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Down-regulation of c-Src/EGFR-mediated signaling activation is involved in the honokiol-induced cell cycle arrest and apoptosis in MDA-MB-231 human breast cancer cells

Eun-Jung Park [a], Hye-Young Min [a], Hwa-Jin Chung [a], Ji-Young Hong [a], You-Jin Kang [a], Tran Manh Hung [b], Ui Joung Youn [b], Yeong Shik Kim [c], KiHwan Bae [b], Sam Sik Kang [c], Sang Kook Lee [a,*]

[a] College of Pharmacy and Division of Life and Pharmaceutical Sciences, Ewha Womans University, 11-1 Daehyun-dong, Seodaemun-gu, Seoul 120-750, Republic of Korea
[b] College of Pharmacy, Chungnam National University, Daejeon 305-764, Republic of Korea
[c] College of Pharmacy, Seoul National University, Seoul 151-742, Republic of Korea

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ABSTRACT

Honokiol is a naturally occurring neolignan abundant in Magnoliae Cortex and has showed anti-proliferative and pro-apoptotic effects in a wide range of human cancer cells. However, the molecular mechanisms on the anti-proliferative activity in cancer cells have been poorly elucidated. In this study, we evaluated the growth inhibitory activity of honokiol in cultured estrogen receptor (ER)-negative MDA-MB-231 human breast cancer cells. Honokiol exerted anti-proliferative activity with the cell cycle arrest at the G0/G1 phase and sequential induction of apoptotic cell death in a concentration-dependent manner. The honokiol-induced cell cycle arrest was well correlated with the suppressive expression of CDK4, cyclin D1, CDK2, cyclin E, c-Myc, and phosphorylated retinoblastoma protein (pRb) at Ser780. Apoptosis caused by honokiol was also concomitant with the cleavage of caspases (caspase-3, -8, and -9) and Bid along with the suppressive expression of Bcl-2, but it was independent on the expression of Bax and p53. In addition, honokiol-treated cells exhibited the cleavage of poly (ADP-ribose) polymerase (PARP) and DNA fragmentation. In the analysis of signal transduction pathway, honokiol down-regulated the expression and phosphorylation of c-Src, epidermal growth factor receptor (EGFR), and Akt, and consequently led to the inactivation of mTOR and its downstream signal molecules including 4E-binding protein (4E-BP) and p70 S6 kinase. These findings suggest that honokiol-mediated inhibitory activity of cancer cell growth might be related with the cell cycle arrest and induction of apoptosis via modulating signal transduction pathways.

1. Introduction

Breast cancer is one of the most common cause of cancer-related death and the second leading incidence of cancer in women [1]. Especially, estradiol-nonresponsive, estrogen receptor (ER)-negative breast cancers showed poor prognosis and less responded toward chemotherapeutic agents [1]. Therefore, continuous efforts to develop chemotherapeutic agents with novel mechanisms of action should be required for managing ER-negative breast cancers.

Honokiol is a naturally occurring neolignan mainly found in Magnoliae Cortex, the bark of *Magnolia obovata* Thunberg (Magnoliaceae), which has been traditionally used to enhance the function of the gastrointestinal tract in Asian countries. Recently, various biological effects of honokiol have been reported including alleviation of

* Corresponding author. Tel.: +82 2 3277 3023; fax: +82 2 3277 2851.
E-mail address: sklee@ewha.ac.kr (S.K. Lee).

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inflammatory arthritis [2], neuroprotection against oxidative stress [3], inhibition of arterial thrombosis [4], growth restraint of microbes [5], and suppression of metastasis [6], angiogenesis [7], invasion [8] and proliferation in a wide range of cancer cell types [9–14].

Although several studies on the anti-proliferative effect of honokiol in breast cancer cells have been reported, the underlying mechanism of action was not fully clarified yet [15–17]. In this study, we showed the growth inhibitory effect of honokiol in MDA-MB-231 ER-negative human breast cancer cells via the induction of cell cycle arrest and apoptosis through the regulation of signal transduction pathway, especially, tyrosine kinases including epidermal growth factor receptor (EGFR) and c-Src and their downstream signaling pathways.

