Polychlorinated biphenyl 153 alters the intestinal epithelial cell transcriptome

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Abstract. Polychlorinated biphenyl 153 (PCB153) is one of the most persistent environmental pollutants and abundant PCB congeners detected in human tissues, primarily acquired through dietary exposure. The intestinal epithelium therefore represents a critical initial target for PCB-related toxicity. However, the molecular mechanisms by which PCB153 disrupts normal intestinal epithelial function remain incompletely understood. The present study investigated the effects of PCB153 exposure on non-transformed human intestinal epithelial cells (IECs) using transcriptomic profiling. The data revealed that PCB153 induced dose-dependent alterations in the IEC transcriptome. Key pathways affected by PCB153 included Wnt signaling, ABC transporters, cGMP-PKG signaling and metallothionein-mediated metal homeostasis. High-dose exposure further activated inflammatory and tumorigenic pathways such as TNF and NF-κB signaling, while suppressing mitochondrial metabolism, oxidative phosphorylation and cellular detoxification processes. To the best of our knowledge, this is the first report that reveals the extensive transcriptomic remodeling in normal human IECs in response to PCB153 exposure, highlighting the disrupted intestinal regeneration, immune response and metabolic regulation. These findings provide novel mechanistic insights into how PCB153 compromises intestinal epithelial health, and establishes a transcriptomic framework for identifying biomarkers and therapeutic targets related to environmental toxicant exposure.

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Abbreviations: DEG, differentially expressed gene; DMSO, dimethyl sulfoxide; IEC, intestinal epithelial cell; PCA, principal component analysis; PCB, polychlorinated biphenyl; PCB153, polychlorinated biphenyl 153

Key words: PCB153, IECs, RNA sequencing, transcriptome, environmental toxicants

Introduction

Polychlorinated biphenyls (PCBs) are synthetic chlorinated compounds that were historically used in a numerous industrial and commercial applications due to their chemical stability, and insulating properties. Although the manufacture and use of PCBs have been banned or severely restricted in many countries, PCBs persist in the environment and continue to raise significant public health concerns due to their bioaccumulative nature and long biological half-life (1,2). Human exposure occurs primarily through the consumption of contaminated food, particularly animal fats, as well as through inhalation and dermal contact (1,3). Among the various PCB congeners, PCB153 is one of the most abundant and persistent in human tissues (2). Its strong lipophilicity and resistance to metabolic degradation facilitate its accumulation in the food chain, especially in lipid-rich food sources such as fish and animal meat (4). The intestinal epithelium, as the first site of dietary exposure and absorption, represents a critical yet understudied target for PCB153 toxicity.

The intestinal epithelium plays a central role in maintaining mucosal homeostasis, acting as both a physical barrier and an active regulator of immune and metabolic processes. Disruption of intestinal epithelial cell (IEC) function by environmental toxicants has been linked to impaired barrier integrity, chronic inflammation, metabolic dysregulation (5,6). Previous studies have begun to elucidate the impact of PCB153 on gut health. For example, Choi *et al* (7) demonstrated that PCBs disrupt intestinal barrier function by inducing oxidative stress via NADPH oxidase activation, which alters tight junction protein expression and compromises epithelial integrity.

Additionally, Phillips *et al* (8) reported that intestinal exposure to PCB153 activates the ATM/NEMO pathway, triggering inflammation and epithelial injury. Together, these findings raise important concerns about the impact of PCB153 on intestinal homeostasis and underscore the need for deeper mechanistic studies into how environmental toxicants drive intestinal dysfunction.

Significant knowledge gaps remain regarding the broader transcriptional and cellular consequences of PCB153 exposure in normal human IECs. Prior studies have relied on colon cancer cell lines or murine exposure models (7,8), while may not accurately reflect the normal epithelial physiology.

To bridge these gaps, we utilized the non-tumorigenic human IEC line NCM460D (9) to evaluate the dose-dependent effects of PCB153 exposure on the profiling of whole transcriptome of IECs. By integrating viability assays with RNA sequencing, we systematically characterized how low and high concentrations of PCB153 alter transcriptional programs and key biological pathways. Our data reveal broad transcriptomic shifts involving inflammation, cellular stress, metabolic disruption, impaired regenerative signaling, and altered gut-brain communication. Notably, we found the suppression of Wnt signaling and proliferation-associated genes, suggesting mechanistic insights into how PCB153 compromises epithelial renewal and barrier maintenance.

This study presents the first comprehensive, dose-dependent transcriptomic analysis of PCB153 exposure in normal human IECs, offering new perspectives on the molecular mechanisms through which PCB153 compromises intestinal health.

Materials and methods

In vitro experiments. NCM460D cell line was purchased from INCELL Corporation (San Antonio, TX). NCM460D is a non-transformed human colonic epithelial cell line originally derived from normal colon tissue of a 68-year-old male donor. The cells were selected for stable *in vitro* growth and were not infected, immortalized, or genetically modified, preserving a normal genotype. Phenotypically, NCM460D cells express key intestinal epithelial markers, including cytokeratins, villin, colonic epithelial antigens, and a subset of cells exhibit mucin production, consistent with differentiated epithelial features (9). NCM460D cells are non-tumorigenic and grown in INCELL's enriched M3:10™ medium (INCELL Corp.) to maintain their physiological phenotype, supplemented with 10% fetal bovine serum (Gibco, Thermo Fisher Scientific, Waltham, MA), 100 U/ml penicillin and 100 μg/ml streptomycin (Gibco), at 37˚C in a humidified atmosphere containing 5% CO_2.

PCB153 (2,2',4,4',5,5'-hexachlorobiphenyl) was purchased from Sigma-Aldrich (St. Louis, MO) and dissolved in dimethyl sulfoxide (DMSO) to generate a 10 mM stock solution. Working concentrations were freshly prepared by diluting the stock in M3:10™ complete medium, ensuring the final DMSO concentration did not exceed 0.1% (v/v) in any treatment. Cells were seeded at $2x10^4$ cells/well in 96-well plates for viability assays or $2x10^5$ cells/well in 6-well plates for RNA extraction. For the cell viability assay, cells were treated with 0.5, 5, 50, or 200 μM PCB153, or an equivalent volume of DMSO (vehicle control), for 6, 24, or 48 h. For transcriptomic analysis and RT-qPCR validation, cells were exposed to PCB153 (0, 5, or 50 μM) or DMSO for 24 h under identical culture conditions in a 37˚C humidified atmosphere containing 5% CO_2.

