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Baohuoside I Suppresses Invasion of Cervical and Breast Cancer Cells through the Downregulation of CXCR4 Chemokine Receptor Expression

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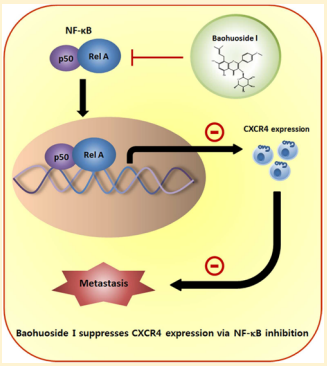
ABSTRACT: More than 90 percent of cancer-mediated deaths are due to metastasis, but the mechanisms that control metastasis remain poorly understood. Thus, the therapy targeting this process has been challenged constantly, but no therapy has yet been approved. CXC chemokine receptor 4 (CXCR4), a Gi protein-coupled receptor for the CXC chemokine ligand (CXCL) 12/stromal cell derived factor (SDF) 1α, is known to be expressed in various tumors. Recently, the CXCL12/CXCR4 axis has emerged as a key mediator of tumor metastasis; therefore, the possibility that identification of CXCR4 inhibitors can be a promising strategy for abrogating metastasis has been considered. In this report, we investigate baohuoside I, a component of *Epimedium koreanum*, as a regulator of CXCR4 expression as well as function in cervical cancer and breast cancer cells. We observed that baohuoside I downregulated CXCR4 expression in a dose- and time-dependent manner in HeLa cells. Treatment with a pharmacological proteasome and lysosomal inhibitors did not have a substantial effect on baohuoside I's ability to suppress CXCR4 expression. When we investigated the molecular mechanism of action, it was observed that the suppression of CXCR4 expression occurred at the level of mRNA. The decrease in the level of CXCR4 expression caused by baohuoside I was correlated with inhibition of the CXCL12-induced invasion of both cervical and breast cancer cells. Overall, our results show that baohuoside I exerts its antimetastatic effect through the downregulation of CXCR4 expression and, thus, has the potential to play a role in the suppression of cancer metastasis



Cervical and breast cancers are the major causes of cancer mortality in women worldwide.[1,2] Although death rates for these cancers have been decreasing, the metastasis of cancer to various organs such as the lung,[3,4] liver,[5] and bones[6,7] is a critical factor that contributes to the mortality rate in cancer patients. This metastatic cascade is initiated by local invasion of tumor cells at the primary site and their subsequent adhesion within vessels of target organs, which is followed by cell migration and the development of tumors in those organs.[8] One of the most generally studied chemokines in tumor cell migration and metastasis is CXC chemokine ligand (CXCL) 12 and its receptor, CXCR4.[9] The CXCR4/CXCL12 axis is involved in inflammatory cell migration processes, including atherogenic T cell recruitment, inflammation, and the metastasis of cancer cells.[10−12] Interestingly, the organs that secrete CXCL12 at a higher level are also the most common secondary metastatic sites.[13] Expression of the CXCR4 has been demonstrated on cells of the major adult epithelial cancers, including HeLa (human cervical carcinoma),[14] MDA-MB-231 (human breast tumor),[15] and CAOV3 (human ovarian carcinoma).[16] This observation has further highlighted the important role played by the CXCR4/CXCL12 signaling axis in cancer metastasis.[17,18]

Anticancer drugs derived from natural plant extracts, generally considered safe, have been shown to mediate anticancer activities against a variety of cell types.[19] Baohuoside I, also known as icariside II, is derived from the stems and leaves of *Epimedium koreanum*, which has been traditionally utilized in Chinese traditional medicine.[20] As a Chinese traditional medicine, it is believed to possess various therapeutic properties that treat osteoporosis, irregular menstruation, and joint pain.[21] Recent studies have demonstrated that baohuoside I appeared to possess anticancer activity against multiple prostate cancers,[22] myeloma,[23] osteosarcoma,[24] and skin cancer cells.[25] Similarly, baohuoside I induced apoptosis by attenuating the hypoxia inducible factor (HIF) 1α protein level in human osteosarcoma cells,[24] suppressing the cyclooxygenease (COX)-2/prostaglandin E2 pathway in prostate cancer PC3 cells,[22] blocking the signal transducer and activator of transcription (STAT) 3 signaling pathway in U266 multiple myeloma cells,[23] and inducing apoptosis in breast cancer MCF7 cells involving both the intrinsic and extrinsic signaling pathways.[26]

There have also been several reports of other flavonoids derived from *Epimedium* that regulate cellular responses. For instance, icariin could stimulate angiogenesis by activating the extracellular signal-related kinase (ERK) and phosphatidylinositol 3-kinase (PI3K)/Akt/endothelial nitric oxide synthase (eNOS)-dependent signal pathways in human endothelial cells.[27] Also, ikarisoside A inhibited osteoclastogenic differentiation via c-Jun N-terminal kinase (JNK) and nuclear factor κB (NF-κB) in RAW 264.7 cells.[28]

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In this study, we determined whether baohuoside I may modulate CXCR4 expression and inhibit tumor cell invasion. Our results demonstrate, for the first time, that baohuoside I downregulates CXCR4 expression in various tumor cells and inhibits CXCL12-induced invasion of cervical and breast cancer cells.

