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Meta-analysis identifies key genes and pathways implicated in Benzo[a]pyrene exposure response

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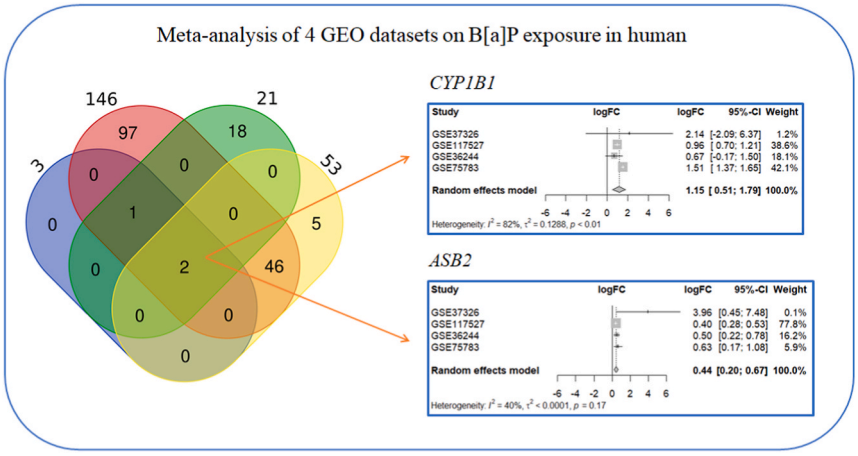
HIGHLIGHTS

- CYP1B1 and ASB2 upregulated by B[a]P.
- Key pathways in B[a]P toxicity revealed.
- Meta-Analysis underscores gene markers for B[a]P exposure.

GRAPHICAL ABSTRACT



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ABSTRACT

Introduction: Benzo[a]pyrene (B[a]P) is a carcinogenic polycyclic aromatic hydrocarbon that poses significant risks to human health. B[a]P influences cellular processes via intricate interactions; however, a comprehensive understanding of B[a]P's effects on the transcriptome remains elusive. This study aimed to conduct a comprehensive analysis focused on identifying relevant genes and signaling pathways affected by B[a]P exposure and their impact on human gene expression.

Methods: We searched the Gene Expression Omnibus database and identified four studies involving B[a]P exposure in human cells (T lymphocytes, hepatocellular carcinoma cells, and C3A cells). We utilized two approaches for differential expression analysis: the LIMMA package and linear regression. A meta-analysis was

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utilized to combine log fold changes (FC) and p-values from the identified studies using a random effects model. We identified significant genes at a Bonferroni-adjusted significance level of 0.05 and determined overlapping genes across datasets. Pathway enrichment analysis elucidated key cellular processes modulated by B[a]P exposure.

Results: The meta-analysis revealed significant upregulation of CYP1B1 (log FC = 1.15, 95% CI: 0.51–1.79, $P < 0.05$, $I^2 = 82\%$) and ASB2 (log FC = 0.44, 95% CI: 0.20–0.67, $P < 0.05$, $I^2 = 40\%$) in response to B[a]P exposure. Pathway analyses identified 26 significantly regulated pathways, with the top including *Aryl Hydrocarbon Receptor Signaling* (P = 0.00214) and *Xenobiotic Metabolism Signaling* (P = 0.00550). Key genes CYP1A1, CYP1B1, and CDKN1A were implicated in multiple pathways, highlighting their roles in xenobiotic metabolism, oxidative stress response, and cell cycle regulation.

Conclusion: The results provided insights into the mechanisms of B[a]P toxicity, highlighting CYP1B1's key role in B[a]P bioactivation. The findings underscored the complexity of B[a]P's mechanisms of action and their potential implications for human health. The identified genes and pathways provided a foundation for further exploration and enhanced our understanding of the multifaceted biological activities associated with B[a]P exposure.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous in the environment due to their presence in combustion byproducts and pyrolyzed organic material (Lawal, 2017). Among PAHs, Benzo[a]pyrene (B[a]P) has been classified by the International Agency for Research on Cancer (IARC) as "Group 1" or "known human carcinogen" (International Agency for Research on Cancer IARC, 2012). B[a]P is carcinogenic due to its involvement in complex metabolic pathways that activate the compound and lead to DNA damage, mutations, and cancer (Xue and Warshawsky, 2005). These impacted pathways are involved in the activation by enzymes, detoxification, DNA binding, repair mechanisms, and the induction of inflammation and oxidative stress (Bukowska et al., 2022; Ewa and Danuta, 2017). Upon entering the human body, B[a]P encounters a series of metabolic transformations, primarily facilitated by the cytochrome P450 (CYP) enzyme family (Singh et al., 2011; Werck-Reichhart and Feyereisen, 2000; Ortiz de Montellano, 2010; Otto et al., 1992). Among these, CYP1A1 and CYP1B1 play pivotal roles in converting B[a]P into its reactive epoxide forms (Shimada et al., 2004). CYP1A1, primarily found in extrahepatic tissues, catalyzes Phase I metabolism reactions, including aromatic ring hydroxylation, which are crucial in carcinogenesis initiation (Androutsopoulos et al., 2009; Moorthy et al., 2015). CYP1A1 converts carcinogens like B[a]P to epoxide intermediates, which are then further activated to diol epoxides by epoxide hydrolase (Androutsopoulos et al., 2009). CYP1B1 emerges as a primary actor in this bioactivation process. This enzyme, detected in human lung epithelial cells, has been localized to mitochondria, implicating it as a central mediator of B[a]P's toxicological outcomes (Spivack et al., 2001). These intermediates possess the capability to bind DNA and proteins, thus laying the groundwork for carcinogenesis (Uppstad et al., 2010). Once these intermediates bind to DNA, they can induce mutations or disrupt normal cell function, both of which are critical events that may lead to the uncontrolled cell growth characteristic of cancer. Understanding the critical role of cytochrome P450 enzymes, particularly CYP1A1 and CYP1B1, in the metabolic activation of B[a]P sheds light on the mechanisms underlying its carcinogenicity.

