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ARTICLE

Estrogenic Activities of Sesame Lignans and Their Metabolites on Human Breast Cancer Cells

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Sesame lignans (sesamin, sesamolin) and their metabolites (enterodiol, ED; enterolactone, EL; and sesamol) have been evaluated for their estrogenic activities. ED and EL have been indicated to have estrogenic/antiestrogenic properties on human breast cancer cells; however the estrogenic activities of sesamin, sesamolin and sesamol have not been reported. In the present study, estrogenic potencies of sesame lignans and their metabolites were determined by estrogen responsive element (ERE) luciferase reporter assay in T47D cells stably transfected with ERE-luc (T47D-KBluc cells) and quantifying *pS2* and progesterone receptor gene expression in T47D cells. All tested compounds except ED possessed ability of ERE activation with a very low potency compared to estradiol (E2). These effects were abolished by coincubating tested compounds with 1 μM ICI 182 780, suggesting that estrogen receptors were directly involved in their ERE activations. Among tested compounds, sesamol showed the highest ability in ERE induction. The coincubation of increasing concentration of E2 (10^{-12}–10^{-6} M) with 10 μM of tested compounds resulted in a downward shift of E2-ERE dose–response curves. In contrast, at the low concentration of E2 (10^{-12} M), sesamin and sesamol significantly exhibited additive effects on the E2 responses. The inhibitory effect in a dose-dependent manner was also observed when 1–100 μM sesamol was coincubated with 1 nM E2. Sesamin, sesamol and EL significantly induced *pS2* gene expression whereas only sesamol could significantly induce progesterone receptor gene. The data obtained in this study suggested that sesame lignans and their metabolites possess weak estrogenic/antiestrogenic activity.

KEYWORDS: Estrogenic activities; sesame lignans; sesamol; human breast cancer cells

INTRODUCTION

Estrogen and metabolites play a crucial role in carcinogenesis in several mammalian organs such as breast, ovary, liver and kidney by the estrogen receptor (ER)-dependent mechanisms (*1*, *2*). Exposure to estrogen throughout a lifetime has been reported to be a risk factor of breast cancer. The occurrence of breast cancer increases with age. Concerning estrogen-related breast cancer, use of phytoestrogens for breast cancer chemoprevention is of interest. Phytoestrogens are a group of compounds contained in plants that possess an array of pharmacological effects including anticancer properties (*3*, *4*). Additionally, epidemiological studies have suggested that dietary intake of phytoestrogens decreased the risk of breast cancer in humans (*5*, *6*). These phytoestrogens possess either weak estrogenic and/or antiestrogenic properties (*7*, *8*). However, their possible preventive effect against human breast cancer is still subject to debate, and remains to be clarified (*9*, *10*). Several lines of evidence demonstrated ability of phytoestrogens in promoting growth of estrogen-dependent breast cancer cells. In contrast, there are experiments and epidemiological studies that suggested cancer preventive effect of phytoestrogens (*3*, *11*–*14*).

Sesame seeds contain substantial amounts of lignans: sesamin and sesamolin (**Figure 1A**,**B**) (*15*–*17*). They are oil soluble and have a structure which contains furofuran and methylenedioxyphenyl group. Sesamol, a phenolic compound, is mainly derived from conversion of sesamolin during roasting sesame seed and processing sesame oil (**Figure 1C**) (*18*). Ingested sesamin is converted by intestinal microflora to the mammalian lignans, enterodiol (ED) (**Figure 1D**) and enterolactone (EL) (**Figure 1E**) (*19*, *20*). In rat

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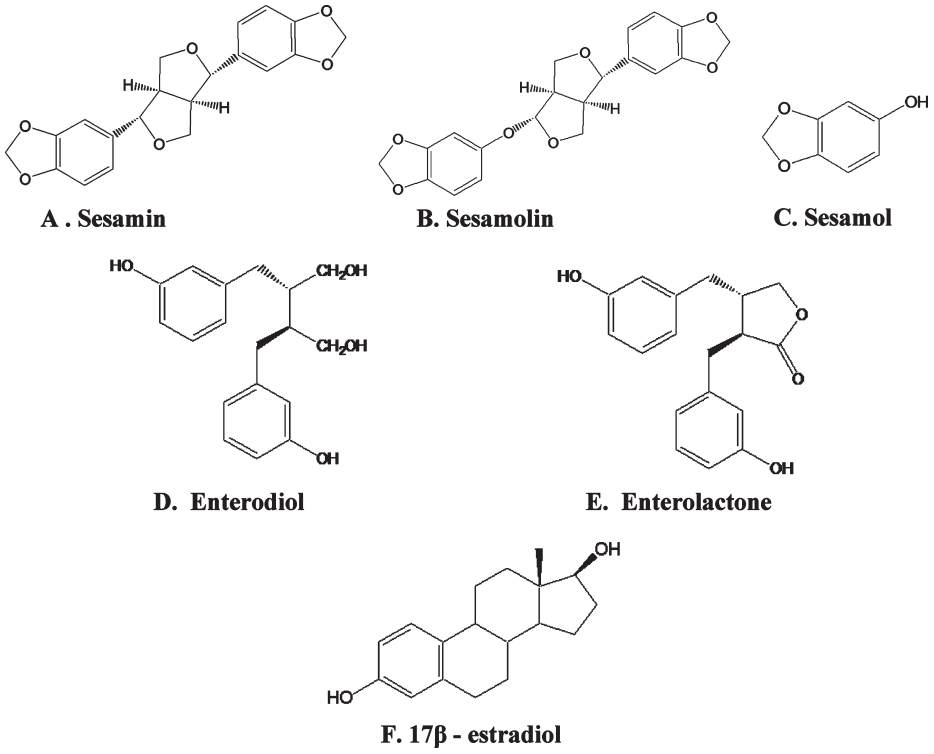

Figure 1. Chemical structures of sesame lignans, their metabolites and 17β-estradiol (E2).

model, it was also reported that sesamolin can be converted to sesamol (*21*).

Studies of estrogenic properties of sesamin, sesamolin and sesamol have not been reported. The estrogenic effect was found mostly from the studies of sesamin's metabolites (ED and EL) which have been reported to have distinct estrogenic effects on breast cancer. ED can activate estrogen targeted genes such as trefoil factor 1 (*pS2*) gene (*22*). Nevertheless, the information regarding estrogenic or antiestrogenic activities of sesame lignans and sesamol is limited. In addition, estrogen mediates several physiological functions by binding to estrogen receptors, interacting with ERE which initiates estrogen regulated gene expression and consequently activates molecular effects in the targeted tissue (*23*, *24*). Utilization of ERE activation and estrogen targeted gene expression have been shown to be a valuable physiological assay for estrogenic activity of compounds like phytoestrogens (*25*). Therefore, in this study, the estrogenic activity of sesame lignans was tested by using the ERE reporter system in hormone-dependent breast cancer cells. Further studies of the expression of estrogen targeted genes *pS2* and progesterone receptor were also performed. However, the limitation of the *in vitro* study is that cell lines used are incapable of converting sesamin to ED and EL. Therefore, the estrogenic activities of sesame lignans, sesamol, ED and EL were investigated to quantify their estrognic potency. In addition, their estrogenic/antiestrogenic effects were also compared to a positive control, estradiol (E2), and the known estrogen antagonists ICI 182 780 and tamoxifen.

MATERIALS AND METHODS

Chemicals and Reagents. Sesamin and sesamolin extracted and isolated from sesame seeds (*Sesamum indicum* L.) were obtained from Laboratories of Natural Products and Pharmacology, Chulabhorn Research Institute. Sesamol, 17β-estradiol (E2), and tamoxifen were purchased from Sigma-Aldrich (St. Louis, MO, USA). Enterodiol (ED) and enterolactone (EL) were purchased from Fluka (Buchs, Switzerland), and ICI 182 780 was purchased from Tocris Bioscience (Ellisville, MO, USA). Cell culture supplies were obtained from Invitrogen Life Technology (Carlsbad, CA, USA). All other reagents and chemicals were of analytical grade and purchased from commercial sources.

