

# The Red Wine Polyphenol Resveratrol Displays Bilevel Inhibition on Aromatase in Breast Cancer Cells

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Estrogen plays a crucial role in the development of breast cancer, and the inhibition of estrogen synthesis has been an important target for the prevention and treatment of this disease. The rate-limiting reaction of the hormone biosynthesis is catalyzed by cytochrome P450 (CYP) 19 enzyme or aromatase. It has been of genuine interest to uncover an aromatase-inhibitory compound from a dietary source. Resveratrol is a polyphenolic compound that can be isolated from grape peel. Because of its structural resemblance to estrogen, resveratrol's agonistic and antagonistic properties on estrogen receptor have been examined and demonstrated. In the present study, the effect of resveratrol on the expression and enzyme activity of aromatase was investigated. By assaying on MCF-7 cells stably transfected with *CYP19* (MCF-7aro cells), resveratrol inhibited the aromatase activity with an  $IC_{50}$  value of  $25\mu\text{M}$ . Kinetic analysis indicated that both competitive and noncompetitive inhibition might be involved. The administration of  $10\text{ nmol/l}$  testosterone—a substrate of aromatase—produced a 50% increase in the MCF-7aro cell number. This cell proliferation specifically induced by testosterone was significantly reduced by  $10\mu\text{M}$  resveratrol. In addition,  $50\mu\text{M}$  resveratrol significantly reduced the *CYP19*-encoding mRNA abundance in SK-BR-3 cells. The transcriptional control of *CYP19* gene is tissue specific, and promoter regions I.3 and II have previously been shown to be responsible for *CYP19* expression in breast cancer cells. Luciferase reporter gene assays revealed that resveratrol could repress the transcriptional control dictated by the promoter regulation. The present study illustrated that pharmacological dosage of resveratrol inhibited aromatase at both the enzyme and mRNA levels.

**Key Words:** resveratrol; aromatase; breast cancer cell proliferation.

Epidemiological studies have shown that the use of exogenous estrogen (Collaborative Group on Hormonal Factors in Breast Cancer, 1997; IARC, 1999; Pike and Spicer, 1993) or augmented endogenous estrogen concentration (Adlercreutz

*et al.*, 1994; Toniolo *et al.*, 1995) is associated with increased breast cancer risk. The causal relationship between estrogen exposure and breast cancer has been reviewed by Colditz (1999).

The cancer-inducing mechanisms of estrogen in the breast can be multifaceted and may participate in either the initiation or the promotion stage. Estrogen can be metabolized into various catechol estrogens, and estrogen-2-hydroxylase and estrogen-4-hydroxylase are cytochrome P450 (CYP) enzymes that hydroxylate estrogen at the C-2 and C-4 positions, respectively (Liehr, 2000). These hydroxylated metabolites can further be converted into quinone and semiquinone structures, which have been shown to be carcinogenic in animal models (Li and Li, 1987; Liehr *et al.*, 1986). In addition, free radicals generated by some of these metabolites may cause oxidative DNA damage (Zhu and Conney, 1998). These genotoxic effects of estrogen have been demonstrated in MCF-7 cells (Yared *et al.*, 2002) and rat mammary tissues (Zhang *et al.*, 2001).

The notion that estrogen promotes breast cancer is reinforced in a transgenic mouse model that develops spontaneous mammary tumors. Treatment of estrogen accelerates the development of neoplastic lesions and carcinomas in these mice (Yoshidome *et al.*, 2000). Estrogen-induced cell proliferation has been a major focus in breast cancer research. The pertained mechanisms lie in the regulation of cell cycle (Dickson *et al.*, 1987; Tsai and O'Malley, 1994), Bcl-2 family protein expression (Leung and Wang, 1999), and the interaction with plasma membrane receptors (Watson *et al.*, 1999).

Estrogen is synthesized from cholesterol in several steps, and *CYP19* (aromatase) catalyzes the final rate-limiting reaction. Aromatase is encoded by a single-copy gene (Means *et al.*, 1989; Toda *et al.*, 1990). The promoter utilization for *CYP19* regulation varies in different tissues, which provides the basis for tissue-specific expression (Harada *et al.*, 2003). Polymorphisms in the *CYP19* gene have been associated with breast cancer risk (Lee *et al.*, 2003). Many aromatase inhibitors have recently been developed, and some of them are promising agents for breast cancer prevention and therapy (Cuzick, 2003).

