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REVIEW

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The AhR pathway regulation in phthalates-induced cancer promotion, progression and metastasis: a scoping review

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Abstract

Background Cancer remains a leading cause of death worldwide. Environmental factors, specifically endocrine-disrupting chemicals (EDCs), like phthalates, are increasingly being linked to cancer development. Phthalates, widely used in consumer products, can activate the aryl hydrocarbon receptor (AhR). This scoping review investigates how phthalate exposure influences cancer-related molecular pathways through the regulation of the AhR pathway to uncover the underlying mechanisms.

Methods We conducted a comprehensive literature search in PubMed, Scopus, and Web of Science (ISI) database up to November 2023. Studies were selected based on peer-reviewed status, focus on phthalates' effects on cancer through the AhR pathway and the availability of full texts. Data extraction emphasized study models, types of phthalates, exposure protocols, and cancer-related signaling pathway outcomes.

Results Out of 108 initial articles, 10 met the inclusion criteria. Di-(2-ethylhexyl) phthalate (DEHP) and its metabolite Mono (2-ethylhexyl) phthalate (MEHP) were found to promote cancer cell proliferation, epithelial-mesenchymal transition (EMT), and chemoresistance through the AhR pathway. Specifically, DEHP activated AhR, leading to elevated expression of EMT markers, increased cancer stem cell populations, and enhanced drug metabolism and resistance. Other phthalates, such as Butyl Benzyl Phthalate (BBP), also activated AhR-mediated pathways, promoting angiogenesis and metastasis.

Conclusion Phthalates activate the AhR pathway, contributing to cancer progression underscoring the need for developing effective interventions against phthalate-induced carcinogenesis. Regulatory measures to minimize phthalate exposure are crucial to preventing harmful health effects and improving cancer treatment outcomes.

Keywords Phthalates, AhR pathway, Metastasis, EMT, Metabolic dysregulation

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Introduction

Cancer continues to be a major cause of death from illness worldwide. Annually, over 19 million individuals are diagnosed with cancer and 10 million die from the disease, accounting for one in six deaths globally [1, 2]. The risk of developing cancer may be increased by exposure to certain environmental chemicals [3]. Endocrine-disrupting chemicals (EDCs) are a growing global concern due to their significant threats to human health and the environment. A study by the FDA discovered over 1800 chemicals that interfere with at least one of the three major endocrine pathways: androgen, estrogen, and thyroid [4]. EDCs include a wide range of chemicals such as pesticides, bisphenols, heavy metals, flame retardants, and phthalates [5]. The rising incidence of hormone-related cancers over recent decades has raised concerns about the potential role of EDCs in cancer development [6]. It is estimated that nearly 80% of EDCs have the potential to cause tumors [7].

Phthalates are esters of 1,2-benzenedicarboxylic acid. The composition of phthalates varies depending on the number of side chains, which may include alkyl, dialkyl, or aryl groups. Phthalates are classified into two categories based on their molecular weight: high-molecular-weight phthalates (HMWP) and low-molecular-weight phthalates (LMWP). HMWPs, such as Di-n-octylphthalate (DnOP), DEHP, Di-(2-propylheptyl) phthalate (DPhP), Diisononyl phthalate (DiNP), and Diisodecyl phthalate (DiDP), are used in the production of polyvinyl chloride (PVC), while LMWPs, such as di-n-butyl phthalate (DnBP), Dimethyl Phthalate (DMP), Diisobutyl phthalate (DiBP), Diethyl phthalate (DEP), and Benzyl butyl phthalate (BBzP), are commonly found in personal care products, solvents, and adhesives [8]. Due to no covalent bonding existing between phthalates and the plastic matrix, these chemicals are easily released and distributed in water, soil, and air, and then exposure occurs in multiple ways (inhalation, ingestion, and dermal contact). The global market share of phthalates was projected to reach 6.76 million tons in 2019, with HMWPs such as DiNP and DEHP accounting for over three-quarters of the total [9].

In 2013, the International Agency for Research on Cancer (IARC) reclassified DEHP from Group 3 to Group 2B, acknowledging the role of DEHP and its metabolite, MEHP, in initiating and supporting cancer growth [10]. The US Environmental Protection Agency (EPA) and the European Union have set an oral reference dose (RfD) of 20 μg/kg body weight/day and a tolerable daily intake (TDI) of 50 μg/kg body weight/day for DEHP [11]. Despite increasing evidence linking phthalates like DEHP and DBP to cancer progression, the precise molecular mechanisms, particularly through AhR-related pathways, remain poorly understood. For instance, high-level DBP exposure has been associated with a nearly two-fold increase in the risk of estrogen receptor-positive breast cancer, underscoring the urgent need for targeted research in this area. Unraveling these pathways could offer novel insights for therapeutic interventions in cancer [12]. Phthalates, found in the environment, can bind to and interact with nuclear receptors including progesterone, estrogen, and aryl hydrocarbon receptors (AhR). AhR, a member of the basic helix-loop-helix/Per-ARNT-SIM (bHLH/PAS) family, plays a key role in various cellular functions and can bind to a broad range of ligands. These ligands include natural molecules like kynurenine and 6-formylindolo[3,2-b]carbazole (FICZ), as well as exogenous compounds like BaP and Benzo(a)pyrene and 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD). The effects of these ligands can vary depending on the tissue. When AhR is not bound to a ligand, it forms a cytosolic complex with Hsp90, c-Src kinase, XAP2, and the chaperone p23 [13]. Upon ligand binding, AhR undergoes conformational changes that result in the dissociation of the protein complex and the translocation of AhR to the nucleus. There, it dimerizes with the AhR nuclear translocator (ARNT) and binds to xenobiotic-responsive elements (XREs) in the regulatory regions of target genes, initiating their transcription [14].

A recent study suggested that the AhR binding site has a promiscuous nature, allowing it to accommodate a wide variety of ligands, including DEHP [15]. DEHP's hydrophobic nature arises primarily from its aromatic ring and branched alkyl chains [16]. Furthermore, phthalates may influence tryptophan metabolism and activate the AhR pathway, both of which are critical in regulating cell growth, apoptosis, differentiation, and tumor formation [17]. This scoping review endeavors to analyze the influence of phthalate exposure on AhR signaling in cancer promotion, progression and metastasis, while also examining the mechanisms by which phthalates influence cancer cell proliferation, metabolism, angiogenesis, and EMT.

