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Robert N. Helsley
University of Kentucky, robert.helsley@uky.edu

Changcheng Zhou
University of Kentucky, c.zhou@uky.edu

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REVIEW ARTICLE

Epigenetic impact of endocrine disrupting chemicals on lipid homeostasis and atherosclerosis: a pregnane X receptor-centric view

Robert N. Helsley[1,2] and Changcheng Zhou[1,*]

[1]Department of Pharmacology and Nutritional Sciences, Center for Metabolic Disease Research, University of Kentucky, Lexington, KY 40536, USA and [2]Department of Cellular and Molecular Medicine, Cleveland Clinic Lerner Research Institute, Cleveland, OH 44195, USA

*Correspondence address. Department of Pharmacology and Nutritional Sciences, Center for Metabolic Disease Research, University of Kentucky, 900 South Limestone, 517 Wethington Building, Lexington, KY 40536, USA. Tel: +1-859-218-1801; Fax: +1-859-257-3646; E-mail: c.zhou@uky.edu

Abstract

Despite the major advances in developing diagnostic techniques and effective treatments, atherosclerotic cardiovascular disease (CVD) is still the leading cause of mortality and morbidity worldwide. While considerable progress has been achieved to identify gene variations and environmental factors that contribute to CVD, much less is known about the role of "gene–environment interactions" in predisposing individuals to CVD. Our chemical environment has significantly changed in the last few decades, and there are more than 100,000 synthetic chemicals in the market. Recent large-scale human population studies have associated exposure to certain chemicals including many endocrine disrupting chemicals (EDCs) with increased CVD risk, and animal studies have also confirmed that some EDCs can cause aberrant lipid homeostasis and increase atherosclerosis. However, the underlying mechanisms of how exposure to those EDCs influences CVD risk remain elusive. Numerous EDCs can activate the nuclear receptor pregnane X receptor (PXR) that functions as a xenobiotic sensor to regulate host xenobiotic metabolism. Recent studies have demonstrated the novel functions of PXR in lipid homeostasis and atherosclerosis. In addition to directly regulating transcription, PXR has been implicated in the epigenetic regulation of gene transcription. Exposure to many EDCs can also induce epigenetic modifications, but little is known about how the changes relate to the onset or progression of CVD. In this review, we will discuss recent research on PXR and EDCs in the context of CVD and propose that PXR may play a previously unrealized role in EDC-mediated epigenetic modifications that affect lipid homeostasis and atherosclerosis.

Key words: pregnane X receptor (PXR); epigenetics; cardiovascular disease (CVD); nuclear receptors; endocrine disrupting chemicals (EDCs)

Introduction

Atherosclerotic cardiovascular disease (CVD) is a leading cause of mortality and morbidity worldwide [1]. Atherosclerosis is a chronic inflammatory disease characterized by the accumulation of lipids and inflammatory molecules in the subendothelium of the artery, which can restrict blood flow and ultimately lead to myocardial infarction and stroke. Atherosclerosis is a complex chronic disease involving the interaction of genetic and environmental factors over multiple years. Some people are at a greater risk for developing CVD, and these risk factors can be classified into either modifiable or non-modifiable. Non-modifiable risk factors for developing CVD are age, gender, and genetic heritability [2]. Modifiable CVD risk factors include smoking, obesity, nutrition, hypertension, sedentary lifestyle, stress, and hyperlipidemia [2]. Despite detection of atherosclerotic lesions in young children, the progression of atherosclerosis was long considered to begin during adolescence [3]. Interestingly, studies have also revealed factors influencing CVD risk of the offspring. The number and size of fatty streaks were significantly increased in fetal aortas from hypercholesterolemia mothers during pregnancy [4]. In the 'Fate of Early Lesions in Children' (FELIC) study, aortic lesions progressed much faster in children from hypercholesterolemic mothers than in normocholesterolemic mothers, even though both groups of children had similar lipid profiles [3, 5]. Furthermore, offspring from smokers had an adverse lipoprotein profile compared with adults who had not been exposed *in utero* [6, 7], suggesting that environmental factors can contribute to the CVD risk of the exposed individuals' offspring. While considerable progress has been achieved to identify risk factors that contribute to CVD, the role played by "gene–environment interactions" in predisposing individuals to CVD remains relatively unexplored.

Endocrine Disrupting Chemicals in CVD

The chemical environment to which we are exposed has significantly changed in the past few decades, and mounting evidence has shown that endocrine disrupting chemicals (EDCs) can interfere with an organism's complex endocrine signaling mechanisms and result in adverse consequences [8–11]. The US Environmental Protection Agency defines an EDC as, "an exogenous agent that interferes with synthesis, secretion, transport, metabolism, binding action, or elimination of natural blood-borne hormones that are present in the body and are responsible for homeostasis, reproduction, and developmental process" [11]. A few examples of compounds that have been identified as EDCs are polychlorinated biphenyls (PCBs), many pesticides, the plastic base compound bisphenol A (BPA) and its structural analogs, and numerous plasticizers such as phthalates and phthalate substitutes [11]. It was originally thought that EDCs act primarily through nuclear hormone receptors; however, it is now widely accepted that EDCs act through a variety of signaling mechanisms, which include but are not limited to nuclear steroid receptors, nonsteroid receptors, orphan receptors, epigenetic modifications, and enzymatic pathways ultimately responsible for maintaining endocrine homeostasis [11].

