Berberine hydrochloride IL-8 dependently inhibits invasion and IL-8-independently promotes cell apoptosis in MDA-MB-231 cells

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Abstract. Breast cancer, the leading cause of cancer-related mortality worldwide in females, has high metastatic and recurrence rates. The aim of the present study was to evaluate the anti-metastatic and anticancer in situ effect of berberine hydrochloride (BER) in MDA-MB-231 cells. BER dose-dependently inhibited proliferation and the IL-8 secretion of MDA-MB-231 cells. Additional experiments revealed that the inactivation of PI3K, JAK2, NF-κB and AP-1 by BER contributed to the decreased IL-8 secretion. BER abrogated cell invasion induced by IL-8 accompanied with the downregulation of the gene expression of MMP-2, EGF, E-cadherin, bFGF and fibronectin.

In addition, BER reduced cell motility but induced G2/M arrest and cell apoptosis in an IL-8-independent manner. BER modulated multiple signaling pathway molecules involved in the regulation of cell apoptosis, including activation of p38 MAPK and JNK and deactivation of JAK2, p85 PI3K, Akt and NF-κB. The enhanced cell apoptosis induced by BER was eliminated by inhibitors of p38 MAPK and JNK but was strengthened by activator of p38 MAPK. Thus, BER inhibited cell metastasis partly through the IL-8 mediated pathway while it induced G2/M arrest and promoted cell apoptosis through the IL-8 independent pathway. Apoptosis induced by BER was mediated by crosstalks of various pathways including activation of p38 MAPK and JNK pathways and inactivation of JAK2/Pi3K/NF-κB/ERK/AKT pathways. The results suggested that BER may be an efficient and safe drug candidate for treating highly metastatic breast cancer.

Introduction

Breast cancer, the leading cause of cancer-related mortality worldwide in females, has high metastatic and recurrence rates following curative resection. Metastasis after resection, is the leading cause of mortality in patients with breast cancer (1,2). Interleukin-8 (IL-8), a cytokine of the CXC chemokine family (3), is highly expressed in many tumor tissues (4) and promotes tumor progression and cancer metastasis (5-10). Findings of recent studies have shown that the overexpression of IL-8 is associated with recurrence and poor prognosis in breast cancer (11-14). The expression of IL-8 in highly metastatic breast cancer MDA-MB-231 cells is much higher than that in the non-metastatic MCF-7 breast cancer cell line (15,16). Recent studies have demonstrated that, during or after treatment, several chemotherapeutic drugs resulted in resistance and cancer metastasis associated with upregulated IL-8 in human breast cancer (17-20). Thus, suppression of IL-8 may be beneficial for breast cancer treatment.

Chemotherapeutic agents, such as 5-fluorouracil, adriamycin, dacarbazine and paclitaxel can induce IL-8 upregulation (5,21-25). Coptis chinensis Franch (Huanglian), a medicinal herb, induces cell growth arrest and apoptosis in human breast cancer cells (26). Berberine, an active isoquino-
line alkaloid in *C. chinensis*, shows anti-metastatic activity in breast cancer cells including MDA-MB-231 and MCF-7 cells (27). In the clinic, berberine hydrochloride (BER) with better water solubility than berberine, is used to treat gastrointestinal inflammation, bacterial diarrhea or infection as well as some gastrointestinal cancers (10,28,29). In our previous studies, BER inhibited the proliferation and IL-8 expression of AGS cells and counteracted enhanced IL-8 induced by evodiamine in AGS cells (10,30). However, the effect of berberine and BER on IL-8 expression and the relationship of IL-8 with migration and cell proliferation in MDA-MB-231 cells remains to be determined.

Induction of apoptosis of cancer cells, mainly through the mitochondrial- and death receptor-dependent pathways, is the principal strategy for chemotherapy. In addition, several other pathways involved in cell apoptosis are influenced by chemotherapeutic drugs (31-33). Berberine has been demonstrated to induce apoptosis of cancer cells including SW60 and HepG2 cells by interfering with the expression of molecules in pathways including Bcl-2, Bax and caspase-3 (34-36). However, whether these pathways play a role in BER-induced apoptosis in breast cancer cells remains to be clarified.

