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Cell Growth of BG-1 Ovarian Cancer Cells was Promoted by 4-Tert-octylphenol and 4-Nonylphenol via Downregulation of TGF-β Receptor 2 and Upregulation of *c-myc*

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Transforming growth factor β (TGF-β) is involved in cellular processes including growth, differentiation, apoptosis, migration, and homeostasis. Generally, TGF-β is the inhibitor of cell cycle progression and plays a role in enhancing the antagonistic effects of many growth factors. Unlike the antiproliferative effect of TGF-β, E2, an endogeneous estrogen, is stimulating cell proliferation in the estrogen-dependent organs, which are mediated via the estrogen receptors, ERα and ERβ, and may be considered as a critical risk factor in tumorigenesis of hormone-responsive cancers. Previous researches reported the cross-talk between estrogen/ERα and TGF-β pathway. Especially, based on the E2-mediated inhibition of TGF-β signaling, we examined the inhibition effect of 4-*tert*-octylphenol (OP) and 4-nonylphenol (NP), which are well known xenoestrogens in endocrine disrupting chemicals (EDCs), on TGF-β signaling via semi-quantitative reverse-transcription PCR. The treatment of E2, OP, or NP resulted in the downregulation of TGF-β receptor2 (TGF-β R2) in TGF-β signaling pathway. However, the expression level of TGF-β1 and TGF-β receptor1 (TGF-β R1) genes was not altered. On the other hand, E2, OP, or NP upregulated the expression of a cell-cycle regulating gene, *c-myc*, which is a oncogene and a downstream target gene of TGF-β signaling pathway. As a result of downregulation of TGF-β R2 and the upregulation of *c-myc*, E2, OP, or NP increased cell proliferation of BG-1 ovarian cancer cells. Taken together, these results suggest that E2 and these two EDCs may mediate cancer cell proliferation by inhibiting TGF-β signaling via the downregulation of TGF-β R2 and the upregulation of *c-myc* oncogene. In addition, it can be inferred that these EDCs have the possibility of tumorigenesis in estrogen-responsive organs by certainly representing estrogenic effect in inhibiting TGF-β signaling.

***Key words*:** Endocrine disrupting chemicals, Estrogen, OP, NP, TGF-β1, *c-myc*, Ovarian cancer cells

INTRODUCTION

Transforming growth factor β (TGF-β) is one of the TGF-β superfamily which contains over 30 different members such as activins/inhibins, bone morphogenetic proteins (BMPs), and others (Matsuda *et al.*, 2001). These factors are involved in cellular processes, including proliferation, differentiation, apoptosis, migration, and other cellular homeostasis (Massague, 2008). TGF-β acts as an antiproliferative factor in normal cells and at early stages of oncogenesis by inducing cell cycle arrest and apoptosis (Hill *et al.*, 2009; Yilmaz *et al.*, 2011). In ovarian cancer, TGF-β1 and activin A decreased cancer cell motility and proliferation. Blocking TGF-β1 and activin signaling resulted in increased proliferation and cell saturation density (Theriault and Nachtigal, 2011). Therefore, TGF-β is regarded as an inhibitor of cell cycle progression, a stimulator of apoptosis, and an enhancer in the antagonistic effects of many growth factors. TGF-β signaling begins with the binding to TGF-β receptor2 (TGF-β R2) which is serine/threonine receptor kinase. On binding TGF-β, TGF-β R2 heterodimerizes with TGF-β receptor1 (TGF-β R1) and then phosphorylates it. Phosphorylated TGF-β R1 recruits and phosphorylates the receptor-activated Smad (R-Smad) transcription factors, Smad2 and Smad3 (Feng and Derynck, 2005; Massague *et al.*, 2005). Activated R-Smads then form complex with the

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co-Smad, Smad4, and then this complex enters the cell nucleus where it acts as a transcription factor for various genes, including *c-myc*, $p21^{CIP1}$, $p27^{Kip1}$, and $p15^{INK4B}$ genes, which are important for cell cycle regulation (Dunfield and Nachtigal, 2003) and for other genes to activate the mitogen-activated protein kinase 8 pathway, which triggers apoptosis (Schuster and Krieglstein, 2002).

Unlike the antiproliferational activity of TGF-β/Smad signaling, estrogen/estrogen receptors (ERs) signaling is typically involved in cell proliferation. An endogenous estrogen, 17-β estradiol (E2), is a primary female sex hormone, which regulates cellular growth, proliferation, and differentiation, and is an important risk factor for the development of estrogen-dependant cancers such as breast, ovarian, and endometrial cancers (Shanle and Xu, 2011). The actions of estrogen are mediated by two types of ERs, ERα and ERβ, which are the nuclear hormone receptors and ligand-activated transcription factors (Choi *et al.*, 2004; Hwang *et al.*, 2011). Among these two receptors, ERα is known to be a primary estrogen receptor for cell proliferation and differentiation and one of the most known oncogenes in estrogen-dependant cancers (Khan *et al.*, 1998; Regan *et al.*, 2006). When activated by estrogen, ERα, as a transcription factor, can induce the expression of cyclin D and *c-myc* to promote cell cycle in its genomic action and can activate multiple pathways such as the ERK and AKT pathways to induce mitogenesis in its non-genomic action (Doisneau-Sixou *et al.*, 2003; Pedram *et al.*, 2006).

