



Unraveling the molecular mechanisms of PFOA in clear cell renal cell carcinoma through network toxicology and molecular docking strategies

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Background: As Perfluorooctanoic Acid (PFOA) has been extensively utilized as a processing aid in the manufacture of non-stick coatings, waterproof materials, and other products, concerns regarding its adverse health effects have emerged. Epidemiological data revealed a strong correlation between renal cell carcinoma (RCC) and PFOA concentration, while animal experimental results also demonstrate the association between PFOA and RCC. However, the key targets and mechanisms underlying PFOA-induced RCC remain elusive. This study utilized network toxicology to elucidate the critical target genes and mechanisms of PFOA-induced clear cell RCC (ccRCC), the most prevalent RCC subtype.

Methods: We retrieved potential PFOA targets from the Swiss Target Prediction database, ChEMBL, and STITCH, and identified RCC-related targets from GeneCards and OMIM. Transcriptomic data for ccRCC patients were obtained from The Cancer Genome Atlas Program (TCGA) to identify differentially expressed genes. We intersected genes from these datasets for constructing a protein-protein interaction (PPI) network. Hub genes were identified from the network using MCODE and cytoHubba plugins in Cytoscape. A risk score based on these hub genes was developed for prognostic analysis, and molecular docking was applied to validate the interactions between PFOA and hub targets.

Results: Intersection genes from these datasets, yielding 70 potential PFOA-induced ccRCC targets. Network analysis identified 7 hub genes—*CYP2C9*, *CYP3A4*, *CYP1A1*, *CYP1A2*, *CYP2B6*, *CYP2C8*, and *ABCB1*, and molecular docking confirmed PFOA's binding affinity to their corresponding proteins. Enrichment analysis using Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG), and Reactome databases on the 70 potential targets and 7 hub genes revealed four potential mechanisms of PFOA-induced ccRCC: abnormal xenobiotic metabolism and accumulation of toxic intermediates, disrupted lipid homeostasis, oxidative stress and reactive oxygen species (ROS) generation, and disrupted steroid hormone signaling.

Conclusion: Our findings provide novel insights into PFOA-induced ccRCC mechanisms, with implications for risk assessment and environmental health.

Keywords: clear cell renal cell carcinoma, cytochrome P450, molecular docking, network toxicology, perfluorooctanoic acid

Introduction

Perfluorooctanoic Acid (PFOA), a member of the perfluoro and polyfluoroalkyl substances (PFAS) family, is a synthetic chemical extensively utilized in human production and daily life^[1]. Characterized by its high-energy carbon-fluorine bonds, PFOA exhibits remarkable chemical and thermal stability, rendering it crucial in applications such as non-stick cookware coatings, fire-fighting foams, waterproof and oil-repellent

HIGHLIGHTS

- Identified 70 common genes linked to PFOA exposure and ccRCC.
- Core targets and potential mechanisms in PFOA-induced ccRCC are uncovered via network toxicology.
- Risk score based on 7 hub genes revealed significant survival differences between high and low risk groups.
- Strong binding between PFOA and hub proteins underscores their pivotal roles in PFOA-induced ccRCC.

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Sponsorships or competing interests that may be relevant to content are disclosed at the end of this article.

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International Journal of Surgery (2025) 111:4842–4853

materials, and military operations^[2]. The widespread utility of PFOA has led to its distribution in numerous global ground-water and drinking water systems, raising concerns about its environmental and health impacts^[3]. In typical watercourses of China, the mean concentration of PFOA reaches 28.79 µg/L, which is approximately 700 times the Chinese drinking water

Received 15 April 2025; Accepted 23 April 2025

Supplemental Digital Content is available for this article. Direct URL citations are provided in the HTML and PDF versions of this article on the journal's website, www.ijl.com/international-journal-of-surgery.

Published online 12 May 2025

<http://dx.doi.org/10.1097/JS9.0000000000002461>

standard (≤ 40 ng/L) and 7000 times the U.S. drinking water standard (≤ 4 ng/L)^[4-6]. PFOA in the environment can enter the human body through various pathways, including water sources, food, and air, with the highest concentration detected in human serum reached 22.4 $\mu\text{g/mL}$ ^[7].

Epidemiological studies have demonstrated correlations between PFOA and various cancers, with the strongest association observed for kidney cancer^[8]. Additionally, elevated PFOA levels in biological systems have been linked to diverse kidney-related health concerns, encompassing renal dysfunction, chronic kidney disease, and kidney cancer^[9]. Animal experiments have demonstrated that PFOA induced sloughing of epithelial cells in renal tubular and collecting ducts within the renal medulla in mice^[10], as well as proximal convoluted tubule epithelial turbidity and tumefaction in rats^[11]. Renal cell carcinoma (RCC), originating from renal tubular epithelial cells, constitutes most of malignant tumors in the kidney and ranks among the top ten most common cancers globally^[12]. Additionally, clear cell RCC (ccRCC) is the most prevalent subtype of RCC, accounting for approximately 75% ~ 80% of diagnosed cases, characterized by its aggressive nature and high metastatic potential, thus garnering significant clinical attention^[13].

The association between PFOA and RCC has emerged as a focal area of research in environmental health. In 2023, the International Agency for Research on Cancer (IARC) classified PFOA as “carcinogenic to humans,” underscoring the critical need to delve deeper into its carcinogenic mechanisms^[14]. While numerous studies have suggested a correlation between PFOA exposure and an increased RCC risk, the precise carcinogenic mechanisms remain incompletely understood^[8,15]. Current investigative efforts primarily concentrate on several mechanisms underlying PFOA-induced RCC. Firstly, epigenetic alterations, including DNA methylation induced by PFOA exposure, may impact gene expression, thereby fostering tumor initiation and progression^[16]. Secondly, PFOA exposure is intimately linked to immunosuppression, manifesting as heightened susceptibility to infectious diseases and reduced vaccine responsiveness^[17]. This immunosuppressive effect could potentially weaken the body’s surveillance against cancer cells, facilitating tumorigenesis. Additionally, PFOA exposure triggers oxidative stress, elevating intracellular reactive oxygen species (ROS) levels, which subsequently damage DNA and other biomolecules, ultimately precipitating cellular mutations and tumor formation^[18]. However, these mechanistic studies have largely been confined to specific targets or biological processes, lacking a comprehensive, non-targeted analysis of the mechanisms underlying PFOA-induced RCC.

