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REVIEW

The Impact of Di(2-ethylhexyl)phthalate on Cancer Progression

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Abstract

Di(2-ethylhexyl)phthalate (DEHP), a widely used plasticizer, mainly serves as an additive to render polyvinyl chloride (PVC) soft and flexible. PVC plastics have become ubiquitous in our modern society. Yet, the leaching of DEHP from PVC-based consumables ultimately results in the deposition in certain tissues via inadvertent applications. Health risks for human populations exposed to DEHP has been assumed by studies on rodents and other species, including the DEHP-induced developmental dysregulation, reproductive impairments, tumorigenesis, and diseases in a transgenerational manner. In this review, we comprehensively summarize the accumulated literature regarding the multifaceted roles of DEHP in the activation of the nuclear receptors, the alteration of the redox homeostasis, epigenetic modifications and the acquisition of chemoresistance.

Keywords DEHP · Tumorigenesis · Nuclear receptors · Redox homeostasis · Epigenetic modifications · Chemoresistance

Introduction

Phthalates, diesters of phthalic acid, are a group of industrial chemicals with endocrine-disruptive properties that disperse ubiquitously in our daily surroundings. Among them, di(2-ethylhexyl)phthalate (DEHP) is one of the most widely used plasticizers, and is primarily responsible for making polyvinyl chloride (PVC) products more flexible (Fay et al. 1999). It is estimated that over 2 million tons of DEHP are produced globally each year (Halden 2010). DEHP is not covalently bound to PVC products, leading to its gradual leaching out of the plastics (Miyamoto and Sasakawa 1987) and hence engendering human exposure. Inadvertent exposure to DEHP is a concern due to its deleterious effects on human health, including liver toxicity, impairment in the reproductive system, decreased spontaneous heart rate, potential carcinogenicity, and epigenetic-based transgenerational diseases (Erkekoglu and Kocer-Gumusel 2014; Gillum et al. 2009; Osumi and Hashimoto 1978; Posnack et al. 2015; Wang et al. 2012). The potential routes of internal exposure to DEHP are often via oral ingestion, dermal absorption, inhalation and intravenous administration during application of a vast range of PVC-based consumables, such as personal-care products (including soaps, cosmetics,

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lotions, and deodorant), food packaging, floor tiles, water pipes, certain toys and teethers, furniture upholstery, as well as certain medical devices such as blood storage bags, parenteral nutrition, catheters, intravenous tubing, dialysis tubing and air tubing (Falconer et al. 2006; Pradeep and Benjamin 2012; Shelby 2006).

DEHP Exposure in the Western Countries and Taiwan

The US Environmental Protection Agency and the European Union have established a respective oral reference dose (RfD) of 20 µg/kg of body weight/day and a tolerable daily intake (TDI) of 50 µg/kg of body weight/day for DEHP. RfD and TDI are estimated doses that show no noticeable adverse effects on human populations during their lifetime (Hines et al. 2011). In the United States and Germany, the estimated exposure of the general populations to DEHP is 1–30 µg/kg body weight (BW)/day (Doull et al. 1999; Shelby 2006) and 13.8 µg/kg BW/day, respectively (Koch et al. 2003a, b). Notably, in May 2011, the Taiwan Food and Drug Administration disclosed that a number of foods and beverages were illegally tainted with DEHP and dibutyl phthalate (DBP) as a substitute for clouding agents (Wu et al. 2012; Yen et al. 2011). Despite the fact that the surveillance of Taiwan foodstuffs has been tightly monitored after the provoking event, the majority of Taiwanese have been exposed to an estimated median DEHP dose of 33.9 µg /kg BW/day for at least 15 years (Chen et al. 2008).

Metabolism of DEHP

The DEHP has an estimated half-life of approximately 12 h for elimination from the body through urine (Schmid and Schlatter 1985). Unlike the low molecular weight phthalates such as DBP and diethyl phthalate (DEP), both of which are able to be excreted quickly in their primary metabolite (hydrolytic monoester), DEHP belongs to high molecular weight phthalates with relatively more hydrophobic properties and is excreted via its secondary metabolites, which are hydrophilic and oxidative monoesters (Barr et al. 2003). Research has shown that after oral administration, DEHP is primarily metabolized to mono-(2-ethylhexyl)phthalate (MEHP) via pancreatic lipase-mediated hydrolysis, predominantly taking place in the intestine. The aliphatic side chain of MEHP then rapidly undergoes ω-1-oxidation, resulting in a generation of 30 or more secondary metabolites (Albro et al. 1982; Albro and Lavenhar 1989).

MEHP and three secondary metabolites, mono(2-ethyl-5-oxo-hexyl)phthalate (MEOHP), mono(2-ethyl-5-hydroxyhexyl)phthalate (MEHHP), and mono-(5-carboxy-2-ethylpentyl)phthalate (MCEPP), are currently measured in human urine samples as an assessment of the body burden of DEHP. Through mass spectrometric measurements, the levels of MEHHP and MEOHP have been shown to be significantly higher than that of MEHP in human urine samples (Barr et al. 2003; Schmid and Schlatter 1985). The urine samples of 62 participants in the United States were assessed. The results showed that MEOHP was detected in 96.8% of the urine samples with a median concentration of 28.3 ng/mL, MEHHP was in 95.2% of the urine samples with a median concentration of 35.9 ng/mL. However, MEHP was detected in 80.6% of the urine samples with a median concentration of only 4.5 ng/mL (Barr et al. 2003). In another study in Germany, non-occupationally exposed subjects to DEHP showed median concentrations of MEHHP, MEOHP and MEHP to be 46.8, 36.5 and 10.3 µg/L in urine, respectively (Koch et al. 2003b). These results led to uncertainty as to whether MEHP is a convincing biomarker for estimating the degree of human exposure to DEHP. Our original understanding regarding the pharmacokinetics of DEHP, including the rates of absorption and elimination as well as the metabolic efficiency (Barr et al. 2003), might even need to be questioned.

Plastics are extensively used in Taiwan and around one million tons of PVC are manufactured every year (Chen et al. 2008); the most concern nowadays is the level of DEHP in take-away foods covered with DEHP-containing PVC films or bags. In 2008, a study evaluating the DEHP exposure in a Taiwan population ($n=60$) showed a remarkably high level of the median urinary MEHP concentration (15.9 µg/L) (Chen et al. 2008). After the event of the illegal use of phthalates as a clouding agent in 2011 (Wu et al. 2012; Yen et al. 2011), a study performed in 2015 indicated that the median levels of metabolites of DEHP in urine samples of 290 adult Taiwanese were 7.9 µg/g creatinine for MEHP, 12.6 µg/g creatinine for MEOHP and 22 µg/g creatinine for MEHHP (Huang et al. 2015). These decreases in the concentrations of DEHP metabolites in the tested Taiwan population suggest that food surveillance has been successfully conducted, although there are still specific populations exposed to high levels of DEHP, such as PVC production workers (Fong et al. 2014).