Previous studies reported that estrogen/ERs signaling can regulate TGF-β/Smad signaling. In MCF-7 breast cancer cells, TGF-β-mediated transcriptional activity was inhibited by estrogen (Goto *et al.*, 2011; Ito *et al.*, 2010). Estrogen treatment inhibited the activity of Smads by reducing the phosphorylation of Smad2 and Smad3 (Band and Laiho, 2011; Ito *et al.*, 2010). ERα suppressed Smad3-dependent transcription by binding to Smad3 and estrogen inhibited TGF-β signaling by promoting Smad2/3 ubiquitination and subsequent degradation (Ito *et al.*, 2010; Matsuda *et al.*, 2001). These observations suggest that the inhibition of TGF-β signaling by estrogen/ERs may contribute to estrogen-mediated cell proliferation.

In this study, we examined the effect of endocrine disrupting chemicals (EDCs) and E2 on TGF-β signaling. EDCs are exogenous chemicals that mimic or inhibit the action of estrogen or other hormones (Choi and Jeung, 2003). They are discharged from numerous industrial products as plastics, pesticides, drugs, detergents, and cosmetics (Choi *et al.*, 2004; Hwang *et al.*, 2011). Among diverse EDCs, xenoestrogens, which are estrogen mimics with chemical structures similar to that of E2, display agonistic and antagonistic effects toward ERs and bring about interfering with the actions of estrogen in endocrine system (Park *et al.*, 2011; Park *et al.*, 2009). Therefore, we hypothesized that some xenoestrogens could have an effect on TGF-β signaling via ERα like E2 in ER positive cancer cell line. Here, we examined whether 4-*tert*-octylphenol (OP) and 4-nonylphenol (NP), well known EDCs, possibly inhibited TGF-β signaling by comparison with E2 in BG-1 ovarian cancer cell line which is a highly E2-responsive cancer cell line by expressing ERα and is estimated to be the best *in vitro* model to detect an estrogenic effect of EDCs (Hwang *et al.*, 2011). To evaluate TGF-β signaling modulation by E2, OP, and NP, we tested their effects on the gene expression of TGF-β, TGF-β receptors and *c-myc*, which is a downstream target gene of TGF-β. In addition, the cancer cell proliferation activity of E2 and two EDCs resulting from their estrogenic activities via inhibition of TGF-β signaling pathway was also identified.

MATERIALS AND METHODS

Cell culture and media. BG-1 human ovarian cancer cells were obtained from Dr. K. S. Korach (National Institute of Environmental Health Sciences, NIH, 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), 100 U/m*l* penicillin G and 100 mg/m*l* streptomycin (Life Technologies, Rockville, MD, USA) at 37°C in a humidified atmosphere of 5% CO_2 containing air. To prevent the effects of the estrogenic components of DMEM and FBS, phenol red-free DMEM supplemented with 5% charcoal-dextran treated FBS was used to detect the estrogenicity of EDCs in BG-1 cells.

Cell proliferation assay. Cell growth was demonstrated by MTT assay as previously demonstrated (Hwang *et al.*, 2011). BG-1 cells were plated at 4,000 cells per well of 96-well plates in 0.1 μ*l* of phenol red-free DMEM supplemented with 5% charcoal-dextran treated FBS medium. After incubation for 48 h, the cells were washed and treated with E2 (Sigma-Aldrich Corp., St. Louis, MO, USA), OP (Sigma-Aldrich Corp.), and NP (Sigma-Aldrich Corp.) at various concentrations in the medium as described above for 5 days. Dimethyl sulfoxide (DMSO; 0.1%) in the same medium was used as a vehicle. Following treatments, the cells were then treated with 10 μ*l* of MTT solution (5 mg/m*l*) and incubated at 37°C for 4 h. MTT-containing medium was removed and the precipitants were solubilized in DMSO (100 μ*l*). An absorbance was measured at 540 nm using an ELISA reader (VERSA man; Molecular Devices, CA, USA).