MATERIALS AND METHODS

Materials. Baohuoside I (99% pure) was purchased from Biopurify Phytochemicals Ltd. (Chengdu, China). Baohuoside I was dissolved in dimethyl sulfoxide (DMSO) as a 10 mM stock solution and stored at 4 °C. Further dilution was done in cell culture medium. RPMI1640, Dulbecco's modified Eagle's medium (DMEM), and fetal bovine serum (FBS) were purchased from Hyclone. The 0.25% trypsin-EDTA and antibiotic-antimycotic were obtained from Gibco by life technologies. Lactacystin and chloroquine were obtained from SantaCruz Biotechnology (Santa Cruz, CA). Antibodies against CXCR4 were obtained from Abcam (Cambridge, MA). CXCL12 was purchased from R&D Systems (Minneapolis, MN).

Cell Culture. The immortalized human cervical cancer HeLa cells were cultured in RPMI 1640 supplemented with 10% FBS and 1% antibiotics. Breast cancer MDA-MB-231, pancreatic cancer HPAC, and liver cancer HepG2 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% FBS and 1% antibiotics. Cells were maintained at 37 °C in an atmosphere of 5% CO_2 and 95% air. All cells were passaged at 80% confluence in 0.25% trypsin-EDTA for 3−5 min.

Western Blotting. For detection of CXCR4, baohuoside I-treated whole cell extracts were lysed with RIPA buffer [150 mM NaCl, 10 mM Tris (pH 7.2), 0.1% sodium dodecyl sulfate (SDS), 1% Triton X-100, 1% deoxycholate, and 5 mM ethylenediaminetetraacetic acid (EDTA)] enriched with a complete protease inhibitor cocktail tablet (Roche Diagnostics, Mannheim, Germany) and then incubated on ice for 30 min with regular vortexing before being centrifuged at 14000 rpm and 4 °C for 15 min. The protein concentration was determined by using the bicinchoninic acid (BCA) protein assay kit (Pierce Biotechnology, Rockford, IL). The protein samples were boiled in SDS sample buffer for 5 min and were resolved on a 10% SDS−polyacrylamide gel. After electrophoresis, proteins were transferred onto a polyvinyl difluoride (PVDF) membrane, which was blocked with 5% nonfat dry milk in Tris-buffered saline with 0.1% Tween 20 (TBST) and incubated with the primary antibody at the appropriate final concentration followed by hybridization with a horseradish peroxidase-conjugated anti-rabbit or anti-mouse secondary antibody. For each step, the membrane was washed with TBST three times for 10 min and the transferred proteins were incubated with supersignal pico-chemiluminescent substrate or dura-luminol substrate (Thermo Scientific, Waltham, MA) for 2 min according to the manufacturer's instructions and visualized with imagequant LAS 4000 (Fujifilm Life Science, Roche Diagnostics).

RNA Analysis and Reverse Transcription Polymerase Chain Reaction (RT-PCR). Total RNA was extracted using Trizol reagent according to the manufacturer's instructions (Invitrogen, Carlsbad, CA), and cDNA synthesis was performed using the AccuPower Rocketscript cycle RT premix (Bioneer, Daejeon, Korea) according to the manufacturer's protocol. The relative expression of CXCR4 was analyzed by quantitative RT-PCR with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as an internal control. The following pairs of forward and reverse primer sets were used: CXCR4, sense (5′-CCG TGG CAA ACT GGT ACT TT-3′) and antisense (5′-TTT CAG CCA ACA GCT TCC TT-3′); GAPDH, sense (5′-CAG CCT CAA GAT CAT CAG CA-3′) and antisense (5′-GTC TTC TGG GTG GCA GTG AT-3′). The RT-PCR mixture contained 2.5 μL of 10× Taq reaction buffer, 0.5 μL of each 10 mM dNTP, 1 μL each of forward and reverse primers, and 2 μL of each template DNA in a final volume of 25 μL. Amplification products were resolved by 1.5% agarose gel electrophoresis stained with safe dye and photographed with an imagequant LAS 4000 instrument.