In the present study, the effects of BER on IL-8 expression, and the migration, invasion and cell proliferation of MDA-MB-231 cells were investigated. Furthermore, the possible molecular mechanisms involved in the anti-metastatic and pro-apoptotic effect of BER were discussed. The results suggested that BER may be an efficient and safe drug candidate for treating highly metastatic breast cancer.

Materials and methods

Materials and chemicals. Berberine hydrochloride (BER, purity: 98%, no. 889210) was purchased from Shanghai Taot biotech Co., Ltd. (Shanghai, China). Dulbecco's modified Eagle's medium (DMEM)/high-glucose medium was obtained from Cellgro (Manassas, VA, USA). Trypsin (no. 1310929) and FBS (no. 1301838) were provided by Gibco (Grand Island, NY, USA). Propidium iodine (PI) (no. 118 K3583) and dimethyl sulfoxide (DMSO) (no. RNBC 3642) were supplied by Sigma Chemical Co. (St. Louis, MO, USA). The primers for qPCR, TRIzol (no. 66205) and Annexin v-FITC (no. 1081948) were from Invitrogen (Carlsbad, CA, USA). Cell Counting Kit-8 (no. ET758) was provided by Dojindo Laboratories (Kumamoto, Japan). SYBR Premix Ex Taq (no. AK2902) and PrimeScript RT reagent kit (no. AK2001) were from Takara (Dalian, China). IL-8 ELISA kit (no. E15164-105) was obtained from AGS (Carlsbad, CA, USA). SYBR Premix Ex Taq (no. AK2902) and PrimeScript RT reagent kit (no. AK2001) were from Takara (Dalian, China). IL-8 ELISA kit (no. E15164-105) was obtained from eBioscience, San Diego, CA, USA. JAK inhibitor I (Jak inhibitor, no. D3010), LY294002 (PI3K inhibitor), and SB203580 (p38 MAPK inhibitor, no. C3110) were purchased from Santa Cruz Biotechnology Inc. (Santa Cruz, CA, USA). Curcumin (AP-1 inhibitor) was obtained from ICM Biomedicals Inc. (Costa Mesa, CA, USA). BAY-11-7082 (NF-κB inhibitor, no. 01), SP600125 (JNK inhibitor, no. 01), PD98059 (ERK1/2 inhibitor, no. 03) were obtained from Selleck Chemicals (Houston, TX, USA). Anisomycin (activitors of p38 MAPK and JNK, no. D00140631) was from Calbiochem (San Diego, CA, USA).

Proliferation assay. MDA-MB-231 cells were cultured in DMEM with 10% fetal bovine serum (FBS). The cells were seeded in 200 µl of medium at 1.0x10^4 cells/ml in 96-well culture plates and grown overnight. Following treatment with BER for 24 or 48 h, respectively, the culture medium was collected for ELISA assay of IL-8. An equal volume of fresh medium was then added back to each well with an additional 20 µl of CCK-8 solution and incubated at 37°C for another 1 h. Absorbance of the dissolved solutions was detected at 450 nm by a Thermo Scientific Varioskan Flash microplate reader (Thermo Fisher Scientific). The cell viability rate was calculated as: (absorbance of drug-treated sample/absorbance of control sample) x 100.

Enzyme-linked immunosorbent assay (ELISA). Cells were seeded in 96-well, 6-well or 35-mm plates and cultured overnight. Following treatment with BER for 24 or 48 h, the culture medium was collected, centrifuged at 3000 rpm for 5 min and subjected to IL-8 assay using an ELISA kit according to the manufacturer's instructions. The absorbance at 450 nm was measured with a microplate reader, and the concentration of IL-8 in medium was determined by the standard curve.

