GPR30 mediates the effect of tetrabromobisphenol A but not tetrachlorobisphenol A on ovarian cancer cell proliferation.

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Introduction

GPR30 (G protein-coupled receptor 30) has been identified as a novel membrane receptor localized on both the plasma membrane and endoplasmic reticulum. It is structurally dissimilar to nuclear estrogen receptors and has ability to bind endogenous and exogenous estrogens (Thomas and Dong, 2006; Revankar et al., 2005). GPR30 plays important role in several cancers including ovarian cancer, by regulating cell proliferation, migration and its expression is associated with lower survival rates for ovarian cancer patients (Smith et al., 2009; Yan et al., 2013; Heublein et al., 2014; François et al., 2015). Exogenous estrogens, such as bisphenol A (BPA) bind to GPR30 and activate signaling pathways (Thomas and Dong, 2006; Dong et al., 2011).

Tetrabromobisphenol A (TBBPA), and tetrachlorobisphenol A (TCBPA), halogenated derivatives of BPA, are used as effective flame retardants in a wide variety of consumer products. The average serum concentration of TBBPA ranges from the lowest detection level to 6.58 ng/g (Xiao et al., 2011), whereas the serum concentration of TCBPA ranges from 4 to 200 pg/g (Thomsen et al., 2001). Studies showed that BPA, a suspected endocrine-disrupting chemical can acts directly on ovarian cancer and promotes the proliferation (Park et al., 2009; Ptak et al., 2011) and migration (Ptak et al., 2014) of ovarian cancer cells. In contrast to BPA, there is little information on the effects of TBBPA and TCBPA on cancers in humans and ability of these compounds to disrupt the endocrine system. However, Olsen et al. (2003) compared the estrogen-like potency of BPA and its analogs TBBPA and TCBPA in the MCF-7 breast cancer cell line and showed their low potency induced an imbalance in the oestrogen response.

Ovarian tumours, the second most common type of gynaecological malignancy (Jemal et al., 2010), are a type of hormone-dependent cancer that can arise from any cell type in the ovary. The majority of the malignant ovarian tumors are epithelial in origin (90%), with approximately 7% being classified as ovarian sex cord tumors (the most common are granulosa tumors) and only 3–5% being classified as germ cell tumors (Smith et al., 2006). OVCAR-3 and KGN cells were used as in vitro models representing epithelial and granulosa ovarian tumors, respectively.

Based on these observations, we investigated whether the haogenated derivatives of BPA, TBBPA and TCBPA, stimulate ovarian cancer cell proliferation. We also examined the potential role of GPR30 in these processes.

Materials and methods

The human ovarian serous carcinoma cell line OVCAR-3 was obtained from the American Type Culture Collection (Manassas, VA, USA), and were cultured in RPMI 1640 medium supplemented with 15% FBS

(Biowest, Nuaillé, France). The human granulosa cell line KGN was obtained from the Riken BioResource Center (RCB1154, Riken Cell Bank, Koyadai, Japan), and were cultured in DMEM/Ham's F-12 medium supplemented with 10% FBS. All cultures were maintained at 37 °C in a humidified atmosphere containing 5% CO2. OVCAR-3 and KGN cells were seeded at 70% confluence in the indicated media for 24 h, and exposed to vehicle (0.1% DMSO or ethanol), BPA (AccuStandard, New Haven, CT, USA), TBBPA and TCBPA (Santa Cruz Biotechnology, CA, USA) (1, 10 and 50 nM), for 48 h. To investigate the involvement of tested compounds cells were pretreated with the selective GPR30 antagonist G15 (1 μM) for 2 h. Cell proliferation was measured using AlamarBlue Cell Viability Reagent (Invitrogen, Paisley, UK), according to the manufacturer's instructions. Total RNA was isolated from control cells, as well as those treated with BPA, TBBPA and TCBPA for 24 h, followed by cDNA synthesis using a TaqMan Gene Expression Cells-to-CT Kit (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions. The TaqMan Gene Expression Assays used for real-time PCR were as follows: GPR30 (Hs01922715_s1) and GADPH (4310884E). Statistical data are presented as the mean ± SD of three independent experiments performed in triplicate. Statistical analysis was performed using one-way ANOVA followed by Tukey's test (GraphPad Software, La Jolla, CA, USA). The level of significance was set at $P < 0.05$.