To overcome these constraints, our study employs network toxicology and molecular docking techniques to thoroughly evaluate the targets and pathways underlying the effects of PFOA in ccRCC (the most common subtype of RCC). Network toxicology, an innovative research approach, offers a unique perspective for dissecting the toxicity mechanisms of pollutants. Furthermore, molecular docking serves as a predictive tool for assessing interactions between chemicals and their targets, with lower binding energies signifying enhanced binding stability^[19]. Through the integration of these two strategies, our research aims to identify pivotal targets underlying PFOA-induced ccRCC, and to explore the mechanisms through which PFOA contributes to ccRCC (Fig. 1). Our results may illuminate new potential therapeutic targets for ccRCC treatment influenced by PFOA exposure and enhance understanding of environmental pollutant-driven

carcinogenesis, offering critical insights for both environmental health policies and cancer research strategies.

Materials and methods

Collection of PFOA and RCC related targets

Human target genes potentially affected by PFOA were retrieved from the Swiss Target Prediction database (<http://www.swisstar.getprediction.ch/>), ChEMBL database (<https://www.ebi.ac.uk/chembl/>), and STITCH database (<http://stitch.embl.de/>). RCC-related genes were collected from the GeneCards database (<https://www.genecards.org/>) and OMIM database (<https://omim.org/>) using the keyword “renal cell carcinoma.” These gene datasets were subsequently merged, deduplicated, and standardized utilizing the UniProt database (<https://www.uniprot.org/>) for the final target sets for PFOA and RCC.

Download and analysis of ccRCC-related data in TCGA database

RNA-seq data from the Cancer Genome Atlas Program (TCGA, <https://portal.gdc.cancer.gov/>) Kidney Clear Cell Carcinoma (KIRC) cohort were utilized. Processing steps included extracting normalized gene expression data along with clinical phenotype information. Only samples categorized as “Primary Tumor” and “Solid Tissue Normal” were selected for subsequent analysis. Differential expression analysis was performed using the DESeq2 package^[20], with criteria for identifying differential expression genes (DEGs) set at an adjusted *P*-value < 0.05 and $\log_2\text{FoldChange} > 1$.

Construction of protein–protein interaction (PPI) network

Protein-protein interaction (PPI) analysis was conducted for the overlapping genes among PFOA targets, RCC targets, and ccRCC-related genes using the STRING database (<https://cn.string-db.org/>) with parameters tailored for “*Homo sapiens*.” A threshold of “Medium Confidence” with an interaction score > 0.4 was applied. The results obtained from STRING were then imported into Cytoscape software (version 3.10.1) for the evaluation of the PPI network’s topological properties.

Identification of hub genes from PPI network

Within Cytoscape, the MCODE plugin identified the most critical module within the PPI network. Additionally, the cytoHubba plugin was utilized to compute “Node Scores” using 9 algorithms (Betweenness, Closeness, Degree, DMNC, EPC, MCC, MNC, Radiality, and Stress). For each algorithm, the top 20 genes with the highest scores were designated as key genes. Subsequently, these algorithm-identified key genes were intersected, and the genes present in all intersections were defined as “hub genes.”

Construction of a PFOA exposure risk model for ccRCC patients

To construct a risk model for ccRCC patients exposed to PFOA, we utilized expression data of hub genes and clinical information from TCGA database. Initially, we conducted multivariate Cox regression analysis on the selected hub genes. Subsequently, a risk score was computed based on the coefficients and expression levels of these hub genes. Using the survminer package, we