Total RNA extraction. BG-1 cells were cultured at 3×10^5 cells per well of 6-well plates and then E2, OP, NP, and DMSO were treated. Total RNA was extracted at various time points (0, 6 and 24 hr) using TriZol reagents (Invitro-

Table 1. Primer sequences and product sizes of PCR products for semi-quantitative reverse-transcription PCR

Target gene	Sequences	Product size
TGF-β1	Sense: 5'-TCCGCAAGGACCTCGGCTGGA-3'	244 bp
	Antisense: 5'-ATCATGTTGGACAGCTGCTCC-3'	
TGF-β R1	Sense: 5'-AAATTGCTCGACGATGTTCC-3'	309 bp
	Antisense: 5'-GGAGAGTTCAGGCAAAGCTG-3'	
TGF-β R2	Sense: 5'-CGCTTTGCTGAGGTCTATAAGGC-3'	395 bp
	Antisense: 5'-GATATTGGAGCTCTTGAGGTCCCT-3'	
c-myc	Sense: 5'-GAATGTGGCTATGGCTGGTG-3'	237 bp
	Antisense: 5'-ACGGGGCCATGCTTTATTAT-3'	
GAPDH	Sense: 5'-ATGTTCGTCATGGGTGTGAACCA-3'	351 bp
	Antisense: 5'-TGGCAGGTTTTTCTAGACGGCAG-3'	

gen Life Technologies, Carlsbad, CA, USA) according to the manufacturer's instructions. The concentration of total RNAs was measured by a spectrophotometer (Optizen, Mecasys, Dea-jeon, Korea) at 260 nm/280 nm. One microgram of total RNA was dissolved in dietyl pyrocarbonate - deionzed water for cDNA synthesis.

Semi-quantitative reverse-transcription PCR. To synthesis cDNAs from total RNAs for reverse transcription PCR, the reaction mixture was consisted with murine leukemia virus reverse transcriptase (M-MLV RT; iNtRON Biotechnology, Sungnam, Kyeonggido, Korea), 200 pM nonamer random primer (iNtRON Biotechnology), dNTPs (iNtRON Biotechnology), RNase inhibitor (iNtRON Biotechnology) and RT buffer (iNtRON Biotechnology). The cDNA synthesis was performed at 37°C for 1 h and 95°C for 5 min. TGF-β1, TGF-β receptor 1, TGF-β receptor 2, *c-myc* and GAPDH mRNAs were amplified by using each forward and reverse primer, Taq polymerase, PCR buffer, dNTP mixture and each cDNA template via PCR process as previously done (Yi *et al.*, 2011). The each forward and reverse primer and the expected size of RT-PCR products were shown in Table 1 (Gantus *et al.*, 2011; Kim *et al.*, 2011; McCaffrey *et al.*, 1995; Osada *et al.*, 2011). PCR products were run on a 1.5% agarose gel and the bands were compared to 100-bp ladders. The gels were scanned and the density of the bands on the gel was quantified using Gel Doc 2000 (Bio-Rad Laboratories, Inc., Hercules, CA, USA).

Data analysis. Data were shown as the mean ± standard deviation (S.D.). A statistical analysis was performed by Student's *t*-test, two-pair comparisons. $P < 0.05$ was considered statistically significant.

RESULTS

Effects of cell proliferation by OP and NP on BG-1 cells. To evaluate the effects of cell proliferation, BG-1 cells were cultured with treatment vehicle (DMSO, 0.1%),

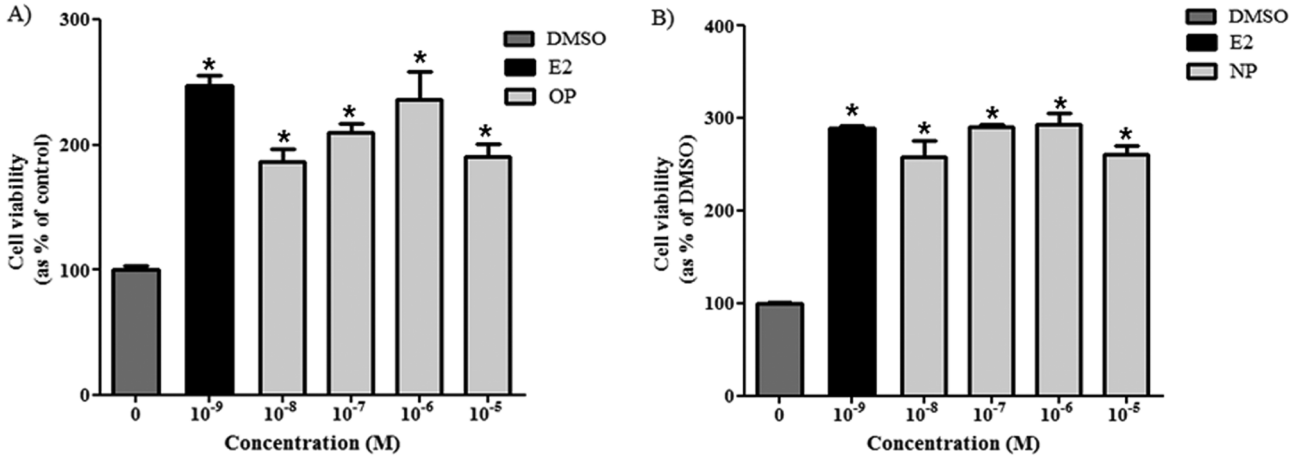

Fig. 1. EDCs-induced cell growth following treatments with E2, OP or NP in BG-1 cells. Cells were treated with DMSO as a vehicle, E2 (10^{-9} M), OP (10^{-8} to 10^{-5} M) or NP (10^{-8} to 10^{-5} M) for five days and viable cells were measured using MTT assay at 540 nm. (**A**) Cell proliferation effects by treatment with E2 or OP. (**B**) Cell proliferation effects by treatment with E2 or NP. Data represent the means ± S.D. of triplicate experiments. *, $p < 0.05$ compared to a vehicle treated with DMSO.

