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Effect of Resveratrol on the Expression of Autocrine Growth Modulators in Human Breast Cancer Cells

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ABSTRACT

The effect of resveratrol on the growth of human breast cancer cells was examined. Resveratrol inhibited the growth of estrogen receptor-positive MCF-7 cells cultivated in the presence of estradiol in a dose-dependent fashion. At 10^{-5} *M*, resveratrol maximally inhibited the growth stimulatory effect mediated by 10^{-9} *M* estradiol without affecting cell viability. At the molecular level, resveratrol in a dose-dependent fashion antagonized the stimulation by estradiol of an estrogen response element reporter gene construct and of progesterone receptor gene expression in MCF-7 cells. Resveratrol also inhibited the proliferation of the estrogen-receptor negative human breast carcinoma cell line MDA-MB-468. These later data suggest that resveratrol can also inhibit breast cancer cell proliferation by another mechanism besides estrogen receptor antagonism. We show here that resveratrol altered the expression of several autocrine growth modulators and their receptors in MCF-7 cells. Resveratrol at 10^{-5} *M* inhibited the expression of the autocrine growth stimulators transforming growth factor-α (TGF-α), PC cell-derived growth factor, and insulin-like growth factor I receptor mRNA. In addition, resveratrol significantly elevated the expression of the growth inhibitor TGF-β2 mRNA without changes in TGF-β1 and TGF-β3 expression. These data suggest that resveratrol inhibits proliferation by altering autocrine growth modulator pathways in breast cancer cells. Antioxid. Redox Signal. 3, 969–979.

INTRODUCTION

RESVERATROL (3,5,4′-TRIHYDROXYSTILBENE), a bioflavonoid found in grapes and mulberries, was first regarded as playing an important role in the host defense mechanism against infection and injury (11). Studies have attributed to resveratrol the cardioprotective effect observed in people with moderate wine consumption as red wine is believed to be the main source of resveratrol in the human diet. Resveratrol has protective effects against oxidation of lipoproteins (16), inhibits platelet aggregation, and alters eicosanoid synthesis (48). Recently, resveratrol has acquired a renewed interest because of its chemopreventive activity and its effect as an inhibitor of tumor cell proliferation, tumor initiation, promotion, and progression (15, 17, 23, 41).

Bioflavonoids are major constituents in plants and vegetables (21). Some of them have been categorized as phytoestrogens because these environmentally derived compounds bind and activate the estrogen receptor (ER) although they are less active than endogenous estrogens (40). Moreover, several flavonoids have

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an antiestrogenic effect by preventing more potent endogenous estrogen from binding to ER, similarly to tamoxifen (9, 36, 37). These properties have been used to explain the chemopreventive effect of this class of compounds and the low incidence of breast cancer and prostate cancer among vegetarians and Orientals, who normally have higher blood levels of phytoestrogens (1, 36, 47).

Recently, it has been reported that resveratrol can bind and activate ER (19). Resveratrol has been shown to act as a mixed agonist/antagonist for ER α and β (5). This would explain the fact that resveratrol can substitute for estrogen in stimulating the proliferation of ER-positive breast cancer cells (5, 32). In the presence of estrogen, resveratrol was shown to act as an estrogen antagonist resulting in the inhibition of proliferation of the ER-positive MCF-7 cells (32). In the present article, we examined the effect of resveratrol in combination with estrogen on the growth of ER-positive breast cancer MCF-7 cells, as well as its effect on the proliferation of ER-negative MDA-MB-468 cells at both the cellular and molecular levels.

MATERIALS AND METHODS

Cell culture

MCF-7 cells and MDA-MB-468 cells were obtained from the American Type Culture Collection (ATCC) and maintained in DME-F12 medium (1:1 mixture of Dulbecco's modified Eagle's medium and Ham's F12 medium) supplemented with 5% fetal bovine serum (FBS) (Life Technologies).

