Environmental Science and Pollution Research (2021) 28:36060–36068
https://doi.org/10.1007/s11356-021-13267-0

RESEARCH ARTICLE

Bisphenol A induces ovarian cancer cell proliferation and metastasis through estrogen receptor-α pathways

Chen Sang[1] · Yu Song[2] · Tong-wang Jin[1] · Shuo Zhang[3] · Linyan Fu[3] · Yi Zhao[3] · Xinxin Zou[3] · Zhe Wang[4] · Hui Gao[3] · Sijin Liu[5]

Received: 21 December 2020 / Accepted: 1 March 2021 / Published online: 8 March 2021
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Abstract

Bisphenol A (BPA) is a widely used raw material that can be detected both in the environment and in the human body. Due to its estrogen-like effects, wide concerns have been raised about the potential role of BPA in the initiation and development of hormone-dependent cancers. Ovarian cancer is the most common reproductive system cancer and has a high mortality rate in women. Despite recent investigations into BPA's carcinogenic effects, studies on its role in ovarian cancer development remain limited. In this study, we aimed to assess the effect of BPA at various environmentally relevant concentrations on proliferation and metastasis of ovarian cancer cells. We discovered that BPA can stimulate proliferation of OVCAR-3 ovarian cancer cells after exposure for up to 5 days. Strikingly, BPA enhanced ovarian cancer cell migration, invasion, and adhesion (to vascular endothelial cells) through upregulation of matrix metalloproteinase-2 (MMP-2), MMP-9, and intercellular cell adhesion molecule-1 (IMAC-1). The stimulatory effects of BPA on cancer cell proliferation and metastasis were reversed by treatment with an ERα inhibitor, but not by treatment with an ERβ inhibitor. Together, these results suggest that BPA induces proliferation and metastasis of ovarian cancer cells through ERα signaling pathways. This study provides new insights into the carcinogenic effects of BPA with regard to ovarian cancer.

Keywords Bisphenol A; · Low concentration; · Ovarian cancer; · Cell proliferation; · Metastasis; · Estrogen receptor-α

Introduction

Bisphenol A (BPA), the most common member of the bisphenol group, is present throughout the environment of human daily life (Giulivo et al. 2016). In addition to being detectable in serum, BPA can be detected in human liver, brain, follicular fluid, and amniotic fluid (Vandenberg et al. 2007; Vandenberg et al. 2010; Genuis et al. 2012; Michałowicz 2014). Different concentrations of BPA can also be detected in umbilical cord blood, breast milk and the urine of newborns (Lee et al. 2018). BPA can bind to the classical nuclear estrogen receptors (ERs) ERα and ERβ, as well as to a cell membrane receptor called G-protein-coupled estrogen receptor (GPER), to disrupt the endocrine system (Safe et al. 2001; Thomas and Dong, 2006; Acconcia et al. 2015). Recent studies have also indicated that BPA plays a role in the carcinogenesis of several hormone-dependent cancers, such as breast cancer and endometrial cancer (Seachrist et al. 2016; Wang et al. 2017). Despite emerging progress toward understanding the health risks of BPA, large knowledge gaps still remain regarding its potential effects on cancer initiation and development.

Chen Sang and Yu Song contributed equally to this work.

Responsible editor: Ludek Blaha

✉ Zhe Wang
hnwzhe@163.com

✉ Hui Gao
gaohuibj2003@163.com

[1] School of Biological Science and Medical Engineering, Beihang University, Beijing 100191, China

[2] Department of Orthopedic Surgery, The Second Affiliated Hospital of Harbin Medical University, Harbin 150086, Heilongjiang Province, China

[3] Department of Obstetrics & Gynecology, Beijing Tian Tan Hospital, Capital Medical University, Beijing 100730, China

[4] School of Public Health, Xinxiang Medical University, Xinxiang 453003, Henan Province, China

[5] State Key Laboratory of Environmental Chemistry and Ecotoxicology, Research Center for Eco-Environment Sciences, Chinese Academy of Sciences, Beijing 100085, China

Ovarian cancer, characterized by tumors of the reproductive system, is a common cancer with a high mortality rate in women. Previous studies have reported that hormones, especially estrogen, play crucial roles in ovarian cancer development; for example, receiving hormone therapy during menopause may increase a patient's risk of developing ovarian cancer, particularly mucinous and endometrioid tumors (Liu et al. 2019). Because of the hormonic effects of BPA, there have been concerns about its potential effects on the development of ovarian cancer. In previous studies by our group and others, BPA has been demonstrated to promote proliferation of various ovarian cancer cells through the exertion of estrogenic effects, increasing risk of ovarian cancer (Shi et al. 2017). In vitro studies have shown that BPA can stimulate migration of ovarian cancer cells by regulating estrogen-dependent pathways (i.e., MAPK and PI3K/Akt signaling pathways) (Ptak et al. 2014). BPA may also promote tumor angiogenesis; a recent study reported that BPA can enhance expression of vascular endothelial growth factor (VEGF) and its receptor (VEGF-R) in ovarian cancer cells (Ptak and Gregoraszczuk, 2015). Despite these investigations, our current understanding of the roles of BPA in ovarian cancer development remains limited.