Cell viability assay. The genotoxicity of PCB153 on intestinal epithelial cells was determined by PrestoBlue™ HS Reagent (Invitrogen, Thermo Fisher Scientific, Waltham, MA). Briefly, NCM460D cells ($2x10^4$ cells/well) were seeded into a 96-well plate and treated with PCB153 at final concentrations of 0, 0.5, 5, 50 and 200 μM, with DMSO serving as the vehicle control. Treatments were performed for 6, 24, and 48 h. After treatment, PrestoBlue reagent was added directly to the culture medium as 1/10th of the total well volume and incubated for 1 h in a cell culture incubator at 37˚C, protected from direct light. Absorbance was measured at 570 nm, using 600 nm as a reference wavelength. Cell viability was calculated as the ratio of OD570/600.

Reverse transcription-quantitative PCR (RT-qPCR). NCM460D cells ($2 x 10^5$ cells/well in 6-well plate) were treated with PCB153 (0, 5, or 50 μM) for 24 h, with DMSO serving as the vehicle control. Total RNA was extracted from the cells using TRIzol reagent (Invitrogen, Thermo Fisher Scientific, Waltham, MA) according to the manufactory protocol. RNA purity and concentration were assessed using the NanoDrop spectrophotometer (Agilent Technologies, Santa Clara, CA). Total 1 μg of RNA was reverse-transcribed to cDNA using high-capacity cDNA reverse transcription kit (Applied Biosystems, Thermo Fisher Scientific, Waltham, MA). Reverse transcription was performed at 25˚C for 10 min, followed by 37˚C for 120 min, 85˚C for 5 min, and then held at 4˚C. Quantitative PCR was performed with SYBR Green master mix (Applied Biosystems) on a QuantStudio 3 Real-Time PCR System (Thermo Fisher Scientific). The thermocycling program consisted of an initial denaturation at 95˚C for 10 min, followed by 40 cycles of 95˚C for 15 sec and 60˚C for 60 sec. The relative expression of target gene was analyzed by the $2^{-\Delta\Delta Cq}$ method (10), normalizing each target to *GAPDH* and expressing data as fold-change relative to the solvent control. Primer sequences of *MT1G*, *MT2A*, *LGR5*, *DACT1*, *LEF1* and *GAPDH* are provided in Table SI.

RNA sequencing. NCM460D cells ($2x10^5$ cells/well in 6-well plate) were treated with PCB153 (5, or 50 μM) for 24 h, with DMSO serving as the vehicle control. Total RNA was extracted from the cells using TRIzol reagent (Invitrogen, Thermo Fisher Scientific, Waltham, MA) as previously described and purified by RNA Clean & Concentrator kit (Zymo Research, Irvine, CA) according to the manufactory protocol. RNA integrity and quantity were further assessed using Agilent Bioanalyzer (Agilent Technologies, Santa Clara, CA) before the library construction for RNA sequencing. Total RNA was processed for Poly(A) mRNA isolation and preparation of cDNA libraries using NEBNext Ultra II RNA Library Prep Kit (New England Biolabs, Ipswich, MA) and barcoded with NEBNext Multiplex Oligos for Illumina (NEB), all according to the manufacturer's manuals. Library concentration was measured by Agilent Bioanalyzer (Agilent Technologies) and $2.6x10^{-10}$ moles desired loading of final

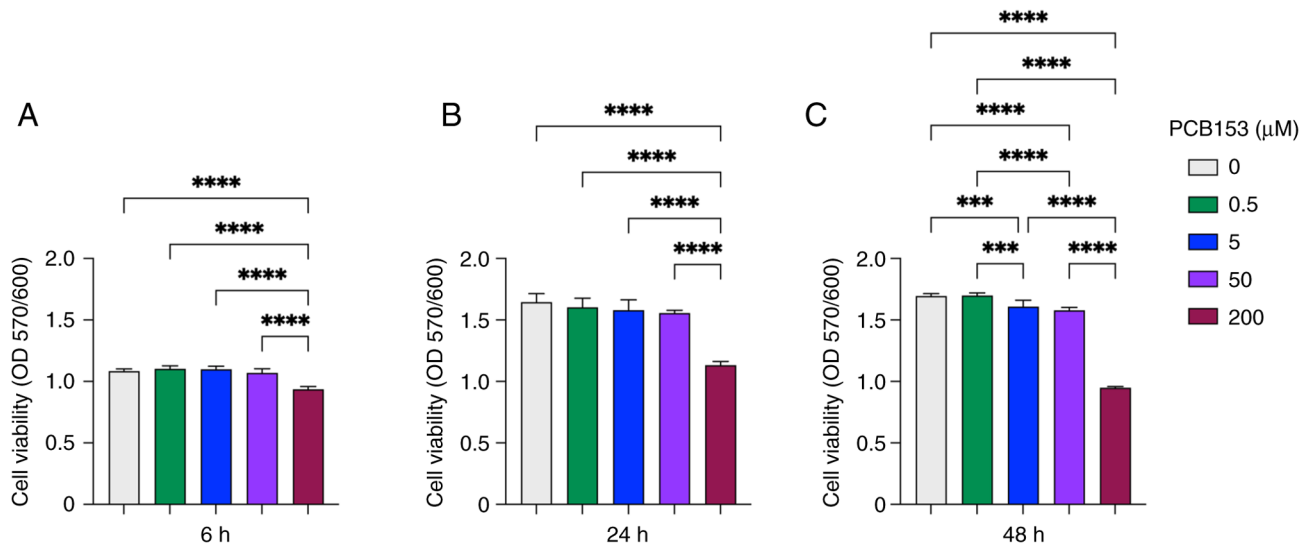

Figure 1. Effects of PCB153 on NCM460D cell viability. NCM460D cells were treated with a series of concentrations of PCB153 (0, 0.5, 5, 50 and 200 μM) for (A) 6, (B) 24 and (C) 48 h. Cell viability was determined using the PrestoBlue™ HS assay. Data are presented as the mean ± SD from six biological repeats. One-way ANOVA with Tukey's post hoc test; ***P<0.001, ****P<0.0001. OD, optical density; PCB153, polychlorinated biphenyl 153.

library were subjected to 100 bp pair-end sequenced (PE100) on the Illumina NovaSeqX platform at the University of Chicago Genomics Core Facility (Chicago, IL). RNA-Seq raw data has been uploaded to the database at the National Center for Biotechnology Information (NCBI) (https://www.ncbi.nlm.nih.gov/bioproject/) under BioProject accession number PRJNA1285092.