Wound-healing assay. MDA-MB-231 cells were seeded in 24-well plates. After the cells reached 90-95% confluence, a scratch was drawn on the cell monolayer with a sterile 100 µl pipette tip. The detached cells were removed by washing with PBS. Treatments of BER (30, 60 and 90 µM) and IL-8 (100 µg/ml) prepared in medium were added onto the cells and the images were captured immediately under an Olympus CKX41 microscope (Olympus, Tokyo, Japan) and denoted as time T0. Following incubation for 24 and 48 h, the cells were photographed again and denoted as time T24 and T48.

Invasion assay. The invasion ability of breast cancer cells was evaluated according to the methods described by Kuo et al (27). Briefly, 200 µl of MDA-MB-231 cells (1x10^6 cells in serum-free medium) were seeded onto the upper part of the 24-well Transwell chambers coated with Matrigel (BD Biosciences, San Jose, CA, USA). FBS (10%) was used as the chemoattractant in the bottom chambers. After incubation with IL-8 (100 ng/ml), BER (90 µM) or their combination at 37°C for 24 h, the non-invaded cells were removed from the top of the Transwell membrane with a cotton swab. The invaded cells were fixed with 4% PFA for 10 min, followed by incubation with 2% crystal violet staining solution for 15 min, and were observed under an Olympus CKX41 microscope.

Quantitative polymerase chain reaction (qPCR). Total RNA was extracted from the MDA-MB-231 cells using TRIzol...
reagent according to the manufacturer’s instructions. Reverse transcription was conducted with a PrimeScript RT reagent kit. Sense and antisense primers used for qPCR were shown in Table I. qPCR was performed with SYBR Premix Ex Taq by using following amplification conditions: 95˚C for 30 sec; followed by 40 cycles (95˚C for 5 sec; 60˚C for 34 sec); and 95˚C for 15 sec; 60˚C for 1 min; and 95˚C for 15 sec. The relative expression level of individual genes was normalized to that of GAPDH in the same sample.

Cell cycle distribution analysis. Cells were seeded in 6-well plates at 6x10^4 cells/well in 3 ml medium and cultured overnight. After serum starvation for 24 h, the cells were incubated with BER (30, 60 and 90 µM), IL-8 (100 ng/ml) or a combination of IL-8 and BER (90 µM) for 24 h. The cells were harvested by trypsinization, washed twice with phosphate-buffered saline (PBS), and fixed with cold 70% ethanol overnight followed by staining with PI solution containing 50 µg/ml RNase A and 0.1% Triton X-100. The distribution of the cell cycle was examined using a Millipore Guava flow cytometer (Millipore, Billerica, MA, USA).

Cell apoptosis detection. Cells were seeded in 6-well plates at 7.5x10^4 cells/well in 3-ml medium and allowed to adhere to plates overnight. After serum starvation for 24 h, the cells were incubated with a range of concentrations of BER (30, 60 and 90 µM) with 10% FBS for another 24 h. In experiments for clarifying cell signaling pathways involved in BER-induced apoptosis, the cells were treated with BER at 90 µM for 24 h after pre-incubation of SB203580 (25 µM), LY294002 (10 µM), SP600125 (20 µM), PD98059 (20 µM), BAY-11-7082 (5 µM), JAK inhibitor I (5 µM), curcumin (8 µM) and anisomycin (10 µg/ml) for 1 h. The cells were subsequently harvested by careful trypsinization, and washed twice with 1X Annexin v binding buffer. After resuspension in 1X Annexin V binding buffer, the cells were stained with Annexin V and PI. Fluorescence of the cells was examined on a Guava flow cytometer.

Western blotting. After incubation with BER, the cells were lysed with lysis buffer and sonicated three times each for 15 sec. The cell lysate was centrifuged at 14,000 g for 15 min at 4°C, and the supernatant was collected. Protein samples were separated by SDS-PAGE (12 or 15%) and transferred onto PVDF membrane by wet transfer. PVDF membranes were blocked with 5% non-fat milk solution and incubated with different primary antibodies overnight at 4°C. After being washed with 1X TBST, PVDF membranes were incubated with respective secondary antibodies. The protein bands were visualized with ECL Prime kit.