In normal conditions, EGFR is usually activated in response to extracellular mitogenic ligands, including epidermal growth factor (EGF), heparin-binding epidermal growth factor (HB-EGF), transforming growth factor-α (TGF-α), amphiregulin, epiregulin, and betacellulin [18]. Ligand binding leads to homo- or heterodimerization with another ligand-bound ErbB receptors, and transmits extracellular mitogenic signals to downstream target signaling cascades which involve cell survival and proliferation such as phosphatidylinositol 3-kinase (PI3K)/Akt, mitogen-activated protein kinase (MAPK), and signal transducer and activator of transcription 3 (STAT3) [18]. Elevated EGFR may cause neoplastic transformation of normal cells in the presence of ligands, and overexpression of EGFR have been observed in a variety of cancer cell lines, including bladder, colon, esophagus, larynx, liver, lung, prostate, ovary, stomach, and breast cancer cells [19]. In human breast cancer cell lines, EGFR expression is relatively elevated in MDA-MB-231, MDA-MB-468, and SK-BR-3 cells compared with other breast cancer cells (MCF7, MDA-MB-453, BT474, UACC-812) or normal epithelial breast cells (HS578Bst) [20]. Moreover, about 30% of breast carcinomas have been found to overexpress EGFR [19], and several clinical data have been demonstrated that EGFR may be a prognostic indicator in human breast cancer [21].

In addition, cellular Src (c-Src), a nonreceptor tyrosine kinase, is also known to be up-regulated in various human cancer cells [19]. c-Src interacts with EGFR, potentiates the activation of mitogenic signaling, and thereby promotes cancer progression in human breast cancer cells [19]. It was also demonstrated that c-Src was overexpressed and associated with EGFR in MDA-MB-231 cells [20].

Based on these findings, in this study, we designed to investigate the cellular mechanism of honokiol-mediated growth inhibition in breast cancer cells in relation to modulate the mitogenic signaling pathways, especially c-Src/EGFR-mediated downstream signaling.

2. Materials and methods

2.1. Chemicals

Dimethyl sulfoxide (DMSO), bicinchoninic acid (BCA), copper sulfate, trichloroacetic acid (TCA), sulforhodamine B (SRB), propidium iodide, RNase A, and anti-β-actin antibody were purchased from Sigma–Aldrich, Inc. (St. Louis, MO, USA). Dulbecco's modified eagle medium (DMEM), fetal bovine serum (FBS), trypsin-EDTA solution (1×) and antibiotic–antimycotic solution (100×), and SYBR® gold staining solution were purchased from Invitrogen (Carlsbad, CA, USA). Anti-p53, anti-c-Myc, anti-Bcl-2, anti-Bax, anti-$p27^{Kip-1}$, and anti-EGFR, and all secondary antibodies were from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Anti-phospho-Akt (Ser473), anti-Akt, anti-phospho-mTOR (Ser2448), anti-mTOR, anti-phospho-pRb (Ser780), anti-pRb, anti-phospho-p53 (Ser15), anti-caspase-3, -8, and -9, anti-phospho-4E-BP1 (Tyr37/46), anti-4E-BP1, anti-phospho-c-Src (Tyr416), and anti-p70 S6 kinase were purchased from Cell Signaling Biotechnology (Danvers, MA, USA).

2.2. Isolation of honokiol

The dried stem bark (2 kg) of *Magnolia obovata* Thunb was extracted with methanol (MeOH) three times under reflux for 4 h. The MeOH solutions were combined, filtered, and concentrated to yield a dried MeOH extract (240 g). The MeOH extract (240 g) was suspended in distilled water and fractionated successively with hexane, EtOAc and BuOH to give hexane (50 g), EtOAc (70 g), and BuOH-soluble fractions (40 g), respectively. The hexane-soluble fraction was chromatographed over a silica gel column, eluting with hexane–EtOAc (100:0–50:50), to afford nine fractions (H1–H9). Fraction H7 was chromatographed on a silica gel column eluting with hexane–EtOAc (50:1–10:1) to obtain crude crystals which were recrystallized from $CHCl_3$ to give honokiol (150 mg). ^{1}H NMR (300 MHz, $CDCl_3$) of honokiol: δ 7.17 (1H, d, J = 2.2 Hz), 7.15 (1H, dd, J = 8.2, 2.2 Hz), 7.00 (1H, d, J = 2.1 Hz), 6.97 (1H, dd, J = 8.0, 2.1 Hz), 6.91 (1H, d, J = 8.2 Hz), 6.74 (1H, d, J = 8.0 Hz), 5.94 (2H, m), 5.15 (4H, m), 3.36 (2H, d, J = 6.6 Hz), 3.27 (2H, d, J = 6.6 Hz) (Fig. 1).

