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Hexachlorobenzene modulates the crosstalk between the aryl hydrocarbon receptor and transforming growth factor-β1 signaling, enhancing human breast cancer cell migration and invasion

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ABSTRACT

Given the number of women affected by breast cancer, considerable interest has been raised in understanding the relationships between environmental chemicals and disease onset. Hexachlorobenzene (HCB) is a dioxin-like compound that is widely distributed in the environment and is a weak ligand of the aryl hydrocarbon receptor (AhR). We previously demonstrated that HCB acts as an endocrine disruptor capable of stimulating cell proliferation, migration, invasion, and metastasis in different breast cancer models. In addition, increasing evidence indicates that transforming growth factor-β1 (TGF-β1) can contribute to tumor maintenance and progression. In this context, this work investigated the effect of HCB (0.005, 0.05, 0.5, and 5 μM) on TGF-β1 signaling and AhR/TGF-β1 crosstalk in the human breast cancer cell line MDA-MB-231 and analyzed whether TGF-β1 pathways are involved in HCB-induced cell migration and invasion. RT-qPCR results indicated that HCB reduces AhR mRNA expression through TGF-β1 signaling but enhances TGF-β1 mRNA levels involving AhR signaling. Western blot analysis demonstrated that HCB could increase TGF-β1 protein levels and activation, as well as Smad3, JNK, and p38 phosphorylation. In addition, low and high doses of HCB were determined to exert differential effects on AhR protein levels, localization, and activation, with a high dose (5 μM) inducing AhR nuclear translocation and AhR-dependent CYP1A1 expression. These findings also revealed that c-Src and AhR are involved in HCB-mediated activation of Smad3. HCB enhances cell migration (scratch motility assay) and invasion (Transwell assay) through the Smad, JNK, and p38 pathways, while ERK1/2 is only involved in HCB-induced cell migration. These results demonstrate that HCB modulates the crosstalk between AhR and TGF-β1 and consequently exacerbates a pro-migratory phenotype in MDA-MB-231 cells, which contributes to a high degree of malignancy. Taken together, our findings help to characterize the molecular mechanism underlying the effects of HCB on breast cancer progression.

Abbreviations: AhR, aryl hydrocarbon receptor; ANF, α-naphthoflavone; EMT, epithelial-mesenchymal transition; ERα, estrogen receptor α; ERK1/2, extracellular signal related kinase 1 and 2; HCB, hexachlorobenzene; JNK, c-jun n-terminal kinase; TCDD, 2,3,7,8-tetrachlorodibenzo-p-dioxin; TGF-β1, transforming growth factor-β1; TβRI, type I TGF-β receptor; TβRII, type II TGF-β receptor.

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1. Introduction

Breast cancer is by far the most frequently diagnosed cancer in women. However, given the low rate of breast cancer etiology being attributed to hereditary origins (WHO, 2015), other causative factors have been postulated, including environmental exposure to persistent organic pollutants. Hexachlorobenzene (HCB) is a widespread environmental pollutant that has detrimental biological effects and the International Agency for Research on Cancer has classified it as a probable human carcinogen (ATSDR, 2002). HCB was used as a fungicide until the 1970's when such use was prohibited. However, considerable amounts are still being generated as waste by-products of industrial processes and released into the environment. Moreover, HCB has been detected in mother's (Der Parsehian, 2008) and cow's milk for human consumption (Maitre et al., 1994). The role of endocrine disruptors in cancer development and progression has been studied over the last few decades. Animal exposure to HCB elicits a number of effects such as thyroid disruption (Chiappini et al., 2009) and co-carcinogenesis in mammary tumors (Randi et al., 2006).

HCB is a "dioxin-like" compound that weakly binds to the aryl hydrocarbon receptor (AhR), a transcription factor that directs the expression of many detoxification genes (Nguyen and Bradfield, 2008). Many of the biological effects of AhR ligands involve multiple interactions between AhR and other signaling pathways. It has been proposed that, upon ligand binding, two cellular signaling events are initiated. Specifically, binding to the AhR can trigger membrane actions, where c-Src release from its cytosolic AhR multiprotein complex and phosphorylates a variety of growth factor receptors (Park et al., 2007). Furthermore, binding to AhR can trigger nuclear actions that modulate the expression of genes involved in the regulation of cell proliferation, differentiation, and/or apoptosis (Matsumura, 1994). The AhR has been shown to exhibit growth-inhibitory activity in MCF-7 breast cancer cells and to promote cell cycle progression in hepatoma cells (Abdelrahim et al., 2003). These results suggest that the AhR could act either as a tumor suppressor or an oncoprotein according to the cell context. Trombino et al. (2000) found higher AhR levels in mammary tumors compared to the corresponding normal mammary tissues. In addition, estrogen receptor α (ERα) loss is tightly associated with AhR overexpression in breast cancer (Bekki et al., 2015).