Although research on the health effects of EDCs in humans initially focused primarily on reproduction and development, recent findings have implicated EDC exposure to cardiometabolic disease risk [11–25]. Sergeev *et al.* demonstrated that people living within close proximity to sites contaminated with organic pollutants compared to sites not containing any

identified hazardous waste had a 20% increase in acute myocardial infarction hospital discharge rates [26]. Exposure to PCBs induced hypercholesterolemia and promoted atherosclerosis in animals [27–29]. Circulating PCB levels have been associated with atherosclerotic plaques in the elderly [30]. BPA, a base chemical used extensively in polycarbonate plastics in many consumer products, has recently been associated with CVD in several large and well-controlled cross-sectional and longitudinal studies [13–15]. Independent studies have associated BPA exposure with coronary atherosclerosis [31], carotid atherosclerosis [32], and peripheral arterial disease [33], a subclinical measure of atherosclerotic vascular disease. Consistently, BPA exposure in elderly patients associates with inflammatory gene expression [34]. These associations are independent of traditional CVD risk factors including body mass index, blood pressure, lipid concentrations, and levels of physical activity [13, 15]. In addition to BPA, several phthalates and phthalate metabolites have been associated with atherosclerosis. Phthalate metabolites are associated with increased atherosclerosis in the elderly [32]. However, the underlying mechanisms responsible for these associations remain elusive, which continues to hamper rational assessment of the health risks of EDC exposure.

A multitude of genetic, nutritional, and environmental factors influence whether an individual is at risk of developing obesity and metabolic disorders that affect CVD risk. Several groups have shown that EDCs may act as "obesogens" to increase the risk of obesity and diabetes [23, 35–38]. For example, organotin compounds such as tributyltin chloride can induce the differentiation of adipocytes *in vitro* and increase adipose mass *in vivo* [22]. Recent studies demonstrated that exposure to benzyl butyl phthalate (BBP) enhanced adipogenesis thereby contributing to EDC-elicited adiposity [36]. Consistently, BPA treatment enhanced hepatic fat deposition accompanied by elevation of hepatic inflammation in CD-1 mice [39]. Exposure of *p,p′*-dichlorodiphenyldichloroethylene in rats exacerbates many aspects of the metabolic syndrome, including dyslipidemia, glucose intolerance, and hypertension [37]. Verbanck *et al.* [35] described a potential role of BPA, bisphenol F, and bisphenol S in altering global RNA expression in human subcutaneous adipocytes. Collectively, these studies clearly demonstrate a potential role for EDCs to contribute to the development of cardiometabolic disease risk in humans.

Xenobiotic Receptor PXR and EDCs

Numerous EDCs including organochlorine and organophosphate pesticides, alkylphenols, phthalates, PCBs, and BPA and its analogs have been identified to activate the nuclear receptor pregnane X receptor (PXR) which functions as a xenobiotic sensor to regulate xenobiotic metabolism [40–46] (Table 1). The ability of organisms to adapt to their surroundings and possess a host defense mechanism against toxic chemicals is essential to maintain life. PXR, also known as steroid and xenobiotic receptor or SXR, was identified by three independent groups in 1998 [47–49] and was subsequently designated as NR1I2 (nuclear receptor subfamily 1, group 1, member 2) in the standard nomenclature [50]. Despite the different designations, this receptor functions as a steroid and xenobiotic sensor to regulate xenobiotic metabolism. Like other nuclear receptors, PXR contains both an N'-terminal DNA-binding domain and a C'-terminal ligand-binding domain (LBD), which are indispensable for DNA binding and ligand binding as well as interaction with co-regulators. PXR is activated by numerous endogenous hormones, dietary steroids, pharmaceutical agents, and xenobiotic

Table 1: EDCs that modulate human and/or rodent PXR activity

EDCs	Human	Rodent	References
2-acetylaminofluorene	+	+	[183]
2-(4′-hydroxyphenyl)-2-phenylpropane	+	−	[42]
4-nonylphenol	+	ND	[184]
4-octylphenol	+	ND	[184]
α-hexachlorocyclohexane	+	ND	[184]
β-napthoflavone	+	ND	[184]
Aldrin	+	−	[185]
Alachlor	+	+	[186, 187]
Acetyl tributyl citrate	+	+	[76, 99]
Acetyl triethyl citrate	+	ND	[99]
Androstanol	+	−	[188]
Bisphenol A (BPA)	+	−	[42, 45]
Bisphenol B (BPB)	+	−	[42]
Benzophenone	−	+	[187]
Bisphenol AF (BPAF)	+	−	[42]
Brominated flame retardants (BDE) 28	+	ND	[184]
BDE 47	+	ND	[184]
BDE 99	+	ND	[184]
BDE 100	+	ND	[184]
BDE 153	+	ND	[184]
BDE 209	+	ND	[184]
Chlordecone	+	−	[185]
Chlorpyrifos	+	−	[185]
Cypermethrin	+	−	[185]
Cyperoterone acetate	−	+	[189]
Decamethylcyclopentasiloxane	+	ND	[184]
Dibutyl phthalate	+	+	[190]
Dichlorodiphenyltrichloroethane	+	−	[184, 185]
o,p′-Dichlorodiphenyltrichloroethane	+	+	[185, 191]
Dichlorodiphenyldichloroethylene	+	+	[184, 192]
di(2-ethylhexyl) phthalate (DEHP)	+	+	[44, 45, 76]
Dieldrin	+	−	[184, 185]
diethyl phthalate (DEP)	+	ND	[182]
Diflubenzuron	+	−	[186]
Digoxin	+	−	[45]
di-isobutyl phthalate (DiBP)	+	ND	[182]
diisononyl phthalate (DiNP)	+	ND	[76, 182]
di-n-butyl phthalate (DnBP)	+	ND	[99, 182]
Endosulfan	+	−	[184, 185]
Endrin	+	−	[185]
Fenarimol	+	−	[186]
Fenbuconazole	+	−	[186]
Fenvalerate	+	−	[185]
Fipronil	+	−	[186]
Hexabromocyclododecane (HBCD) mix: α-HBCD (10%); β-HBCD (9%); γ-HBCD (81%)	+	ND	[184]
Hexamethylcyclotrisiloxane	+	ND	[184]
Imazalil	+	−	[186]
Isoproturon	+	−	[186]
Lindane	+	+	[184, 185]
MEHP	+	+	[193]
Methoxychlor	−	+	[184, 185, 187]
Metolachlor	+	−	[186]
Myclobutanil	−	+	[194]
Nonylphenol	−	+	[44]
Octamethylcyclotetrasiloxane	+	ND	[184]
Oxadiazon	+	−	[186]
Permethrin	+	−	[186]
PentaBDE mix: BDE 47 (42%); BDE99 (34%); BDE100 (9%); BDE153 (2%); BDE154 (2%)	+	ND	[184]
Pentachlorophenol	+	−	[185]
Perflurononanoic acid	+	ND	[184]
Polybrominated diphenyl ether (PBDE) (47, 99, and 209)	+	+	[195, 196]
Polychlorinated biphenyl (PCB) 47	+	+	[184, 189]

