Aloe-Emodin Enhances Tamoxifen Cytotoxicity by Suppressing Ras/ERK and PI3K/mTOR in Breast Cancer Cells


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Abstract: Aloe-emodin (AE) is derived from Aloe vera and rhubarb (Rheum palmatum) and exhibits anticancer activities via multiple regulatory mechanisms in various cancers. AE can also enhance the anticancer efficacy of cisplatin, doxorubicin, docetaxel, and 5-fluorouracil; however, its effects remain poorly characterized. MCF-7, MDA-MB-231, MDA-MB-468, BT-474, and HCC-1954 breast cancer cell lines were treated with the indicated conditions of AE, and cell viability assays were performed. The expression levels of signaling proteins were determined by western blot analysis, intracellular reactive oxygen species (ROS), cell cycle distributions, and rates of apoptosis as estimated by flow cytometry. In comparison with other cells, MCF-7 cells were more sensitive to AE

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treatment; AE enhanced the cytotoxicity of 9 μg/ml tamoxifen by reducing EGFR, ERα, Ras, ERK, c-Myc, and mTOR protein expression and blocking PI3K and mTOR activation. Finally, although co-treatment of AE with tamoxifen increased intracellular ROS, there were no effects on cell cycle progression. Besides facilitating tamoxifen-induced cell death, AE also enhanced the antiproliferative activity of tamoxifen by blocking Ras/ERK and PI3K/mTOR pathways in breast cancer cells, thus demonstrating the chemosensitizing potential of AE.

Keywords: Breast Cancer; Aloe-Emodin; Tamoxifen; Chemosensitization; ERK; PI3K; mTOR.

Introduction

In Taiwan, breast cancer is the most common cancer among women and the fourth most common cause of death from cancer (Health Promotion Administration, 2016). In 2012, 10,525 Taiwanese women were diagnosed with breast cancer, and the age-standardized mortality rate of 11.9 per 100,000 people was similar to that reported globally (Sanguinetti et al., 2014). Breast cancer is treated with chemo-, radio-, anti-hormonal, and targeted therapies. However, incidences of breast cancer continue to increase, and alternative therapies with improved efficacy are required. The anticancer properties of various plant compounds have been identified; for example, paclitaxel from the bark of Pacific yew trees is widely used to treat ovarian, breast, lung, pancreatic, and various other cancers (Horwitz, 1992).

Plant anthraquinones are known to inhibit the growth of several cancer cells and their proliferation in vivo and in vitro (Huang et al., 2007). Among these, the anthraquinone emodin is abundant in rhubarb, and its anticancer activities are similar to those of breast cancer chemotherapies (Huang et al., 2007). However, although emodin reportedly inhibits cancer invasion, migration, and metastasis in human breast cancer cells (Zu et al., 2015), the liver is a major target organ for drug-induced toxicology (Guo et al., 2008).

Aloe-emodin (1,8-dihydroxy-3-hydroxymethyl-9,10-anthracenedione; AE) is derived from Aloe vera and rhubarb (Rheum palmatum); the aloe component is found as a gel in the sap and leaves of the plant. Aloe vera is widely used as a cosmetic and in Chinese alternative medicine because of its wound healing, skin smoothing, and rejuvenating effects, and emodin can induce apoptosis in some human breast cancer cells (Huang et al., 2013; Sui et al., 2014; Zu et al., 2015). Although the anti-breast cancer effects of AE are rarely reported, the low renal and liver toxicities of AE compared with those of emodin and rhein have been demonstrated through assays of antiviral, antibacterial, liver-protective, and blood pressure-modulating activities. Moreover, AE increases chemotherapeutic efficacy in Merkel skin carcinoma cells (Fenig et al., 2004), inhibits hepatoma cancer cell growth (Zhang et al., 2015), and markedly inhibits the proliferation of MKN45 gastric cancer cells (Chihara et al., 2015).

Previous studies have reported the antiproliferative mechanisms of AE in various cancers. However, these mechanisms have not been demonstrated for breast cancer; thus,
studies are warranted on the effects of AE in breast cancer cells with and without other anticancer drugs. Here, we showed the synergistic effects of tamoxifen and AE (Tam-AE) in breast cancer cells and evaluated the AE-mediated clinical pathways.

Materials and Methods

Materials

All cell culture media and additives were purchased from Gibco (Thermo Fisher Scientific Inc., Waltham, MA, USA), and chemicals were purchased from Merck Millipore (Billerica, MA, USA), unless otherwise indicated.

