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Research Article

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Analysis of the effects of N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine Quinone on lung disorders using computational toxicology and molecular docking

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Abstract

This study aimed to investigate the potential molecular mechanisms by which N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine Quinone (6PPD-Q), the oxidative metabolite of a tire antioxidant, induces human pulmonary diseases. It focused on three representative lung disorders: lung cancer, chronic obstructive pulmonary disease (COPD), and pulmonary fibrosis. A systematic analysis was conducted to identify key molecular pathways that 6PPD-Q potentially disrupts. Network toxicology and molecular docking approaches were employed. Disease-associated targets were screened using databases such as PubChem, SwissADME, and GeneCards. A protein–protein interaction network was constructed using the STRING database and Cytoscape to identify core targets. GO and KEGG enrichment analyses were performed using DAVID. Protein structural information was retrieved from UniProt and the PDB. Molecular docking simulations were carried out with AutoDock Vina to validate binding affinity. Results indicated that 6PPD-Q may induce lung cancer, COPD, and pulmonary fibrosis through synergistic effects involving multiple interconnected pathways. Specifically, it triggers oxidative stress and chronic inflammation, impairing innate immune function in the lung. It disrupts proteostasis, including

protein synthesis and folding homeostasis. Additionally, it alters apoptosis and cellular proliferation, interferes with critical signal transduction pathways, and modulates cytoskeleton rearrangement. Molecular docking results validated the stable binding of 6PPD-Q to core targets, including ACTB and RPS27A. Moreover, this study revealed intrinsic molecular associations among the three diseases. Collectively, this work provided novel insights into the molecular mechanisms by which the environmental pollutant 6PPD-Q induces multiple pulmonary diseases and establishes inter-disease molecular connections. This establishes a theoretical foundation for the prevention and treatment of pulmonary diseases.

Keywords: 6PPD-Q, network toxicology, molecular docking, lung disorders

Introduction

With rapid industrial development, numerous chemical substances are widely used across various sectors. One significant substance is N-(1,3-dimethylbutyl)-N'-phenyl-p-benzoquinone diamine (6PPD-Q), an oxidation product of the antioxidant N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD), which is commonly used in rubber tires[1]. 6PPD-Q has gained public attention because it readily oxidizes in ozone-rich environments. Tire wear particles release 6PPD-Q into aquatic environments via stormwater runoff, raising concerns about its toxicity and health risks. Studies show that 6PPD-Q exhibits strong teratogenic and mutagenic toxicity, being a primary cause of acute mortality in coho salmon exposed to urban stormwater runoff[2]. Therefore, it is recognized as an environmental pollutant that threatens aquatic organisms and ecosystems. Both 6PPD and 6PPD-Q are commonly found in the environment, including road dust, tunnel PM_{2.5}, and surface water, with dermal contact being the main exposure route. They have also been detected in human urine, indicating internal exposure [3]. This suggests that chronic exposure to these pollutants may significantly impact human health. Consequently, 6PPD-Q, particularly regarding its effects on human health and its potential link to pulmonary diseases, is the focus of this study. Confirming a mechanistic link between 6PPD-Q exposure and pulmonary disease could lead to new prevention and treatment strategies.

Lung cancer is a malignant tumor from bronchial or alveolar epithelium, resulting from long-term synergistic effects of multiple factors. Its core processes include abnormal cell proliferation and enhanced invasive capacity, leading to local tumor lesions that can spread. Smoking, air pollution, occupational exposure, and genetics significantly increase lung cancer risk. Tumor cell destruction of lung tissue can cause cough, hemoptysis, and dyspnea, severely impairing respiratory function.

COPD is a preventable and treatable chronic respiratory disease that features persistent airflow

limitation. Its pathogenesis is linked to chronic inflammatory responses in the airways and lung tissue. Prolonged exposure to harmful gases or particles, such as tobacco smoke and dust, causes airway epithelial injury. This activates inflammatory cells, which release a large amount of inflammatory mediators. These changes lead to airway wall thickening, excessive mucus secretion, and decreased lung elasticity, resulting in persistent airflow limitation that worsens over time. COPD is commonly associated with long-term smoking, air pollution, recurrent pulmonary infections, and genetic factors. Pulmonary fibrosis is a chronic, progressive lung disease characterized by pulmonary interstitial fibrosis and disrupted lung structure. It reflects an abnormal repair process following lung injury. Multiple triggers exist, including idiopathic factors, connective tissue diseases, drug-induced injury, radiation injury, and chronic infections. These factors lead to the abnormal activation of pulmonary interstitial fibroblasts, resulting in excessive collagen deposition that replaces normal lung tissue. This reduces lung compliance and impairs gas exchange. The etiology of pulmonary fibrosis is multifactorial; genetic susceptibility and environmental exposure increase the risk of developing the disease.

