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Exposure to coplanar PCBs induces endothelial cell inflammation through epigenetic regulation of NF-κB subunit p65

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ABSTRACT

Epigenetic modifications of DNA and histones alter cellular phenotypes without changing genetic codes. Alterations of epigenetic marks can be induced by exposure to environmental pollutants and may contribute to associated disease risks. Here we test the hypothesis that endothelial cell dysfunction induced by exposure to polychlorinated biphenyls (PCBs) is mediated in part though histone modifications. In this study, human vascular endothelial cells were exposed to physiologically relevant concentrations of several PCBs congeners (e.g., PCBs 77, 118, 126 and 153) followed by quantification of inflammatory gene expression and changes of histone methylation. Only exposure to coplanar PCBs 77 and 126 induced the expression of histone H3K9 trimethyl demethylase jumonji domain-containing protein 2B (JMJD2B) and nuclear factor-kappa B (NF-κB) subunit p65, activated NF-κB signaling as evidenced by nuclear translocation of p65, and up-regulated p65 target inflammatory genes, such as interleukin (IL)-6, C-reactive protein (CRP), intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and IL-1α/β. The increased accumulation of JMJD2B in the p65 promoter led to a depletion of H3K9me3 repression mark, which accounts for the observed up-regulation of p65 and associated inflammatory genes. JMJD2B gene knockdown confirmed a critical role for this histone demethylase in mediating PCB-induced inflammation of the vascular endothelium. Finally, it was determined, via chemical inhibition, that PCB-induced up-regulation of JMJD2B was estrogen receptor-alpha (ER-α) dependent. These data suggest that coplanar PCBs may exert endothelial cell toxicity through changes in histone modifications.

1. Introduction

Epigenetic marks, such as DNA methylation and histone modifications, control gene expression capable of being passed on to daughter cells upon cell division without changing the genetic code (Fraga et al., 2005; Kaminsky et al., 2009; Heijmans et al., 2008). Changes in epigenetic marks have been shown to be induced by exposure to various environmental pollutants. Some of these alterations are associated with a broad range of diseases, including cancer, cardiovascular diseases (CVD), or metabolic and reproductive disorders (Cortessis et al., 2012; Baccarelli and Bollati, 2009; Lind et al., 2012). Evidence suggests that persistent organic pollutants such as polychlorinated biphenyls (PCBs), and especially dioxin like (or coplanar) PCBs, can cause epigenetic changes in global DNA methylation, histone modifications in the gene promoters, and expression of corresponding histone modifying enzymes (Desaulniers et al., 2009; Ovesen et al., 2011; Casati et al., 2012, 2013). These epigenetic mechanisms may link the environmental exposures to disease outcomes by shaping the genome into active or inactive structures based on endogenous and exogenous environmental changes.

Evidence suggests that the pathology of CVD is associated with exposure to environmental pollutants (Goncharov et al., 2008; Sergeev and Carpenter, 2010; Perkins et al., 2015). For example, increased incidence rates of CVD have been demonstrated in populations exposed to high levels of dioxin and PCBs (Dalton et al., 2001; Gustavsson and Hogstedt, 1997; Ha et al., 2007). Endothelial dysfunction is believed to be an underlying cause of the initiation of CVD such as atherosclerosis (Ross, 1999). Exposure to PCBs has also been shown to cause inflammation of the vascular endothelium via expression of several inflammatory markers, cytokines and adhesion molecules (Eske et al., 2014; Han et al., 2010; Majkova et al., 2009). Thus, it is of great importance to investigate the underlying mechanisms in the regulation of these inflammatory markers.

Abbreviations: AP-1, activator protein-1; ARs, androgen receptors; ChIP, chromatin immunoprecipitation; CRP, C-reactive protein; CVD, cardiovascular disease; DAPI, 4,6-diamidino-2-phenylindole; EDCs, endocrine-disrupting chemicals; ERs, estrogen receptors; ERE, estrogen responsive element; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; H3K4me3, K4-trimethylated histone H3; H3K9me3, K9-trimethylated histone H3; ICAM-1, intercellular adhesion molecule-1; IL, interleukin; JMJD2B, jumonji domain-containing protein 2B; K, lysine; MLL2, mixed-lineage leukemia 2; NF-κB, nuclear factor-kappa B; PBS, phosphate buffered saline; PCBs, polychlorinated biphenyls; qRT-PCR, quantitative real-time PCR; siRNA, small interfering RNA; TNF-α, tumor necrosis factor alpha; VCAM-1, vascular cell adhesion molecule-1.