Resveratrol is a nonflavonoid phytoestrogen and can be isolated from grape peel. Previous epidemiological studies

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have shown that resveratrol intake is inversely associated with breast cancer risk (Levi *et al.*, 2005; Simopoulos, 2004). Because the CYP19-inhibitory property was previously demonstrated in red wine extract (Eng *et al.*, 2001, 2002), we would like to evaluate whether resveratrol was one of the active ingredients for the inhibition in the present study. Two breast cancer cell lines, MCF-7aro and SK-BR-3, were used, respectively, for enzyme kinetic measurement and mRNA expression analyses.

## MATERIALS AND METHODS

### Chemicals

Resveratrol (Cat. No. R-5010) was obtained from Sigma Chemicals (St Louis, MO). All chemicals, if not stated, were purchased from Sigma Chemicals.

### Cell Culture

The breast cancer cell line SK-BR-3 was a generous gift from Dr Richard K. W. Choy (Department of Obstetrics and Gynaecology, the Chinese University of Hong Kong, Kowloon, Hong Kong), and the parental breast cancer MCF-7 cell line, from which the stable transfected cell lines were derived, were obtained from American Tissue Culture Collection (Rockville, MD). The MCF-7 cells were stably transfected with human *CYP19* (MCF-7aro), which were used for an "in-cell" aromatase assay as previously described (Zhou *et al.*, 1990).

The stably transfected MCF-7 cells were maintained in Eagle's minimum essential medium (Invitrogen, Grand Island, NY) supplemented with 10% fetal bovine serum (FBS) (Invitrogen Life Technology, Rockville, MD) and the selection antibiotic 500 µg/ml G418 (USB Corporation, Cleveland, OH). SK-BR-3 cells were cultured in McCoy's 5A medium (Sigma Chemicals) with 10% FBS. Cells were incubated at 37°C, 5% carbon dioxide, and routinely subcultured when reaching 80% of confluency. Resveratrol was administered in the solvent vehicle dimethyl sulfoxide (DMSO), and the concentration was kept constant at 0.1% vol/vol for all treatments. Control or 0µM resveratrol groups received DMSO only. Cell density was seeded uniformly at  $5 \times 10^2$  cells/mm<sup>2</sup> in experiments. All experiments were repeated, and the results shown are representations of similar findings.

### "In-Cell" Aromatase Assays

The assays were performed as previously described (Grube *et al.*, 2001). In brief, MCF-7aro cells were seeded and allowed 1 day for attachment. Assays were started by replacing the culture medium with serum-free medium containing 1β-<sup>3</sup>H-androstenedione and resveratrol. The final concentration of androstenedione was controlled at 25nM, and the reaction was incubated at 37°C for 1 h. An aliquot of the medium was then mixed with equal volume of chloroform, followed by a 10,000 × g centrifugation at 4°C for 10 min. The aqueous phase was removed into a new tube containing 500 µl of 5% activated charcoal suspension. After a 30-min incubation, an aliquot of the supernatant fraction was taken out for scintillation counting. The protein content of the cells, on the other hand, was determined by using a bicinchoninic acid kit (Sigma Chemicals) after dissolving the cells in 0.5 mol/l NaOH.

A similar protocol was applied to assays performed on SK-BR-3 cells, except that resveratrol was administered in the cell cultures after the attachment period and incubated for another 24 h before assay.

### Quantitative Real-Time RT-PCR Assay

In order to quantify the suppression of mRNA abundance, a cell line with a reasonable amount of aromatase expression had to be used. Because aromatase mRNA was barely detectable in wild-type MCF-7 cells, we employed an aromatase-expressing cell line SK-BR-3 for this assay. The

real-time quantitative PCR was carried out as previously described (Giulietti *et al.*, 2001).

CYP19 and β-actin cDNA fragments were amplified and cloned into pGEMT-Easy vector (Promega Corp, Madison, WI) as templates for quantifying the absolute amount of mRNA expression. Plasmids containing the respective amplicon—pGEMT-CYP19 and pGEMT-β-actin—were sequenced and stored at -20°C until use.