AE was kindly provided by Prof. Hui-Yi Lin (School of Pharmacy, China Medical University, Taichung, Taiwan). AE was prepared as a 20 mg/ml stock solution and diluted with fresh cell culture medium with the indicated concentration before use.

Anti-caspase 7, anti-cleaved PARP, anti-Bim, anti-Bcl-xl, anti-Estrogen Receptor α (ERα), anti-ERK1/2, anti-phospho-ERK1/2, anti-PI3K, anti-phospho-PI3K, anti-mTOR, and anti-phospho-mTOR were purchased from Cell Signaling Technology (Danvers, MA, USA). Anti-Bax and anti-EGFR were purchased from Santa Cruz Biotechnology (Dallas, TX, USA). Anti-Ras was purchased from Merck Millipore (Billerica, MA, USA). Anti-C-Myc was purchased from Abcam (Cambridge, MA, USA).

Cell Cultures

Human breast cancer cell lines were purchased from American Type Culture Collection (ATCC). MDA-MB-231 cells were cultured in L15 medium containing 10% fetal bovine serum (FBS), 50 unit/ml penicillin, and 50 unit/ml streptomycin at 37°C in a humidified atmosphere containing 100% air. MCF-7 cells were maintained in RPMI medium containing 10% FBS, 50 unit/ml penicillin, and 50 unit/ml streptomycin at 37°C in a humidified atmosphere containing 5% CO₂.

Cell Viability

Following cell treatments with the indicated conditions, MTT proliferation assays were performed according to standard procedures. Briefly, the tetrazolium salt MTT (5 mg/ml) was added to culture media and was metabolized by active mitochondria to the insoluble formazan salt after 4 h at 37°C. MTT-containing media were then removed by aspiration, and intracellular formazan crystals were dissolved in DMSO (150 μl/well) and quantified spectrophotometrically at 540 nm using an automatic spectrophotometer (Multiskan, Thermo Scientific, Hudson, NH, USA).

Further cell proliferation assays were performed by counting viable and dead cells using an automated cell analyzer (Muse; Merck Millipore, Billerica, MA, USA) and by fluorescence staining using the Muse Count & Viability Kit (Merck Millipore, Billerica, MA, USA) according to the manufacturer’s instructions.
Cell Cycle Analysis

Cells were seeded into 12-well plates (6 × 10^5/well) and treated with the indicated conditions. Cells were then harvested and fixed in 70% ethanol (in deionized water) at 4°C and were assayed using the Muse Cell Cycle Assay Kit (Merck Millipore, Billerica, MA, USA). Briefly, 200 μl of fixed cells in a 12 × 75 mm polystyrene test tube were centrifuged at 300 × g for 5 min at room temperature. Supernatants were then removed, and cell pellets were resuspended in 0.25 ml PBS per 5 × 10^5 cells. Then, the samples were centrifuged at 300 × g for 5 min at room temperature. Subsequently, 200 μl aliquots of ice-cold 70% ethanol were slowly added to the samples, mixed, and incubated for at least 3 h at −20°C. After removing and discarding the supernatants, cell pellets were resuspended and incubated for 30 min in 200 μl Muse Cell Cycle Reagent at room temperature in the dark, and cell cycle phases were determined using the Muse Cell Analyzer.

Apoptosis Analyses

Cells were harvested, suspended, and processed using the Muse Annexin V & Dead Cell Assay Kit (Merck Millipore, Billerica, MA, USA) according to the manufacturer’s instructions. Briefly, 100 μl aliquots of the Muse Annexin V & Dead Cell Reagent were added and mixed with 100 μl cell suspensions, and they were incubated for 20 min at room temperature in the dark. Cell apoptosis percentage was then determined using the Muse Cell Analyzer.

Oxidative Stress Detection

Intracellular oxidative stress was analyzed using the Muse Oxidative Stress Kit (Merck Millipore, Billerica, MA, USA) according to the manufacturer’s instructions. Briefly, after the indicated treatments, cells were suspended in 1 × Assay Buffer at 1 × 10^6–10^7 cells/ml, and Muse Oxidative Stress Reagent was diluted (1:100) in 1 × Assay Buffer (intermediate solution). The diluted Muse Oxidative Stress intermediate solution was then added at 1:80 to 1 × Assay Buffer, and 190 μl aliquots of the resulting Muse Oxidative Stress working solution were added to 10 μl cell suspensions and incubated at 37°C for 30 min. Finally, samples were mixed thoroughly and analyzed using a Muse Cell Analyzer.