E_2 (1×10^{-9} M), OP, or NP (1×10^{-5} to 1×10^{-8} M) for 5 days. The results indicated that E2 as a positive control markedly increased the BG-1 cell proliferation in comparison with DMSO as shown in Fig. 1A and 1B ($p < 0.05$). OP and NP also considerably increased the proliferation of BG-1 cells compared to DMSO (Fig. 1A and B; $p < 0.05$). Particular, both OP and NP showed a potent cell proliferation activity at 1×10^{-6} M.

TGF-β1 gene expression by OP and NP. To evaluate the effect on the expression level of genes related with TGF-β pathway, we treated the BG-1 cells with both OP, or NP at 1×10^{-6} M at which the cell growth showed maximum proliferation. In semi-quantitative RT-PCR experiment, the gene expression of TGF-β1 was not significantly changed by the treatments of E2, OP, and NP compared to a vehicle (DMSO) at all time points as shown in Fig. 2 ($p < 0.05$).

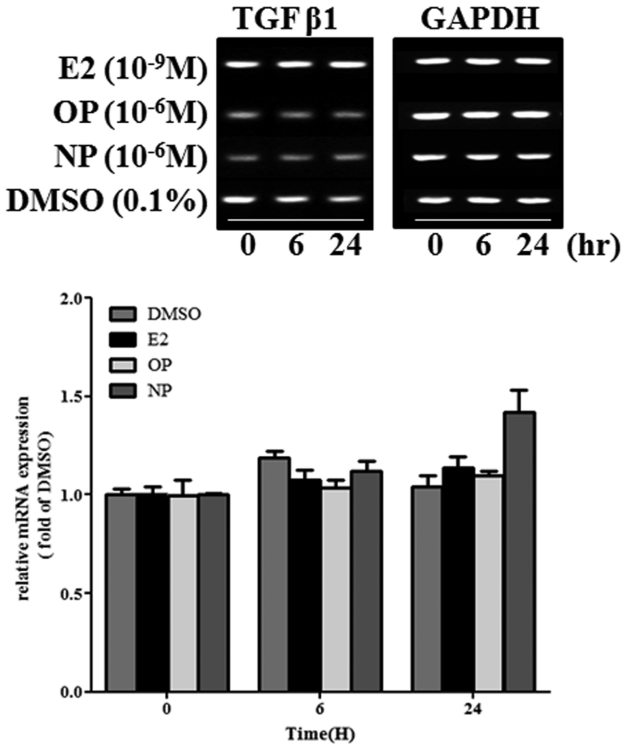

Fig. 2. Altered expression levels of TGF-β1 gene following treatments with E2, OP or NP. BG-1 cells were seeded in 6-well plates and treated with E2 (10^{-9} M), OP (10^{-6} M) or NP (10^{-6} M). Total RNAs were extracted in a time-dependent manner (0, 6, and 24 h). Expression level of TGF-β1 was detected by using semi-quantitative reverse-transcription PCR. PCR products were run on a 1.5% agarose gel, bands were scanned and the density of the bands on the gel was quantified using Gel Doc 2000 as described in Materials and Methods. Data represent the means ± S.D. of triplicate experiments. *, $p < 0.05$ compared to a vehicle treated with DMSO.

TGF-β receptor 1 and 2 gene expressions by OP and NP. In parallel with no alteration in the expression level of TGF-β1 by E2, OP, and NP, the expression of TGF-β R1 gene was also not changed at all time points (Fig. 3A). However, the expression of TGF-β R2 gene was significantly decreased by the treatments of E2, NP, and OP compared with a vehicle (DMSO) after 24 h treatment as seen in Fig. 3B ($p<0.05$). In particular, the gene expression of TGF-β R2 was similarly inhibited by NP or E2 treatment at 6 h (Fig. 3B; $p < 0.05$).

c-myc expression by OP and NP. The expression level of *c-myc* was further examined following treatment with E2, OP, and NP. The expression of *c-myc* was significantly enhanced by E2 compared to DMSO treatment for 6 h and 24 h as demonstrated in Fig. 4 ($p < 0.05$). In addition, its expression was also significantly enhanced by OP or NP treatment for 6 h and 24 h in BG-1 ovarian cancer cells (Fig. 4).

DISCUSSION

While estrogen is required for normal reproductive development, cumulative exposure to estrogen is a high risk factor in tumorigenesis of the estrogen-dependent organs such as breast, endometrium, and ovary (Park *et al.*, 2011; Watanabe *et al.*, 2007).