<div style="text-align: right;">continued</div>

Table 1: Continued

EDCs	Human	Rodent	References
PCB 97	+	ND	[184]
PCB 101	+	ND	[184]
PCB 118	+	ND	[184, 197]
PCB 151	+	ND	[184]
PCB 153	+	+	[43, 184, 197, 198]
PCB 170	+	ND	[184]
PCB 183	+	ND	[184]
PCB 184	+	+	[43, 184, 189]
PCB 188	ND	+	[189]
PCB 190	+	ND	[184]
PCB 194	+	ND	[198]
PCB 196	−	+	[43, 189]
PCB 197	+	+	[43]
PCB 200	ND	+	[189]
PCB 201	+	+	[43]
PCB 209	+	+	[43]
Prochloraz	+	−	[186]
Propiconazole	+	+	[186, 194]
Pretilachlor	+	−	[186]
Tetrabromobisphenol A	+	ND	[184]
Trans-nonachlor	+	+	[185, 189, 199]
Triadimefon	−	+	[194]
Tributyl citrate	+	+	[76, 99]
Triclosan	+	−	[197]
Trifluralin	−	+	[187]
Vinclozolin	−	+	[184, 187]

EDCs that activate human or rodent PXR are indicated by "+" and EDCs that do not modulate PXR activity are indicated by "−." ND, not determined.

chemicals (Table 1) [40, 41, 46]. It functions as a heterodimer with the retinoid X receptor (RXR) to modulate gene transcription [51, 52]. PXR was initially characterized based on the observation that ligands activating the receptor can induce cytochrome P450 monooxygenase 3A4 (CYP3A4) expression. Consequently, it was further identified that PXR transcriptionally regulates CYP3A4 [48, 49, 53]. Similar to the CYP enzymes, PXR is highly expressed in the liver and intestine [47–49, 53], two main sites of the host defense against toxic xenobiotics. The discovery of PXR led to the identification of many novel target genes involved in detoxification and transport of lipophilic compounds, such as genes that encode Phase I (e.g. CYP450s) and Phase II (e.g. glutathione transferase) detoxification enzymes, as well as the ABC family transporters [e.g. multidrug resistance 1 (MDR1)]. Collectively, PXR has also been shown to be a critical regulator for other processes, such as modulating lipid levels, atherogenesis, and inflammation.

Since the initial discovery of PXR, many orthologs have been cloned from a wide variety of species, including *Drosophila* [54], *Caenorhabditis elegans* [55], and different types of mammals (e.g. human, mouse, rat, rabbit, monkey) [47–49, 53, 55–58]. In *Drosophila*, the PXR ortholog was identified as DHR96 [54]. In C. *elegans*, three orthologs of PXR are represented as DAF12, NHR-8, and NHR-48 [55]. Compared with most other nuclear receptors, PXR is remarkably divergent across mammalian species with the LBDs sharing only ∼60–80% identity compared with the ∼90% typically exhibited by orthologous nuclear receptors [41, 46]. PXR exhibits significant differences in its pharmacology across species (e.g. mouse vs. human) [41, 48, 59, 60]. The known species differences in CYP3A induction observed *in vivo* appears to be partially attributed to the different pharmacology of PXR. For example, pregnenolone 16α-carbonitrile (PCN), a known CYP3A inducer in rodents, acts as a potent agonist of mouse

and rat PXR but does not activate human PXR [46, 60]. By contrast, rifampicin, a known inducer of CYP3A in humans and rabbits, does not activate mouse PXR [46, 59, 60]. In addition to its species-specific responses, some ligands can activate PXR in a tissue-specific manner [46, 61–63]. For example, rifaximin, a clinically used antibiotic drug, can only activate human PXR in the intestine but not in the liver [62]. Some forms of vitamin E, such as tocotrienols, can selectively regulate PXR target genes in hepatic and intestinal cell lines [46, 61]. Collectively, these studies suggest that PXR can regulate species- and tissue-specific responses to xenobiotic exposure.

A fundamental question concerning all EDC studies is whether low-dose exposure to EDCs can affect human endocrine functions, leading to adverse effects [42]. For example, recent studies have demonstrated that BPA can activate human PXR *in vitro* at relatively high doses (∼2 μM) [42]. However, BPA and analogues have been shown to be able to synergistically activate human PXR *in vitro* [42]. Therefore, combinations of BPA and other EDCs may activate human PXR *in vivo* at a much lower dose. In addition, several groups have measured unconjugated BPA in human urine samples and found that BPA concentrations range from undetectable to 2.5 ng/ml [64–67]. By comparison, mice fed diet supplemented with BPA at a dose of 50 mg/kg for 12 weeks had approximately 2 ng/ml of unconjugated BPA in the urine [66], well within the human range. These animals also exhibited an elevated xenobiotic response with BPA exposure [66]. Many of these EDCs are lipophilic compounds, which can accumulate in adipose tissue; BPA and its analogues, for example, were found in 55% of breast adipose tissue samples from women [68]. It has been predicted that many classes of EDCs accumulate in the lipid droplet of adipocytes, which may result in high concentrations in the fat depot [69, 70]. Therefore, even if an individual EDC may not reach sufficient concentrations to

activate PXR in some tissues, the accumulation of this EDC in certain tissues and the synergistic effects of this and other EDCs may still lead to the activation of PXR.