Cell Lines. The hormone-dependent T47D and T47D-KBluc human breast cancer cells were purchased from the American Type Culture Collection (ATCC) (Rockville, MD, USA). T47D-KBluc cells were stably transfected with luciferase reporter plasmid, pGL2.TATA.Inr.luc.neo, containing three estrogen response elements (EREs) which resulted in the expression of an estrogen-responsive luciferase reporter system. This reporter system is sensitive for the assessment of compounds with estrogenic or antiestrogenic activities and provides an *in vitro* system that can be used to evaluate the effect of compounds which modulate the activation of ERE (*24*). Both cell lines were grown and maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS), 2 mM glutamine, 1 mM sodium pyruvate, 20 mM D-glucose, 100 U/mL of penicillin and 172 μM streptomycin and 13.9 μM insulin.

Cell Viability Assay. To determine whether sesame lignans cause alteration in cell viability of T47D-KBluc cells, sulforhodamine B (SRB) assay was used for cell viability assessment as previously described (*26*). T47D-KBluc cells were cultured and maintained in RPMI 1640 medium supplemented with 10% FBS (a steroid containing cell cultured medium) (*27*), and all supplemented chemicals (2 mM glutamine, 1 mM sodium pyruvate, 20 mM D-glucose, 100 U/mL of penicillin and 172 μM streptomycin and 13.9 μM insulin). To lower the level of estrogen in the medium, cells were cultured in a non phenol-red RPMI containing 10% steroid-free (charcoal-dextran stripped serum, CSS) medium and supplemented chemicals for 5 days prior to the assay. Subsequently, cells were plated at 1×10^4 cells/100 μL/well into a 96-well culture plate and allowed to attach overnight. Cells were treated with several ranges of concentrations of sesame lignans and their metabolites. After treatment, cells were fixed with 4% ethanol in 1% acetic acid and incubated at 20 °C for 10 min. Then cells were washed with tap water 4−5 times, and the plates were air-dried at room temperature. The fixed cells were stained with 0.4% SRB in 1% acetic acid for 30 min. The excess SRB was removed by rinsing 4−5 times with 1% acetic acid, and the plates were dried for 30 min. The dye was solubilized with 10 mM Tris base (pH 10.5) for 5 min on a shaker. The color intensity was measured at 564 nm by using a microplate spectrophotometer (Spectramax plus 384, Molecular Devices, Sunnyvale, CA, USA).

Table 1. Primers Utilized for Real-Time RT-PCR Analysis

gene	primer sequence	PCR product size (bp)	gene accession no.
pS2 forward	5′-AGAGACGTGTACAGTGGCCC-3′	245	NM_003225.2
pS2 reverse	5′-CCGAGCTCTGGGACTAATCA-3′		
progesterone receptor forward	5′-CGCGCTCTACCCTGCACTC-3′	121	NM_000926.4
progesterone receptor reverse	5′-TGAATCCGGCCTCAGGTAGTT-3′		
GAPDH forward	5′-GAAGGTGAAGGTCGGAGTC-3′	226	NM_002046
GAPDH reverse	5′-GAAGATGGTGATGGGATTTC-3′		

Estrogen-Responsive Luciferase Reporter Assay. The method was based on Wilson et al. (*24*). Briefly, after 4 days of estrogen withdrawal, T47D-KBluc cells were seeded at 1×10^4 cells per well in a 96-well opaque plate and allowed to attach overnight. Medium in each well was replaced with 100 μL/well of dosing medium 5% CSS which contained the tested chemicals and incubated for 24 h. After the treatment period, dosing medium was removed and cells were washed with PBS at room temperature and then harvested in 25 μL of lysis buffer (Promega, Madison, WI, USA) per well. Luciferase activity assay was started by adding 50 μL of reaction buffer (25 mM glycylglycine, 15 mM $MgCl_2$, 5 mM ATP, 1.49 μM BSA, pH 7.8) and 50 μL of 1 mM D-luciferin (Promega, Madison, WI, USA) to each well using microinjector equipped in microplate luminometer (Spectramax L, Molecular Devices, Sunnyvale, CA, USA). Luciferase activity was determined and quantified as relative light units (RLU). Each treatment was performed in triplicate, and data were collected from at least three independent experiments.

Cells were tested with 17β-estradiol (E2) or estradiol antagonists (ICI 182 780 and tamoxifen) for the control of estrogenic and antiestrogenic activities, respectively. Sesame lignans and their metabolites were tested alone to determine their estrogenic activities and in combination with progressively increasing concentrations of E2 to test their antiestrogenic activities.

Determination of ERE Activity of Sesame Lignans and Their Metabolites. To assess sesamin, sesamolin, sesamol, ED and EL estrogenic response, T47D-KBluc breast cancer cells were treated with these compounds dissolved in vehicle (DMSO) for 24 h. The doses of sesame lignans and their metabolites were selected on the basis of our preliminary study. In all experiments, final concentration of DMSO did not exceed 0.1% (v/v). The ERE-luciferase activity was assessed by luminometer and determined as relative light units (RLU). The ERE luciferase activity of each treatment was calculated to fold induction of vehicle control.

To determine whether estrogen receptor (ER) was involved in the ERE activation, 1 nM E2 or 10 μM tested compounds (except ED) were coincubated with 1 μM ICI 182 780 for 24 h. The ERE luciferase activity of each treatment was calculated to fold induction of vehicle control.

To determine the potency of sesame lignans and their metabolites in comparison with a full natural agonist, 17β-estradiol (E2) dissolved in vehicle (ethanol) was used as a positive control. The response of T47D-KBluc cells to E2 was assessed by treating cells with progressively increasing concentrations of E2 from 1 pM (10^{-12} M) to 1 μM (10^{-6} M) and the final concentrations of vehicle (ethanol or DMSO) did not exceed 0.1% (v/v). The magnitude of ERE activation was calculated as fold induction of vehicle control.

Inhibitory Effect of Sesame Lignans on E2-Induced ERE Activation. To determine whether sesame lignans and their metabolites exhibit antiestrogenic effects, T47D-KBluc breast cancer cells were coincubated for 24 h with 10 μM of these substances and increasing concentrations of E2 from 1 pM to 1 μM. The degree of luciferase induction was plotted as fold induction over vehicle control. The known estrogen antagonists tamoxifen and ICI 182 780 were used as positive controls for antiestrogenic activity.

To confirm whether the tested compounds exhibited the inhibitory effect on E2-ERE activation in a dose-dependent manner, various concentrations of tested compound were coincubated with 1 nM E2 for 24 h. The degree of luciferase induction was plotted as fold induction over vehicle control.

Real-Time Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR). Real-time RT-PCR was used to determine the expression levels of trefoil factor 1 (*pS2*) and progesterone receptor mRNA in T47D cells. Cells were treated with sesame lignans and their metabolites for 24 h in 5% CSS medium. Concentrations of sesame lignans and their metabolites will be selected based on the concentrations that induced ERE activation. Cells treated with 1 nM E2 were used as positive control.

The sequences of specific primers for trefoil factor 1 (*pS2*) and progesterone receptor genes are shown in **Table 1**. The primers were checked by using the primer design tool program of the US National Center for Biotechnology Information (NCBI) to confirm the total gene specificity of the nucleotide sequences. For analysis of mRNA expression, total RNA was isolated from 5×10^6 cells using RNA purification cell kit (5 PRIME GmbH, Hamburg, Germany) according to the manufacturer's recommendations. In brief, cell culture medium was removed and cells were lysed in a detergent/salt solution (4.5 M guanidine hydrochloride, 50 mM Tris-HCl and 30% Triton X-100 [w/v], pH 6.6). Nucleotides were collected on a silica filter by high-speed centrifugation at 15000*g*. To remove contaminating DNA, nucleotides were treated with 200 U of DNase for 15 min and washed in a buffer consisting of 5 M guanidine hydrochloride and 20 mM Tris-HCl, pH 6.6 in ethanol. The filter was washed twice in a buffer consisting of 20 mM NaCl and 2 mM Tris-HCl, pH 7.5 in ethanol. Finally, RNA was eluted in 50 μL of sterile, nuclease-free, double-distilled water. Total RNA concentration was determined by absorbance measurement on a NanoDrop spectrophotometer (NanoDrop-ND1000, NanoDrop Technologies, Wilmington, DE, USA) at 260 nm. The ratio of absorbance at 260 and 280 nm was calculated to determine RNA purity. The integrity of the isolated RNA was determined by 1% (w/v) agarose gel electrophoresis.