Statistical analysis. Each value was presented as means ± SEM. Differences between two groups were analyzed using Student’s t-test. Pairwise comparisons among groups were conducted by one-way ANOVA with Dunnett’s analysis using PrismDemo 4. P<0.05 was considered statistically significant.

Results

BER inhibits proliferation and IL-8 secretion of MDA-MB-231 cells. To examine the efficacy of BER on cell growth of breast cancer, MDA-MB-231 cells were treated with a range of concentrations of BER for 24 and 48 h, respectively. As shown in Fig. 1B, BER dose-dependently inhibited the proliferation of MDA-MB-231 cells at 24 or 48 h. When used at concentrations of >90 µM for 24 h, BER suppressed the growth of cells, and the growth inhibitory rate of was >44.18%. By contrast, when treated for 48 h, at lower concentrations, such as 60 µM, BER prevented 38.94% of cells from proliferation. Accordingly, the IC50 of BER was 78.21 µM for 24-h treatment, while that for 48 h was 71.87 µM.

Further analysis demonstrated that BER significantly decreased the IL-8 secretion of MDA-MB-231 cells in a dose-dependent manner (Fig. 1C). To determine cellular signaling molecules involved in the modulation of IL-8 secretion of MDA-MB-231 cells, multiple pathway inhibitors were used, including LY294002 (10 µM), SB203580 (25 µM), SP600125 (20 µM), PD98059 (20 µM), BAY-11-7082 (5 µM), JAK inhibitor I (5 µM), curcumin (8 µM) and anisomycin (10 µg/ml) for 1 h. The cells were subsequently harvested by careful trypsinization, and washed twice with 1X Annexin V binding buffer. After resuspension in 1X Annexin V binding buffer, the cells were stained with Annexin V and PI. Fluorescence of the cells was examined on a Guava flow cytometer.

Table I. Primer sequences used in qPCR.

<table>
<thead>
<tr>
<th>Genes</th>
<th>Forward primer</th>
<th>Reverse primer</th>
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<tr>
<td>GAPDH</td>
<td>GCACCGTCAAGGCTGAAGAAC</td>
<td>TGGTGAAAGCAGCCAGTGGGA</td>
</tr>
<tr>
<td>E-cadherin</td>
<td>CGAGAGCTACACTGTCACGGG</td>
<td>GGGTGTCAGGGGAAATAGG</td>
</tr>
<tr>
<td>Fibronectin</td>
<td>TGAGCTGCACTGTCAGTGGTA</td>
<td>TCCTACGTGAGATGCTCCTG</td>
</tr>
<tr>
<td>bFGF</td>
<td>GCAGTGGTATTTGACGAGTGA</td>
<td>TATGCGTACTGACACCTTGA</td>
</tr>
<tr>
<td>EGF</td>
<td>GACTTGGGAGCCTTGAGCAGAA</td>
<td>CATGCAAAAGTGTGACTGAGGAGAGG</td>
</tr>
<tr>
<td>MMP-2</td>
<td>TGGCAAGTACTGGCTTGTCTG</td>
<td>TTCTGTGCGGTAGTGTGATC</td>
</tr>
<tr>
<td>Jak 2</td>
<td>TCTGGGGGATGTATGGTCAGAA</td>
<td>AGACATGGTTGGGAGATACC</td>
</tr>
<tr>
<td>Akt1</td>
<td>CCTCCACGACATCCGACTG</td>
<td>TCACAAAGAGCCTCCATTATCA</td>
</tr>
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BER decreases cell invasion and migration of MDA-MB-231 cells. In order to clarify the relationship between BER and cell metastasis, invasion and wound-healing assays were carried out. As shown in Fig. 2A, IL-8 increased the invasion of MDA-MB-231 cells as more cells stained by crystal violet were found on the bottom chambers of the Transwell membranes when compared with those in the control groups. BER (90 µM) inhibited the invasion of MDA-MB-231 cells, and could abolish the increased cell invasion induced by IL-8.