Role of PXR in Mediating the Adverse Effects of EDCs on CVD

The identification of PXR as a xenobiotic sensor has provided an important tool for the study of new mechanisms through which EDC exposure impacts disease development. While the role of PXR in xenobiotic metabolism has been well studied by many laboratories, recent studies have demonstrated novel functions of PXR in CVD [46, 66, 71–76]. Many clinically relevant PXR ligands have been shown to elevate plasma lipid levels in patients and increase their CVD risk [71, 77–81]. For example, treatment with rifampicin, a PXR ligand used in the clinic for the treatment of tuberculosis, can cause hyperlipidemia [77], and short-term treatment increased the ratio of lathosterol to cholesterol, indicative of increased cholesterol synthesis [82]. Treatment with ritonavir and amprenavir, HIV protease inhibitors and potent PXR activators [75, 83], induced hyperlipidemia and was also associated with increased risk of CVD in HIV patients [78, 79, 84, 85]. Furthermore, a meta-analysis of seven genome-wide association studies found that the common genetic variants in PXR are associated with plasma low-density lipoprotein (LDL) cholesterol levels in humans [73].

PXR has also been demonstrated to have pro-atherogenic effects in multiple animal models [71, 72]. Chronic activation of PXR elicited by feeding mice the mouse PXR ligand PCN led to increased levels of total plasma cholesterol and atherogenic lipoproteins very low-density lipoprotein and LDL in wild-type (WT) mice, but not in PXR-deficient ($PXR^{-/-}$) mice [71], suggesting that the PCN-mediated effects were through PXR signaling. de Haan et al. [74] demonstrated that activation of PXR by PCN treatment increased plasma total cholesterol and very low-density lipoprotein levels in apolipoprotein E (ApoE)*3-Leiden mice which exhibit a human-like lipoprotein distribution on a cholesterol-rich diet. PCN-mediated PXR activation also decreased high-density lipoprotein cholesterol levels in ApoE*3-Leiden cholesteryl ester transfer protein (CETP) transgenic mice [74]. Another study showed that short-term exposure to the HIV protease inhibitor, amprenavir, which activates PXR by oral delivery, significantly increases plasma total cholesterol and LDL cholesterol levels in WT mice [75]. By contrast, amprenavir did not affect plasma cholesterol levels in $PXR^{-/-}$ mice [75], indicating a potential role of PXR in mediating adverse effects on lipid homeostasis of relevant antiretroviral drugs. In addition to dyslipidemia, chronic PXR activation also significantly increased atherosclerotic lesion sizes in $ApoE^{-/-}$ mice [71]. By contrast, PXR loss-of-function decreased atherosclerosis in $ApoE^{-/-}$ mice in part due to decreased fatty acid transporter CD36 expression and CD36-mediated lipid uptake in macrophages [72]. All of the evidence suggests that modulation of PXR can affect lipid metabolism and atherosclerosis in different animal models.

In addition to clinically used drugs such as rifampicin and amprenavir, recent studies have also revealed the role of PXR in mediating EDCs' impact on lipid homeostasis and atherosclerosis. For example, several large-scale human studies indicate that exposure to BPA may increase CVD risk or atherosclerosis development [13–15, 31, 32]. BPA is a weak agonist of the estrogen receptor (ER), and most studies on BPA have focused on its estrogenic effects [86, 87]. However, the estrogenic effects of

BPA may not explain the link between BPA exposure and CVD risk since numerous human and animal studies have demonstrated the protective effects of estrogen against atherosclerosis or CVD [11, 88–91]. In addition to activating ER, BPA and several of its analogs have been identified as potent agonists for PXR [42]. Interestingly, BPA can strongly activate human PXR but does not affect mouse or rat PXR activity [42], indicating the importance of utilizing appropriate animal models to evaluate BPA's risk assessment in humans. To investigate the effects of BPA exposure on atherosclerosis development, a PXR-humanized ApoE-deficient mouse model has been generated [66] and exposure to BPA significantly increased atherosclerosis in mice expressing human PXR, but not in littermates without human PXR [66]. BPA-mediated human PXR activation also increased CD36 expression, lipid accumulation, and foam cell formation in macrophages of PXR-humanized ApoE-deficient mice [66]. In addition to mouse models, Fang et al. also demonstrated that chronic BPA exposure induced metabolic disease and atherosclerosis in hyperlipidemic rabbits [92, 93]. Since BPA can activate rabbit PXR as well as human PXR, it is plausible that PXR signaling may also contribute to those adverse effects in rabbits. Overall, these studies suggest that BPA-mediated PXR activation may potentially accelerate atherosclerosis development in humans, which provide a potential molecular mechanism that links BPA exposure to increased risk of CVD in exposed individuals.

The adverse effects of BPA and several phthalate plasticizers [e.g. di-(2-ethylhexyl)phthalate, DEHP] on human health have attracted considerable attention and engendered controversy in the past few decades, partly due to their endocrine-disrupting properties. In addition to these well-known EDCs, there are many phthalate substitute plasticizers that have not been well studied for endocrine disruption [17, 94–96]. For example, citrate esters, including TBC, acetyl tributyl citrate (ATBC), and triethyl citrate, represent a large group of plasticizers that have been approved by the FDA to be used in food packaging materials, vinyl toys, medical devices, cosmetics, and pharmaceutical drugs [94, 95, 97, 98]. Several citrate esters including TBC and ATBC have been identified as potent and selective PXR agonists [76, 99] (Table 1). Similar to rifaximin, TBC activates intestinal PXR but does not affect hepatic PXR activity [76]. Interestingly, short-term TBC treatment increased plasma total cholesterol and atherogenic LDL cholesterol levels in WT mice but did not affect lipid levels in PXR-deficient mice [76]. In addition to fatty acid transporter CD36, activation of PXR by TBC stimulated intestinal expression of Niemann–Pick C1-Like 1 (NPC1L1), an essential transporter in mediating intestinal cholesterol uptake in the gut [100–102]. TBC promoted cholesterol uptake by both murine and human intestinal cells in a PXR-dependent manner [76]. In addition to NPC1L1, several other studies demonstrated that PXR might regulate other key intestinal genes mediating lipid uptake and transportation including CYP27A1, ABCA1, gastric lipase, lysosomal lipase, and diacylglycerol acyltransferase 1 and 2 [46, 75, 103–106]. Therefore, activation of PXR by exposure to EDCs such as TBC may lead to aberrant lipid homeostasis, thereby inducing dyslipidemia and increasing the risk of CVD in exposed individuals.