RNA sequencing data analysis. For transcriptomic analysis, we performed two independent biological experiments, each containing duplicate cultures per treatment condition (0, 5, and 50 μM PCB153). To minimize technical variation and maximize consistency across experiments, the duplicate cultures within each treatment group were pooled prior to RNA extraction, resulting in two biological replicates per treatment group used for RNA-seq. The output FASTQ sequences were aligned to reference human genome annotation (GENCODE version 48) using STAR software (version 2.7) (11). The aligned sequences were further sorted with Samtools (version 1.18) (12). Counting reads in features with htseq-count (version 2.0.2) (13). The count matrix was then normalized and differentially expressed were identified (adjusted P-value <0.05) with the DESeq2 package (version 1.46) (14) in R software (version 4.4). Gene Ontology (GO) was identified by clusterProfiler (version 4.14) (15) to discover the functional enrichment of pathways comparing experimental groups. ShinyGO 0.82 was used to visualize the pathway networks (16).

Statistical analysis. All statistical analyses were performed using GraphPad Prism (version 10.5). For cell viability assays (n=6) and RT-qPCR (n=3) experiments, differences among treatment groups were analyzed using one-way ANOVA, followed by Tukey's post hoc test for multiple comparisons. Data are presented as mean ± standard deviation (SD). A P-value of P<0.05 was considered statistically significant. Significance levels are indicated as *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001.

Results

Establishing RNA-seq compatible PCB153 exposure conditions. To evaluate the cellular consequences of PCB153 exposure in a physiologically relevant intestinal model, we first established an *in vitro* system using the non-tumorigenic human intestinal epithelial cell line NCM460D. This line is derived from normal colonic mucosa and retains key features of differentiated intestinal epithelial cells, including expression of cytokeratins, villin, and other colonic epithelial markers, without exogenous genetic modifications (9). Due to these characteristics, NCM460D cells provide an appropriate model for examining transcriptomic disruptions in normal IECs.

To determine suitable exposure conditions for transcriptomic profiling, we next assessed the effects of PCB153 on NCM460D cell viability across a range of concentrations (0.5, 5, 50, and 200 μM) and exposure times (6, 24, and 48 h) (Fig. 1). This analysis was conducted to identify non-cytotoxic conditions appropriate for detection of meaningful alterations by RNA-seq, rather than to define a full cytotoxicity curve. At 6 h, cells exposed to 0.5-50 μM PCB153 showed little change in viability, indicating that this short exposure primarily captures immediate early-response genes and is not ideal for broader transcriptional profiling (Fig. 1A). In contrast, 200 μM PCB153 caused acute viability loss at this early time point, making it unsuitable for mechanistic studies (Fig. 1A). Cells were maintained >90% viability when exposed with both 5 and 50 μM PCB153 at 24 h, providing sufficient time for primary transcriptomic responses to develop while avoiding overt cytotoxicity (Fig. 1B). By comparison, 0.5 μM PCB153 produced minimal biological impact on NCM460D cells and was unlikely to yield detectable transcriptional changes (Fig. 1A-C). However, subtle but statistically significant reductions in cell viability emerged at 48 h, even at 5 and 50 μM intermediate doses, indicating that longer exposures may introduce nonspecific cytotoxic effects (Fig. 1C). Together, these findings identify 5 and 50 μM PCB153 for 24 h as optimal

exposure conditions that balance IEC cellular integrity with meaningful transcriptional alterations while minimizing confounding cytotoxic effects and thus were selected for subsequent RNA-seq experiments.

Transcriptome changes in IECs exposed to PCB153. Although recent studies have identified various adverse effects of PCB153 exposure, significant knowledge gaps remain regarding its impact and underlying mechanisms of cytotoxicity in normal intestinal epithelial cells. In addition, no studies have systematically investigated the dose-dependent effects of PCB153 exposure on normal IECs. To address these gaps, this study aimed to characterize the transcriptional responses and signaling pathways affected by PCB153 in human normal IECs using RNA-seq analysis. Principal component analysis (PCA) revealed a significant transcriptomic shift between control and PCB153-treated cells. More importantly, the transcriptomic changes followed a dose-dependent pattern in IECs exposed to PCB153 (Fig. 2A). Further analysis of differentially expressed genes (DEGs), using an adjusted P-value cutoff of <0.05, identified 329 upregulated and 246 downregulated genes in IECs treated with 5 μM of PCB153 compared to controls (Fig. 2B). Moreover, exposure to 50 μM of PCB153 resulted in 4,006 upregulated and 3,070 downregulated genes (Fig. 2C). Among these significantly differentially expressed genes, 136 were commonly downregulated, and 181 were commonly upregulated in both the 5 and 50 μM PCB153 treatment groups compared to controls (Fig. 2D). These results suggest that acute PCB153 exposure significantly alters the transcriptome of IECs, with higher doses exerting more pronounced effects than lower doses. The higher PCB153 exposure resulted in thousands of gene expression changes, indicating a dose-dependent impact on the IEC transcriptome. To validate the RNA-seq results, RT-qPCR was performed for several representative differentially expressed genes, including *MT1G*, *MT2A*, *LGR5*, *DACT1*, and *LEF1*. The RT-qPCR results confirmed a strong induction of *MT1G* and *MT2A*, and a marked suppression of *LGR5*, *DACT1*, and *LEF1* following PCB153 exposure, consistent with the RNA-seq data (Fig. 2E). Taken together, these findings suggest that PCB153 exposure may profoundly influence IEC cellular functions and biological processes.

PCB153 exposure leads to intestinal epithelial cell dysfunction, metabolic disruption, and tumorigenic potential. Through DEG analysis, we identified a core set of 317 genes (136 downregulated and 181 upregulated) shared between two doses of PCB153 exposure. Pathway analysis of this core set revealed significant alterations in several key biological processes. GO terms analysis of the 181 upregulated genes indicated significant enrichment in pathways related to mineral absorption, ribosome function, coronavirus disease, and axon guidance (Fig. 3A and B). The upregulation of mineral absorption pathways suggests that PCB153 disrupts metal homeostasis, particularly affecting zinc (Zn) and copper (Cu) transport. Metallothionein (MT) family genes (*MT1E*, *MT1F*, *MT1G* and *MT2A*), regulate essential metal ions (17,18), dramatically induced, suggesting an adaptive response to PCB153-induced metal transporter disruption, potentially leading to increased cellular stress due to metal accumulation or dysregulation of Zn/Cu levels. Additionally, the increased ribosome activity observed following PCB153 exposure suggests enhanced protein synthesis, which may burden cellular machinery, triggering ER stress, misfolded proteins, and conditions favoring tumorigenesis (19,20). The upregulation of coronavirus disease pathway (Path:hsa05171, aggregates 232 pathway genes) likely reflects the activation of a general 'viral response-related pathways' or inflammatory stress response, suggesting PCB153 exposure alters immune responses in the IECs, potentially increasing susceptibility to viral infections and chronic inflammation. Taken together, network analysis further revealed a potential connection between ribosome and coronavirus disease pathways in response to PCB153, suggesting that ribosome dysregulation may exacerbate gut inflammation during infections (Fig. 3B). Interestingly, PCB153 exposure also disrupted axon guidance signaling, suggesting potential alterations in gut-brain axis communication. Axon guidance genes, such as *NTN4* [a member of the netrin family (21)], regulate neuronal connectivity, and their dysregulation may impair gut-brain signaling, potentially affecting gut motility and neurological function.