Mitochondrial Membrane Potential Analysis

Following the indicated treatments, cells were harvested, centrifuged, and resuspended at 1 × 10^5 cells/ml in 1 × Assay Buffer for assays using the Muse MitoPotential Assay Kit (Merck Millipore, Billerica, MA, USA) according to the manufacturer’s instructions. Briefly, 100 μl cell suspensions were mixed with 95 μl aliquots of MitoPotential working solution and incubated for 20 min at 37°C in a CO2 incubator. Finally, 5 μl Muse Mito-Potential 7-AAD reagent was added, mixed thoroughly, and incubated for 5 min at room temperature. Mitochondrial membrane potential (MMP) analyses were performed using a Muse Cell Analyzer.
**Western Blot Analysis**

Treated cells were harvested in lysis buffer containing 20 mm Tris (pH 7.5), 150 mm NaCl, 1 mm EDTA, 1 mm EGTA, 1% Triton X-100, 2.5 mm sodium pyrophosphate, 1 mm glycerophosphate, and 1 mm Na$_3$VO$_4$ with a protease inhibitor mixture of 1 mm phenylmethylsulfonyl fluoride, 10 mg/ml leupeptin, and 10 mg/ml aprotinin. Protein concentrations in the lysates were quantified using the Bradford assay after heating at 95°C for 5 min in sample buffer, and 50 μg protein samples were separated on 8% polyacrylamide gels and transferred to PVDF membranes (Merck Millipore, Billerica, MA, USA).

Membranes were then blocked for 1 h using 5% non-fat dried milk in 0.1% PBST (500 ml of 1 × PBS with 0.5 ml of Tween-20) at room temperature and incubated with specific primary antibodies overnight at 4°C. Subsequently, membranes were washed three times with PBST and incubated with horseradish peroxidase-conjugated secondary antibody for 1 h at room temperature.

After washing with PBS three times, protein signals were detected using an enhanced chemiluminescence detection system (Amersham Biosciences, Piscataway, NJ, USA). The Coomassie dye staining in each lane was used as an internal control. Protein signal was determined using Image J software (http://rsb.info.nih.gov/ij/).

**Dose-Effect Analysis**

CompuSyn Software (ComboSyn Inc., Paramus, NJ) was used to analyze the drug interaction between different drugs according to the manufacturer’s instructions. The resulting combination index (CI) was used to indicate the dose-effect. A CI value of less than 1 indicates a synergism of Tam-AE, whereas a CI value above 1 indicates an antagonistic effect.

**Statistical Analysis**

Analyses were performed using the Statistical Analysis System (SAS 9.1). Data are presented as mean ± standard deviation (SD) except where indicated otherwise. Comparisons between groups were analyzed using Student’s two-tailed t-test. A value of $P < 0.05$ is considered statistically significant.

**Results**

MTT results showed that AE treatments decreased the survival of various breast cancer cell lines; these effects were the greatest in MCF-7 cells (Fig. 1), suggesting that MCF-7 cells are the most sensitive to AE among the tested cell lines. To reveal the changes in absolute cell viability after treating MCF-7 cells with the Tam-AE combination, cell viability assay was performed. There was no significant change in MCF-7 cell viability after 24 h of treatment; however, cell viability significantly decreased after 72 h of Tam-AE treatment ($P < 0.001$) (Fig. 2A) and was accompanied by cell shrinking and cell rupture (Fig. 2B).
Figure 1. Effects of AE on MCF-7, BT-474, and HCC1954 breast cancer cell viability. Cells were treated with the indicated doses of AE for (A) 24 h and (B) 72 h. Cell viability was then determined using MTT assays. Data are expressed as mean ± SD of three independent experiments.