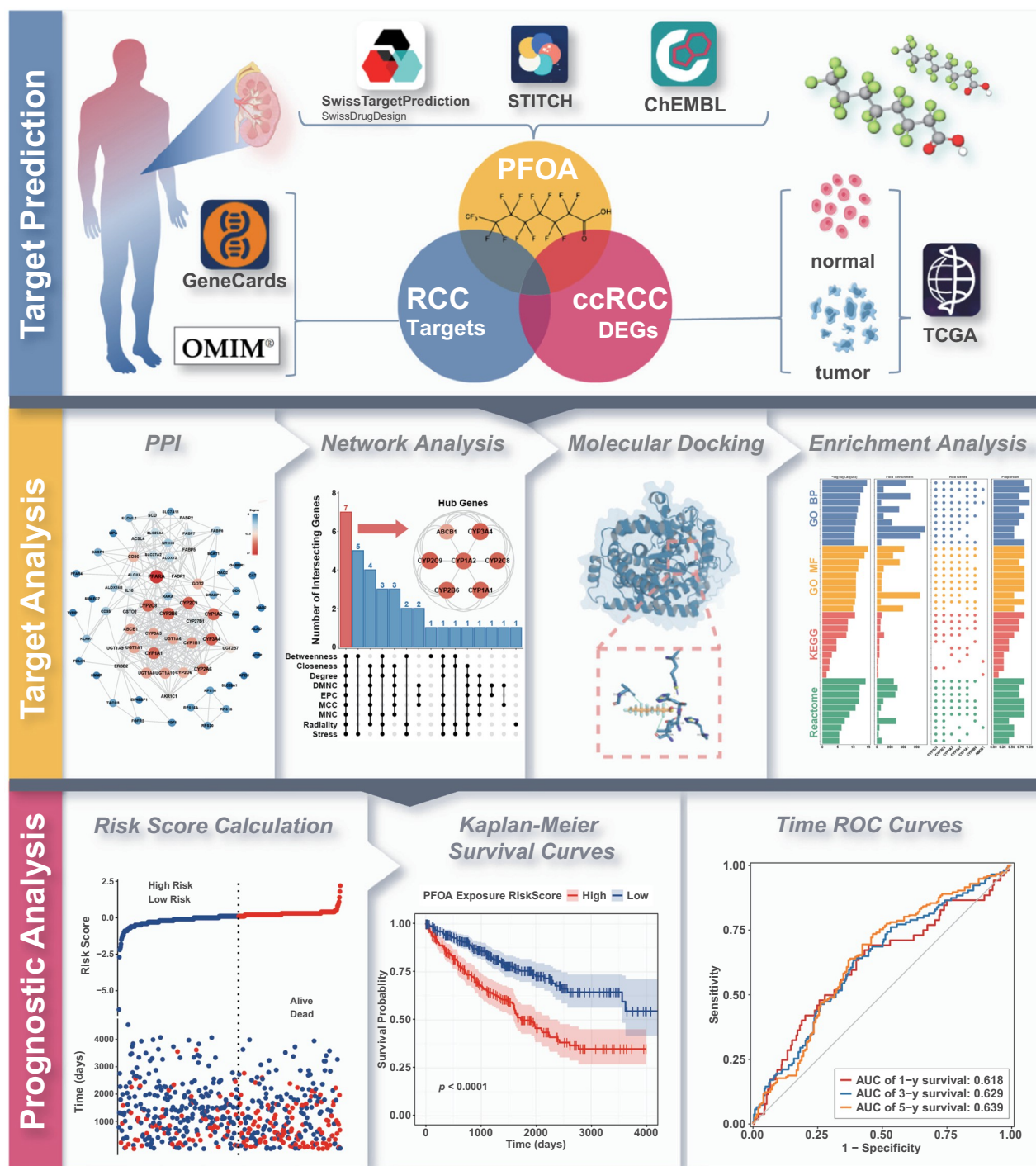


Figure 1. Workflow outlines the network toxicology analysis conducted to investigate PFOA-induced ccRCC, followed by validation through molecular docking and prognostic analysis

determined the optimal cutoff point to categorize patients into high-risk and low-risk groups^[21]. Kaplan–Meier curves and log-rank tests were employed to evaluate the prognostic significance of the risk score by comparing overall survival (OS) between the two risk groups. Furthermore, multivariate Cox regression

analysis was performed to ascertain whether the risk score served as an independent prognostic factor for ccRCC patients. Additionally, Receiver Operating Characteristic (ROC) curve analysis was conducted to assess the predictive accuracy of our risk model.

Functional enrichment analysis for target genes

Enrichment analyses of potential or hub genes were conducted using the clusterProfiler package to investigate Gene Ontology (GO) terms, encompassing biological processes (BP), molecular functions (MF), and cellular components (CC), as well as Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways^[22], alongside the ReactomePA package for Reactome pathway analysis^[23]. Significant enrichment of terms or pathways was determined based on an adjusted *P*-value threshold of <0.05, corrected using the Benjamini-Hochberg approach.

Molecular docking validation

To gain deeper insights into the intermolecular interactions between PFOA and the hub proteins identified in our study, we utilized a molecular docking methodology. The structures of these proteins were predicted using AlphaFold 3^[24]. Preparation of the target proteins involved the removal of water molecules and native ligands using PyMOL. Subsequently, the proteins were introduced into AutoDock Tools (v1.5.6) for hydrogenation, charge assignment, and refinement of non-polar hydrogen bonds. Docking simulations were conducted using AutoDock Vina (v1.2.6)^[25], with the results being subsequently visualized through PyMOL for interpretation. The following protein-ligand interactions were identified using PLIP^[26].

Results

Identification of potential targets

RNA-seq data for ccRCC, encompassing 541 primary tumor and 72 solid tissue normal samples, were obtained from TCGA. Principal component analysis (PCA) effectively discriminated between the Normal and Tumor groups (Fig. 2A). Differential analysis between the tumor and normal groups yielded 13,531 differentially expressed genes, with 10,208 upregulated and 3323 downregulated (Fig. 2B). We curated a total of 377 PFOA human target genes from Swiss Target Prediction, ChEMBL, and STITCH databases. Additionally, 4,783 RCC-related genes were retrieved from GeneCards and OMIM databases. By intersecting the differentially expressed genes in ccRCC patients from TCGA, predicted PFOA target genes, and RCC-related genes, we identified 70 potential targets implicated in PFOA-induced ccRCC (Fig. 2C). A PPI network was constructed for these 70 targets, consisting of 70 nodes and 303 edges. Network analysis was then performed using Cytoscape, where the size and color gradient (from blue to red) of each target in the PPI network correlated with its degree (Fig. 2D). Specifically, targets with higher degree values exhibited larger sizes and redder colors.

Functional enrichment analysis of all potential targets

Enrichment analysis was performed on 70 potential targets, and the results, sorted in ascending order by adjusted *P*-value, revealed the following results (Table S1-S3. <http://links.lww.com/JIS9/E135>). Among the GO BP terms, the top three enriched processes were “fatty acid metabolic process,” “xenobiotic metabolic process,” and “cellular response to xenobiotic stimulus.” In terms of GO CC, the top enriched components were “peroxisome,” “microbody,” and “cytosolic small ribosomal subunit.”

Among the GO MF, the top three functions were “monocarboxylic acid binding,” “carboxylic acid binding,” and “organic acid binding” (Fig. 3A). In the KEGG pathways, the top three enriched pathways were “Metabolism of xenobiotics by cytochrome P450,” “Drug metabolism—cytochrome P450,” and “Retinol metabolism” (Fig. 3B). Additionally, in the Reactome pathways, the top three enriched processes were “Xenobiotics,” “Biological oxidations,” and “Biosynthesis of DHA-derived specialized proresolving mediators (SPMs)” (Fig. 3C).

Identification of hub genes

To further explore the core network within the PPI, we employed MCODE to extract the highest-scoring subnetwork, which comprised 16 nodes and 117 edges, with all members possessing degree values ranging from 16 to 22 in the parent network (Fig. 4A). Subsequently, using nine algorithms available in Cytoscape, we identified the top 20 genes for each algorithm (Table S4. <http://links.lww.com/JIS9/E135>). An upset plot analysis of these genes revealed 7 hub genes that were consistently highlighted across all nine algorithms: CYP2C9, CYP3A4, CYP1A1, CYP1A2, CYP2B6, CYP2C8, and ABCB1 (Fig. 4B). Based on the PFOA exposure risk score derived from the 7 hub genes, patients were stratified into low-risk and high-risk groups for survival analysis (Fig. 4C).

Notably, all 7 hub genes were present within the highest-scoring subnetwork identified by MCODE, further emphasizing their significance. Expression analysis of these hub genes showing that, except for CYP2C8, the expression of which was up-regulated in ccRCC, all other hub genes were down-regulated in ccRCC (Fig. 4D). Kaplan-Meier survival curves demonstrated significantly poorer overall survival in the high-risk group compared to the low-risk group ($p < 0.0001$) (Fig. 4E). Multivariate Cox regression analysis, incorporating age, stage, gender, and risk group, confirmed that the risk score served as an independent prognostic factor for ccRCC patients (Fig. 4F). The time-dependent ROC curves for the risk score model, with AUC values of 0.618, 0.629, and 0.639 for 1-year, 3-year, and 5-year survival predictions, respectively, indicates moderate predictive accuracy (Fig. 4G). Overall, the PFOA exposure risk score model, constructed using the 7 hub genes identified from the PPI network, provides a robust framework for predicting the prognosis of ccRCC patients.