Pulmonary diseases arise from functional and structural abnormalities in lung tissue and the respiratory tract. They are typically caused by environmental exposures, pathogen infections, immune response dysregulation, genetic susceptibility, or lifestyle factors like smoking. As industrial pollution increases, global populations age, and unhealthy habits spread, pulmonary disease incidence is rising worldwide, becoming a leading threat to human health. This study discusses three representative pulmonary diseases based on pathway enrichment analysis: lung cancer, chronic obstructive pulmonary disease (COPD), and pulmonary fibrosis.

Network toxicology, a developing interdisciplinary field, has advanced compound toxicity research significantly[4]. By integrating technologies like bioinformatics and big data analytics, it systematically elucidates compound toxicity mechanisms. For instance, studies on polycyclic aromatic hydrocarbons (PAHs) show that they exert toxic effects through multiple pathways, including cell cycle interference and oxidative stress induction. This provides robust support for environmental risk assessment. In drug development, network toxicology aids in understanding adverse reactions and offers insights for drug safety evaluation. Molecular docking is crucial for studying compound-target interactions at the molecular level. It uses computational simulations to predict the binding modes and affinities of compounds with their targets, providing evidence for understanding compound mechanisms of action. In anticancer drug development, researchers use molecular docking to screen and refine candidate molecules, evaluating their binding capacity and specificity. This leads to the identification of compounds with potential anticancer activity and

clarifies their interaction mechanisms with targets. Research on 6PPD-Q has mainly focused on its toxicity to aquatic organisms. Studies show that 6PPD-Q is highly toxic to aquatic life, causing physiological dysfunction, impaired growth, and even mortality. Its environmental fate has been partially characterized; 6PPD-Q displays moderate stability in water and can biomagnify through food chains. However, research on its potential health impacts on humans, particularly its links to pulmonary diseases, is limited, with no in-depth studies reported.

This study aims to analyze the toxicity of 6PPD-quinone (6PPD-Q) and its mechanisms in lung diseases using network toxicology and molecular docking. We hypothesize that 6PPD-Q may induce pulmonary disease. To explore how 6PPD-Q contributes to pulmonary disease, we integrated network toxicology with molecular docking. We retrieved potential targets of 6PPD-Q from the ChEMBL and SwissTargetPrediction databases, while disease-associated targets for pulmonary disease came from the CTD, DisGeNET, DrugBank, GeneCards, and OMIM databases. We constructed a network prediction model to systematically identify and analyze toxic pathways related to 6PPD-Q-induced pulmonary disease. This analysis aims to clarify the potential toxicological characteristics of 6PPD-Q and predict its toxicity and underlying molecular mechanisms. Furthermore, this study serves as a comprehensive reference for assessing the toxicity of common chemical substances encountered in daily life and provides a scientific basis for diagnosing diseases linked to exposure to these toxic compounds.

Method

Target identification

The acquisition of 6PPD-Q-related targets began with a search in the PubChem database for "N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine quinone" to retrieve its standard structure and Simplified Molecular Input Line Entry System (SMILES) representation. This workflow commenced with a PubChem database query to identify and confirm the correct chemical name and molecular formula of 6PPD-Q. An SDF file was then uploaded to the SwissTargetPrediction website, where predicted targets with a probability greater than zero were filtered and downloaded. Targets associated with a probability value exceeding zero were classified as 6PPD-Q targets. Additionally, potential targets were specifically retrieved from the ChEMBL database for the species "Homo sapiens". ChEMBL identifiers and SwissTargetPrediction predictions were integrated, deduplicated, and standardized using the UniProt database. Following integration and deduplication, the final 6PPD-Q target library was constructed.

