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Anticancer effect of genistein on BG-1 ovarian cancer growth induced by 17 β-estradiol or bisphenol A via the suppression of the crosstalk between estrogen receptor alpha and insulin-like growth factor-1 receptor signaling pathways

Kyung-A Hwang, Min-Ah Park, Nam-Hee Kang, Bo-Rim Yi, Sang-Hwan Hyun, Eui-Bae Jeung, Kyung-Chul Choi *

Laboratory of Veterinary Biochemistry and Immunology, College of Veterinary Medicine, Chungbuk National University, Cheongju, Chungbuk 361-763, Korea

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ABSTRACT

The interaction between estrogen receptor (ER) and insulin-like growth factor-1 receptor (IGF-1R) signaling pathway plays an important role in proliferation of and resistance to endocrine therapy to estrogen dependent cancers. Estrogen (E2) upregulates the expression of components of IGF-1 system and induces the downstream of mitogenic signaling cascades via phosphorylation of insulin receptor substrate-1 (IRS-1). In the present study, we evaluated the xenoestrogenic effect of bisphenol A (BPA) and antiproliferative activity of genistein (GEN) in accordance with the influence on this crosstalk. BPA was determined to affect this crosstalk by upregulating mRNA expressions of ERα and IGF-1R and inducing phosphorylation of IRS-1 and Akt in protein level in BG-1 ovarian cancer cells as E2 did. In the mouse model xenografted with BG-1 cells, BPA significantly increased a tumor burden of mice and expressions of ERα, pIRS-1, and cyclin D1 in tumor mass compared to vehicle, indicating that BPA induces ovarian cancer growth by promoting the crosstalk between ER and IGF-1R signals. On the other hand, GEN effectively reversed estrogenicity of BPA by reversing mRNA and protein expressions of ERα, IGF-1R, pIRS-1, and pAkt induced by BPA in cellular model and also significantly decreased tumor growth and in vivo expressions of ERα, pIRS-1, and pAkt in xenografted mouse model. Also, GEN was confirmed to have an antiproliferative effect by inducing apoptotic signaling cascades. Taken together, these results suggest that GEN effectively reversed the increased proliferation of BG-1 ovarian cancer by suppressing the crosstalk between ERα and IGF-1R signaling pathways upregulated by BPA or E2.

Introduction

Estrogens are the primary steroidal sex hormones that promote development of female secondary sexual characteristics and regulate growth and function of reproductive organs such as endometrium, uterine, and vagina (Lee et al., 2012b; Park et al., 2011). They also play important roles in fetal development, bone growth, various metabolic processes, and women's mental health (Douma et al., 2005; Wu et al., 2009). On the other hand, estrogens were proven to pose a risk of development and progression of several cancers including breast (Santen et al., 2007), ovarian (Giacalone et al., 2003), endometrium (Grady et al., 1995), and cervical carcinomas (Chung et al., 2010). These types of cancers are referred to estrogen-responsive or estrogen-dependent cancers since they usually express the receptor for estrogen (ER) and antiestrogen therapy has been an effective therapeutic treatment.

The molecular events caused by estrogens via ER in these cancers are mediated in genomic or nongenomic signal transduction pathway (Lee et al., 2013). In genomic pathway, estrogen binding to ERs located in cytosol triggers a series of events such as migration of the receptor from cytosol into nucleus, dimerization, and subsequent binding of ER dimer to specific sequences of DNA with high affinity. The DNA/receptor complex then recruits other proteins that are responsible for transcription of downstream target genes (Tsai and O'Malley, 1994), whose products are mainly involved in proliferative signaling via cell cycle progression, i.e. cyclin D1, c-fos, and c-myc (Altucci et al., 1996; Loose-Mitchell et al., 1988). Some ERs, meanwhile, may mediate cytoplasmic signaling cascades via nongenomic pathways by interacting with receptor tyrosine kinases in a cell membrane. Epidermal growth factor receptor (EGFR) and insulin-like growth factor-1 receptor (IGF-1R) are typical receptor tyrosine kinases that provoke a mitogenic cytoplasmic signaling by activating extracellular signaling-regulated kinase (ERK) cascade (Improta-Brears et al., 1999) and phosphatidylinositol 3-kinase (PI3-kinase) route (Castoria et al., 2001; Sun et al., 2001) and they are also closely linked to ER signaling, thus providing an intricate pathogenesis in estrogen-dependent cancers (Massarweh and Schiff, 2006).