Recent studies have expanded the understanding of B[a]P's health impacts beyond carcinogenesis, highlighting its association with several other health issues. For example, B[a]P exposure has been linked to respiratory problems, metabolic abnormalities, and immunotoxic effects (Venkatraman et al., 2024). Long-term exposure to PAHs, including B[a]P, which can induce oxidative stress and inflammation, has been associated with cardiovascular diseases such as atherosclerosis and hypertension (Lee et al., 2020; Mallah et al., 2021). Moreover, B[a]P exposure has been shown to interfere with endocrine functions, leading to reproductive health issues and developmental abnormalities in offspring (Rafiee et al., 2024; Zhang et al., 2022). B[a]P exposure, which is found not only in environmental pollutants but also in food, poses significant health risks. A recent meta-analysis found no significant health risks from B[a]P in infant foods but emphasized the importance of monitoring environmental contaminants in baby food (Sadighara et al., 2023).

On the other hand, gaps remain in our understanding of B[a]P's intricate interaction with the human genome. Previous research has often focused on isolated pathways or specific cellular responses. In contrast, a holistic view that encompasses the genomic responses remains elusive. A recent study explored the transcriptomic impacts of various endocrine disrupting chemicals (EDCs), including bisphenol A (BPA), bis(2-ethylhexyl) phthalate (DEHP), tributyltin (TBT), and perfluorooctanoic acid (PFOA) across multiple species including humans, mice, and rats (Zamora et al., 2024). However, B[a]P was not studied.

In the present study, we employed an integrative meta-analysis approach, leveraging publicly available gene expression datasets to identify key genes and pathways consistently influenced by B[a]P across a spectrum of human cell models. Our primary goal was to provide a comprehensive view of the molecular networks and biological processes that B[a]P perturbs. This study showed B[a]P's mechanisms in toxicity and carcinogenesis, as well as identified biomarkers for B[a]P exposure. Recognizing the inherent limitations and potential discrepancies among individual studies, our meta-analysis was structured to minimize random errors that could lead to false associations between genes or pathways and B[a]P exposure. This approach enabled us to discern differentially expressed genes and key biological processes associated with B[a]P-induced gene expression alterations.

2. Methods

2.1. Study identification

A preliminary search was conducted using the National Center for Biotechnology Information's Gene Expression Omnibus (GEO) database on November 26, 2021, to identify relevant studies (Fig. 1). Our research was conducted in accordance with the requirements of Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA, 2020) (Page et al., 2021). The search utilized the following query terms: ((benzoapyrene) AND "*Homo sapiens*"[porgn: _txid9606]) or ((polycyclic aromatic hydrocarbons) AND "*Homo sapiens*"[porgn: _txid9606]). Initially, a total of 147 studies were retrieved. To meet the inclusion criteria, studies were required to fulfill the following conditions: i) the study had to involve expression profiling by microarray, ii) the study had to be conducted on human tissues, iii) each dataset had to include both a B[a]P-exposed group and a corresponding unexposed group as a control, and iv) in cases where a study included multiple groups, only the B[a]P-exposed group and its corresponding control were selected for analysis. Following a thorough evaluation of each study against these criteria, a refined selection process identified a total of 4 studies that met the stringent requirements.

These studies (Table 1) are described as follows: Dataset GSE117527 comprises 8 samples, including 4 replicates of control and 4 replicates of

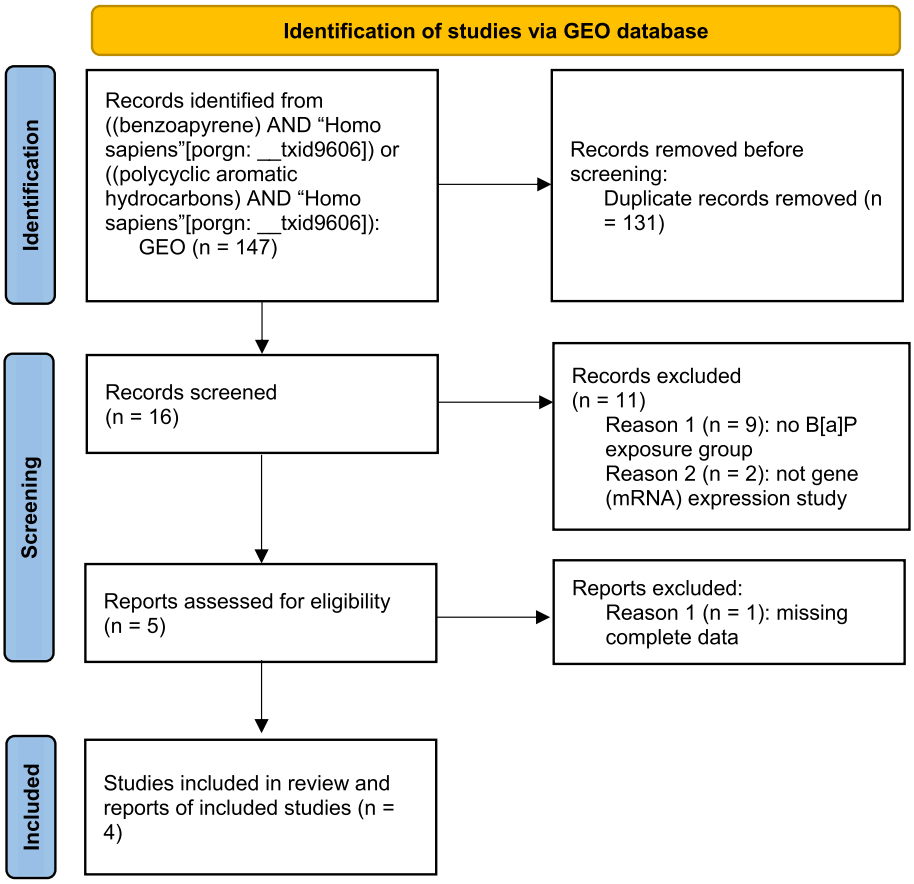

Fig. 1. Flowchart of study inclusion and exclusion.