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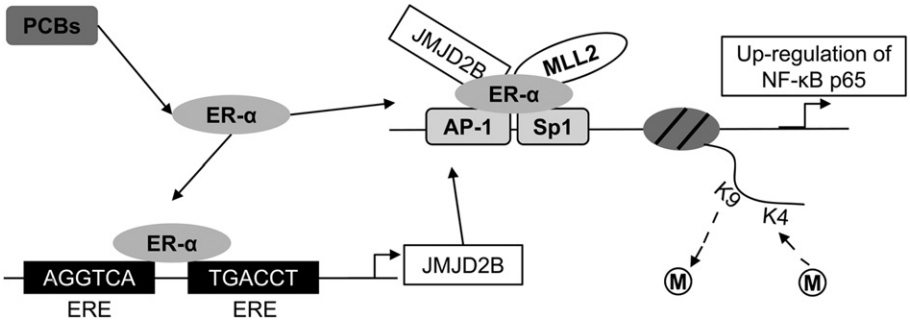

Fig. 1. PCB-induced epigenetic regulation of NF-κB subunit p65. This diagram illustrates the pathways for PCB-induced epigenetic regulation of NF-κB subunit p65. In response to PCBs, ER-α is activated to bind to the EREs in the first intron of *JMJD2B* and induce its expression. JMJD2B can physically interact with ER-α and MLL2 to form a protein complex and be further recruited to the *p65* promoter through AP-1 or Sp1 mediated chromatin binding. This ER-α/JMJD2B/MLL2 complex modifies histone methylation patterns in the promoter region.

Moreover, PCBs act as endocrine-disrupting chemicals (EDCs), which interfere with the endocrine system by acting as agonists or antagonists to hormone receptors, such as estrogen receptors (ERs), androgen receptors (ARs), or thyroid hormone receptors (Connor et al., 1997; Jansen et al., 1993; Kester et al., 2000; Schrader and Cooke, 2003; Zoeller et al., 2002). Previous studies indicate that PCBs have an affinity for ERs and exhibit significant biological activity and disrupt endogenous ER signaling (Bergeron et al., 1994; Gierthy et al., 1997; Sumpter, 1998; Matthews et al., 2007; Abdelrahim et al., 2006). For example, PCB 77 is capable of binding to ER-α and exerting agonistic or antagonistic effects in endothelial cells while PCB 126 exhibits a strong estrogen-like effect by inducing the expression of estrogen receptor α (ER-α) and promoting ER-α-mediated transcription (Abdelrahim et al., 2006; Tavolari et al., 2006; Gjernes et al., 2012). Importantly, ER-α mediates the biological functions of estrogen and regulates transcriptional expression of ER target genes through binding to estrogen responsive elements (EREs) (Robinson-Rechavi et al., 2003; Klinge, 2001). The jumonji domain-containing protein 2B (JMJD2B), a histone H3K9 trimethyl demethylase, contains two half-ERE sites located in the first intron of its gene, suggesting that *JMJD2B* itself is transcriptionally targeted by ER-α (Carroll et al., 2006; Fodor et al., 2006; Yoshioka et al., 2009; Shi et al., 2011). In addition, JMJD2B is physically associated with ER-α and mixed-lineage leukemia 2 (MLL2), a methyltransferase required for H3K4 trimethylation (Shi et al., 2011; Denissov et al., 2014). It also has been shown that ER-α/JMJD2B/MLL2 complex defines H3K4 and K9 methylation patterns in transcription of target genes (Shi et al., 2011). Nuclear factor-kappa B (NF-κB) subunit *p65* may be a transcriptional target of ER-α/JMJD2B/MLL2 complex as well, although its promoter does not contain EREs. This transcriptional regulation may be due to ER-α's ability to interact with regulatory elements of target genes indirectly by associating with activator protein-1 (AP-1) and Sp1 transcription factor complexes and their respective binding sites (Robinson-Rechavi et al., 2003; Klinge, 2001; Dahlman-Wright et al., 2012; Kushner et al., 2000; Li et al., 2001; Saville et al., 2000). Importantly, there are consensus binding sites of AP-1 and Sp1 transcription factors in the *p65* promoter (Ueberla et al., 1993). Thus, ER-α may function as a mediator in the formation of complexes by histone modifying enzymes and transcription factors. The inflammatory target genes of p65 subunit, such as *interleukin* (*IL-6*), *C-reactive protein* (*CRP*), *intercellular adhesion molecule-1* (*ICAM-1*), *vascular cell adhesion molecule-1* (*VCAM-1*), *IL-1α*, and *IL-1β* (Mori and Prager, 1996; Hiscott et al., 1993; Son et al., 2008; Libermann and Baltimore, 1990; Shimizu et al., 1990; van de Stolpe et al., 1994; Bunting et al., 2007; Iademarco et al., 1992; Zhang et al., 1995; Agrawal et al., 2003a, 2003b), produce critical inflammatory cytokines that are involved in the initiation and progression of CVD (Incalcaterra et al., 2013). Taken together, a coherent model has been proposed to implicate PCBs in inflammatory gene regulation through ER-α-mediated epigenetic mechanisms (Fig. 1).