Real-Time Quantitative PCR. Real-time PCR was performed on the cDNA using the selective primers for CXCR4 [5′-CCG TGG CAA ACT GGT ACT TT-3′ (sense) and 5′-TTT CAG CCA ACA GCT TCC TT-3′ (antisense)] and GAPDH [5′-CAG CCT CAA GAT CAT CAG CA-3′ (sense) and 5′-GTC TTC TGG GTG GCA GTG AT-3′ (antisense)]. PCR was performed in a Light Cycler 480 (Roche Diagnostics, Indianapolis, IN) using the Light Cycler DNA Master SYBR Green Kit (Roche Diagnostics) according to the manufacturer's instructions. The PCR thermal profile was 95 °C for 10 min and 40 cycles of 95 °C for 10 s and 55 °C for 30 s followed by a cooling step at 40 °C for 30 s. For relative quantification, the crossing point (Cp) value of CXCR4 was normalized by keeping the Cp value of GAPDH as a control.

Invasion Assay. The *in vitro* invasion assay was conducted using the Bio-Coat Matrigel invasion assay system (BD Biosciences, Lexington, KY) according to the manufacturer's instructions. Cancer cells (5×10^4 per milliliter) were suspended in medium and seeded into the Matrigel-precoated transwell chambers with polycarbonate membranes with a pore size of 8 μm. After preincubation with or without baohuoside I (25 μM), transwell chambers were then placed into 24-well plates, to which was added the basal medium only or basal medium containing 100 ng/mL CXCL12. After incubation (24 h for HeLa and MDA-MB-231), the upper surface of transwell chambers was wiped off with a cotton swab and invading cells were fixed and stained with a Diff-Quick stain. The invading cell numbers were counted in five randomly selected microscope fields (100×).

Electrophoretic Mobility Shift Assay. A DIG Gel Shift kit (Roche) was used for the electrophoretic mobility shift assay (EMSA). The NF-κB oligonucleotide probe [5′-CTT GAA GGG ATT TCC CTG GCT TGA AGG GAT TTC CCT GG-3′ (only sense strands are shown; consensus sequences for NF-κB are underlined)] containing the NF-κB binding motif was end-labeled with DIG-ddUTP. For the binding reaction, 10 μg of the sample protein was incubated at room temperature for 30 min with a DIG-labeled probe. The DNA−protein complexes were separated by electrophoresis in 6% non-denatured polyacrylamide gels using 0.5× TBE as a running buffer. After electrophoresis, the gels were transferred to nylon membranes and detected chemiluminescently. Signal intensity was quantified with an image analyzer (Las4000).

Statistical Analysis. The results obtained from each experiment are expressed as means ± the standard deviation (SD) from at least three independent experiments. The significance level was set at $p < 0.05$ for each analysis using a Student's t test.

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RESULTS

Baohuoside I Suppresses CXCR4 Protein Expression in HeLa Cells. The chemokine receptor CXCR4 is strongly expressed in human cervix adenocarcinoma (HeLa) cells[29] and is widely known to play a biologically relevant role in tumor growth and spread. Thus, the CXCR4/CXCL12 axis has been considered as an important mediator of proliferation and invasion in cervical cancer cells.[30]

Therefore, we investigated whether baohuoside I can modulate the expression of CXCR4 in HeLa cells. When cells were incubated with different concentrations of baohuoside I for 24 h or with 25 μM baohuoside I for different periods of time, baohuoside I suppressed the expression of CXCR4 in both dose- and time-dependent manners (Figure 1B,C).