Quantifications of *pS2* and progesterone receptor mRNA were performed by real-time RT-PCR (LightCycler 1.5, Roche Molecular Biochemicals, Mannheim, Germany). A master mixture containing DNA polymerase enzyme, mixed dNTP, SYBR Green dye, reverse transcriptase enzyme, and specific primers were prepared as recommended by the manufacturer (TOYOBO, Osaka, Japan). The final concentration of magnesium in the reaction mixture was 2.5 mM; forward and reverse primers were 0.25 μM each, and the amount of template RNA was 250 ng. The real-time RT-PCR cycle for *pS2* and progesterone receptor mRNA analysis started with 1 cycle of reverse transcription at 50 °C for 20 min, 1 cycle of PCR initial activation at 95 °C for 15 min, followed by 45 cycles of amplification consisting of denaturation at 94 °C for 15 s, annealing at 60 °C for 20 s, and extension at 72 °C for 20 s. The fluorescence was measured after the annealing step in every cycle. A negative control without RNA template was run to assess overall specificity. Melting curve analysis and 1% (w/v) agarose gel electrophoresis of products were performed to validate the reaction products. The housekeeping gene, glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*), was used as a reference for normalization. The crossing point value, which is defined as the point at which the fluorescence rises appreciably above the background fluorescence, was determined for the targeted and reference genes. The calculation of relative quantification of the targeted gene compared to the reference gene was performed using the Lightcycler Relative Quantification Software (Roche Molecular Biochemicals, Mannheim, Germany) based on the $2^{-\Delta\Delta Cp}$ method (*28*), resulting in the relative transcription level of the targeted genes in treated cells compared to control cells.

Statistical Analysis. Data are expressed as mean ± standard error of the mean (SEM) from at least three independent experiments. Student's *t* tests were used to compare between two groups. Statistically significant difference was defined by $p < 0.05$. The EC_{50} values were calculated using linear regression analysis. The E_{max} values of cotreatment were derived from the maximal luciferase induction over vehicle control.

RESULTS

The Effects of Sesame Lignans and Their Metabolites on Cell Viability. T47D-KBluc cells cultured in FBS and CSS were treated

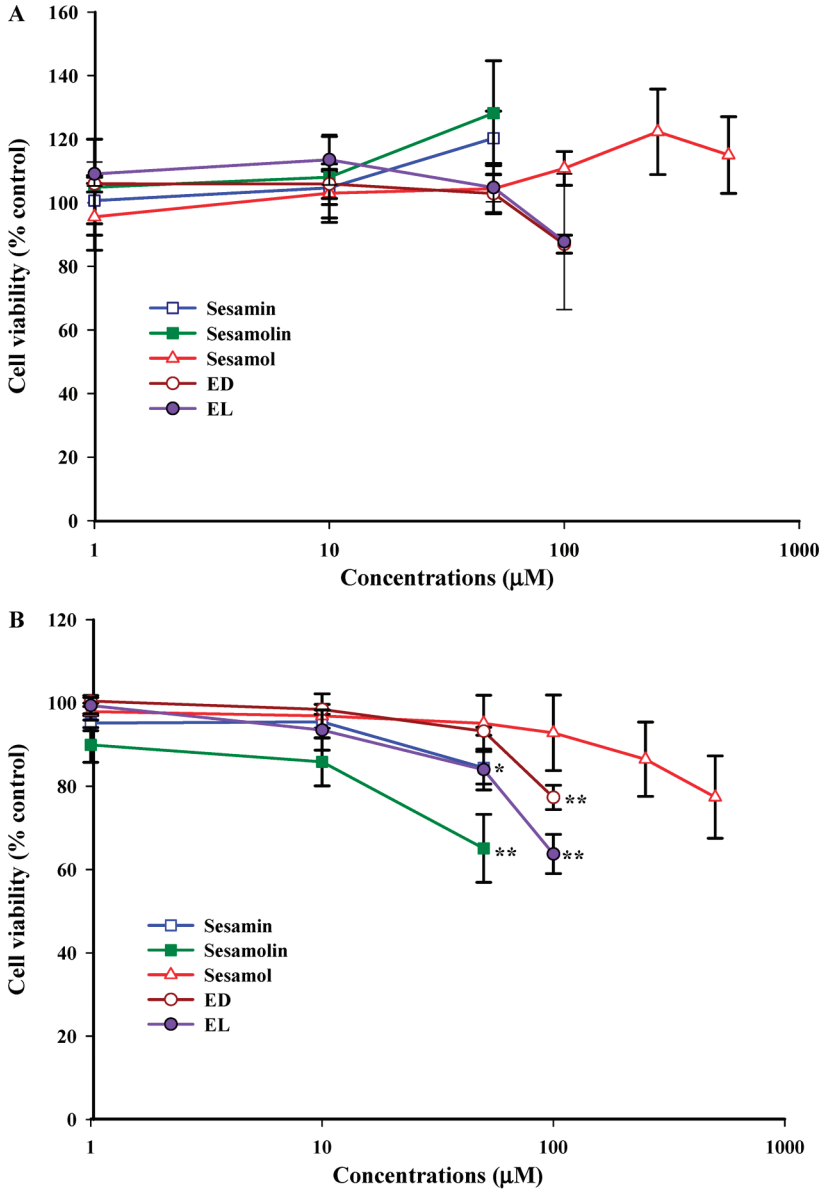

Figure 2. Effects of sesame lignans and their metabolites on cell viability. Data are represented as the mean of percent viability of vehicle control from three replicates ± standard error of the mean. (**A**) Cells were cultured in CSS medium and treated with sesame lignans and their metabolites for 24 h. (**B**) Cells were cultured in FBS medium and treated with sesame lignans and their metabolites for 24 h. * and ** represent statistically significant differences from control at $p < 0.05$ and 0.01.

with various concentrations of sesamin, sesamolin, sesamol, ED, or EL. The concentrations of sesame lignans and their metabolites were selected on the basis of our preliminary study, which revealed that sesamin and sesamolin precipitated at concentrations exceeding 50 μM; therefore, maximum concentration of these compounds in this study was 50 μM. However, sesamol, ED and EL dissolved in DMSO better than sesamolin and sesamin, therefore, these compounds were able to be tested at higher concentrations. After 24 h of exposure, cell viability was measured by using the SRB method. In CSS medium, tested compounds at a concentration of 50 μM did not cause cytotoxic effects (**Figure 2A**). In contrast, a slight increase in cell viability was observed when T47D-KBluc cells were treated with sesamin and sesamolin while sesamol showed a slight increase in cell viability at concentrations of 100−500 μM. Distinct responses of T47D-KBluc cells to lignans were observed when cultured in FBS medium. In the culture medium containing E2, all tested compounds exhibited cytotoxic effects on T47D-KBluc cells in a dose-dependent manner (**Figure 2B**). However, at the tested doses, significant reductions in cell viability ($p < 0.05$) were evidenced when tested with sesamin, sesamolin or EL at concentration 50 μM. These data suggested that the cytotoxic effects of sesame lignans and their metabolites were related to the level of E2 present in the cell cultured medium and may function via an interference with the modulatory activity of E2 on cell growth.