SK-BR-3 cells were cultured and treated as described above. After 24 h of treatment, total RNA was extracted from the cells using TRIzol reagent (Invitrogen, Carlsbad, CA). The concentration and purity of the isolated RNA were determined by the absorbance reading observed at 260 and 280 nm. Three micrograms of total RNA, oligo-dT, and M-MLV Reverse Transcriptase (USB Corporation) were used for the first strand synthesis. Target fragments were quantified by real-time PCR and an Opticon 2 system (MJ Research, Waltham, MA). CYP19 copy number was determined by absolute quantification. A standard curve was constructed by 10-fold serial dilutions from 10 to 10<sup>7</sup> copies amplified from pGEMT-CYP19 or pGEMT-β-actin. Sample copy number was read from the standard curve. An SYBR green PCR Master Mix Reagent kit was obtained from Applied Biosystems (Foster City, CA), and PCR reactions were set up as described in the manual. A typical reaction contained 200 nmol/l of forward and reverse primer and 2 µl of cDNA, and the final reaction volume was 20 µl. The reaction was initiated by preheating at 50°C for 2 min, followed by 95°C for 10 min. Subsequently, 45 amplification cycles were then carried out with a 15-s denaturation at 95°C and 1 min annealing and extension at 58°C. Copies of β-actin RNA were also determined and used for normalization. The forward and reverse primers designed for CYP19 were 5'-ATC TCT GGA GAG GAA ACA CTC ATTA-3' and 5'-CTG ACA GAG CTT TCA TAA AGA AGGG-3' and those for β-actin were 5'-CAC CAA CTG GGA CGA CAT-3' and 5'-AGG CGT ACA GGG ATA GCA-3', respectively. Dissociation curve and gel image analysis did not reveal nonspecific amplifications generated from these primers.

### Measurement of Cell Viability

Previous studies (Grube *et al.*, 2001; Sun *et al.*, 1997; Wang *et al.*, 2005) have shown that MCF-7aro cell proliferation is specifically induced by testosterone through aromatase-mediated estrogen synthesis. By comparing the positive and negative testosterone treatments, the contribution of aromatase on cell proliferation and the inhibition of cell proliferation through counteracting aromatase can be estimated.

Cell number was assessed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) staining as described by Mosmann (1983). Briefly, MCF-7aro cells were seeded in 96-well plates and maintained in EMEM supplemented with 10% charcoal dextran-treated serum (Hyclone, Logan, UT). The cells were allowed 24 h for attachment and treated with testosterone and/or resveratrol for 48 h. At the end of the treatment, 50 µl of 1 mg/ml of MTT was added to the cells and incubated at 37°C for 4 h. Cell viability was assessed with respect to the absorbance at 544 nm.

### Luciferase Gene Reporter Assay

**Construction of CYP19 promoter-driven reporter plasmid.** As reviewed by Simpson *et al.* (1994), human aromatase is encoded by a single-copy gene with 10 exons. The expression of CYP19 is tissue specific and also regulated by alternate promoter usage. A nascent mRNA of aromatase comprises a protein-coding region coming from exons II to X and a untranslated subfraction coming from exon I located upstream of the coding region. There are different subfractions on the first exon that are controlled by various tissue-specific promoters. These subfractions of exon I are spliced onto a common acceptor site in exon II to form the nascent mRNA. The promoter usage, thus, dictates the expression of this gene.

A gene fragment (-446/+118) from human *CYP19* upstream to exon II was amplified from genomic DNA isolated from SK-BR-3 cells. This fragment contained promoters I.3 and II that were specifically associated with expression in breast cancer cells (Zhou and Chen, 1999; Zhou *et al.*, 1996). Primers were designed with the incorporation of *Kpn*II and *Xho*I restriction sites.

The amplified product was then digested and subcloned into a firefly luciferase reporter vector pGL3 basic (Promega Corp) and the sequence verified.

**Dual luciferase assays.** SK-BR-3 cells were plated in 24-well dishes at  $1 \times 10^5$  per well. After 24 h, transient transfection was performed using 1  $\mu$ l Lipofectamine and 2  $\mu$ l Plus reagent (Invitrogen Life Technologies) per well. Reporter plasmids (0.25  $\mu$ g, containing DNA fragments derived from aromatase promoter I.3 and II regions) were transfected into cells in serum-free medium. The renilla luciferase vector pRL-CMV (Promega Corp) was cotransfected as an internal correction for transfection efficiency. After a 5-h incubation, cells were cultured in phenol red-free PRMI 1640 supplemented with 5% charcoal dextran-treated FBS. After 1 day, the medium was removed, and the cells were treated with resveratrol for 24 h. The cells were lysed, and the activities of the luciferases were determined using Dual-Luciferase Assay Kit (Promega Corp). The luciferase bioluminescence was quantified by using a FLUOstar Galaxy plate reader. The transactivation activities of the *CYP19* promoter represented by firefly luciferase light units were then normalized with that of renilla luciferase.