On the other hand, PCB153 exposure led to the downregulation of 136 core genes, which were significantly enriched in pathways related to DNA replication, ABC transporters, digestive secretions (gastric acid, pancreatic, and salivary), cGMP-PKG signaling, cell cycle, estrogen signaling, and Wnt signaling (Fig. 3C and D). Among these, the suppression of DNA replication and cell cycle genes suggests reduced IEC proliferation. Network analysis highlighted a core connection between gastric acid secretion, pancreatic secretion, and salivary secretion, indicating that PCB153 may significantly disrupt digestive processes (Fig. 3D). The downregulation of these pathways may impair protein, fat, and carbohydrate digestion, leading to malabsorption disorders (22). Furthermore, the cGMP-PKG signaling pathway is also connected to the digestive secretion function, which plays a role in intestinal motility and cellular homeostasis (23,24), was also downregulated (Fig. 3C and D). The suppression of ABC transporters (e.g., *ABCA1*, *ABCG1*) impairs cholesterol efflux, leading to lipid accumulation and oxidative stress (25). Similarly, downregulation of estrogen signaling in IECs may contribute to the disruptions in intestinal lipid homeostasis. Since PCBs are lipophilic and accumulate in lipid membranes, the inhibition of ABC transporters may increase intracellular PCB retention, exacerbating cellular toxicity and bioaccumulation. Most importantly, PCB153 exposure significantly disrupted the Wnt signaling pathway, which is critical for intestinal stem cell renewal and epithelial integrity (26,27) (Fig. 3C). Notably, due to the downregulation of WNT signaling, *LGR5*, a stemness-associated molecule, was significantly downregulated (Fig. 2D), suggesting impaired intestinal regeneration and homeostasis. Additionally, *TCF4* and *LEF1*, two major Wnt transcription factors essential for IEC proliferation, ranked among the top 20 downregulated genes (Fig. 2D). Their suppression directly contributes the disrupts of WNT signaling linking to IECs renewal and regeneration, highlighting the detrimental impact of PCB153 on intestinal epithelial integrity.

Taken together, PCB153 exposure profoundly alters multiple essential signaling pathways in normal IECs, disrupting cell proliferation, lipid metabolism, immune