B[a]P-exposed independent T lymphocyte cultures isolated from human blood donors. GSE37326-GPL1708 encompasses 27 samples, consisting of 3 B[a]P-exposed samples and 24 samples subjected to other PAH-exposed human hepatocellular carcinoma (HepG2) cells, all of which served as controls. GSE36244-GPL13695 includes 16 samples, comprising 8 B[a]P-exposed and 8 dimethyl sulfoxide (DMSO)-exposed controls using HepG2 cells. GSE75783 includes 5 samples, with 2 B[a]P-exposed and 3 DMSO controls utilized for analysis involving C3A cells.

2.2. Data pre-processing and identification of common genes with summary statistics

All raw gene expression files meeting the inclusion criteria were obtained. In cases where a gene had multiple probes on the same microarray chip, the gene expression value was calculated as the average of all corresponding probe values. To ensure uniformity and ease of interpretation, the gene IDs were converted to gene symbols using the online gene annotation software DAVID (Database for Annotation, Visualization, and Integrated Discovery, available at https://david.ncifcrf.gov/) (Huang da et al., 2009a; Huang da et al., 2009b).

To obtain all relevant datasets, we initiated the data acquisition process by employing the *getGEO* function from the *GEOquery* R package, version 2.40.0 (Davis and Meltzer, 2007). Notably, all raw gene expression profiling sets retrieved from three of the studies had undergone prior log2 transformation. We also applied log2 transformation to the raw gene expression profiles from the remaining study to ensure uniform data processing.

We utilized two approaches to calculate the summary statistics for the meta-analysis, aiming to enhance the robustness against heterogeneity in study design and data analysis methods. For Approach 1, we implemented default analytic method provided by GEO2R using the Linear Models for Microarray Data (LIMMA) package version 3.26.8 (Smyth, 2004). The rationale behind choosing LIMMA lies in its comprehensive and powerful statistical analysis framework, specifically designed for handling gene expression data generated by RNA sequencing and microarray. LIMMA can also handle complex experimental designs with small sample sizes by leveraging linear models and empirical Bayes methods, which ensure reliable statistical inferences by

Table 1
Summary of included GEO datasets/studies.

GEO accession	Platform	Study year	Included sample size	Experiment design
GSE117527	GPL25336	2018	8	This experiment involved isolating T lymphocyte cultures from blood donors, treating them differently, pooling their RNAs, and then performing gene expression analysis.
GSE37326	GPL1708	2012	27	Human hepatocellular carcinoma (HepG2) cells were treated with various PAHs, and their gene expression was analyzed using a whole human genome microarray.
GSE36244	GPL13695	2012	16	This study used RNA sequencing and microarray analysis to characterize transcriptomic changes in HepG2 human liver cells treated with the carcinogen benzo[a]pyrene versus controls over 12 and 24 h.
GSE75783	GPL13607	2015	5	C3A cells were treated with substances, and RNA was extracted, processed, and analyzed using microarrays with specific replicates for both treatments and controls.

moderating gene-wise variances (Ritchie et al., 2015). For each GEO dataset, the *lmFit* function from the LIMMA package was used to analyze the association of each gene with B[a]P exposure and estimate the log fold change (FC), standard error (SE), and p-value for each gene. Subsequently, we extracted the common genes across the analyzed datasets for the following meta-analysis.

Approach 2 was implemented to analyze the association of each gene with B[a]P exposure in each individual GEO dataset using linear regression or linear mixed modeling depending on the study type (i.e., independent samples or repeated design). Unlike LIMMA, simple linear regression is more straightforward and flexible for various designs and analyses, such as repeated measurements, which is advantageous when dealing with complicated longitudinal data. This approach complements the results obtained from LIMMA by offering a different perspective on the data and enhancing the robustness of our findings through methodological diversity. This procedure also entailed the collection of log FC, SE, and p-values of the overlapped genes across all datasets for the downstream meta-analysis.

2.3. Meta-analysis

To construct a comprehensive meta-signature of gene expression associated with B[a]P exposure, a systematic approach was employed, as illustrated in Fig. 2. Following the identification of gene-specific p-values, log FC, and SE estimates within each individual dataset, a set of common genes consistently observed across all datasets was selected for meta-analysis. Further, we used two meta-analysis methods in the *MetaVolcanoR* package (version 1.8.0) (Prada et al., 2019). One method is an effect size based meta-analysis using log FC and SE, which yielded the combined log FC and corresponding meta p-value for each gene. A random effects model was applied to account for potential heterogeneity across the four included GEO datasets. The other method is a p-value based method, which only produced a meta p-value for each gene. The meta p-values from these two methods were both adjusted using the Bonferroni correction with a significance threshold set at 0.05. The codes utilized in this study have been deposited on GitHub at https://github.com/MZ666Z/Meta-analysis-Identifies-Key-Genes-and-Pathways-Implicated-in-Benzo-a-pyrene-Exposure-Response.

The two meta-analysis methods and the two data preprocessing approaches resulted in four sets of significant genes as is shown in Fig. 2. Subsequently, from the four sets of significant genes, we identified the common genes shared among them. Lastly, a detailed examination was carried out to scrutinize the directionality of gene expression changes observed in the meta-analysis and individual dataset analyses. This analysis pipeline highlighted genes exhibiting significant and consistent directional changes across multiple datasets.

