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Effect of benzophenone-1 and octylphenol on the regulation of epithelial-mesenchymal transition via an estrogen receptor-dependent pathway in estrogen receptor expressing ovarian cancer cells

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ABSTRACT

Epithelial-mesenchymal transition (EMT) is an important process in embryonic development and cancer progression and metastasis. EMT is influenced by 17β-estradiol (E2), an endogenous estrogen. Benzophenone-1 (2,4-dihydroxybenzophenone, BP-1) and 4-*tert*-octylphenol (OP) are suspected endocrine disrupting chemicals (EDCs) because they can exhibit estrogenic properties. In this study, we examined whether BP-1 and OP can lead to EMT of BG-1 ovarian cancer cells expressing estrogen receptors (ERs). A wound healing assay and western blot assay were conducted to show the effect of BP-1 and OP on the migration of BG-1 cells and protein expression of EMT-related genes. BP-1 (10^{-6} M) and OP (10^{-6} M) significantly enhanced the migration capability of BG-1 cells by reducing the wounded area in the cell monolayer relative to the control, similar to E2 (10^{-9} M). However, when BG-1 cells were co-treated with ICI 182,780, an ER antagonist, the uncovered area was maintained at the level of the control. N-cadherin, snail, and slug were increased by BP-1 and OP while E-cadherin was reduced compared to the control. However, this effect was also restored by co-treatment with ICI 182,780. Taken together, these results indicate that BP-1 and OP, the potential EDCs, may have the ability to induce ovarian cancer metastasis via regulation of the expression of EMT markers and migration of ER-expressing BG-1 ovarian cancer cells.

1. Introduction

Ovarian cancer is a highly metastatic disease with the highest fatality-to-case ratio of all gynecological cancers. Two-thirds of patients are diagnosed with advanced-stage ovarian cancer because it is asymptomatic in its early stages. The molecular changes associated with ovarian cancer metastasis still need to be identified to enhance therapeutic effects.

Endocrine-disrupting chemicals (EDCs) are exogenous agents that interfere with the normal actions of hormones through competitive binding to diverse hormone receptors including estrogen and androgen receptors (ER and AR). EDCs influence development and reproduction; metabolism and obesity; breast, prostate, and thyroid cancer; neuroendocrinology; and cardiovascular endocrinology (Diamanti-Kandarakis et al., 2009; Shanle and Xu, 2011). Exposure to EDCs in our daily lives is nearly inevitable because they are used in a wide variety of industrial products including plastics, pesticides, drugs, detergents, and cosmetics (Park et al., 2013).

2,4-dihydroxybenzophenone (benzophenone-1; BP-1) is a primary UV stabilizer used to prevent polymer degradation and deterioration in quality due to UV irradiation. Recently, BP-1 was reported to bioaccumulate in humans via absorption through the skin and to have the potential to induce health problems including endocrine disruption (Park et al., 2013). 4-*tert*-octylphenol (OP)

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belongs to a family of alkylphenols commonly produced by industrial activities (Kim et al., 2011). The duration of OP exposure has been linked to the development of ER-positive breast cancer because OP can bind to the ER and induce abnormal activation of ER-mediated signaling, thereby upregulating oncogene expression (Pisapia et al., 2012). Thus, OP induces cancer cell growth via increased oncogene expression (Hwang et al., 2011; Jung et al., 2012). It has been reported that BP-1 and OP have estrogenic effects in the body by mimicking the actions of estrogen (Lee and Choi, 2013; Park et al., 2013). 17β-Estradiol (E2), a potent endogenous estrogen, can affect the growth and migration of estrogen dependent cancer cells through diverse functions (Bai et al., 2000; Liu et al., 2014; Yan et al., 2013). Previous studies also demonstrated that BP-1 and OP may cause cancer proliferation effects in estrogen dependent cancers such as breast and ovarian cancers in a similar fashion to E2 (Lee and Choi, 2013; Park et al., 2013).

In this study, the effect of BP-1 and OP on the migration of BG-1 ovarian cancer cells relative to E2 was investigated. As an estrogen responsive cancer model, the BG-1 ovarian cancer cell line was adopted because ERα and ERβ genes were found to be expressed in BG-1 cells in our previous study (Hwang et al., 2011). The migration of cancer cells is accompanied by an epithelial-mesenchymal transition (EMT). During EMT, the expression of cell adhesion proteins such as E-cadherin and desmosome decreases while the expression of intermediate filamentous proteins such as vimentin and fibronectin increases (Mohamet et al., 2011). In addition, the expression of transcription factors involved in EMT including E box-binding protein, snail family, ZEB family, and helix–loop–helix (HLH) family is increased (Peinado et al., 2007). Among these, snail is a very important factor because it can block E-cadherin expression and induce expression of mesenchymal markers (Nieto, 2002). As a result, the morphology of cancer cells is changed enough to allow for easy invasion to other places through the EMT process. Therefore, changes in the expression of E-cadherin, N-cadherin, and snail family members in response to treatment with E2, BP-1, or OP were examined along with measuring altered migration ability of BG-1 ovarian cancer cells to clarify their effects on ovarian cancer progression, including metastasis via EMT.