Rodent Data and Human Epidemiological Data upon DEHP Exposure

The acquisition of tumorigenic potential contributed by environmental pollutants may proceed over a period of at least a decade. There is cumulative evidence for the carcinogenic effects of long-term exposure to DEHP on rodent species, as shown through the lowest observed adverse-effect level (LOAEL) studies, which is the lowest concentration of

substance obtained from experimental studies that causes an adverse alteration (David et al. 1999, 2000a, b; Voss et al. 2005). The LOAEL for the increased incidence of hepatocellular tumors in Fischer 334 rats was 2500 ppm (147 mg/kg/day for males), while the LOAEL for the increased incidence of hepatocellular tumors in B6C3F1 mice was 1500 ppm (292 mg/kg/day for male). These data were obtained from studies in which rats and mice were fed diets containing DEHP at doses ranging 100–12,500 and 100–6000 ppm for 104 weeks, respectively.

Furthermore, in an independent chronic study, diets containing 6000 or 12,000 and 3000 or 6000 ppm DEHP were given to Fischer 334 rats and B6C3F1 mice, respectively. The results showed that there was a significant dose–response relationship between DEHP exposure and the incidence of hepatocellular carcinomas in female rats and in mice of both sexes (National Toxicology 1982). The causal associations between the DEHP exposure and the risk of hepatocellular tumors are difficult to interpret. It is possible that the increased rate of hepatocarcinogenesis observed in these rodents is due to increase peroxisomal enzyme activity (David et al. 1999). However, several studies have consistently reported that a dietary concentration of 500 ppm (28.9–36.1 mg/kg/day) for at least an 1-year administration of DEHP to rodents was considered to be the no-observable-effect level (NOEL); that is, the highest dose of a poison that does not cause noticeable adverse effect on animals. This reported NOEL for DEHP was based on the absence of detectable adverse effects, such as peroxisome proliferation, increased liver weight and increased incidence of liver tumors (Cattley et al. 1987; David et al. 1999; Mitchell et al. 1985b). On the contrary, the study of PPAR-null mice exposed to DEHP showed that there was also a substantial increase in the incidence of hepatocellular tumors (Ito et al. 2007), indicating that DEHP-induced hepatocarcinogenesis is not entirely, but predominantly, due to peroxisome proliferation in rodent livers.

In most of these studies, hepatocarcinogenesis was documented in the rodent species after 2 years of oral administration of DEHP. In another study, long-term feeding of DEHP to Fischer 334 rats showed that at least 78 weeks were required for the development of DEHP-induced hepatocellular carcinoma, since no histologically detectable hepatocellular carcinoma was detected at week 52 (Hayashi et al. 1994). Apart from livers being most susceptible to DEHP-induced carcinogenesis, rodent studies indicated the pancreas (pancreatic acinar cell adenomas) and testes (Leydig cell tumors) being the relevant target organs for DEHP (David et al. 2000b).

There was also evidence indicating DEHP's absence of carcinogenic activity. Sherman rats of both sexes were given diets containing DEHP at 400, 1300 or 4000 ppm, respectively, for 2 years. At termination, the sample sizes were small, but eventually, no carcinogenic effects were observed (Carpenter et al. 1953). Consistent findings of the absence of hepatocarcinogenic potential of DEHP were also reported from a chronic study in rats (Harris et al. 1956). Such negative association is probably due to the exposure concentration being below the hazard threshold.

At present, little epidemiological evidence is available to support the carcinogenic effects on humans after DEHP exposure. An alternative way to infer the relationship between the leakage of DEHP from PVC-containing materials and the risk of cancer incidence is to examine human populations who have been immersed for long periods of time in a region with high quantities of PVC, such as PVC-processing industries and hospitals (Chiazze and Ference 1981; Hagmar et al. 1990; Lee and Harry 1974; Monson et al. 1974; Tabershaw and Gaffey 1974). As first demonstrated in 1974, male workers occupationally exposed to PVC have been associated with the increased incidence of hepatic angiosarcoma (Lee and Harry 1974). Later, in a historical prospective mortality study, male workers ($n=8384$) who had been occupationally exposed to PVC for at least 1 year showed an increased incidence of cancer arising in the digestive and respiratory systems, as well as in the brain (Tabershaw and Gaffey 1974). This is inconsistent with another study that used proportionate mortality ratio to calculate the correlation between white employees of the PVC industry ($n=3847$) and the causes of their death. The results showed that mortality was significantly related to digestive cancers and respiratory cancers in both genders, whereas mortality numbers for the female employees were significantly related to urinary and breast cancers (Chiazze and Ference 1981).

A Swedish cohort study on PVC workers ($n=2031$) also exhibited a significant increase in the incidence of respiratory cancer in male populations (Hagmar et al. 1990). Furthermore, increased risk of pancreatic and testicular cancers was also shown to be linked to employees who had been occupationally exposed to PVC for over 10 years (Hardell et al. 1997; Selenskas et al. 1995). Such phenomena implicate but hardly prove that such PVC workers have definitively been exposed to cancer-causing or -promoting chemicals.

As a major additive of PVC, the impact of DEHP on human health could not be ruled out. The urinary concentrations of phthalate metabolites have been used to calculate the human body burden of phthalates, and to estimate whether that burden is associated with the occurrence of a disease. Epidemiologic reports on a sample of Mexican women showed that the risk of developing breast cancer was linked to urinary concentrations of certain phthalate metabolite—monoethyl phthalate—suggesting that exposure to its parent DEP would pose a risk of breast cancer to women (Lopez-Carrillo et al. 2010). Despite DEHP exposure being

common in the general population, the risk of breast cancer observed from the human data was limited and the DEHP-promoted progression of breast cancer was only clarified by in vitro studies. The academic trends regarding rodent data reflected that the liver was the most responsive target of DEHP-induced carcinogenic effects. However, accumulating epidemiologic studies in human populations indicate that DEHP might affect multiple organs. This sheds light on our knowledge that the potential carcinogenicity of DEHP is not specific to the liver.

The Action of DEHP on Nuclear Receptors

Besides estrogen receptor, little is known regarding whether DEHP directly interacts with other sex hormone-related receptors. Recently, Sarath Josh et al. (2014) predicted the interaction of DEHP and peroxisome proliferator-activated receptor (PPAR) and retinoid X receptor (RXR) using in silico approaches. However, DEHP has been described to exert its pleiotropic effects predominantly by mediating the nuclear receptor called the PPARα, which binds to peroxisome proliferator response elements (PPREs) located at the target gene promoters as a heterodimer with its partner RXR. However, changes in the transcriptional profiling in response to DEHP exposure indicated that DEHP might possibly recognize other nuclear receptors apart from PPARα. Considering that DEHP possesses a benzene ring with lipophilic characteristics, several nuclear receptors are thought to be the primary targets for DEHP contributing to its adverse effects, such as estrogen receptor α (ERα), androgen receptor, aryl hydrocarbon receptor (AhR) and constitutive androstane receptor (CAR). Even so, it is proposed that the DEHP-induced impact, including liver carcinogenesis, might be due to an interplay of multiple molecular pathways rather than a single pathway.

