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Environmental estrogen exposures alter molecular signaling in immune cells that promote the development of childhood asthma

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Abstract

Environmental estrogens (EEs) are associated with an increased prevalence of asthma. These epigenetic alterations of the immune cells may explain the multigenerational effects on asthma development. We hypothesized that exposure to immune cells enhances allergic sensitization by initiating signaling in these cells. Human T cell lines (TIB-152, CCL-119) were exposed to varying concentrations of estradiol, bisphenol A, bisphenol S, or bisphenol A + estradiol. H3K27me3, phosphorylations of EZH2 (pEZH2), AKT (pAKT), and phosphatidylinositol 3-kinase (pPI3K) were assessed. pAKT and pPI3K were decreased in response to some of the concentrations of these exposures in both cell lines. It is likely that EEs exposure to immune cells is one of the factors in the increase in the prevalence of asthma.

Keywords

allergy; asthma; bisphenol A; bisphenol S; environmental estrogens; immune signaling

1. INTRODUCTION

The prevalence of asthma, particularly in children in developed countries, has been increasing rapidly over the last 30 years (Borna et al., 2019). Perinatal exposures have a significant role in the current asthma epidemic. Asthma is more prevalent in females than

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males from puberty to menopause (Borna et al., 2019). Asthma can be reactivated in women taking estrogen-dominant hormone replacement therapy (Bonds and Midoro-Horiuti, 2013; Choi, 2011; Dratva, 2010). These findings strongly suggest a role for endogenous and exogenous estrogens in the development of asthma. Therefore, we elucidated the potential roles of environmental estrogen (EE) exposure in the development and morbidity of asthma.

We reported that perinatal exposure to bisphenol A (BPA), a common EE, which is detectable in the majority of humans (Kovacic et al., 2020), promotes the development of an asthma-like phenotype in our mouse model (Midoro-Horiuti et al., 2010; Nakajima et al., 2012). We also noted that this phenotype was perpetuated in the 2nd and 3rd generations, after eliminating the BPA exposure after the weaning of 1st generations (Sowers et al., 2020). Epidemiologic studies also describe associations between maternal BPA exposures with wheezing among infants and decreased pulmonary function (Donohue et al., 2013; Gascon et al., 2015; Spanier et al., 2012). Also, associations between personal BPA urinary excretion and allergic asthma later in life have been described (Vaidya and Kulkarni, 2012). Even after the US Food and Drug Administration stopped approval of BPA usage in consumer products, BPA containing micro- and nano-plastics in the environment can be an important source of BPA (Chen et al., 2017; Kwon et al., 2020). Bisphenol S (BPS), the substitute for BPA, which is now detected in about 80% of humans, may have similar effects to BPA (Dualde et al., 2021; Gys et al., 2021; Vinas and Watson, 2013).

The effects of steroid hormones that occur independently from widely known gene transcription have been termed nongenomic to distinguish them from the direct, or genomic effects of their receptors as transcription factors in the nucleus (Vinas and Watson, 2013; Watson et al., 2012). Walker et al. have identified a novel role for this nongenomic signaling by xenoestrogens via activation of membrane-associated ER and regulation of enhancer of zeste homolog 2 (EZH2) to reprogram the developing uterus (Walker, 2011). They found that in response to both 17- β estradiol (E_2) and the xenoestrogen diethylstilbestrol (DES), ER signaling via PI3K/AKT phosphorylates EZH2 at S21, and reducing H3K27me3 levels in hormone-responsive uterine myometrial (Bredfeldt et al., 2010) and prostate cancer cells (Wang et al., 2016). Although ER α expression is reported in the T cells (Kassi et al., 2001; Rider et al., 2000), the specific mechanisms by which EEs reprogram the developing epigenome were unknown in immune cells, including T cells. We recently reported that *in vitro* and *in vivo* exposures to EEs induce the overexpression of zinc finger DHHC domain-containing (ZDHHC)1 and stimulators of interferon genes (STING) at the mRNA and protein levels (Sowers et al., 2020). Based on these findings, we propose this study to understand the intracellular signaling pathways by which EE alters the expression of the immune-modulating genes and the epigenetic mechanisms that may perpetuate these pathologic responses.