Figure 2. MCF-7 cells after treatment with Tam-AE. Tamoxifen was used to treat cells in the presence or absence of AE. (A) Cell viability was determined after 24 h and 72 h of treatment. Data are expressed as mean ± SD of four independent experiments. * and ** represent $P < 0.05$ and $P < 0.001$, respectively, when compared with the tamoxifen-only treatment group. (B) Morphological changes in MCF-7 cells treated with Tam-AE. The morphology of cells following 72 h of treatment was photographed using a phase contrast microscope. The lower right panel of the Tam-AE (Tamoxifen + AE) group is a 2× magnified view of the center box.
To identify Tam-AE synergism in MCF-7 cells, dose-effect analysis was performed. Despite antagonism in the 24-h treatment group, the results demonstrated the synergistic interaction of Tam-AE in the 72-h treatment group (Table 1). In the 24-h treatment group, the higher the treatment concentration of tamoxifen and/or AE, the more antagonistic the drug interaction; however, the trend was reversed in the 72-h treatment group.

To determine whether AE can enhance the cytotoxic activity of tamoxifen through regulation of cell cycle progression, MCF-7 cells were treated with 9 μg/ml tamoxifen in the presence or absence of 4 μg/ml AE for 24 h and 72 h. However, no significant differences were observed between the AE, tamoxifen, or Tam-AE groups (Fig. 3A), suggesting that AE effects on tamoxifen-induced cell death were not mediated by cell cycle arrest. Furthermore, the apoptotic rates of cells with Tam-AE treatment were higher (P < 0.05) than those with tamoxifen-only treatment, and the rates were greatest with 4 μg/ml AE, indicating the synergistic effects of AE on tamoxifen-induced apoptosis in MCF-7 cells (Fig. 3B). Caspases and PARPs play key roles in apoptosis induction. Western blot

**Table 1. CI Calculated Using CompuSyn Software**

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<tr>
<th>Tamoxifen (μg/ml)</th>
<th>AE (μg/ml)</th>
<th>Fa</th>
<th>CI</th>
<th>Effect</th>
<th>Fa</th>
<th>CI</th>
<th>Effect</th>
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<td>Antagonistic</td>
<td>0.79</td>
<td>0.54</td>
<td>Synergistic</td>
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</table>

*Note: A CI value of less than 1 indicates synergism of Tam-AE. A CI value above 1 indicates an antagonistic effect. Fa, fraction affected; CI, combination index.*

To identify Tam-AE synergism in MCF-7 cells, dose-effect analysis was performed. Despite antagonism in the 24-h treatment group, the results demonstrated the synergistic interaction of Tam-AE in the 72-h treatment group (Table 1). In the 24-h treatment group, the higher the treatment concentration of tamoxifen and/or AE, the more antagonistic the drug interaction; however, the trend was reversed in the 72-h treatment group.

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**Figure 3. Effects of AE on cell cycle progression and apoptosis in tamoxifen-treated MCF-7 cells.** (A) Cell cycle. (B) Apoptosis after 72 h of treatment. (C) Cleavage of caspase 7 and PARP. Cells were treated with the indicated conditions. * and ** represent P < 0.05 and P < 0.001, respectively, when compared with the tamoxifen-only treatment group.
analysis showed significant increases in the cleavage of caspase 7 and PARP in MCF-7 cells (Fig. 3C) after Tam-AE treatment for 24 h and 72 h, suggesting that AE may strengthen tamoxifen-induced apoptosis via cleavage of caspase 7 and PARP. To verify whether the effects of AE on tamoxifen-induced apoptosis were mediated through the mitochondrial-dependent pathway, post-treatment MMP was measured. In comparison with tamoxifen-only treatment, Tam-AE treatment reduced MMP. In the tamoxifen-only group, the ratio of dead depolarized cells was 15.64% and 29.55% after 16 h and 24 h of treatment, respectively, whereas in the Tam-AE group, the higher ratio of dead depolarized cells was 22.81% and 36.45% after 16 h and 24 h of treatment, respectively (Figs. 4A and 4B). Tam-AE treatment also downregulated Bcl-xl and upregulated Bim and Bax in MCF-7 cells without affecting Bcl-2 protein expression (Fig. 4C).

Intracellular reactive oxygen species (ROS) level was also measured after the indicated treatment for 3 h. In the absence of AE, tamoxifen induced intracellular ROS up to 1.82 times as high as determined in the control group; in the presence of AE, higher ROS levels of 4.48 and 2.46 folds were detected in the Tam-AE group compared with the control and tamoxifen-only groups, respectively (Fig. 5). Hence, AE-induced ROS may contribute to apoptosis in tamoxifen-treated MCF-7 cells.