Results and discussion

Previous studies indicate that BPA can binds to GPR30, which mediate rapid actions at the cellular and organism level by activation of signaling cascades (Thomas and Dong, 2006; Filardo et al., 2002; Revankar et al., 2005). Moreover, the binding affinity of BPA to GPR30 is 8–50 times higher than its affinity to the estrogen receptors (Hu and Aizawa, 2003; Blair et al., 2000). It was also shown that BPA can activates intracellular signaling pathways through binding to GPR30 in the OVCAR-3 and BG-1 cell lines (Park et al., 2009; Ptak and Gregoraszczuk, 2012). Thus, we examined basal GPR30 expression levels in OVCAR-3 and KGN cells. We demonstrated that GPR30 receptor expression does not differ between OVCAR-3 and KGN cells at both the mRNA and protein level (Fig. 1 A, B). Also, BPA, TBBPA and TCBPA does not change the GPR30 mRNA expression after 24 h treatment in OVCAR-3 and KGN cell lines (Fig. 1C).

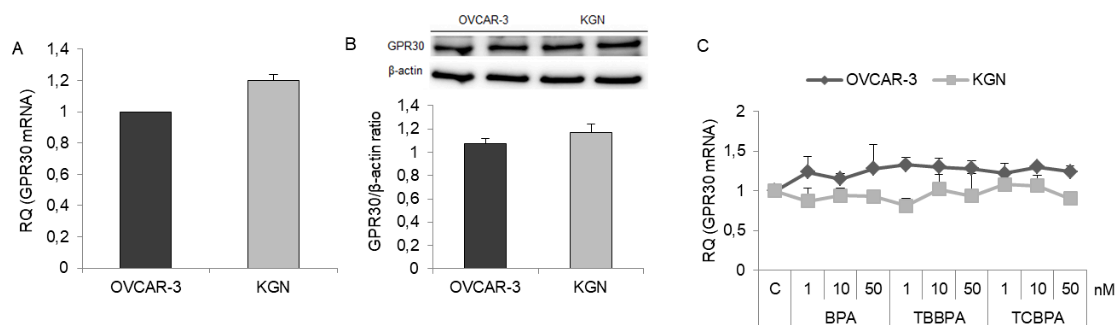

Fig. 1. Basal GPR30 gene (A) and protein (B) expression in OVCAR-3 and KGN cells. The relative quantity (RQ) of GPR30 obtained for OVCAR-3 cells was arbitrarily set as 1. Effects of BPA, TBBPA and TCBPA (1, 10, and 50 nM) on GPR30 gene expression at 24 h in OVCAR-3 and KGN cells. RQ, relative quantity. Data represent means ± SD of three independent experiments.

It was shown that nanomolar concentrations of BPA have significant proliferative effects on in vitro cultured ovarian cancer cells (Park et al., 2009; Ptak et al., 2011). Thus, we analyzed the effect of the BPA analogs, TBBPA and TCBPA, on human ovarian cell proliferation for 48 h and compared this effect with that in BPA-treated cells. In OVCAR-3 cells, TBBPA increased cell proliferation at 10 and 50 nM (118% and 129% of control after 48 h), (Fig. 2A, $P < 0.05$, $P < 0.001$). TCBPA at all concentrations tested had no effect on OVCAR-3 cell proliferation (Fig. 2A). We observed that TBBPA proliferative effects were lower than those observed in BPA-treated cells (126% and 142% of control after 48 h), (Fig. 2A, $P < 0.05$, $P < 0.001$). In KGN cells, increased effects on cell proliferation were observed following treatment with BPA and TBBPA (Fig. 2B). BPA stimulated KGN cell proliferation (128% and 145% of control after 48 h), (Fig. 2B, $P < 0.01$, $P < 0.001$). Treatment with TBBPA resulted in a proliferative effect (122% and 126% of control after 48 h) (Fig. 2B, $P < 0.05$, $P < 0.01$). TCBPA at all concentrations tested had no effect on KGN cell proliferation (Fig. 2B).