Effect of resveratrol on the proliferation of MCF-7 cells and MDA-MB-468 cells

Experiments were carried out with cells cultivated in six-well plates (10^5 cells per well) in DME-F12 medium supplemented with 5% FBS in the presence or absence of increasing concentrations of resveratrol (Sigma, St. Louis, MO, U.S.A.). To measure the effect of resveratrol in estrogen-depleted medium, the cells were plated in 24-well plates at 5×10^4 cells per well in 1 ml of phenol red-free α-modified Eagle's medium (α-MEM) supplemented with 5% charcoal-stripped FBS (PFMEM) for 24 h. The medium was then removed and replaced with fresh PFMEM medium in the presence or absence of various concentrations of resveratrol and 17β-estradiol (E_2). Cells were counted with a hemocytometer after the cells were detached with trypsin-EDTA.

RNA isolation and reverse transcriptase–polymerase chain reaction (RT-PCR)

mRNA expression for transforming growth factor-α (TGF-α), transforming growth factor-β1, -β2, and -β3 (TGF-β1, -β2, and -β3), insulin-like growth factor I (IGF-I), insulin-like growth factor II (IGF-II), PC cell-derived growth factor (PCDGF), or growth factor receptors such as IGF-I receptor (IGF-IR), TGF-β receptors, and epidermal growth factor receptor were measured by RT-PCR of RNA prepared from cells treated or not with E_2 and/or resveratrol. Progesterone receptor (PR) mRNA expression were measured as described previously (35). Specific mRNA expression was examined by RT-PCR using human β-actin mRNA expression as an internal control for RNA equal loading. Five micrograms each of total RNA from resveratrol-treated cells and control cells was reverse-transcribed by random primer and Superscript II reverse transcriptase. The resulting cDNA was subjected to PCR with the appropriate set of primers. The primers used for amplification of PR, IGF-IR, and PCDGF were synthesized according to published sequences (4, 22, 43). The primers for TGF-α, TGF-β2, and β-actin were obtained from Clontech (Palo Alto, CA, U.S.A.). The amplification reactions were performed with an initial incubation step at 94°C for 3 min followed by 25 cycles each (30 cycles for TGF-β2, TGF-β) at 94°C for 1 min, 60°C for 45 s, 72°C for 2 min. These cycles were followed by a final incubation step at 72°C for 7 min. The samples were subjected to electrophoresis in 1.2% agarose gel and stained with ethidium bromide. The identities of PCR products were confirmed by restriction enzyme digestion and by southern blot analysis using the corresponding sense primer for each PCR product as a probe that had been end-labeled with [γ-^{32}P]ATP using T4 polynucleotide kinase. The RT-PCR analysis was repeated at

least three times for each of two independent experiments. The sizes of amplified PCR fragments were as follows: 297 bp for TGF-α, 755 bp for IGF-IR, 838 bp for β-actin, 415 bp for TGF-β2, 742 bp for PR, and 750 bp for PCDGF.

For estrogen responsive element–luciferase (ERE-LUC) activity reporter gene assay, MCF-7 cells were transfected with ERE-LUC gene construct as described previously (35) for 6 h prior to treatment with E_2 (10^{-9} *M*) and resveratrol for an additional 24 h. Transfection efficiency was determined by cotransfecting the cells with plasmid DNA from a β-galactosidase reporter gene construct containing a cytomegalovirus promoter used as a standard. ERE-LUC activity and β-galactosidase activity were measured with a luminometer. Values for ERE-LUC activity were normalized to the β-galactosidase values.

Statistics

Experiments were carried out in triplicate and data were expressed as means ± SD. Two-tailed Student's *t* test was used for statistical analysis of the data. $p < 0.05$ was taken as the level of significance.