To identify the mechanisms involved in the effects of AE on the growth inhibition activity of tamoxifen in breast cancer, western blot analysis was used to determine the
Figure 4. Enhancement of tamoxifen-induced apoptosis through the mitochondrion-dependent pathway by AE. 
(A) Representative data of MMP changes in MCF-7 cells after Tam-AE (Tamoxifen + AE) treatment. Cells were treated with the indicated conditions. After cell harvesting, three independent MMP analyses were performed, and the quantitative results are shown in (B). (C) The altered protein expression of Bcl-2 family members. Following the indicated treatments, cells were harvested and subjected to protein extraction for western blot analysis. The Coomassie dye staining in each lane was used as an internal control.
Figure 5. Representative data of cellular ROS level change in Tam-AE-treated MCF-7 cells. Cells were treated with a combination of 9 µg/ml tamoxifen and 4 µg/ml AE for 3 h, and cellular ROS level was detected. Three independent experiments were performed.

![Fluorescence Count](image)

<table>
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<th></th>
<th>24 hours</th>
<th>72 hours</th>
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<tr>
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<td>9 9 9</td>
</tr>
<tr>
<td>AE (µg/mL)</td>
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<td>0 0.25 4</td>
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<tr>
<td>EGFR</td>
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<tr>
<td>p-mTOR</td>
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Figure 6. Representative data of the protein expression of Ras, ERK, PI3K, and mTOR after Tam-AE treatment in MCF-7 cells. Three independent experiments were performed. The Coomassie dye staining in each lane was used as an internal control.
expression of several proteins in Tam-AE-treated MCF-7 cells. In comparison with tamoxifen-only treatment, Tam-AE treatment for 72 h suppressed the expression of ERα, Ras, and ERK1/2 and the phosphorylation of ERK1/2, PI3K, and mTOR (Fig. 6), suggesting that AE could suppress breast cancer proliferation via the ERK and PI3K/mTOR pathways, respectively.

**Discussion**

Estrogen and ERs play important roles in tumor proliferation and progression; since more than 60% of Taiwanese breast cancer patients are hormone receptor-positive, hormone therapy is the standard treatment method for breast cancer. AE has estrogenic activity, leading to increased proliferation of breast cancer cells (Huang et al., 2013; Kang et al., 2008; Matsuda et al., 2001). However, the effects of AE in tamoxifen-treated breast cancer cells have not been previously investigated, and its potential anticancer properties remain unclear.

The estrogen antagonist tamoxifen is used for the treatment of early and advanced ER-positive breast cancers in pre- and post-menopausal women. Tamoxifen also reduces contralateral breast cancer rates in these women by preventing estrogen binding to ERs, leading to the blockade of ERα transcriptional activity. AE decreases ERα protein expression through proteasome degradation via an ERα-dependent pathway in breast cancer cells (Huang et al., 2013). Accordingly, 4 μg/ml AE inhibited ERα activation in the presence of 9 μg/ml tamoxifen (Fig. 6), indicating that AE may inhibit ERα-positive cancer cell growth.

The cellular receptor-mediated Ras/ERK pathway transduces signals for nuclear DNA transcription and is associated with increased cell proliferation. Our results showed decreased Ras, ERK1/2, and phosphorylated ERK activity following Tam-AE treatment, suggesting that AE exhibits its chemosensitization ability through inhibiting cell proliferation. This result is consistent with that of a study on glioma cells (Mijatovic et al., 2005).

AE-induced cell cycle arrest occurs in various cancer cells. In particular, AE was previously found to arrest cell proliferation at the G2/M phase in gastric cancer cells (Guo et al., 2008), delay DNA synthesis in U-373MG glioma cells (Acevedo-Duncan et al., 2004), induce in vitro G2/M arrest in human oral cancer cells (Xiao et al., 2007), and lead to G2/M arrest and differentiation in cervical cancer cells (Guo et al., 2007). AE can also induce G2/M arrest in human leukemia cells and have limited toxicity in normal human cells (Chen et al., 2004). In the present study, Tam-AE co-treatment inhibited cell proliferation and induced apoptosis; however, the cell cycle progression of treated cells was not affected compared with that of untreated cells. Hence, AE anticancer effects may be mediated by apoptosis pathways that are independent of the cell cycle.