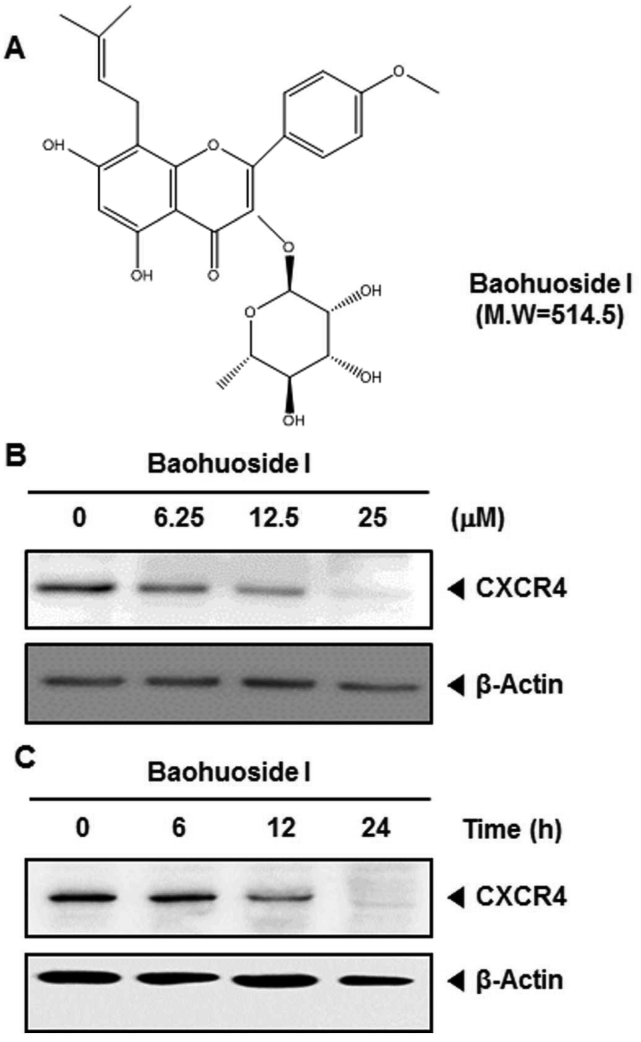

Figure 1. Baohuoside I suppresses CXCR4 expression in HeLa cells. (A) Chemical structure of baohuoside I. (B) Baohuoside I suppresses CXCR4 levels in dose-dependent manner. HeLa cells (1×10^6) were treated with the indicated concentrations of baohuoside I for 24 h. Whole cell extracts were then prepared, and 20 μg of protein was resolved via SDS−PAGE, electrotransferred onto PVDF membranes, and probed for CXCR4. (C) Baohuoside I suppresses CXCR4 levels in time-dependent manner. HeLa cells (1×10^6) were treated with 25 μM baohuoside I for the indicated times, after which Western blotting was conducted as described above. The same blots were stripped and reprobed with a β-actin antibody to show equal protein loading. Representative results of three independent experiments are shown.

Baohuoside I-induced suppression could be observed as early as 12 h after incubation with a concentration as low as 12.5 μM. The exposure of cells to 25 μM baohuoside I for 24 h significantly inhibited CXCR4 expression in HeLa cells.

Downregulation of CXCR4 by Baohuoside I Is Not Mediated through Its Degradation. Because CXCR4 has been shown to undergo ubiquitination at its lysine residue followed by degradation,[31] we explored the possibility that baohuoside I enhances the rate of degradation through proteasomal activation. To determine this, we first examined whether lactacystin, a proteasome inhibitor, promotes CXCL12-induced expression of CXCR4 in HeLa cells. As shown in Figure 2A (left), the CXCR4 expression level showed no change when cells were cotreated with CXCL12 and lactacystin. Then we treated HeLa cells with lactacystin 1 h before being exposing them to baohuoside I. As shown in Figure 2A (right), lactacystin had no effect on baohuoside I-induced degradation of CXCR4, suggesting that this is an unlikely mechanism by which baohuoside I downregulates CXCR4.

Because CXCR4 has also been shown to undergo ligand-dependent lysosomal degradation,[32] we next examined the ability of chloroquine, a lysosomal inhibitor, to promote CXCL12-induced expression of CXCR4 in HeLa cells. Like lactacystin, the CXCR4 expression level showed no change when it was cotreated with CXCL12 and chloroquine (Figure 2B, left). Then we investigated the ability of chloroquine to block baohuoside I-induced degradation of CXCR4. To examine this, cells were pretreated with chloroquine 1 h before being exposed to baohuoside I. Like lactacystin, chloroquine also had no influence on the degradation of CXCR4 (Figure 2B, right), indicating that this was not the primary pathway for suppressing CXCR4 expression.

Downregulation of CXCR4 by Baohuoside I Occurs at the Transcriptional Level. Because baohuoside I did not downregulate CXCR4 expression by enhancing its degradation, we further investigated whether suppression occurs at the transcriptional level using both RT-PCR and quantitative PCR (real-time PCR). HeLa cells were treated with baohuoside I at different concentrations, and the mRNA level was measured. As shown in panels C and D of Figure 2, baohuoside I promoted the downregulation of CXCR4 mRNA in a dose-dependent manner.

Baohuoside I Suppresses the Constitutive Activation of NF-κB in HeLa Cells. Previously, Penzo et al.[33] reported that the promoter of CXCR4 contains several NF-κB binding sites. Thus, it is possible that baohuoside I exerts its effect on CXCR4 by suppressing NF-κB activation. We used a DNA binding assay to explore whether baohuoside I could affect the constitutive NF-κB activation in HeLa cells. We found that treatment of HeLa cells with baohuoside I for 24 h suppressed NF-κB activation in a dose-dependent manner (Figure 2E). Thus, these results suggest that baohuoside I may downregulate CXCR4 expression by suppressing NF-κB activation.