Methods

Information sources and search strategy

A comprehensive literature search was conducted according to the PRISMA extension for scoping reviews (PRISMA-ScR) [18] to obtain all relevant studies on "the role of AhR pathway in phthalates-induced cancer metastasis". The search used both Medical Subject Headings (MeSH) terms and advanced search options in the electronic database PubMed, Scopus, and Web of Science (ISI). The following keywords were used: ("Aril hydrocarbon receptor" OR "Aryl Hydrocarbon Receptor" OR "Receptor, Aryl Hydrocarbon" OR "Aryl Hydrocarbon Receptors" OR "Dioxin Receptor" OR "Receptor, Dioxin" OR "Dioxin Receptors" OR "Receptors, Dioxin"

OR "Polyaromatic Hydrocarbon Receptors" OR "Polyaromatic Hydrocarbon Receptor" OR "Receptor, Polyaromatic Hydrocarbon" OR "Receptors, Polyaromatic Hydrocarbon" OR "TCDD Receptor" OR "Receptor, TCDD" OR "Receptors, TCDD" OR "TCDD Receptors" OR "ash receptor" OR "receptor, ash" OR "ash receptors" OR "receptors, ash" OR "Receptors, 2,3,7,8-Tetrachlorodibenzo-p-dioxin") AND (phthalate*) AND ("cancer progression" OR metastasis OR EMT OR "Epithelial-Mesenchymal Transition" OR "Epithelial Mesenchymal Transition") up to November 2023. The syntax of each database included as supplementary.

Eligibility criteria and study selection

After acquiring and organizing the articles, we removed any duplicates using the reference manager tool. Following that, two independent reviewers (H. H-A and A. SH) conducted a two-stage screening process. In the first stage, the reviewers assessed the relevance of each study by examining titles and abstracts in line with the objectives of this study. In the second stage, the remaining articles were evaluated for inclusion or exclusion based on predefined criteria. Studies were included if they: [1] were peer-reviewed; [2] specifically investigated the effects of phthalates on cancer progression and metastasis through the AhR pathway; [3] provided sufficient data; [4] had accessible full texts; and [5] had no restrictions based on the year of publication.

Studies were excluded if they were: (a) unrelated research; (b) case reports; (c) letters to the editor; (d) book chapters; (e) posters; (f) editorials; (g) review articles; or (h) conference papers and abstracts.

Data extraction process

Two researchers (H. H-A and A. SH) assessed each eligible study and subsequently obtained the following information: [1] author name and year of publication; [2] types of models used (in vivo or *in vitro);* [3] type and dosage of phthalates, along with the exposure protocol; [4] the effects of phthalates on cancer progression; and [5] the proposed signaling pathway.

Results

Literature search and screening

Figure 1 illustrates the study selection process. We applied the elimination process according to the methods described by Bramer et al. [19]. A comprehensive search of the listed electronic database yielded 108 articles until November 2023. After removing duplicates ($n = 46$), we assessed the remaining articles ($n = 62$) by reviewing their titles and abstracts, eliminating 38 articles based on relevance. Of the 24 full-text articles, 14 were removed (not related = 10, incomplete date = 1, and non-original = 3), and 10 studies met the inclusion and exclusion criteria. Table 1 summarizes the key information from the selected studies.

Data extraction

Table 1 presents key data extracted from each paper, using the ASH and MA techniques. Two researchers conducted a thorough and independent evaluation, reaching a consensus on any discrepancies.

Di-(2-ethylhexyl) phthalate (DEHP)

DEHP and AhR are both involved in drug resistance in breast cancer cells. Specifically, DEHP blocked the effects of paclitaxel and doxorubicin in MDA-MB-231 and MCF-7 cells, as well as in zebrafish and mouse tumor initiation models. DEHP activated the vinculin/AhR/ERK signaling pathway, leading to increased expression of trefoil factor 3 (TFF3). Additionally, through the AhR genomic pathway, DEHP upregulated CYP450 isoforms, including CYP2D6, CYP2C8, and CYP3A4, in the MCF-7 and MDA-MB-231 breast cancer cell lines, which contributed to enhanced EMT and doxorubicin metabolism. In AhR knockout mice, DEHP-induced changes in ER expression through ubiquitination mechanisms reduced tamoxifen efficacy [20]. Short-term exposure (5 days) to low levels (33–400 μg/L) of DEHP increased AhR activity in Tg (cyp1a) zebrafish in a dose-dependent manner [21]. DEHP also enhanced vitality and ATP production in pituitary cells, increasing cell proliferation and the expression of Ccnd1 and PTTG mRNA, along with elevated AhR and AIP expression [22].

At a dose of 0.1 μM, DEHP significantly promoted migration and invasion in PLC and Huh7 cells. Following a 24-hour exposure to DEHP, the expression of the epithelial marker E-cadherin decreased considerably, while the expression of the mesenchymal indicators N-cadherin and vimentin increased. In addition, DEHP enhanced the proportion of cancer stem cells ($CD44^+$, CD133+, and SP cells). Suppression of AhR via shRNA inhibited ERK phosphorylation and reduced DEHP-induced SK1 activity. Transfection with S1PR3 shRNA reduced the CD44+/CD133 + cell population stimulated by DEHP, indicating that DEHP regulates HCC CSC-like cells via S1PR3, not S1PR1. Overall, DEHP promotes migration, invasion, and CSC-like cell populations in HCC through the activation of the AhR/ERK/SK1 pathway via S1PR3 signaling [23].

Mono(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP) and Mono(2-ethyl-5-hexyl) phthalate (MEHP)

MEHHP, a metabolite of DEHP, significantly increased cell viability and decreased apoptosis in leiomyoma (LM) cells. It also elevated intracellular levels of tryptophan and kynurenine and upregulated the expression of SLC7A5, SLC7A8, and tryptophan 2,3-dioxygenase (TDO2). MEHHP induced nuclear translocation of AhR