* Corresponding author at: Laboratory of Veterinary Biochemistry and Immunology, College of Veterinary Medicine, Chungbuk National University, Cheongju, Chungbuk 361-763, Republic of Korea. Fax: +82 43 267 3150.
E-mail address: kchoi@cbu.ac.kr (K.-C. Choi).

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In the present study, we focused on the specific crosstalk between ER and IGF-1R signaling pathways in estrogen-responsive BG-1 ovarian cancer. A growing body of studies suggests the role of interactions between two pathways as a potent mitogen promoting cancer proliferation in breast carcinomas (Massarweh and Schiff, 2006; Pivot et al., 2009), but limited data are available in other malignancies including ovarian cancer (Spentzos et al., 2007). Based on the action of 17β-estradiol (E2), the strongest estrogen in physiological estrogens, in the crosstalk between ER and IGF-1R signal transductions, we also examined the potential role of bisphenol A (BPA). BPA is a key monomer compound used in the production of epoxy resins and polycarbonate plastics and a well-known chemical classified as a xenoestrogen, an endocrine disrupting chemical (EDC) with estrogenic activity (Aksglaede et al., 2006; Danzo, 1998). Many studies published over the past few decades have demonstrated the hazardous health effects of BPA as an endocrine disruptor which originate from its structural and functional similarity to E2 (Lee et al., 2012a; Wolstenholme et al., 2011). Especially, children may be more susceptible to BPA exposure than adults because detoxification pathway is not fully developed. For instance, the previous study has found that fetuses and young children exposed to BPA are at risk for brain and behavior changes and immune disorders (Erler and Novak, 2010). For the risk of carcinogenesis of BPA, human perinatal exposure to environmentally relevant BPA doses was reported to result in morphological and functional alterations of the male and female genital tract and mammary glands that may give rise to an earlier onset of disease such as mammary and prostate cancer (Maffini et al., 2006). Although the connections between BPA and cancers have been identified, the pathogenic mechanism of BPA in cancer progression has not been fully elucidated. In the present study, we investigated the effect of BPA on nongenomic pathway of E2 signals, especially on the crosstalk between ERα, an isoform of ERs (ERα and ERβ), and IGF-1R signaling pathways in cellular and animal models of BG-1 ovarian cancer.

As another keynote of the present study, we also explored the antiproliferative effect of genistein (GEN) to reverse the cancer proliferation promoted by E2 or BPA via suppression of the crosstalk between ERα and IGF-1R signaling pathways. GEN, which is the most abundant isoflavone in soybean products and a classical dietary phytoestrogen, influences multiple biochemical functions including a chemopreventive efficacy against diverse cancers (Q.S. Li et al., 2012; Mense et al., 2008). From the epidemiologic observations indicating that cancer incidences are much lower in Asian populations that consume considerably higher amounts of phytoestogens including GEN through diet compared to Western individuals, the chemopreventive activity of GEN have been extensively studied although the anticancer property of GEN remains unclear (H.Q. Li et al., 2012; Mense et al., 2008; Ravindranath et al., 2004). In our previous study, we already elucidated that GEN acts as an effective antiproliferative agent abolishing BG-1 ovarian cancer cell proliferation associated with BPA and E2 by reversing cell cycle progression (Hwang et al., 2013). Therefore, the present study may provide more detailed information about the chemopreventive mechanism(s) of GEN by elucidating its role, which may have a beneficial effect on the crosstalk between ERα and IGF-1R signaling pathways in both cellular and animal models.

Materials and methods

Reagents and chemicals. 17β-estradiol (E2) and bisphenol A (BPA) were purchased from Sigma-Aldrich Corp. (St. Louis, MO, USA). Genistein (GEN) was obtained from LC Laboratories (Woburn, MA, USA). All chemicals were dissolved in 100% dimethyl sulfoxide (DMSO; Junsei Chemical Co., Tokyo, Japan) and stored as stock solutions at 4 °C.