RESULTS

Effect of resveratrol on the growth of MCF-7 cells

MCF-7 cells were plated in DME-F12 medium supplemented with 5% FBS in the absence or presence of increasing concentrations of resveratrol. As shown in Fig. 1A, resveratrol inhibited the growth of MCF-7 cells in a dose-dependent fashion. After 6 days, 10^{-5} *M* resveratrol inhibited by 80% MCF-7 cell growth. The cells had a doubling time of 50 h, whereas control cells doubled every 24 h. We examined whether the growth inhibitory effect of resveratrol was reversible. After removal and extensive washing of resveratrol from the medium, the MCF-7 cells did not show any stimulation of proliferation, suggesting that the effect of resveratrol was irreversible (Fig. 1B). In addition, cells that had been incubated for 3 days with resveratrol (10^{-5} *M*) and were replated in medium without resveratrol, still continued to grow at a reduced rate (data not shown). These data would provide additional support to the irreversibility of the growth inhibitory effect of resveratrol. One possible explanation is that resveratrol may have induced differentiation of the MCF-7 cells leading to a reduced growth rate. However, the expression of lactalbumin, a differentiation marker of mammary cells (45) measured by immunocytochemistry, was inhibited rather than elevated in the resveratrol-treated cells, suggesting that resveratrol did not stimulate MCF-7 cell differentiation. Resveratrol did not change the expression of a MUC-1 mucin gene, a marker associated with the degree of malignancy of

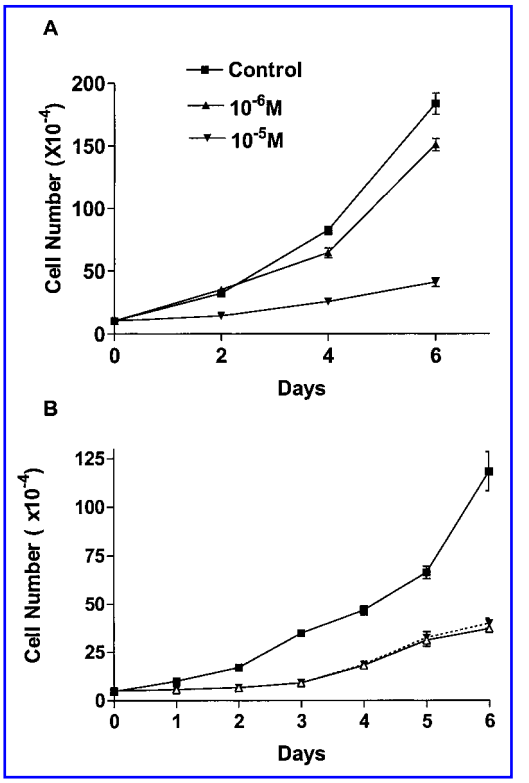

FIG. 1. Effect of resveratrol on the growth of MCF-7 cells cultivated in regular medium. (**A**) MCF-7 cells were plated at 10^5 cells per well in 24-well plates in DME-F12 medium plus 5% FBS either in the absence (0.1% ethanol only) or in the presence of the indicated concentrations of resveratrol. Cell number per well was determined every other day until day 6. Values are expressed as means ± SD of triplicate determinations. (**B**) MCF-7 cells were plated as described before in the absence (■) or presence (△) of 10^{-5} *M* resveratrol. After 3 days, resveratrol was removed from half of the plates that had been treated with resveratrol since day 1, whereas the other half continued to be treated with resveratrol (▲). Cells were counted every day. Values are expressed as means ± SD of triplicate experiments.

breast cancer (49). Apparently, the irreversible inhibition by resveratrol of the growth of MCF-7 cell was due to reasons other than induction of differentiation.

No evidence of apoptosis measured with the ApoAlert Annexin V Apoptosis kit was observed in cells treated with 10^{-5} *M* resveratrol, although a significant inhibition of cell growth was observed. Cell viability was not affected by resveratrol except at 10^{-4} *M*, where only ~50% of the treated cells remained viable (data not shown).

Resveratrol antagonizes E_2-mediated stimulation of MCF-7 cell growth

As E_2 is the major stimulator of MCF-7 cell growth, we next examined the effect of resveratrol on E_2-mediated MCF-7 cell growth. MCF-7 cells were seeded in PFMEM medium. Twenty-four hours later, MCF-7 cells were cultivated in the presence of 10^{-9} *M* E_2 concentration and increasing concentrations of resveratrol in order to examine the antiestrogenic potential of different concentrations of resveratrol. As shown in Fig. 2, resveratrol antagonized the effect of E_2 in a dose-dependent manner, starting from 10^{-6} *M*. A 50% inhibition of the E_2 effect was observed at a resveratrol concentration of 5×10^{-6} *M*. Maximal growth inhibition was achieved in the presence of 10^{-5} *M* resveratrol.