The primary objective of the current study was to investigate the potential effects of BPA on the progression of ovarian cancer. We recently demonstrated that proliferation of the human ovarian cancer cell line OVCAR-3 was promoted by exposure to low concentrations of BPA for 24 h (Shi et al. 2017). Importantly, OVCAR-3 is considered a highly metastatic human ovarian carcinoma cell line, as well as an ideal model in which to evaluate tumor cell metastasis (Hamilton et al. 1983). To this end, the OVCAR-3 cell line was used as an in vitro model of ovarian cancer. Our results show that low concentrations of BPA stimulate proliferation and metastasis of OVCAR-3 ovarian cancer cells, primarily through ERα signaling pathways.

Materials and methods

Reagents

RPMI-1640 and phenol red-free RPMI-1640 culture media were purchased from Invitrogen (USA). Fetal bovine and dextran-activated carbon adsorbed fetal bovine sera were purchased from Tianhang Biotechnology (China). BPA, DMSO, and agarose were purchased from Sigma (USA). ERα inhibitor MPP and ERβ inhibitor PHTPP were purchased from Santa Cruz (USA). Trizol was purchased from Ambion (USA). Matrigel was purchased from Corning (MA, USA). CCK-8 Kit was purchased from Dongren Chemical (China). Revert Aid First Strand cDNA was purchased from Thermo (USA). 2Taq PCR Master MixKit was purchased from Genview (USA), and primers used in the experiment were synthesized by Dingguo (China).

Cell culture and treatment

Human ovarian cancer cells (OVCAR-3) and human umbilical vein endothelial cells (HUVEC) were obtained from the American Type Culture Collection (Manassas, VA, USA). Cells were cultured in RPMI-1640 medium supplemented with 10% FBS and 1% penicillin-streptomycin in a humidified incubator with 5% CO_2 at 37 °C. Prior to BPA treatment, culture medium was removed and replaced with phenol red-free medium containing 10% dextran-coated charcoal-stripped FBS, after which cells were cultured for an additional 24 h. Cells were treated with vehicle or with various concentrations of BPA (ranging from 1 to 100 nM).

Cell proliferation assay

OVCRA-3 cells were cultured in 96-well plates and treated with different concentrations of BPA for five days. Culture medium was changed every two days to maintain BPA concentration. CCK-8 cell activity assay kit was used to determine cell viability, according to manufacturer's instructions. For each well, 10 μL CCK-8 regent was added, and the plate was incubated for an additional hour. The optical density (OD) value was measured at 450 nm using a microplate reader (Tecan, Mannedorf, Switzerland). To evaluate effects of ERs, cells were pre-treated for 30 min with ER inhibitors MPP (5 μM) or PHTPP (5 μM), then co-treated with BPA for 5 days.

Migration assay

Migration of OVCAR-3 cells was measured using a Transwell assay. Cells in serum-free medium were added into the 8 μm pore chamber. Phenol red-free RPMI-1640 medium containing 5% activated carbon-glucan-treated serum was added to the lower chamber. BPA was added to the culture medium in the lower chamber to a final concentration of 1, 10 or 100 nM. After 24 h, OVCAR-3 cells that passed through the membrane were fixed and stained with 0.5% crystal violet. Then, the numbers of migrated cells in ten randomly selected visual fields were counted under 10× magnification. The migration capacity of the control group was set as 100%, and the increased migratory rate of cells treated with different concentrations of BPA was calculated. When using ER inhibitors, cells were pretreated with MPP or PHTPP for 30 min, after which BPA was added for the migration experiment.

Invasion assay

The upper chamber of the Transwell plate was layered with Matrigel and incubated at 37 °C for 4 h until the Matrigel

solidified. OVCAR-3 cells were pre-treated with ERα inhibitor MPP, ERβ inhibitor PHTPP, or DMSO for 30 min, after which 4×10^4 cells were seeded into the upper chamber. The lower chamber contained BPA at a concentration of 10 nM. After 24 h, the matrix glue of the upper chamber was removed, the invaded OVCAR-3 cells were stained with 0.5% crystal violet. The numbers of invasive cells in ten randomly selected visual fields were counted under 10× magnification, and the average number of invasive cells in each visual field was calculated (the control group was set at 100%).

Adhesion of OVCAR-3 cells to vascular endothelial cells

OVCAR-3 cells were treated by a combination of BPA and ER inhibitor (MPP or PHTPP) for 24 h, then co-incubated with HUVECs for 2 h. Nonadherent HUVECs were washed away with PBS buffer. The numbers of adherent endothelial cells in ten randomly selected visual fields were counted using an optical microscope.