Cell culture. BG-1 ovarian adenocarcinoma cells were obtained from Dr. K. S. Korach (National Institute of Environmental Health Sciences, 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 Inc), 1% penicillin G and streptomycin (Cellgro Mediatech, Inc., Manassas, VA, USA), and 1% antifungal HEPES (Invitrogen Life Technologies, Carlsbad, CA, USA) at 37 °C in humidified atmosphere of 5% CO_2-95% air. To prevent effects of estrogenic components in DMEM and FBS, BG-1 cells were also cultured in phenol red-free DMEM supplemented with 5% charcoal-dextran treated FBS (CD-FBS) to measure estrogenicity of BPA as previously described (Kang et al., 2012a; Lee and Choi, 2012; Park et al., 2012, 2013). The cells were detached with 0.05% Trypsin/0.02% EDTA in Mg^{2+}/Ca^{2+}-free Hank's balanced salt solution (PAA Laboratories, Pasching, Austria).

Total RNA extraction. BG-1 cells were seeded at a density of 3.0×10^5 cells per well in a 6-well plate, and then treated with DMSO, E2, BPA, or a combination of GEN and E2 or BPA. The concentrations of E2, BPA, and GEN were 10^{-9}, 10^{-5}, and 10^{-4} M, respectively. Total RNA was extracted at various time points (0, 6, and 24 h) using TRIzol reagent (Invitrogen Life Technologies) according to the manufacturer's instructions. The concentration of total RNA was measured with a spectrophotometer (Optizen, Mecasys, Deajeon, Korea) at 260 nm/280 nm. One microgram of total RNA was then dissolved in dietyl pyrocarbonated–deionzed water (DEPC–DW) for cDNA synthesis.

Semi-quantitative reverse transcription (RT) PCR. cDNA was synthesized from total RNA by reverse transcription (RT) PCR. The reaction mixture contained Moloney-murine leukemia virus reverse transcriptase (M-MLV RT; iNtRON Biotechnology, Sungnam, Republic of Korea), 200 pM nonamer random primer (iNtRON Biotechnology), dNTPs (iNtRON Biotechnology), RNase inhibitor (iNtRON Biotechnology), and RT buffer (iNtRON Biotechnology). cDNA synthesis was performed at 37 °C for 1 h and 95 °C for 5 min. cDNAs for ERα, IGF-1R, and GAPDH were amplified by PCR with specific forward and reverse primers, Taq polymerase, PCR buffer, dNTP mixture, and each cDNA template as previously described (Hwang et al., 2013). Sequences of forward and reverse primers along with the predicted sizes of each gene product are shown in Table 1. The RT-PCR products were separated on 1.5% agarose gel and the size of each gene band was estimated by comparison with 100-bp size ladders (iNtRON Biotechnology). The gels were scanned and the band densities were quantified using Gel Doc 2000 (Bio-Rad Laboratories, Inc., Hercules, CA, USA).

Western blot analysis. Western blotting was performed to assess the protein expression of pIRS-1 and pAkt in BG-1 cells. The cells were cultured to a density of 1.0×10^6 cells per of 100-mm dish and then treated with DMSO, E2, BPA, or combinations of GEN and E2 or BPA for 24 and 48 h. The concentrations of E2, BPA, and GEN were 10^{-9}, 10^{-5},

Table 1
Primer sequences and sizes for the semi-quantitative reverse-transcription PCR.

Target gene	Sequences	Product size
ERα	Sense: 5′-AGACATGAGAGCTGCCAACC-3′ Antisense: 5′-GCCAGGCACATTCTAGAAGG-3′	299 bp
IGF-1R	Sense: 5′-TCCAACACAACACTGAAGAATC-3′ Antisense: 5′-ACCATATTCCAGCTATTGGAGC-5′	185 bp
GAPDH	Sense: 5′-ATGTTCGTCATGGGTGTGAACCA-3′ Antisense: 5′-TGGCAGGTTTTTCTAGACGGCAG-3′	351 bp