2.3. Cell culture

ER-negative human breast adenocarcinoma MDA-MB-231 cells were obtained from the Korean Cell Line Bank (Seoul, Korea) and were cultured in DMEM supplemented with 10% heat-inactivated fetal bovine serum, 100 units/ml penicillin, 100 μg/ml streptomycin, and 250 ng/ml amphotericin B at 37 °C in a humidified atmosphere with 5% CO_2.

2.4. Evaluation of anti-proliferative potential

The effect of honokiol on the cell proliferation was evaluated by sulforhodamine B (SRB) cellular protein-staining method with some modifications [22]. Briefly, MDA-MB-231 cells (1×10^4 cells in 190 μl of complete DMEM) were seeded in 96-well plate with various concentrations of honokiol and incubated at 37 °C in a humidified atmosphere with 5% CO_2. After 72 h of honokiol treatment, cells were fixed with 10% TCA solution for 1 h and stained cellular proteins with 0.4% SRB in 1% acetic acid solution. Stained cells were dissolved in 10 mM Tris buffer (pH 10.0). The effect of honokiol on cell viability was calculated as a percentage, relative to solvent-treated control, and the

IC_{50} values were calculated using non-linear regression analysis (percent survival versus concentration).

2.5. Analysis of cell cycle distribution

Cell cycle distribution was assessed by staining DNA content with propidium iodide as previously described method with some modifications [23]. Briefly, MDA-MB-231 cells were plated at a density of 1×10^6 cells per 100-mm culture dish and incubated for 24 h. Fresh complete media containing serially diluted honokiol were replaced to culture dishes and further incubated for 24 h. After incubation, both adherent and floating cells were harvested and fixed with 80% ethanol in PBS overnight. Fixed cells were incubated with RNase A (50 μg/ml) for 25 min prior to staining nucleic acid with propidium iodide (50 μg/ml) for 5 min. The DNA content of 2×10^4 cells in each group was analyzed by flow cytometer (Becton Dickinson) and the results were demonstrated as histograms of DNA content. The distribution of cells in each phase of cell cycle was calculated using ModFit LT 2.0 program.

2.6. DNA fragmentation

Cellular DNA was extracted after treatment of indicated concentrations of honokiol for 24 h [24]. Briefly, whole cells were washed with PBS and resuspended with lysis buffer containing 50 mM Tris–HCl (pH 7.5), 20 mM EDTA, and 1% NP-40. After centrifugation at 3,000 rpm for 5 min, 10 μl of 10% SDS and 10 μl of 50 mg/ml RNase A were added to the supernatants, and then incubated at 56 °C for 2 h. Subsequently, 10 μl of proteinase K (2.5 mg/ml) was added and further incubated at 37 °C for 2 h. DNA was allowed to precipitate with 0.5 volume of 10 M ammonium acetate and 2.5 volume of cold ethanol of total volume at −70 °C overnight. Extracted DNA was dissolved in 25 μl of 10 mM Tris–HCl buffer (pH 8.0) containing 1 mM EDTA. 2 μg of DNA samples were resolved electrophoretically on a 2% agarose gel, stained with SYBR® gold, and visualized under UV transilluminator.

2.7. Evaluation of the protein expression by western blot

Cells exposed with various concentrations of honokiol for 24 h were lysed and protein concentrations were determined by BCA method [25]. Total proteins (30 μg) in each cell lysate were subjected to resolution on various concentrations (6–15%) of sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS–PAGE) and electro-transferred onto PVDF membranes. The membranes were incubated with blocking buffer (5% non-fat dry milk in phosphate-buffered saline-0.1% Tween 20 (PBST)) for 1 h at room temperature and then further incubated with specific antibodies diluted in PBS overnight at 4 °C. After washing with PBST, membranes were incubated with horseradish peroxidase (HRP)-conjugated secondary antibodies for 2 h at room temperature and visualized by HRP-chemiluminescent detection kit (Lab Frontier, Seoul, Korea) using LAS-3000 Imager (Fuji Film Corp., Japan).

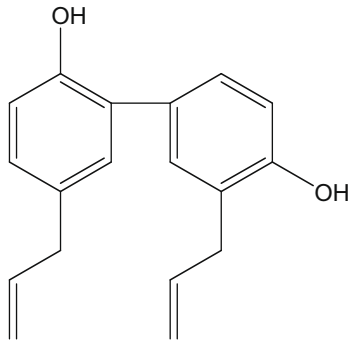

Fig. 1. Chemical structure of honokiol.

2.8. Statistical analysis

Data were expressed as means ± standard deviation (SD) for the indicated number of independently performed experiments. Statistical significance ($P < 0.05$) was assessed by *t*-test.