Real–time PCR

OVCAR-3 cells were co-treated with BPA and ER inhibitor (MPP or PHTPP) for 24 h. Total RNA was extracted and reverse-transcribed to cDNA using revert Aid first strand cDNA (Thermo, USA). mRNA expression levels were quantified with polymerase chain reaction (PCR) using the 2Taq PCR Master Mix Kit (Genview, USA).

Statistical analysis

Experimental data were expressed as mean ± standard error. Differences between groups were assessed by *t*-test, with $P<0.05$ indicating significant difference. The experiment was conducted independently for three times.

Results

BPA stimulates proliferation of OVCAR-3 cells

Proliferation is an important hallmark of cancer cells. Our previous study demonstrated that exposure to BPA at low, environmentally relevant concentrations (1–100 nM) promotes proliferation of OVCAR-3 ovarian cancer cells (Shi et al. 2017). Here, we further examined the long-term effects of this concentration range of BPA on ovarian cancer cell proliferation by extending the exposure duration from 24 h to 5 days. As shown in Fig. 1, cell growth was increased in BPA-treated OVCAR-3 cells, with a 20% increase at 10 nM ($P<0.01$) compared to the control. This was similar to findings after 24 h of BPA exposure (Shi et al. 2017). These results confirm that long-term exposure to low concentrations of BPA stimulates proliferation of ovarian cancer cells.

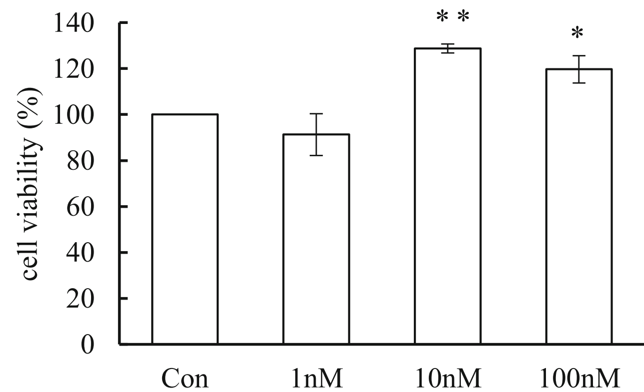

Fig. 1 Effects of different concentrations of BPA on OVCAR-3 cell proliferation. OVCAR-3 cells were treated with different concentrations of BPA for 5 days, and culture medium was changed every 2 days. Viability of OVCAR-3 cells was assessed using a CCK-8 kit. *, Compared with the control group, $P<0.05$. **, Compared with the control group, $P<0.01$. $n=3$

Given that BPA exerts its biological effects through multiple hormone receptors, we selected ER inhibitors to investigate whether classical nuclear receptors play roles in BPA-induced proliferation. The ERα inhibitor MPP (5 μM) and the ERβ inhibitor PHTPP (5 μM) had no effect on growth of OVCAR-3 cells (data not shown). As shown in Fig. 2, proliferation of OVCAR-3 cells was reduced by about 70% after co-treatment with 10 nM BPA and 5 μM MPP compared to BPA-treated cells ($P<0.05$). In contrast, co-treatment with PHTPP only slightly reduced BPA-stimulated proliferation (Fig. 2). These findings suggest that low-concentration BPA exposure stimulates ovarian cancer cell proliferation through ERα, rather than ERβ, pathways. Because long-term exposure (5 days) of BPA tends to have similar effects on cell proliferation as short-term exposure (24 h), we next

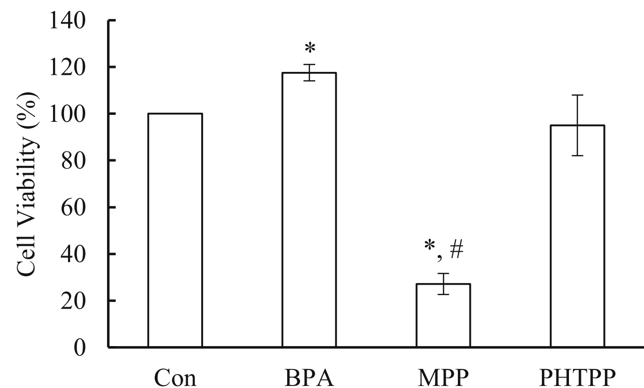

Fig. 2 Effects of ER inhibitors on OVCAR-3 cell viability. OVCAR-3 cells were treated with 10 nM BPA for 5 days. Before BPA was added for the first time, cells were treated with 5 μM MPP or PHTPP for 30 min. Culture media were changed every 2 days. CCK-8 was used to assess cell viability. *Compared with the control group, $P<0.05$; #Compared with the BPA group, $P<0.05$. $n = 3$