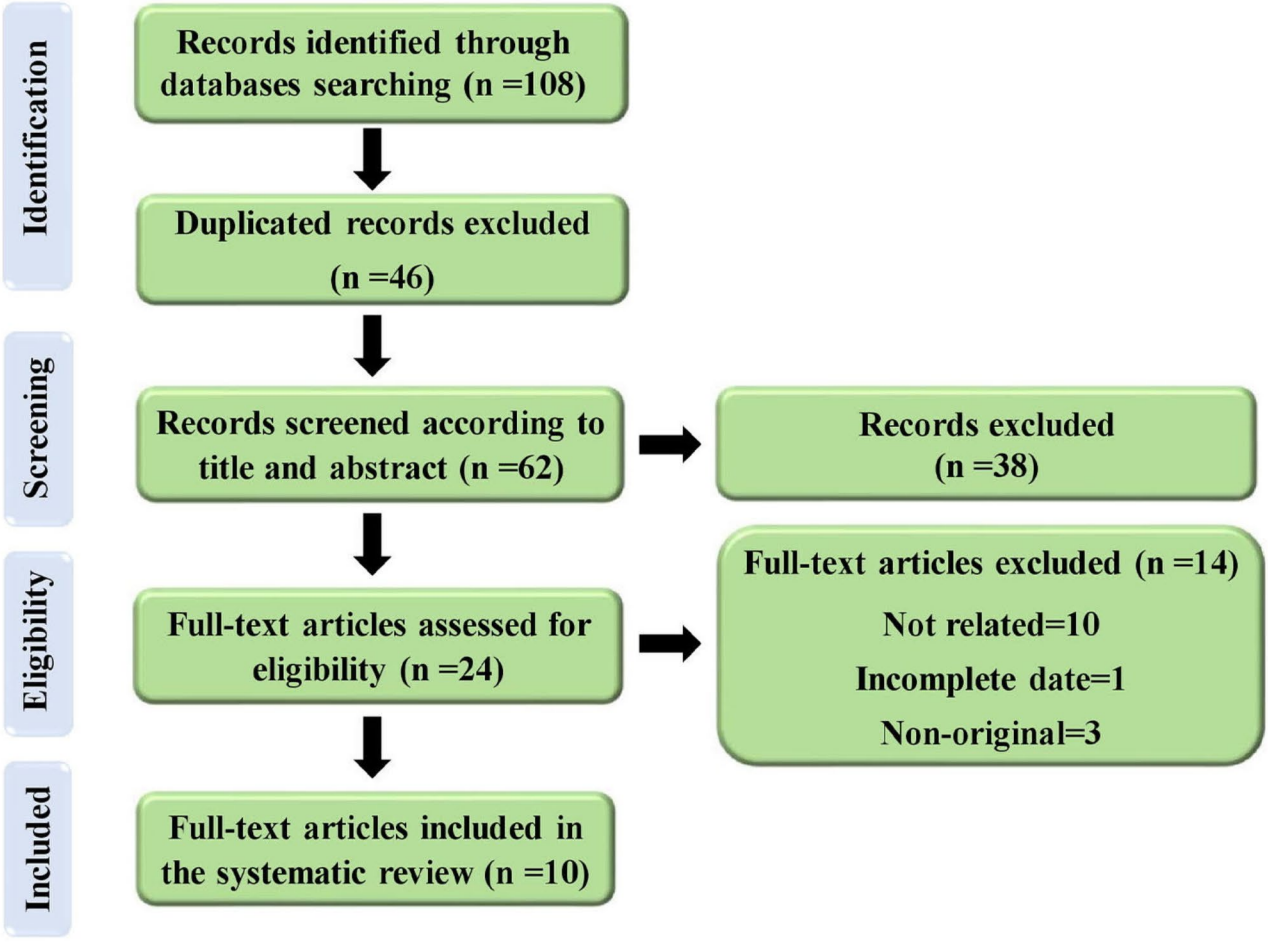

Fig. 1 Flow diagram of the current study's selection process

and increased the expression of CYP1A1 and CYP1B1. Knocking down siRNA or inhibition of SLC7A5/SLC7A8, TDO2, or AhR with drugs eliminated the effects of MEHHP on leiomyoma cell viability [17].

Both MEHP and TCDD can increase MCF7 cell migration and invasion in a dose-dependent manner. However, when administered together, MEHP and TCDD negated each other's effects. MEHP also induced AhR-DRE binding and increased CYP1A1 expression, while decreasing TCDD-induced CYP1A1 levels. By binding to AhR, MEHP acted as a competitive ligand to TCDD, inhibiting the EMT process that MEHP alone initiates in MCF7 cells [24].

Benzyl butyl phthalate (BBP)

BBP stimulated AhR in Huh7 cells via a non-genomic mechanism that involved G-protein signaling, promoting cell migration and invasion through the AhR/Gβ/PI3K/Akt/NF-κB pathway. Furthermore, BBP stimulated angiogenesis, both in vitro and in vivo, by activating the AhR/ERK/VEGF pathway. BBP also increased COX-2 production through the AhR/Gαq/11/calcium signaling pathway [25].

In breast cancer cells, BBP activated AhR, which accelerated the SPHK1/S1P/S1PR3 signaling pathway, facilitating the formation of metastatic breast cancer stem cells. BBP induced histone modifications in S1PR3-expressing side population (SP) cells, but no similar changes were observed in non-SP cells. Suppression of SPHK1 or S1PR3 in breast cancer cells significantly reduced tumor development and lung metastasis in vivo [26].

Activation of cell surface AhR by BBP also triggered the cyclic AMP (cAMP)/PKA/CREB1 signaling pathway, leading to the upregulation of HDAC6 expression. This promoted the formation of the β-catenin-LEF1/TCF4 transcriptional complex within the nucleus, thereby activating the c-Myc oncogene [27]. Furthermore, BBP demonstrated the ability to modify the expression of the aryl-hydrocarbon receptor nuclear translocator 2 (ARNT2), showing a dose-dependent reduction in ARNT2 expression in ESR1-positive cells (MCF-7 and

Table 1 Summary of studies on phthalates and AhR correlation in cancer promotion, progression and metastasis

Author & year	Models		Type of Phthalates (dosage) / Exposure protocol	Outcomes of Phthalates on Promotion and Progression of Cancer	Proposed signaling pathway
Hsieh et al., [20]	In vitro	MCF-7, MDA-MB-231	DEHP (1 μM), for 24 h (CCK-8 assay) and 14 days (clonogenic assay). DEHP metabolites (1 μM) on cell growth	↓ paclitaxel and doxorubicin effects on cell growth ↑ Cell viability & colony number (DEHP resulted in higher cell numbers relative to its metabolites)	DEHP directly targeted AhR and affected its downstream signaling pathway (↑ CYP1A1 and CYP1B1 protein expression). ↑ TFF3 expression through the vinculin/AhR/ERK ↑CYP family members and ↑ doxorubicin metabolism ↓ER expression (AhR-mediated and through the ubiquitination system). ↓ Tamoxifen effects in $AhR^{+/+}$ transgenic mice ↓ Paclitaxel & doxorubicin effects
	In vivo	AhR-null mice were treated with DMBA for 6 weeks to initiate mammary tumor formation	DEHP (1 μM), paclitaxel, doxorubicin, DEHP + paclitaxel, or DEHP + doxorubicin for 4 weeks.	↑ pulmonary metastasis ↑ mammary tumor formation	
		Zebrafish xenotransplantation (1×10^4 MDA-MB-231–GFP)	MDA-MB-231–GFP cells were treated with different groups of DEHP (1 μM), paclitaxel (1 μM), or doxorubicin (1 μM) for 24 h, then implanted into the yolk sac of each zebrafish embryo. whole-body fluorescence was performed imaging 48 h after transplantation.	↓ paclitaxel and doxorubicin effects on cell growth	
	Clinical	457 breast cancer patients (79.9% in Early (0/I/II) stage, and 20.1% in Advanced (III/IV) stage)	urine concentrations of DEHP metabolites, reflecting exposure levels	Σ4MEHP were positively correlated with tumor stage, lymph node status, estrogen receptor status, Her2/Neu status, recurrence, tumor size.	
Hsieh et al., [20]	In vitro	MDAMB-231	BBP, DBP, DMF (0.1–10 μM)/ for 24–72 h (XTT and BrdU assay), 24 h (Wound-healing and invasion assays), or 21 days (Colony formation assay)	↑ Proliferation, Migration, Invasion & Colony formation ↑AhR membrane localization	phthalates induce gene expression of HDAC6 through the AhR-PKA-CREB1 cascade ⟨ facilitated nuclear assembly of the β-catenin-LEF1/TCF4 ⟨ c-Myc activation
	In vivo	2×10^6 MDA-MB-231-GFP cells were Sc injected into the flanks of female nude mice	BBP & DBP (800 mg/kg/d)/ were IP injected for 45 days	↑tumor growth ↑HDAC6 and c-Myc	
Junaid et al., [21]	In vitro	MDA-MB-231	DEHP (10, 33, 100, 400, 800, 1600, 3200 μg/L) for 24–96 h (MTT assay), 24 h (Comet assay)	↑DNA damage & ↓Cell viability,	Activated PI3K-AKT-mTOR pathway
	In vivo (Migration assay)	xenotransplantation of CM-DiI labelled MDA-231 into yolk of zebrafish	DEHP (400–1600 μg/L) for 6 h	↑ Migration	
	In vivo (Acute)	zebrafish embryos	DEHP (0, 10, 33, 100, 200–400 μg/L) for 7 days	↑ Cellular Growth and Proliferation	
		Tg(cyp1a: gfp) zebrafish	DEHP (10, 33, 100, 400 μg/L) for 5 days	Dose-dependent AhR activation in zebrafish embryos, confirmed by fluorescence.	
	In vivo (Chronic)	healthy 5-month-old adult male zebrafish	DEHP (10, 33, 100 μg/L) for 3 months	↓Most of the HPT associated genes (tshβ, nis, tg, nkx2.1, ugt1ab, dio2, trα, and traβ)	environmental levels disturbed the endocrine system through the HPT axis pathway suppression