Enrichment analysis of hub genes

Enrichment analysis was conducted for seven hub genes, and the results, sorted in ascending order based on adjusted *P*-values were displayed (Fig. 5, Table S5-S7. <http://links.lww.com/JIS9/E135>). Among the GO BP, the top three terms were “xenobiotic catabolic process,” “xenobiotic metabolic process,” and “epoxygenase P450 pathway.” In terms of GO MF, the top enriched functions were “steroid hydroxylase activity,” “oxidoreductase activity acting on paired donors, with incorporation or reduction of molecular oxygen, reduced flavin or flavoprotein as one donor, and incorporation of one atom of oxygen,” and “aromatase activity.” In the KEGG pathways, the top three enriched pathways were “Retinol metabolism,” “Drug metabolism—cytochrome P450,” and “Metabolism of xenobiotics by cytochrome P450.” While in the Reactome pathways, the top three enriched processes were “Xenobiotics,” “Biosynthesis of DHA-derived specialized proresolving mediators (SPMs),” and “Biosynthesis of specialized proresolving mediators (SPMs).”

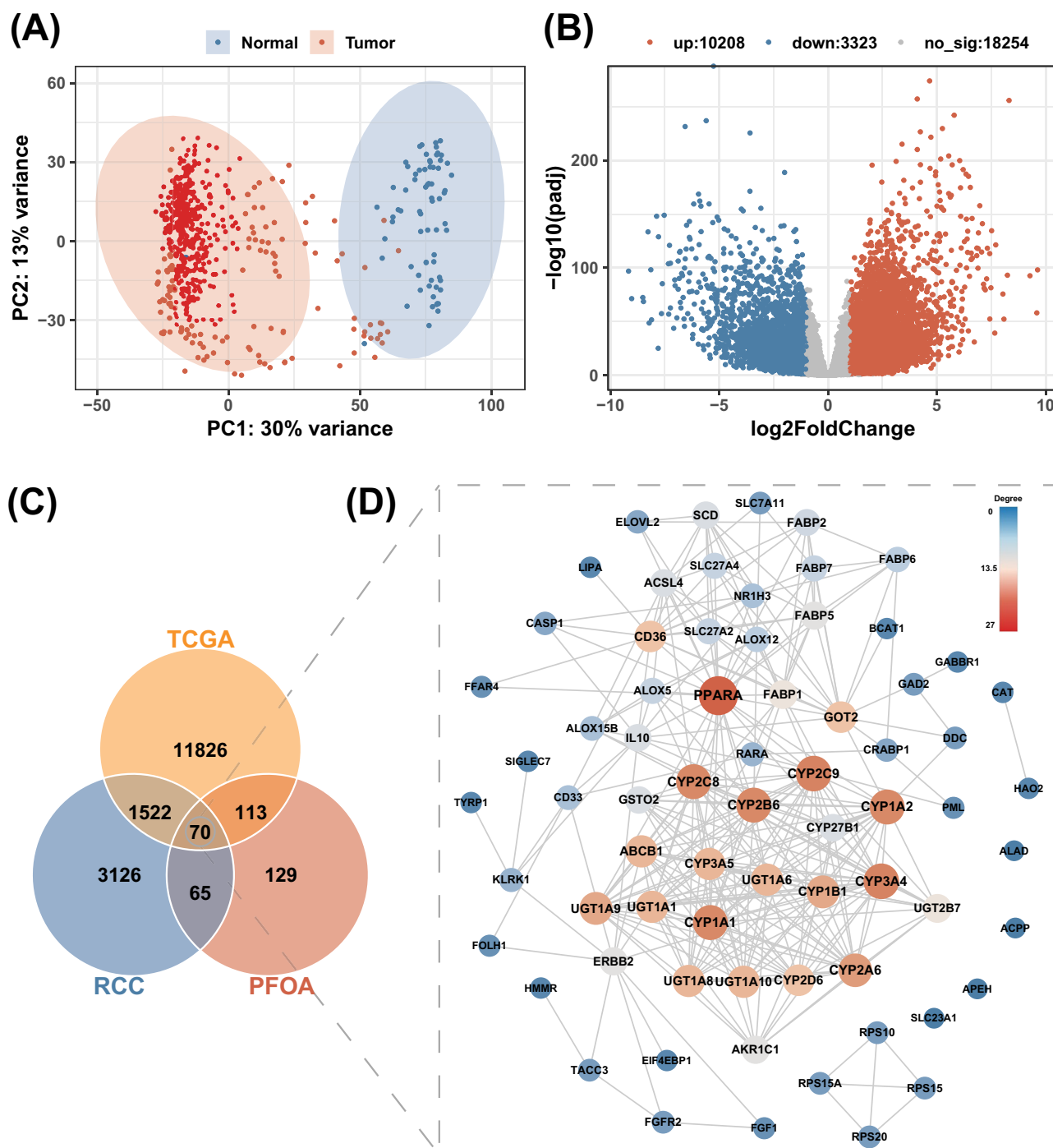


Figure 2. (A) PCA of transcriptomic sequencing data from clear cell renal cell carcinoma (ccRCC) tissues and their corresponding normal tissues in the TCGA database, with 95% confidence ellipses provided for clarity. (B) Volcano plot displaying differential expression genes between ccRCC and normal tissues, with genes screened based on an adjusted P -value < 0.05 and $|\log_2\text{FoldChange}| > 1$. (C) Venn diagram illustrating the intersection between PFOA targets, renal cell carcinoma (RCC) targets and ccRCC-associated genes. (D) PPI network of commonly shared targets

Molecular docking validation

To decipher the interaction paradigms between PFOA and 7 key hub genes, molecular docking analyses were undertaken. In the framework of molecular docking, spontaneous binding of the receptor and ligand, devoid of external energy input, is implied

by a binding energy below 0 kcal/mol. Notably, binding energies of less than -5 kcal/mol suggest excellent binding, whereas those lower than -7.2 kcal/mol emphasize strong binding with high affinity^[27]. Docking simulations using AutoDock vina yielded remarkably low binding energies. Subsequently, these docking outcomes were visualized using PyMOL (Fig. 6A-G). Among the