#### Western Analysis

Cells were washed once with PBS (pH 7.4) and harvested into a 1.5-ml microtube with 0.5 ml of lysis buffer (PBS, 1% NP40, 0.5% sodium deoxycholate, 0.1% SDS). The lysis buffer contained protease inhibitors (40 mg/l phenylmethylsulphonyl fluoride, 0.5 mg/l aprotinin, 0.5 mg/l leupeptin, 1.1 mmol/l EDTA, and 0.7 mg/l pepstatin). The harvested cells were then lysed with a cell disruptor (Branson Ultrasonics Corp., Danbury, CT) on ice for 30 s. The protein concentration of cell lysate was determined by Dc protein assay (BioRad, Richmond, CA). Fifty micrograms of lysate protein was separated on 10% SDS-PAGE and transferred onto an Immobilon PVDF membrane (Millipore, Bedford, MA). Primary antibody raised against aromatase (Abcam plc, Cambridge, United Kingdom) and  $\beta$ -actin (Sigma Chemicals), and secondary antibodies conjugated with horseradish peroxidase (HRP) (Santa Cruz Biotechnology, Santa Cruz, CA) were used for protein detection. An ECL Detection Kit (Amersham, Arlington Heights, IL) provided the chemiluminescence substrate for HRP, and the targeted protein was visualized by autoradiography.

#### Statistical Methods

A Prism 3.0 (GraphPad Software, Inc., San Diego, CA) software package was utilized for statistical analysis. The results were analyzed by one-way ANOVA followed by Bonferroni's multiple comparison test if significant differences ( $p < 0.05$ ) were observed. A *t* test was employed for comparison of the means between resveratrol-treated and control cultures in Figure 3. Another software package SigmaPlot (SPSS Inc., Chicago, IL) was used for graphing the Lineweaver-Burk plots.

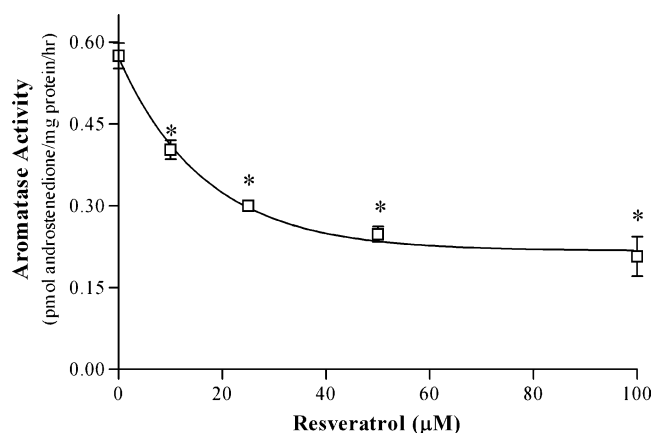
## RESULTS

### Enzyme Inhibition Assay Performed on MCF-7aro Cells

A previous study (Wang *et al.*, 2005) has indicated that MCF-7aro cells have an aromatase activity 200 times higher than that assayed in the control cells and can be substituted for recombinant enzyme assay. In the present study, resveratrol displayed an inhibitory effect with an  $IC_{50}$  value about 25  $\mu$ M in the MCF-7aro cell system (Fig. 1).

#### Enzyme Kinetic Assay

Four concentrations, i.e., 0, 10, 25, and 100  $\mu$ M, of resveratrol were selected for testing the enzyme kinetic response. The Lineweaver-Burk plot revealed that the presence of resveratrol



**FIG. 1.** Inhibitory effect of resveratrol on CYP19 in MCF-7aro cells. MCF-7aro cells were maintained in EMEM medium and switched to serum-free medium upon assay. Tritiated androstenedione and resveratrol were administered and incubated for 1 h. Significant inhibition was seen at 12.5  $\mu$ M or more. The  $IC_{50}$  value of resveratrol was determined to be 25  $\mu$ M. Values are means  $\pm$  SEMs,  $n = 3$ . \*Means were significantly ( $p < 0.05$ ) different from those of the control.

increase the values of  $K_m$  and slope accompanying a reduction in  $V_{max}$ . The plot showed that resveratrol had a mixed type of inhibition on CYP19. In a subplot of slope values against various resveratrol concentrations, a  $K_i$  value of 25  $\mu$ M was determined (Fig. 2).

### Specific Inhibition on Testosterone-Induced Proliferation in MCF-7aro Cells

Resveratrol was able to reduce the testosterone-induced proliferation of MCF-7aro cells through the inhibition of aromatase (Fig. 3). The administration of 10nM testosterone increased the cell number by 50% as shown at 0  $\mu$ M of resveratrol. Ten micromoles of resveratrol could significantly ( $p < 0.05$ ) reduce the cell proliferation. At 50  $\mu$ M, resveratrol reduced the testosterone-induced cell viability to the same level as their counterparts without testosterone treatment.