Table 1 (continued)

Author & year	Models		Type of Phthalates (dosage) / Exposure protocol	Outcomes of Phthalates on Promotion and Progression of Cancer	Proposed signaling pathway
Iizuka, [17]	In vitro	Primary cell culture of leiomyoma tissue patients	MEHHP (0.16, 1.6, & 16 µM) & DEHP (1, 10, 100 µM)/ for 48–72 h	↑ Cell viability & ↓Apoptosis. ↑AhR Nuclear translocation ⟨ ↑CYP1A1 & CYP1B1 gene expression. ↑ SLC7A5, SLC7A8, &TDO2 gene expression⟨ ↑Tryptophan uptake & kynurenine production	MEHHP promotes leiomyoma cell survival through increasing cellular tryptophan uptake, kynurenine production, and AhR pathway activation.
	Clinical	712 leiomyoma patients	MEHHP urine levels	↑ risk of uterine leiomyoma diagnosis	
Qin, [28]	In vitro	MCF-7, LNCaP, & BG1Luc4E2	BBP, DBP, & DEHP (10^{-14}-10^{-4} M)/for 24 h	↓ ARNT2 gene & protein expression in ESR1-positive MCF-7 & BG1Luc4E2 cells, but not in ESR1-negative LNCaP cells	ARNT2 expression is modulated by BBP and DBP by an ESR1-dependent mechanism in MCF-7 breast cancer cells.
Shan, [24]	In vitro	MCF-7	MEHP (1, 10, & 100 µM)/ for 12, 24, and 48 h	MEHP or TCDD: ↑Migration & Invasion TCDD ↓ Migration & invasion induction by MEHP MEHP ↑MMP2 & MMP9 & Vimentin & ↓E-cadherin (No significant difference in the TCDD-treated group) ↑Nuclear translocation of AhR ⟨ CYP1A1 gene expression	MEHP or TCDD can promote migration and invasion in MCF7 cells in a dose-dependent manner, and these effects are AhR dependent. This promotion is antagonized by cotreatment with MEHP and TCDD. MEHP is a competitive antagonist against TCDD binding to AhR, and this competitive binding effect inhibits the MCF7 human breast cancer cell EMT process induced by MEHP alone. MEHP is a potential AhR agonist with less potency than TCDD but promotes migration or invasion more than TCDD.
	In vivo	Xenotransplantation of 1×10^6 MCF7 cells in the mammary fat pad of BALB/c nude mice	1000 mg/kg of DEHP/ ip administration for 3 days	TCDD ↑AhR & CYP1A1 gene expression in vivo. DEHP antagonizes TCDD to reduce AhR and CYP1A1 mRNA expression in vivo.	
Tapella, [22]	In vitro	Pituitary primary cells of male Sprague–Dawley rats	DEHP (250, 650, 1250 pM)/ for 3, 24–96 h	↑ATP, Ccnd1, Pttg1 gene expression⟨ ↑ proliferation ↑AhR & AIP gene expression	DEHP activate AhR/AIP expression and stimulate proliferation in normal rat pituitary cells.
Tsai, [23]	In vitro	Huh7, PLC/PRF/5 cells	DEHP (0.1-1 µM), DBP, BBP (0.1 µM)/ for 24–48 h	↓E-cadherin, ↑Vimentin & N-cadherin ⟨ ↑Migration & Invasion ↑SP cells, $CD44^+$/ $CD133^+$ cells in HCC through activation of AhR/ERK/SK1/S1P3 signaling pathway.	DEHP-induced AhR expression, migration, invasion, & EMT in cells through ERK/SK1 signaling in vitro. DEHP promotion of lung metastasis may be related to the increase in CSCs-like and through AhR pathway Curcumin (1.25-5µM) suppressed phthalate-induced migration, invasion, & CSC-like cell maintenance through inhibition of the AhR/ERK/SK1/S1P3 signaling pathway
	In Vivo	direct intrahepatic injection of Huh7-GFP cells (1×10^6) into the male BALB/cA-nu nu/nu mice	DEHP (60 mg/kg)/ IP and every 2 days to 1 month	↑Tumor Growth, and lung metastasis	
		microinjection of Huh7-GFP (DEHP treated) cells into embryos Tg(fli1:EGFP)y1 zebrafish	Huh7-GFP treated with DEHP (0.1 µM) for 24 h	↑HCC Stemness and Maintenance	

Table 1 (continued)