EDCs having similar structures to E2 may show estrogenic activity and disrupt a general estrogen signaling, which is mediated via two ERs (Diamanti-Kandarakis *et al.*, 2009), leading to the increase of human health risk by interfering with hormone balance in endocrine system of body organs (Choi *et al.*, 2004). Furthermore, the cellular mutation caused by steady exposure to EDCs may also increase the risk of cancer (Hwang *et al.*, 2011). OP and NP are alkyphenols and are known as typical EDCs. Previous studies reported that they directly interacted with ERs and caused estrogenic responses (Asimakopoulos *et al.*, 2011; Hagiwara *et al.*, 2008). In MCF-7 breast cancer cells, the expression of estrogen-responsive genes highly increased when treated with treated with E2, NP or OP (Terasaka *et al.*, 2006). Also, NP and OP induced ERE promoter activity in ovarian cancer (Kochukov *et al.*, 2009; Shanle and Xu, 2011). In our previous study, we also showed that treatment of the BG-1 cells with bis-phenol A (BPA), a classical alkylphenol, obviously increased the cell proliferation as E2 did and changed the expression level of cell cycle regulating genes such as cyclin D, cdk-4 and p21 (Hwang *et al.*, 2011; Park *et al.*, 2011).

In the present study, we determined the estrogenic effect of two EDCs, NP and OP, by investigating their effect on TGF-β signaling. Although TGF-β has paradoxical aspects in tumorigenesis such as prevention of early stage tumor growth and promotion of growth and invasion of cancer in

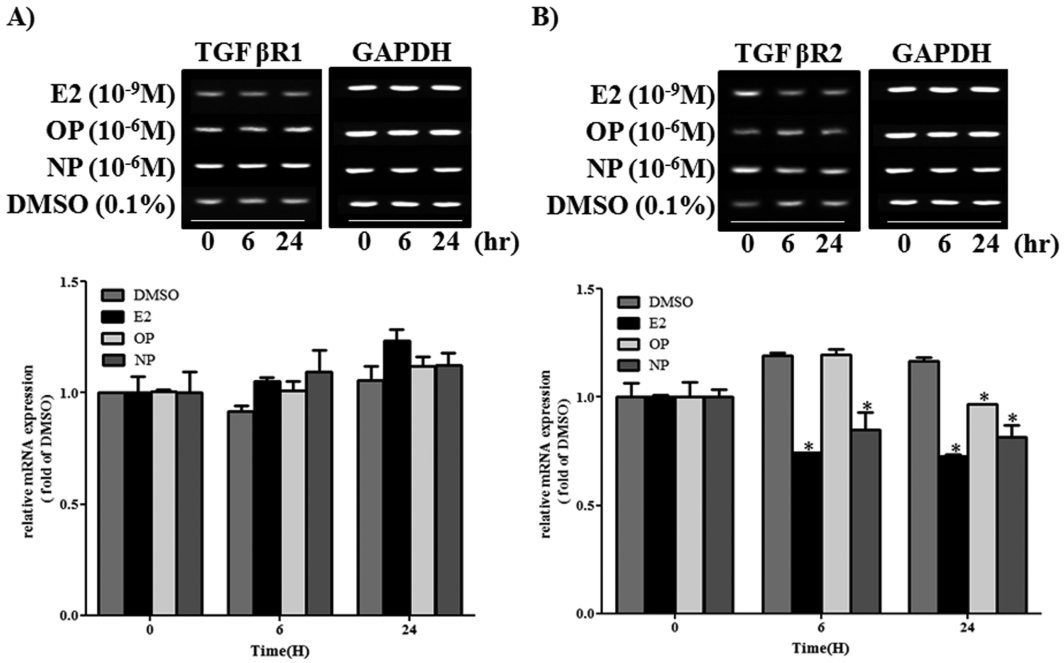

Fig. 3. Altered expression levels of TGF-βR1 gene and TGF-βR2 gene following treatments with E2, OP or NP. BG-1 cells were seeded in 6-well plates and treated with E2 (10^{-9} M), OP (10^{-6} M) or NP (10^{-6} M). Total RNAs were extracted in a time-dependent manner (0, 6, and 24 h). (**A**) Expression levels of TGF-βR1 by treatment with E2, OP or NP. (**B**) Expression levels of TGF-βR2 by treatment with E2, OP or NP. Expression level of TGF-βR1 and TGF-βR2 was detected by using semi-quantitative reverse-transcription PCR. Data represent the means ± S.D. of triplicate experiments. *, $p < 0.05$ compared to a vehicle treated with DMSO.

late stage (Elliott and Blobe, 2005; Nilsson and Skinner, 2002), it is generally known to inhibit cell cycle progression and to enhance the antagonistic effects of many growth factors. Recent studies showed that estrogen/ERs signaling can regulate TGF-β/Smad signaling in various steps, leading to restoration of cell cyle progression and cell proliferation which were suppressed by TGF-β/Smad signaling. In this study, we examined the effect of NP, OP, and E2 on the BG-1 cell proliferation and the inhibition of TGF-β signaling. As a result, NP and OP significantly increased cell proliferation like E2. The expression of TGF-β1 gene or TGF-β R1 gene is not changed by E2, OP, and NP in all time points. But, the expression of TGF-β R2 gene significantly decreased by E2 and NP at 6 h, and OP at 24 h. TGF-β R2 is a first receptor to bind TGF-β dimers and then to recruit and phosphorylate TGF-β R1.