Peroxisome Proliferator-Activated Receptor α

The phenomenon of hepatic abnormalities has been observed in nearly all studies with phthalates. The long-branched phthalates, particularly DEHP, are reported to be more potent than the other phthalates with shorter and straight ester chains (Barber et al. 1987; Mann et al. 1985). Laboratory rodents exhibited a strong hepatic response to high doses of DEHP, including hepatomegaly, peroxisome proliferation, increased liver weight, and increased activity of peroxisomal enzymes (Moody et al. 1991; Rao and Reddy 1987). However, a potential mechanism by which DEHP causes such hepatic abnormalities involves the activation of PPARs, which are ligand-activated transcription factors belonging to the superfamily of nuclear receptor (Ward et al. 1998). There are three isoforms for PPARs: PPARα, PPARβ and PPARγ. These isoforms have been identified in various tissues (Lemberger et al. 1996; Wang et al. 2002), with PPARα predominately found in the liver of rats (Braissant et al. 1996). PPARα has been implicated in a number of physiological processes such as lipid homeostasis and inflammatory responses (Schoonjans et al. 1996). The hepatocarcinogenesis associated with long-term exposure to DEHP in the rodent model is PPARα dependent (Corton et al. 2000). Binding of ligands to PPARα is the initiating event in the downstream transcriptional responses. The ligand-bound PPARα forms a heterodimeric complex with its partner RXR (Knapp et al. 2006). The subsequent binding of this heterodimeric complex to the PPREs located at the target gene promoters results in the transcription of mRNAs for peroxisomal β-oxidation of fatty acids, including acyl-CoA oxidase (ACOX), peroxisomal 3-ketoacetyl-CoA thiolase (PTL) and peroxisomal bifunctional enzyme (BIEN), and for microsomal arachidonic acid ω-hydroxylation, including CYP 4A1 and CYP 4A3 (Reddy and Hashimoto 2001).

Maloney and Waxman (1999) studied the PPARα-mediated transactivation potential using COS-1 monkey kidney cells transfected with human and mouse PPARα expression plasmids. Mouse PPARα was found to be more responsive to MEHP than human PPARα in the micromolar range, whereas the transcriptional activities of neither mouse PPARα nor human PPARα were affected by the parent DEHP at concentrations up to 2 mM (Maloney and Waxman 1999). These data were consistent with the results of Hurst and Waxman (2003), who demonstrated that mouse and human PPARα were both activated by MEHP with an effective concentration for half-maximal response (EC50) values of 0.6 and 3.2 μM, respectively. The same research also demonstrated that in cultured rat hepatoma FaO cells stably transfected with mouse PPARα, MEHP treatment resulted in increased protein expression of PPARα target gene, ACOX, and PTL (Bility et al. 2004). Collectively, it is possible to conclude that PPARα is stimulated in response to MEHP, but whether it can directly bind to PPAR is still ambiguous. Scintillation proximity binding assay using purified human PPAR ligand-binding domains showed that MEHP was able to directly bind to the PPARα and PPARγ, with a similar affinity to WY-14,643, a hypolipidemic agent and well-established activator of PPARα (Lapinskas et al. 2005).

Even though MEHP activates PPARα in transactivation assays and its downstream-regulated genes, only a little or even no significant changes in those parameters were observed after DEHP treatment in vitro (Gray et al. 1982; Lake et al. 1987; Lapinskas et al. 2005; Lhuguenot et al. 1985; Maloney and Waxman 1999; Mitchell et al. 1985a). This may be due in part to the cultured cell lines that are unable to enzymatically hydrolyze phthalate diesters to

monoesters (Gray et al. 1983), which are more biologically active than their parent diesters. Ward et al. (1998) first reported that an increased incidence of peroxisome proliferation in wild-type mice administered with DEHP in the diet at 12,000 ppm for 8 weeks was observed, as judged by the eosinophilic granular appearance. These effects were absent in PPARα-null mice treated with DEHP (Ward et al. 1998). Moreover, the increases in hepatic mRNA of peroxisomal enzymes ACOX, PTL and BIEN, and the microsomal enzymes CYP 4A1 and CYP 4A3 were observed in DEHP-treated mice (Ito et al. 2012), suggesting a possible role for DEHP in activating the PPARα signaling. However, the activation of PPARα signaling seen in response to DEHP exposure in vivo resulted in a great production of hepatic lesion. It has been proposed that these adverse effects in rodent models lead to hepatocyte proliferation and possibly hepatocarcinogenesis.

The hepatocarcinogenicity of DEHP has been questioned by the demonstration that DEHP is not suitable as a peroxisome proliferation inducer for humans (Melnick 2001). However, a study comparing the species differences in DEHP-induced activation of PPARα between wild-type (mPPARα) and PPARα-humanized mice reported that after 2 weeks of feeding, mPPARα was preferentially activated by DEHP (Ito et al. 2012). This suggests that human PPARα is distinct from rodent types. These potency differences were also supported by the observation that the apparent low expression of PPARα was detected in the human liver (Palmer et al. 1998; Tugwood et al. 1996). Given the limited epidemiological data and species differences in susceptibility to DEHP exposure, DEHP seems not to be a human carcinogen to cause liver cancers.

Estrogen Receptor α

DEHP is suspected to be an endocrine disruptor chemical predominantly due to its agonist or antagonist role in interfering with the nuclear receptors (Eagon et al. 1994; Melnick 2001; Wang et al. 2012). DEHP at a concentration range of 0.1–100 μM was shown to promote the cell proliferation of ERα-positive breast cancer MCF-7 cells (Blom et al. 1998; Chen and Chien 2014; Okubo et al. 2003). The full name for this cancer cell line is "Michigan Cancer Foundation-7", which was originally derived via the pleural effusion from a 69-year-old Caucasian woman (Soule et al. 1973). This phenomenon indicates the ability of DEHP to activate ERα through mimicking the action of estrogen 17β-estradiol (E2). Moreover, changes in gene profiling in response to DEHP exposure has been observed in MCF-7 cells, and the altered genes appear mostly related to fetal development (Hokanson et al. 2006). The data imply that DEHP causes an adverse effect on fetal development probably via disturbing the ERα activity.

Other phthalate esters, including benzyl butyl phthalate, possess estrogenic activity about 100,000-fold less than E2. In contrast, the estrogenic activity of DEHP was undetectable in several in vitro assays (Harris et al. 1997; Lee et al. 2012). DEHP's lack of estrogenic activity was also supported by in vivo studies. Daily gavage of DEHP at the dosage of 600 mg/kg BW to immature female rats for 3 days did not affect the expression of Calbindin-D9k (CaBP-9k), a downstream target gene of E2 in the uterus (Hong et al. 2005). Besides, male Fischer 344 rats exposed to DEHP for more than 4 weeks showed a significant decrease in the activity of estrogen receptor and the expression of ceruloplasmin, a protein synthesized in response to estrogen levels (Eagon et al. 1994).