3. Results

3.1. The anti-proliferative effect of honokiol in MDA-MB-231 human breast cancer cells

The anti-proliferative potential of honokiol in MDA-MB-231 breast cancer cells was assessed by SRB assay. As shown in Fig. 2A, cells treated with various concentrations of honokiol (0–100 μM) exhibited a dose-dependent growth inhibition with an IC_{50} value of 59.5 μM. In particular, cells exposed to the highest concentration (100 μM) exerted remarkable decrease in cell survival.

In the observation of morphological changes by honokiol, vehicle (DMSO)-treated control cells were maintained their original morphology and adherent to the plates, whereas honokiol-treated cells were less grown and polarized at 60 μM and detached from the plate and floated with round-up shapes at higher concentrations (80 and 100 μM) (Fig. 2B).

3.2. Effect of honokiol on the cell cycle distribution

To determine the effect of honokiol on the cell cycle distribution, flow cytometric analysis was performed after treatment with indicated concentrations of honokiol for 24 h. Cells exposed to 60 μM honokiol were accumulated in the G0/G1 phase by approximately 20% increase compared with control cells. In addition, cells treated with higher concentrations of honokiol (80 and 100 μM) were significantly accumulated in the sub-G1 phase by 30% and 40% of total cell population, respectively (Fig. 3).

3.3. Induction of DNA fragmentation by honokiol in MDA-MB-231 cells

DNA fragmentation has been considered as a hallmark of apoptosis, a programmed cell death [26]. To determine whether the cytotoxic effect of honokiol was associated with the induction of apoptosis, DNA was isolated and visualized on a 2% agarose gel. The non-degraded high molecular weight DNA was detected in control cells and cells treated with 60 μM honokiol, while fragmented DNA (DNA ladder) was clearly observed in cells treated with 80 or 100 μM honokiol (Fig. 4).

3.4. Effect of honokiol on the expression of cell cycle checkpoint and apoptosis-related proteins

Based on the results of cell cycle distribution and DNA fragmentation, in order to investigate whether the cell cycle arrest and induction of apoptosis mediated by honokiol were related to the expression of cell cycle-regulatory proteins, the expression level of cell cycle checkpoint and apoptotic biomarker proteins were observed by Western blot analysis. Honokiol dose-dependently suppressed the expression of G1 cell cycle ar-