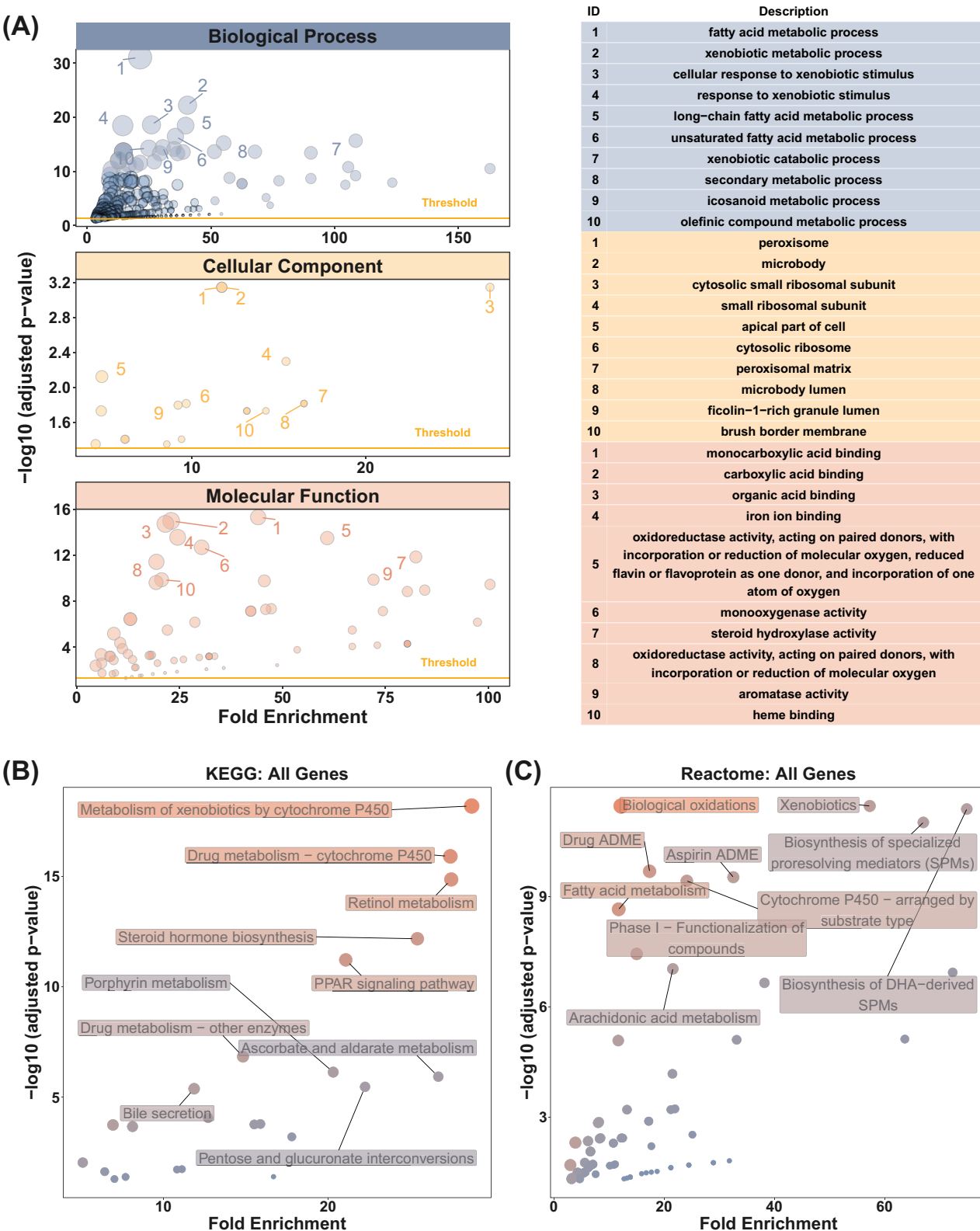


Figure 3. Dot plots present the enrichment analysis of all potential targets shared between PFOA exposure and ccRCC, with fold enrichment on the x-axis, $-\log_{10}(\text{adjusted } P\text{-value})$ on the y-axis, and the size of dots representing the count of genes enriched in each term. (A) GO enrichment analysis is shown, with the top 10 terms of each ontology listed in the table (different colors representing different GO ontologies). (B) KEGG and (C) Reactome enrichment analysis is displayed, with the names of the top 10 pathways are labeled in the plot (the gradient color from blue to red is used to represent the increasing counts)