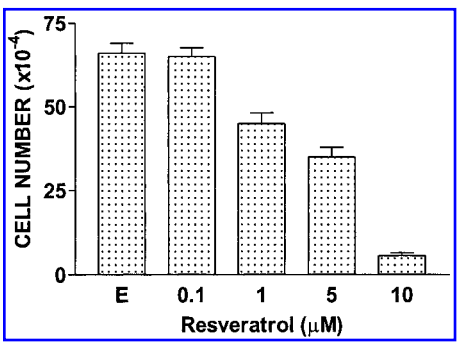

FIG. 2. Resveratrol antagonizes the E_2-mediated growth in MCF-7 cells. MCF-7 cells were cultivated in estrogen-depleted medium consisting of α-MEM supplemented with 5% charcoal-extracted FBS (PFMEM medium) with 10^{-9} *M* E_2 and increasing concentrations of resveratrol. The control cells received 10^{-9} *M* E_2 only (E). Cells were counted at day 6. Values are means ± SD of triplicate determinations.

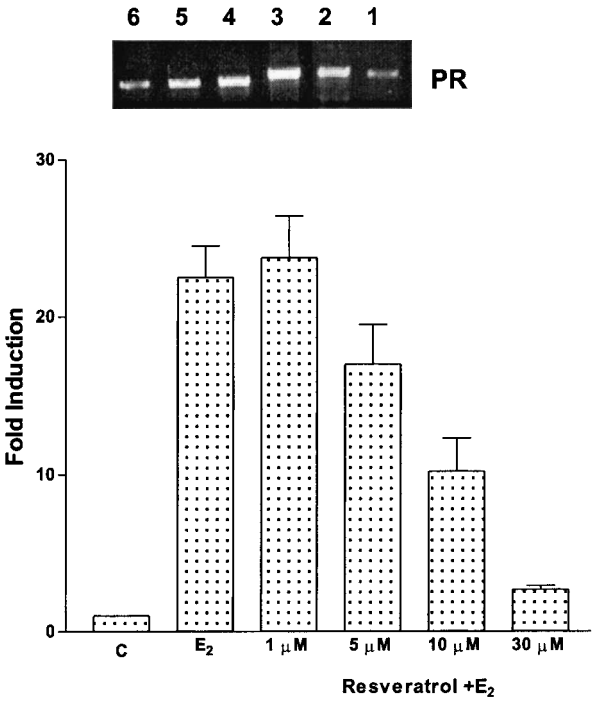

FIG. 3. Effect of resveratrol on PR mRNA expression stimulated by E_2. MCF-7 cells were plated in DME-F12 medium supplemented with 5% FBS. Two days later, cells were starved in PFMEM medium for 24 h. Cells were treated either with 10^{-9} *M* E_2 alone or or with increasing concentrations of resveratrol. Total RNA was isolated 24 h later to measure PR mRNA expression by RT-PCR as described in Materials and Methods. **Top panel:** Samples were analyzed by agarose gel electrophoresis followed by ethidium bromide staining. 1: untreated MCF-7 cell control; 2: cells treated with 10^{-9} *M* E_2; 3–6: cells treated with E_2 and resveratrol at 1, 5, 10, and 30 μM, respectively. **Bottom panel:** The amplified bands were detected by Southern blot using a radiolabeled PR probe followed by autoradiography. Data were analyzed by densitometric scanning, normalized to β-actin internal controls (data not shown), and the results expressed as fold stimulation above untreated control. Values correspond to means ± SD.

Resveratrol antagonizes the PR expression stimulated by E_2

To demonstrate further the antiestrogenic action of resveratrol, we examined whether resveratrol antagonized the effect of E_2 on the activity of a luciferase reporter gene construct containing the estrogen responsive element (ERE-LUC) and on the stimulation of the mRNA expression of the E_2-inducible PR. PR is the most responsive of all the estrogen-regulated RNAs studied so far (39). As shown in Fig. 3, E_2 stimulated by 20-fold the PR mRNA expression measured by RT-PCR. Addition of

