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BISPHENOL A AND ITS HALOGENATED DERIVATIVES INDUCE APELIN EXPRESSION WHICH ACTS AS MITOGENIC FACTOR IN HUMAN EPITHELIAL OVARIAN CANCER CELL.

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Introduction

Epidemiological studies reported that adults have detectable levels not only bisphenol A (BPA, 0.2 to 20 ng/ml) but also its halogenated derivatives tetrabromobisphenol A (TBBPA, 6.58 ng/g) and tetrachlorobisphenol A (TCBPA, 4-200 pg/g) in serum [1-3]. Our previous study has shown that BPA may act directly on ovarian cancer progression by induction of proliferation, migration and angiogenesis [4-6], or indirectly by increased leptin receptor expression creating more binding sites for leptin action [7]. Leptin belongs to the adipokines (adipose tissue hormones) which act via their receptors on tumour cells and regulate cell proliferation, apoptosis, and invasion [8, 9]. Another adipokine which has been shown to exhibit tumorigenic properties is apelin [10]. However, no information is currently available regarding to the role of BPA, TBBPA and TCBPA on apelin expression in ovarian cancer. Thus, we investigated if apelin and its receptor expression may be modulated by BPA and its analogs or whether a change in the expression of apelin translates into stimulatory action on epithelial cell tumor proliferation.

Materials and Methods

Cell culture The human ovarian surface epithelial cells HOSEpiC (ScienCell) were routinely cultured in OEpiCM medium, and the human ovarian serous carcinoma cell line OVCAR-3 (ATCC) was propagated in RPMI 1640 medium with 10% fetal bovine serum (FBS, Sigma-Aldrich). Apelin-13 (A6469) and 17β-estradiol (E2785) were obtained from Sigma. BPA (AccuStandard), TBBPA, TCBPA (SantaCruz Biotechnology) were dissolved in DMSO. Real-time PCR analysis: The OVCAR-3 cells were exposed to vehicle (DMSO, 0.1 %), BPA, TBBPA, TCBPA (1, 10, 50 and 100 nM) or E2 (0.1, 1 nM) for 24 h. Total RNA isolation, cDNA synthesis and real-time PCR analysis were performed using the TaqMan Gene Expression Cells-to-CT Kit (Applied Biosystems), according to the manufacturer's instructions. The expression of apelin (APLN; Hs00936329_m1) and its receptor (APLNR, Hs00270873) was normalised to that of GADPH (4310554E). Relative expression was quantified using the $2^{-\Delta\Delta}$Ct method [11]. The apelin secretion was assessed using human Apelin-36 EIA kit (Phoenix Pharmaceuticals Inc.) and cell proliferation was measured using the alamarBlue cell viability reagent (Invitrogen) according to the manufacturer's instructions. The OVCAR-3 and HOSEpiC cells were exposed to vehicle or with four different doses (0.02, 0.2, 2, and 20 ng/ml) of apelin for 48 h. Statistical analysis was carried out using the one-way ANOVA followed by Tukey's test (GraphPad Software), and the level of significance was set at a $P< 0.05$. Statistical data are presented as the mean ± SD of three individual experiments performed in triplicate.

Results and discussion

Because, no information is available in the literature about the apelin and its receptor expression in human ovary, first we analysed basal APLN and APLNR expression in non-cancer HOSEpiC and cancer OVCAR-3 ovarian cells. We demonstrated, for the first time, that apelin mRNA level was similar in non-cancer and cancer cells (Fig. 1). While, basal APLNR expression was 34-fold higher in the cancer cells, than in the non-cancer cells (Fig. 1, $P<0.001$). So far, apelin and its receptor expression have been demonstrated only in bovine ovary [12].

Previously published data showed that BPA may change adipokines expression in the 3T3-L1 adipocyte cells [13-15] as well as in ovarian cancer cells [7]. We demonstrated that, BPA, TBBPA and TCBPA, increased apelin gene expression at all concentrations tested (~2.5-fold), but had no effect on apelin receptor expression after 24h (Fig 2a, $P<0.001$; Fig 2b). Moreover, the apelin secretion pattern correlates with the APLN expression pattern and its higher in the BPA, TBBPA, and TCBPA treated cells (Fig. 3, $P<0.05$). E2 used as a control, had no effect on apelin and its receptor expression in OVCAR-3 cells (Fig. 2a, b). The fact that BPA increases apelin expression, suggests a possibility increase apelin bioavailability in ovarian cancer cells.