### Resveratrol Reduced Aromatase mRNA

#### Expression in SK-BR-3 Cells

Because aromatase expression in MCF-7 cells was low, we employed the breast cancer cell line SK-BR-3 for the assessment of mRNA expression levels. Quantitative RT-PCR indicated that the mRNA abundance of aromatase was reduced by resveratrol. Cultures treated with 50  $\mu$ M resveratrol revealed a 55% drop in aromatase expression, and 100  $\mu$ M of the phytochemical could decrease the expression by about 75% (Fig. 4).

#### Aromatase Activity in SK-BR-3 Cells

##### Treated with Resveratrol

Since the mRNA abundance could be suppressed by resveratrol, we measured the aromatase activity as an indication

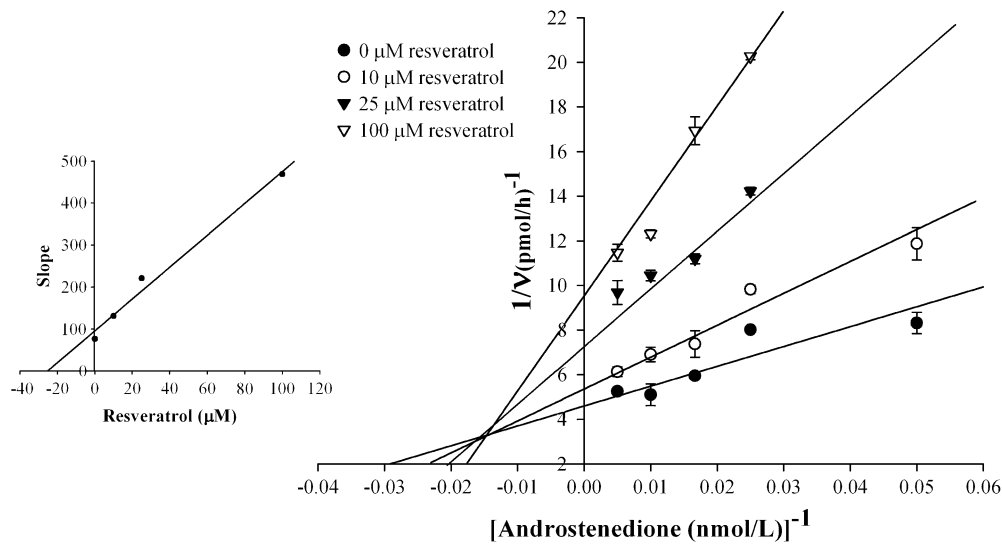


FIG. 2. Kinetic analysis of resveratrol inhibition on CYP19. MCF-7aro cells were cultured and assayed for aromatase activity. Four concentrations, 0, 10, 25, and 100  $\mu\text{M}$ , of resveratrol were coadministered to the cells for the enzyme kinetic assay. Values are means  $\pm$  SEMs,  $n = 3$ . Lineweaver-Burk plot showed that resveratrol had a mixed type of inhibition on CYP19 with a  $K_i$  value of 25.4  $\mu\text{M}$ .

for reduced expression. After 24 h of treatment, the aromatase activity was found to be significantly reduced by 25  $\mu\text{M}$  resveratrol, and the  $\text{IC}_{50}$  value was estimated to be 43  $\mu\text{M}$  (Fig. 5).

#### Effect of Resveratrol on Promoter Activity of CYP19 in SK-BR-3 Cells

As the mRNA expression is reduced by resveratrol, we subsequently determined the transcriptional control driven by promoter regions I.3 and II. Figure 6A demonstrated that the reporter plasmid was actively transcribed in SK-BR-3 cells.