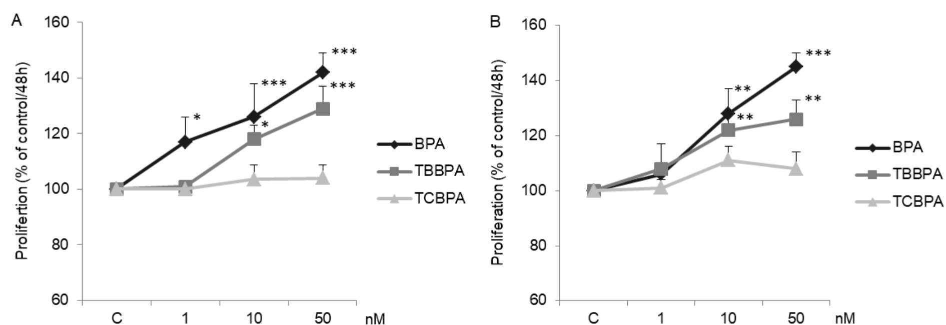

Fig. 2. Effects of BPA (1, 10, and 50 nM), TBBPA (1, 10, and 50 nM), and TCBPA (1, 10, and 50 nM) on OVCAR-3 (A) and KGN (B) cell proliferation after 48 h. Data represent means ± SD of three independent experiments. $P < 0.05$*, $P < 0.01$**, $P < 0.001$*** vs. control cells.

Our study for the first time showed that low nanomolar concentrations of TBBPA, but not TCBPA, possess the ability to stimulate proliferation in ovarian cancer cell lines. Moreover, TBBPA potency was lower than that of BPA, which was used as a positive control for this study. Similarly, Olsen et al. (2003) indicating that TBBPA and TCBPA induce less cell growth than BPA in MCF-7 breast cancer cells (Olsen et al., 2003).

Next, we examined whether the GPR30 receptor is involved in BPA- and TBBPA-induced proliferation in OVCAR-3 and KGN cells. Pretreatment with the GPR30 antagonist G15 (1 μM) prior to BPA and TBBPA treatment abolished cell proliferation to control levels in both cell lines (Fig. 3, $a < b < c$, $P < 0.05$). This finding suggested that BPA and TBBPA stimulate epithelial and granulosa tumor cell proliferation through the GPR30. Previous published data shows that activation of GPR30 promote cell motility and invasiveness in the ovarian cancer cell line OVCAR-5 (Yan et al., 2013), but inhibit the migration and invasion of the granulosa tumor cell lines KGN and COV434 (François et al., 2015).

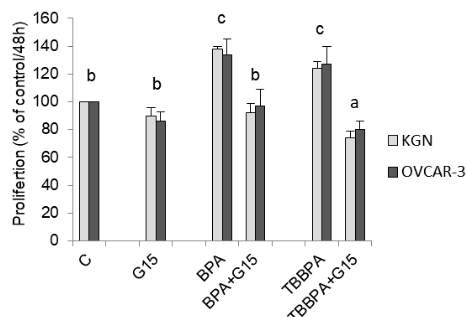

Fig. 3. Involvement of GPR30 in the proliferatory effects of BPA (10 nM) and TBBPA (10 nM) on OVCAR-3 and KGN cells at 48 h. Cells were pretreated with the GPR30 inhibitor G15 (1 μM) followed by treatment with BPA or TBBPA in OVCAR-3 and KGN cells. Data represent means ± SD of three independent experiments. a < b < c, P < 0.05 vs. control cells.

In conclusion, our results provides evidence that nanomolar concentrations of TBBPA, which has lower potency than BPA, stimulates ovarian cancer cell proliferation through the GPR30 pathway.

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