A study pointed out that DEHP needed to reach a dose of 100 μM for binding with ERα at extremely low affinity (around 1000-fold lower than E2). This concentration seems to be unreachable in our daily surroundings, except for the case where patients received the extracorporeal circulation therapy with the use of DEHP-containing PVC tubing (Ohashi et al. 2005). DEHP is assumed to share the structural similarity with E2, as it possesses a phthalic benzene ring that may function as an A ring moiety of E2. The much lower binding affinity of DEHP to ERα might be due to the absence of a phenolic hydroxyl group that E2 possesses in that functional group (Asai et al. 2000). Although DEHP might exert effects that are similar to those of E2, the mechanisms of how DEHP activates the estrogen receptor pathway and promotes the cell proliferation of MCF-7 cells are still a puzzle.

Aryl Hydrocarbon Receptor

The aryl AhR, a cytosolic ligand-activated transcription factor, belongs to the member of the basic helix–loop–helix (bHLH)-Per-ARNT-Sim family of the nuclear receptor. AhR is able to detoxify a broad range of xenobiotics including halogenated aromatic hydrocarbons, nonhalogenated polycyclic aromatic hydrocarbons, and related chemicals (Denison and Nagy 2003). Naturally occurring compounds are also reported to serve as endogenous AhR ligands that can mediate the AhR activity, such as tryptophan photoproducts, tetrapyrroles, and arachidonic acid metabolites (Abel and Haarmann-Stemmann 2010). However, these AhR ligands display a structural distinguishing feature, but, at least, conforming with one or more aromatic ring backbones with the hydrophobic character (Denison and Nagy 2003). A study indicated that the cavity for AhR ligand binding preserves a promiscuity property, allowing the accommodation of a broad range of AhR ligands (Denison and Nagy 2003). This behavior hinted that a set of phthalates, as well as DEHP, might be involved in activating the AhR pathway, as DEHP

consists of an aromatic ring with two carboxylic acid groups on the adjacent carbon that displays a hydrophobic character.

There are two AhR-responsive genes, cytochrome P450 (CYP)1A1 and CYP1B1, which are encoded for the lipophilic compound metabolism and largely contribute to the conversion of exogenous polycyclic hydrocarbons into a soluble form via oxidation (Go et al. 2015; Omiecinski et al. 1999). Both CYP1A1 and CYP1B1 can inactivate a set of therapeutic agents including caffeine, theophylline, phenacetin, and R-warfarin despite a difference in their metabolic rate (Yamazaki and Shimada 1999). CYP enzyme often accidentally converts certain xenobiotics to form carcinogens mainly by DNA-adduct formation, including benzo[a]pyrene, aflatoxin B1 and 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (Wang and Zhou 2009). CYP1B1 is physiologically important and ubiquitously expressed (although at a very low level) in many types of human tissues. Large amounts of CYP1B1 mRNA have been detected in numerous types of cancers, including lung, colon, breast, skin, lymph node, brain and testicular cancers (Murray et al. 1997). However, mouse hepatoma Hepa1.12cR cells exposed to DEHP were shown to weakly induce AhR transactivation via the regulation of dioxin response element, which is located in the promoter region of AhR-responsive genes (Kruger et al. 2008).

The role of DEHP involved in AhR activation is also supported by a study with humanized transgenic mice, in which DEHP increased the CYP1B1 expression driven by tetracycline in a dose-dependent manner (Hwang et al. 2005). Furthermore, human granulosa cells co-exposed to DEHP and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), a well-characterized agonist of AhR, showed an induction of the CYP1B1 expression, which was found to be more potent than that in the cells exposed to TCDD alone (Ernst et al. 2014). This suggested that exposure to DEHP tends to promote the AhR-mediated CYP1B1 expression as one of the possible mechanisms of DEHP-induced adverse effects, including developmental abnormalities, tumorigenesis caused by mutagens, as well as therapeutic resistance in cancers.

The Interplay Between DEHP and Redox Alteration on Carcinogenesis

In vivo experiments have confirmed that continuous administration of DEHP causes hepatocarcinogenesis in rodents (Kluwe et al. 1982). After performing the rodent cancer bioassays, DEHP was shown to disturb the redox homeostasis during tumorigenesis (Osumi and Hashimoto 1978). One postulated mechanism is the action of PPARα, which is supported by the fact that DEHP induces an increased transcription of the PPAR downstream targets, especially the enzymes in the CYP4A subclass (Johnson et al. 2002). This receptor belongs to a nuclear hormone receptor superfamily that is presumably stimulated in response to hypolipidemic agents, including the mono- and di-carboxylic acid metabolites of DEHP (Dzhekova-Stojkova et al. 2001). PPARα plays a critical role in modulating the lipid homeostasis including lipid transport, β-oxidation of fatty acids, and cholesterol metabolism.

Under normal circumstances for lipid metabolism, peroxisomal β-oxidation leads to the production of hydrogen peroxide (H_2O_2) as a side product in hepatocytes, constituting approximately one-third of the total H_2O_2 generated in the body (Dansen and Wirtz 2001; Palut 1997). However, DEHP is often best described as an arch criminal to induce a massive proliferation of peroxisomes which leads to the bulk generation of H_2O_2, suggesting DEHP may exert its tumorigenic action in an indirect genotoxic manner. Controversially, recent data have shown that mice exposed to DEHP in the absence of PPARα exhibited 8-hydroxy-2′-deoxyguanosine (8-OHdG) generation, implicating the occurrence of oxidative DNA damage, and increased incidence of liver tumors. Furthermore, the induction of 8-OHdG was more potently induced in PPARα-null mice than in the mice with PPARα (Ito et al. 2007). The results suggest that DEHP caused oxidative DNA damage both in the presence and absence of PPARα in rodents, which subsequently led to hepatic damage.

In addition, previous findings have yielded consistent results of DEHP causing redox perturbation; several anti-oxidants were mechanistically perturbed by DEHP in defense against the oxidative DNA damage. Xeroderma pigmentosum group C (XPC) gene is required for nucleotide excision repair. XPC gene also alleviates the burden of the oxidative DNA damage generated by the prolonged exposure of the cells, especially lung tissues and mouse embryonic fibroblasts, to atmospheric oxygen (20%), which contributes to mutagenesis. However, $XPC^{-/-}$ transgenic mice that were long-term exposed to DEHP (39 weeks) were predisposed to hepatocarcinoma developing with an increased frequency of liver mutations, as well as elevated lipofuscin levels, an index of oxidative stress in vivo (Melis et al. 2013). In addition, DEHP exposure for less than 60 days was sufficient for hepatic hyperplasia induction. This phenomenon was probably due to the diminished activity of the copper metabolic enzyme, ceruloplasmin oxidase, which is responsible for copper transportation from the liver to the blood circulatory system. Therefore, the loss of ceruloplasmin oxidase activity might have increased the oxidative damage generated by excessive copper accumulating in the liver (Eagon et al. 1999).