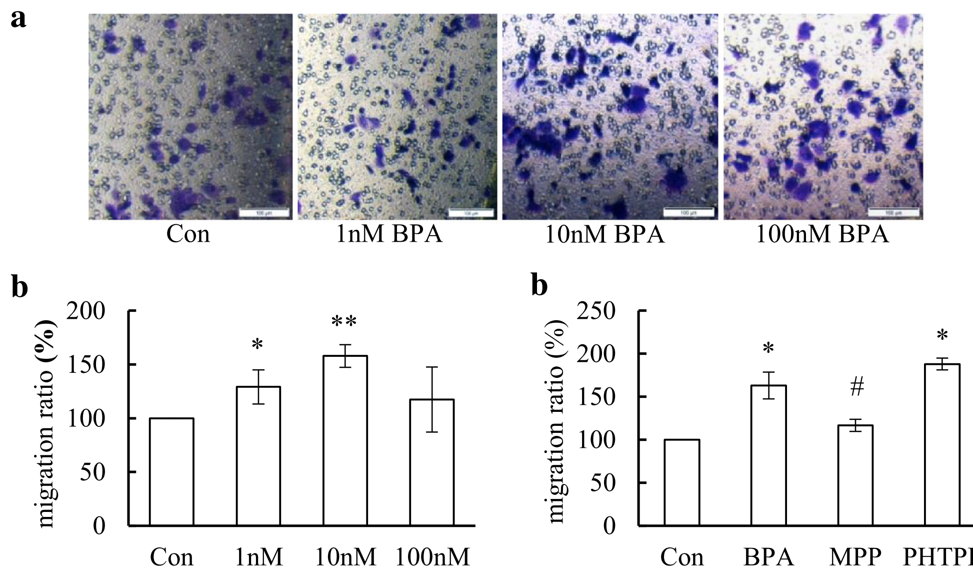

Fig. 3 Effect of BPA on OVCAR-3 cell migration. Cell migration was detected using a Transwell chamber. To the lower chamber, 1, 10, and 100 nM BPA or 5 μM ER inhibitors were added. OVCAR-3 cells were added into the upper chamber. After 24 h, cells were fixed and stained, and the number of migrated cells was counted under a microscope. The increased ratio of migrating cells after treatment with different concentrations of BPA was calculated. **a** The migrated cells were stained with crystal violet. **b** The ratio of migrating cells for groups treated with different concentrations of BPA compared to the control. **c** OVCAR-3 cell migration is affected by ER inhibitors. **P*<0.05 compared with the control group; ***P*<0.01 compared with the control group; #*P*<0.05 compared with the BPA group. *n* = 3. Error bar=100 μm

investigated the effects of BPA on ovarian cancer cell metastasis after 24 h of exposure.

BPA induces migration of OVCAR-3 cells

Metastasis is another important hallmark of cancer cell. Tumor metastasis involves two critical steps, migration and invasion from the primary site to the blood steam, as well as extravasation to the target organs. First, we used a Transwell assay to assess the effects of different concentrations of BPA on ovarian cancer cell migration. As shown in Fig. 3a, migration of OVCAR-3 cells was increased by about 30% (*P*<0.05) and 60% (*P*<0.01) compared to the control group after treatment with 1 and 10 nM BPA, respectively. In contrast, migration was only slightly increased in OVCAR-3 cells treated with 100 nM BPA (Fig. 3a). To determine the role of ERs in BPA-induced migration, OVCAR-3 cells were co-treated with ER inhibitors for 24 h. As shown in Fig. 3b, treatment with an ERα inhibitor (5 μM) significantly reduced BPA-induced cell migration to approach control levels (*P*<0.05). This result is consistent with previous studies showing that BPA can promote migration of MCF-7 CV breast cancer cells (Kim et al. 2017). In contrast, treatment with an ER β inhibitor (5 μM) had no effect on BPA-induced migration (Fig. 3b). Additionally, proliferation of OVCAR-3 cells was not inhibited after treatment with this combination of BPA and ER inhibitors for 24 h (data not shown). Together, these results indicate that low concentrations of BPA induce ovarian cancer cell migration, and this effect is mediated by ERα, not ERβ, signaling. For subsequent experiments to investigate BPA-induced metastasis of ovarian cancer cells, we treated cells with 10 nM BPA, as this concentration showed the most significant effects on cell migration.

BPA promotes invasion of OVCAR-3 cells

We then examined invasion of ovarian cancer cells using a Transwell assay. Matrigel (equivalent to extracellular matrix) was added into the upper chamber. As shown in Fig. 4, the number of OVCAR-3 cells that passed through the Matrigel was approximately 20% higher in the 10 nM BPA-treated group than in the control group (*P*<0.05), indicating that BPA promotes invasion of ovarian cancer cells. Moreover, BPA-induced cell invasion was significantly reduced by co-treatment with the ERα inhibitor MPP (*P*<0.05), while ERβ inhibitor PHTPP showed no inhibitory effects (Fig. 4). Considering that ER inhibitors did not inhibit OVCAR-3 cell proliferation after 24 h of exposure, these results indicate that at low concentrations, BPA promotes ovarian cancer cell invasion primarily through ERα signaling pathways.