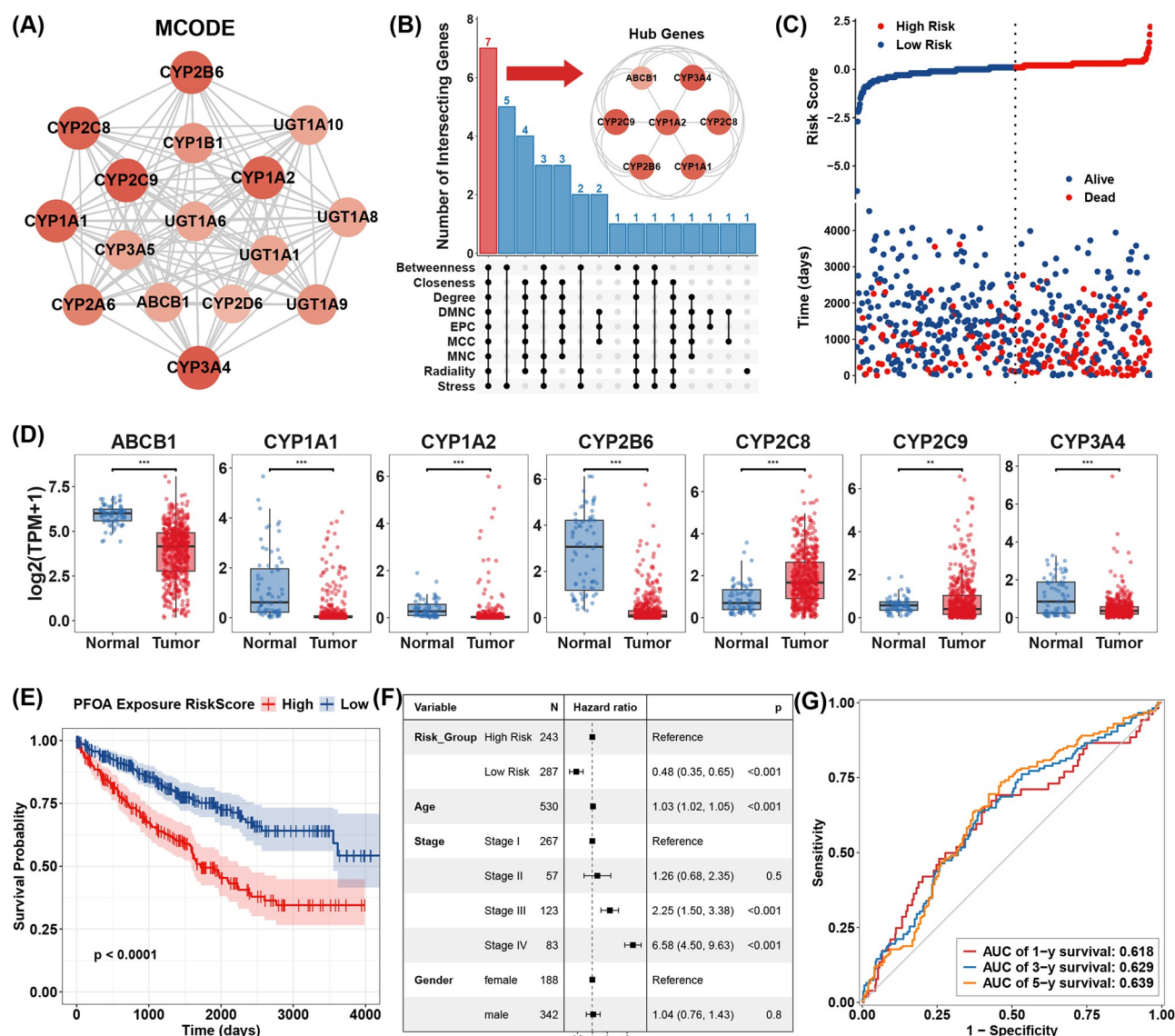


Figure 4. (A) The top-ranked module identified using the MCODE plugin in Cytoscape. (B) An upset plot of the top 20 genes calculated using nine algorithms in Cytoscape, where the seven genes in the intersection are highlighted, and a subnetwork extracted from the parent PPI network featuring these genes is displayed. (C) Distribution plot of PFOA exposure risk scores and survival time. (D) Box plot representing the transcripts per million (TPM) of hub genes. Statistical significance between tumor and normal was determined using the student's *t* test, and the corresponding *p* values are indicated (***P* < 0.01, ****P* < 0.001). (E) Kaplan-Meier survival curves for ccRCC patients stratified into high-risk and low-risk groups based on the risk score. (F) Multivariate Cox regression analyses of age, stage, gender and risk group. (G) Time-dependent ROC curves with AUC values indicating the predictive accuracy of the risk score model for 1-year, 3-year, and 5-year survival

7 hub proteins investigated, all exhibited binding energies surpassing the -7.2 kcal/mol threshold, indicating robust binding stability and high affinity (Fig. 6H). These observations offer profound insights into the molecular interactions between PFOA and these hub proteins, contributing to a better understanding of their biological significance.

Discussion

PFOA, a ubiquitous and persistent environmental contaminant, accumulates in organisms and elicits multiple adverse effects, including cancer. In this study, we comprehensively investigated the potential mechanisms underlying PFOA-induced ccRCC.

Specifically, we identified 70 PFOA-related ccRCC targets and constructed a PPI network (Fig. 2), from which we recognized seven hub genes: *CYP2C9*, *CYP3A4*, *CYP1A1*, *CYP1A2*, *CYP2B6*, *CYP2C8*, and *ABCB1* (Fig. 4). Molecular docking analysis confirmed the stable binding of PFOA to the proteins encoded by these hub genes (Fig. 6).

Among the hub genes identified, *CYP2C9*, *CYP3A4*, *CYP1A1*, *CYP1A2*, *CYP2B6*, and *CYP2C8* belong to the cytochrome P450 (CYP450) superfamily. Previous studies have demonstrated that PFOA can induce aberrant expression of CYP450 enzymes by activating peroxisome proliferator-activated receptors (PPAR) and the constitutive androstane receptor (CAR) signaling pathways^[28,29]. Notably, *CYP1A1* and