TABLE 1. EFFECT OF RESVERATROL ON ERE-LUC EXPRESSION AND PRmRNA EXPRESSION IN MCF-7 CELLS STIMULATED BY E_2

Culture conditions	*PR mRNA expression*	*ERE-Luc activity*
E_2 only (10^{-9} *M*)	100	100
Resveratrol (10^{-5} *M*)	50	60
Resveratrol (3×10^{-5} *M*)	25	20

MCF-7 cells were plated in DME-F12 medium supplemented with 5% FBS. Two days later, cells were starved in PFMEM medium for 24 h. Cells were treated either with 10^{-9} *M* E_2 alone or with increasing concentrations of resveratrol. Total RNA was isolated 24 h later to measure PR mRNA expression as described in Materials and Methods. The PR signals were scanned and normalized to β-actin internal control. For the ERE-LUC activity reporter gene assay, the MCF-7 cells were transfected with plasmid DNa from the ERE-LUC gene construct as described in Materials and Methods prior to being treated with E_2 or resveratrol. Values corresponding to the average of three independent experiments are expressed as % of control corresponding to ERE-LUC activity and PR mRNA expression in MCF-7 cells treated with E_2 only.

resveratrol resulted in a dose-dependent inhibition of the E_2 effect. Table 1 summarizes the effect of resveratrol on the E_2 stimulation of PR mRNA expression and ERE-LUC activity. When added in the presence of E_2 (10^{-9} *M*), resveratrol inhibited ERE-LUC reporter gene activity, as well as PR expression in a dose-dependent manner. At 10^{-5} *M*, resveratrol inhibited PR mRNA expression and ERE-LUC activity by 75% and 80%, respectively ($p < 0.02$). Resveratrol (10^{-5} *M*) had no effect on ER mRNA expression (data not shown), indicating that the inhibitory effect observed on ERE-LUC and PR mRNA expression was not due to a decrease in receptor expression, but rather to the fact that resveratrol acted as an estradiol antagonist.

Resveratrol inhibits the proliferation of the ER-negative human breast carcinoma cell line MDA-MB-468

In order to determine whether the effect of resveratrol on inhibiting the proliferation of human mammary epithelial cells was solely dependent on its ability to antagonize the E_2 effect, we examined whether resveratrol could affect the proliferation of the ER-negative breast carcinoma MDA-MB-468 cells (44). These cells are unable to respond to E_2 as they harbor a mutation on the ER receptors and proliferate in estrogen depleted medium. As shown in Fig. 4, resveratrol inhibited the growth of MDA-MB-468 cells. However, the effect was reduced when compared with the one observed with MCF-7 cells. At day 6, inhibition by resveratrol was 45% on MDA-MB-468 cells, whereas the same concentration of resveratrol inhibited MCF-7 cell growth by 70%. These data would suggest that resveratrol can inhibit proliferation of human breast cancer cells independently from solely antagonizing the E_2 effect, although with a reduced efficiency.

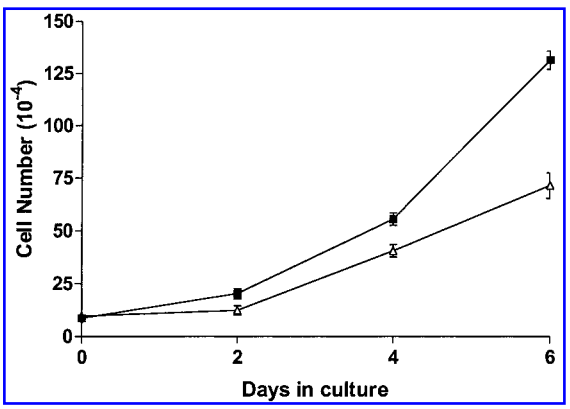

FIG. 4. Effect of resveratrol on the proliferation of the ER-negative breast carcinoma cell line MDA-MB-468. The culture conditions of MDA-MB-468 cells were the same as the ones described in the legend of Fig. 1 for MCF-7 cells. Resveratrol was added to the cells at a concentration of 10^{-5} *M* after the cells had attached to the plates (△). Control cells were maintained in the absence of resveratrol and in the presence of 0.1% ethanol as vehicle only (■). Values are means ± SD of triplicate determinations.