Baohuoside I Suppresses CXCL12-Induced Cervical Cancer Cell Invasion. Several lines of evidence implicate the role of CXCR4 in cervical cancer metastasis,[34,35] so CXCR4 silencing could be a potential strategy for preventing the metastasis of cervical cancer. Whether the downregulation of CXCR4 by baohuoside I correlates with cervical cancer cell migration was examined using an *in vitro* invasion assay. As a result, we found that CXCL12 induced the invasion of cervical

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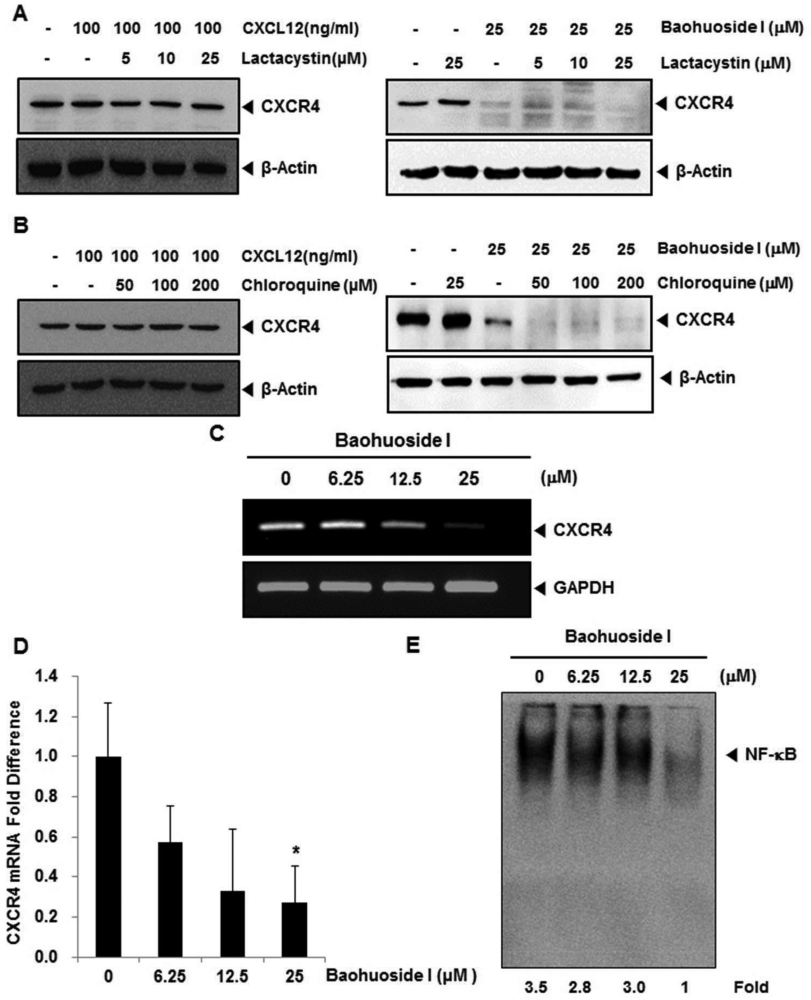

Figure 2. Baohuoside I suppresses CXCR4 through the mRNA level. (A and B) Baohuoside I does not suppress CXCR4 through lysosomal and proteasomal degradation. HeLa cells were treated with the indicated concentration of lactacystin or chloroquine for 1 h at 37 °C followed by treatment with 100 ng/mL CXCL12 for 24 h (left). HeLa cells were treated with the indicated concentration of lactacystin or chloroquine for 1 h at 37 °C followed by treatment with 25 μM baohuoside I for 24 h (right). Whole cell extracts were prepared and analyzed by Western blot analysis with antibodies against CXCR4. The same blots were stripped and reproved with a β-actin antibody to show equal protein loading. (C and D) Baohuoside I suppresses expression of CXCR4 mRNA levels. (C) HeLa cells were treated with baohuoside I at the indicated concentrations. Total RNA was isolated and analyzed by RT-PCR assays described in Materials and Methods. GAPDH was used to show equal loading of total RNA. (D)HeLa cells were treated with baohuoside I at the indicated concentrations. Total RNA was isolated and analyzed by real-time PCR assays described in Materials and Methods. The crossing point (Cp) value of CXCR4 was normalized by keeping the Cp value of GAPDH as a control; bars show the standard error (*P < 0.05). (E) Baohuoside I inhibits NF-κB activation in HeLa cells. HeLa cells were incubated with the indicated concentrations of baohuoside I for 24 h, and then the nuclear extracts were assayed for NF-κB activation in HeLa cells.

cancer cells and that baohuoside I effectively abrogated it (Figure 3A,B).