2.4. Pathway, disease, and biofunction enrichment analysis

To elucidate the potential biological implications of genes associated with B[a]P exposure identified from the meta-analysis, we conducted comprehensive pathway enrichment analysis using both the DAVID and QIAGEN Ingenuity Pathway Analysis tools (QIAGEN IPA) (QIAGEN Inc., https://digitalinsights.qiagen.com/IPA) (Krämer et al., 2014). This analysis encompassed Gene Ontology (GO) (https://geneontology.org/) (Ashburner et al., 2000; Aleksander et al., 2023) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway assessments (http://www.genome.ad.jp/kegg/) (Kanehisa and Goto, 2000). Within the IPA

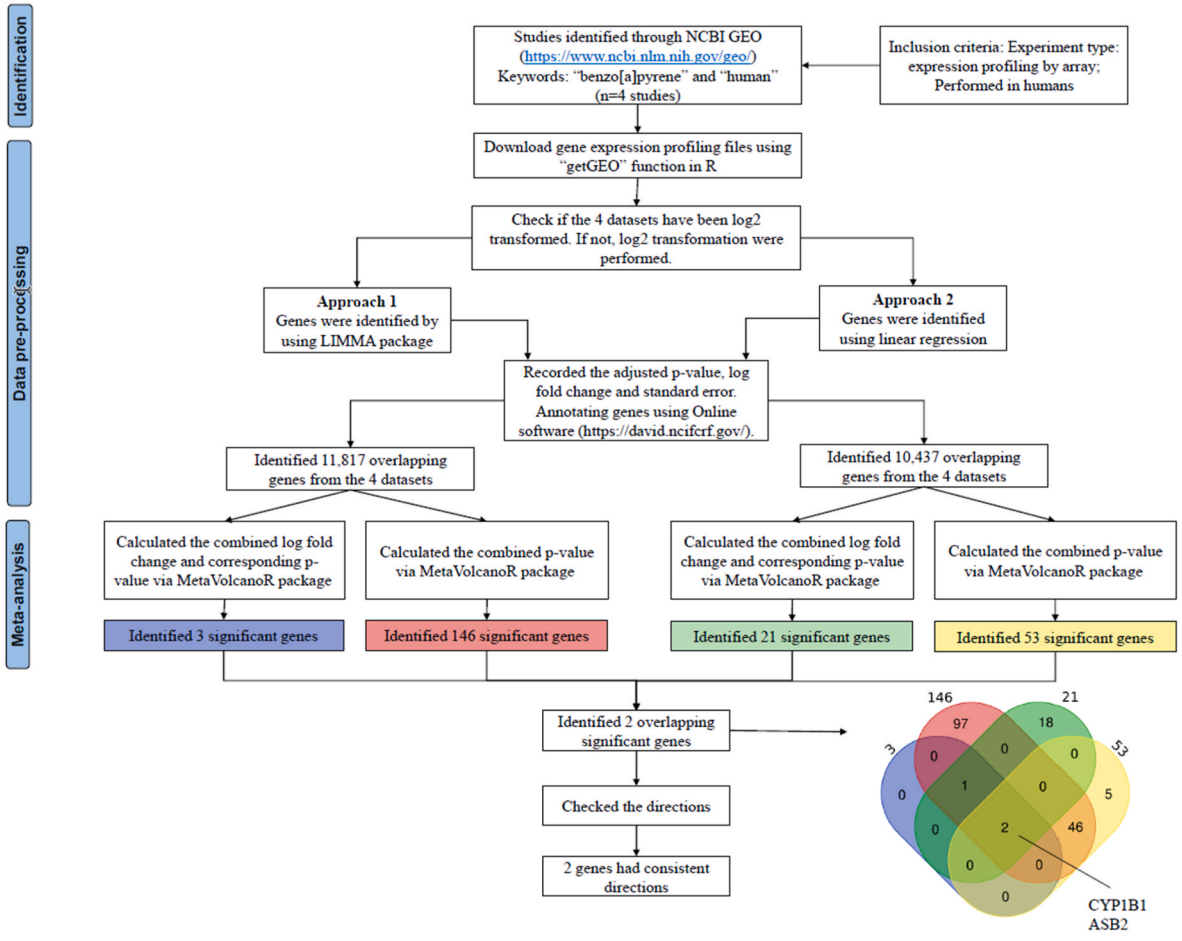

Fig. 2. The workflow of the meta-analysis.

software, we leveraged the "core analysis" function, including an exploration of biological processes, canonical pathways, upstream transcriptional regulators, and gene networks. Each gene identifier was mapped to its corresponding gene object in the Ingenuity Pathway Knowledge Base (IPKB). We applied a Benjamini-Hochberg (BH) False Discovery Rate (FDR) threshold of <0.05 to control for false positives when identifying significantly enriched KEGG pathways and GO functional categories. The BH method was used to address the issue of multiple hypothesis testing, which reduces the likelihood of false discoveries.

3. Results

3.1. Study selection

A comprehensive keyword search initially yielded a total of 147 studies for consideration. Following screening and removal of duplicates, 16 unique studies remained in the dataset, as is illustrated in Fig. 1. Subsequently, the titles and abstracts of these 16 articles underwent rigorous review against the predetermined study selection criteria, leading to the exclusion of 11 studies. Among the excluded studies, 9 did not include a designated B[a]P exposure group, while 2 were not gene expression studies. Of the remaining 5 studies, one was excluded due to incomplete data, resulting in a final set of 4 studies eligible for subsequent analysis.

3.2. Data preprocessing and identification of common genes

We identified 11,817 common genes across the four datasets in Approach 1. Subsequently, while employing a random effects model for meta-analyses on these common genes, we computed combined log FC values along with their corresponding p-values. Following this, we applied the same random effects model to analyze the identical set of common genes, leading to the identification of 146 significant genes using the combined p-value method and 3 significant genes using the combined log FC method.

In Approach 2, linear regression was employed, which resulted in the identification of 10,437 common genes present in all four datasets. This approach revealed 21 significant genes using the combined log FC method and 53 significant genes using the combined p-value method. The difference in the number of genes obtained from these two approaches stems from the fact that each approach may have failed to estimate the effect size of different genes due to ill-fitting in regression, which were subsequently filtered out. Thus, the final number of genes obtained from these two approaches varied.

From these results, we identified a total of 2 overlapping significant genes (CYP1B1 and ASB2). We verified the effect size direction of these 2 genes in each individual dataset and different meta-analysis methods. Remarkably, these genes exhibited consistent directional changes across all results (Fig. 3), providing robust evidence of their involvement in the observed biological responses.