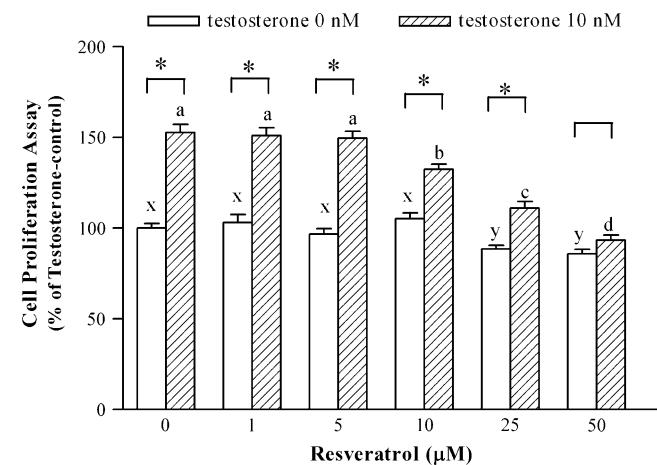


FIG. 3. Reversal effect of resveratrol on testosterone-induced MCF-7aro cell proliferation. MCF-7aro cells were seeded in 96-well plates and maintained in EMEM supplemented with 10% charcoal dextran-treated serum. Cell number was quantitated after 48 h under the influence of testosterone administration. Values are means  $\pm$  SEMs,  $n = 8$ . (\*) Mean values of testosterone-treated cultures were significantly ( $p < 0.05$ ) higher than those of the cultures without testosterone treatment. Means labeled with x differ ( $p < 0.05$ ) from those labeled with y; means labeled with a, b, c, or d are also significantly different.

Twenty-five micromoles of resveratrol was able to repress the transactivation of *CYP19* promoter activity (Fig. 6B) ( $p < 0.05$ ). This reporter gene assay result suggested that the decrease in mRNA abundance could be caused by inhibition at the transcriptional level.

#### Effect of Resveratrol on CYP19 at the Protein Level

Since all experiments indicated that the transcription of *CYP19* was reduced by resveratrol, we further examined the

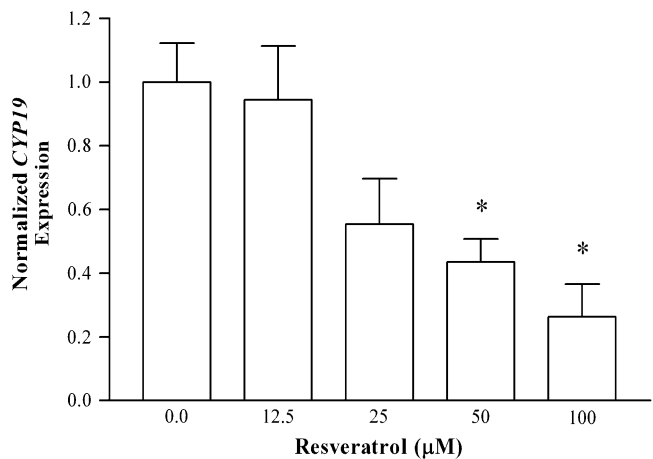
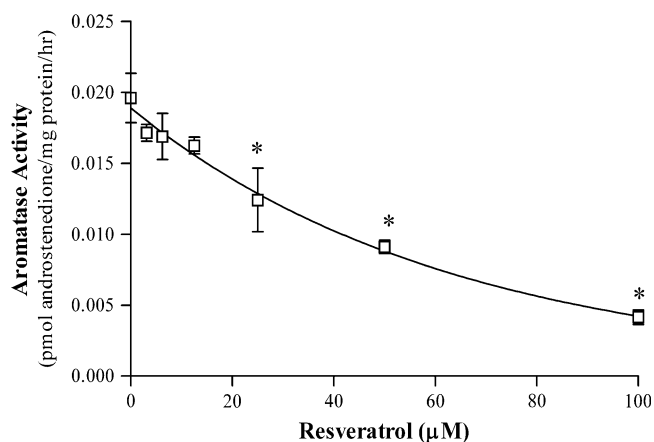


FIG. 4. Message RNA expressions of aromatase in SK-BR-3 cells treated with resveratrol. SK-BR-3 cells were seeded in six-well plates and maintained in McCoy's 5A medium supplemented with 10% charcoal dextran-treated serum. Resveratrol was administered to the cultures for 24 h. The effect of resveratrol on the *CYP19* expression was determined by real-time RT-PCR, and the *CYP19* expression was normalized to  $\beta$ -actin. Values are means  $\pm$  SEMs,  $n = 3$ , and samples are isolated from independent cultures. \*Mean values were significantly ( $p < 0.05$ ) different from those of the control cultures with no resveratrol treatment.



**FIG. 5.** Inhibitory effect of resveratrol on CYP19 in SK-BR-3 cells. SK-BR-3 cells were seeded in six-well plates and maintained in McCoy's 5A medium supplemented with 10% charcoal dextran-treated serum. Resveratrol was administered to the cultures for 24 h. The cultures were switched to serum-free medium upon assay. Tritiated androstenedione was administered and incubated for 1 h. Significant inhibition was seen at 25µM and more. The  $IC_{50}$  value was determined to be 40µM. Values are means  $\pm$  SEMs,  $n = 3$ . \*Means were significantly ( $p < 0.05$ ) different from those of the control.