Activation of ERE by Sesame Lignans and Their Metabolites. The observed involvement of E2 in the cytotoxic activity of sesame lignans and their metabolites suggested the possibility that the tested compounds may possess estrogenic or antiestrogenic properties. To directly address this hypothesis, the effect of tested compounds on activation of ERE was studied using T47D-KBluc cells. The cells were treated for 24 h with each sesame lignan and its metabolites. The results showed that all compounds except ED could induce ERE-luciferase expression above the vehicle control. Among tested compounds, sesamol possessed the highest ability in ERE induction. The significant ERE induction

by sesamol can be observed at all tested concentrations of 1−500 μM ($p < 0.01$). The ERE induction by sesamol peaked at sesamol concentration of 10 μM (16.42 ± 2.67 fold of control, $p < 0.01$), and the level was maintained at concentration 50 μM (16.60 ± 1.18 fold of control, $p < 0.01$) before declining at higher concentrations of 100 μM to 500 μM (**Figure 3A**). For sesamin, sesamolin and EL, significant ERE inductions were observed at 10 μM (10.15 ± 0.59, 6.03 ± 0.48 and 7.79 ± 0.11, respectively) (**Figure 3A**). The results indicated that sesame lignans and their metabolites have variable efficacy in ERE induction in human breast cancer cells.

Further investigation was conducted to test whether estrogen receptors are directly involved in the ERE activation by E2, sesame lignans and their metabolites. The known estrogen receptors' competitive antagonist, ICI 182 780, was used at concentration 1 μM, a concentration which has been shown to completely inhibit E2-induced ERE (*24*). Our results showed that ICI 182 780 at this concentration almost completely inhibits E2-ERE induction. The luciferase responses to 1 nM E2, 10 μM of tested compounds except ED were significantly reduced by coincubation with 1 μM ICI 182 780 ($p < 0.05$, **Figure 3B**), indicating that estrogen receptors were directly involved in this response. When comparing the potency of sesame lignans and their metabolites to E2, the degree of ERE inductions by tested compounds was significantly less than those attained by E2 (**Figure 3C**). These results indicated that sesame lignans and their metabolites except ED were weak activators of ERE as compared to endogenous activator, E2, in human breast cancer cells.

Sesame Lignans and Their Metabolites Decreased E2-Induced ERE Activation. As the previous experiment showed that sesame lignans and their metabolites possessed weak estrogenic activity in the absence of E2, their effects in the presence of E2 were investigated. The results showed that coincubation of E2 with 10 μM of each compound led to a significant decrease in the maximal responses of E2-induced ERE activation ($p < 0.05$, **Figure 4A**). EL showed the highest inhibitory effect on ERE activation by E2. It should be noted that at the lowest concentration of E2 tested (10^{-12} M), 10 μM sesamin and sesamol significantly ($p < 0.01$) increased the responses of E2 when compared to E2 alone, suggesting that these compounds exhibited an additive estrogenic effect when coincubated with low concentrations of E2. From **Figure 4A**, a downward trend of maximum luciferase induction was observed in all coincubation treatments when compared to E2 alone. This result differed from the coincubated E2 with the known antagonists tamoxifen and ICI 182 780. From **Figure 4B**, tamoxifen and ICI 182 780 shifted the dose−response curves of T47D-KBluc cells to E2 to the right without decreasing the maximum response. This characteristic differed from the coincubation of sesame lignans and their metabolites with E2 (**Figure 4A**), which did not shift the dose−response curve to the right but significantly decreased maximal responses of E2. The obtained data suggested that the inhibitory effect of sesame lignans and their metabolites on E2-induced ERE activation is different from estrogen antagonists, tamoxifen and ICI 182 780.

To confirm that the observed antagonistic effect of tested compounds exhibited a dose-dependent manner in fixed E2 concentration, sesamol was selected as a representative compound. Sesamol at concentrations of 1−100 μM was coincubated with 1 nM E2, a physiological concentration, for 24 h. The result revealed that sesamol 1−100 μM decreased the E2-ERE activation in a dose-dependent manner at which the significant inhibitory effect of sesamol could be observed at concentrations 10 μM and 100 μM ($p < 0.05$) (**Figure 4 C**).

In order to determine the E2 antagonistic potency of sesame lignans and their metabolites, the EC_{50} values of each compound were calculated. The EC_{50} values are defined as the concentrations of test compounds giving 50% of the maximum responses which were calculated from the dose−response curves. The EC_{50} values of E2 coincubated with 10 μM sesame lignans and their metabolites were slightly different. The slope of concentration response curve of E2 alone was steeper than the coincubation with sesame lignans. The coincubation of E2 with all tested compounds exhibited a significantly lower E_{max} than E2 alone ($p < 0.05$ and 0.01). The coincubation of E2 with 10 μM EL exhibited the lowest EC_{50} (10.77 ± 0.61, $p < 0.01$) (**Table 2**).

Sesame Lignans and Their Metabolites Induced the Expression of *pS2* and Progesterone Receptor Genes. To investigate whether sesame lignans and their metabolites can induce the expression of targeted genes of estrogen, 10 μM of each compound or 1 nM E2 was used to treat T47D cells for 24 h and mRNA levels of *pS2* and progesterone receptor were quantified by real time RT-PCR. Sesamin, sesamol, and EL significantly induced *pS2* gene expression whereas sesamolin and ED did not (**Figure 5**). Among all tested compounds, 10 μM sesamol exhibited the highest ability to induce *pS2* gene, and this induction was similar to that of E2 (14.00 ± 2.93 and 15.43 ± 1.64 fold induction of control, respectively). For progesterone receptor gene stimulation, all the tested compounds except ED could induce the expression of this gene but only E2 and sesamol exhibited statistically significant induction over control (**Figure 5**). These results indicated that most sesame lignans and their metabolites have ability in induction of some targeted genes of estrogen which confirms their estrogenic activity in the absence of E2.

DISCUSSION

This study aimed to determine the estrogenic properties of several sesame lignans and their metabolites. To address this issue, their effects in the presence or absence of estrogen on the ERE of hormone-dependent human breast cancer cells were investigated. Moreover, their effects on the viability of T47D-KBluc cells in the presence or absence of steroid hormone in cultured medium were also examined. From the results, effects of sesame compounds on growth of T47D-KBluc cells depended on level of estrogen in cultured medium (**Figure 2A**,**B**). Sesamin, sesamolin and enterolactone exhibited the cytotoxic effect in the presence of estrogen in cultured medium (FBS) (**Figure 2B**) while the opposite effect was observed in estrogen depleted medium (CSS) (**Figure 2A**). These results are similar to the previous *in vitro* study which has demonstrated that EL could inhibit E2-stimulated MCF-7 cell growth (*29*). An increase in cell growth by sesamin, sesamolin and EL may be due to their estrogenic effects (*30*). In the presence of estrogen, sesame lignans may exert antiestrogenic activity by reducing the growth of T47D-KBluc cells. These results led us to hypothesize that sesame lignans and their metabolites may possess estrogenic or antiestrogenic activities in hormone-dependent breast cancer cells. Therefore, in this study, the human breast cancer, T47D-KBluc cells, which is transfected with the ERE luciferase reporter system, was used to test whether selected sesame lignans and their metabolites have estrogenic/antiestrogenic properties.

The data of ERE induction indicated that all of the tested compounds except ED possessed estrogenic activity. However, when compared to E2, sesame lignans and their metabolites are weak estrogen agonists. Moreover, sesame lignans and their metabolites showed lower affinity to ERE activation when compared to E2 (**Figure 3C**). This effect can be abolished by coincubation of 1 nM E2, sesame lignans and their metabolites with 1 μM ICI 182 780, suggesting that the estrogenic activities of these compounds are activated via estrogen receptors. It is well-known that estrogen mediates its function on ER as ligand-inducible transcription factors.