3.3. CYP1B1 and ASB2

In Fig. 2, our meta-analysis highlighted two genes, CYP1B1 and ASB2, that emerged as the significantly over-expressed genes following exposure to B[a]P. Specifically, the pooled estimate of the log FC for CYP1B1 indicated a significant increase in expression upon B[a]P exposure, with a log FC of 1.15 (95% CI: 0.51–1.79, $P < 0.05$). Notably, the analysis revealed considerable heterogeneity across studies ($I^2 = 82\%$, $\tau^2 = 0.1288$, $P < 0.01$). This substantial heterogeneity suggests that factors such as diverse experimental conditions and variations in sample sizes, as well as other unidentified influences, may impact the degree of CYP1B1 expression in response to B[a]P exposure. For ASB2, the pooled log FC was 0.44 (95% CI: 0.20–0.67, $P < 0.05$), indicating a statistically significant upregulation. The heterogeneity for ASB2 was moderate ($I^2 = 40\%$, $\tau^2 < 0.0001$, $P = 0.17$), suggesting that while there is variability in the expression response of ASB2 to B[a]P among the datasets, the differences were not statistically significant. The τ^2 value being less than 0.0001 indicates a relatively small variance between

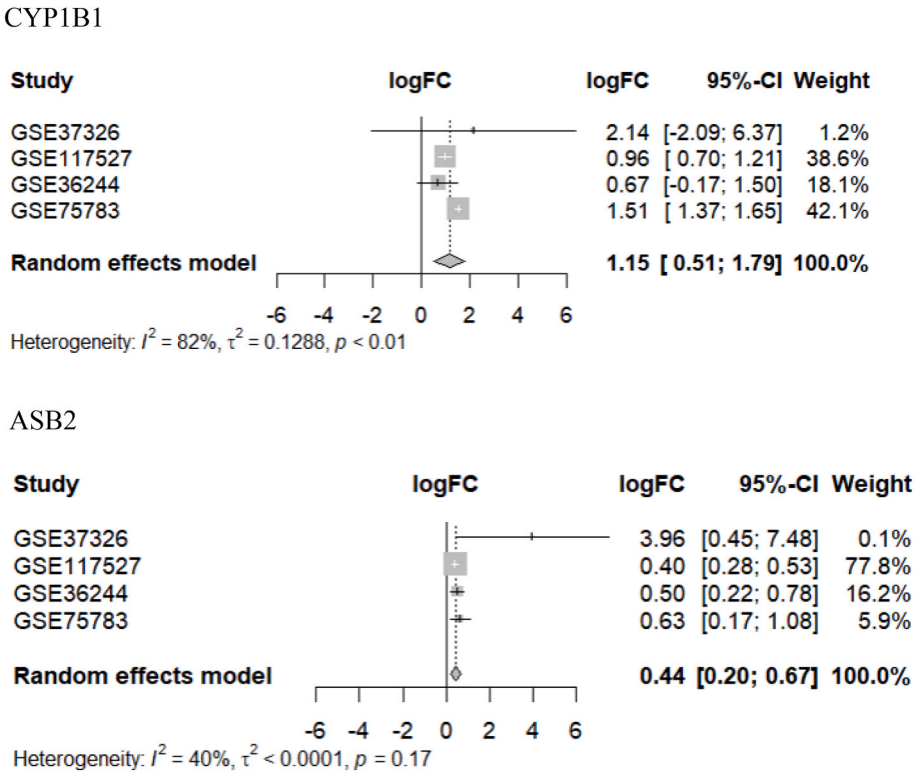

Fig. 3. Forest plot of the top two upregulated genes across all studies.

Table 2
Canonical pathways regulated by B[a]P.

Ingenuity Canonical Pathways	BH p-value	Regulated Genes
Aryl Hydrocarbon Receptor Signaling	0.00214	AHRR, CDKN1A,CYP1A1,CYP1B1,GSTA3,MDM2,NQO1
Xenobiotic Metabolism AHR Signaling Pathway	0.00550	AHRR,CYP1A1,CYP1B1,GSTA3,NQO1
Molecular Mechanisms of Cancer	0.00550	CDKN1A,GNAI1,LRP5,MDM2,PMAIP1,RALGDS,RAP1A,SMAD9,SOS1,WNT11
p53 Signaling	0.00851	CDKN1A,MDM2,PMAIP1,RRM2B,TP53I3
Role of NANOG in Mammalian Embryonic Stem Cell Pluripotency	0.01585	LIF,RAP1A,SMAD9,SOS1,WNT11
Xenobiotic Metabolism Signaling	0.01585	AHRR,CYP1A1,CYP1B1,FTL,GSTA3,NQO1,RAP1A
Human Embryonic Stem Cell Pluripotency	0.01585	LIF,RAP1A,SMAD9,SOS1,TBX3,WNT11
ID1 Signaling Pathway	0.01585	CDKN1A,LIF,MDM2,PLXNB2,RAP1A,SMAD9
NRF2-mediated Oxidative Stress Response	0.01738	CYP1A1,FTL,GSTA3,MAFG,NQO1,RAP1A
Hereditary Breast Cancer Signaling	0.01738	CDKN1A,FANCB,RAP1A,UBC,XPC
HER-2 Signaling in Breast Cancer	0.02042	CDKN1A,ELF4,MDM2,RAP1A,RPS6,SOS1
Glioblastoma Multiforme Signaling	0.02818	CDKN1A,MDM2,RAP1A,SOS1,WNT11
DNA damage-induced 14-3-3σ Signaling	0.02818	CCNB2,CDKN1A,MDM2
WNT/β-catenin Signaling	0.02818	DKK1,LRP5,MDM2,UBC,WNT11
Mouse Embryonic Stem Cell Pluripotency	0.02818	LIF,RAP1A,SMAD9,SOS1
Telomerase Signaling	0.02818	CDKN1A,ELF4,RAP1A,SOS1
Chronic Myeloid Leukemia Signaling	0.02818	CDKN1A,MDM2,RAP1A,SOS1
Colorectal Cancer Metastasis Signaling	0.02818	GNAI1,LRP5,RALGDS,RAP1A,SOS1,WNT11
Melanoma Signaling	0.02818	CDKN1A,MDM2,RAP1A
Cell Cycle: G2/M DNA Damage Checkpoint Regulation	0.02818	CCNB2,CDKN1A,MDM2
Prostate Cancer Signaling	0.03020	CDKN1A,MDM2,RAP1A,SOS1
Neuregulin Signaling	0.03311	EREG,RAP1A,RPS6,SOS1
Glioma Signaling	0.03802	CDKN1A,MDM2,RAP1A,SOS1
Senescence Pathway	0.03802	CCNB2,CDKN1A,ELF4,MDM2,RAP1A,SMAD9
Ferroptosis Signaling Pathway	0.04169	CDKN1A,FTL,GLS2,RAP1A
HGF Signaling	0.04467	CDKN1A,ELF4,RAP1A,SOS1