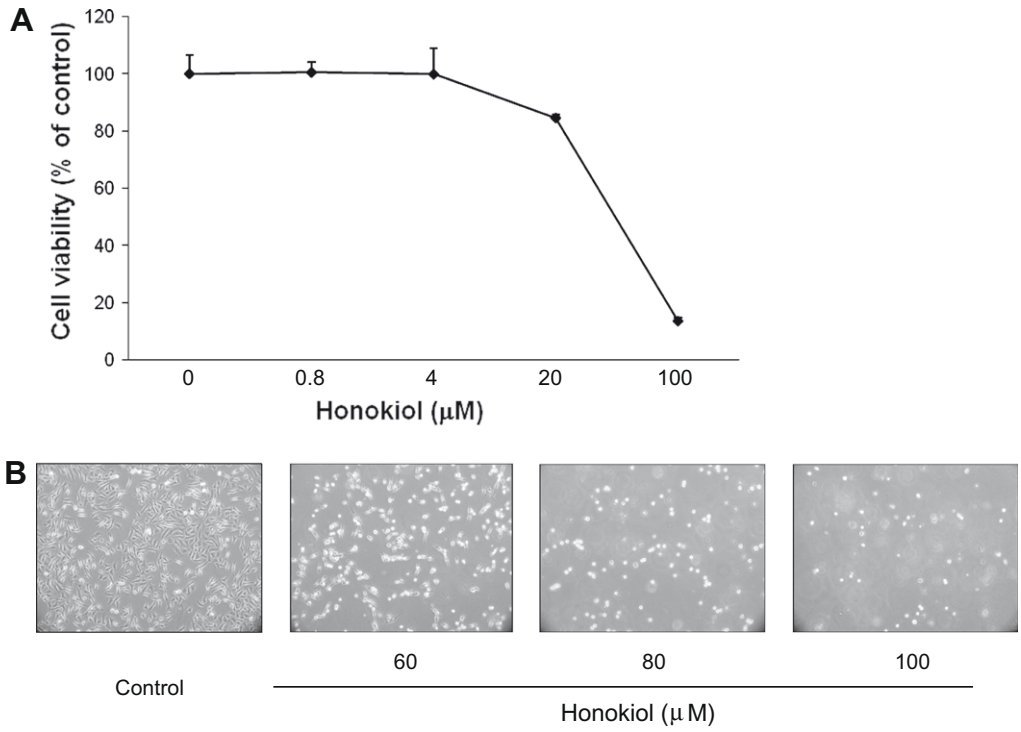

Fig. 2. Inhibitory effect of honokiol on the proliferation of MDA-MD-231 human breast cancer cells. (A) Cells (1×10^4 cells) were seeded in 96-well plates and incubated with honokiol as the indicated concentrations for 72 h. The effect on the cell proliferation was examined by SRB assay. Data are represented as the means ± SD ($n = 3$). (B) Morphological changes of MDA-MB-231 cells by treatment with honokiol. Cells were incubated with vehicle alone (control) or various concentrations of honokiol (60, 80, and 100 μM) for 24 h. Morphological changes were observed under the inverted phase-contrast microscope and photographed.

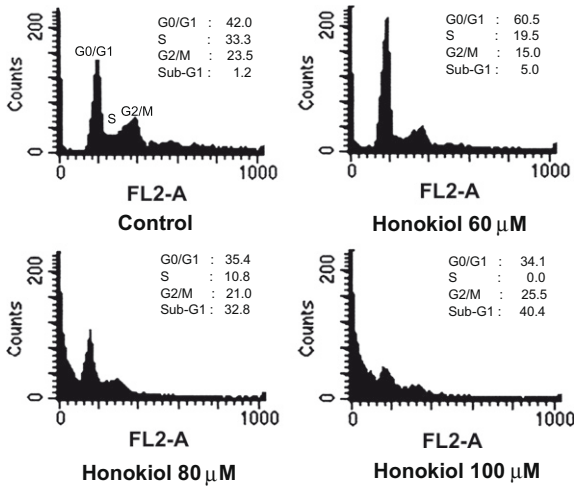

Fig. 3. Effect of honokiol on cell cycle distribution in cultured MDA-MB-231 cells. Cells were treated with various concentrations of honokiol (0, 60, 80, or 100 μM) for 24 h and then DNA content was analyzed by flow cytometry.

rest biomarkers such as cyclin D1, CDK2, and CDK4 (Fig. 5A). The transition of G1 to S phase was also blocked by honokiol with the suppressive expression of cyclin A and phospho-pRb (Ser780) and the induction of $p27^{Kip-1}$. c-Myc, a protein regulates cell cycle progression and cell proliferation [27], was also down-regulated and thus this event might affect to arrest the growth of cancer cells. Furthermore, the apoptotic cell death by honokiol was well correlated with the activation of caspase cascade (caspase-3, -8, and -9) through the cleavage of pro-form and the formation of cleaved active form of corresponding active caspases at the higher concentrations of honokiol (80 or 100 μM) (Fig. 5B). Down-regulation of Bcl-2 and Bid expression was also considered to be involved in the apoptotic cell death. In addition, PARP was also cleaved by honokiol, and this result was concomitant with the DNA fragmentation data as shown in Fig. 4. These results indicate that down-regulation of cyclins, CDKs, c-myc, and pRb phosphorylation and activation of caspases might be involved in the honokiol-mediated growth arrest and apoptotic cell death in MDA-MB-231 cells.

3.5. Effect of honokiol on the activation of signal transduction

To further determine the mechanisms of action underlying honokiol-mediated anti-proliferation activity on ER-negative human breast cancer cells, relevant overexpressed or activated signaling proteins in the cancer cells were examined. In MDA-MB-231 cells, c-Src and EGFR are aberrantly overexpressed and well associated with each other compared to normal cells [20]. Honokiol exhibited a dose-dependent suppression in the level of c-Src and phospho-c-Src (Tyr416) as well as markedly decrease in the protein expression of EGFR (Fig. 6). Consequently, the phosphorylation and activation of downstream kinases such as PKB/Akt and ERK were also down-regulated by treatment with honokiol. It is known that PKB/Akt promotes cell proliferation and survival via modulating several downstream signaling molecules including mTOR, FKHR, GSK-3β, MDM2, and NF-κB [28]. Among them, mTOR regulates protein translation and thereby enhance cell growth, proliferation, and survival [29]. To examine the effect of honokiol on the activation of Akt-mediated signaling, the expression level of the phosphorylated form of mTOR and its downstream molecules 4E-BP1 and p70 S6 kinase was evaluated. As shown in Fig. 6, honokiol suppressed the expression of total and phosphoylated Akt (Ser473), which in turn gave rise to downregulate the expression of phos-