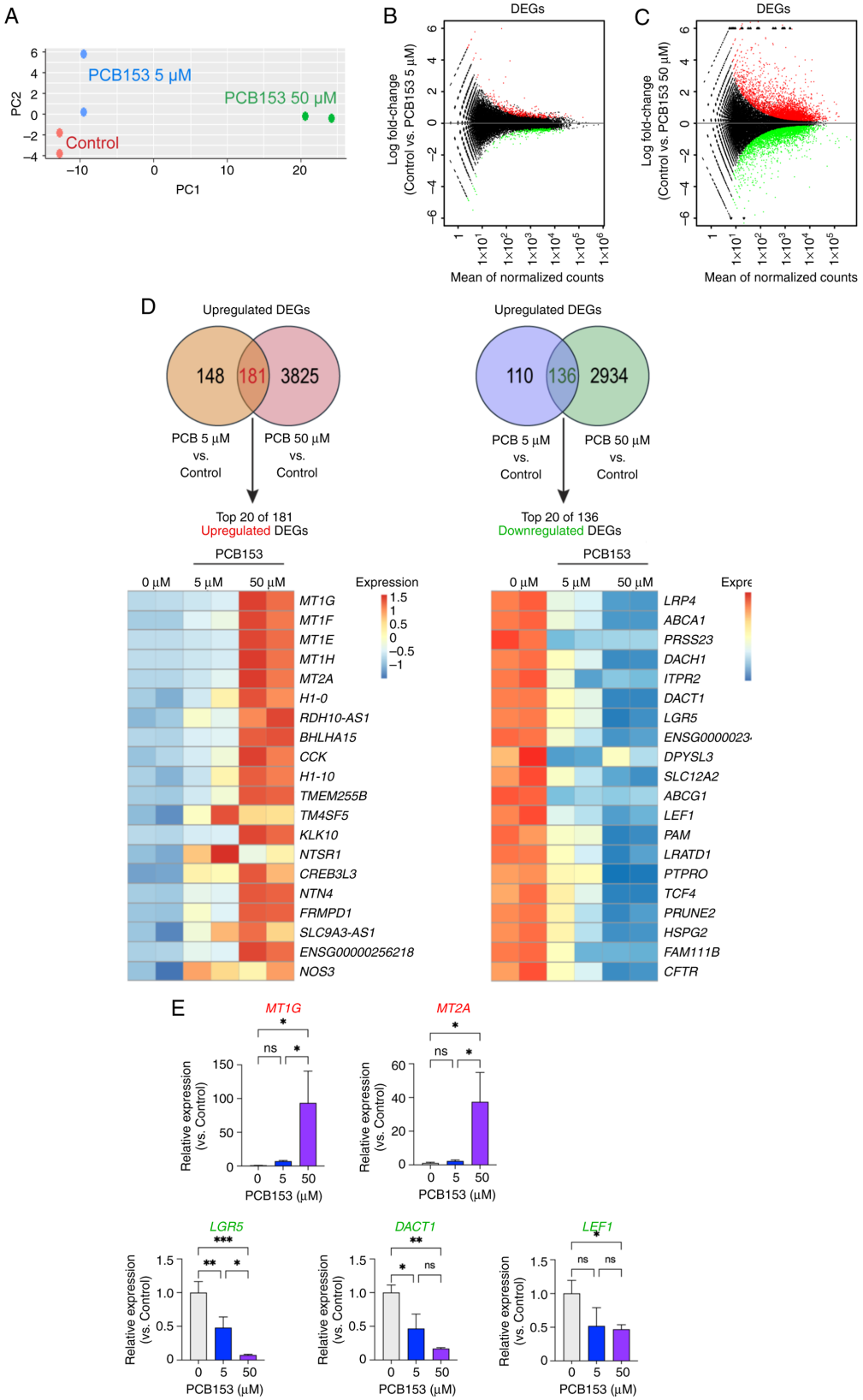

Figure 2. Dose-dependent transcriptomic alterations in human IECs following PCB153 exposure. (A) PC analysis of RNA-seq data showing distinct clustering of control and PCB153-treated IECs, with a clear dose-dependent separation between the 5 and 50 μM exposure groups. (B) MA plot of DEGs in IECs treated with 5 μM PCB153 compared with the control group, highlighting 329 upregulated and 246 downregulated genes (adjusted P<0.05). (C) MA plot of DEGs in IECs treated with 50 μM PCB153 compared with the control group, showing 4,006 upregulated and 3,070 downregulated genes (adjusted P<0.05). (D) Venn diagram illustrating the overlap of DEGs between the 5 and 50 μM PCB153 treatment groups, identifying 181 commonly upregulated and 136 commonly downregulated genes, with the top 20 DEGs (ranked by P-value) visualized in a heatmap. RNA-seq data from two independent biological experiments, each performed in duplicate are presented. (E) Reverse transcription-quantitative PCR validation of selected representative DEGs (*MT1G*, *MT2A*, *LGR5*, *DACT1* and *LEF1*) confirming induction of metallothionein genes and suppression of Wnt-related targets, consistent with RNA-seq results. n=3 for each group. Statistical significance was determined using one-way ANOVA. *P<0.05, **P<0.01, ***P<0.001. DEG, differentially expressed gene; IEC, intestinal epithelial cell; ns, not significant; PC, principal component; PCB153, polychlorinated biphenyl 153; RNA-seq, RNA sequencing; MA plot, minus-average plot.

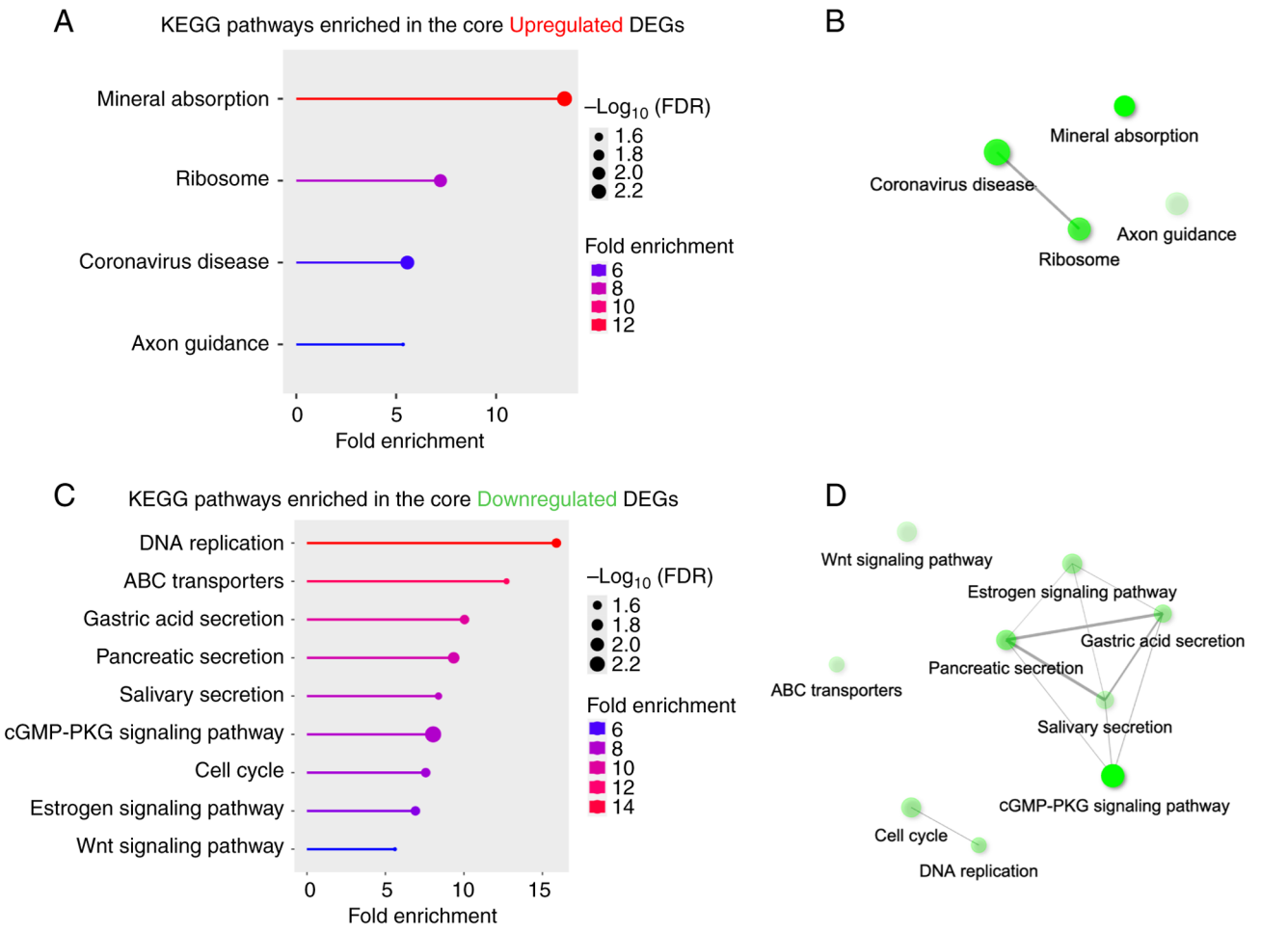

Figure 3. Pathway enrichment analysis of commonly dysregulated genes in IECs following PCB153 exposure. (A) KEGG pathway enrichment analysis of 181 commonly upregulated genes revealed significant enrichment in pathways related to 'mineral absorption', 'ribosome', 'coronavirus disease' and 'axon guidance'. (B) Network analysis of upregulated pathways highlighted potential interactions between 'ribosome' and viral response-related pathways ('coronavirus disease'). (C) KEGG pathway enrichment analysis of 136 commonly downregulated genes identified significant suppression of pathways involved in 'DNA replication', 'ABC transporters', digestive secretions ('gastric acid secretion', 'pancreatic secretion' and 'salivary secretion'), 'cGMP-PKG signaling', 'cell cycle', 'estrogen signaling' and 'Wnt signaling'. (D) Network analysis of downregulated pathways showed interconnected suppression of digestive secretion pathways and the 'cGMP-PKG signaling pathway', indicating impaired intestinal epithelial physiology. (B and D) Interactive plot shows the relationship between enriched pathways. Two pathways (nodes) are connected if they share ≥20% of genes. Darker nodes are more significantly enriched gene sets. Bigger nodes represent larger gene sets. Thicker edges represent more overlapped genes. DEG, differentially expressed gene; FDR, false discovery rate; IEC, intestinal epithelial cell; KEGG, Kyoto Encyclopedia of Genes and Genomes; PCB153, polychlorinated biphenyl 153.

responses, and gut-brain axis communication. These findings suggest that PCB153 exposure not only impairs gut homeostasis but may also contribute to tumorigenic potential and long-term metabolic dysfunction.