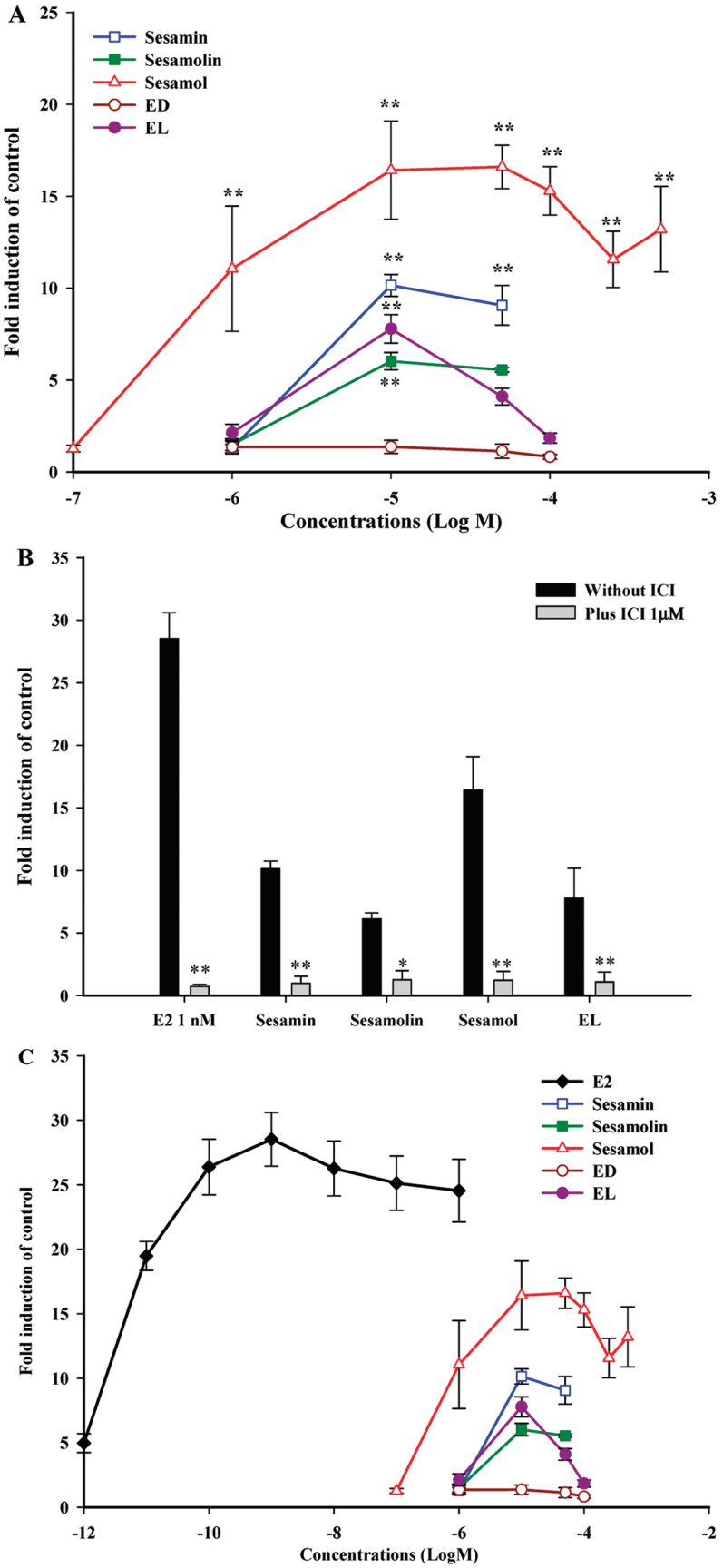

Figure 3. ERE activation by sesame lignans and their metabolites. (**A**) T47D-KBluc cells were treated with increasing concentrations of sesamin, sesamolin, sesamol, ED and EL in 100 μL of medium in a 96-well opaque plate for 24 h. (**B**) The inhibitory effect of 1 μM ICI 182 780 on 1 nM E2 and sesame lignans and their metabolites induced ERE activation. (**C**) Comparison of the potency of sesame lignans and their metabolites with 17β-estradiol (E2). Data are represented as the mean of fold induction of vehicle controls of three replicated assays $\pm$ standard error of the mean. * and ** represent statistically significant differences from control at $p < 0.05$ and 0.01, respectively.

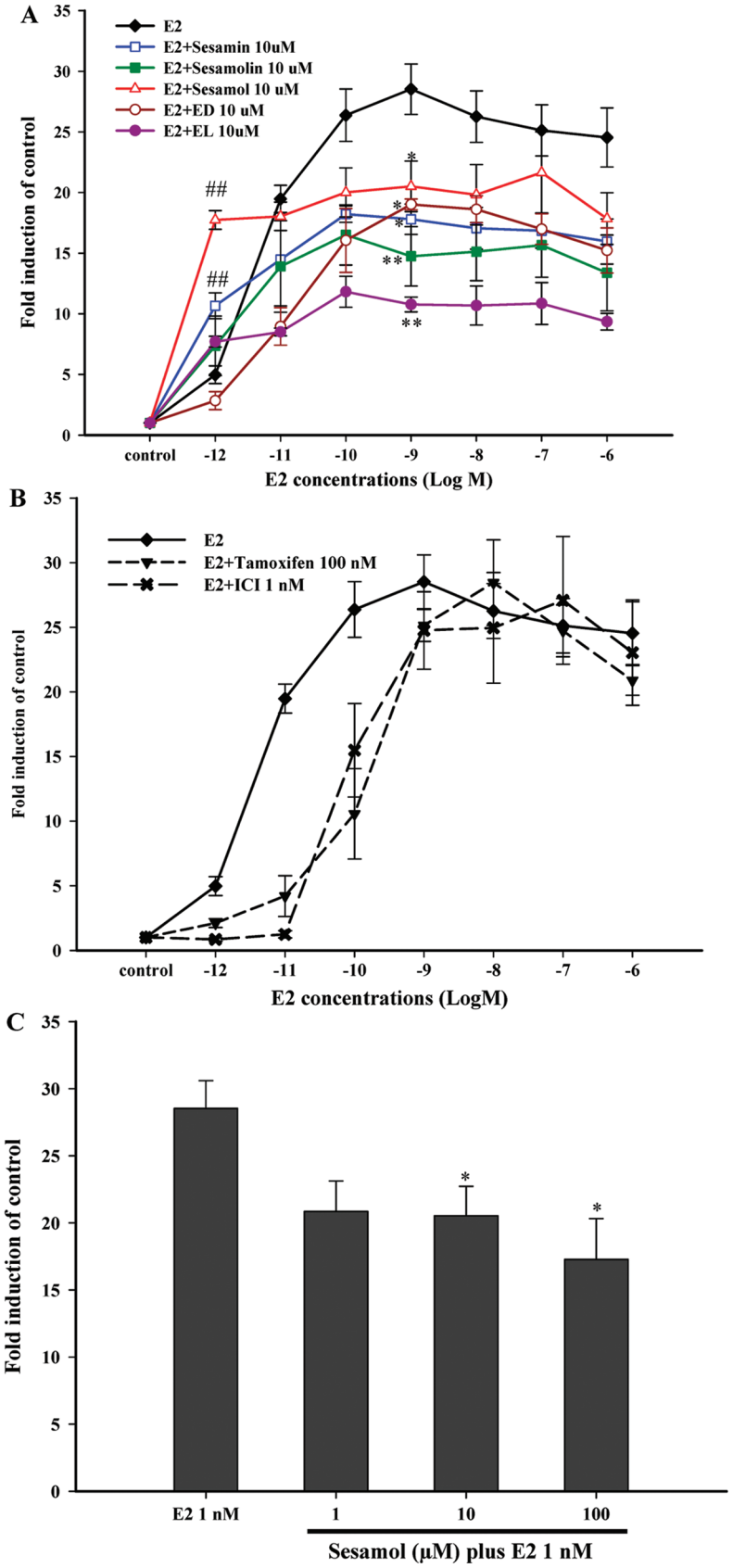

Figure 4. Modulatory effects of sesame lignans, their metabolites, and E2 antagonists on E2-induced ERE activation in T47D-KBluc cells. (**A**) Dose−response curves of ERE activation by E2 alone and in combination with 10 μM sesamin, sesmolin, sesamol, ED and EL for 24 h. (**B**) Comparison of the inhibitory effect of 100 nM tamoxifen and 1 nM ICI 182 780 on luciferase activity of E2- induced ERE activation. (**C**) The effect of sesamol on 1 nM E2-induced ERE activation. Data are represented as the mean of fold induction of vehicle controls of three replicated assays ± standard error of the mean. * and ** represent statistically significant differences from 10^{-9} M E2 at $p < 0.05$ and 0.01, respectively. ## represents statistically significant differences from 10^{-12} M E2 at $p < 0.01$.