Author & year	Models		Type of Phthalates (dosage) / Exposure protocol	Outcomes of Phthalates on Promotion and Progression of Cancer	Proposed signaling pathway
Tsai, [25]	In vitro	Huh7, PLC/PRF/5 HepG2, and HUVEC cells.	BBP (1 μM) for 0–120 min (AhR analysis) 1 day for (VEGF Measurement)	↑AhR gene & protein expression ↑$G\alpha_{q/11}$ and G_{β} ⟨↓PIP2, ↑IP3R, & ↑internal release of calcium⟨ ↑COX-2 protein expression ↑binding of Gβ protein to PI3K⟨ ↑p-Akt and NF-κB nucleus translocation⟨ Migration & Invasion ↑ p-ERK⟨ ↑VEGF & angiogenesis	BBP induces membrane translocation of AhR (nongenomic) & the initial activation of COX-2 by AhR/$G\alpha_{q/11}$ signaling. In addition, BBP promotes angiogenesis via the AhR/ERK/VEGF pathway. cell migration & invasion are also increased through AhR/Gβ/PI3K/Akt/NF-κB signaling.
	In vivo	Huh7-IFP cells (1×10^6) injected into the upper left lobe of the liver of male BALB/cA-nu nu/nu	BBP (500 mg/kg)/ IP every 2 days to 1 month	↑lungs, kidneys, and spleen metastasis ↑angiogenesis	
Wang, [26]	In vitro	MDA-MB-231, MCF-7	BBP (1 μM) for 24 h	↑Proportion of SP cells ↑Vimentin, ↓ E-cadherin⟨ EMT in SP and non-SP cells ↑Doxorubicin and paclitaxel chemoresistant ↑Nuclear translocation of AhR and ARNT-binding⟨↑ p-ERK & SPHK1/S1P/S1PR3⟨↑metastasis-initiating BCSCs ↑SPHK1 & S1PR3⟨ ↑Akt ⟨ metastasis-initiating BCSCs & $CD44^{high}/CD24^{low}$ MCF-7 cell populations	BBP activated AhR in breast cancer cells to stimulate SPHK1/S1P/S1PR3 signaling & enhance the formation of metastasis-initiating BCSCs
	In vivo	MCF-7_{shGFP}, MCF-$7_{shSPHK1}$ or MCF-$7_{shS1PR3}$ cells (1×10^6) were injected into mammary fat pads of female nude mice.	Subcutaneous BBP (200 mg/kg.bw)/IP and 3 times per week up to 5 weeks	↑metastatic lung lesions SP cells induced tumor formation more frequently than non-SP cells Implanted MCF-$7_{shS1PR3}$ cells eliminate BBP-induced breast tumors⟨ ↑survival rate	

↑, Increase; ↓, Decease; &, and; p-, phosphorylated; EMT, Epithelial-mesenchymal transition; DEHP, Di(2-ethylhexyl)phthalate; GFP, green fluorescent protein; MEHHP, mono-(2-ethyl-5-hydroxyhexyl) phthalate; Akt, Protein Kinase B; ERK, extracellular signal-regulated kinase; MEHP, mono(2-ethylhexyl) phthalate; MECPP, mono (2-ethyl-5-carboxypentyl) phthalate; MMP, matrix metalloproteinase; mTOR, Mammalian Target Of Rapamycin; PI3K, phosphatidylinositol-3 kinase; MEOHP, mono(2-ethyl-5-oxohexyl) phthalate; LC-MS/MS, Liquid Chromatography with tandem mass spectrometry; TFF3, Trefoil factor 3; CYP, Cytochromes P450; AhR, Aryl hydrocarbon Receptor; ER, Estrogen receptor; Sc, Subcutaneous; ip, Intraperitoneal injection; BBP, n-butyl benzyl phthalate; DBP, dibutyl phthalate; DMF, 3,4-dimethoxyflavone; CSC, cancer stem cell; BrdU, bromodeoxyuridine; cAMP, cyclic AMP; PKA, Protein kinase A; CREB, cAMP-response element-binding protein; HDAC, Histone deacetylase; LEF-1, Lymphoid enhancer-binding factor 1; TCF4, Transcription factor 4; HPF, hours post-fertilization; HPT, hypothalamus-pituitary-thyroid; SLC, Solute carrier; TDO2, Tryptophan 2,3-dioxygenase; ARNT2, Aryl hydrocarbon receptor nuclear translocator 2; PDZK1, PDZ domain containing 1; HFD, high fat diet; TCDD, 2,3,7,8-tetrachlorodibenzo-p-dioxin; Ccnd1, cyclin D1; PTTG, pituitary tumor transforming gene; AMPK, 5′ AMP-activated protein kinase; siRNA, Small interfering RNA; DRE, dioxin response element; AIP, aryl hydrocarbon receptor (AhR) interacting protein (AIP); SP, Side population; SK1, Sphingosine kinase 1; SIRP-1, Sphingosine-1-phosphate receptor 1; ESR, Estrogen receptor; p38, p38 mitogen-activated protein kinases; HER2, human epidermal growth factor receptor 2; HCC, hepatocellular carcinoma; PIP2, Phosphatidylinositol 4,5-bisphosphate; NF-?B, Nuclear factor kappa-light-chain-enhancer of activated B cells; COX-2, cyclooxygenase-2; BCS, Breast Cancer Stem; VEGF, Vascular endothelial growth factor; S1PR3, Sphingosine-1-phosphate receptor 3, ?4MEHP, The sum of DEHP metabolites; ESR1, estrogen receptor 1; SPHK1, sphingosine kinase 1; S1P, sphingosine 1-phosphate; S1PR3, sphingosine-1-phosphate receptor 3; BCSCs, Breast cancer stem cells

BG1Luc4E2), while having no effect on ESR1-negative LNCaP cells [28].

Discussion

Cancer continues to be a major global health issue, with around 20 million new cases reported every year and 9.7 million cancer-related deaths in 2022, accounting for approximately 1 in 6 deaths worldwide [29, 30]. This imposes a substantial burden on healthcare systems and economies, with estimated costs exceeding 25.2 trillion international dollars from 2020 to 2050 [31]. Environmental factors, including phthalates, are implicated in cancer risk. Phthalates are environmental contaminants that have been associated with an increased risk of cancer at different stages of the disease [32]. Exposure to phthalates through multiple routes, including ingestion, inhalation, and medical procedures, can potentially cause carcinogenic effects [33]. These contaminants interact with nuclear receptors like the AhR, triggering complex signaling pathways that contribute to tumor initiation, progression, and metastasis. As AhR becomes increasingly recognized as a key regulator in