The aim of the current study is to investigate the epigenetic mechanisms that link PCBs and vascular inflammation. In particular, we have demonstrated (a) PCB-induced expression of *JMJD2B*; (b) PCB-activated NF-κB signaling; (c) the role of JMJD2B in PCB-induced *p65* transcription; and (d) implication of ER-α in mediating PCB toxicity.

2. Material and Methods

2.1. Cell cultures and treatments

EA.hy926 human endothelial cells (ATCC, Manassas, VA) were cultured in Dulbecco's Modified Eagle's Medium (DMEM; Invitrogen, Carlsbad, CA) supplemented with 10% fetal bovine serum (FBS; Hyclone, Logan, UT) as described previously (Lim et al., 2007). Cells were

Table 1
Oligonucleotide primers used for quantitative real-time PCR.

Gene[a]	Sequence (5′–3′)	F/R[b]	Template
CRP	GCCCTTCAGTCCTAATGTCCTG	F	cDNA
	AGCATAGTTAACGAGCTCCCAGA	R	
ER-α	CTTAATTCTGGAGTGTACACAT	F	cDNA
	CTCCATGCCTTTGTTACTCAT	R	
GAPDH	TCCACTGGCGTCTTCACC	F	cDNA
	GGCAGAGATGATGACCCTTT	R	
GAPDH	CAAGACCTTGGGCTGGGACTGGCTGA	F	gDNA
	GATGCGGCTGACTGTCGAACAGGAGGA	R	
ICAM-1	GCCACCCCAGAGGACAA	F	cDNA
	CCATTATGACTGCGGCTGCTA	R	
IL-1α	ACAAAAGGCGAAGAAGACTGA	F	cDNA
	GGAACTTTGGCCATCTTGAC	R	
IL-1β	CTGTCCTGCGTGTTGAAAGA	F	cDNA
	TTGGGTAATTTTTGGGATCTACA	R	
IL-6	CAATGAGGAGACTTGCCTGGTGA	F	cDNA
	TGGCATTTGTGGTTGGGTCAG	R	
JMJD2B	CCAACAGCGAGAAGTACTGTA	F	cDNA
	CCACGTCGTCATCATACAA	R	
JMJD2B	TCACAGCTGGAATGGTGGT	F	gDNA
	CACCTCAGGCCCTCAACA	R	
p65	GGCCATGGACGAACTGTTCC	F	cDNA
	GAGGGTCCTTGGTGACCAG	R	
p65	CAGTTTCCCCTCTGGGTGGA	F	gDNA
	CCCACCTCCCTCCAGAGA	R	
VCAM-1	GTCTTGGTCAGCCCTTCCT	F	cDNA
	ACATTCATATACTCCCGCATCCTTC	R	

[a] Genbank entries: CRP = *Homo sapiens c-reactive protein* (NM_000567.2 for cDNA); ER-α = *Homo sapiens estrogen receptor alpha* (NM_000125.3 for cDNA); GAPDH = *Homo sapiens glyceraldehyde-3-phosphate dehydrogenase* (NM_002046.5 for cDNA, NC_000012.11 for gDNA); ICAM-1 = *Homo sapiens intercellular adhesion molecule 1* (NM_000201.2 for cDNA); IL-1α = *Homo sapiens interleukin-1 alpha* (NM_000575.3 for cDNA); IL-1β = *Homo sapiens interleukin-1 beta* (NM_000576.2 for cDNA); IL-6 = *Homo sapiens interleukin-6* (NM_000600.3 for cDNA); JMJD2B (also known as KDM4B) = *Homo sapiens lysine (K)-specific demethylase 4B* (NM_015015.2 for cDNA, NC_000019.10 for gDNA); p65 (also known as RelA) = *Homo sapiens v-rel avian reticuloendotheliosis viral oncogene homolog A* (NM_021975.3 for cDNA, NC_000011.9 for gDNA); VCAM-1 = *Homo sapiens vascular cell adhesion molecule 1* (NM_001078.3 for cDNA).

[b] Abbreviations: cDNA, complementary DNA (for mRNA quantification); F, forward; gDNA, genomic DNA (for ChIP assays); R, reverse.