Apelin is known as a potent activator of tumour angiogenesis in endothelial cell [16,17] and migration in lung, oral squamous and colon cancer [10,18,19]. Because, no information is available in the literature about the apelin action in human ovary, we analysed its action on non-cancer and cancer ovarian cell proliferation. In HOSEpiC cells, apelin had no effect on cell proliferation (Fig. 4a), whereas increased cancer cells proliferation (Fig. 4b, $P<0.05$, $P<0.01$). This observation indicated that apelin may acts in paracrine manner through increased cell proliferation.

Conclusions

These finding suggest that the human epithelial cancer cells produce apelin which induce cells proliferation. Moreover, BPA, TBBPA, and TCBPA by increasing apelin secretion, may enhanced apelin action on ovarian cancer cells.

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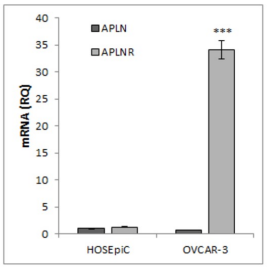

Figure 1. Basal apelin (*APLN*) and its receptor (*APLNR*) mRNA expression in HOSEpiC and OVCAR-3 cell lines. ***P<0.001 compared to HOSEpiC cells.

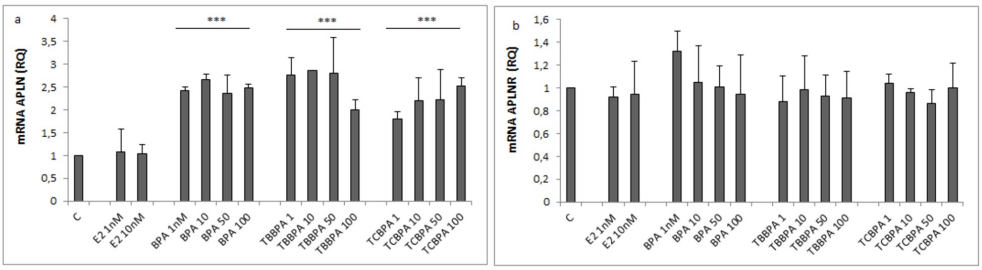

Figure 2. Effect of BPA, TBBPA, TCBPA (1, 10, 50, and 100 nM) and E2 (1, 10 nM) on a) apelin, and b) apelin receptor expression in OVCAR-3 cells. RQ relative quantity. Control value=1.0. ***P<0.001 compared to control cells.

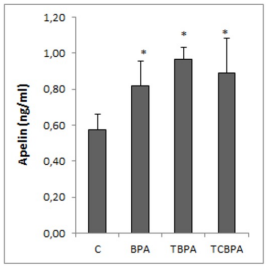

Figure 3. Effect of BPA, TBBPA, and TCBPA (1, 10, 50, and 100 nM) on apelin secretion in OVCAR-3 cells for 48 h. C control (untreated cells). *P<0.05 compared to control cells.

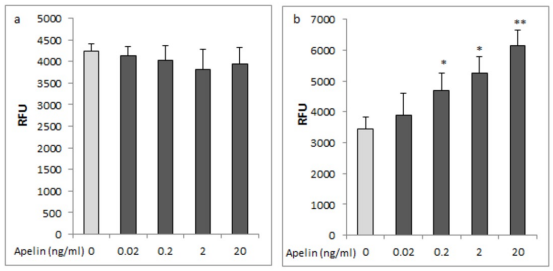

Figure 4. Effects of apelin at concentrations of 0.02, 0.2, 2 and 20 ng/ml on a) HOSEpiC, and B) OVACR-3 cell proliferation for 48 h. C, control (untreated cells). *P<0.05 and **P<0.01 compared to control cells.