In the wound-healing assays (Fig. 2B), BER (30, 60 and 90 µM) appeared to dose-dependently prevent the motility of MDA-MB-231 cells after treatment for 24 and 48 h. However, IL-8 (100 ng/ml) treatment did not affect cell motility (Fig. 2C) at 24 or 48 h and showed no interaction with BER treatment.

BER inhibits gene expression of metastasis-related molecules in MDA-MB-231 cells. To confirm the anti-metastatic effect of BER, the mRNA expression of MMP-2, EGF, E-cadherin, bFGF and fibronectin was quantified by qPCR. BER at 90 µM decreased the mRNA expression of the measured molecules significantly (P<0.05 or P<0.001) (Fig. 3). By contrast, when used <90 µM, BER only reduced the mRNA expression of MMP-2, EGF and fibronectin.

BER induces G2/M phase arrest and apoptosis in MDA-MB-231 cells. BER induced the G2/M arrest of MDA-MB-231 cells in a dose-dependent manner (Fig. 4). IL-8 (100 ng/ml) had no effect on cell cycle distribution. When combined with IL-8, BER (90 µM) induced G2/M arrest significantly, although no difference with that induced by BER alone was observed.

BER appeared to dose-dependently induce the apoptosis of MDA-MB-231 cells as the ratio of early apoptotic cells increased with the elevation of BER concentrations (Fig. 5I). By contrast, as shown in Fig. 5II, IL-8 did not markedly affect cell apoptosis. To our surprise, it also did not influence cell apoptosis induced by BER (90 µM).

Consistent with the results from flow cytometry, BER modulated the expression of apoptotic proteins. BER dose-dependently increased the amount of cleaved caspase-3 (Fig. 5I). By contrast, it also downregulated the protein expression of Bcl-2 in a dose-dependent manner but showed no effect on the expression of Bax.

Cellular signaling pathways are involved in BER-induced apoptosis in MDA-MB-231 cells. To determine the effect of BER on the protein expression of the cellular signaling molecules, MDA-MB-231 cells were treated with BER (30, 60 and 90 µM) for 24 h and then subjected to western blot analysis. Fig. 6A shows that BER dose-dependently increased the phosphorylation of p38 and SAPK/JNK MAPKs but did not affect that of ERK. By contrast, BER treatment decreased the phosphorylation of Jak2, p85 PI3K, Akt and p65 NF-κB in
a dose-dependent manner (Fig. 6B). Additionally, BER inhibited the mRNA expression of Jak2 and Akt1 (Fig. 6C and D).

To verify the involvement of p38 and JNK MAPKs in the BER-induced apoptosis, MAPK pathway inhibitors were used together with BER (90 µM). As shown in Fig. 7A-G, the elevated apoptosis induced by BER was significantly abrogated by SB203580 and SP600125. Moreover, the addition of SB203580 and SP600125 reduced the cellular cleaved caspase-3 compared with that treated with BER (Fig. 7H). To confirm the effect of p38 MAPK and JNK on BER-induced apoptosis, anisomycin, the p38 MAPK and JNK activator, was used. As shown in Fig. 8A, anisomycin (10 µg/ml) significantly increased the activation of p38 MAPK, which enhanced the cleavage of caspase-3. When co-treated with anisomycin, BER
(90 µM) induced the increased phosphorylation of p38 MAPK compared with BER treatment alone, leading to an increased production of cleaved caspase-3. Consistent with the western blot results, anisomycin and BER induced significant apoptosis compared with the control (P<0.001) (Fig. 8B-F). When combined together, anisomycin and BER enhanced apoptosis as compared to that induced individually.

In the present study, the effect of NF-κB, PI3K, AP-1 and JAK2 on BER-induced apoptosis was clarified. As shown in Fig. 9, inhibitors of NF-κB and AP-1 increased the rates of

Figure 3. BER inhibits gene expression of metastasis-related proteins for 24 h. Gene expression of MMP-2, EGF, E-cadherin, bFGF and fibronectin were all significantly downregulated by BER. Data are presented as means ± SEM, *P<0.05, ***P<0.001 vs. control. B, berberine hydrochloride.

