Y., and Yamori, Y. Resveratrol attenuates ovariectomy-induced hypertension and bone loss in stroke-prone spontaneously hypertensive rats. J. Nutr. Sci. Vitaminol. (Kyoto), *46:* 78–83, 2000.
24. Turner, R. T., Evans, G. L., Zhang, M., Maran, A., and Sibonga, J. D. Is resveratrol an estrogen agonist in growing rats? Endocrinology, *140:* 50–54, 1999.
25. Freyberger, A., Hartmann, E., Hildebrand, H., and Krotlinger, F. Differential response of immature rat uterine tissue to ethinylestradiol and the red wine constituent resveratrol. Arch. Toxicol., *11:* 709–715, 2001.
26. Bhat, K. P. L., and Pezzuto, J. M. Resveratrol exhibits cytostatic and antiestrogenic properties with human endometrial adenocarcinoma (Ishikawa) cells. Cancer Res., *61:* 6137–6144, 2001.
27. Mehta, R. G., Hawthorne, M. E., and Steele, V. E. Induction and prevention of carcinogen induced precancerous lesions in mouse mammary gland organ culture. Methods Cell Sci., *19:* 19–24, 1997.
28. Hata, H., and Kuramoto, H. Immunocytochemical determination of estrogen and progesterone receptors in human endometrial adenocarcinoma cells (Ishikawa cells). J. Steroid Biochem. Mol. Biol., *42:* 201–210, 1992.
29. Stahl, S., Chun, T-Y., and Gray, W. G. Phytoestrogens act as estogen agonists in an estrogen-responsive pituitary cell line. Toxicol. Appl. Pharmacol., *152:* 41–48, 1998.
30. Ashby, J., Tinwell, H., Pennie, W., Brooks, A. N., Lefevere, P. A., Beresford, N., and Sumpter, J. P. Partial and weak oestrogenecity of the red wine constituent resveratrol: consideration of its superagonist activity in MCF-7 cells and its suggested cardiovascular protective effects. J. Appl. Toxicol., *19:* 39–45, 1999.
31. Fan, S., Meng, Q., Gao, B., Grossman, J., Yadegari, M., Goldberg, I. D., and Rosen, E. M. Alcohol stimulates estrogen receptor signaling in human breast cancer cell lines. Cancer Res., *60:* 5635–5639, 2000.
32. Singletary, K. W., Frey, R. S., and Yan, W. Effect of ethanol on proliferation and estrogen receptor-α expression in human breast cancer cells. Cancer Lett., *165:* 131–137, 2001.
33. Trela, B. C., and Waterhouse, A. L. Resveratrol: isomeric molar absorptivities and stability. J. Agric. Food Chem., *44:* 1253–1257, 1996.
34. Bhat, K. P. L., Kosmeder, J. W., II, and Pezzuto, J. M. Biological effects of resveratrol. Antioxid. Redox Signal., in press, 2001.
35. Schutze, N., Schneider, M. R., Knuppen, R., and Vollmer, G. Tamoxifen and ZK 119010 exert mixed agonistic and antagonistic effects on pS2 expression in MCF-7 cells. Cancer Detect. Prev., *18:* 479–483, 1994.
36. Berry, M., Metzger, D., and Chambon, P. Role of the two activating domains of the oestrogen receptor in the cell-type and promoter-context dependent agonistic activity of the anti-oestrogen 4-hydroxytamoxifen. EMBO J., *9:* 2811–2818, 1990.
37. Tzukerman, M. T., Esty, A., Santiso-Mere, D., Danielian, P., Parker, M. G., Stein, R. B., Pike, J. W., and McDonnell, D. P. Human estrogen receptor transactivational capacity is determined by both cellular and promoter context and mediated by two functionally distinct intramolecular regions. Mol. Endocrinol., *8:* 21–30, 1994.
38. Pettersson, K., Delaunay, F., and Gustafsson, J. A. Estrogen receptor β acts as a dominant regulator of estrogen signaling. Oncogene, *19:* 4970–4978, 2000.