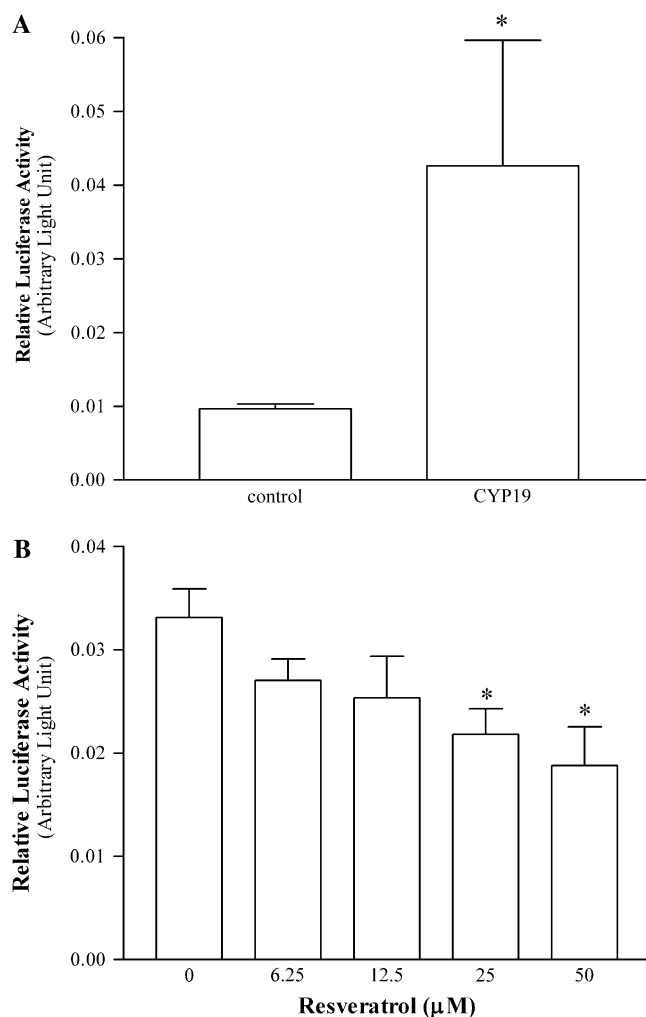
enzyme expression at the protein level. Figure 7 revealed that aromatase was reduced by resveratrol at the concentration of 25µM or more. The data represented one of two experiments with same result and were consistent with the mRNA and enzyme activity data.

## DISCUSSION

In this study, we found that resveratrol inhibited aromatase activity in MCF-7aro cells, and the enzyme kinetic result revealed that both competitive and noncompetitive inhibitions were involved. A previous study from our laboratory has illustrated that MCF-7aro cells are a viable model for the screening of aromatase inhibitor from phytochemicals, and the differential growth rates observed in MCF-7aro and MCF-7vec cells under testosterone administration are attributed to aromatase activity (Wang *et al.*, 2005). In the present study, resveratrol could inhibit both aromatase activity and testosterone-induced MCF-7aro cell proliferation. These results indicated that resveratrol was a CYP19 inhibitor at the enzyme level.

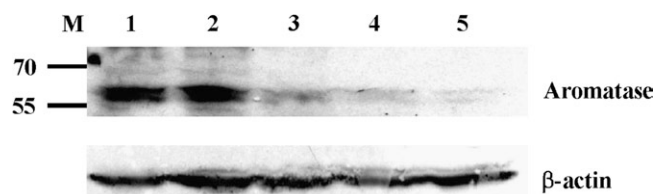
Resveratrol also reduced the aromatase mRNA abundance in another breast cancer cell line SK-BR-3. We illustrated that the decreased mRNA expression could occur at the transcription level. The promoter utilization of human aromatase gene is tissue specific, and promoters I.3 and II have been identified to be responsible for the expression in breast cancer cells (Chen *et al.*, 1999). In the present study, we demonstrated that the transactivation activity of the gene fragment containing promoters I.3 and II was inhibited by resveratrol.