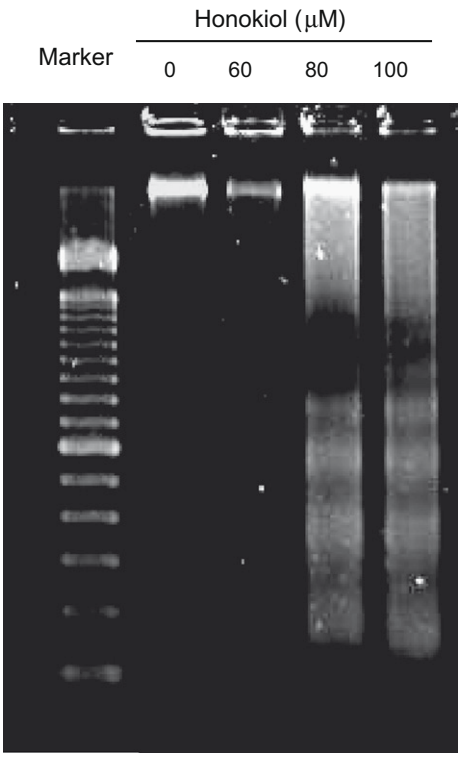

Fig. 4. Effect of honokiol on DNA fragmentation in cultured MDA-MB-231 cells. Cells were incubated for up to 24 h in the presence or absence of honokiol. DNA was extracted and separated using 2% agarose gel electrophoresis.

pho-mTOR (Ser2448). Honokiol also affected to the activation of 4E-BP and p70 S6 kinase via reduction of their total protein expression and inhibition of the phosphorylation of 4E-BP1 at Thr37/46 residue. Heat shock protein 90 (Hsp90), which is known to control the stability of EGFR and Akt [30], was also decreased by honokiol in a dose-dependent manner (Fig. 6).

4. Discussion

Many clinically used cancer chemotherapeutic agents are derived from natural products or semi-synthetic compounds of natural origins including topotecan, irinotecan, etoposide, teniposide, vinblastine, vincristine, docetaxel, and paclitaxel. Studies, therefore, on the evaluation of anticancer properties of natural products have been considered to play an important role in the development of anticancer agents [31]. On this line, we evaluated the anticancer effect of honokiol, a neolignan isolated from the traditional medicine Magnoliae Cortex. Honokiol has been reported to inhibit the proliferation of a wide variety of cancer cell types via induction of cell cycle arrest, apoptosis, or necrosis [5–13,15–17]. However, the mechanisms of action of honokiol on cancer cells have not been fully elucidated yet. In the present study, we focused on the effect of honokiol on the regulation of biomarkers related to signal transduction pathways as well as cell cycle and apoptosis regulatory proteins in ER-negative MDA-MB-231 human breast cancer cells. ER-negative breast cancer cells are known to have highly tumorigenic, invasive, metastatic, and proliferative characteristics and less respond toward currently applying anticancer agents [14]. Therefore, alternative targets for coping with ER-negative cells are needed to be identified.