2. Materials and methods

2.1. Reagents and chemicals

E2, fulvestrant (ICI 182,780), BP-1, and OP were purchased from Sigma–Aldrich Corp. (St. Louis, MO, USA). All chemicals were dissolved in 100% dimethyl sulfoxide (DMSO; Junsei Chemical Co., Tokyo, Japan). The final concentration of DMSO was 0.1% in the cell culture media.

2.2. Cell culture and media

BG-1 human ovarian cancer cells were obtained from Dr. K. S. Korach (National Institute of Environmental Health Sciences, National Institutes of Health, Research Triangle Park, NC, USA). The cells were cultured in Dulbecco's modified Eagle's medium (DMEM; Hyclone Laboratories, Inc., Logan, UT, USA) supplemented with 10% heat-inactivated fetal bovine serum (FBS; Hyclone Laboratories), 200 U/mL penicillin, and 200 mg/mL streptomycin (A&E Scientific, Logan, UT, USA) at 37 °C in a humidified atmosphere of 5% CO_2. To exclude the estrogenic compounds in DMEM and FBS, phenol red-free DMEM (Sigma–Aldrich Corp.) supplemented with 5% charcoal/dextran-treated FBS (CD-FBS) was used to measure an estrogenicity of each EDC in BG-1 cells as indicated previously (Kang et al., 2013). Charcoal/dextran-treated FBS was produced by exposure to 56 °C for 30 min followed by incubation for 1 h under charcoal/dextran-treated conditions to reduce levels of many hormones, steroids, and growth factors. The cells were detached with 0.05% trypsin-EDTA (Life Technologies, Carlsbad, CA, USA).

2.3. Wound healing assay

BG-1 ovarian cancer cells were cultured to more than 70% confluent growth (about 1.0×10^6 cells) in each well of 6-well plates at 37 °C in a humidified atmosphere containing 5% CO_2 air. The cell monolayer was scratched with a sterilized 1 ml micropipette tip to create wounds with the same length and width then washed with PBS to remove cell debris. Next, BG-1 cells were treated with 0.1% DMSO as a control, E2 (10^{-9} M) as a positive control, BP-1 (10^{-5} M), or OP (10^{-5} M) in media containing 5% charcoal/dextran-treated FBS for 48 h and 96 h. Images of each group were captured at 40× magnification using an Olympus CKX 41 microscope (Olympus Corp., Tokyo, Japan). Quantification of uncovered areas was carried out using the eXcope Lite 5.0.1 software (DIXI Optics, Dae-Jeon, Republic of Korea). Uncovered areas at 0, 48, and 96 h were calculated by converting each area into a percentage of the area at 0 h.

2.4. Western blot assay

After treatment with E2 or the two EDCs for 24 and 72 h, total proteins from the BG-1 cells were harvested with radioimmunoprecipitation assay (RIPA) buffer (50 mM Tris–HCl, pH 8.0; 150 mM NaCl, 1% NP-40; Sigma–Aldrich Corp, 0.5% deoxycholic acid; Sigma–Aldrich Corp, and 0.1% SDS). Bicinchoninic acid (BCA; Sigma–Aldrich Corp.) was used to measure the protein concentrations. Total cell proteins (50 μg) were separated in a 10% SDS-PAGE gel then transferred to a polyvinylidene fluoride (PVDF) membrane (BioRad Laboratories, Hercules, CA, USA). Membranes were then blocked with 5% skim-milk (BioRad Laboratories Inc.) for 2 h at room temperature and incubated with mouse monoclonal anti-GAPDH antibody (Abcam, Cambridge, UK), mouse monoclonal anti-SNAIL antibody (Cell Signaling Technology, Inc.), mouse monoclonal anti-SLUG antibody (Abcam), mouse monoclonal anti-N-cadherin antibody (Abcam), and rabbit polyclonal anti-E-cadherin antibody (Abcam) at 4 °C. The blot was then incubated with horseradish peroxidase (HRP)-conjugated secondary antibody for 2 h at room temperature (goat anti-mouse lgG [H + L] or goat anti-mouse lgG [H + L], 1:5000 dilution; BioRad Laboratories). Antibody binding was detected using a West-Q Chemiluminescent Substrate Plus kit (GenDEPOT, Barker, TX, USA). Band density was estimated using Gel Doc 2000. Quantification of protein bands was conducted by measuring band densities using Quantity One (BioRad Laboratories). Protein expression levels of snail, slug, N-cadherin, and E-cadherin were normalized by the band size of GAPDH protein.

2.5. Data analysis

All experiments were performed at least three times to ensure consistent results. All data were analyzed using the GraphPad Prism software (San Diego, CA, USA) and expressed as the mean ± standard deviation (S.D.). Results were analyzed by one-way analysis of variance (ANOVA) followed by a *post hoc* Dunnett's multiple comparison test for three-pair comparisons and Student's *t*-test. *P*-values < 0.05 were considered statistically significant.

3. Results

3.1. Effects of BP-1 and OP on BG-1 cell migration

The EMT process is associated with cell migration. To investigate