BPA increases adhesion of OVCAR-3 cells to vascular endothelial cells

Binding to the vascular endothelium is a key step of cancer cell extravasation to the target organs (Shenoy and Lu, 2016). Therefore, we evaluated the effects of BPA on the adhesion of ovarian cancer cells to endothelial cells. As shown in Fig. 5, 24 h of treatment with BPA at 10 nM significantly increased the adhesion of OVCAR-3 cells to HUVECs by nearly 30%

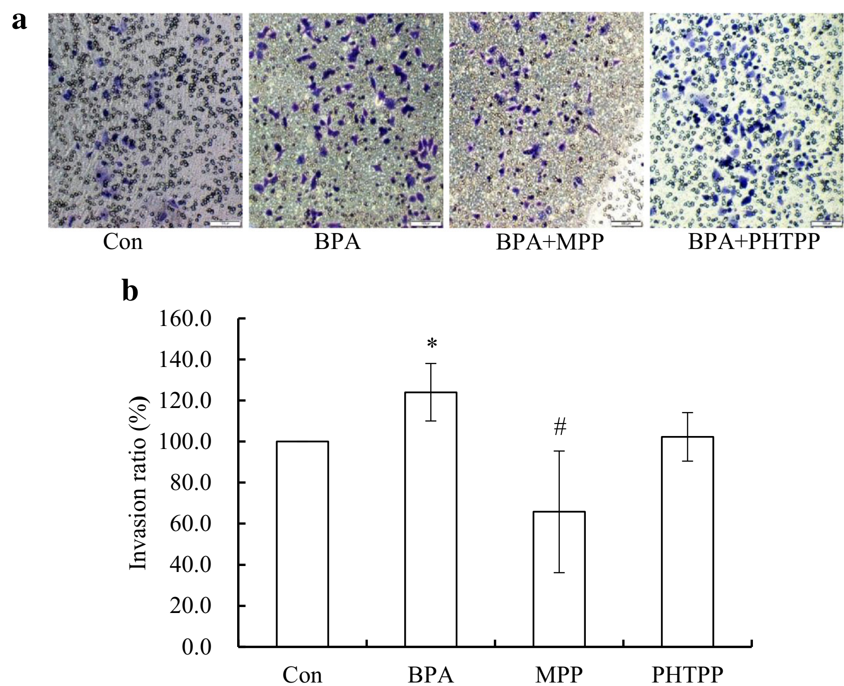

Fig. 4 Effects of BPA and estrogen receptor inhibitors on invasion of OVCAR-3 cells. Cell invasion was detected using a Transwell chamber and Matrigel. To each compartment, 4×10^4 cells were added. After 24 h, the matrix glue of the upper chamber was removed and stained with 0.5% crystal violet. The number of invasive cells was randomly counted, and the average number of invasive cells was calculated. **a** The invaded cells were stained with crystal violet. **b** OVCAR-3 cell invasion is affected by ER inhibitors. *P<0.05 compared with the control group; #P<0.05 compared with the BPA group. n = 3. Error bar=100 μm

compared to the control group (P<0.05). These stimulatory effects of BPA on cell adhesion were reversed by treatment with an ERα inhibitor (Fig. 5). In addition, we also examined the ability of OVCAR-3 cells to adhere to HUVECs pretreated with 10 nM BPA for 24 h. We found no obvious differences between ovarian cancer cell adhesion to HUVECs with and without BPA pretreatment (data not shown). These results suggest that BPA increase the adhesion of ovarian cancer cells to cells in the vascular endothelium via ERα pathways.

Effect of BPA on factors related to migration, invasion, and adhesion

Matrix metalloproteinases MMP-2 and MMP-9 are important factors that regulate tumor metastasis by degrading extracellular matrices and basement membranes (Klein et al. 2004). The effects of BPA on expression levels of MMP-2 and MMP-9 were determined through RT-PCR. As shown in Fig. 6a, expression levels of MMP-2 and MMP-9 were upregulated by 2.2 and 1.2 times, respectively, in OVCRA-3 ovarian cancer cells treated with BPA, compared to the control group. This result was consistent with a previous study in which BPA was found to promote the expression of MMP-2 and MMP-9 in ER-negative breast cancer cells (Zhang et al. 2016). This BPA-induced upregulation of MMP-2 and MMP-9 was reduced by treatment with an ERα inhibitor, suggesting, once again, that ERα pathways play key roles in BPA-mediated ovarian cancer cell migration. BPA might induce expression of MMPs through mitogen-activated protein kinase (MAPK) and phosphatidylinositol 3-kinase (PI3K) pathways (Ptak et al. 2014) or through the IKK- β/NF-κ B signal pathway (Ma et al. 2015). Additionally, we examined expression of epithelial mesenchymal transformation (EMT)-related factors that are crucial for cancer cell metastasis (Kalluri and Weinberg, 2009). No significant differences were observed in the expression of E-Cadherin or Vimentin in BPA-treated OVCAR-3 cells compared to the control (Fig. 6b), consistent with previous studies (Ptak et al. 2014; Zhang et al. 2016).