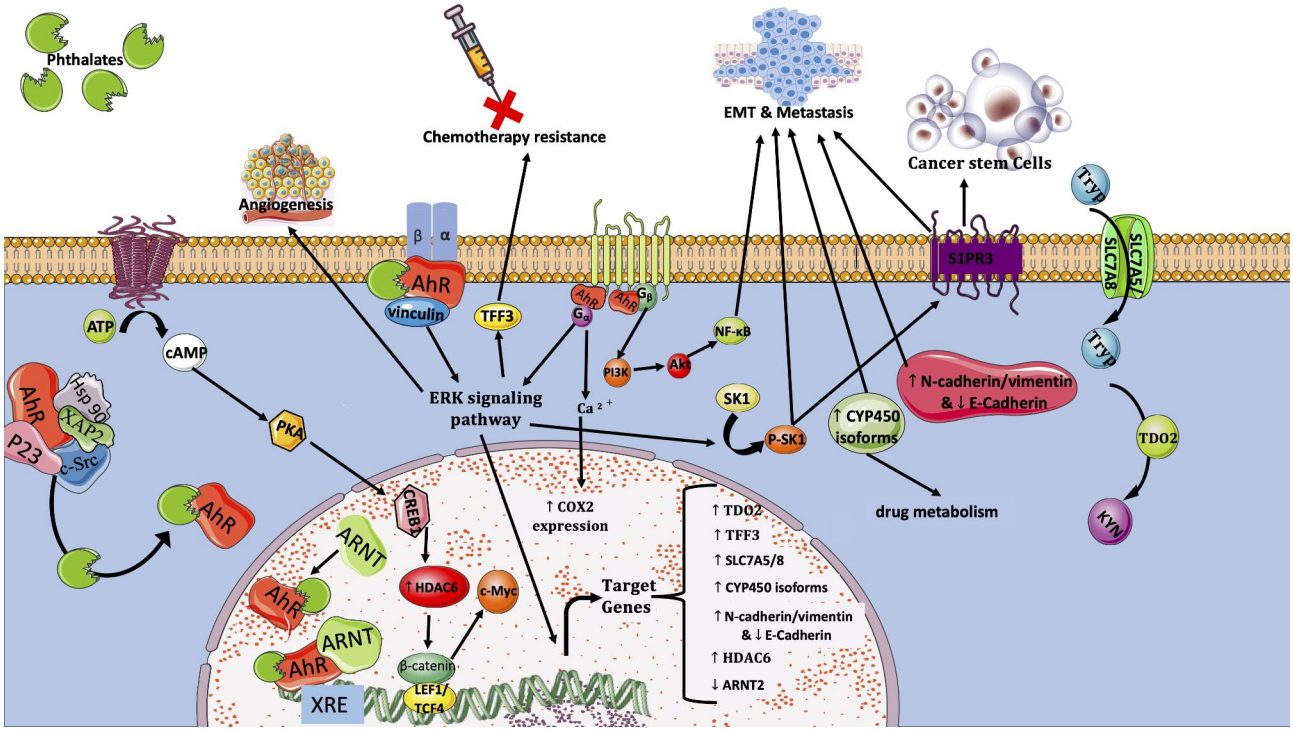

Fig. 2 The basic mechanisms underlying the AhR pathway regulation in phthalates-induced cancer promotion, progression and metastasis

cancer, influencing cell proliferation, survival, and metastasis, understanding the complex interaction between phthalates, AhR, and cancer biology is crucial for developing effective preventative and therapeutic approaches against cancer [34].

During tumor progression, phthalates can contribute to abnormal cell proliferation, angiogenesis, and evasion of the immune system through dysregulation of signaling pathways [33]. Additionally, phthalate exposure may enhance the migration and invasion potential of cancer cells by modulating EMT processes and extracellular matrix remodeling [35]. Moreover, phthalates can confer resistance to chemotherapy and targeted therapies, complicating treatment strategies and exacerbating disease outcomes [36].

Our research focused primarily on investigating the mechanical process by which phthalates induce cancer progression and metastasis through the AhR pathway. We also analyzed how phthalates activate AhR and their role in carcinogenesis. Our study summarizes the key findings in Table 1, highlighting our research's crucial aspects. Furthermore, in Fig. 2, we have presented the significant role of phthalates in cancer progression.

Cell proliferation is a complex process in cancer that is regulated by various molecular pathways. AhR plays a pivotal role in promoting cell proliferation and survival through diverse mechanisms. It modulates receptor expression crucial for cell differentiation and survival, participates in growth factor signaling pathways inducing cell proliferation, and exhibits anti-apoptotic effects by controlling apoptosis-related gene expression. Moreover, AhR regulates the cell cycle and promotes cell cytokine expression [37, 38]. Phthalates, such as DEHP, exert significant influence on these pathways, initiating cellular responses associated with cancer proliferation and triggering DNA damage. Phthalates also exhibit regulatory effects on genes and proteins closely associated with cancer cell proliferation, such as Ccnd1 and Pttg1, pivotal in G1/S cell cycle transition and pituitary tumorigenesis, respectively [22]. Additionally, Barhoover et al. illustrated that in the absence of exogenous ligands, AhR interaction with CDK4 and CCND1 facilitates cell cycle progression, indicating a potential interplay between AhR and phthalates in driving cancer progression [39]. Furthermore, various phthalate exposures enhance cell viability and colony formation in breast cancer cell lines, reinforcing their role in fueling cancer proliferation [20].

EMT is a critical process in cancer metastasis, characterized by epithelial cells acquiring a mesenchymal phenotype, enhancing their ability to migrate and invade surrounding tissues. Key regulators of EMT include the transcription factors Slug, Snail, Twist, and the loss of E-cadherin expression [40]. AhR activation promotes EMT in many cancers. In triple-negative and breast cancer cell lines, AhR activation leads to EMT-like morphological changes and upregulates EMT-associated genes like Twist, vimentin, and Snail1 [41]. Clinical evidence reveals a correlation between high AhR expression in

inflammatory breast cancer and aggressive metastatic behavior, indicating AhR's role in EMT induction and metastasis [42]. AhR activation by phthalates promotes EMT, enhancing invasiveness and metastasis in cancer cells [43]. Phthalate exposure triggers cancer cell migration and invasion, altering EMT marker expression. This involves decreased E-cadherin and increased MMP2, MMP9, and vimentin expression via AhR pathway activation [24]. Inhibition of AhR signaling pathways emerges as a promising therapeutic approach to counteract phthalate-induced migration, invasion, and maintenance of cancer stem cell populations [25]. Studies have demonstrated that curcumin can suppress phthalate-induced migration, invasion, and maintenance of cancer stem-like cells through inhibition of the AhR/ERK/SK1/S1P3 signaling pathway [23].

Moreover, investigations into other phthalates, such as BBP, reveal intricate signaling pathways mediated by AhR activation. BBP exposure activates AhR signaling, leading to the stimulation of signaling pathways that enhance the formation of metastasis-initiating breast cancer stem cells and promote breast cancer metastasis [26].

Angiogenesis, crucial for tumor survival and metastasis, is regulated by AhR activation [44]. AhR-ARNT heterodimers interact with HIF-1α, leading to increased expression of angiogenic factors like interleukin-8 and VEGF while downregulating anti-angiogenic factors [45]. Phthalates, through non-genomic AhR mechanisms, promote angiogenesis by upregulating VEGF expression via ERK1/2 phosphorylation [25]. This highlights the link between AhR activation, angiogenesis, and tumor progression, with phthalates exacerbating this process by enhancing VEGF expression.

Dysregulation of metabolic pathways is a hallmark of cancer, and AhR emerges as a central player in cancer cell metabolism, exerting profound effects on lipid metabolism, glycolysis, and nucleotide metabolism [46]. In the glycolytic pathway, AhR activation, facilitated by the kynurenine pathway, upregulates glycolysis-promoting CXCL5 in cancer cells. Also, AhR directly manages the activity of genes that make glycolytic enzymes, such as hexokinase 2 (HK2). This speeds up the breakdown of glycogen and helps tumors grow [46]. AhR also regulates lipid metabolism by modulating fatty acid transporter proteins and key de novo lipogenesis regulators. AhR also affects nucleotide metabolism, which could change the production of pyrimidine and control the levels of reactive oxygen species (ROS), which can affect the growth of tumors [47]. Also, AhR controls the increase of CYP enzymes like CYP2D6, 2C8, and 3A4, which speeds up EMT and the breakdown of chemotherapy drugs like doxorubicin, making breast cancer treatment less effective. When phthalates activate AhR, they change the levels of estrogen receptors, affect the targets of tamoxifen, and raise the level of ABCG2, which makes breast cancer less sensitive to chemotherapy [20].