The mechanisms through which the DEHP-induced accumulation of hepatic copper may contribute to hepatocarcinogenesis remain unclear. It is possible that the accumulated copper in the liver may work in concert with

H_2O_2 to generate reactive oxygen species (ROS) as well as a metal–oxygen complex that gradually increases the mutational load. Furthermore, the activities of peroxide-destroying enzymes in F-344 male rats exposed to DEHP for 1 year were examined. The results showed that glutathione peroxidase and glutathione (GSH) were decreased in activities, except for catalase (Hayashi et al. 1994). We hypothesize that although catalase, the H_2O_2-destroying enzyme, was increased after 8 days of DEHP exposure, it was insufficient to compensate for the oxidative stress generated by the action of H_2O_2-generating peroxisomal fatty acyl-CoA oxidase and the hepatic copper-mediated ROS induction. Based on the above studies, the phenomenon of peroxisome proliferation is not the only hypothesis to describe the underlying pathway of DEHP-induced hepatocarcinogenesis. Changes in antioxidant defense mechanisms can also be used to illustrate the genesis of DEHP-induced tumorigenesis.

The Exposure of Growing Male Rats to DEHP Disturbs the Reproductive Development and Deregulates the Testosterone Biosynthesis

The notion that DEHP possesses anti-androgen properties is suggested by the observations that the reproductive malformations present in the male rat offspring are similar to those observed with well-studied anti-androgens, including vinclozolin, procymidone, and flutamide (Parks et al. 2000). In contrast, the dose of DEHP up to 10 μM showed no binding affinity for human androgen receptors in vitro (Gray et al. 2000). The observed malformations included hypospadias, undescended testes, shortened anogenital distance, and nipple retention, which were found in male rat offspring after prenatal and/or lactational exposure to excess DEHP, particularly in the period of masculinization. Yet, these mal-development syndromes were no longer apparent in adult male rats (15-week-old rats) (Gray and Gangolli 1986; Gray et al. 2000). This critical period of sensitivity to DEHP conforms to the timing of the androgen-driven genital developments in fetuses, notably testosterone. This particular androgen surges on gestation day (GD) 16 and reaches a peak on GD 19, playing a pivotal role in maintaining male fertility (Parks et al. 2000; Zhang et al. 2008). However, the reduction in testosterone levels in fetal and prepubertal male rats has been reported more frequently in the groups excessively exposed to DEHP during the late gestation and/or the early lactation periods, whereas the testosterone levels were not consistently reduced when those rat offspring reached the post-pubertal stage. During the fetal life of rodents, the testosterone is released into the fetal circulation primarily by the fetal-type Leydig cells residing in the interstitial space of testis (Svechnikov et al. 2010). Histological analyses of the testis from the male rat fetuses exposed to excess DEHP during late gestational and/or early postnatal periods have shown that the Leydig cells scattered within the testicular interstitium became hyperplastic as a large clump with the presence of multinucleated gonocytes in seminiferous cords (Andrade et al. 2006; Borch et al. 2005, 2006; Parks et al. 2000). The increased number of Leydig cells in DEHP-exposed male rat offspring is observed through the enhancement in immunohistochemical staining of 3β-hydroxysteroid dehydrogenase (3β-HSD), a Leydig cell-specific gene for catalyzing the bio-conversion of androgens (Borch et al. 2005). Examples include the conversion of androstenedione from dehydroepiandrosterone and testosterone from androstenediol (Simard et al. 2005). This increased number has also been confirmed by the results of upregulation of the proliferative markers, proliferating cell nuclear antigen (PCNA) and Cyclin D3, in the Leydig cells isolated from the testes of the DEHP-exposed male rat offspring (Akingbemi et al. 2004). Paradoxically, even though the massive proliferation of Leydig cells was observed in DEHP-exposed male rats, the testosterone production by Leydig cell was significantly decreased rather than increased (Parks et al. 2000).

The evidence supporting the role of DEHP in inhibiting the testosterone biosynthesis in Leydig cells after prenatal exposure (GD 7–21) comes from the observations demonstrating that the number of genes related to cholesterol uptake into the Leydig cells was decreased in the testes of the DEHP-exposed male fetuses. The genes included scavenger receptor B-1, steroidogenic acute regulated protein (StAR) and peripheral benzodiazepine receptor, a gene called P450scc (also termed Cyp11a1) responsible for the conversion of cholesterol to the precursor of all androgens, pregnenolone, and P450c17 for further conversion of progesterone into androstenedione (Borch et al. 2006).

A theory from a study of DEHP on castrated male rats at the pubertal stage suggested that malformations in the androgen-dependent organs were attributed to a DEHP-induced disturbance in testosterone production rather than to 5-reductase, which converts testosterone to dihydrotestosterone. This was because not only the development of dihydrotestosterone-dependent organs, for instance, prostate and seminal vesicles, were affected by DEHP treatment, but also the testosterone-dependent organs, especially the prostate, was affected by the lower doses of DEHP (Stroheker et al. 2005). Several studies have reported that a decrease in the expression of insulin-like factor-3 (Insl-3), a hormone secreted by Leydig cells for regulating gubernacular differentiation, was observed when fetal testes were prenatally exposed to DEHP (Borch et al. 2006; Howdeshell et al. 2007; Wilson et al. 2007).

Furthermore, extensive molecular analyses for the prenatal exposure to DEHP (GD 11–21) have demonstrated a decline in the expression of genes involved in

the steroidogenic pathway, including Cyp11a1, StAR, and Hsd3b1 (Howdeshell et al. 2007; Vo et al. 2009). Microarray approach also showed a significant increase in the expression of genes involved in the steroid metabolic process including Cyp11b1, Cyp11b2, and nr1h4, and a decrease in paraoxonase 1 (Vo et al. 2009). Studies of prenatal exposure to DEHP have shown that those newborn male rats had a reduction of serum levels of luteinizing hormone, a hormone released from the anterior pituitary glands and primarily stimulated testosterone production by Leydig cell (Akingbemi et al. 2001). The majority of evidence links maternal exposure of male fetuses to excess DEHP with reduced Leydig cell-mediated testosterone biosynthesis at GD 21 after the prenatal exposure. However, the adverse effects contributed by this exposure period were not observed in the DEHP-exposed male rats after growing to adult rats (PND 90) (Akingbemi et al. 2001).

