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Interleukin-24 as a target cytokine of environmental aryl hydrocarbon receptor agonist exposure in the lung

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ABSTRACT

Exposure to environmental aryl hydrocarbon receptor (AhR) agonists, such as halogenated aromatic hydrocarbons and polycyclic aromatic hydrocarbons (PAHs), has great impacts on the development of various lung diseases. As emerging molecular targets for AhR agonists, cytokines may contribute to the inflammatory or immunotoxic effects of environmental AhR agonists. However, general cytokine expression may not specifically indicate environmental AhR agonist exposure. By comparing cytokine and chemokine expression profiles in human lung adenocarcinoma cell line CL5 treated with AhR agonists and the non-AhR agonist polychlorinated biphenyl (PCB) 39, we identified a target cytokine of environmental AhR agonist exposure of in the lungs. Thirteen cytokine and chemokine genes were altered in the AhR agonists-treated cells, but none were altered in the PCB39-treated cells. Interleukin (IL)-24 was the most highly induced gene among AhR-modulated cytokines. Cotreatment with AhR antagonist completely prevented IL-24 induction by AhR agonists in the CL5 cells. Knockdown AhR expression with short-hairpin RNA (shRNA) significantly reduced benzo[*a*]pyrene (BaP)-induced *IL-24* mRNA levels. We further confirmed that gene transcription, but not mRNA stability, was involved in *IL-24* upregulation by BaP. Particulate matter (PM) in the ambient air contains some PAHs and is reported to activate AhR. Oropharyngeal aspiration of PM significantly increased IL-24 levels in lung epithelia and in bronchoalveolar lavage fluid of mice 4 weeks after treatment. Thus, our data suggests that IL-24 is a pulmonary exposure target cytokine of environmental AhR agonists.

1. Introduction

Some environmental toxicants, such as halogenated aromatic hydrocarbons and polycyclic aromatic hydrocarbons (PAHs), are aryl hydrocarbon receptor (AhR) agonists, which can activate the AhR pathway. Particulate matter (PM) in the air contains various PAHs (Brook et al., 2010), some of which are AhR agonists. PM exposure could increase AhR activity in a cell-based reporter assay (Kennedy et al., 2010). Certain environmental AhR agonists have caused particular concern because of their association with lung cancer risk (Shen et al., 2014).

AhR, a ligand-activated member of the Per-Arnt-Sim family of basic helix-loop-helix transcription factors (Bersten et al., 2013), is a receptor for environmental toxicants such as dioxins, polychlorinated biphenyls (PCBs), and PAHs. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) and benzo[*a*]pyrene (BaP) are the most well-studied environmental AhR agonists. PCBs are mainly divided into two categories: coplanar and noncoplanar. Coplanar PCBs (e.g., PCB126) are AhR agonists, whereas noncoplanar PCBs (e.g., PCB39) are not. Lipophilic AhR ligands enter cells through simple diffusion. After binding to the AhR occurs in the cytosol, the liganded AhR dissociates from heat shock protein 90 and p23, translocates from the cytosol to the nucleus, heterodimerizes with the AhR nuclear translocator, and binds to an enhancer sequence—referred to as a dioxin response elements (DREs)—within an array of target genes encoding for phase I and II metabolizing enzymes (e.g., CYP1A1) (Murray et al., 2014). The pleiotropic toxicities of PAHs are associated with AhR activation, which leads to altered expression of target genes (N'Diaye et al., 2006).

Several studies have reported that the respiratory system is sensitive to AhR agonists, which might cause chronic bronchitis, allergic asthma, COPD, and lung cancer (Brandt, 1975; Chiba et al., 2011, 2012). AhR is highly expressed in bronchial epithelial cells; hence, AhR has many physiological roles in the lung, modulating cell proliferation and differentiation, cell–cell adhesion interaction, cytokine expression, mucin production, and xenobiotic metabolism (Wong et al., 2010; Chiba et al., 2011). On the other hand, the activation of AhR by environmental AhR agonists has various downstream effectors that influence lung

Abbreviations: AhR, aryl hydrocarbon receptor; PAHs, polycyclic aromatic hydrocarbons; PCB, polychlorinated biphenyl; IL, interleukin; BaP, benzo[*a*]pyrene; shRNA, short-hairpin RNA; PM, particulate matter.

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tumorigenesis, inflammation, DNA adducts formation, cell proliferation, and cell–cell adhesion loss (Tsay et al., 2013). Furthermore, some cytokines are emerging as molecular targets for AhR agonists; these cytokines may contribute to the adverse inflammatory or immunotoxic effects of exposure to PAHs (Vandebriel et al., 1998; Lecureur et al., 2005; Fardel, 2013). However, these cytokines expression, in general, may not represent specific indicators of environmental AhR agonist exposure. Although the CYP1 family is relatively specifically induced through AhR activation, CYP1 proteins cannot be easily detected in human specimens such as bronchial exhaled breath condensate, serum, or urine. By contrast, cytokines can be readily detected in bronchial exhaled breath condensate or serum. This study aimed to identify a cytokine specific for environmental AhR agonist exposure in human lung cells. By using cytokine and chemokine real-time reverse transcription polymerase chain reaction (RT-PCR) array, we identified that, among AhR agonist-modulated cytokines, *IL-24* mRNA showed the highest increase in human lung cancer and macrophage cell lines. Moreover, we observed that PM increased IL-24 expression and secretion in vitro and in vivo. Our results suggest that IL-24 can be applied as a target cytokine of pulmonary exposure to environmental AhR agonists.