Table 2. EC_{50} Values of E2 Coincubated with Sesame Lignans and Their Metabolites[a]

treatment	EC_{50} (M)	slope factor	R^2	E_{max} (fold induction of control)
E2 (1 nM)	1.35×10^{-12}	38.50	0.957	28.52 ± 2.08
E2 + sesamin 10 μM	1.06×10^{-12}	30.49	0.933	17.80 ± 1.25*
E2 + sesamolin 10 μM	1.13×10^{-12}	31.96	0.981	14.75 ± 2.45**
E2 + sesamol 10 μM	9.9×10^{-13}	32.39	0.896	20.51 ± 2.08*
E2 + ED 10 μM	1.32×10^{-12}	31.94	0.946	19.02 ± 0.45*
E2 + EL10 μM	1.04×10^{-12}	28.13	0.896	10.77 ± 0.61**

[a] Values of EC_{50}, slope factor and R^2 are calculated from linear regression fitting of concentration−response curve. E_{max} values are represented as mean ± standard error of the mean. * and ** represent statistically significant differences from E2 at $p < 0.05$ and 0.01, respectively.

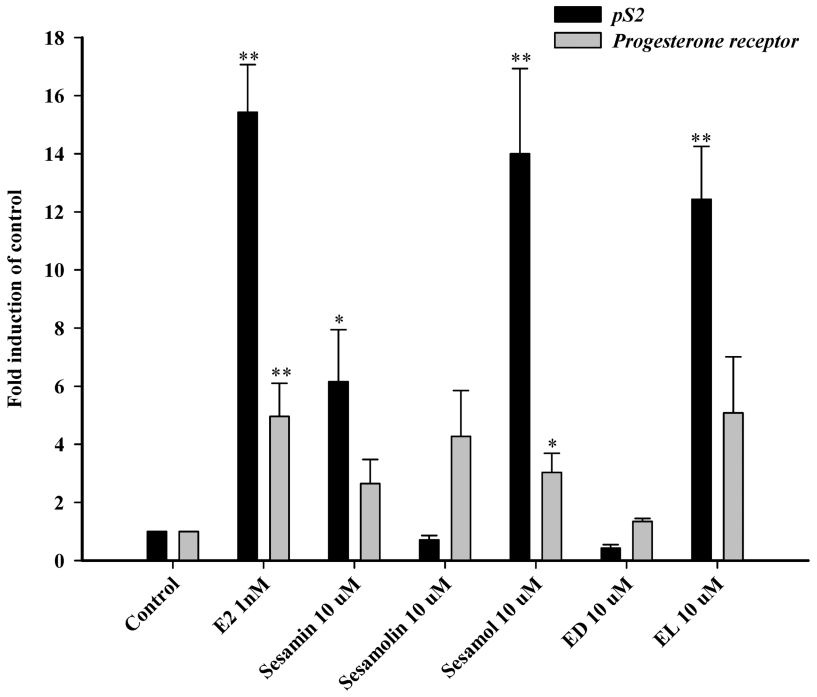

Figure 5. The relative mRNA levels of *pS2* and progesterone receptor in T47D cells after incubation with 1 nM 17β-estradiol (E2) and 10 μM sesame lignans and their metabolites. Data are represented as the mean of fold induction of vehicle controls of three replicated assays ± standard error of the mean. * and ** represent statistically significant differences from control at $p < 0.05$ and 0.01, respectively.

The ligand binding domain (LBD) of ER contains a specific ligand binding cavity which will recognize a certain structural feature such as E2 which contains a steroidal skeleton including four aromatic rings and two OH groups (**Figure 1**). The precise spacing of two OH groups at both ends of the E2 structure and its four aromatic rings is considered as the structural basis of ER-LBD (*31*). However, based on the principle that chemicals with similar biological activity share common structural features, ER-LBD can recognize any chemicals that possess either steroid skeletal or aromatic ring containing OH group (phenolic ring). Regarding chemical structures of sesame lignans and their metabolites, all tested compounds except sesamol are grouped as lignans which are based on the coupling of two propylbenzene (C_6C_3) units (**Figure 1**) (*32*), while sesamol contains a phenolic ring. In the present study, estrogenic activity of tested compounds may be related to their aromatic ring and phenolic ring which are likely to be ER ligand (*31*). However, their binding specificity may be different depending on the nature of their structures. It has been reported that the remaining phenolic ring has stronger binding affinity to ER than an aromatic ring that did not contain any OH group. However, it also depends on the rest of the functional groups that may interact to the adjacent binding cavity of ER-LBD (*31*, *33*). Therefore, in the present study, sesamol exhibited the highest agonistic effect on ERE activation among the tested compounds, which may be due to its containing a phenolic hydroxyl ring that is similar to the OH group attaching to a C3 unit of the E2 structure (*33*,*34*). However, because this cell naturally expresses both estrogen receptor subtypes (ERα and ERβ), we could not identify which subtype was responsible for the ERE activation.

Interestingly, although sesamin and sesamol exhibited low estrogenic activity in the absence of E2 (**Figure 3C**), coincubating of these compounds with various concentrations of E2 resulted in biphasic effects on E2-ERE activation. The concentrations of E2 selected ranged from high concentrations (10^{-7} to 10^{-6} M) to physiological concentrations (10^{-8} to 10^{-9} M) and low circulating concentrations in postmenopausal women (10^{-10} to 10^{-12} M). It has been reported that the concentrations at 10^{-12} to 10^{-10} M E2 were sensitive enough to produce the maximal response of E2 (*35*). Sesamin and sesamol exhibited stimulatory effects on E2-ERE induction at low E2 concentration (10^{-12} M) and inhibitory effects at high concentrations of E2 (10^{-9}−10^{-6} M) (**Figure 4A**). These results were consistent with the cytotoxic effects of tested compound on T47D-KBluc cells. In the absence of E2, sesamin and sesamol slightly induced cell proliferation, which may reflect their weak estrogen agonistic effect (**Figure 2A**). At the low level of E2, sesamol and sesamin may mimic the E2 effect, hence, additive effects on E2-ERE activation were observed (**Figure 4A**). In contrast, when the tested compounds were coincubated with physiological and high concentrations of E2, inhibitory effects were

observed. Moreover, at a fixed concentration of E2, sesamol 1−100 μM showed an inhibitory effect on E2-induced ERE activation in a dose-dependent manner (**Figure 4C**). This result was consistent with the cytotoxic effect of tested compounds on T47D-KBluc cells which exhibited more toxicity in the presence of E2 (**Figure 2B**). At physiological and high concentrations of E2, 10 μM sesame lignans and their metabolites exert antiestrogenic activities on ERE activation, which may result in a cytotoxic effect on T47D-KBluc cells (**Figure 2B**). Taken together, the results demonstrated that sesame lignans and their metabolites exhibited estrogenic or antiestrogenic effects depending on E2 levels. Although this present study showed stimulation of ERE and activation of E2-regulated gene expression, direct evidence of sesame lignans and their metabolites bound with ER is still needed. Studies by others as discussed previously suggested that the tested compounds are likely to be ER ligand with regard to their structural features (*33*, *34*). Sesame lignans and their metabolites may induce conformational changes of ER which affected the LBD and consequently altered the binding of E2. The ERE activation may be decreased by the imperfect binding of E2 on ER and further induced incomplete recruitment of coregulators (*23*).