IECs displays a dose-dependent toxicant response to PCB153 exposure. In this study, we further evaluated the dose-dependent effects of PCB153 on cell fate and signaling in IECs. We found that the 5 μM of PCB153 exposure resulted in the upregulation of the same cellular pathways as identified with the common core upregulated gene set, suggesting the lower dose of PCB153 exposure can initiate the cellular changes of mineral absorption, ribosome function, coronavirus disease, and axon guidance (Fig. 4A). We found that relatively low PCB153 exposure in IECs led to the downregulation in DNA replication, mismatch repair, homologous recombination, ABC transporters, cell cycle regulation, and cGMP-PKG signaling, effects that would be predicted to result in severe gut dysfunction (Fig. 4B). Impaired DNA repair and cell cycle arrest increase genomic instability, raising the risk of mutations and colorectal cancer (28,29). Downregulated ABC transporters reduce toxin efflux, suggesting that PCB153 exposure could augment further cellular PCB153 retention. Additionally, weakened intestinal regeneration can impair the barrier integrity and contribute to chronic inflammation and disrupted homeostasis. These changes collectively are predicted to compromise gut health, increasing susceptibility to disease, oxidative stress, and metabolic imbalances.

On the other hand, 50 μM of PCB153 exposure in IECs resulted in dramatical impairments of multiple signaling pathways. Using an FDR <0.05 as the significance cutoff, a total of 111 pathways were significantly upregulated and 68 were downregulated. The top 20 pathways are shown in Fig. 4C and D. To enhance interpretability, the enriched pathways were consolidated into functional modules based on shared biological relevance. Upregulated pathways were grouped into four major categories: 'Immune & Host-Defense', 'Proteostasis & Translation', 'Proliferation/Oncogenic',

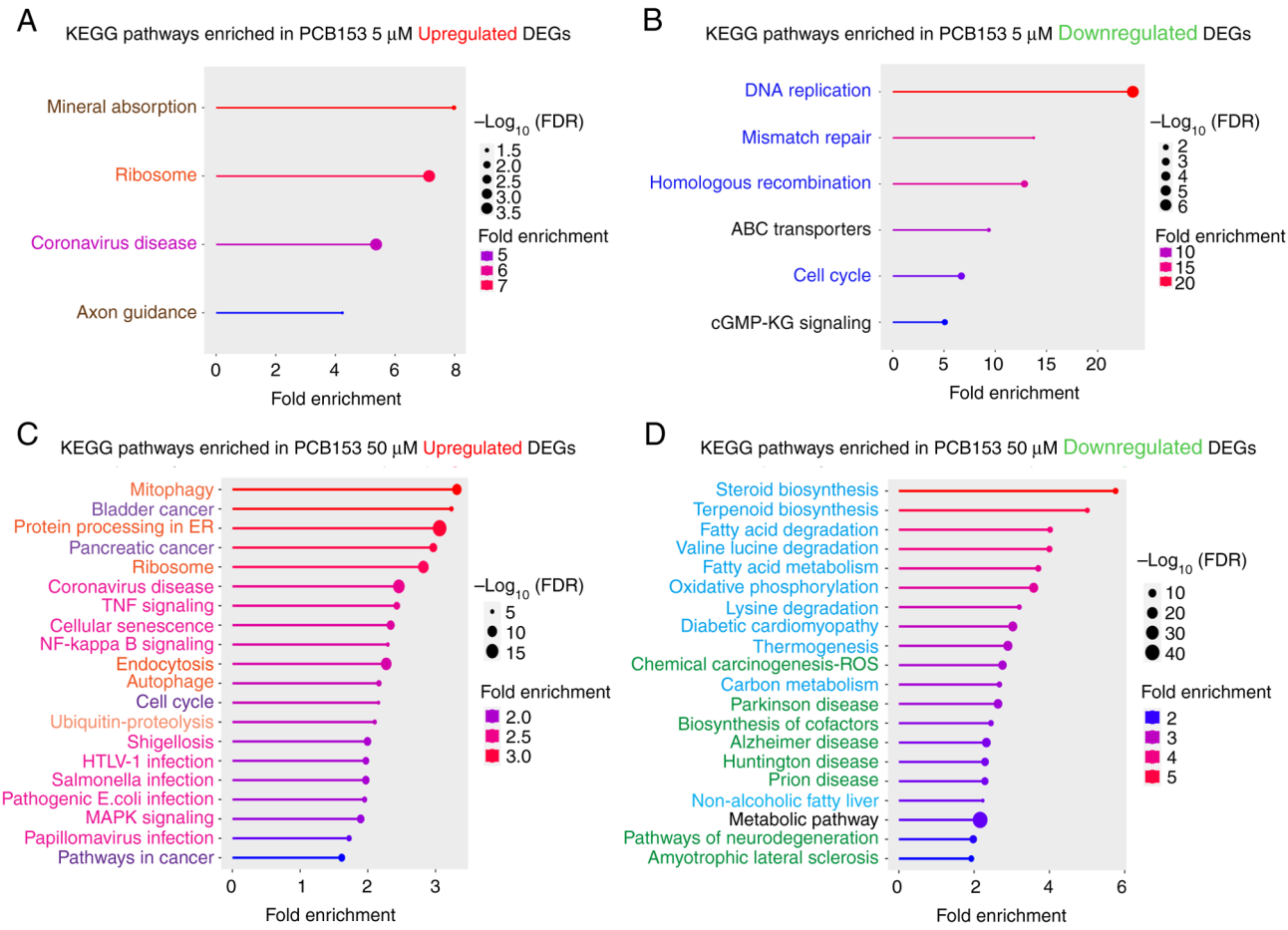

Figure 4. Dose-dependent pathway alterations in IECs following PCB153 exposure. KEGG pathway enrichment analysis of (A) upregulated genes and (B) downregulated genes in IECs treated with 5 μM PCB compared with the control group. (C and D) The top 20 enriched pathways in IECs treated with 50 μM PCB153 were ranked by fold enrichment and reorganized into functional modules (indicated by the font color) to improve clarity and interpretability. (A-D) Upregulated pathways were grouped into 'Immune & Host-Defense' (magenta), 'Proteostasis & Translation' (orange), 'Proliferation/Oncogenic' (violet) and 'Others' (brown) categories, whereas downregulated pathways were grouped into 'Core Energy & Lipid Metabolism' (light blue), 'Genome Maintenance & Proliferation' (dark blue), 'Neurodegeneration & Proteostasis Stress' (green) and 'Transport/Signaling & Global' (black) categories. The significance of pathway enrichment is shown as -$\log_{10}$(FDR), with higher values indicating stronger statistical enrichment. DEG, differentially expressed gene; FDR, false discovery rate; IEC, intestinal epithelial cell; KEGG, Kyoto Encyclopedia of Genes and Genomes; PCB153, polychlorinated biphenyl 153.

and 'Others'. Downregulated pathways were classified into 'Core Energy & Lipid Metabolism', 'Genome Maintenance & Proliferation', 'Neurodegeneration & Proteostasis Stress', and 'Transport/Signaling & Global' modules. This modular grouping highlights the extensive remodeling of immune, metabolic, and stress-related processes in intestinal epithelial cells following high-dose PCB153 exposure.