2. Materials and methods

2.1. Materials

The AhR ligand BaP, actinomycin D and thiazolyl blue tetrazolium blue (MTT) were purchased from Sigma-Aldrich, Inc. (St Louis, MO, USA). TCDD (ULTRA Scientific, Kingston, RI, USA) was dissolved in dimethyl sulfoxide (DMSO) and stored in the dark at −20 °C until use. The AhR ligand PCB126 and non-AhR ligand PCB39 were obtained from AccuStandard, Inc. Standard Reference Material 2786 (SRM2786) for fine PM was purchased from the National Institute of Standard and Technology (NIST; Gaithersburg, MD, USA). Bovine serum albumin, RPMI1640 medium, BEBM medium, penicillin-streptomycin, L-glutamine, nonessential amino acids (NEAAs), and sodium pyruvate were obtained from Invitrogen, Inc. (Hayward, CA, USA). IL-24 antibody and IL-24 enzyme-linked immunosorbent assay (ELISA) were acquired from R&D System, Inc. (Minneapolis, MN, USA). CYP1A1 antibody was purchased from Santa Cruz Biotechnology, Inc. (Dallas, TX, USA). IL-24 was obtained from PEPROTECH (Rocky Hill, NJ, USA). The cytokine and chemokine RT2 profiler PCR array was purchased from QIAGEN, Inc. (Valencia, CA, USA).

2.2. Cell culture

The human lung adenocarcinoma cell lines CL5 and H1355, human bronchial epithelial cell line BEAS-2B, and human acute monocytic leukemia cell line THP-1 were used in this study. CL5 cells were grown as a monolayer with an epithelial morphology and cultured in RPMI1640 medium with 2 mM L-glutamine, 1.5 g/L sodium bicarbonate, 0.1 mM NEAAs, 1.0 mM sodium pyruvate, and 10% fetal bovine serum (FBS). H1355 cells were cultured in RPMI-1640 with 5% FBS. BEAS-2B immortalized with SV40 (American Type Culture Collection, Manassas, VA, USA) were maintained in serum-free LHC-9 medium (BioSource

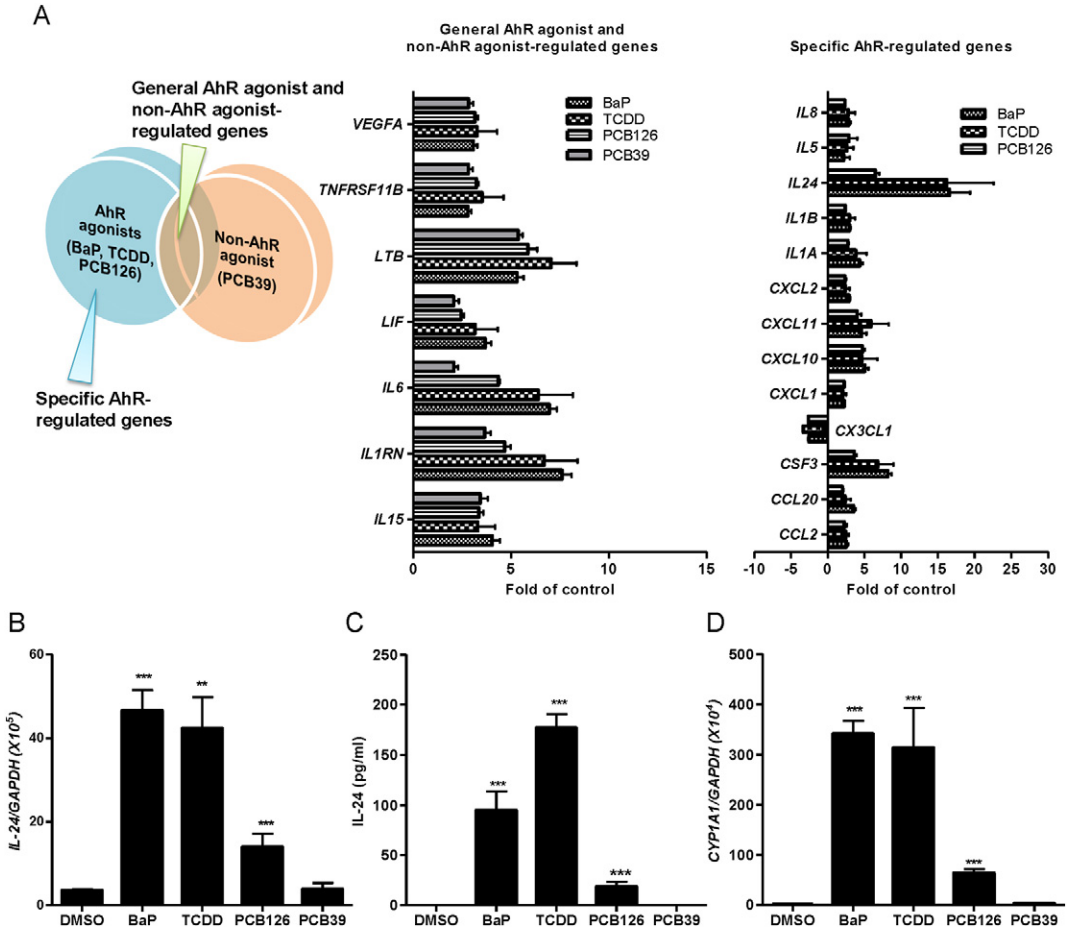

Fig. 1. Identification of specific AhR-regulated cytokine and chemokine genes in human lung cancer cells. CL5 cells were treated with AhR agonists with four repetitive treatments for 24 h. RT-PCR array (A), real-time RT-PCR (B and D), and ELISA (C) results, compared with control group and AhR agonists. (A) The general AhR agonist and non-AhR agonist-regulated genes and specific AhR-regulated genes changed were more than two folds relative to the DMSO vehicle control and p value < 0.05. $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$, compared with DMSO control group.