In addition, we further investigated the expression level of *c-myc* gene, whch is a TGF-β1 target gene and a regulator gene that codes for a transcription factor. In general, TGF-β causes cell cycle arrest by reducing the expression level of *c-myc* and by inhibiting cyclin dependent kinase (Cdk) activities (Chen *et al.*, 2002; Gomis *et al.*, 2006). The increased expression of *c-myc* gene leads to the upregulation of many genes involved in cell proliferation and the cancer formation. As expected from cell proliferation activity for BG-1 cells, E2, OP and NP significantly increased the expression of *c-myc* gene. From these results, we also confirmed that E2 inhibits TGF-β signaling in estrogen-dependant BG-1 ovarian cancer cell line. Unlike other studies in which E2 mainly inhibits TGF-β signaling by suppressing the activity of Smads, the transcription factors in the downstream pathway of TGF-β, we identified that E2 inhibits TGF-β signaling by reducing the expression of TGF-β R2, not TGF-β R1. Furthermore, we demonstrated the E2-mediated inhibition of TGF-β signaling by identifying the increased expression of *c-myc*, a downstream target gene repressed by TGF-β signaling, and the BG-1 cell proliferation by E2. These results were shared by the treatment OP and NP, well known xenoestrogens. In other words, these EDCs certainly represent estrogenic effect in associated with TGF-β pathway and they have the possibility of tumor growth in ovarian cancer via the increase of the expression of *c-myc* as a result of inhibiting TGF-β signaling. However, the interfering mechanism of EDCs on TGF-β pathway and their effects are not certain for now.

In conclusion, these results suggest that these EDCs and E2 may mediate cancer cell proliferation by inhibiting TGF-β signaling via the downregulation of TGF-β R2 and by the upregulation of *c-myc* oncogene. In addition, it can be inferred that these EDCs have the possibility of tumorigenesis in estrogen-responsive organs by certainly representing estrogenic effect in inhibiting TGF-β signaling. A further study is required to verify more profound mechanism for disturbance of TGF-β signaling by EDCs as well

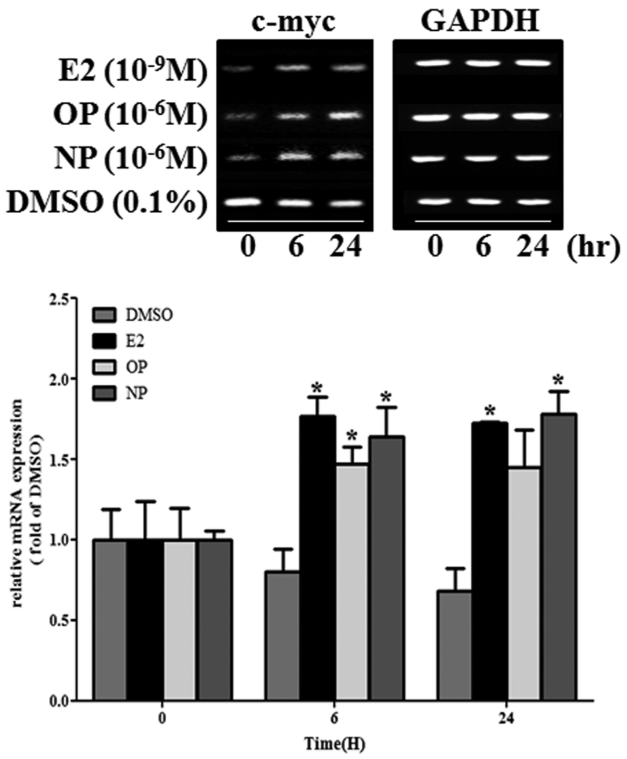

Fig. 4. Altered expression levels of *c-myc* gene following treatments with E2, OP or NP. BG-1 cells were seeded in 6-well plates and treated E2 (10^{-9} M), OP (10^{-6} M) or NP (10^{-6} M). Total RNAs were extracted in a time-dependent manner (0, 6, and 24 h). Expression level of *c-myc* was detected by using semi-quantitative reverse-transcription PCR. Data represent the means ± S.D. of triplicate experiments. *, $p < 0.05$ compared to a vehicle treated with DMSO.

as estrogen.

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REFERENCES

Asimakopoulos, A.G., Thomaidis, N.S. and Koupparis, M.A. (2011). Recent trends in biomonitoring of bisphenol A, 4-t-octylphenol, and 4-nonylphenol. *Toxicol. Lett*. In press.

Band, A.M. and Laiho, M. (2011). Crosstalk of TGF-beta and estrogen receptor signaling in breast cancer. *J. Mammary Gland Biol. Neoplasia*, **16**, 109-115.