ERE activation by E2 and ER agonists induce ERE activation leading to the stimulation of the relevant breast cancer genes such as *pS2* (*14*, *36*). In the present study, we investigated the expression of two E2-regulated genes which are *pS2* and progesterone receptor in T47D cells after incubation with tested compounds. Sesamol, sesamin and EL stimulated the expression of *pS2* while sesamolin and ED did not have this effect (**Figure 5B**). For the progesterone receptor gene, only sesamol exhibited significant induction. Among the tested compounds, sesamol was the most prominent compound with regard to its ERE activation and the activation of *pS2* and progesterone receptor genes. Although the ERE activations of tested compounds were lower than that of E2, the *pS2* gene activation by some tested compounds were at a similar level activated by E2. This is probably because the expression of estrogen targeted genes depends on the promoter of ERE sequence context. In addition, estrogen receptor binding affinity of each compound also has an impact on the stimulation of estrogen targeted genes (*37*). In the present study, the ERE induction was obtained from the three EREs which had been transfected into T47D cells. This could reflect the high sensitivity of ERE response even though the tested compounds possessed a low estrogenic activity that may lack measurable activity in endogenous single ERE (*38*). Thus, the degree of gene expression induction of endogenous ERE does not always account for the degree of induction from exogenous ERE (*37*). Therefore, it could be possible that the level of the estrogen targeted gene induction of sesame lignans and their metabolites could be different from the potency of ERE activations. However, it should be noted that sesame lignans, sesamol as well as mammalian lignans, have the ability to induce transcriptional activation of hormone-dependent breast cancer and subsequently induce the estrogen-dependent genes.

The coincubation of E2 with lignans responded differently from the coincubation with tamoxifen and ICI 182 780. The dose−response curves of coincubation with tested compound showed definite characteristics of a noncompetitive antagonism. It is well documented that there are two important types of antagonism, competitive and noncompetitive. Competitive antagonism is characterized by the parallel shift of dose−response curve to the right without changing the maximal response of the agonist, whereas noncompetitive antagonism shows the suppression of the maximal response of dose−response curve and a nonparallel shift of dose−response curve to the right. Likewise, the noncompetitive characteristics of sesame lignans and their metabolites are shown by the reduction of maximal E2 responses even when the concentrations of E2 were highly increased. Furthermore, a significant decrease of E_{max} responses of E2 in the tested compounds' coincubation was also observed (**Table 2**). This antagonism may be attributable to a partial allosteric conformation of estrogen receptors. Since sesame lignans may not activate estrogen receptors at the same site as estradiol, they may partially interfere with events downstream from the receptors such as activation of ERE transcription.

Unlike sesame lignans and their metabolites, antiestrogens such as tamoxifen and ICI 182 780 differently inhibited the ERE activation in a competitive manner. Tamoxifen is classified as a type I antiestrogen compound which has mixed estrogenic/antiestrogenic action while ICI 182 780 is a type II or pure antiestrogen which has no estrogenic properties (*35*). Both types of antiestrogen compounds are competitive antagonists on estrogen action which interact at the same site of the ligand binding domain (*23*). However, since the compounds are in competition, increasing E2 concentrations still allows the achievement of maximum response. In this experiment, different doses of ICI 182 780 and tamoxifen were selected from our preliminary study. ICI 182 780 has a higher inhibitory effect than tamoxifen (data not shown), therefore we selected the IC_{50} of each compound to demonstrate its inhibitory effect on the E2 response. The parallel shifts of dose−response curves to the right in **Figure 4B** were consistent with the competitive antagonistic characteristic (*39*). The mechanisms of action of these two compounds were reported to inhibit estrogenic effect by disrupting the translocation of estrogen receptors and causing changes in shape of the receptor complex which prevented ERE activation (*35*, *40*).

This present study explored a new aspect of estrogenic activity of sesame seed constituents and their metabolites. The selected compounds found in sesame seed and oil exhibited an estrogenic or antiestrogenic activity on ERE activation in hormone-dependent human breast cancer cells depending on the E2 conditions. This evidence suggests that the endogenous E2 status is important for determining the estrogenic/antiestrogenic properties of sesame lignans and their metabolites. The estrogen agonistic effect of sesame lignan information may be useful for conventional hormone replacement in postmenopausal women. However, due to a potential tumor growth stimulation of sesame lignans and their ability to induce some estrogen related genes, oral supplementation of sesame seed should be prescribed with caution particularly in postmenopausal women and hormone-dependent breast cancer patients. It should also be emphasized that sesame seeds available in the market have a wide variation of sesame lignan contents (*17*); therefore, their estrogenic and antiestrogenic effects may not be observed in those consumers taking sesame products containing very low sesame lignan contents. Furthermore, the molecular mechanism relevant to estrogenic effect of these selected lignans should be studied further in detail to gain a better understanding of their potential health benefits and contraindication in hormone-dependent breast cancers.

ABBREVIATIONS USED

ED, enterodiol; EL, enterolactone; ERE, estrogen response element; E2, 17β-estradiol; SRB, sulforhodamine B; FBS, fetal bovine serum; CSS, charcoal-dextran stripped serum; PBS, phosphate buffer saline; DMSO, dimethyl sulfoxide; RLU, relative light unit; h, hour; min; minute, RT-PCR, reverse transcriptase-polymerase chain reaction; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; v/v, volume by volume; w/v, weight by volume; LBD, ligand binding domain.

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LITERATURE CITED

(1) Liehr, J. Is estradiol a genotoxic mutagenic carcinogen? *Endocr. Rev.* **2000**, *21*, 40−54.

(2) Yager, J. D.; Davidson, N. E. Estrogen carcinogenesis in breast cancer. *N. Engl. J. Med.* **2006**, *354*, 270−282.

(3) Sotoca, A. M.; Ratman, D.; van der Saag, P.; Strom, A.; Gustafsson, J. A.; Vervoort, J.; Rietjens, I. M.; Murk, A. J. Phytoestrogen-mediated inhibition of proliferation of the human T47D breast cancer cells depends on the ERalpha/ERbeta ratio. *J. Steroid Biochem. Mol. Biol.* **2008**, *112*, 171−178.

(4) Mak, P.; Leung, Y. K.; Tang, W. Y.; Harwood, C.; Ho, S. M. Apigenin suppresses cancer cell growth through ERbeta. *Neoplasia* **2006**, *8*, 896−904.

(5) 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.; Adlercreutz, T. Soybean phytoestrogen intake and cancer risk. *J. Nutr.* **1995**, *125*, 757−770.

(6) dos Santos Silva, I.; Mangtani, P.; McCormack, V.; Bhakta, D.; McMichael, A. J.; Sevak, L. Phyto-oestrogen intake and breast cancer risk in South Asian women in England: findings from a population-based case-control study. *Cancer Causes Control* **2004**, *15*, 805−818.

(7) Morito, K.; Hirose, T.; Kinjo, J.; Hirakawa, T.; Okawa, M.; Nohara, T.; S, O.; Inoue, S.; Muramatsu, M.; Masamune, Y. Interaction of phytoestrogens with estrogen receptors α and β. *Biol. Pharm. Bull.* **2001**, *24*, 351−356.

(8) Kuiper, G. G.; Lemmen, J. G.; Carlsson, B.; Corton, J. C.; Safe, S. H.; van der Saag, P. T.; van der Burg, B.; Gustafsson, J. A. Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor beta. *Endocrinology* **1998**, *139*, 4252−4263.

(9) Boyapati, S. M.; Shu, X. O.; Ruan, Z. X.; Dai, Q.; Cai, Q.; Gao, Y. T.; Zheng, W. Soyfood intake and breast cancer survival: a followup of the Shanghai Breast Cancer Study. *Breast Cancer Res. Treat.* **2005**, *92*, 11−17.

(10) Keinan-Boker, L.; van Der Schouw, Y. T.; Grobbee, D. E.; Peeters, P. H. Dietary phytoestrogens and breast cancer risk. *Am. J. Clin. Nutr.* **2004**, *79*, 282−288.