EDC-Elicited Epigenetic Modifications That Influence CVD Risk

The term epigenetics refers to the reversible heritable alterations in gene expression that are not due to changes in the DNA

sequence. Epigenetic modifications, such as DNA methylation, histone modifications, and noncoding RNAs, alter chromatin structure and/or DNA accessibility therefore regulating gene expression [107]. These processes, regulated by environmental and dietary cues, are essential for proper development. Recent studies have revealed an important role of epigenetic modification in CVD [108–110]. In this section, we will mainly discuss the impact of EDC exposure on epigenetic modifications in the context of CVD.

DNA Methylation

DNA methylation is an epigenetic mark that adds a covalently bound methyl group (-CH3) to sites in the genome where cytosine is followed by guanine to generate "CpG" islands. Most CpG dinucleotides in the human genome are methylated and are mostly associated with a reduction in gene expression [111]. Changes in DNA methylation patterns have emerged in patients and animal models of CVD.

Differential methylation patterns were first discovered in atherosclerotic lesions of rabbits in the late 1990s. Laukkanen *et al.* [112] discovered marked hypomethylation of the extracellular superoxide dismutase gene in atherosclerotic aortas of rabbits. Consistently, the same group also identified a decrease in genomic 5-methylcytosine content in advanced human, mouse, and rabbit atherosclerotic lesions [113]. Both hypermethylation and hypomethylation were detected in mice prior to no detectable changes in atherosclerotic lesions [114]. Lund *et al.* [114] also discovered that treatment with atherogenic lipoproteins promote global DNA hypermethylation in human monocytes. Differential methylation patterns have been identified on CVD-associated genes, such as ERα [115], ERβ [116], Niemann–Pick type C1 [117], ATP-binding cassette A1 [118], sterol regulatory element–binding protein 1 [119], LDL receptor [119], and the monocarboxylate transporter gene [120]. The impact DNA methylation has on CVD including cardiac hypertrophy, heart failure, and arrhythmias has been discussed in detail in another review [108]. While these experimental observations link methylation pattern changes to CVD, the contribution of EDC exposure to these changes is not well defined.

Several PXR agonistic EDCs have also been demonstrated to cause epigenetic changes that may increase CVD risk. For example, *in utero* exposure to the PXR agonist DEHP [76, 121] results in hypermethylation of the glucose transporter 4 in F1 offspring of rats, which likely contributes to the exacerbated cardiometabolic disease state of those animals [122]. It was later discovered by the same group that gestational feeding of DEHP also caused global DNA hypermethylation of genes involved in the development and function of pancreatic β-cells [123], which may also lead to increased CVD risk. Higher urinary concentrations of another EDC, BPA, in prepubescent girls were associated with decreased global methylation, which corresponded to changes in the expression of genes involved in inflammation and metabolism [124]. In an HFD-driven rat steatosis model, *in utero* BPA exposure altered methylation within the carnitine palmitoyltransferase (CPT) 1a gene leading to hepatic deposition of triglycerides [125]. Garcia-Arevalo *et al.* [126] investigated the impact of exposure to BPA during pregnancy on body weight, glucose homeostasis, and gene expression in several major organs in male offspring. They found that *in utero* exposure to BPA elicited hyperglycemia, glucose intolerance, increased plasma non-esterified fatty acids and hepatic triglyceride levels [126]. BPA treatment also affected the expression of the several key genes involved in lipid and fatty acid

metabolism including SREBP-1c, PPARα, and CD36 in the offspring [126]. In another study, the same group found that *in utero* BPA exposure influenced the expression of genes involved in β-cell development, which may contribute to the impaired glucose tolerance observed in adult offspring [127]. Although the DNA methylation status of those genes was not investigated, it is likely that *in utero* BPA exposure affects the DNA methylation to induce those changes. There are many more studies investigating the impact of BPA exposure on epigenetic changes and metabolic diseases, which have been discussed in detail in a comprehensive review [128]. In addition to these genes related to CVD risk, PXR promoter methylation can also be modulated by ligands or during pathophysiological disease conditions [129–131]. However, it remains to be determined whether those PXR agonist EDCs can mediate epigenetic modulation of the PXR gene itself to influence CVD risk.

Histone Acetylation

Lysine acetylation, controlled by histone acetyltransferases (HATs) and histone deacetylases (HDACs), was first identified as histone modifiers that regulate DNA accessibility and chromatin structure to control gene transcription [132, 133]. This process is now known to occur in more than 80 transcription factors and various cytoplasmic proteins [132, 133]. The acetylation pattern varies from one protein to another, and the effects can be multifaceted for one protein [132, 133]. Many studies have revealed a role for both HATs and HDACs in regulating genes that are involved in CVD initiation and progression [134, 135], and we highlight a few of these studies below.

HDACs are closely linked to the progression of atherosclerosis, and inhibition of HDACs prevents the progression of atherosclerosis [136]. Local administration of the HDAC inhibitor, trichostatin A (TSA), significantly suppressed neointimal hyperplasia [136, 137]. HDAC inhibition has also been shown to suppress vascular smooth muscle cell proliferation and ultimately neointima formation [138, 139]. In addition, common genetic variants of some HDACs may also increase genetic susceptibility to ischemic stroke and CVD [140]. For example, HDAC9 was upregulated in human atherosclerotic plaques [141], and deletion of HDAC9 alleviated atherosclerosis in mice [142, 143]. In contrast, Choi *et al.* [144] demonstrated that HDAC inhibition with TSA exacerbated atherosclerosis in LDL receptor–deficient mice.