Baohuoside I Downmodulates CXCR4 in Different Cell Types. CXCR4 is overexpressed on a wide variety of tumor cells. Thus, we investigated whether baohuoside I downregulates the expression of CXCR4 in breast (MDA-MB-231), liver (HepG2), and pancreatic (HPAC) cancer cell lines. Cells were treated with 25 μM baohuoside I for 24 h, and CXCR4 expression was measured. Figure 4A shows that baohuoside I downregulated CXCR4 in all these cell lines, and its effect was most dramatic in breast cancer cells (MDA-MB-231). To confirm this, MDA-MB-231 cells were incubated with different concentrations of baohuoside I for 24 h and CXCR4 levels were measured. The result showed that baohuoside I suppressed the expression of CXCR4 in a dose-dependent manner (Figure 4B).

Downregulation of CXCR4 by Baohuoside I Occurs at the Transcriptional Level. Next, we further investigated whether suppression of CXCR4 in MDA-MB-231 cells occurs at the transcriptional level as in HeLa cells. MDA-MB-231 cells were treated with baohuoside I at different concentrations, and the CXCR4 mRNA levels were measured using both RT-PCR and quantitative PCR (real-time PCR). As shown in panels C and D of Figure 4, baohuoside I induced the downregulation of CXCR4 mRNA in a dose-dependent manner.

Baohuoside I Suppresses CXCL12-Induced Breast Cancer Cell Invasion. We determined if the suppression of invasion by baohuoside I is tumor cell specific. CXCL12/CXCR4 signaling has been also shown to play a critical role in breast cancer metastasis.[36,37] To elucidate whether baohuoside I has an effect on breast cancer cell metastasis, we examined the effect of baohuoside I on CXCL12-induced cell invasion. As shown in panels A and B of Figure 5, treatment of baohuoside I

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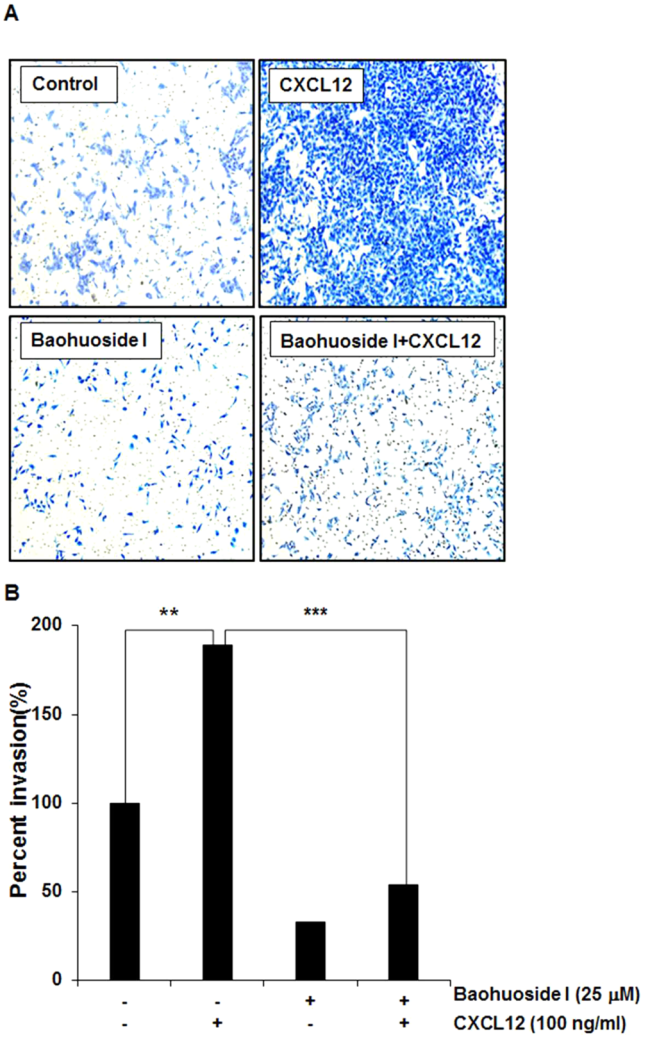

Figure 3. Baohuoside I suppresses invasion of cervical cancer cells. (A and B) HeLa cells (5×10^4 per milliliter) were seeded on the top chamber of matrigel. After incubation with or without baohuoside I (25 μM) for 24 h, transwell chambers were then placed in 24-well plates, to which we added the basal medium only or basal medium containing 100 ng/mL CXCL12. After incubation, the invasion assay was conducted as described in Materials and Methods. (B) Columns give the mean numbers of invaded cells; bars give the standard error (*P < 0.05; **P < 0.005; ***P < 0.0001).