Tryptophan metabolites like kynurenine (Kyn) serve as potent AhR ligands, being produced abundantly by malignant cells in cancers such as glioblastoma, head and neck, and breast. These endogenous AhR ligands drive chronic AhR activity, contributing to metabolic reprogramming and tumor progression. Phthalate metabolites, particularly MEHHP, promote leiomyoma cell survival and inhibit apoptosis through the tryptophan-kynurenine-AhR pathway [48]. These findings elucidate the intricate interplay between phthalates, AhR activation, cancer progression, and drug resistance. Identifying specific gene targets affected by phthalate exposure may offer novel avenues for therapeutic intervention in phthalate-associated cancers.

Conclusion

This review highlights the growing evidence linking phthalate exposure to cancer progression, primarily through the activation of the AhR signaling pathway. While experimental studies underscore the ability of phthalates to modulate tumor-related processes such as proliferation, migration, and immune evasion, significant knowledge gaps remain. The differential effects of individual phthalates, their dose-response relationships, and long-term impacts on tumor dynamics remain poorly understood. Furthermore, existing studies lack comprehensive in vivo models that capture the complexity of human cancer progression, particularly in advanced stages like metastasis.

Addressing these gaps is crucial for a deeper understanding of the carcinogenic potential of phthalates and their role in environmental cancer risk. Future research should prioritize: [1] developing standardized methodologies to evaluate phthalate-induced AhR activation in diverse biological contexts [2], investigating genetic and environmental factors that may modulate these effects, and [3] employing advanced models, including patient-derived organoids and animal studies, to elucidate the interplay between phthalates and cancer biology. These steps will enable the design of targeted interventions and regulatory policies to mitigate the potential health risks associated with phthalate exposure.

Supplementary Information

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Supplementary Material 1

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Author contributions
Mostafa Akbariani: Conceptualization, Writing-original draft, Writing-review & editing. Mahmoud Omidi: Conceptualization, Writing-original draft, Writing – review & editing. Zohreh Shahabi: Conceptualization; Writing-original draft; Writing - review & editing. Hamed Haghi-Aminjan: Conceptualization; Writing - original draft; Writing - review & editing. Amir Shadboorestan: Conceptualization, Writing-original draft, Writing - review & editing.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethical approval
This study has only been a scoping review and no need to ethics approval.

Competing interests
The authors declare no competing interests.