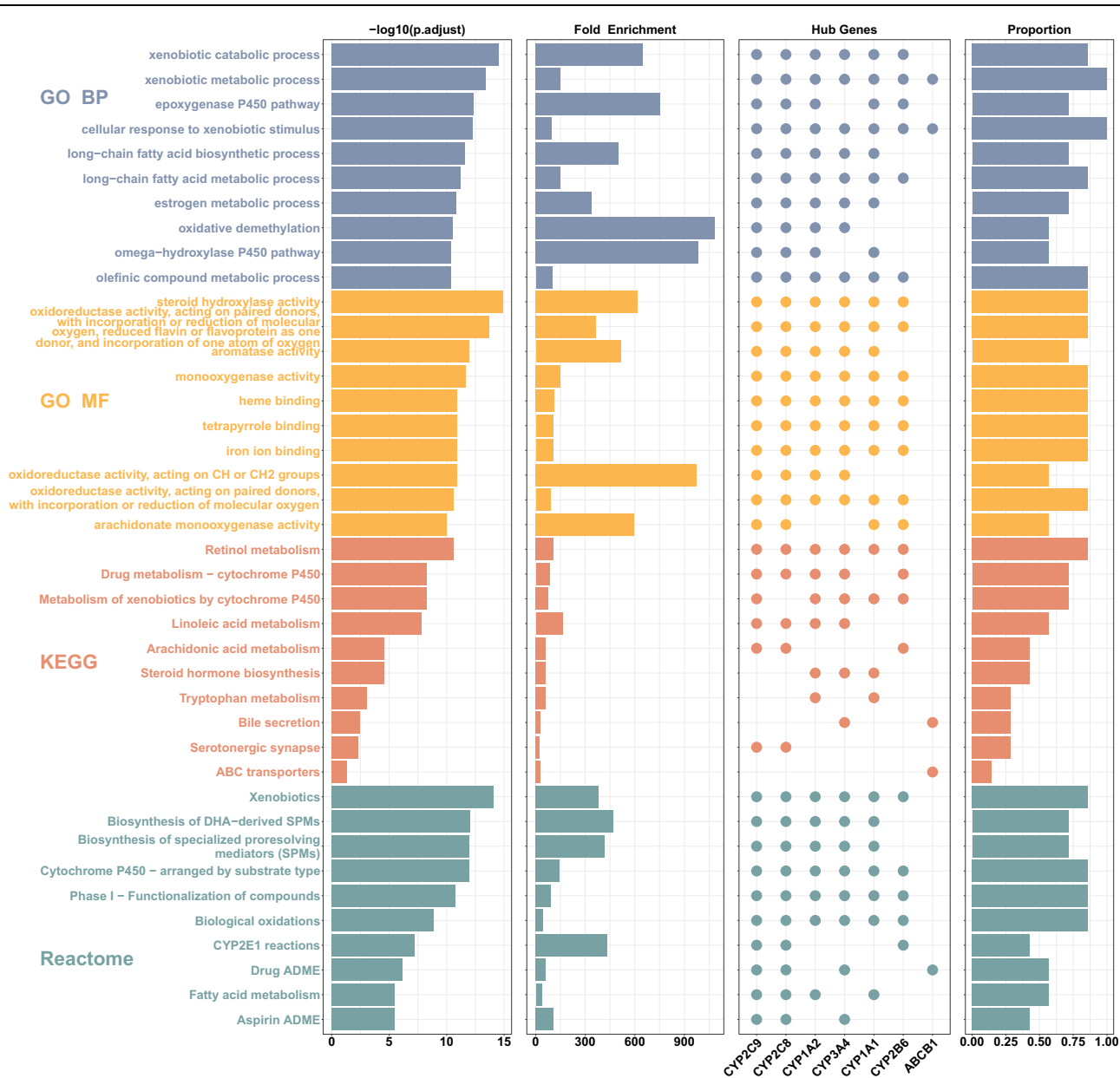


Figure 5. Enrichment analysis results for seven hub genes, with different colors representing various databases. The top 10 terms from each database are displayed. Bar plots illustrate the $-\log_{10}(\text{adjusted } P\text{-value})$, fold enrichment, and the proportion of hub genes enriched in each pathway. Dot plots show the distribution of the hub genes across the corresponding terms

CYP1A2, as key effector molecules of the aryl hydrocarbon receptor (AhR), their overexpression may lead to the metabolic activation of procarcinogens^[30]. In renal proximal tubular cells, this sustained metabolic stress may cause oxidative renal injury through the excessive generation of ROS, which may constitute a crucial molecular basis for PFOA-induced ccRCC^[31].

CYP2C9 and *CYP3A4*, as the most important drug-metabolizing enzymes in humans, their deregulated expression may impair the renal detoxification process of PFOA. Animal studies have shown that PFOA exposure significantly decreases renal *CYP3A4* activity, resulting in the accumulation of electrophilic metabolites^[32]. It is noteworthy that *CYP2C8* plays a pivotal

role in eicosanoid metabolism, and its dysfunction may disrupt the arachidonic acid metabolic balance in the kidney, promoting the synthesis of proinflammatory prostaglandins^[33]. This metabolic imbalance may synergistically promote inflammation in the tumor microenvironment, accelerating the malignant transformation of renal tubular epithelial cells^[34].

ABCB1 (P-glycoprotein), a transmembrane transporter, its upregulation may have dual implications. On one hand, it may reduce PFOA accumulation by enhancing efflux during early exposure stages^[35]; however, long-term activation may lead to a multidrug-resistant phenotype, which is closely associated with chemotherapy resistance in advanced ccRCC^[36]. Notably,

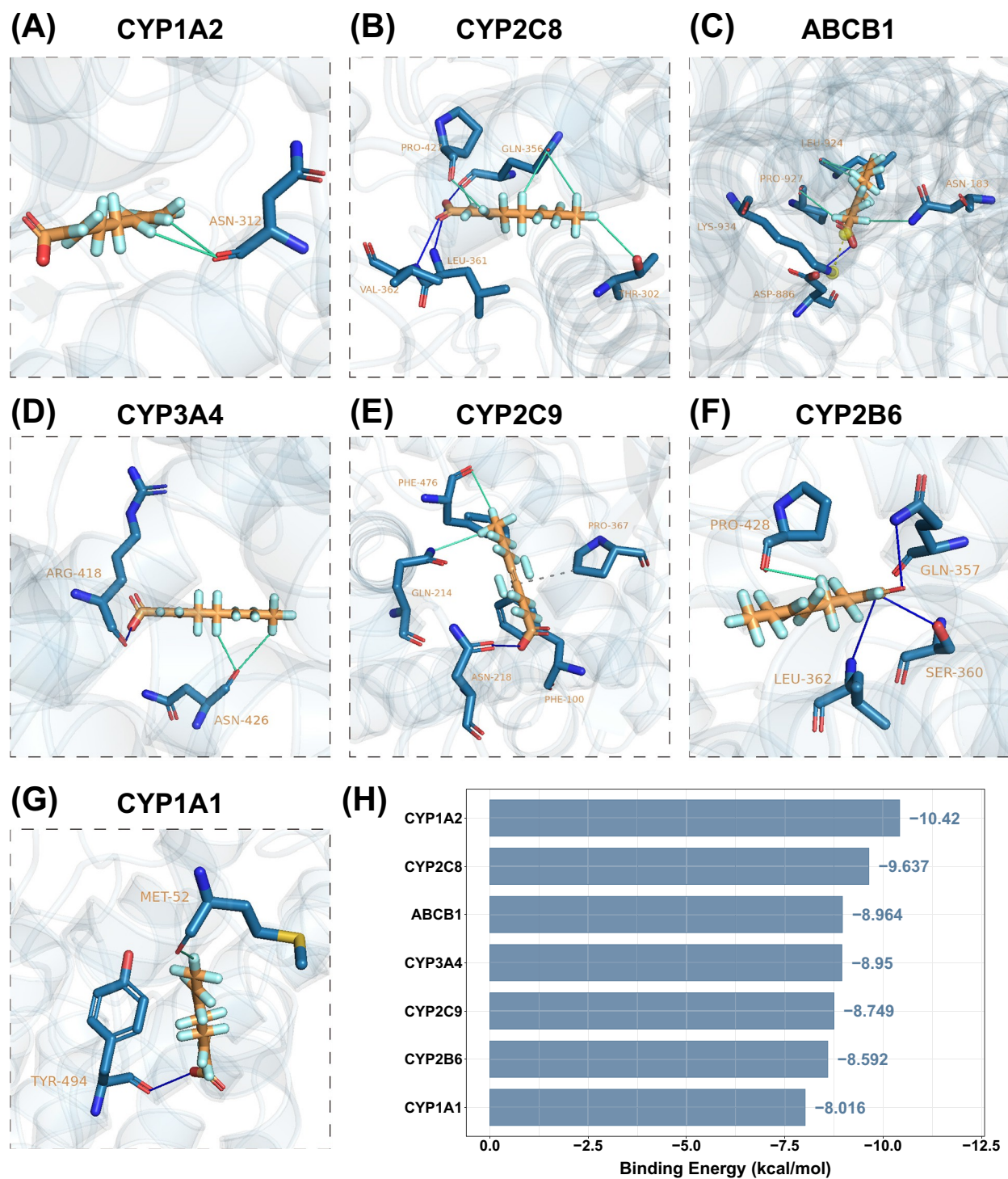


Figure 6. (A-G) Molecular docking result of PFOA with the proteins corresponding to the hub genes. (H) Binding energy of PFOA with corresponding proteins

PPI network analysis revealed functional coupling between ABCB1 and multiple CYP450 enzymes, suggesting that PFOA may establish a tumor-permissive microenvironment by affecting the drug metabolism-transporter axis.