2. Material and Methods

2.1. Reagents and antibodies

We obtained cell culture reagents from ThermoFisher Scientific (Pittsburgh, PA) and Sigma-Aldrich (St. Louis, MO). Anti-H3K27me3 and anti-EZH2 antibodies were from Active Motif (Carlsbad, CA), anti-phosphorylated AKT (S473) and anti-phosphorylated PI3K

[pPI3K(p85), PI3K(p55)] were from Cell Signaling Technology (Danvers, MA), and anti-phosphorylated EZH2 (S21) was from Bethyl, Inc. (Montgomery, TX).

2.2. Cell culture

Since we detected altered ZDHHC-1 expression in human T cell lines (Sowers et al., 2020), we used ER α -positive human T cell lines CCL-119 and TIB-152 (ATCC, Manassas, VA). Cells were grown and maintained as we described.

2.3. Estrogen treatments

Cells were starved in a phenol red-free RPMI with 1% charcoal-stripped fetal calf serum before estrogen treatment for 15–24 h. Cells were plated and exposed to various concentrations of EE or combination with 1×10^{-9} M E₂ (human physiological concentration) for 5 or 120 min.

2.4. Preparation for cell lysates for Western blot (WB) analysis

After exposure to estrogens, cells were lysed in RIPA buffer containing protease inhibitor cocktail (Roche Applied Science, Indianapolis, IN)]. The protein concentrations of supernatants of the cell lysates were determined using a DeNovix DS-11 Spectrophotometer (New England BioGroup, Atkinson, NH). Equal amounts of denatured proteins were separated in 4–25% SDS-PAGE (Bio-Rad, Hercules, CA) and electrotransferred onto polyvinylidene difluoride membranes. The membrane was incubated with a primary antibody and then with an appropriate horseradish peroxidase-conjugated secondary antibody (SantaCruzBiotechnology, Santa Cruz, CA). Proteins or posttranslational modifications of interest were visualized using Amersham enhanced chemiluminescence start (GE Healthcare, Chicago, IL) or Super Signal West Dura Extended Duration Substrate (ThermoFisher). We used β -actin expression in each well for signal normalization. Experiments were repeated at least three times using different passages of cells. We used a 0.001% ethanol vehicle as a negative control.

2.5. Statistical analyses

The relative intensities were compared against the intensities generated to vehicle-exposed samples. Statistical analyses were performed using one-way analysis of variance. Where the differences between EE- and vehicle-exposed groups was present, the elements that differed were identified by the Student's t-test. Differences at $p < 0.05$ were defined as statistically significant.

3. Results and Discussion

3.1. Exposure to EE alters H3K27me3 in T cell lines

We tested various concentrations of the estrogens to adequately describe the effects they might have at different human contamination levels and to account for their nonmonotonic behavior. We chose the 120 min time point for histone modification because this time point would result in a distinctly nongenomic response (Bredfeldt et al., 2010; Trevino et al., 2015). In the TIB-152 cell line, H3K27me3 was not decreased in response to BPA

alone, but was significantly decreased in response to 1×10^{-11} - 1×10^{-10} M BPA + 10^{-9} M E₂ (Fig. 1A left). H3K27me3 was decreased in response to 10^{-10} - 10^{-9} M E₂ in TIB-152. In response to BPS alone, H3K27me3 was decreased with 10^{-11} M in TIB-152. In the CCL-119, H3K27me3 was not decreased significantly (Fig. 1A right). Our study suggested that exposure to EE reduced H3K27me3 in T cells. We observed reduced H3K27me3 in the mouse splenic CD4⁺ cells from the F0 to F3 mice, from which F0 were exposed to BPA during pregnancy and the nursing period (Sowers et al., 2020). Others showed that BPA exposure induced enhanced secretion of both Th1 and Th2 cytokines in a mouse model (Yoshino et al., 2004; Yoshino et al., 2003). Also, using histone H3K27 methyltransferase Ezh2-deficient cells, others show the increased production of both Th1 and Th2 cytokines (Tumes et al., 2013). This reduction of H3K27me3 may explain one of the mechanisms of this EE-induced increased cytokine production.

3.2. Exposure to EE alters pEZH2 in T cell lines

Phosphorylation of EZH2 was not significantly changed in either cell line at 120 min exposure, except significantly decreased ($p < 0.05$) at 1×10^{-12} M BPS and 1×10^{-9} M E₂ to TIB-152, but not CCL-119 (Data not shown).