Resveratrol effect on mRNA expression of autocrine growth modulators and their receptors

Experiments were then performed to investigate the effect of resveratrol on the mRNA expression of growth factors and growth factor receptors synthesized by MCF-7 cells and known to be important for their growth. Using RT-PCR, the expression of mRNA for TGF-α, TGF-βs, IGF-I, and IGF-II, as well as their receptors, was examined in MCF-7 cells treated for 6 days with 10^{-5} *M* resveratrol. As shown in Fig. 5, resveratrol inhibited the TGF-α and IGF-IR mRNA expression in a dose-dependent fashion. Resveratrol at 10^{-5} *M* inhibited the expression of TGF-α mRNA by 87% ($p < 0.001$) and IGF-IR mRNA by 90% ($p < 0.002$). There was a slight increase in IGF-II mRNA expression, whereas IGF-I mRNA was not detected by RT-PCR. No changes were observed in the mRNA expression of IGF-II receptor and epidermal growth factor after resveratrol treatment (data not shown).

Recently, we have characterized a novel autocrine growth factor named PC cell-derived growth factor (PCDGF) overexpressed in breast cancer cells that mediates some of the mitogenic effect of estrogen in ER-positive breast cancer cells and that is transcriptionally activated by E_2 in these cells (33–35). We show that resveratrol also inhibited by 80% ($p < 0.001$) the expression of PCDGF in MCF-7 cells measured by RT-PCR. This indicates that resveratrol inhibited the expression of several autocrine growth factors known to be under the control of estrogen.

TGF-β2 is a potent growth inhibitor for breast cancer cells and a marker for measuring antiestrogenic action *in vitro* and *in vivo* because its expression is stimulated by antiestrogen (7, 26, 28, 42). A six-fold increase of TGF-β2 mRNA expression was observed after MCF-7 cells were treated with 5×10^{-6} *M* resveratrol ($p < 0.01$). At 10^{-5} *M*, resveratrol significantly stimulated TGF-β2 mRNA expression up to 15-fold above the control ($p < 0.02$). In contrast to TGF-β2, no change in the expression of TGF-β1, TGF-β3, and TGF-β receptor mRNAs was observed in these conditions (data not shown).

In contrast, resveratrol had no effect on TGF-β2 expression in MDA-MB-468 cells. This result is in agreement with the fact that TGF-β2 is under the control of estrogen in MCF-7 cells and could be explained by the antiestrogenic effect of resveratrol.

DISCUSSION

The results presented in this article demonstrate that resveratrol inhibits the growth of ER-positive human breast cancer MCF-7 cells. We show that resveratrol, itself a partial ER agonist, antagonizes the growth stimulatory effect of E_2 at both the cellular level (cell growth)

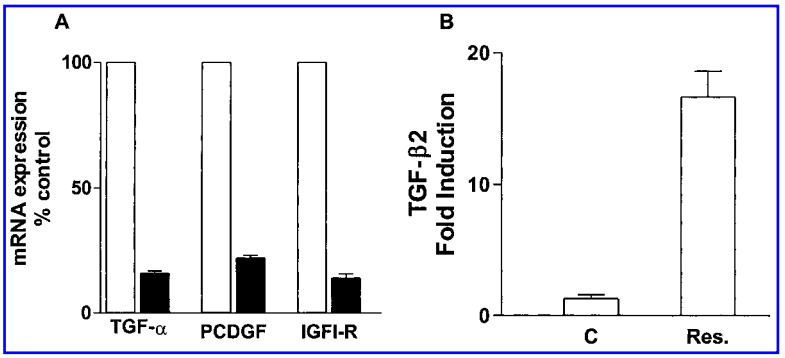

FIG. 5. Effect of resveratrol on the mRNA expressions of TGF-α, PCDGF, IGF-IR, and TGF-β2 in MCF-7 cells. MCF-7 cells were plated in DME-F12 medium plus 5% FBS in the absence (0.1% ethanol only, control cells, C) or in the presence of 10^{-5} *M* resveratrol (res.). Total RNAs were isolated at day 6 and examined for (**A**) the expressions of TGF-α, IGF-IR, and PCDGF and (**B**) the expression of TGF-β2 mRNA by RT-PCR. The signals were normalized to β-actin internal control, and the results were expressed as either fold induction or percentage of inhibition in comparison with control. Values are means ± SD of three independent experiments.