studies, which, in conjunction with the p-value, points to a more consistent response among the studies compared to CYP1B1.

3.4. Functional enrichment analysis

To elucidate the impact of B[a]P exposure on cellular signaling pathways, we leveraged the 169 genes identified from the union of the 4 results to conduct a comprehensive canonical pathway analysis using IPA. Our analysis unveiled a total of 26 canonical pathways that were significantly regulated by B[a]P exposure (Table 2). The most significantly enriched pathways included *Aryl Hydrocarbon Receptor (AhR) Signaling* (BH adjusted P = 0.00214), *Xenobiotic Metabolism Signaling* (BH adjusted P = 0.00550), *Molecular Mechanisms of Cancer* (BH adjusted P = 0.00550) and *p53 Signaling pathways* (BH adjusted P = 0.00851). Multiple genes involved in xenobiotic metabolism and oxidative stress response, such as CYP1A1, CYP1B1, GSTA3, and NQO1, were altered by B[a]P exposure. This suggests that B[a]P metabolism and subsequent reactive oxygen species generation may play an important role in its toxic effects.

Several stem cell pluripotency pathways were also affected, including the *Role of NANOG in Mammalian Embryonic Stem Cell Pluripotency* and *Human Embryonic Stem Cell Pluripotency pathways*. Key pluripotency genes like LIF, RAP1A, SMAD9, SOS1, and WNT11 were changed with B[a]P-exposure. Disruption of stem cell function by B[a]P could impact embryonic development and differentiation. Additionally, the *p53, HER2, and Wnt/β-catenin signaling pathways*, known to regulate cell proliferation and apoptosis, were significantly enriched. Specific genes altered in these pathways, such as CDKN1A, MDM2, and LRP5, indicate B[a]P may promote aberrant cell growth through effects on cell cycle control and survival.

3.5. Overlapping genes among significant pathways

Several genes appear across multiple pathways, indicating their central role in mediating B[a]P's effects. For example, CDKN1A is involved in *AhR Signaling*, *p53 Signaling*, *HER-2 Signaling in Breast Cancer*, and several other pathways. This gene plays a crucial role in cell cycle regulation and DNA damage response, suggesting that B[a]P exposure may lead to coordinated regulation of these processes. Similarly, MDM2 is another gene often appearing in pathways such as *Molecular Mechanisms of Cancer*, *p53 Signaling*, and *Glioblastoma Multiforme Signaling*. MDM2 is known to negatively regulate p53, implicating a tightly controlled balance between cell survival and apoptosis upon B[a]P exposure.

The canonical pathway analysis also revealed several overarching biological themes, primarily centered around cancer-related pathways and stem cell pluripotency. For example, pathways such as *Molecular Mechanisms of Cancer*, *Chronic Myeloid Leukemia Signaling*, and *Colorectal Cancer Metastasis Signaling* share genes like CDKN1A and RAP1A, indicating that B[a]P may influence multiple aspects of oncogenic transformation and tumor progression. In addition, the role of stem cell pluripotency is highlighted by pathways such as *Role of NANOG in Mammalian Embryonic Stem Cell Pluripotency* and *Human Embryonic Stem Cell Pluripotency*. Genes like LIF and WNT11 are key regulators in these pathways, suggesting that B[a]P exposure might impact stem cell maintenance and differentiation, potentially contributing to its carcinogenic effects.

3.6. Diseases and biofunctions

IPA predicted the activation of numerous cancer-related diseases and functions following B[a]P exposure (Table 3). The top enriched categories included *genitourinary tumor* (BH adjusted P = 0.00193), *malignant genitourinary solid tumors* (BH adjusted P = 0.00197), and *anogenital cancer* (BH adjusted P = 0.00224), with over 80 genes dysregulated in

Table 3
Top diseases and biological functions regulated by B[a]P.

Diseases or Functions	BH p-value	# Genes
Genitourinary tumor	0.00193	86
Malignant genitourinary solid tumor	0.00197	85
Anogenital cancer	0.00224	81
Malignant neoplasm of respiratory system	0.00269	57
Cell viability of tumor cell lines	0.00302	22
Metastasis	0.00302	17
Cell death of lung cancer cell lines	0.00058	13
HER2 negative hormone receptor negative breast cancer	0.00302	11
Apoptosis of lung cancer cell lines	0.00302	10
Cell survival of cervical cancer cell lines	0.00269	3

each category. Respiratory malignancies were also significantly altered, including *lung cancer* (BH adjusted P = 0.00058) and *malignant neoplasms of the respiratory system* (BH adjusted P = 0.00269). Furthermore, our results suggest that B[a]P exposure may influence critical cellular functions, such as cell viability in tumor cell lines, metastasis, apoptosis in lung cancer cell lines, and even specific breast cancer subtypes, particularly HER2-negative and hormone receptor-negative breast cancer.