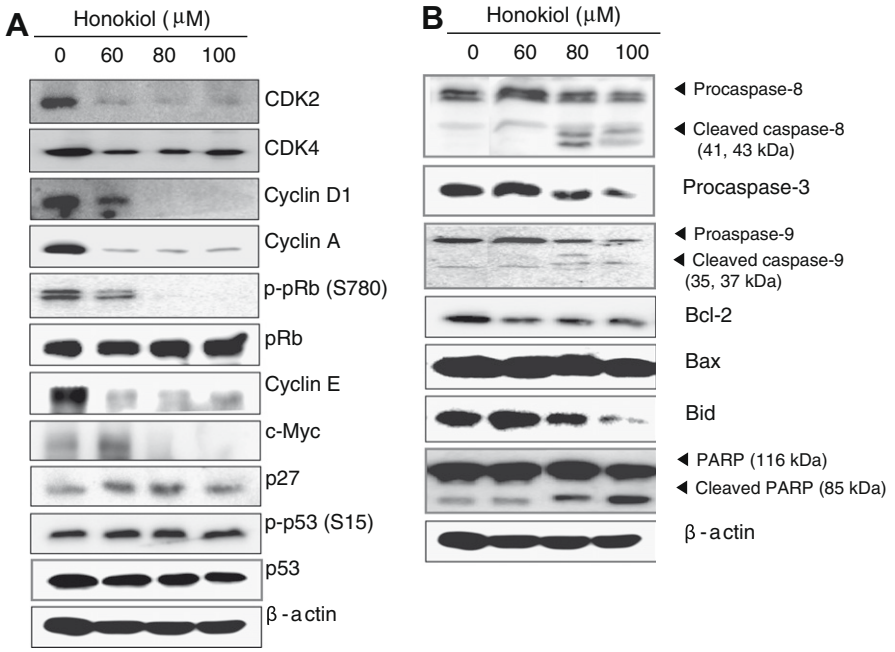

Fig. 5. Effects on the expression of cell cycle (A) and apoptosis (B)-related proteins. MDA-MB-231 cells were treated for 24 h with various concentrations of honokiol, and the protein expressions were measured by Western blot analysis.

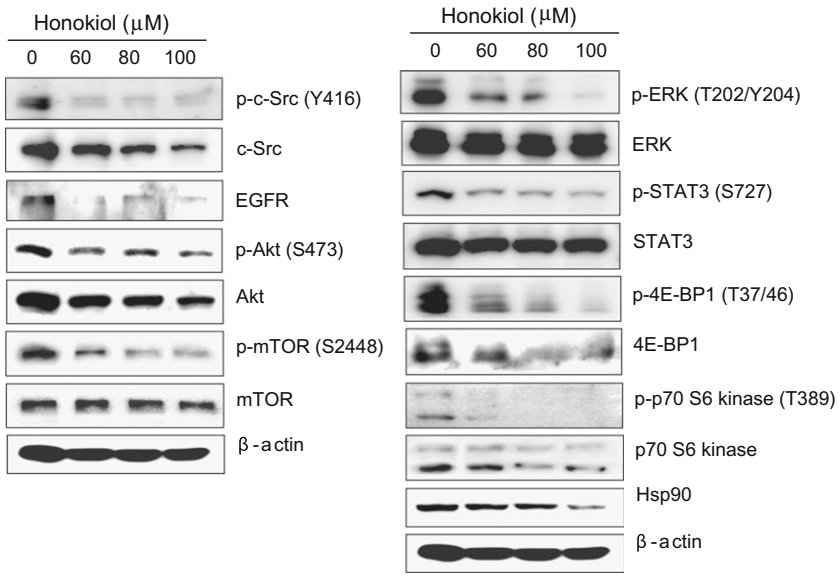

Fig. 6. Effects on the expression of signal transduction proteins. MDA-MB-231 cells were treated for 24 h with various concentrations of honokiol, and the protein expressions were measured by western blot analysis.

In our program to develop anticancer agents from natural products we identified honokiol as one of lead candidate for the inhibition of ER-negative breast cancer cell growth. Honokiol inhibited the proliferation of MDA-MB-231 cells through the G0/G1 phase cell cycle arrest at the low concentration and the induction of apoptosis at high concentrations (Figs. 2 and 3).

Cyclins and CDKs are considered as cell cycle regulators and initiate their functions via the formation of cyclin-CDK complexes [32]. The functions are also regulated by CDK inhibitors. However, cancer cells exhibit deregulation of these proteins and continuously proceed cell division by bypassing restriction points or repair systems, leading to abnormal proliferation [32]. Cyclin D1-CDK4 and cyclin E-CDK2 complexes play a crucial role in the transition of cells from G_1 phase to S phase through inactivation of pRb tumor suppressor by phosphorylation at serine residues, and their functions are negatively regulated by CDK inhibitor, including $p16^{INK4A}$, $p21^{WAF1}$, and $p27^{Kip-1}$ [32]. Inactivated pRb results in the detachment from elongation factor-2 (E2F), allowing E2F-dependent transcription. On this line, the present data of honokiol-mediated cell cycle arrest at the G0/G1 phase were well correlated with the up-regulation of one of CDK inhibitor $p27^{Kip-1}$ and the down-regulation of CDK4, cyclin D1, CDK2, cyclin A and cyclin E (Fig. 4). Along with the cell cycle arrest the apoptosis induced by honokiol was confirmed by the observation of cleavage of PARP and DNA fragmentation (Figs. 4 and 5). The induction of apoptosis by honokiol was also associated with the activation of caspase cascade and this event leads to PARP cleavage and DNA fragmentation.