Many phytochemicals have also been documented to be aromatase inhibitors. By employing human placental micro-



**FIG. 6.** Resveratrol suppressed *CYP19* promoter-driven luciferase activity in SK-BR-3 cells. SK-BR-3 cells were seeded in 24-well plates. After 24 h, the cells were transiently transfected with 0.25 µg of the *CYP19* reporter plasmid and 2.0 ng of renilla luciferase control plasmid and the activities of the luciferases determined in the cell lysate. Reporter plasmids with (*CYP19*) or without (control) *CYP19* insert were transfected and compared in (A). (B) represents luciferase activities determined from cells transfected with *CYP19* and under a 24-h resveratrol treatment. Values are means  $\pm$  SEMs,  $n = 4$ . \*Means were significantly ( $p < 0.05$ ) different from those of the control.

somes, Le Bail *et al.* (2001) found that chalcones exhibit inhibitory actions on aromatase with  $IC_{50}$  values ranging from 34.6µM to concentrations beyond 50µM. The phytochemical naringenin (Kao *et al.*, 1998), on the other hand, is of greater potency than the chalcones. Red wine extract can inhibit aromatase in the same placental assay system (Eng *et al.*, 2001), and administration of the extract to transgenic mice overexpressing CYP19 in the mammary tissues abrogates mammary hyperplasia. Subsequently, the same group has identified one of the active ingredients in the extract to be procyanidin B dimers (Eng *et al.*, 2003). Because the major source of procyanidin B is in grape seeds, the concentration may not be significant in red wine. In the present study,



**FIG. 7.** Western analysis of aromatase in SK-BR-3 cells treated with resveratrol. SK-BR-3 cells were seeded in six-well plates and maintained in McCoy's 5A medium supplemented with 10% charcoal dextran-treated serum. Resveratrol was administered to the cultures for 24 h. The effect of resveratrol on the CYP19 expression at the protein level was compared by immunoblotting; lane M, protein marker, lane 1, control (0 μM resveratrol); lane 2, 12.5 μM resveratrol; lane 3, 25 μM resveratrol; lane 4, 50 μM resveratrol; and lane 5, 100 μM resveratrol.

resveratrol was demonstrated to inhibit CYP19 at the expression and enzyme levels and could be a contributor for the CYP19-inhibitory effect of red wine extract. This bi-level suppression of resveratrol might have advantages over procyanidin.

Many factors have been described for the regulation of the transcription of aromatase. Cyclic AMP, phorbol esters, dexamethasone, prostaglandin E<sub>2</sub>, transforming growth factor β, and γ-interferon are documented compounds that increase the transcriptional activity (Simpson *et al.*, 1997), whereas cyclooxygenase (COX) inhibitors exhibit inhibitory effect on CYP19 expression and enzyme activity (Diaz-Cruz *et al.*, 2005). Since resveratrol can suppress COX-2 expression induced by phorbol ester (Subbaramaiah *et al.*, 1998), we speculated that this could be an inactivation mechanism of CYP19 transcription observed in the present study.

Resveratrol has been proposed to be a potential chemopreventive agent against breast cancer. It can protect against 7,12-dimethylbenz[*a*]anthracene- or N-methyl-N-nitrosourea-induced mammary tumorigenesis, and the protein expression of some downstream genes of estrogen receptor transactivation are also altered (Bhat *et al.*, 2001). The reduction in CYP1A1 expression and enzyme activity may contribute to the anti-initiation activities of resveratrol (Ciolino and Yeh, 1999; Lee and Safe, 2001). Moreover, resveratrol may interrupt the promotion or progression phase by inhibiting COX-2 (Subbaramaiah *et al.*, 1998). Resveratrol's agonist/antagonist activity toward estrogen receptor binding may contribute to the antagonism of estradiol-induced MCF-7 cell growth, the expression of transforming growth factor α, and insulin-like growth factor I receptor (Lu and Serrero, 1999). The minimum concentration at which aromatase inhibition was observed in the present study was comparable to these studies.

The physiological relevance of resveratrol concentration in human has not been clearly established. The total resveratrol concentration in human plasma is estimated to be around 40 μM after an ingestion of 1 mg of resveratrol per kg body weight at 1.5 h (Meng *et al.*, 2004). In rats, a dosage of 2 mg/kg administered intragastric (ig) gives 1.2 μM resveratrol in blood.

In a pharmacokinetic study of resveratrol, Sale *et al.* (2004) have demonstrated that giving 240 mg/kg ig to mice would result in a peak plasma concentration of 32 μM in 2 h. Therefore, the effective CYP19-suppressing concentrations observed in the current study are most likely falling into the pharmacological range.

In summary, we demonstrated that resveratrol inhibited the enzyme activities and reduced the mRNA abundance of CYP19. This study suggested that resveratrol could reduce localized estrogen production in breast cancer cells.

## ACKNOWLEDGMENTS

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