While these studies revealed an important regulatory role for HDACs in CVD, only a few studies have investigated the impact of EDCs on histone acetylation related to CVD. Yeh *et al.* [145] demonstrated that exposure to EDCs, nonylphenol, and 4-octylphenol suppressed LPS-induced inflammatory gene expression in human-derived THP-1 monocytes. Furthermore, LPS-elicited histone 4 acetylation at the IFN-α-inducible protein 10 (IP10) and the monocyte-derived chemokine gene loci were reduced after exposure to these EDCs [145]. It is worth noting that nonylphenol is also a PXR agonist [44] (Table 1), and PXR activation has been well documented to suppress inflammation [146]. Therefore, it would be interesting to determine whether EDC-mediated activation of PXR can affect histone acetylation to influence CVD risk in the future.

Noncoding RNA

While the majority of the human genome is transcribed, only ~2% of these transcripts are translated into protein [147]. For many years, the scientific community regarded the other 98% of

non-translated transcripts to be "junk," but the functions of these non–protein-coding RNAs (ncRNAs) are now being revealed in many diseases, including cancer [148] and CVD [149]. The ncRNAs can be classified into microRNAs (miRNAs), short interfering RNAs (siRNAs), PIWI-interacting RNAs (piRNAs), XCL inactivation-linked small RNAs (XiRNAs), and long ncRNAs. These different ncRNAs can be defined based on their length, target of interest, and function. Long ncRNAs, for example, are longer than 200 nucleotides, whereas the other ncRNAs are less than 45 nucleotides. There is a host of literature surrounding the influence of long ncRNAs on epigenetic processes [150], but this review will focus on the most widely studied ncRNA in CVD, miRNAs. miRNAs are short ncRNAs that bind to complementary regions in their target genes, often in the 3′-UTR regions and suppress their translation and/or facilitate degradation [151, 152]. miRNAs control epigenetic modifications, such as establishing DNA methylation through regulation of key DNA methylation enzymes [153–158]. Moreover, miRNAs may control DNA accessibility via regulation of chromatin structure by regulating histone acetylase/deacetylase enzymes [155, 159, 160]. Mounting evidence has identified several key miRNAs in the development of CVD (for a thorough review on all miRNAs in CVD, see Barwari *et al.* [161]); however, little is known about the environmental factors influencing miRNA-mediated CVD risk.

Evidence linking EDC exposure to differential miRNA expression and CVD risk has been recently reported. Wahlang *et al.* [162] demonstrated that human endothelial cell exposed to the PCB mixture Aroclor 1260 significantly altered 557 out of 6658 miRNAs, and 21 of these miRNAs were found to be associated with vascular diseases. Aroclor 1260 exposure increased miR-21, miR-31, miR-126, miR-221, and miR-222 expression levels [162]. Interestingly, elevated miR-21 levels have been reported in cardiac injury [163] and miR-126 and miR-31 levels have been demonstrated to alter inflammation [164]. Other groups have demonstrated the impact of EDC exposures on differential miRNA expression in the central nervous system, reproductive axis, as well as metabolic regulation [165]. However, more work is required to understand how EDCs alter the miRNA profile in atherosclerosis and hyperlipidemia.

Epigenetic Regulation of PXR Expression

In addition to the well-described ligand-dependent mechanism, transcriptional activity of PXR can also be epigenetically regulated. Studies have demonstrated the impact of EDC exposure on the epigenetic modification of genes related to cardiometabolic disease [122, 123, 125, 145, 162]. It is plausible that these EDCs can also epigenetically modulate PXR expression to influence CVD risk. For example, changes of PXR promoter methylation has been demonstrated under different conditions in mice and humans [129–131]. In females with intrahepatic cholestasis of pregnancy compared to healthy pregnant women, the distal PXR promoter (−1224 bp) was significantly hypomethylated, which was positively correlated with bile acid composition [129]. Moreover, differential PXR promoter methylation was observed in a variety of colon cancer cells, which might be associated with interindividual variability of drug toxicity [130].

PXR has also been demonstrated to be epigenetically regulated by microRNAs. Takagi *et al.* [166] measured PXR messenger RNA (mRNA) and protein levels in 25 human livers and found that mRNA and protein levels were not correlated, suggesting posttranscriptional regulation of PXR. Furthermore, 16 miRNA binding sites were found in the 3′-UTR in human PXR

[166]. MiR-148a was then identified as a critical regulator of human PXR expression [166]. Overexpression of miR-148a suppressed PXR protein levels and, in contrast, inhibition of miR-148a elicited increases in PXR protein expression [166]. A similar binding site was discovered in human CYP3A4; however, CYP3A4 was unresponsive to miR-148a overexpression [166]. Although the 3′-UTR of PXR is not well conserved between species, the miR-148a element has been identified in both mice and rats, suggesting that this specific miRNA may control PXR gene expression in other mammals [166].

In another human cohort study, genetic variants in the 3′-UTR of PXR were identified in specific genes involved in xenobiotic metabolism [152]. For example, bioinformatics analysis revealed that 1025 unique miRNAs were predicted to bind to the 3′-UTR of hPXR mRNA [152]. Furthermore, the PXR target gene CYP3A4 was predicted to be regulated by 889 unique miRNAs [152]. The variant *rs1054190C* of PXR may result in a new binding site for miR1250-5p [152]. In contrast, the variant *rs1054191G* was predicted to result in the loss of a binding site for miR-371 b-3p, miR-4258, and miR-4707 [152]. More recently, Vachirayonstien and Yan [167] tested 58 miRNAs and identified miR-30c-1-3p as a silencer of PXR expression. Many PXR SNPs have been identified to be significantly associated with plasma LDL cholesterol levels in humans, many of which were found in the 3′-UTR of PXR mRNA [73]. It is possible that these SNPs affect miRNA binding to PXR mRNA, leading to altered lipid homeostasis and changed LDL levels. Although there may be other regulatory miRNAs targeting PXR, the physiological consequence on PXR stability and the impact of these identified miRNAs and other potential miRNAs on lipid metabolism have not been investigated.