Apoptotic pathways can be classified as intrinsic (mitochondrion-dependent) or extrinsic (receptor-dependent); both pathways activate caspases to initiate apoptosis. However, the present analysis of caspase 8 found no significant differences between untreated and treated cells (data not shown), suggesting the predominant role of intrinsic...
apoptosis in AE mechanisms. In comparison with tamoxifen-only treatment, Tam-AE treatment in MCF-7 cells resulted in a series of distinct apoptosis-related events, including initial induction of ROS, inhibition of anti-apoptotic Bcl-xL expression, and increased pro-apoptotic Bax and Bim protein expression, followed by permeabilization of the mitochondrial outer membrane and cytochrome c release from the mitochondria.

Bcl-2 family members reportedly play vital roles in cell death and survival by regulating anti-apoptotic proteins, pro-apoptotic effectors, and pro-apoptotic activators. In particular, the anti-apoptotic protein Bcl-xL, which prevents the release of mitochondrial cytochrome c, was downregulated following Tam-AE treatment, whereas the pro-apoptotic proteins Bim and Bax were upregulated. Mitochondrial dysfunction was revealed in the analyses of MMP and ROS. Among the critical functions of the mitochondria, apoptosis (Gottlieb, 2000) is regulated by mitochondrial proteases of the caspase family (Wang, 2001). On the other hand, the absence of growth factors or extracellular glucose can decrease MMP and lead to apoptosis (Vander Heiden et al., 2001). Hence, the decreases in MMP following Tam-AE exposure indicate apoptosis induction by AE. In agreement, the protein expression of cleaved caspase 7 and cleaved PARP was significantly increased in response to 72 h of Tam-AE treatment (Fig. 3C), further demonstrating the roles of mitochondrial apoptotic pathways.

Multiple extracellular signaling molecules can induce cell growth and differentiation via activation of the MAPK/ERK kinase cascade. Specifically, the ERK cascade promotes cell growth and differentiation, and over-activation is common in human cancers. Emodin is an active ingredient of various Chinese herbs; its anti-inflammatory effects have been exploited in treatments for inflammation and infection (Muto et al., 2007). Emodin suppresses multiple cell signaling pathways and inhibits cell growth (Tan et al., 2011). In a previous study, emodin treatments increased the phosphorylation of AMPKα and ERK1/2 and inhibited growth in NSCLC cells; they also inhibited lung cancer cell growth via AMPKα-mediated reduction of ILK expression (Tang et al., 2015).

ERK phosphorylation promotes cell survival, and Ras signals are associated with cell growth. In the present study, decreased ERK and Ras expressions were accompanied by suppression of total and phosphorylated ERK, suggesting that cell survival pathways are blocked by Tam-AE in MCF-7 cells. Furthermore, PI3K-Akt-mTORC signaling pathway activation has been associated with increased cell proliferation, and over-activation in various cancers reportedly results in uncontrolled cell growth. Previous studies have demonstrated that emodin inhibits the PI3K-Cdc42/Rac-1-PAK and p53 signaling pathways, thereby regulating Bcl-2 proteins in prostate cancer and human hepatoma cells (Cha et al., 2005; Shieh et al., 2004). However, estradiol was found to suppress apoptosis following PI3K/mTOR blockade, and combinations of endocrine therapy and PI3K inhibitors have been successful in ER-positive breast cancer cells (Crowder et al., 2009). Moreover, patients with hormone receptor-positive cancers have high PI3K signaling activity and may benefit from agents targeting the ER and PI3K pathways (Miller et al., 2010). The PI3K/mTOR pathway was found to involve in the anticancer mechanisms of AE in the present breast cancer cell lines, with significantly reduced phosphorylation of PI3K and mTOR following Tam-AE treatment. This data strongly indicates the
significance of these pathways in the observed AE-induced chemosensitivity to tamoxifen in breast cancer cells, and warrant consideration of AE as a co-treatment for circumventing drug resistance in hormone receptor-sensitive breast cancer cells.

In conclusion, a combined treatment of AE and the anticancer drug tamoxifen significantly inhibited proliferation and induced apoptosis in human breast cancer cells. Subsequent experiments demonstrated the roles of mitochondrion-dependent apoptosis pathways in MCF-7 cells, including inhibition of proliferation via PI3k/mTOR and Ras/ERK signaling. We also observed concomitant downregulation of ERα activity following Tam-AE treatment. These data provide evidence that AE induces chemosensitivity to tamoxifen and warrant consideration of AE as a clinical agent that increases the efficacy of anticancer drugs in breast cancer patients.

Acknowledgments

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References