Transforming growth factor-β1 (TGF-β1) has been hypothesized to participate in breast cancer development (Moses and Barcellos-Hoff, 2011). TGF-β1 has an anti-proliferative effect on most epithelial cells and in the early-stages of tumorigenesis, whereas it accelerates cancer progression and metastasis in the late stages of cancer (Humbert et al., 2010). High TGF-β1 levels in tumor cells correlate with a higher risk of metastasis and poor prognosis (Dumont and Arteaga, 2003). TGF-β1 is secreted into the extracellular medium and forms a latent complex with accessory proteins. After activation, it binds to the type II TGF-β receptor (TβRII), which phosphorylates the type I TGF-β receptor (TβRI) that in turn phosphorylates Smad2 and Smad3. Phosphorylated Smad2/3 forms a complex with Smad4 and is subsequently transferred to the nucleus where it regulates gene transcription (Massagué et al., 2005). In addition, it is well known that TGF-β1 also induces other pathways, which include activation of extracellular signal related kinase 1 and 2 (ERK1/2), c-jun N-terminal kinase (JNK), and p38 MAPK (Derynck and Zhang, 2003).

Crosstalk between TGF-β1 and AhR signaling has been shown to have common targets involved in processes such as cell cycle control (Haarmann-Stemmann et al., 2009). Analyses in cell systems and in mouse models lacking AhR expression have demonstrated that AhR represses TGF-β1 activity and that certain of the phenotype identified in AhR-depleted mice may involve the up-regulation of this cytokine (Gómez-Durán et al., 2009). Moreover, TGF-β1 triggers cell type-specific effects on AhR by inhibiting receptor expression and activation in lung cancer cells, while the receptor function is enhanced in hepatoma cells (Döhr et al., 1997; Wolff et al., 2001). Although AhR is not as well-characterized as TGF-β1 regarding its contribution to human disease, it is reasonable to assume that low or high AhR expression could differentially affect disease progression and involve TGF-β1 signaling.

We previously demonstrated that HCB is a mammary tumor co-carcinogen in rats (Randi et al., 2006) and an inducer of proliferation in MCF-7 cells that acts in an ERα-dependent manner (García et al., 2010). Evidence in the human breast cancer cell line MDA-MB-231 has also shown that HCB can induce cell migration and invasion through c-Src, epidermal growth factor receptor, and AhR (Pontillo et al., 2011, 2013). Therefore, the aim of the present study was to examine the effect of HCB on the crosstalk between AhR and TGF-β1 pathways in MDA-MB-231 cells and analyze the role of TGF-β1 in HCB-induced cell migration and invasion.

2. Materials and methods

2.1. Chemicals

HCB (>99% purity, commercial grade) was obtained from Aldrich-Chemie GmbH & Co. (Steinheim, Germany). Anti-TGF-β1 and anti-AhR antibodies were purchased from Abcam, Ltd. (Cambridge, UK). Anti-Smad3, anti-phospho-Smad3, anti-JNK, anti-phospho-JNK, anti-p38, and anti-phospho-p38 were obtained from Cell Signaling Technology, Inc. (MA, USA). Anti-β-Actin antibody, TGF-β1, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), antibiotic–antimycotic, dimethyl sulphoxide (DMSO), trypsin, glutamine, and the inhibitors α-naphthoflavone (ANF), SB431542, SB203580, SP600125, and PD98059 were purchased from Sigma-Aldrich Chemical, Co. (St. Louis, MO, USA). The 4-Amino-5-(4-chlorophenyl)-7-(t-butyl) pyrazolo[3,4-d]pyrimidine (PP2) inhibitor was obtained from Calbiochem (La Jolla, CA, USA). The enhanced chemiluminescence kit (ECL) and Alexa 488 anti-mouse IgG were obtained from GE Healthcare Life Sciences (Buckinghamshire, UK). The Alexa 488 anti-rabbit IgG was purchased from Thermo Fisher Scientific, Inc. (Waltham, MA, USA). RPMI-1640 culture medium was obtained from HyClone Laboratories, Inc. (Logan, UT, USA). The random primers were purchased from Biodynamics (Argentina). Moloney Murine Leukemia Virus Reverse Transcriptase (M-MLV RT) and cofactors for reverse transcription were obtained from Promega Corporation (Madison, WI, USA). The kit Hot FirepolEvaGreen qPCR Mix Plus (ROX) was purchased from Solis Biodyne (Estonia), and the specific oligonucleotides for human AhR and TGF-β1 were obtained from Invitrogen Life Technologies (UK). Matrigel was purchased from Becton Dickinson Biosciences (San José, CA, USA). All of the other reagents that were used were of analytical grade.

2.2. Cell culture and treatment

The MDA-MB-231 (-ERα) cell line (American Type Culture Collection) was derived from a metastatic site (pleural effusion) of a human breast adenocarcinoma. This cell line represents a less differentiated tumor phenotype, with a great degree of malignancy. The cells were cultured at 37 °C in a 5% CO_2 incubator with RPMI-1640 supplemented with 10% fetal bovine serum (FBS), 1% antibiotic–antimycotic mixture (10,000 Units/ml penicillin, 10 mg/ml streptomycin sulfate, and 25 μg/ml amphotericin B), and 1% glutamine. After 24 h of starvation, the cells at 70–80% confluence were exposed to HCB dissolved in ethanol (EtOH). For time-course studies, the cells were treated with HCB (0.05 μM) or vehicle in RPMI supplemented with 5% FBS for 5, 15, and 30 min, as well as 2,