Potential Role of PXR in Epigenetic Regulation of Gene Transcription

In addition to binding to its responsive motifs and transcriptionally regulating target gene expression, a few studies have indicated a potential role of PXR in epigenetic regulation of multiple targets. Cui *et al.* [168] performed an unbiased investigation of PXR DNA-binding motifs and their genomic locations by using chromatin immunoprecipitation (ChIP)-Seq approach in mouse livers. Surprisingly, PXR can bind to similar regions of DNA as the epigenetic mark of gene activation, H3K4Me2 [168]. By contrast, PXR binding did not overlap with H3K27Me3, an epigenetic mark of gene repression. Unlike strong enrichment of H3K4Me2 on the prototypical PXR target gene Cyp3A11, another PXR-regulated gene involved in lipid metabolism, CD36, was not enriched with H3K4Me2. Cui *et al.* [168] suggested that a permissive epigenetic mark is absent, and thus, PXR binding is masked in the chromatin and binding is unable to occur. A more recent study demonstrated that PXR ligand rifampicin treatment can lead to increased levels of H3K4me3 and H3 acetylation and decreased levels of H3K27me3 in the CYP3A4 promoter in human intestinal LS174T cells [169]. Consistently, knockdown of PXR abolished the changes of those epigenetic changes in the promoter [169]. In another study using human primary hepatocytes, Smith *et al.* [170] found that PXR was significantly recruited to its DNA binding motifs after rifampicin treatment. As expected, ligand-activated PXR increased binding to promoters of target genes. Interestingly, rifampicin also increased PXR binding to intronic and exonic regions of target genes [170]. There was a large overlap of PXR binding sites with that of p300, an HAT that gives an epigenetic tag for transcriptional

activation. The authors then discovered that rifampicin treatment enhanced PXR/p300 occupancy at regions near genes involved in xenobiotic metabolism [170]. Yan *et al.* [169] also found that rifampicin enhanced the recruitment of both p300 and nuclear receptor coactivator 6 (NCOA6) to the CYP3A4 promoter and knockdown of PXR suppressed their recruitment to the promoter. p300 acetylation is also critical for ligand activation of another nuclear receptor, FXR [171], and PXR has been reported to be a direct target gene of FXR [172]. Therefore, p300 acetylation may not only enhance the PXR-mediated gene transcription but also facilitate the transcription of PXR gene itself. In addition to p300, PXR can also directly interact with arginine methyltransferase 1 (PRMT1), the primary methyltransferase that deposits the asymmetric dimethylarginine mark [173]. ChIP analysis revealed a ligand-dependent recruitment of PRMT1 to PXR regulatory regions within CYP3A4 promoter [173].

Aside from PXR's ability to influence recruitment of proteins involved in epigenetically tagging genomic loci, PXR itself has been established to undergo a host of posttranslational modifications including phosphorylation, SUMOylation, ubiquitination, and acetylation [174]. These posttranslational mechanisms that impact PXR stability may be elicited through ligand-independent interactions. For example, the deacetylase sirtuin-1 (SIRT1) can interact with PXR both in the presence and absence of RXR and PXR ligands [175]. Interestingly, resveratrol treatment (a SIRT1 activator) promotes lipogenesis in murine primary hepatocytes, which is similar to the effects of PCN treatment in mouse liver [175]. Furthermore, this phenotype was alleviated by nicotinaminde treatment, an HDAC inhibitor [175]. These data indicate that deacetylation of PXR may promote lipogenesis independent of canonical ligand binding. It is clear that PXR stability is governed by a host of posttranslational modifications, but whether the function of these modifications are initiated by EDCs and how PXR stability influences the epigenome remain to be determined.

Ligand-activated PXR also suppresses the transcription of CYP7A1, a rate-limiting enzyme involved in bile acid synthesis [176, 177]. CYP7A1 therefore plays an important role in the regulation of lipid, glucose, and energy homeostasis. PXR can interact with hepatic nuclear factor 4α (HNF4α), which is critical for CYP7A1 transcription. PXR ligands can promote this interaction and block PPARγ coactivator α (PGC-1α) activation with HNF4α, thereby inhibiting CYP7A1 gene transcription [176]. Interestingly, CYP7A1 transcription can also be epigenetically regulated by some stimulators such as glucose [178]. Glucose can affect histone acetylation (H3 and H4), H3K9Me2, and H3K9Me3 of the CYP7A1 promoter [178]. Although PXR signaling has been implicated in glucose metabolism [46, 179], it is not clear whether PXR can also regulate CYP7A1 transcription through epigenetic mechanisms and future works are required to explore this possibility.

The DNA methylation and histone modification profiles of tissue-specific transporters including OATP1B2, NTCP, BSEP, and ABCG5/8 have also been investigated in mice [180]. Many of these transporters including NTCP and BSEP are PXR target genes [181]. Genome-wide DNA methylation profiling revealed distinct hypomethylation around the transcriptional start site of these transporters in the liver, but those regions were hypermethylated in the kidney and cerebrum [180]. These methylation patterns were also consistent with the tissue distribution pattern of these transporters [180]. Histone 3 was hyperacetylated at their genetic loci in the liver but was hypoacetylated in the kidney and cerebrum [180]. It would be interesting to compare the methylation and acetylation patterns in WT and PXR-deficient mice and to investigate the impact of PXR signaling on epigenetic systems in different tissues in the future. While all of these studies indicate a role of PXR in epigenetic regulation of gene transcription, to the best of our knowledge, there is no study investigating the effects of EDC-mediated PXR activation on epigenetic changes that influence CVD risk and future studies are urgently needed to address this important topic.