Cell adhesion molecules (CAMs) play important roles in the metastasis of multiple cancers (Makrilia et al. 2009). Intracellular cell adhesion molecule-1 (ICAM-1) mediates the adhesion of cancer cells to vascular endothelial cells

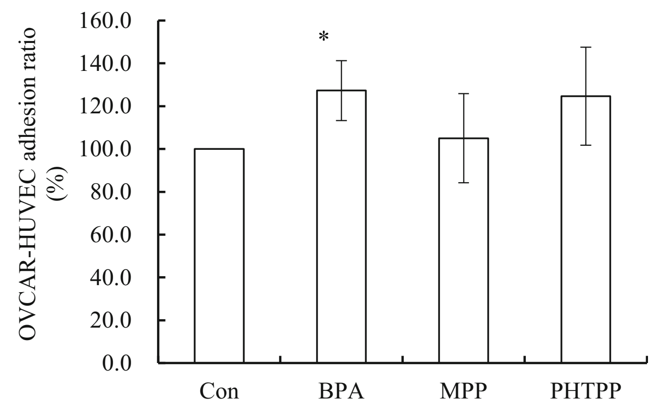

Fig. 5 Effects of BPA and estrogen receptor inhibitors on the adhesion of OVCAR-3 cells to vascular endothelial cells. OVCAR-3 cells were treated with 10 nM BPA for 24 h, then co-incubated with HUVECs for 2 h. The number of HUVECs adhering to OVCRA-3 cells was counted under a microscope (the control group was set at 100%). *P<0.05 compared with the control group. n = 3

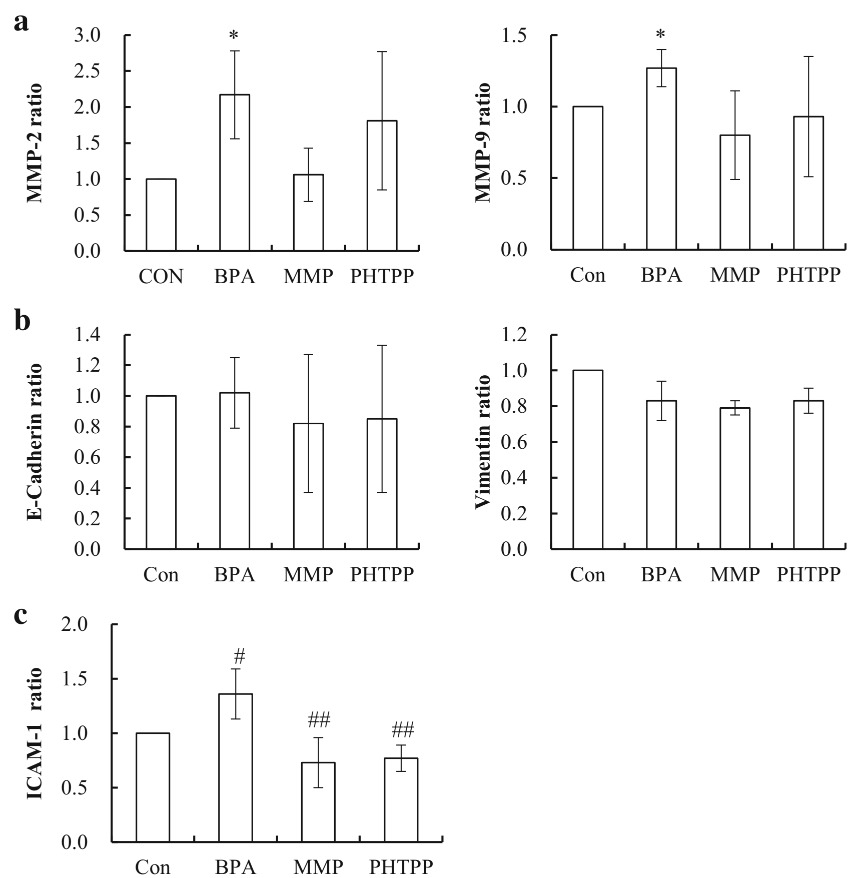

Fig. 6 Effects of BPA and ER inhibitors on the expression of genes related to cell adhesion, migration, and invasion. OVCAR-3 cells were co-treated with 10 nM BPA and ER inhibitors for 24 h, and RNA was extracted from collected cells. The mRNA expression levels of related factors were detected by RT-PCR, and GAPDH was used as the internal reference gene. *$P<0.05$ compared with the control group; #$P=0.05$ compared with the control group; ##$P<0.05$ compared with the BPA group. $n = 3$