In addition to the clearly documented contributions of in utero DEHP exposure to the reduction of testosterone biosynthesis, there are stark discrepancies in the testosterone levels altered by the male rats exposed to DEHP during the peri-pubertal period. For instance, exposure to 100 mg/kg/day DEHP starting at PND 21 for 28 days (PND 21 to PND 48) increased the serum testosterone and luteinizing hormone levels with the concurrent increase in Leydig cell-mediated testosterone biosynthesis (Akingbemi et al. 2001). Instead, those exposed to the same dose on PND 21 but extending the exposure period to 70 or 100 days (PND 21–90 or 120) consistently increased the serum testosterone and luteinizing hormone levels but failed to increase the testosterone biosynthesis, and even decreased dramatically (Akingbemi et al. 2004). The identical increases in the serum testosterone and luteinizing hormone levels observed in rats with the same DEHP doses for different periods of exposure were probably due to a compensatory mechanism. However, the decreased testosterone biosynthesis in male rats subjected to long-term exposure of 70 or 100 days was explained by the induction of aromatase, which is responsible for the conversion of testosterone to estrogen that, therefore, elevates the content of estradiol (Akingbemi et al. 2004).

Yet another study indicated that increasing the dose of DEHP to 750 mg/kg/day while preserving the exposure period to 28 days resulted in a decrease in the serum testosterone levels and was associated with delayed onset of preputial separation from PND 41.5 in the controls to PND 46.3 in DEHP-exposed groups. However, the effects of low-dose DEHP exposure appeared to differ from those of exposure to high doses. For instance, male rats exposed to 10 mg/kg/day DEHP for the same 28-day treatment displayed increased serum testosterone levels in company with the advanced onset of preputial separation to PND 39.7 (Ge et al. 2007). In consistency with this outcome, male rats exposed to low-dose DEHP (5–135 mg/kg/day) were reported to increase the testis weight on PND 22 after exposure from GD 6 to PND 21 (Andrade et al. 2006). An in vitro study reinforced these differences by showing that murine Leydig tumor MLTC-1 cells subjected to 0.001 μmol/L demonstrated a significant increase in the expression of P450scc, P450c17, 3β-HSD, and Insl-3, but no similar changes were seen at higher doses (10 μmol/L) (Chen et al. 2013). Hence, the DEHP-induced alterations in the male reproductive functions and the testosterone production seemed to depend heavily on the dose of administration, the duration of exposure, and the exposure period that are taking place during the fetal or the pubertal development.

DEHP Causes Epigenetic Events in a Transgenerational Manner

An epigenetic event is defined as reversible changes in gene phenotypes (either switching on or off certain genes) without changes in DNA sequences. There are several types of epigenetic modification, including DNA methylation, histone modifications, and nucleosome composition. A unique feature of epigenetic modification is that gene expressions could be reversibly regulated. However, epigenetic modification is heritable. That is, the loci of epigenetic silencing can be preserved through many rounds of DNA replication (Tammen et al. 2013). Methylation of CpGs (also termed CpG islands), which are genomic regions arranged in an order of 5′ cytosine base to 3′ guanine base frequently displaying the methylation status, and is more compatible upon circumstance since there are a class of DNA methyltransferases (DNMTs) responsible for maintaining the methylated CpGs in a newly synthesized strand of DNA, at the same time, can shut down specific genes effectively via deletion of these nucleotide sequences (Feng et al. 2010).

Some findings of the phthalate-associated adverse effects from the rodent studies have shed light on genomic imprinting, which refers to genes that are epigenetically marked in a parent-of-origin-specific manner, generally achieved through CpG methylation to maintain the silenced status (Doyle et al. 2013; Kang et al. 2011; LaRocca et al. 2014; Li et al. 2014; Manikkam et al. 2013; Parks et al. 2000). After gestating rodents (F0 generation) were exposed to DEHP during the embryonic stage 7–14 days, their unexposed descendants (F2 and F3 generations) showed an increased risk of adult-onset disease in the absence of further insults. This suggests the changes in a transgenerational manner and is termed transgenerational diseases. These studies further indicated that DEHP has a profound impact on epigenetic aberration affecting the gene expression in the unexposed offsprings, called epimutation. Notably, the germ cells of the unexposed F3 generation were shown to be altered in their epigenetic

profile at the 197 DNA methylation regions; especially, a gene encoding for the glial cell line-derived neurotrophic factor, showing an approximately 38-fold increase in expression (Manikkam et al. 2013).

In reproductive diseases, DEHP not only has been reported to reduce the anogenital distance, but also to decrease the number of spermatogenic cells. Both of these adverse effects are displayed in a transgenerational manner (Dalsenter et al. 2006; Doyle et al. 2013; Parks et al. 2000). In the case of obesity, exposure of gestating female rats to DEHP resulted in glucose metabolic dysfunction in their offsprings. Concomitantly, the down-regulation of glucose transporter 4 (GLUT4) gene expression, via DNA methylation of the GLUT4 promoter switch at the bHLH transcription factors (MYOD)-binding site, and increased DNMT levels were observed (Rajesh and Balasubramanian 2014). These observations provide a clue that one of the possible actions of DEHP was to cause changes in DNA methylation, leading to transgenerational diseases. And these changes, at least in part, preserved the altered epigenetic pattern that will pass from one generation to the next.

Genomic imprinting within the developing germ cells is critical for giving rise to a new organism. Apart from the DNA genetic code of germ cells that is inherited from both paternal and maternal origins, there are about a 100 epigenetic imprints donated by a father or mother (Morison et al. 2005). After fertilization, the developing germ cells arising in the embryo undergo an epigenetic reprogramming of imprinted loci during embryonic days 7 to the newborn stage (Sasaki and Matsui 2008). This reprogramming, in which a set of DNA methylation imprints are erased and further reestablished, is critical for the fetal germ cells to obtain totipotency and permit the systematic process of fetal development after fertilization to form a zygote (Hajkova et al. 2008; Kono et al. 2004). This erasure of methyl imprints creates an instant de-repressing of chromatins in fetal germ cells but not in somatic cells. However, the re-establishment of the erased imprints occurs at the stage of sexual determination (prenatal) in the male fetus, but after birth (postnatal) in the females. This difference in the imprinting period between males and females provides a clue that exposure of the F0 generation to DEHP tends to produce transgenerational diseases in the male offsprings. A recent study showed that exposure of pregnant mice to DEHP caused the epigenetic perturbations in 4 of the 38 representative imprints in the early developing embryo. These altered imprints include Igf2r in the yolk sac, Rasgrf1 in the placenta, Osbpl5 in the yolk sac, and impact in the intestines (Kang et al. 2011).

Therefore, contact with DEHP during the period of pregnancy (especially at the stage at which fetal germ cells undergo epigenetic reprogramming) should be considered a risk for humans predisposed to developing the transgenerational diseases. Although DEHP is not working exclusively in such circumstances, at least, it offers a slight prospect of identified imprinted genes, the properties of which could be used to support the transgenerational phenomenon.