Chen, C.R., Kang, Y., Siegel, P.M. and Massague, J. (2002). E2F4/5 and p107 as Smad cofactors linking the TGFbeta receptor to c-myc repression. *Cell*, **110**, 19-32.

Choi, K.C. and Jeung, E.B. (2003). The biomarker and endocrine disruptors in mammals. *J. Reprod. Dev.*, **49**, 337-345.

Choi, S.M., Yoo, S.D. and Lee, B.M. (2004). Toxicological characteristics of endocrine-disrupting chemicals: developmental toxicity, carcinogenicity, and mutagenicity. *J. Toxicol. Environ. Health B Crit. Rev.*, **7**, 1-24.

Diamanti-Kandarakis, E., Bourguignon, J.P., Giudice, L.C., Hauser, R., Prins, G.S., Soto, A.M., Zoeller, R.T. and Gore, A.C. (2009). Endocrine-disrupting chemicals: an Endocrine Society scientific statement. *Endocr. Rev.*, **30**, 293-342.

Doisneau-Sixou, S.F., Sergio, C.M., Carroll, J.S., Hui, R., Musgrove, E.A. and Sutherland, R.L. (2003). Estrogen and antiestrogen regulation of cell cycle progression in breast cancer cells. *Endocr. Relat. Cancer*, **10**, 179-186.

Dunfield, L.D. and Nachtigal, M.W. (2003). Inhibition of the antiproliferative effect of TGFbeta by EGF in primary human ovarian cancer cells. *Oncogene*, **22**, 4745-4751.

Elliott, R.L. and Blobe, G.C. (2005). Role of transforming growth factor Beta in human cancer. *J. Clin. Oncol.*, **23**, 2078-2093.

Feng, X.H. and Derynck, R. (2005). Specificity and versatility in tgf-beta signaling through Smads. *Annu. Rev. Cell Dev. Biol.*, **21**, 659-693.

Gantus, M.A., Alves, L.M., Stipursky, J., Souza, E.C., Teodoro, A.J., Alves, T.R., Carvalho, D.P., Martinez, A.M., Gomes, F.C. and Nasciutti, L.E. (2011). Estradiol modulates TGF-beta1 expression and its signaling pathway in thyroid stromal cells. *Mol. Cell Endocrinol.*, **337**, 71-79.

Gomis, R.R., Alarcon, C., Nadal, C., Van Poznak, C. and Massague, J. (2006). C/EBPbeta at the core of the TGFbeta cytostatic response and its evasion in metastatic breast cancer cells. *Cancer Cell*, **10**, 203-214.

Goto, N., Hiyoshi, H., Ito, I., Tsuchiya, M., Nakajima, Y. and Yanagisawa, J. (2011). Estrogen and antiestrogens alter breast cancer invasiveness by modulating the transforming growth factor-beta signaling pathway. *Cancer Sci.*, **102**, 1501-1508.

Hagiwara, H., Sugizaki, T., Tsukamoto, Y., Senoh, E., Goto, T. and Ishihara, Y. (2008). Effects of alkylphenols on bone metabolism in vivo and in vitro. *Toxicol. Lett.*, **181**, 13-18.

Hill, J.J., Tremblay, T.L., Cantin, C., O'Connor-McCourt, M., Kelly, J.F. and Lenferink, A.E. (2009). Glycoproteomic analysis of two mouse mammary cell lines during transforming growth factor (TGF)-beta induced epithelial to mesenchymal transition. *Proteome. Sci.*, **7**, 2.

Hwang, K.A., Park, S.H., Yi, B.R. and Choi, K.C. (2011). Gene alterations of ovarian cancer cells expressing estrogen receptors by estrogen and bisphenol a using microarray analysis. *Lab. Anim. Res.*, **27**, 99-107.

Ito, I., Hanyu, A., Wayama, M., Goto, N., Katsuno, Y., Kawasaki, S., Nakajima, Y., Kajiro, M., Komatsu, Y., Fujimura, A., Hirota, R., Murayama, A., Kimura, K., Imamura, T. and Yanagisawa, J. (2010). Estrogen inhibits transforming growth factor beta signaling by promoting Smad2/3 degradation. *J. Biol. Chem.*, **285**, 14747-14755.

Khan, S.A., Rogers, M.A., Khurana, K.K., Meguid, M.M. and Numann, P.J. (1998). Estrogen receptor expression in benign breast epithelium and breast cancer risk. *J. Natl. Cancer Inst.*, **90**, 37-42.

Kim, D.Y., Kim, M.J., Kim, H.B., Lee, J.W., Bae, J.H., Kim, D.W., Kang, C.D. and Kim, S.H. (2011). Suppression of multidrug resistance by treatment with TRAIL in human ovarian and breast cancer cells with high level of c-Myc. *Biochim. Biophys.*

Acta., **1812**, 796-805.

Kochukov, M.Y., Jeng, Y.J. and Watson, C.S. (2009). Alkylphenol xenoestrogens with varying carbon chain lengths differentially and potently activate signaling and functional responses in GH3/B6/F10 somatomammotropes. *Environ Health Perspect.*, **117**, 723-730.