Figure 4. BER induces G2/M phase arrest in an IL-8 independent manner in MDA-MB-231 cells. (A) Typical images from flow cytometry with PI staining showed G2/M arrest. (B) BER induced G2/M arrest after treatment for 24 h detected by flow cytometry with PI staining. (C) BER induced G2/M phase arrest in an IL-8-independent manner. Data are presented as means ± SEM; *P<0.05, **P<0.01 vs. control. B, berberine hydrochloride.
cell apoptosis. Pre-incubation with the inhibitors of NF-κB, PI3K, AP-1 and JAK2 significantly increased cell apoptosis induced by BER. Moreover, the protein expression of cleaved caspase-3 was increased by the pre-incubation of inhibitors of PI3K, JAK2 and p65 NF-κB, compared with BER used alone.

Discussion

The results of the present study have demonstrated that BER significantly inhibited IL-8 secretion, invasion and migration of MDA-MB-231 cells and induced cell apoptosis. Additional experiments revealed that BER suppressed cell proliferation through G2/M arrest and promoted apoptosis of MDA-MB-231 cells by modulation of various signaling pathway molecules such as MAPKs, JAK2, PI3K, Akt and NF-κB.

Chemotherapeutic agents are known to induce IL-8 upregulation in tumor cells, which is closely associated with chemotherapy resistance and cancer metastasis (3,5-7,10,18,21-25,37-40). Depletion of IL-8 induces cell cycle arrest and increases the efficacy of chemotherapeutic agents in breast cancer cells (41). Therefore, chemotherapeutic agents with an inhibitory effect on IL-8 production may be more efficacious in treating breast cancer. MDA-MB-231 is one of the breast cancer cell lines constitutively expressing a high level of IL-8 (15,16). Consistent with previous studies (41), IL-8 enhanced the invasive ability of MDA-MB-231 cells in our...
experiments (Fig. 2A). However, IL-8 did not interfere with cell migration (Fig. 2C) or cell cycle distribution (Fig. 4). To the best of our knowledge, IL-8 mainly increases the risk of breast cancer metastasis through the enhancement of invasive ability, at least, for MDA-MB-231 cells. In the present study, BER dose-dependently inhibited the proliferation and IL-8.
secretion of MDA-MB-231 cells (Fig. 1B and C). Furthermore, BER abrogated the increased invasion induced by IL-8. Thus, BER counteracted the metastasis of MDA-MB-231 cells at least partly in an IL-8 dependent manner.

A number of pathways have been found to be actively involved in the modulation of IL-8 production, including MAPKs, JAK2, and PI3K/Akt pathways (21,25,42-44). In agreement with those studies, our results showed that the activation of ERK1/2, SAPK/JNK, p38 MAPK, AP-1 and p65 NF-κB pathways was closely associated with the constitutive IL-8 secretion in MDA-MB-231 cells (Fig. 1D). Correspondingly, BER was able to modulate the activation of those molecules. Notably, BER only deactivated JAK2, p85 PI3K and p65 NF-κB signaling but activated that of MAPK pathway molecules (Fig. 6). Therefore, the inhibition of BER on IL-8 production might occur mainly through JAK2/PI3K/NF-κB pathways.

IL-8 can activate PI3K, protein kinase B (PKB and Akt), mammalian target of rapamycin (mTOR), ERK1/2, p38 MAPK and JAK2 pathways to regulate numerous gene and protein expressions involved in cell proliferation, survival, invasion and migration (25,45). Activation of PI3K and JAK2 pathways has been reported to modulate cell invasion and angiogenesis (25). In experiments of the present study, we found that BER inactivated the PI3K/Akt, JAK2 and NF-κB pathways. The results also showed that many metastasis-related genes, including MMP-2, EGF, E-cadherin, bFGF and fibronectin, were downregulated by BER, suggesting the anti-invasive effect of BER was partly, if not all, mediated in an IL-8 dependent manner.