The Potential Contributors Induced by DEHP for Acquired Chemoresistance

As far as the leakage of DEHP from medical devices is concerned, patients continuously exposed to DEHP daily or during therapies seem to be inevitable. DEHP is considered either acting as a suspected carcinogen or cancer promoter, but its role in chemoresistance has long been overlooked. Recently, accumulating findings reported that both short- and long-term exposure to DEHP were correlated to chemosensitivity via diverse mechanisms (Angelini et al. 2011; Kim et al. 2004; Sims et al. 2014). The issues of chemoresistance have been studied for over three decades. It is a major obstacle when the effectiveness of chemotherapy is limited in malignant tumors. According to the previous studies, tumor cells are resistant to chemotherapy intrinsically or acquire resistance following chemotherapeutic treatments (Abdullah and Chow 2013; Holohan et al. 2013; Wang et al. 2011). Cancer cells can acquire chemoresistance by several known mechanisms, including delayed drug delivery, activation/inactivation of exogenous chemicals, and reducing anticancer drug-induced cellular stress (Fojo and Bates 2003; Longley and Johnston 2005).

Delayed Drug Delivery Caused by DEHP Exposure

Chemoresistance developed via impaired drug delivery is mediated by ATP-binding cassette (ABC) transporter pumps, including multidrug resistance protein 1 (MDR1, also known as ABCB1), multidrug resistance-associated protein 1 (MRP1, also known as ABCC1) and ATP-binding cassette subfamily G member 2 (ABCG2, also known as BCRP) (Gottesman 2002). Activation of these proteins can enhance the activities of efflux pumps for anticancer drugs. In other words, the increased expression of these proteins leads to decreased intracellular drug accumulation, hence limiting the effectiveness of the chemotherapeutic agents. Takeshita has found that DEHP could activate MDR1 gene expression via steroid and xenobiotic receptor in a human colon cancer cell line (Takeshita et al. 2006).

The Activation/Inactivation of Exogenous Chemicals in Response to the DEHP-Induced Alteration of the CYP and GST Systems

There are a large number of enzymes belonging to the class of cytochrome P-450 (CYPs) known to detoxify a wide range of exogenous chemicals. While DEHP was originally

classified as a ligand of PPARα, supported by its adverse effects on hepatic peroxisomes and increasing the transcription of the PPAR downstream targets, particularly CYP4A1 and CYP4A3 (Johnson and Klaassen 2002; Ward et al. 1998), DEHP effects on other subclasses of CYP enzymes were gradually identified. For example, oral administration of DEHP to male Sprague–Dawley rats at 500 mg/kg/day for 28 days significantly increased the protein expression of CYP2B1/2B2, both of which were reported to be transcriptionally regulated by the CAR (Kim et al. 2010). This is consistent with an in vitro study in which exposure of humans to DEHP increased the target genes of CAR, particularly the transcripts of CYP2B6 and CYP3A4 (DeKeyser et al. 2009). Nevertheless, the beliefs regarding the DEHP-induced CYP enzymes linked with the metabolisms of DEHP into readily excreted metabolites are confounded by the finding: all of the DEHP-induced CYP enzymes (CYP1B1, CYP2B6, CYP3A4, and CYP4A2/3) are not involved in the majority of CYP enzymes that are responsible for DEHP metabolisms, such as CYP2C9 and CYP2C19, and CYP2C6 (Choi et al. 2012; Hwang et al. 2005). Alternatively, a study suggested the possibility that DEHP may serve as a promoter in hepatocarcinogenesis. The detoxification process of these CYP enzymes can sometimes inadvertently contribute to the metabolic conversion of procarcinogens into active ultimate carcinogens, such as the formation of (+)-anti-BP7,8-dihydrodiol-9,10-epoxide (BPDE) from benzo[a]pyrene. Treatment of male Wistar rats with DEHP for 14 days showed an increased formation of BPDE–DNA adducts in isolated hepatocytes partially via the enhancement in CYP1A1/2 activity (Voskoboinik et al. 1997). Furthermore, male Wistar rats intraperitoneally administered with DEHP and its metabolites MEHP for a month have been shown to be more effective in their hepatic CYP-dependent metabolism of propranolol, an approved drug used for antianginal antihypertensive and antiarrhythmic conditions (Kambia et al. 2003). Most antitumor agents can induce ROS-dependent apoptotic cell death (Trachootham et al. 2009), including motexafin gadolinium (Magda and Miller 2006), elesclomol (Kirshner et al. 2008; Tuma 2008), doxorubicin and daunorubicin (Vasquez-Vivar et al. 1997). However, cancer cells may utilize metabolic mechanisms by which the cytotoxicity of antitumor agents can be neutralized by certain CYP detoxification enzymes with accompanying decreases in ROS induction (Michael and Doherty 2005). These studies provide a cue that DEHP may be involved in promoting hepatocarcinogenesis and possibly neutralizing chemotherapeutic agents.

The other superfamily of detoxifying enzymes affected by DEHP is the hepatic glutathione S-transferases (GSTs), which catalyze the attachment of GSH to a variety of electrophilic compounds inclusive of potent carcinogens, thereby preventing the danger posed by these compounds. More than 5% of the total proteins in rat liver are GSTs (van Ommen et al. 1990), and moderate inhibition of GST has been suggested as adequate enough to increase the formation of covalent adducts with DNA. Nevertheless, inhibition of the GST activity appears to be a major phenomenon induced by DEHP (Botelho et al. 2009; Erkekoglu et al. 2014; Law and Moody 1991; Tamura et al. 1990). When tested in vitro on hepatocytes, both DEHP and its metabolite MEHP were reported to reduce the GST activity as measured by the substrates of GST, 1,2-dichloro-4-nitrobenzene (DCNB) and 1,2-epoxy-3-(*p*-nitro-phenoxy)propane (ENPP) (Law and Moody 1991). In line with this outcome, the effect of the prolonged administration of male rats with DEHP was also shown to alter the proportion of GST isoenzymes isolated from liver. Although the hepatic contents of the analyzed GST isoenzymes were not wholly suppressed in the DEHP-treated rats; particularly, a decrease in GST 4-4 and an increase in GST 2-2 were noted, and there was an overall trend of reduction in the GST activity as observed in most cases (Voskoboinik et al. 1996). Notably, GST 4-4 is cited as one of the prominent detoxifying enzymes involving in the conjugation of BPDE, an ultimate carcinogen generated through the cytochrome P450 pathway, with GSH to eliminate this toxin (Voskoboinik et al. 1997). Therefore, this DEHP-mediated loss of GST 4-4 suggests that DEHP may render an organism susceptible to chemical toxicity via weakening some parts of the CYP system.