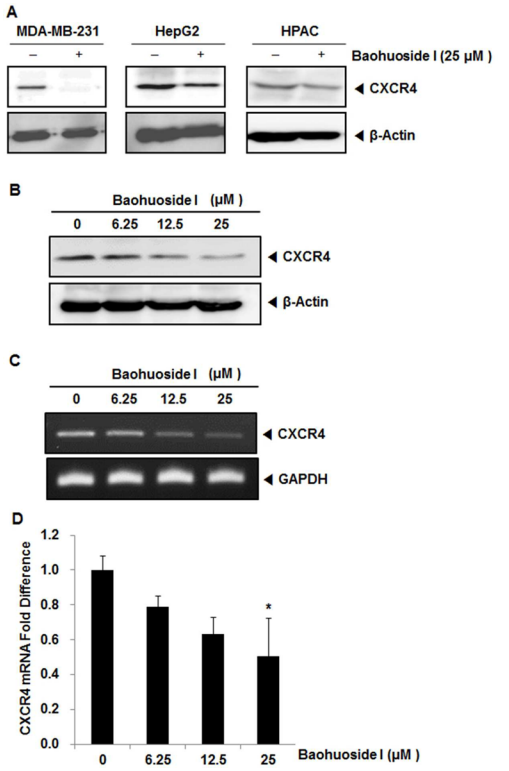

Figure 4. Baohuoside I suppresses CXCR4 in breast cancer cells. (A) Baohuoside I downregulates CXCR4 in different cell types. Cells were incubated with 25 μM baohuoside I for 24 h. Whole cell extracts were prepared and analyzed by Western blot analysis with antibodies against CXCR4. The same blots were stripped and reprobed with a β-actin antibody to show equal protein loading. Representative results of three independent experiments are shown. (B) Baohuoside I suppresses CXCR4 levels in dose-dependent manner. MDA-MB-231 cells (1×10^6) were treated with the indicated concentrations of baohuoside I for 24 h. Whole cell extracts were then prepared, and 20 μg of protein was resolved via SDS−PAGE, electrotransferred onto PVDF membranes, and probed for CXCR4. (C and D) Baohuoside I suppresses expression of CXCR4 mRNA levels. (C) MDA-MB-231cells were treated with baohuoside I for the indicated concentrations. Total RNA was isolated and analyzed by the RT-PCR assay as described in Materials and Methods. GAPDH was used to show equal loading of total RNA. (D) MDA-MB-231 cells were treated with baohuoside I at the indicated concentrations. Total RNA was isolated and analyzed by real-time PCR assays as described in Materials and Methods. The crossing point (Cp) value of CXCR4 was normalized by keeping the Cp value of GAPDH as a control; bars show the standard error (*P < 0.05).

suppressed CXCL12-induced invasion of breast cancer MDA-MB-231 cells.

DISCUSSION

The goal of this study was to determine whether baohuoside I could modulate the expression of CXCR4, a chemokine receptor that has been linked with tumor cell growth, angiogenesis, invasion, and metastasis. Our results indicate for the first time that baohuoside I abolishes the expression of CXCR4 in a wide variety of tumor cells, especially in cervical and breast cancer cells. Our results also demonstrate that downregulation of CXCR4 does not primarily occur through proteolytic degradation of the receptor but rather through the suppression of transcription. Furthermore, downregulation of CXCR4 reduced the extent of cell invasion induced by CXCL12.

The CXCR4 chemokine receptor has been found to be overexpressed in different tumors, including breast cancer, pancreatic cancer, prostate cancer, cervical cancer, and lung cancer, compared to normal cells that show little or no CXCR4 expression.[30,36,38−40]

What leads to overexpression of this receptor in tumor cells is not fully understood; however, previous reports have implicated the involvement of various genetic and epigenetic factors,[41] including the protein degradation pathway,[32,42] hypoxic conditions in the tumor microenvironment,[43] NF-κB,[44] and vascular endothelial growth factor (VEGF).[45] Given that CXCR4 has been linked with the metastasis of various cancers and CXCR4 expression has been correlated with a poor prognosis,[46] CXCR4 seems to be an ideal therapeutic target for the prevention of metastatic cancer.