39. Vladusic, E. A., Hornby, A. E., Guerra-Vladusic, F. K., Lakins, J., and Lupu, R. Expression and regulation of estrogen receptor β in human breast tumors and cell lines. Oncol. Rep., *7:* 157–167, 2000.
40. Dotzlaw, H., Leygue, E., Watson, P. H., and Murphy, L. C. Estrogen receptor-β messenger RNA expression in human breast tumor biopsies: relationship to steroid receptor status and regulation by progestins. Cancer Res., *59:* 529–532, 1999.
41. Kurebayashi, J., Otsuki, T., Kunisue, H., Tanaka, K., Yamamoto, S., and Sonoo, H. Expression levels of estrogen receptor-α, estrogen receptor-β, coactivators, and corepressors in breast cancer. Clin. Cancer Res., *6:* 512–518, 2000.
42. Jiang, S. Y., Langan-Fahey, S. M., Stella, A. L., McCague, R., and Jordan, V. C. Point mutation of estrogen receptor (ER) in the ligand-binding domain changes the pharmacology of antiestrogens in ER-negative breast cancer cells stably expressing complementary DNAs for ER. Mol. Endocrinol., *6:* 2167–2174, 1992.
43. Mullick, A., and Chambon, P. Characterization of the estrogen receptor in two antiestrogen-resistant cell lines, LY2 and T47D. Cancer Res., *50:* 333–338, 1990.
44. Nikov, G. N., Hopkins, N. E., Boue, S., and Alworth, W. L. Interactions of dietary estrogens with human estrogen receptors and the effect on estrogen receptor-estrogen response element complex formation. Environ. Health Perspect., *108:* 867–872, 2000.
45. Mehta, R. G., Bhat, K. P. L., Hawthorne, M. E., Kopelovich, L., Mehta, R. R., Christov, K., Kelloff, G. J., Steele, V. E., and Pezzuto, J. M. Induction of atypical ductal hyperplasia in mouse mammary gland organ culture. J. Natl. Cancer Inst. (Bethesda), *93:* 1103–1106, 2001.
46. Bellamy, C. O. C., McDonald, C., Salter, D. M., Chetty, U., and Anderson, T. J. Noninvasive ductal cacinoma of the breast: the relevance of histological categorization Hum. Pathol., *24:* 16–23, 1993.
47. Brown, M. Estrogen receptor molecular biology. Hematol. Oncol. Clin. N. Am., *8:* 101–112, 1994.
48. Lydon, J. P., Ge, G., Kittrell, F. S., Medina, D., and O'Malley, B. W. Murine mammary gland carcinogenesis is critically dependent on progesterone receptor function. Cancer Res., *59:* 4276–4284, 1999.
49. Moon, R. C., and Mehta, R. G. Cancer chemoprevention by retinoids: animal models. Methods Enzymol., *190:* 395–406, 1990.
50. Moon, R. C., Mehta, R. G., Detrisac, C. J., and Ratko, T. A. Chemoprevention of experimental breast cancer. *In:* U. Pastorino and W. K. Hong (eds.), Chemoimmunoprevention of Cancer. First International Conference, Vienna, Austria, pp. 57–63. New York: Thieme Medical Publishers, Inc., 1991.
51. Moon, R. C., and Mehta, R. G. Retinoid binding in normal and neoplastic mammary tissue. *In:* W. L. Leavitt (ed.), Hormones and Cancer (Advances in Experimental Medicine and Biology), pp. 231–234. New York: Plenum Press, 1982.
52. McCormick, D. L., Adamowski, C. B., Fiks, A., and Moon, R. C. Lifetime dose-response relationships or mammary tumor induction by a single administration of *N*-methyl-*N*-nitrosourea. Cancer Res., *41:* 1690–1694, 1981.
53. De Santi, C., Pietrabissa, A., Spisni, R., Mosca, F., and Pacifici, G. M. Sulphation of resveratrol, a natural product present in grapes and wine, in the human liver and duodenum. Xenobiotica, *30:* 609–617, 2000.

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Estrogenic and Antiestrogenic Properties of Resveratrol in Mammary Tumor Models

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