4. Discussion

We employed an integrative meta-analysis approach to elucidate conserved genomic responses to B[a]P exposure across multiple human cell models exposed to this environmental pollutant and carcinogen. Through a rigorous analytical pipeline, we obtained two significant genes (CYP1B1 and ASB2) from publicly available GEO datasets. CYP1B1 was identified as the consistently upregulated gene in response to B[a]P exposure, underscoring its pivotal role in the metabolic activation of this carcinogenic compound. CYP1B1, a member of the cytochrome P450 enzyme family, plays a crucial role in the conversion of B[a]P into DNA-reactive epoxide intermediates, which subsequently form mutagenic DNA adducts and contributes to carcinogenesis (Uppstad et al., 2010; Šmerdová et al., 2014; Malaplate-Armand et al., 2003). Notably, CYP1B1 exhibits high expression across various human tissues and is implicated in numerous cancers, including lung, breast, prostate, bladder, and others (Murray et al., 2001; Androutsopoulos et al., 2013; Bandiera et al., 2005; McKay et al., 1995). Moreover, our findings corroborate the association between polymorphisms in CYP1B1 and interindividual variations in susceptibility to B[a]P toxicity, potentially mediated by their impact on catalytic activity (Aklillu et al., 2005; Li et al., 2015). This substantiates CYP1B1 as a plausible biomarker for B[a]P exposure and reaffirms its pivotal role in B[a]P-induced carcinogenicity. Overexpression of CYP1B1 has been suggested as a potential biomarker for predicting prognosis in patients with glioblastoma multiforme (GBM) (Ye et al., 2021). This link to cancer prognosis may add context to the observed increase in expression upon B[a]P exposure and could suggest a pathway through which B[a]P contributes to carcinogenicity. The study also found a significant correlation between the expressions of CYP1B1-AS1, a non-coding RNA, and CYP1B1, hinting at a complex regulatory mechanism that might influence gene expression in cancer (Ye et al., 2021). The versatility of CYP1B1 as a heme-thiolate monooxygenase capable of metabolizing xenobiotics is noteworthy. Its involvement in the bioactivation of pro-carcinogens accentuates its significance in the context of chemical carcinogenesis (Šmerdová et al., 2014; Roos and Bolt, 2005; Vaclavikova et al., 2007). Importantly, CYP1B1 is implicated in metabolizing PAHs, including those found in tobacco. The presence of CYP1B1 in blood vessel walls and its association with the risk of myocardial infarction (MI) and cardiovascular diseases (CVD) emphasize its broader impact on human health (Kaur--Knudsen et al., 2009). Also, the presence of CYP1B1 in lung tissue implicates its potential role in chronic obstructive pulmonary disease (COPD), warranting further investigation into the contribution of CYP1B1 with regard to respiratory health (Yang et al., 2020).

In addition to CYP1B1, our study unveiled ASB2 as another noteworthy gene consistently upregulated in response to B[a]P exposure across multiple datasets. ASB2 plays a multifaceted role in cellular processes. It is implicated in hematopoietic cell differentiation, modulating cell spreading and actin remodeling by targeting filamins for degradation. Moreover, it contributes to this differentiation by promoting MLL degradation and down-regulating HOX genes (Heuzé et al., 2008; Wang et al., 2012). Additionally, in leukemia cells, ASB2 targets specific proteins for proteasomal destruction upon induced differentiation (Heuzé et al., 2005). Beyond hematopoiesis, ASB2 is a pivotal regulator of cardiac cell differentiation, influencing myocardial cell architecture and its early function (Métais et al., 2018). In the muscular system, increased ASB2 expression has been associated with reduced muscle mass, suggesting its role as a negative regulator in response to the TGF-β network (Davey et al., 2016). Furthermore, ASB2's induction by the Th2 master regulator, Gata3, hints at its potential involvement in antitumor immunity in colorectal cancer (Spinner et al., 2019). Given these diverse roles, the consistent upregulation of ASB2 due to B[a]P exposure could have broad implications, ranging from immune response alterations to cardiovascular health impacts. Given the moderate heterogeneity and statistically significant upregulation observed in our meta-analysis, it could be suggested that ASB2 plays a consistent role in the cellular response to B[a]P exposure. This could be indicative of a mechanism by which B[a]P exposure alters cellular processes, potentially through disruption of ubiquitin-mediated degradation pathways, where ASB2 is known to function. While the direct health hazards of this upregulation remain to be fully understood, it's conceivable that such perturbations could contribute to or exacerbate various disease states. Further research is paramount to pinpoint the precise health implications, especially in the context of B[a]P exposure.