3.3. Exposure to EE alters the phosphorylation of AKT in T cell lines (Fig. 1B).

Cells were exposed to various concentrations of estrogens for 5 min to examine the rapid phosphorylation of AKT and PI3K. pAKT was not significantly altered in TIB-152 exposed to BPA, but was decreased at 1×10^{-10} - 10^{-9} M BPS and 1×10^{-8} - 10^{-7} M BPA + E₂. In CCL-119, pAKT was decreased at 1×10^{-11} - 10^{-9} M and 1×10^{-7} M BPA, 1×10^{-10} M E₂, and 1×10^{-10} - 10^{-9} M BPA + E₂.

3.4. Exposure to EE alters pPI3K in T cell line (Fig. 1C)

Regarding the phosphorylation of PI3K, there was a different response in TIB-152 compared to CCL-119. In TIB-152, pPI3K was decreased in response to 1×10^{-10} - 10^{-8} M BPA and 10^{-8} M E₂, but not in CCL-119. pPI3K(P55) was significantly decreased in TIB-152 in almost all conditions except 10^{-12} M BPA and BPS, but not in CCL-119. One of the possible reasons for these different responses is the expression levels of ER α on these cells, as might be expected in cells isolated from patients of different sexes and ages. They are leukemia cell lines derived from two different patients: TIB-152 from a 14 year-old boy and CCL-119 from a 4-year-old girl.

Activation of nongenomic ER signaling via the PI3K pathway activates the kinase AKT/PKB in the developing reproductive tract, which phosphorylates the histone lysine methyltransferase (HKMT/EZH2, the key installer of H3K27me3. pAKT inactivates EZH2, decreasing levels of H3K27 methylation. There are eight isoforms of PI3K in mammals. Class I PI3Ks are activated by membrane-bound receptors. The class IA PI3Ks catalytic unit (p110 α , p110 β , or p110 δ) is complexed to one of five regulatory subunits, p85s. The p85s have SH2 domains, which allow the p85/p110 complex to become recruited to phosphor-tyrosine residues upon activation by tyrosine kinase signaling. Walker proposed signaling streams leading EE-induced epigenetic changes that alter gene expression (Walker 2011). Bredfeldt et al. have identified a novel role for rapid ER signaling whereby

PI3K/AKT signaling to EZH2 modulates the epigenome through phosphorylation of the histone methyltransferase and reduction of H3K27me3 methylation (Bredfeldt et al., 2010). They reported a novel role for rapid ER signaling whereby PI3K/AKT signaling reduces H3K27me3 methylation in the MCF-7 breast cancer cell line. They also reported increased pAKT in response to 1×10^{-8} M BPA exposure on human prostate cells (Wang et al., 2016). The pathway of rapid ER signaling via PI3K/AKT was decreased in TIB-152 cells and, to some extent, in CCL-119 cells in our analyses. We examined the alterations of PI3K/AKT and EZH2 signaling with phosphorylation and epigenetic histone regulation by EE exposure on these T cell lines, and we observed decreased pPI3K/pAKT and altered pEZH2 and H3K27me3 in response to the various concentrations of EEs, and in combinations with E₂. It seems the pathway is not the same pathway in the T cell line as described previously in other cell types, such as MCF-7 cells, especially in the early phase. It opens the possibility of different signaling pathways in human T lymphocytes in the response of estrogens to cause alteration of pZDH2 and H3K27me3.

Conclusion

We observed the altered pAKT/pPI3K, pEZH2, and H3K27me3 in ER positive human T cell lines in response to estrogens. The pathways of rapid ER signaling on human T cells may differ from those on reproductive tissue cells, and further details need to be examined.

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Declarations of interests:

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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Highlights

- We observed the altered pAKT/pPI3K and H3K27me3 in human T cell lines with estrogen receptor α (ER α) in response to endogenous and exogenous estrogens.
- The pathway of rapid ER signaling on these cells is likely different from reproductive cells.