The identification of targets associated with respiratory diseases primarily relies on disease databases such as GeneCards, DisGeNET, and OMIM. Searches were conducted using keywords

including "lung cancer," "chronic obstructive pulmonary disease," and "pulmonary fibrosis" to collect gene targets closely linked to the pathogenesis of these diseases. These databases integrate extensive published research findings and clinical data, providing a rich resource for the accurate identification of disease targets.

Target screening and integration

The 6PPD-Q targets retrieved from the ChEMBL and SwissTargetPrediction databases were integrated and subjected to deduplication to generate a unique collection of 6PPD-Q targets, comprising 576 targets. Similarly, targets associated with diseases obtained from disease databases underwent deduplication. Venn analysis identified intersections between the 6PPD-Q targets and targets linked to individual diseases to identify overlapping targets between 6PPD-Q and respiratory diseases.

Construction of protein-protein interaction networks and screening of core targets

The common targets of 6PPD-Q and the disease were input into the STRING database for protein-protein interaction analysis. During the analysis, the species was specified as "Homo sapiens", the minimum interaction score required for medium confidence was set to >0.4 , and free unconnected single genes were removed to ensure high reliability of the resulting protein-protein interactions.

The data obtained from the STRING database analysis were imported into Cytoscape software for visualization processing. Using Cytoscape, each node in the PPI network was scored based on its degree. Potential targets were then identified using the following criteria: betweenness centrality index $>$ median, closeness centrality $>$ median, and degree value $>$ twice the median. The resulting potential targets were imported back into Cytoscape for visualization modification of the protein-protein interaction (PPI) network. Subsequently, these genes were arranged in a circular layout in clockwise order according to their degree values, where degree reflects the importance of a node within the network—higher degree values indicate greater numbers of connections to other nodes and thus a more central functional role in the network. The MCODE plugin in Cytoscape software was used to analyze the PPI network and identify core functional subclusters composed of highly interconnected nodes. Concurrently, the CytoHubba plugin was applied to rank hub genes based on the Degree algorithm, and the top 10 core genes were selected. Subsequently, a re-screening step was performed using the following criteria: betweenness centrality index $>$ median, closeness centrality $>$ median, and degree value $>$ median, yielding a set of candidate genes. Finally, hub genes were defined as the intersection of three gene sets: (1) genes within the core subclusters identified by MCODE, (2) the top 10 core genes predicted by CytoHubba, and (3) genes retained after median-based filtering—thereby identifying core targets

associated with 6PPD-Q and respiratory system disease.

Gene function and pathway enrichment analysis

To explore the biological roles of potential targets implicated in 6PPD-Q-induced diseases, Gene Ontology (GO) functional enrichment analysis was performed on the identified potential targets using the DAVID database. This analysis comprehensively covered three GO domains: biological processes (BP), molecular functions (MF), and cellular components (CC). In-6PPD-Qth interpretation of the GO functional enrichment results clarified the underlying biological processes through which 6PPD-Q may influence disease development. Concurrently, KEGG pathway enrichment analysis was conducted on the same set of potential targets using the DAVID database. This analysis revealed the primary signaling pathways associated with the potential targets and thereby identified the key signal transduction pathways mediating 6PPD-Q's effects on disease mechanisms. During the analysis, the false discovery rate (FDR) threshold was set to less than 0.001 to ensure that the identified signaling pathways exhibit high statistical significance. Visualization of the KEGG pathway enrichment analysis results—including bubble plots and bar charts—intuitively illustrates the enrichment patterns of potential targets across distinct signaling pathways, thereby providing critical insights into the mechanistic impact of 6PPD-Q on disease pathogenesis.

Molecular docking

Molecular docking analysis was performed to investigate the interactions between 6PPD-Q and core target proteins. The three-dimensional structure of the small molecule ligand was retrieved from the PubChem database and saved in SDF file format; it was subsequently converted to Mol2 format using OpenBabel for subsequent docking simulations. Protein crystal structures of the core targets were obtained from the UniProt database, with selection criteria emphasizing high resolution and favorable R-values to ensure the accuracy of docking results. Additionally, the three-dimensional conformation of 6PPD-Q was retrieved from the Protein Data Bank (PDB) and saved in an appropriate file format to facilitate subsequent molecular docking analysis with the protein crystal structures.