To provide deeper biological context, we next analyzed the pathways driving these functional modules to delineate the specific cellular processes affected by PCB153 exposure. Exposure to 50 μM PCB153 in IECs induces significant cellular stress, inflammation, and cancer-related responses. Upregulation of mitophagy, autophagy, and protein processing in the ER suggests heightened stress adaptation mechanisms, while activation of TNF and NF-kB signaling points to chronic inflammation and immune dysregulation (30). Increased expression of cancer-related pathways, including bladder and pancreatic cancer, as well as cell cycle dysregulation and senescence, implies a potential pro-tumorigenic environment. Additionally, pathways linked to bacterial and viral infections (*Shigellosis*, *Salmonella*, *E. coli*, and *Papillomavirus*) are upregulated, suggesting gut barrier dysfunction and increased pathogen susceptibility (31). Thus, these alterations indicate that PCB153 compromises intestinal homeostasis, promotes inflammatory and oncogenic signaling, and weakens immune defenses, increasing the risk of gut-related diseases and malignancies. Conversely, we found that exposure to 50 μM of PCB153 in IECs intensively downregulates key metabolic, mitochondrial, and detoxification pathways, effects likely leading to impaired cellular function. Suppression of steroid and terpenoid biosynthesis, fatty acid metabolism, and amino acid degradation (valine, leucine, lysine) suggests disrupted lipid and energy homeostasis, which may contribute to metabolic disorders. Downregulation of oxidative phosphorylation and thermogenesis points to mitochondrial dysfunction and reduced energy production, potentially compromising cellular survival (32). Inhibition of chemical carcinogenesis (ROS detoxification) and carbon metabolism indicates reduced detoxification capacity, likely rendering PCB153-exposed cells more vulnerable to oxidative stress and secondary toxic insults. Furthermore, repression of pathways related to neurodegenerative diseases (Alzheimer's, Parkinson's, Huntington's,

and ALS) raises concerns about systemic neurotoxic effects of PCB153 exposure. Collectively, these alterations suggest that high dose of PCB153 disrupts cellular metabolism, mitochondrial function, and detoxification mechanisms, potentially contributing to epithelial cell dysfunction, metabolic disorders, and increased disease susceptibility.

Taken together, both concentrations of PCB153 disrupt IEC homeostasis. The exposure to PCB153 exhibits dose-dependent transcriptomic shifts in IECs. The higher dose of PCB153 (50 μM) leads to a broader range of cellular effects, exacerbating cellular stress, metabolic disruption, and cancer-related signaling compared to lower doses (5 μM). These findings highlight the potential role of PCB153 in promoting epithelial cell dysfunction, immune dysregulation, and disease susceptibility.

Discussion

This study provides new insights into the cellular and transcriptomic responses of normal human intestinal epithelial cells to PCB153 exposure, advancing our understanding of how environmental toxicants disrupt intestinal epithelial cell homeostasis. Our data demonstrate that PCB153 exposure leads to broad transcriptional reprogramming that impacts key biological pathways related to epithelial renewal, detoxification, metabolism, immune regulation, and gut homeostasis.

The non-tumorigenic NCM460D cells express key intestinal epithelial markers with differentiated epithelial features, which is widely regarded as a physiologically relevant model for studying normal intestinal epithelial cell biology, though not a model of intestinal stem cells. Therefore, the reductions in stemness-associated transcripts (e.g., *LGR5*) observed after PCB153 exposure should be interpreted as suppression of stemness-related gene programs, rather than loss of *bona fide* intestinal stem cells. Several pathways altered in this study align with previously reported mechanisms of PCB-induced intestinal toxicity. Choi *et al* (7) demonstrated that PCBs compromise intestinal barrier integrity by inducing oxidative stress via NADPH oxidase activation, resulting in altered expression of tight junction proteins. While our study did not directly assess tight junction components, we identified the downregulation of genes involved in cell adhesion and epithelial barrier regulation, including key metabolic and signaling pathways that support epithelial integrity, such as cGMP-PKG signaling and Wnt signaling. The repression of Wnt pathway components (e.g., LEF1, DACT1, and LGR5) in particular points to impaired epithelial renewal and homeostatic maintenance, effects predicted to contribute to barrier dysfunction as reported in Choi's study. Our data support the hypothesis that PCB153 compromises intestinal barrier function through multiple molecular mechanisms, including both oxidative stress and suppression of regenerative signaling. Similarly, Phillips *et al* (8) previously reported that PCB153 activates the ATM/NEMO inflammatory axis leading to intestinal inflammation in mouse models. In our transcriptomic analysis, we observed upregulation of immune-related pathways, including TNF signaling, NF-κB signaling, and genes associated with viral response and inflammation. These changes suggest that PCB153 elicits an epithelial inflammatory response, even in the absence of immune cell stimulation, which may act as a priming signal for more robust inflammation *in vivo*. The upregulation of coronavirus disease-related genes and ribosomal genes may also reflect a heightened stress and immune surveillance state, consistent with the immune-activating effects described by Phillips *et al* (8). Thus, our findings in human IECs support and expand upon the inflammation-related mechanisms impaired by PCB153 exposure.

Importantly, our study also revealed novel transcriptomic responses to PCB153 exposure, particularly in pathways related to axon guidance, metallothionein expression, and lipid metabolism, which have not been widely reported in the context of intestinal toxicology. These alterations suggest that PCB153 may affect not only local gut epithelial function but also broader intercellular communication processes, including gut-brain signaling and systemic metabolic regulation. In particular, enrichment of the axon guidance pathway points to potential modulation of neuro-epithelial communication, as several axon guidance molecules (e.g., semaphorins, netrins) are increasingly recognized for their roles in regulating epithelial integrity, inflammation, and neuronal-immune interactions along the gut-brain axis (33,34), which represents an intriguing direction for future research. Metallothioneins, MT1G and MT2A are regulators of intracellular divalent metal ions, with their highest binding affinity for zinc (Zn^{2+}) and copper (Cu^{+}/Cu^{2+}). MT1 and MT2 isoforms also participate in buffering cadmium (Cd^{2+}), mercury (Hg^{2+}), and other heavy metals when present. In the context of intestinal epithelial cells, the PCB-induced upregulation of *MT1G* and *MT2A* most likely reflects the dysregulation of zinc homeostasis, with potential secondary effects on copper handling (17,18). Additionally, the downregulation of ABC transporters and estrogen signaling pathways points to impaired lipid handling and detoxification, mechanisms with potential implications for both epithelial toxicity and systemic exposure burden (25).