Massague, J., Seoane, J. and Wotton, D. (2005). Smad transcription factors. *Genes. Dev.*, **19**, 2783-2810.

Massague, J. (2008). TGFbeta in Cancer. *Cell*, **134**, 215-230.

Matsuda, T., Yamamoto, T., Muraguchi, A. and Saatcioglu, F. (2001). Cross-talk between transforming growth factor-beta and estrogen receptor signaling through Smad3. *J. Biol. Chem.*, **276**, 42908-42914.

McCaffrey, T.A., Consigli, S., Du, B., Falcone, D.J., Sanborn, T.A., Spokojny, A.M. and Bush, H.L., Jr. (1995). Decreased type II/type I TGF-beta receptor ratio in cells derived from human atherosclerotic lesions. Conversion from an antiproliferative to profibrotic response to TGF-beta1. *J. Clin. Invest.*, **96**, 2667-2675.

Nilsson, E.E. and Skinner, M.K. (2002). Role of transforming growth factor beta in ovarian surface epithelium biology and ovarian cancer. *Reprod. Biomed. Online*, **5**, 254-258.

Osada, H., Yoshitake, Y., Ikeda, T., Ishigaki, Y., Takata, T., Tomosugi, N., Sasaki, H. and Yonekura, H. (2011). Ultraviolet B-induced expression of amphiregulin and growth differentiation factor 15 in human lens epithelial cells. *Mol. Vis.*, **17**, 159-169.

Park, M.A., Hwang, K.A., Lee, H.R., Yi, B.R., Jeung, E.B. and Choi, K.C. (2011). Cell growth of BG-1 ovarian cancer cells was promoted by di-n-butyl phthalate and hexaboromocyclododecane via upregulation of cyclin D and cell cycle-dependent kinase-4 genes. *Mol. Med. Report*. In press.

Park, S.H., Kim, K.Y., An, B.S., Choi, J.H., Jeung, E.B., Leung, P.C. and Choi, K.C. (2009). Cell growth of ovarian cancer cells is stimulated by xenoestrogens through an estrogen-dependent pathway, but their stimulation of cell growth appears not to be involved in the activation of the mitogen-activated protein kinases ERK-1 and p38. *J. Reprod. Dev.*, **55**, 23-29.

Pedram, A., Razandi, M. and Levin, E.R. (2006). Nature of functional estrogen receptors at the plasma membrane. *Mol. Endocrinol.*, **20**, 1996-2009.

Regan, M.M., Viale, G., Mastropasqua, M.G., Maiorano, E., Golouh, R., Carbone, A., Brown, B., Suurkula, M., Langman, G., Mazzucchelli, L., Braye, S., Grigolato, P., Gelber, R.D., Castiglione-Gertsch, M., Price, K.N., Coates, A.S., Goldhirsch, A. and Gusterson, B. (2006). Re-evaluating adjuvant breast cancer trials: assessing hormone receptor status by immunohistochemical versus extraction assays. *J. Natl. Cancer Inst.*, **98**, 1571-1581.

Schuster, N. and Krieglstein, K. (2002). Mechanisms of TGF-beta-mediated apoptosis. *Cell Tissue Res.*, **307**, 1-14.

Shanle, E.K. and Xu, W. (2011). Endocrine disrupting chemicals targeting estrogen receptor signaling: identification and mechanisms of action. *Chem. Res. Toxicol.*, **24**, 6-19.

Terasaka, S., Inoue, A., Tanji, M. and Kiyama, R. (2006). Expression profiling of estrogen-responsive genes in breast cancer cells treated with alkylphenols, chlorinated phenols, parabens, or bis- and benzoylphenols for evaluation of estrogenic activity. *Toxicol. Lett.*, **163**, 130-141.

Theriault, B.L. and Nachtigal, M.W. (2011). Human ovarian cancer cell morphology, motility, and proliferation are differentially influenced by autocrine TGFbeta superfamily signalling. *Cancer Lett.*, **313**, 108-121.

Watanabe, J., Kamata, Y., Seo, N., Okayasu, I. and Kuramoto, H. (2007). Stimulatory effect of estrogen on the growth of endometrial cancer cells is regulated by cell-cycle regulators. *J. Steroid. Biochem. Mol. Biol.*, **107**, 163-171.

Yi, B.R., Kang, N.H., Hwang, K.A., Kim, S.U., Jung, E.B. and Choi, K.C. (2011). Antitumor Therapeuic Effects of Cytosinf Deaminase and Interferon-â Against Endometrial Cancer Cells Using Genetically Ebngineered Stem Cells *In Vitro*. *Anticancer Reserch*, **31**, 2853-2861.

Yilmaz, M., Maass, D., Tiwari, N., Waldmeier, L., Schmidt, P., Lehembre, F. and Christofori, G. (2011). Transcription factor Dlx2 protects from TGFbeta-induced cell-cycle arrest and apoptosis. *EMBO. J.*, **30**, 4489-4499.