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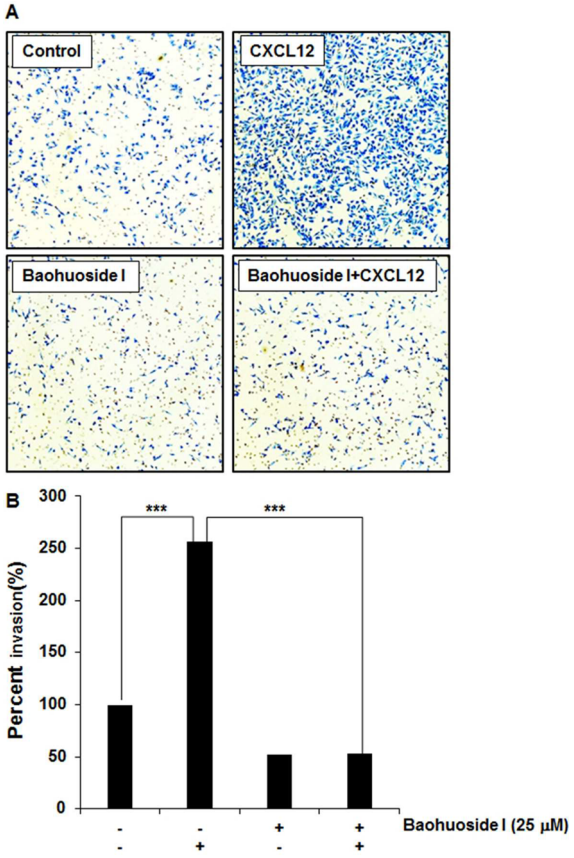

Figure 5. Baohuoside I suppresses invasion of breast cancer cells. (A and B) MDA-MB-231 cells (5×10^4 per milliliter) were seeded in the top chamber of matrigel. After preincubation with or without baohuoside I (25 μM) for 24 h, transwell chambers were then placed in 24-well plates, to which we added the basal medium only or basal medium containing 100 ng/mL CXCL12. After incubation, the invasion assay was conducted as described in Materials and Methods. (B) Columns shows the mean number of invaded cells; bars show the standard error (*$P < 0.05$; **$P < 0.005$; ***$P < 0.0001$).

Our results clearly indicate that baohuoside I suppressed CXCR4 expression in cervical cancer HeLa cells in a dose- and time-dependent manner. Our data also showed that baohuoside I suppressed CXCR4 expression in various tumor cell lines, including breast cancer, liver cancer, and pancreatic cancer, thereby indicating that the effect of baohuoside I on CXCR4 is not limited to a single cell type. The ligand-dependent downregulation of the CXCR4 receptor by lysosomal degradation is well-documented.[42] Other reports also suggest that atrophin-interacting protein (AIP) 4 mediated ubiquitination and degradation of chemokine receptor CXCR4.[32] However, our data suggest that baohuoside I does not downregulate CXCR4 through this mechanism. We found that downregulation of CXCR4 by baohuoside I occurred at the transcriptional level. Transcription factor NF-κB has been implicated in the regulation of CXCR4;[33] therefore, it is possible that downregulation of CXCR4 mRNA occurs through suppression of NF-κB activation. Indeed, the occupancy of NF-κB at the CXCR4 promoter was reduced by baohuoside I. In addition, besides CXCR4, the activation of NF-κB also induces the expression of various molecules, including matrix metalloproteinase (MMP) 9, COX-2, and adhesion molecules such as vascular cell adhesion molecule, intracellular adhesion molecule 1, and endothelial-leukocyte adhesion molecule 1, all of which have been linked with cancer cell metastasis and invasion.[47] Because baohuoside I can inhibit transcriptional activation of NF-κB, as shown in this study, it is possible that baohuoside I suppressed the expression of other NF-κB-regulated molecules and that in HeLa cells.

When we further investigated the effect of baohuoside I on CXCL12-induced invasion of both cervical and breast cancer cells, we found that preincubating cells with baohuoside I could inhibit ligand-induced invasion in both cancer cells.

Traditionally, baohuoside I has been shown to inhibit tumor growth,[48] induce apoptosis,[49] and possess antioxidant and antitumor activities.[50] It is possible that some of these antitumor effects of baohuoside I are also mediated through CXCR4 regulation.

Taken together, our results propose that baohuoside I downregulates the expression of CXCR4, a key receptor involved in the cross-talk between cancer cells and its microenvironment, and that some of the antitumor effects of baohuoside I are possibly mediated through CXCR4 regulation. Further *in vivo* studies are needed to demonstrate the relevance of these observations to cancer treatment.

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Notes

The authors declare no competing financial interest.

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ABBREVIATIONS

CXCR, CXC chemokine receptor; SDF, stromal cell-derived factor; CXCL, CXC chemokine ligand; HIF, hypoxia inducible factor; COX, cyclooxygenease; STAT, signal transducer and activator of transcription; ERK, extracellular signal-related kinase; PI3K, phosphatidylinositol 3-kinase; eNOS, endothelial nitric oxide synthase; JNK, c-Jun N-terminal kinase; NF-κB, nuclear factor κB; VEGF, vascular endothelial growth factor; AIP, atrophin-interacting protein; MMP, matrix metalloproteinase; PAGE, polyacrylamide gel electrophoresis.

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