(Huang et al. 2016). Expression of ICAM-1 was also upregulated in BPA-treated ovarian cancer cells compared to the control group ($P=0.05$, Fig. 6c). The ER inhibitors MPP and PHTPP significantly reduced this BPA-induced upregulation of ICAM-1 (Fig. 6c). Combined with our findings regarding adhesive ability, our data suggest that BPA might promote adhesion of OVCAR-3 cells to vascular endothelial cells by promoting expression of ICAM-1 through ER signaling pathways.

Discussion

BPA is one of the most commonly used endocrine disrupting chemicals (EDCs) and has been suggested to increase the risk of female hormone-dependent cancers, especially breast and endometrial cancers (Rutkowska et al. 2016; Scsukova et al. 2016). Ovarian cancer is a reproductive system tumor that seriously threatens women's health (Runnebaum and Stickeler, 2001). Although previous studies have reported the effects of BPA on cancer development, little research has been conducted specifically regarding its effects on ovarian cancer, a highly lethal cancer in women. In the current study, we attempt to investigate the potential effects of BPA on proliferation and metastasis of ovarian cancer cells. Our results revealed that BPA stimulates proliferation and induces migration, invasion, and adhesion of ovarian cancer cells through estrogen signaling pathways.

Cellular responses to BPA are largely dependent on the exposure concentration (Castillo Sanchez et al. 2016). High-concentration exposure to BPA usually leads to cytotoxicity. For example, treatment with BPA at 50-200 μM could inhibit the proliferation of colonic epithelium and prostate cancer cells (Bilancio et al. 2017; Qu et al. 2018). In contrast, low-concentration exposure to BPA can stimulate cell proliferation and enhance cell migration (Ptak et al. 2014; Song et al. 2015). Considering the low concentrations (i.e., nanomole levels) of BPA in human tissues (Dekant and Völkel, 2008), it is necessary to pay more attention to the effects of low-concentration BPA on cancer development. Proliferation is an important hallmark of cancer cells. Our previous work showed that exposure to BPA for 24 h at low, environmentally relevant concentrations (1–100 nM) stimulates ovarian cancer cell proliferation (Shi et al. 2017). In this study, we found that, at the same concentrations as we used in our previous work, BPA exposure for 5 days could induce OVCAR-3 cell proliferation. This finding indicates that long-term exposure to low concentrations of BPA could result in ovarian cancer development by stimulating cancer cell proliferation. BPA increased estrogen response element (ERE) activity through ER, implicated that

BPA induced proliferation related to a genomic effect through ERs and ERE (Park et al. 2009), furthermore, BPA upregulated ERα and IGF-1R mRNA, the activation of IRS-1 and Akt was induced in BG-1 ovarian cancer cells, indicated that ER and IGF-1R signals crosstalk played a significant role in BPA induced ovarian cancer growth (Hwang et al. 2013).

Invasion and metastasis are also important hallmarks of cancer cells and are a major cause of cancer mortality (Hanahan and Weinberg Robert, 2011). Ovarian cancer has a high mortality rate and is often diagnosed at an advanced stage with extensive metastasis (Liang et al. 2018). Thus, in this study, we investigated the roles of BPA in the invasion and metastasis of ovarian cancer cells. We found that exposure to low concentrations of BPA could induce cell migration and invasion of OVCAR-3 cells, especially at 10 nM (Figs. 3 and 4). MMP-2 and MMP-9 have been shown to be upregulated and activated in ovarian cancer and are associated with the migration and invasion of cancer cells (Rasool et al. 2016; Sun et al. 2016). A recent study by Ptak and colleagues demonstrated that BPA upregulates MMP-2 and MMP-9 in OVCAR-3 human ovarian cancer cells (Ptak et al. 2014). Consistent with this finding, we observed that the stimulatory effects of BPA on cell migration were associated with upregulation of the migration-related molecules MMP-2 and MMP-9 (Fig. 6). Moreover, the epithelial-mesenchymal transition (EMT) plays a crucial role in cancer cell metastasis, and decreased expression of epithelial E-cadherin is considered a typical characteristic of EMT (Kalluri and Weinberg, 2009). BPA has been documented to reduce E-cadherin expression and increase vimentin expression, leading to EMT progress in BG-1 ovarian cancer cells (Kim et al. 2015). Nevertheless, we did not find remarkable changes in the expression of E-cadherin or vimentin in this study (Fig. 6). This discrepancy may result from the use of a different ovarian cancer cell line (epithelial ovarian cancer) than in previous studies (ovarian adenocarcinoma cells). Overall, our findings suggest that BPA might stimulate ovarian cancer cell migration through influencing expression levels of MMP-2 and MMP-9. Otherwise, the increased proliferation and migration in OVCAR-3 cells was induced by 10 nM BPA in previous studies and this study (Ptak et al. 2014; Shi et al. 2017), the increased proliferation and migration was observed in BG-1 ovarian cancer cells exposed to 10 μM BPA (Park et al. 2009; Hwang et al. 2011; Kim et al. 2015), these results implicated that different Ovarian cancer cells may show distinct effects according to the BPA concentration, the further study should pay attention on the tumor cell heterogeneity and the relation with BPA.