Further study was designed to identify the growth inhibitory activity by honokiol with the modulation of cell signal transduction pathway. In general, EGFR, one of receptor tyrosine kinase ErbB family, is up-regulated in breast cancer cells including ER-negative MDA-MB-231 cells compared with normal breast cells [20]. Activated EGFR mediates its proliferative and proangiogenic effects in part through activating signaling cascades which involve PI3K/Akt and mitogen-activated protein kinase (MAPK)/ERK [18]. In addition, c-Src, which activates EGFR by the phosphorylation of the receptor at Tyr845 position [19], is also elevated in MDA-MB-231 cells [20]. Our present results demonstrated that honokiol suppressed the protein expression of both EGFR and c-Src and the phosphorylation of c-Src in MDA-MB-231 cells (Fig. 6), suggesting anti-proliferative effect of honokiol on MDA-MB-231 cells might be mediated in part by the regulation of c-Src/EGFR-mediated signaling.

To further characterize the effect of honokiol on the activation of EGFR-mediated signaling, the downstream effectors of EGFR were investigated. ERK/MAPK is a typical downstream kinase of growth factor-mediated signaling which promotes cell cycle progression and cell proliferation [18]. Akt is serine/threonine kinase that promotes cell survival and blocks apoptosis and consequently activates one of the downstream effectors mTOR by the phosphorylation of mTOR at Ser2448 [28]. mTOR has been known to be involved in the translational initiation of many survival proteins via activating p70 S6 kinase (S6K) and inhibiting eIF4E inhibitor 4E-BP1 [29]. A large body of evidence suggests that mTOR is highly correlated in the carcinogenesis, and mTOR signaling pathway is dysregulated in premalignant or early malignant human tissues [29,33]. As shown in Fig. 6, honokiol dose-dependently suppressed the phosphorylation of ERK, Akt and mTOR, which, in turn, modulated the activation of signaling mediators of mTOR such as 4E-BP1 and p70 S6 kinase. These results further support that honokiol might inhibit the activation of EGFR-mediated signaling pathway and, finally, cancer cell growth in MDA-MB-231 cells.

In addition, honokiol induced the decrease of total Akt and c-Src expression. It is known that heat shock protein 90 (Hsp90), one of chaperone proteins, might be involved in folding and stabilizing certain proteins related to tumorigenesis such as HER2, Src, EGFR, Akt and CDK4 [30]. In this study, honokiol suppressed the expression of Hsp90 in a concentration-dependent manner (Fig. 6). Therefore, suppression of Akt and c-Src expression by honokiol might be mediated by the regulation of Hsp90 expression and also involved in the honokiol-mediated inhibition of EGFR signaling activation in MDA-MB-231 cells.

The present study suggests honokiol inhibited the proliferation of MDA-MB-231 cells through inhibiting c-Src/EGFR-mediated signaling pathway. Recently, it was reported that honokiol suppressed phospholipase D activity, a survival signal, in cancer cells [34]. The various molecular mechanisms on the honokiol-mediated apoptosis induction were also demonstrated in the previous literatures [9–12,35]. Therefore, these findings imply honokiol might possess various cellular targets for its anti-proliferative and pro-apoptotic activities. Further studies including the comparison of its effects between c-Src/EGFR-hyperactivated cancer cells and their inactivated counterparts or the investigation of its effects on the activation of growth factor-mediated c-Src/EGFR signaling might be required to better understand the effect and selectivity of honokiol on the activation of c-Src/EGFR-mediated signaling.

In addition, previous studies demonstrated the anti-proliferative effect of other related neolignans derived from *Magnolia obovata* in human cancer cells. For example, magnolol suppressed the growth of various human cancer cells through inducing cell cycle arrest and apoptosis [36–38]. In H460 human lung cancer cells, magnolol induced cell death through autophagy induction [39]. Another neolignan obovatol also exhibited cell cycle arrest and apoptotic cell death in human colon and prostate cancer cells [40]. These findings and our results collectively suggest the inhibition of cancer cell proliferation by neolignans might share similar and/or common mode of actions. More studies would be necessary to clarify the molecular mechanisms of plant-derived neolignans on the modulation of cancer cell proliferation.

In summary, this study presents an additional mechanism of action in the growth inhibitory activity of honokiol against ER-negative human breast cancer cells. The growth inhibition of honokiol is highly related to cell cycle arrest at the G0/G1 phase and induction of apoptosis. The growth inhibition is also associated with the modulation of signal transduction pathway in the c-Src/EGFR and Akt/mTOR cascade signaling. As currently reported with human breast cancer cells, the potential growth inhibitory activity of the honokiol should be taken into account when considering the further development and prioritization as a cancer chemotherapeutic agent in either alone or combination with other anticancer agents.

Conflict of interest statement

None declared.

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