Conclusion

This review aims to introduce a new, understudied role for PXR in mediating the impact of EDC exposure on epigenetic changes that may influence CVD risk. Numerous EDCs have been identified as PXR ligands, and we have provided an up-to-date table including most if not all EDCs that modulate PXR activity in humans and rodents (Table 1). However, how EDCs influence the epigenome through PXR-dependent mechanisms is poorly understood. In addition to the well-characterized ligand-dependent transcriptional regulation of its target gene expression, we propose a new mechanism by which PXR may act as a "chaperone" guiding epigenetic modifiers to the locus, thereby allowing for transcriptional modulation (Fig. 1). EDC-mediated epigenetic changes may not only affect the exposed individuals but also influence diseases in successive generations. Several lines of evidence suggest that EDCs predispose rodent and human offspring to cardiometabolic disease risk [122, 123, 162, 165]. Since most of the gene–environment interactions do not directly alter the DNA sequence (i.e. initiation of mutations), these phenotypes are likely due to the passing of heritable "tag" that can influence gene expression in the offspring. It would be interesting to investigate the contribution of PXR to the intergenerational and transgenerational effects of EDCs on the development of diseases such as CVD in the offspring in the future.

We now know that EDCs influence a wide range of disease states, including male and female reproduction, breast development and cancer, metabolism and obesity, and CVD [11]. These EDCs, however, are constantly evolving; for example, the Consumer Product Safety Improvement Act now prohibits the sale of children's toys containing more than 0.1% of phthalates such as DEHP, DBP, and BBP. Although phthalate substitutes have emerged as "safe alternatives" to those EDCs, we have not appropriately tested these substitutes for their endocrine-disrupting potential. Our laboratory, as well as others, has demonstrated that both phthalates and phthalate substitutes have the potential to activate PXR and induce hyperlipidemia in animal models [76, 99, 182]. Due to the growing concern posed by EDCs, it is important for us to understand the impact of all these EDCs on human health.

Acknowledgements

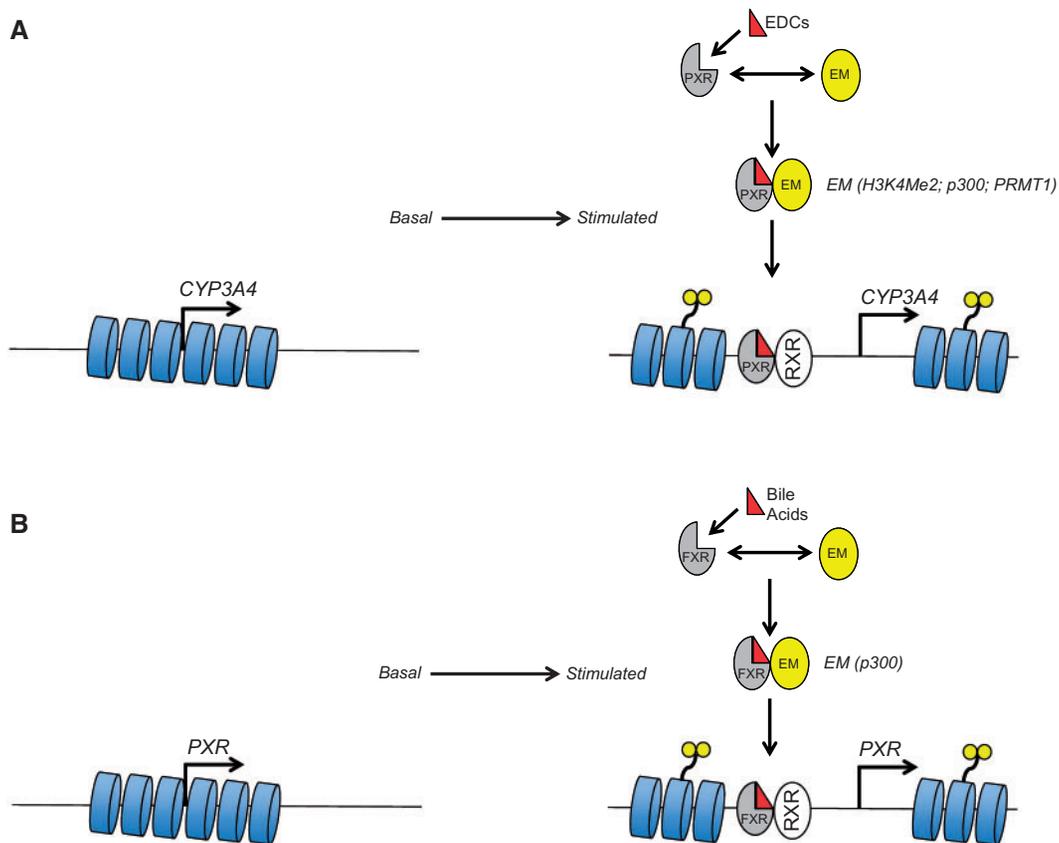## Conflict of interest statement

None declared.

Figure 1: Schematic description of potential epigenetic modifications regulating PXR and its target gene transcription. (**A**) Potential role of PXR in epigenetic regulation of target genes. Upon ligand activation, PXR can bind to DNA regions enriched with epigenetic modifiers (EM) that are considered to be transcriptional activators. This schematic proposes a scenario where EDCs bind to PXR and promote PXR interaction with EMs such as H3K4Me2, p300, and PRMT1. In addition to CYP3A4 locus showed here, PXR also regulates genes involved in lipid homeostasis and atherogenesis (e.g. NPC1L1, CD36). It is highly likely that similar mechanisms also work at specific loci harboring those genes, thereby linking EDC exposure to CVD risk. (**B**) Epigenetic regulation of PXR gene transcription. Since PXR is also an FXR target gene, we propose a scenario where FXR ligands such as bile acids bind to FXR to serve as a "chaperone" guiding these EMs to the PXR locus, leading to increased PXR transcription. Although we used FXR signaling as an example, PXR transcription may be epigenetic regulated by EDCs and other chemicals through different signaling pathways. These two models provide an overview of how PXR regulates gene expression through epigenetic modifications in response to EDC exposure and how PXR itself is epigenetic regulated.

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