In this study, we performed two independent biological experiments for the RNA-seq transcriptomic profiling. Although this replicate number is at the lower end of standard RNA-seq designs, several factors support that it is sufficient for robust bioinformatic analysis and downstream DEG identification. First, the two biological replicates showed excellent concordance, as demonstrated by the tight clustering in the PCA plot (Fig. 2A), which indicates low within-group variability and strong separation among treatment conditions. Second, despite the modest replicate number, the RNA-seq dataset yielded the coherent sets of differentially expressed genes and enriched pathways, including metallothionein genes, Wnt signaling, metabolism, and inflammatory pathways. Third, we independently validated several representative DEGs (*MT1G*, *MT2A*, *LGR5*, *DACT1*, *LEF1*) by RT-qPCR in a separate experiment with biological triplicates, all of which confirmed the direction and magnitude of RNA-seq changes.

While the findings of this study significantly enhance our understanding of PCB153-induced transcriptomic changes in human IECs, several limitations should be acknowledged. The aim of the cell viability assay in our study was not to establish a full pharmacological dose response relationship for PCB153, but rather to identify treatment conditions that allow detectable transcriptomic changes without inducing overt cytotoxicity. We found the sharp viability decline observed at 200 μM compared with 0-50 μM does not imply

a mechanistically meaningful PCB153 dose response inflection point. Instead, it reflects that 200 μM PCB153 exceeds the cytotoxic threshold for the NCM460D intestinal epithelial cells. This abrupt reduction may correlate with the physicochemical properties of PCB153, such as its hydrophobicity and membrane-associated toxicity. In addition, although the concentrations used in this study (5 and 50 μM) exceed typical human serum PCB153 levels reported in epidemiological studies (approximately 0.01-0.1 μM) (35-38), these exposures remain relevant for *in vitro* mechanistic investigation. PCB153 exhibits strong lipophilicity and binds extensively to serum proteins, lipids, and culture plastics, substantially reducing the freely bioavailable fraction in cell culture systems. Therefore, higher concentrations in cell culture system are often required to achieve intracellular levels comparable to chronic low-dose exposures *in vivo*, but does not fully reflect the chronic, low-level exposures experienced by most human populations. Future studies incorporating multiple time points or repeated lower-dose exposure models will be important for capturing more physiologically relevant outcomes. Furthermore, although our results align with previous studies in animal models and provide new mechanistic insights, the use of a single *in vitro* cell culture system inherently limits biological complexity and does not fully capture the multicellular complexity of the intestinal environment, including stromal, immune, and microbial components. In whole organisms, compensatory mechanisms such as epithelial regeneration and systemic detoxification processes may partially counterbalance or adapt to such cellular perturbations, potentially attenuating the magnitude of transcriptomic changes observed *in vitro*. Validation in intestinal organoids or *in vivo* models will be critical to determine the functional relevance of the pathways identified here. In addition, while RNA-sequencing offers a powerful tool for global transcriptomic profiling, it is limited to changes at the mRNA level. Because transcriptional responses do not always correlate with protein expression or cellular function, additional validation is warranted. Future work will focus on confirming major differentially expressed genes at the protein level, assessing key signaling activity (i.e. Wnt/cGMP-PKG) and performing functional assays such as transepithelial electrical resistance (TEER), oxidative stress measurements, and inflammatory cytokine release, to determine whether the observed molecular changes translate into physiological effects and strengthen the mechanistic interpretation of the RNA-seq findings. These future directions will allow us to build upon the transcriptomic signatures identified in this study and more fully delineate the molecular consequences of PCB153 exposure in the intestinal epithelium. Nevertheless, the findings from this study have important implications for populations exposed to PCB153 through dietary or environmental sources. By uncovering specific gene networks and signaling pathways disrupted by PCB153 in normal intestinal epithelial cells, this research offers a molecular framework that may aid in the development of early biomarkers for exposure-related gut dysfunction. Pathways such as Wnt signaling, ABC transporter activity, and metallothionein regulation could serve as candidate targets for monitoring epithelial health or assessing individual susceptibility to PCB-related toxicity. Furthermore, understanding how even low-dose PCB exposure can impair epithelial regeneration, metabolism, and immune regulation supports the need for health surveillance in exposed populations, particularly those with high dietary intake of contaminated fish or those residing in regions with persistent PCB contamination. In this aspect, the *in vivo* toxicokinetic studies of PCB153 bioaccumulation, including absorption, distribution, tissue persistence, and dose-time relationships in animal models, would indeed be highly valuable for future work. Such studies would help define physiologically relevant exposure ranges in the intestine and provide quantitative context for interpreting the transcriptomic responses observed *in vitro*. While toxicokinetic modeling and *in vivo* bioaccumulation analyses are beyond the scope of the present study, we believe that integrating these approaches in future investigations would strengthen translational interpretation and help bridge *in vitro* responses with real world exposure scenarios. These mechanistic insights may also inform future strategies to mitigate toxicant effects, such as dietary or pharmacological interventions that restore epithelial integrity or enhance detoxification capacity, eventually offering a strategy toward personalized approaches to environmental health protection.

In conclusion, our findings support and extend prior research indicating that PCB153 exposure adversely affects intestinal epithelial function. We demonstrate that PCB153 disrupts multiple pathways essential for epithelial renewal, immune regulation, and metabolic balance. These findings emphasize the gut epithelium as a critical and sensitive target of environmental toxicants and underscore the need for further research using physiologically relevant exposure models and mechanistic validation to better assess the health risks associated with PCB exposure.

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Availability of data and materials

The RNA sequencing data generated in the present study may be found in the National Center for Biotechnology Information database under accession number PRJNA1285092 or at the following URL: https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1285092. The other data generated in the present study may be requested from the corresponding author.

Authors' contributions

HH and PS conceived the study, analyzed data and wrote the manuscript. SCT analyzed data and edited the manuscript. RMS and GSP acquired funding, interpreted data, and reviewed and edited the manuscript. HG supervised the study, designed the methodology, interpreted data, and wrote, reviewed and edited the manuscript. HH and HG confirm the authenticity of all the raw data. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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