and the molecular level (gene expression). MCF-7 cells from which resveratrol (10^{-5} *M*) had been removed after 3 days of incubation failed to regain any growth advantage over the cells continuously maintained in the presence of resveratrol, indicating that the effect of resveratrol was irreversible. This was also shown by the fact that cells cultivated in the presence of resveratrol failed to regain growth advantage when they were replated in the absence of resveratrol. At the molecular level, we had shown previously that resveratrol antagonized the stimulatory effect of E_2 on PR mRNA expression in a dose-dependent manner. Resveratrol combined with E_2 (10^{-9} *M*) inhibited PR mRNA expression, pointing out to the antiestrogenic effect of resveratrol (32). These data are confirmed by the fact that resveratrol inhibited the activation by E_2 of the activity of an ERE-LUC gene construct without affecting expression of the ER.

As resveratrol inhibited the growth of MCF-7 cells in the presence of E_2, it was interesting to examine whether it would affect the expression of several autocrine growth factors and their receptors. We show that resveratrol inhibited the expression of several autocrine growth factors known to be stimulated by estrogens and known to stimulate the proliferation of MCF-7 cells (25, 29, 30, 38, 55). Among them, we show that resveratrol inhibited the expression of TGF-α and PCDGF. TGF-α has a growth-promoting effect on human breast cancer cells in culture. Its expression is induced by E_2 and suppressed by antiestrogen (12, 13, 30). PCDGF is a novel autocrine growth factor overexpressed in human breast cancer required for their tumorigenicity (34). We have shown that PCDGF expression is transcriptionally activated by estrogen (33) and that PCDGF mediates the estrogen effect in MCF-7 cells (35). We show here that resveratrol inhibits by 75% the expression of PCDGF in MCF-7 cells. In addition, cells treated with resveratrol displayed a 90% inhibition of IGF-IR mRNA expression. IGF-IR has also been described as a target of estrogen regulation, and its induction is important for the E_2-mediated proliferative effect in MCF-7 cells (50). As the growth-promoting effect of E_2 requires the presence of TGF-α, PCDGF, and IGF-IR, the inhibition of their expression by resveratrol might result in an inhibition of cell growth.

Our results also showed that resveratrol dramatically stimulated TGF-β2 mRNA expression (15-fold) in MCF-7 cells without any change in TGF-β1 and TGF-β3. TGF-βs have been shown to act as negative autocrine regulators that inhibit the growth of most breast cancer cell lines (26). Growth stimulation of estrogen-dependent breast cancer cells with E_2 is associated with down-regulation of TGF-β2 and TGF-β3 mRNAs, whereas growth inhibition of these cell lines by the antiestrogen (tamoxifen) is associated with elevated TGF-β2 mRNA expression (24). In fact, part of the growth inhibitory effect of antiestrogen is thought to be mediated through the induction of TGF-βs (2, 3, 24, 56). Recently, Koli *et al.* (27) have reported that blockade of TGF-β signaling using dominant negative TGF-β2 receptor in combination with neutralization assay failed to prevent antiestrogen-mediated growth inhibition of MCF-7 cells. Regardless of these conflicting reports about its importance for the regulation of breast cancer cells growth, TGF-β2 has been widely accepted as a marker of antiestrogen action *in vitro* and *in vivo* (26, 27, 42). Thus, the stimulation of TGF-β2 mRNA expression by resveratrol is in agreement with its antiestrogenic effect, which is also shown by its ability to inhibit PR mRNA expression stimulated by E_2. This also provides a possible mechanism for the effect of resveratrol as a growth inhibitor for MCF-7 cells. In contrast, resveratrol failed to stimulate TGF-β2 expression in the ER-negative MDA-MB-468 breast carcinoma cell line in spite of the fact that it inhibited its proliferation.