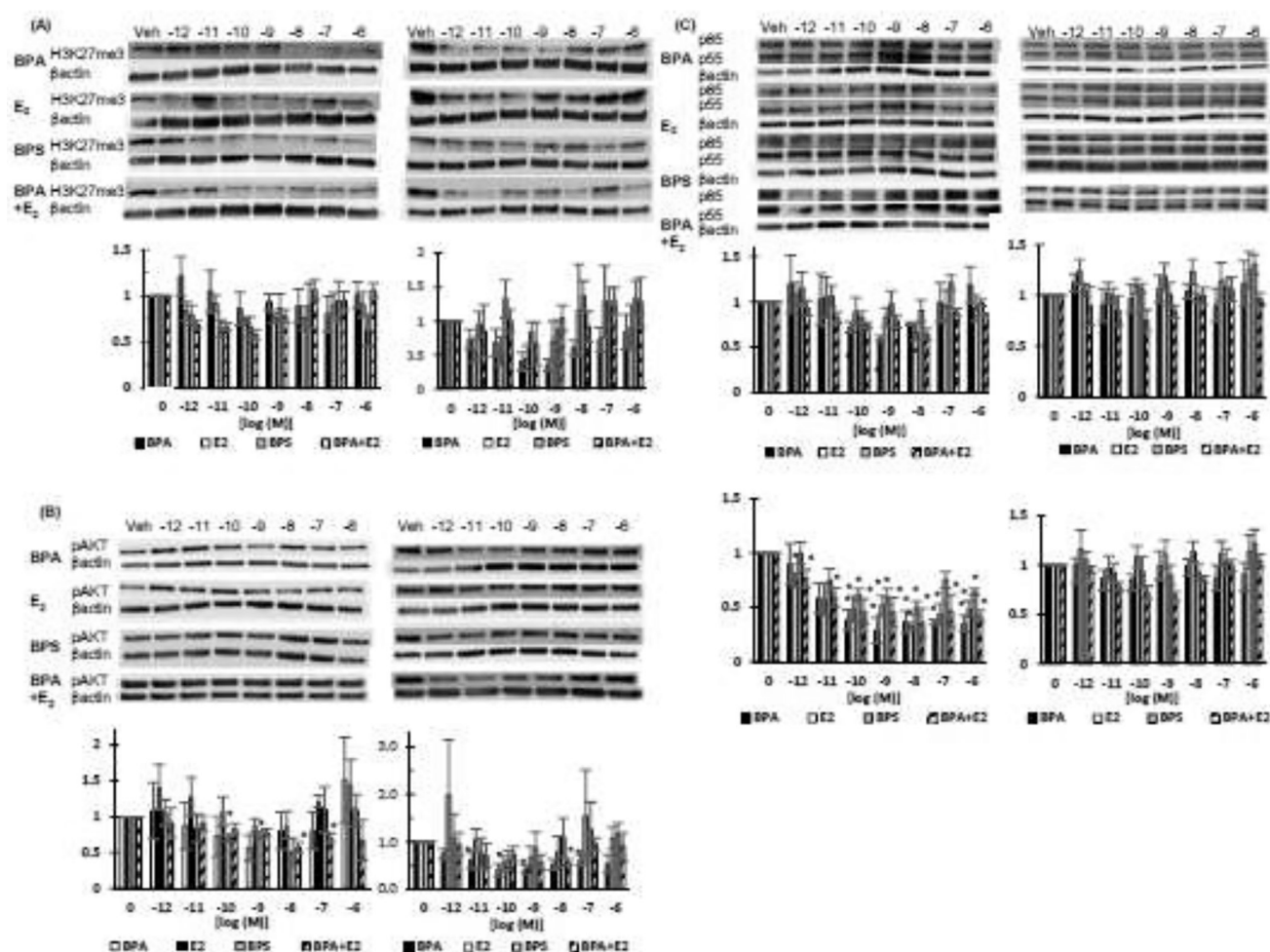


Fig. 1. Exposure to BPA, BPS and E₂ alters (A) H3K27me3, (B) pAKT, and (C) pPI3K. Left: TIB-152 and Right: CCL-cell lines. The alteration after exposure to various concentrations of estrogens for H3K27me3 (120 min), pAKT (5 min) and pPI3K (5 min) were analyzed by WB. The representative image and summary of 6–7 separate experiments were shown. Bar graphs were relative intensity of target protein/β-actin/Vehicle. Mean ± SE were shown. *: $p < 0.05$.