Furthermore, we conducted enrichment analyses using three databases (GO, KEGG, and Reactome) to delve into the molecular mechanisms underlying PFOA-induced ccRCC (Fig. 3,

Fig. 5). By integrating the top ten pathways from each database in the enrichment results of all targets and hub targets, we summarized the following potential mechanisms. (1) Abnormal xenobiotic metabolism and accumulation of toxic intermediates: As an exogenous perfluorinated compound, PFOA may competitively inhibit the detoxification function of CYP450 enzymes towards carcinogens, leading to the accumulation of toxic

intermediates (e.g., epoxides, quinones), which may subsequently induce DNA adduct formation or abnormal epigenetic modifications^[37]. The terms “xenobiotic metabolic process” and “oxidative demethylation” in GO BP further suggest that this mechanism may be associated with gene mutations and epigenetic dysregulation in ccRCC^[38]. (2) Disrupted lipid homeostasis: The intersection targets of PFOA and ccRCC were significantly enriched in fatty acid metabolism (long-chain/unsaturated fatty acid metabolism, arachidonic acid metabolism) and PPAR signaling pathways, implying that PFOA may interfere with fatty acid oxidation and lipid anabolic metabolism by activating PPAR receptors, resulting in lipid accumulation and abnormal energy metabolism^[39], thereby promoting the formation of the tumor microenvironment^[40]. Additionally, “fatty acid metabolism” in Reactome and “retinoid metabolism” in KEGG suggest that PFOA may disrupt the metabolism of fat-soluble vitamins, further affecting cell differentiation and proliferation regulation^[41]. (3) Oxidative stress and ROS generation: In GO MF and Reactome, activities related to CYP450, such as monooxygenases and hydroxylases, and oxidoreductase functions were significantly enriched. PFOA may induce abnormal expression of CYP450 enzymes (e.g., CYP2E1, CYP1A1), leading to excessive ROS generation, which triggers DNA oxidative damage and mitochondrial dysfunction^[42]. The “arachidonic acid metabolism” in KEGG and “biological oxidations” in Reactome further support the role of ROS-mediated lipid peroxidation and inflammation in ccRCC progression^[43]. Additionally, GO CC analysis revealed significant enrichment in peroxisome-related structures. PFOA may disrupt the β -oxidation function of peroxisomes, leading to the accumulation of long-chain fatty acids and decreased ROS scavenging capacity^[44], eventually promoting ccRCC development. (4) Disrupted steroid hormone signaling: The steroid hydroxylase activity in MF and the “steroid hormone biosynthesis” pathway in KEGG suggest that PFOA may interfere with sex hormone metabolism. The hydrophobic tail of PFOA may nonspecifically bind to sex hormone receptors^[45], leading to abnormal activation of hormone-dependent signaling pathways, thereby promoting tubular epithelial cell proliferation and anti-apoptotic effects, driving ccRCC onset^[46].

Our study comprehensively investigated the potential targets and underlying mechanisms of PFOA-induced ccRCC through network toxicology. However, it had some inherent limitations. Firstly, the quality of raw target data is constrained by variations across different databases, algorithms, and screening criteria, lacking a unified and standardized framework in this field. Secondly, the binding data between PFOA and identified key target proteins rely on computer simulations, necessitating molecular experiments to confirm their binding affinities. Lastly, not all significant targets and potential mechanisms identified in our study have definitive experimental evidence linking them to PFOA-induced ccRCC, and the mechanisms discovered lack a clear, sequential connection. Therefore, future research should validate the identified hub genes and mechanisms and elucidate their roles in PFOA-induced ccRCC.

Conclusion

In conclusion, our study employed network toxicology to investigate the potential mechanisms underlying PFOA-induced

ccRCC. Initially, public databases were utilized to identify potential PFOA targets, RCC-related targets, and differentially expressed genes in ccRCC patients. The intersection of these datasets yielded 70 potential targets, which were subsequently used to construct a PPI network. Network analysis identified 7 hub genes, and a risk score based on these hub genes was developed for prognostic analysis. Molecular docking confirmed the binding affinity of PFOA to the proteins corresponding to these hub genes. Functional enrichment analysis of all potential and hub genes using GO, KEGG, and Reactome databases revealed four potential mechanisms of PFOA-induced ccRCC: abnormal xenobiotic metabolism and accumulation of toxic intermediates, disrupted lipid homeostasis, oxidative stress and ROS generation, and disrupted steroid hormone signaling. Our study contributes to the expanding body of evidence linking PFOA to ccRCC by elucidating key targets and underlying mechanisms, offering novel insights into environmental health and cancer research.

Ethical approval

Not necessary. The data used in this study were all sourced from freely accessible public databases.

Consent

Not necessary. This study did not involve patients or volunteers.

Sources of funding

This study was supported by grant from National Natural Science Foundation of China under Grant No. 42207324 (M. G.).

Author contributions

L.W., L.Z.: conceptualization, methodology, software, formal analysis, investigation, data curation, visualization, writing – original draft; D.C.: visualization, writing – review & editing; M.G.: writing – review & editing, supervision, investigation, funding acquisition, conceptualization.

Conflicts of interest disclosure

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.

Guarantor

Miao Guan

Research registration unique identifying number (UIN)

Not necessary. This study does not involve human subjects.

Ethical approval

All approaches were implemented as per associated instructions and regulations. Ethical approval is not applicable to our research.

Consent

Consent is not applicable to our research.

Data availability

Data are available in methods and results of our research.

Provenance and peer review

This paper is not commissioned, externally peer-reviewed.

Data availability statement

Data will be available upon reasonable request.

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