Gehm *et al.* (19) have shown by competitive binding of radiolabeled E_2 in the presence of resveratrol with a clone of MCF-7 cells that resveratrol was a weak ligand for ER. The IC_{50} for resveratrol to inhibit 0.1 n*M* ^{125}I-E_2 binding to ER was $\sim 10^{-5}$ *M*. They also indicated that resveratrol, acting as a pure ER agonist, stimulated the growth of a clone of MCF-7 cells in the absence of estrogen. However, the effect of resveratrol on cell growth in the presence of E_2 was not examined in this study. In addition to initial studies, several other groups have investigated the growth inhibitory effect of

resveratrol on breast cancer cells (10, 41, 51). In our studies, we propose that this growth inhibition may be due to the ability of resveratrol to modulate the expression of several autocrine growth modulators and/or their receptors in the breast cancer cells. As these growth modulators appear to be under estrogen regulation, the most likely mechanism for these effects could be an antiestrogenic effect of resveratrol. Direct competition of E_2 for binding to ER has been demonstrated for other phytochemicals (1, 9). But other mechanisms might also be involved, such as prevention of ER binding to ERE or of ER-mediated transactivation. Finally, our data also suggest that the antiestrogenic effect of resveratrol may not be the sole mechanism for its growth inhibitory effect on breast cancer cells. We have shown that resveratrol is also capable of inhibiting the growth of the ER-negative MDA-MB-468 cells, although less effectively than on the ER-positive MCF-7 cells. Resveratrol has been reported to be a potent inhibitor of ribonucleotide reductase and cyclooxygenase-2 (15, 51), and can also inhibit NADH:ubiquinone oxidoreductase (14) and DNA polymerase (52). The inhibition of these key enzymes involved in cell metabolism may explain some of the antiproliferative effects of resveratrol, particularly observed in ER-negative cells. The inhibitory effect of resveratrol on cyclooxygenase expression (51) is of particular interest because expression of this enzyme has been hypothesized as playing an important role in breast cancer progression as the enzyme becomes constitutively elevated in cells from advanced breast carcinoma (6). In particular, it has been shown that the ER-negative breast carcinoma cell line MDA-MB-231 has a high constitutive level of cyclooxygenase-2 activity (31). One could then hypothesize that inhibition of cyclooxygenase in these ER-negative cells may be involved in the ability of resveratrol to inhibit the proliferation of these cells independently of interaction with the ER. Future experiments will allow us to investigate this possibility in MDA-MB-468 cells.

In addition to these reported effects on human breast cancer cells, resveratrol has been shown to inhibit the proliferation of other types of tumors cells, including oral squamous epithelial, prostate, colon, and leukemia (8, 17, 18, 23, 46, 48, 53). These findings raise an interesting question about the potential role of resveratrol as a chemopreventive agent. In terms of cancer prevention, reports in the literature have also indicated an inverse relationship between breast cancer and wine consumption (not simply alcohol consumption) (17, 20). There is no pharmacokinetics information available about resveratrol metabolism in the human body. But it is believed that a couple of glasses of wine could provide a two-digit micromolar concentration of resveratrol where most of the pharmacological effects of resveratrol are observed (23). Based on these reports and the data presented here, resveratrol is definitely an interesting compound worthy of further investigation for its chemopreventive potential. The fact that resveratrol affects the expression of several endogenously produced growth modulators is of interest because autocrine growth loops are often turned on in cancer cells and play a major role for cancer cells becoming autonomous. This observation suggests that resveratrol may be useful in restoring the cells' ability to respond to the extracellular environment.

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ABBREVIATIONS

DME-F12, Dulbecco's modified Eagle's medium and Ham's F12 medium; E_2, 17β-estradiol; ER, estrogen receptor; ERE, estrogen response element; ERE-LUC, estrogen responsive element–luciferase activity; FBS, fetal bovine serum; IGF-I, insulin-like growth factor I; IGF-II, insulin-like growth factor II; IGF-IR, insulin-like growth factor I receptor; α-MEM, α-modified Eagle's medium; PCDGF, PC cell-derived growth factor; PFMEM, phenol red-free α-

modified Eagle's medium; PR, progesterone receptor; RT-PCR, reverse transcriptase–polymerase chain reaction; TGF-α, transforming growth factor-α; TGF-β, transforming growth factor-β.

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