Our findings revealed enrichment in several critical pathways, including cell cycle regulation, survival signaling, oxidative stress response, and xenobiotic metabolism. These results align closely with potential mechanisms underlying B[a]P cytotoxicity and carcinogenicity, as documented in previous studies (Dautel et al., 2011; Gao et al., 2007). Our analysis identified alterations in pathways associated with cancer, such as p53, HER2, and Wnt/β-catenin, which are frequently dysregulated in various cancer types (Rosenbluh et al., 2014; Ying and Tao, 2009; Rogers et al., 2013; Roh et al., 2004; Duffy et al., 2016; Sahlberg et al., 2013; Hollstein et al., 1991). The upregulation of oxidative stress response genes, including CYP1A1, NQO1, and NFE2L2, was consistent with the well-documented ability of B[a]P to induce the formation of reactive oxygen species during its metabolism (Fabiani et al., 1999; Shukla et al., 2020; Ross et al., 2011; Palikaras et al., 2015). The presence of these known B[a]P-associated pathways provides robust validation of our meta-analysis approach and results. Furthermore, our study goes beyond the confirmation of previously established pathways and offers a broader overview of coordinated gene expression changes that govern key toxicity processes. The identification of genetic networks and biological pathways with both up- and down-regulated gene expression levels expands our understanding of the complex molecular responses to B[a]P exposure. Additionally, it's worth noting that the AhR, a major regulator of xenobiotic-induced carcinogenesis, has been associated with abnormal AhR function and cancer in epidemiological and experimental animal data (Androutsopoulos et al., 2009; Gasiewicz et al., 2008). The expression of CDKN1A protein plays an important role in the suppression of cancer cell proliferation (Souza et al., 2016). NANOG has been implicated in cell-fate determination in cancer stem cells, playing a role in cancer stem cell formation and mediating communication between cancer cells and the surrounding immune microenvironment (Wang et al., 2013). Moreover, the role of the p53 protein, which exhibits differential expression in normal cells and cancer cells, is crucial in cancer progression and oncogenic function due to its overexpression and mutant forms (Kanapathipillai, 2018). The recent meta-analysis study on the transcriptomic effects of various EDCs, not including B[a]P, also highlighted the perturbation of p53 signaling pathways, particularly in response to tributyltin (TBT) exposure (Zamora et al., 2024). This shared pathway enrichment underscores a potential common mechanism through which different environmental carcinogens and EDCs contribute to cellular stress responses and potentially to tumorigenesis. The presence of the p53 signaling pathway activation in both studies suggests a conserved cellular response to chemical stresses, irrespective of the chemical nature of the exposure. This reinforces the role of p53 as a key tumor suppressor activated in response to genomic damage. Given the centrality of p53 in cell cycle regulation and apoptosis, our findings, alongside those of Zamora et al. provide a broader molecular basis for understanding the carcinogenic potential of environmental contaminants. Taken together, these results suggest B[a]P elicits broad effects on cell homeostasis through impacts

on xenobiotic metabolism, oxidative stress, stem cell function, proliferation, and survival. Dysregulation of these key signaling networks provides insight into the mechanisms underlying the cytotoxicity and carcinogenicity of B[a]P exposure.

While our study provides valuable insights into the effects of B[a]P exposure, several limitations should be acknowledged. Our analysis relies on existing datasets, and variations in experimental designs, sample sizes, and platforms across studies may introduce heterogeneity. Variations in experimental designs, including differences in exposure durations, concentrations of B[a]P, and methods of gene expression analysis, likely contributed to the observed diversity in results. For example, studies with longer exposure durations or higher concentrations of B[a]P may elicit different gene expression patterns compared to those with shorter exposures or lower doses. The observed heterogeneity in the gene responses to B[a]P might also stem from complex biological variability. The inclusion of various cell types—T lymphocytes, HepG2, and C3A—each with distinct metabolic and regulatory pathways, further contributes to the observed heterogeneity. The variability in CYP1B1 expression levels across different cell types and experimental conditions underscores the complex regulatory mechanisms governing its induction in response to B[a]P exposure. ASB2's role in protein degradation suggests its variability might reflect different cellular stress responses or protein turnover rates. Statistically, the low τ^2 for ASB2 implies minor overall variance despite some heterogeneity. In contrast, CYP1B1's high I^2, coupled with a significant p-value, signals considerable variance, warranting deeper investigation into the underlying causes.

Our methodological approach to analyzing datasets involving B[a]P-exposed human cells aimed to accommodate studies with varying control group specifications, ensuring comprehensive inclusion of available data. This strategy allowed us to maximize data utilization and statistical power by treating unspecified experimental groups as controls across studies where explicit control groups were not defined. While this approach facilitated broader comparisons between B[a]P-exposed groups and a standardized control set, it also introduced potential variability in the interpretation of the results due to assumptions of uniformity among non-specified control groups.

The identified common genes and pathways represent a subset of potential responses to B[a]P exposure, and other relevant genes and pathways may exist as well. Our study does not consider potential interactions with other environmental factors, which could influence the observed gene expression changes. Nevertheless, the systematic integration of these diverse datasets enabled a comprehensive assessment of gene expression changes and pathway enrichment associated with B[a]P exposure across different cell types.

To address the limitations and risks identified in our study, future research should focus on standardizing experimental protocols, including consistent control definitions and exposure parameters. Incorporating in vivo models and comprehensive exposure assessments that consider interactions with other contaminants will provide a broader understanding of the impact of B[a]P exposure as well. Longitudinal studies and the inclusion of diverse cell types and tissues are essential for identifying long-term and tissue-specific responses. Mechanistic studies and advanced analytical techniques will enhance our understanding of the underlying pathways.

5. Conclusions

Our meta-analysis highlights CYP1B1 and ASB2 as key genes upregulated by B[a]P exposure, suggesting their pivotal roles in carcinogenesis and cellular response processes. CYP1B1's involvement in converting B[a]P into carcinogenic compounds and its association with various cancers underscore the importance of CYP1B1 as a potential biomarker for exposure and risk. Similarly, ASB2's consistent upregulation across studies points to its significant role in cellular differentiation and response to environmental pollutants. Canonical pathway analysis showed 26 enriched pathways, with the most significant being *AhR Signaling*, *Xenobiotic Metabolism Signaling*, *Molecular Mechanisms of Cancer*, and *p53 Signaling*. Pathways related to cell cycle regulation, survival signaling, oxidative stress response, and xenobiotic metabolism were enriched, aligning with known mechanisms of B[a]P toxicity and carcinogenicity. Despite limitations such as dataset variability and unexplored environmental interactions, this study provides a foundation for future research into the biological impact of B[a]P exposure and potential strategies for mitigating its health risks.

CRediT authorship contribution statement

Mingze Zhu: Writing – review & editing, Writing – original draft, Visualization, Formal analysis, Data curation. **Jooyeon Hwang:** Writing – review & editing, Validation, Project administration, Investigation, Funding acquisition. **Chao Xu:** Writing – review & editing, Software, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data utilized in this study was sourced from the Gene Expression Omnibus (GEO). The codes employed in our analysis have been deposited on GitHub at https://github.com/MZ666Z/Meta-analysis-Identifies-Key-Genes-and-Pathways-Implicated-in-Benzo-a-pyrene-Exposure-Response.

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