The adhesion of cancer cells to endothelial cells is a key step in the process of cancer metastasis (Tang and Honn, 1994). CAMs, such as ICAM-1 and vascular endothelial cell adhesion molecule-1 (VCAM-1), have been implicated to play a major role in the cellular interactions underlying the progression and metastasis of multiple cancers (Rokhlin and Cohen, 1996). In ovarian cancer patients, serum levels of soluble ICAM-1 and VCAM-1 are higher than in normal healthy subjects (Banks et al. 1993). However, the effects of BPA on the adhesion of ovarian cancer cells to endothelial cells are still unclear. In this study, we observed a significant increase in expression of ICAM-1 in OVCAR-3 ovarian cancer cells, associated with increased adhesion to vascular endothelial cells (Figs. 5 and 6). A previous study reported that BPA upregulates VCAM-1 expression in HUVECs (Fang et al. 2014).

BPA has been considered to have an estrogenic effect and is demonstrated to bind nuclear ERs (Safe et al. 2001). To investigate whether the roles of BPA in ovarian cancer development are mediated by ER-dependent pathways, we used ER inhibitors (MPP for ERα and PHTPP for ERβ) to pretreat ovarian cancer cells. Consistent with the results of our previous study, proliferation of OVCAR-3 cells was significantly reduced after pretreatment with an ERα inhibitor (Fig. 2). Strikingly, the stimulatory effects of BPA on migration, invasion, and adhesion to endothelial cells were reduced in ERα inhibitor-pretreated ovarian cancer cells (Figs. 3, 4, 5). Intriguingly, upregulation of MPP-2, MPP-9, and ICAM-1 was reversed after pre-treatment with the ERα inhibitor (Fig. 6). Although the affinity of BPA for ERβ is 10-fold higher than for ERα (Wang et al. 2017), treatment with the ERβ inhibitor only slightly reduced the stimulatory effects of BPA on proliferation, invasion, and metastasis of ovarian cancer cells. Nevertheless, the ERβ inhibitor could also reduce, to a certain extent, expression of MMP-9 and ICAM-1. Together, these results indicate that BPA likely promotes ovarian cancer development and progression predominantly through ERα-dependent pathways. However, BPA has been reported to bind other estrogen receptors, including GPER (e.g., G-protein coupled receptor 30, GPR30) and ER-related receptor γ (ERR-γ), to stimulate proliferation, migration, and invasion of breast cancer cells (Dong et al. 2011; Castillo Sanchez et al. 2016; Zhang et al. 2016). Studies have also shown that BPA may bind and activate non-estrogen receptors, such as integrin β1, inducing EMT and migration of ER-negative breast cancer cells (Jia et al. 2019).

Conclusions

This study investigated the potential effects of BPA on ovarian cancer development and progression. We found that long-term exposure to low concentrations of BPA stimulated proliferation of OVCAR-3 cells. Our work also highlights the important roles that BPA plays in ovarian cancer cell metastasis through regulating expression of migration- and adhesion-related factors such as MMP-2, MMP-9, and IMAC-1. The mechanisms responsible for BPA-induced

ovarian cancer proliferation and metastasis primarily involve ERα signaling pathways. This study will assist the field in better understanding the carcinogenic effects of BPA with respect to ovarian cancer.

Author contribution Z.W., H.G., and S.J.L. designed this study. C.S. and Y.S. carried out the main experiments and analyzed the data. T.-W.J., S.Z., and L.Y.F. collected the results of the migration and invasion assay. Y.Z. and X.X.Z. measured the proliferation of cells. C.S., Y.S., Z.W., H.G., and S.J.L. co-wrote the manuscript. All the authors read and approved the final manuscript.

Funding The work in this paper was supported by the open fund of the State Key Laboratory for Environmental Chemistry and Ecotoxicology (grant no. KF2012-4) and grants from the National Natural Science Foundation of China (grant no: 21407172, 21577095).

Data availability All data generated or analyzed during this study are included in this published article.

Declarations

Ethics approval, consent to participate, and consent for publication Not applicable

Conflict of interest The authors declare that they have no conflict of interest.

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