IL-8 was also found to be independent of cell migration, cell cycle distribution and apoptosis of MDA-MB-231 cells. By contrast, BER was actively engaged in these processes, which inhibited cell migration, and induced G2/M arrest and cell apoptosis in an IL-8 independent manner.

The mitogen-activated protein kinases (MAPK) pathways, i.e., ERK, SAPK/JNK and p38 MAPK, are actively involved in drug-induced cell apoptosis of numerous cancer cells (46). Activation of JNK and p38 MAPK pathways results in enhanced apoptosis induced by berberine in human hepatoma and colon carcinoma cells (34,35). When the MAPKs are inhibited, the apoptosis and caspase-3 cleavage in tumor cells are abrogated (46-48). Moreover, inhibitors of MAPKs slightly increase cell viability in MDA-MB-231 cells (49). In the present study, the phosphorylation of p38 MAPK and SAPK/JNK was enhanced by BER treatment. In agreement with the previous studies (31,34), the inhibitors of p38 MAPK and SAPK/JNK attenuated the apoptosis enhanced by BER treatment in a caspase-3-dependent manner, while the inhibitors of p38 MAPK and SAPK/JNK...
Let al: BER IL-8 DEPENDENTLY INHIBITS INVASION AND IL-8-INDEPENDENTLY PROMOTES APOPTOSIS

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Figure 9. Blockage of PI3K, Jak2, NF-κB and AP-1 elevates cell apoptosis induced by BER in MDA-MB-231 cells. (A-J) Typical images from flow cytometry with Annexin V/PI double staining. (K) Blockage of PI3K, Jak2, NF-κB and AP-1 elevated cell apoptosis induced by BER in MDA-MB-231 cells. (I) Western blotting results showed that the blockage of PI3K, Jak2 and NF-κB increased cleaved caspase-3 expression induced by BER. Data are presented as means ± SEM; **P<0.01 vs. control; ***P<0.001 vs. B90. B90, berberine hydrochloride (90 µM). A, 0; B, JAK I, 5 µ; C, LY294002, 10 µM; D, BAY-11-7082, 5 µM; E, Curcumin, 8 µM; F, B90; G, JAKI+B90; H, LY294002+B90; I, BAY-11-7082+B90; J, Curcumin+B90.

alone showed no obvious effect on cell apoptosis in MDA-MB-231 cells. Furthermore, anisomycin, an activator of p38 MAPK and JNK, induced cell apoptosis and significantly elevated cell apoptosis induced by BER in a caspase-3-dependent manner. Thus, the activation of p38 MAPK and SAPK/JNK was involved in the cell apoptosis induced by BER.

In breast cancer cells, activation of the PI3K/Akt pathway has already been found to prevent cell apoptosis (27,36). By contrast, inhibition of janus kinase 2 (JAK2) promotes cell apoptosis (51,52). However, the effect of JAK2 on apoptosis induced by BER remains to be clarified. In the present study, BER inhibited the activation of JAK2, p85 PI3K and Akt by reducing the phosphorylation of Jak2, p85 PI3K and Akt1. Moreover, the inhibitors of NF-κB, PI3K and JAK2 enhanced the cell apoptosis induced by BER, suggesting the involvement of these three pathways in the BER-induced apoptosis of MDA-MB-231 cells. The gene expression of total Jak2 and Akt1 was also decreased by BER. Therefore, the enhanced apoptosis induced by BER resulted from crosstalk among multiple cell signaling pathways including p38 MAPK, JNK, PI3K/Akt/NF-κB and JAK2.

In conclusion, BER inhibited cell metastasis partly through the downregulation of IL-8 and enhanced cell apoptosis by activating MAPKs and deactivating the JAK2/PI3K/Akt/NF-κB pathways. This was different from other chemotherapeutic drugs that induce apoptosis but simultaneously increase IL-8 expression, therefore, promote cancer metastasis. Thus, BER showed simultaneous anti-carcinoma in situ and anti-
metastatic effects in MDA-MB-231 cells, suggesting the effective and safe potential of BER as a therapeutic candidate to treat highly metastatic breast cancer.

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