(11) Lin, C.-C.; Tsai, L.-Y.; Ho, T.-C.; Teng, S.-C. Determination of differential estrogenicity of isoflavonoids by E2-ER-ERE-dependent gene expression in recombinant yeast and MCF-7 human breast cancer cells. *Food Chem.* **2008**, *108*, 719−726.

(12) Benassayag, C.; Perrot-Applanat, M.; Ferre, F. Phytoestrogens as modulators of steroid action in target cells. *J. Chromatogr., B: Anal. Technol. Biomed. Life Sci.* **2002**, *777*, 233−248.

(13) Brooks, J. D.; Thompson, L. U. Mammalian lignans and genistein decrease the activities of aromatase and 17beta-hydroxysteroid dehydrogenase in MCF-7 cells. *J. Steroid Biochem. Mol. Biol.* **2005**, *94*, 461−467.

(14) van Meeuwen, J. A.; Korthagen, N.; de Jong, P. C.; Piersma, A. H.; van den Berg, M. (Anti)estrogenic effects of phytochemicals on human primary mammary fibroblasts, MCF-7 cells and their co-culture. *Toxicol. Appl. Pharmacol.* **2007**, *221*, 372−383.

(15) Tashiro, T.; Fukuda, Y.; Osawa, T.; Namiki, M. Oil and minor components of sesame (*Sesamum indicum* L.) strains. *J. Am. Oil Chem. Soc.* **1990**, *67*, 508−511.

(16) Hemalatha, S. Ghafoorunissa. Lignans and tocopherols in Indian sesame cultivars. *J. Am. Oil Chem. Soc.* **2004**, *81*, 467−470.

(17) Rangkadilok, N.; Pholphana, N.; Mahidol, C.; Wongyai, W.; Saengsooksree, K.; Nookabkaew, S.; Satayavivad, J. Variation of sesamin, sesamolin and tocopherols in sesame (*Sesamum indicum* L.) seeds and oil products in Thailand. *Food Chem.* **2010**, *122*, 724−730.

(18) Elleuch, M.; Besbes, S.; Roiseux, O.; Blecker, C.; Attia, H. Quality characteristics of sesame seeds and by-products. *Food Chem.* **2007**, *103*, 641−650.

(19) Liu, Z.; Saarinen, N. M.; Thompson, L. U. Sesamin is one of the major precursors of mammalian lignans in sesame seed (*Sesamum indicum*) as observed *in vitro* and in rats. *J. Nutr.* **2006**, *136*, 906−912.

(20) Penalvo, J. L.; Heinonen, S. M.; Aura, A. M.; Adlercreutz, H. Dietary sesamin is converted to enterolactone in humans. *J. Nutr.* **2005**, *135*, 1056−1062.

(21) Kang, M. H.; Naito, M.; Tsujihara, N.; Osawa, T. Sesamolin inhibits lipid peroxidation in rat liver and kidney. *J. Nutr.* **1998**, *128*, 1018−1022.

(22) Carreau, C.; Flouriot, G.; Bennetau-Pelissero, C.; Potier, M. Enterodiol and enterolactone, two major diet-derived polyphenol metabolites have different impact on ERalpha transcriptional activation in human breast cancer cells. *J. Steroid Biochem. Mol. Biol.* **2008**, *110*, 176−185.

(23) Brzozowski, A. M.; Pike, A. C.; Dauter, Z.; Hubbard, R. E.; Bonn, T.; Engstrom, O.; Ohman, L.; Greene, G. L.; Gustafsson, J. A.; Carlquist, M. Molecular basis of agonism and antagonism in the oestrogen receptor. *Nature* **1997**, *389*, 753−758.

(24) Wilson, V. S.; Bobseine, K.; Gray, L. E., Jr. Development and characterization of a cell line that stably expresses an estrogen-responsive luciferase reporter for the detection of estrogen receptor agonist and antagonists. *Toxicol. Sci.* **2004**, *81*, 69−77.

(25) Meuller, S. Overview of *in vitro* tool to assess the estrogenic and antiestrogenic activity of phytoestrogens. *J. Chromatogr., B* **2002**, *777*, 155−165.

(26) Tanmahasamut, P.; Liu, J.; Hendry, L. B.; Sidell, N. Conjugated linoleic acid blocks estrogen signaling in human breast cancer cells. *J. Nutr.* **2004**, *134*, 674−680.

(27) Thiantanawat, A.; Long, B.; Brodie, A. Signaling pathways of apoptosis activated by aromatase inhibitors and antiestrogens. *Cancer Res.* **2003**, *63*, 8037−8050.

(28) Latil, A.; Bieche, I.; Vidaud, D.; Lidereau, R.; Berthon, P.; Cussenot, O.; Vidaud, M. Evaluation of androgen, estrogen (ER alpha and ER beta), and progesterone receptor expression in human prostate cancer by real-time quantitative reverse transcription-polymerase chain reaction assays. *Cancer Res.* **2001**, *61*, 1919−1926.

(29) Mousavi, Y.; Adlercreutz, H. Enterolactone and estradiol inhibit each other's proliferative effect on MCF-7 breast cancer cells in culture. *J. Steroid Biochem. Mol. Biol.* **1992**, *41*, 615−629.

(30) Wang, C.; Kurzer, M. S. Phytoestrogen concentration determines effects on DNA synthesis in human breast cancer cells. *Nutr. Cancer* **1997**, *28*, 236−247.

(31) Fang, H.; Tong, W.; Shi, L. M.; Blair, R.; Perkins, R.; Branham, W.; Hass, B. S.; Xie, Q.; Dial, S. L.; Moland, C. L.; Sheehan, D. M. Structure-activity relationships for a large diverse set of natural, synthetic, and environmental estrogens. *Chem. Res. Toxicol.* **2001**, *14*, 280−294.

(32) Moazzami, A. A.; Eldin-Kamal, A. Sesame seed is a rich source of dietary lignans. *J. Am. Oil Chem. Soc.* **2006**, *83*, 719−723.

(33) Vaya, J.; Tamir, S. The relation between the chemical structure of flavonoids and their estrogen-like activities. *Curr. Med. Chem.* **2004**, *11*, 1333−1343.

(34) Miksicek, R. J. Estrogenic flavonoids: structural requirements for biological activity. *Proc. Soc. Exp. Biol. Med.* **1995**, *208*, 44−50.

(35) MacGregor, J. I.; Jordan, V. C. Basic guide to the mechanisms of antiestrogen action. *Pharmacol. Rev.* **1998**, *50*, 151−196.

(36) Willard, T. S.; Frawley, S. L. Phytoestrogens have agonistic and combinatorial effects on estrogen-responsive gene expression in MCF-7 human breast cancer cells. *Endocrine* **1998**, *8*, 117−121.

(37) Hall, J. M.; Korach, K. S. Analysis of the molecular mechanisms of human estrogen receptors alpha and beta reveals differential specificity in target promoter regulation by xenoestrogens. *J. Biol. Chem.* **2002**, *277*, 44455−44461.

(38) Mueller, S. O.; Simon, S.; Chae, K.; Metzler, M.; Korach, K. S. Phytoestrogens and their human metabolites show distinct agonistic and antagonistic properties on estrogen receptor alpha (ERalpha) and ERbeta in human cells. *Toxicol. Sci.* **2004**, *80*, 14−25.

(39) Buxton, O. L. I. Pharmacokinetics and pharmacodynamics. The dynamics of drug absorption, distribution, action and elimination. In *Goodman and Gilman's the pharmacological basis of therapeutics*; Brunton, L. L., Lazo, S. J., Parker, L. K., Eds.; McGraw-Hill: New York, 2006; pp 1−39.

(40) Dauvois, S.; White, R.; Parker, G. M. The antiestrogen ICI 182 780 disrupts estrogen receptor nucleocytoplasmic shuttling. *J. Cell Sci.* **1993**, *106*, 1377−1388.

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