DEHP-Mediated Reducing Anticancer Drug-Induced Cellular Stress

When the drug is switched on after binding with the drug target, the downstream signaling can be activated to promote the generation of cellular stress and then inhibit cancer cell growth via inducing cell apoptosis. In addition, some anticancer drugs compete with growth factors for a specific receptor, for example, tamoxifen. However, Kim has demonstrated that DEHP could inhibit tamoxifen-induced apoptosis in human MCF-7 breast cancer cells (Kim et al. 2004). Furthermore, DEHP exposure was reported to induce the proliferation of the MCF-7 breast cancer cells and promote the metastasis of neuroblastoma cells by activating the Akt/PI3K signaling pathway (Chen and Chien 2014; Zhu et al. 2010). Yet another study indicated that exposure of ERα-negative breast cancer cells MDA-MB-231 to DEHP leads to increased cell invasiveness, as well as an increased expression of matrix metalloproteinases-2/-9 via NF-κB pathway (Zhang et al. 2016). The activation of proliferation and metastasis signaling pathways caused by DEHP exposure may decrease the anticancer drug efficacy and confer the acquisition of chemoresistance. In conclusion, DEHP exposure may result in reducing the clinical anticancer drug efficacy through activating the drug efflux pump protein, the

drug metabolism system, and the anti-apoptotic or the pro-survival signaling pathways during chemotherapies (Fig. 1).

Conclusions

The data collected from the epidemiological and the rodent studies are quite sufficient to raise concerns about the potential carcinogenicity of DEHP, yet the epidemiological evidence in humans seems inadequate to support an attribution of carcinogenicity to DEHP exposure. It also remains unclear which of the cellular processes, including activation of the nuclear receptors such as PPARα, ERα, and AhR, redox alteration, and epigenetic disturbance, act as the major pathway contributing to the DEHP-induced tumorigenesis and reduced chemosensitivity. Nonetheless, health concerns should be raised regarding the wide applications of DEHP as plasticizers and in food products.

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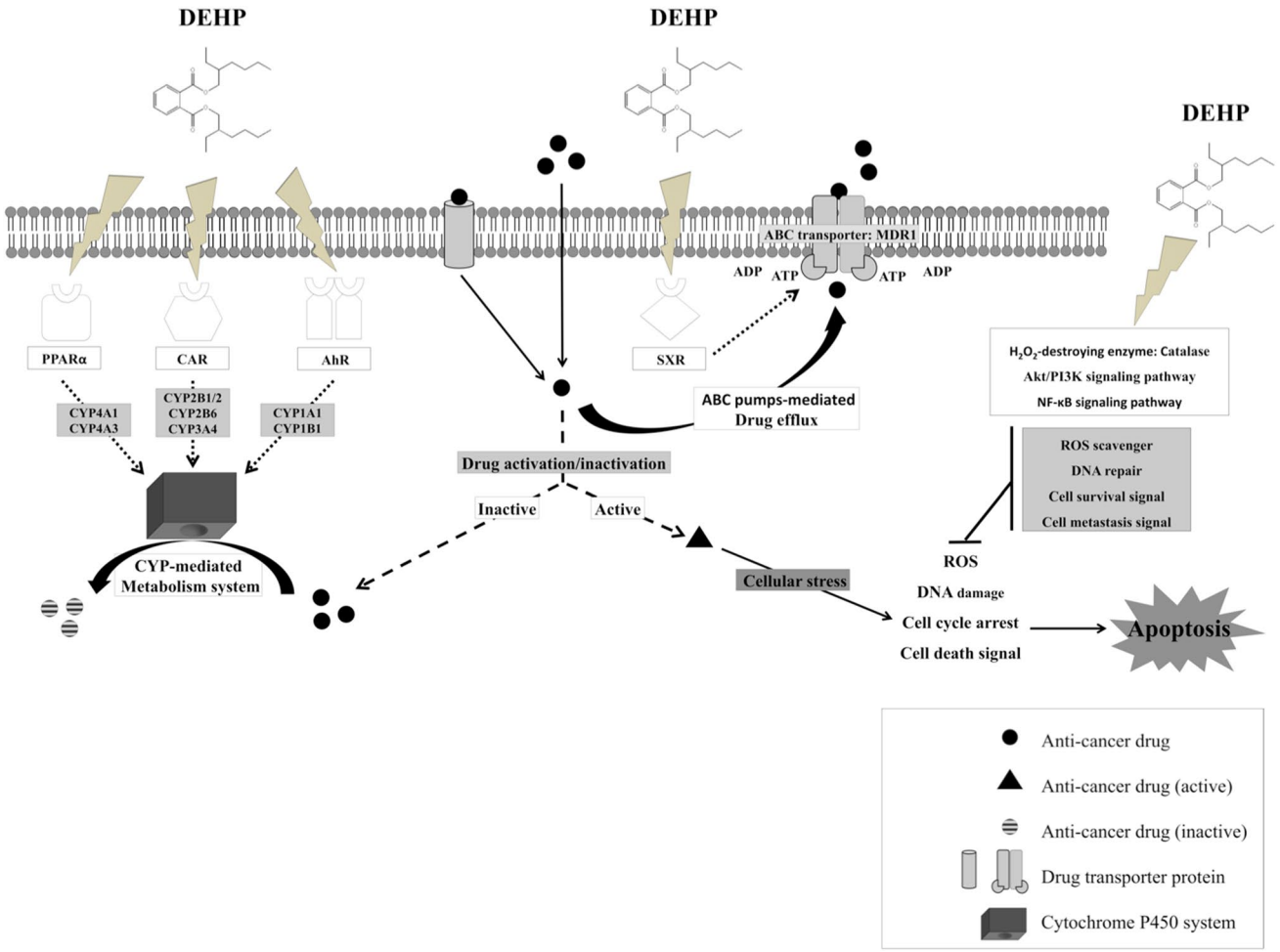

Fig. 1 The model for DEHP-mediated acquisition of chemoresistance. This diagram schematically depicts the possible pathways of DEHP-promoted resistance to anticancer drugs. After the influx of anticancer drugs into cancer cells, the ATP-binding cassette (ABC) transporters that reside in the plasma membranes pump the influxed drugs out of the cancer cells, notably via the MDR1. The remaining drugs would be retarded in their pro-form (inactive form) possibly due to the DEHP-mediated decrease of the drug activation enzymes or the metabolic inactivation of the drugs mediated by the CYP system. These remaining drugs could lead to different cellular stress, such as ROS attack, DNA damage, cell cycle arrest and cell death signaling. At the same time, catalase, an endogenous scavenger of free radicals, may undergo a DEHP-induced increase for neutralizing the oxidative DNA damage. In addition, the drug-induced damage in cancer cells that have previously been exposed to DEHP might be attenuated by the oncogenic signaling pathways, such as the Akt/PI3K and the NF-κB signaling pathways, thereby improving the survival of these cancer cells. *PPAR* peroxisome proliferator-activated receptor, *AhR* aryl hydrocarbon receptor, *CAR* constitutive androstane receptor, *SXR* xenobiotic receptor

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