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References

1. Jassim A, Rahrmann EP, Simons BD, Gilbertson RJ. Cancers make their own luck: theories of cancer origins. Nat Rev Cancer. 2023;23(10):710–24.
2. Samadi M, Haghi-Aminjan H, Sattari M, Hooshangi Shayesteh MR, Bameri B, Armandeh M, et al. The role of taurine on chemotherapy-induced cardiotoxicity: a systematic review of non-clinical study. Life Sci. 2021;265:118813.
3. Iwasaki M, Itoh H, Sawada N, Tsugane S. Exposure to environmental chemicals and cancer risk: epidemiological evidence from Japanese studies. Genes Environ. 2023;45(1):10.
4. De Falco M, Laforgia V. Combined effects of different endocrine-disrupting chemicals (EDCs) on prostate gland. Int J Environ Res Public Health. 2021;18(18):9772.
5. Ahn C, Jeung E-B. Endocrine-disrupting chemicals and Disease endpoints. Int J Mol Sci. 2023;24(6):5342.
6. Pierozan P, Cattani D, Karlsson O. Perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) induce epigenetic alterations and promote human breast cell carcinogenesis in vitro. Arch Toxicol. 2020;94:3893–906.
7. Macedo S, Teixeira E, Gaspar TB, Boaventura P, Soares MA, Miranda-Alves L et al. Endocrine-disrupting chemicals and endocrine neoplasia: a forty-year systematic review. Environ Res. 2022:114869.
8. Liu G, Cai W, Liu H, Jiang H, Bi Y, Wang H. The association of bisphenol A and phthalates with risk of breast cancer: a meta-analysis. Int J Environ Res Public Health. 2021;18(5):2375.
9. Benjamin S, Masai E, Kamimura N, Takahashi K, Anderson RC, Faisal PA. Phthalates impact human health: epidemiological evidences and plausible mechanism of action. J Hazard Mater. 2017;340:360–83.
10. Leng J, Li H, Niu Y, Chen K, Yuan X, Chen H, et al. Low-dose mono (2-ethylhexyl) phthalate promotes ovarian cancer development through PPARα-dependent PI3K/Akt/NF-κB pathway. Sci Total Environ. 2021;790:147990.
11. Chou C-K, Yang Y-T, Yang H-C, Liang S-S, Wang T-N, Kuo P-L, et al. The impact of di (2-ethylhexyl) phthalate on cancer progression. Arch Immunol Ther Exp. 2018;66:183–97.
12. Ahern TP, Broe A, Lash TL, Cronin-Fenton DP, Ulrichsen SP, Christiansen PM, et al. Phthalate exposure and breast Cancer incidence: a Danish Nationwide Cohort Study. J Clin Oncol. 2019;37(21):1800–9.
13. Shadboorestan A, Koual M, Dairou J, Coumoul X. The role of the Kynurenine/AhR pathway in diseases related to metabolism and Cancer. Int J Tryptophan Res. 2023;16:11786469231185102.
14. Larigot L, Juricek L, Dairou J, Coumoul X. AhR signaling pathways and regulatory functions. Biochimie open. 2018;7:1–9.
15. Denison MS, Nagy SR. Activation of the aryl hydrocarbon receptor by structurally diverse exogenous and endogenous chemicals. Annu Rev Pharmacol Toxicol. 2003;43(1):309–34.
16. Larsen ST, Nielsen GD. Structure-activity relationship of immunostimulatory effects of phthalates. BMC Immunol. 2008;9:61.
17. Iizuka T, Yin P, Zuberi A, Kujawa S, Coon JS, Björvang RD, et al. Mono-(2-ethyl-5-hydroxyhexyl) phthalate promotes uterine leiomyoma cell survival through tryptophan-kynurenine-AHR pathway activation. Proc Natl Acad Sci. 2022;119(47):e2208886119.
18. Tricco AC, Lillie E, Zarin W, O'Brien KK, Colquhoun H, Levac D, et al. PRISMA Extension for scoping reviews (PRISMA-ScR): Checklist and Explanation. Ann Intern Med. 2018;169(7):467–73.
19. Bramer WM, De Jonge GB, Rethlefsen ML, Mast F, Kleijnen J. A systematic approach to searching: an efficient and complete method to develop literature searches. J Med Libr Association: JMLA. 2018;106(4):531.
20. Hsieh T-H, Hsu C-Y, Yang P-J, Chiu C-C, Liang S-S, Ou-Yang F, et al. DEHP mediates drug resistance by directly targeting AhR in human breast cancer. Biomed Pharmacother. 2022;145:112400.
21. Junaid M, Jia PP, Tang YM, Xiong WX, Huang HY, Strauss PR et al. Mechanistic toxicity of DEHP at environmentally relevant concentrations (ERCs) and ecological risk assessment in the Three Gorges Reservoir Area, China. Environmental pollution (Barking, Essex: 1987). 2018;242(Pt B):1939-49.
22. Tapella L, Sesta A, Cassarino MF, Zunino V, Catalano MG, Pecori Giraldi F. Benzene and 2-ethyl-phthalate induce proliferation in normal rat pituitary cells. Pituitary. 2017;20(3):311–8.
23. Tsai CF, Hsieh TH, Lee JN, Hsu CY, Wang YC, Kuo KK, et al. Curcumin suppresses Phthalate-Induced Metastasis and the proportion of Cancer Stem Cell (CSC)-like cells via the inhibition of AhR/ERK/SK1 signaling in Hepatocellular Carcinoma. J Agric Food Chem. 2015;63(48):10388–98.
24. Shan A, Leng L, Li J, Luo XM, Fan YJ, Yang Q, et al. TCDD-induced antagonism of MEHP-mediated migration and invasion partly involves aryl hydrocarbon receptor in MCF7 breast cancer cells. J Hazard Mater. 2020;398:122869.
25. Tsai C-F, Hsieh T-H, Lee J-N, Hsu C-Y, Wang Y-C, Lai F-J, et al. Benzyl butyl phthalate induces migration, invasion, and angiogenesis of Huh7 hepatocellular carcinoma cells through nongenomic AhR/G-protein signaling. BMC Cancer. 2014;14(1):1–13.
26. Wang YC, Tsai CF, Chuang HL, Chang YC, Chen HS, Lee JN, et al. Benzyl butyl phthalate promotes breast cancer stem cell expansion via SPHK1/S1P/S1PR3 signaling. Oncotarget. 2016;7(20):29563–76.
27. Hsieh TH, Tsai CF, Hsu CY, Kuo PL, Lee JN, Chai CY, et al. Phthalates induce proliferation and invasiveness of estrogen receptor-negative breast cancer through the AhR/HDAC6/c-Myc signaling pathway. FASEB J. 2012;26(2):778–87.
28. Qin XY, Zaha H, Nagano R, Yoshinaga J, Yonemoto J, Sone H. Xenoestrogens down-regulate aryl-hydrocarbon receptor nuclear translocator 2 mRNA expression in human breast cancer cells via an estrogen receptor alpha-dependent mechanism. Toxicol Lett. 2011;206(2):152–7.
29. Bray F, Laversanne M, Sung H, Ferlay J, Siegel RL, Soerjomataram I, et al. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. Cancer J Clin. 2024;74(3):229–63.
30. Samadi M, Haghi-Aminjan H, Sattari M, Shayesteh MRH, Bameri B, Armandeh M, et al. The role of taurine on chemotherapy-induced cardiotoxicity: a systematic review of non-clinical study. Life Sci. 2021;265:118813.
31. Kreier F. Cancer will cost the world $25 trillion over next 30 years. Nature Publishing Group; 2023.
32. Wang Y, Qian H, editors. Phthalates and their impacts on human health. Healthcare. Multidisciplinary Digital Publishing Institute; 2021.
33. Giuliani A, Zuccarini M, Cichelli A, Khan H, Reale M. Critical review on the presence of phthalates in food and evidence of their biological impact. Int J Environ Res Public Health. 2020;17(16):5655.
34. Kolluri SK, Jin U-H, Safe S. Role of the aryl hydrocarbon receptor in carcinogenesis and potential as an anti-cancer drug target. Arch Toxicol. 2017;91:2497–513.
35. Kim HG, Lim YS, Hwang S, Kim H-Y, Moon Y, Song YJ, et al. Di-(2-ethylhexyl) phthalate triggers proliferation, Migration, Stemness, and epithelial–mesenchymal transition in human endometrial and endometriotic epithelial cells via the transforming growth Factor-β/Smad signaling pathway. Int J Mol Sci. 2022;23(7):3938.
36. Chen H-P, Lee Y-K, Huang SY, Shi P-C, Hsu P-C, Chang C-F. Phthalate exposure promotes chemotherapeutic drug resistance in colon cancer cells. Oncotarget. 2018;9(17):13167.
37. Dietrich C, Kaina B. The aryl hydrocarbon receptor (AhR) in the regulation of cell–cell contact and tumor growth. Carcinogenesis. 2010;31(8):1319–28.
38. Yin J, Sheng B, Qiu Y, Yang K, Xiao W, Yang H. Role of AhR in positive regulation of cell proliferation and survival. Cell Prolif. 2016;49(5):554–60.

39. Barhoover MA, Hall JM, Greenlee WF, Thomas RS. Aryl hydrocarbon receptor regulates cell cycle progression in human breast cancer cells via a functional interaction with cyclin-dependent kinase 4. Mol Pharmacol. 2010;77(2):195–201.
40. Debnath P, Huirem RS, Dutta P, Palchaudhuri S. Epithelial–mesenchymal transition and its transcription factors. Biosci Rep. 2022;42(1):BSR20211754.
41. Stanford EA, Wang Z, Novikov O, Mulas F, Landesman-Bollag E, Monti S, et al. The role of the aryl hydrocarbon receptor in the development of cells with the molecular and functional characteristics of cancer stem-like cells. BMC Biol. 2016;14:1–22.
42. Guarnieri T. Aryl hydrocarbon receptor connects inflammation to breast cancer. Int J Mol Sci. 2020;21(15):5264.
43. Wang Z, Snyder M, Kenison JE, Yang K, Lara B, Lydell E et al. How the AHR became important in Cancer: the role of chronically active AHR in Cancer Aggression. Int J Mol Sci. 2020;22(1).
44. Li Y, Zhou C, Lei W, Wang K, Zheng J. Roles of aryl hydrocarbon receptor in endothelial angiogenic responses†. Biol Reprod. 2020;103(5):927–37.
45. Griffith BD, Frankel TL. The Aryl Hydrocarbon receptor: impact on the Tumor Immune Microenvironment and Modulation as a potential therapy. Cancers. 2024;16(3):472.
46. Wang Z, Zhang Y, Liao Z, Huang M, Shui X. The potential of aryl hydrocarbon receptor as receptors for metabolic changes in tumors. Front Oncol. 2024;14:1328606.
47. Tanos R, Murray IA, Smith PB, Patterson A, Perdew GH. Role of the Ah receptor in homeostatic control of fatty acid synthesis in the liver. Toxicol Sci. 2012;129(2):372–9.
48. Iizuka T, Yin P, Zuberi A, Kujawa S, Coon JSt, Björvang RD, et al. Mono-(2-ethyl-5-hydroxyhexyl) phthalate promotes uterine leiomyoma cell survival through tryptophan-kynurenine-AHR pathway activation. Proc Natl Acad Sci U S A. 2022;119(47):e2208886119.

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