The protein crystal structures obtained were preprocessed using PyMOL to remove water molecules and the original ligand, thereby eliminating potential interference with molecular docking results. Subsequently, the processed protein structures were imported into AutoDockTools for hydrogenation and charge calculation, preparing them for molecular docking. The three-dimensional conformation of 6PPD-Q was similarly processed to meet the requirements of molecular docking.

In AutoDock, the grid box size was defined to fully encompass the protein's active site and the potential binding region of 6PPD-Q; an appropriate genetic algorithm was selected to enhance the accuracy and reliability of the docking results. Molecular docking analysis was performed using AutoDock Vina 1.1.2 via CMD commands. Upon program execution, docking results for 6PPD-Q against its core target were generated. The docking results include critical information such as binding affinity and binding mode; analysis of these parameters enables evaluation of the interaction between 6PPD-Q and its core target. The molecular docking results are visualized using PyMOL to clearly illustrate the binding mode and interaction sites of 6PPD-Q with the core target.

Results

The impact of 6PPD-Q on respiratory system diseases

We conducted a comprehensive screening of 576 6PPD-Q targets from the SwissTargetPrediction website and the ChEMBL database, generating a large-scale associated dataset; concurrently, we identified 21,103 targets from the GeneCards and OMIM databases. By integrating these target datasets, we generated a Venn diagram (Figure 1A) to visually illustrate the overlap between 6PPD-Q targets and respiratory system disease-associated targets, identifying 526 cross-targets implicated in 6PPD-Q-induced respiratory system diseases within the overlapping region. Subsequently, we constructed a PPI network for these 526 cross-targets using the STRING database and imported the network analysis results into Cytoscape software for further refinement. Applying predefined criteria for core target selection, we identified 90 potential targets and generated the corresponding PPI network (Figure 1B). Finally, we performed GO and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses on the 90 potential targets using the DAVID database with Homo sapiens as the species of interest. This yielded a total of 81 KEGG terms and 268 GO terms, comprising 161 biological process (BP) terms, 43 cellular component (CC) terms, and 64 molecular function (MF) terms. From the GO enrichment results, we extracted the top 10 terms with the highest gene representation in each of the BP, CC, and MF categories and generated a multi-category GO enrichment bubble chart (Figure 1C). For the KEGG enrichment analysis, we selected the top 20 terms to generate the corresponding chart (Figure 1D). As shown in Figure 1C, the top BP terms were primarily associated with cytoplasmic translation, translation, and ribosomal small subunit biogenesis; the top CC terms were predominantly linked to the cytosolic ribosome, ribosome, and ribonucleoprotein complex; and the top MF terms were mainly enriched for structural constituents of the ribosome, RNA binding, and rRNA binding. Therefore, we selected lung cancer, pulmonary fibrosis, and COPD—diseases whose core pathological mechanisms involve the aforementioned pathways—for further in-depth analysis.

Accordingly, these three diseases were chosen for subsequent detailed investigation. All pathways included in the visualization met the threshold criterion of $p\text{-value} < 0.05$. A composite target-pathway network was constructed in Cytoscape, visually illustrating the intricate relationships among respiratory diseases (red circles), three pathways (lung cancer, pulmonary fibrosis, and COPD—yellow circles), and core target genes of respiratory system diseases (Figure 2).

The impact of 6PPD-Q on lung cancer

Through searching, integrating, and deduplicating data from the ChEMBL and SwissTargetPrediction databases, a total of 576 6PPD-Q target genes were identified. Using disease databases such as DisGeNET and OMIM, 11,179 unique lung cancer-related targets were obtained. Venn diagram analysis revealed 408 common targets between 6PPD-Q and lung cancer; these common targets were considered potential key targets for the mechanism by which 6PPD-Q influences lung cancer (Figure 3A). Next, we used the STRING database to construct a protein-protein interaction (PPI) network for these potential targets. The network analysis results were imported into Cytoscape software for further optimization. Based on the screening criteria, we selected key targets and identified a total of 71 potential targets.

Using the STRING database and the submitted potential targets for 6PPD-Q, a PPI network was constructed, resulting in a total of 71 nodes and 1,437 edges. We analyzed the topological properties of the network nodes using Cytoscape software and generated a visual PPI network diagram (Figure 3B). Node size and color correspond to their respective degree values; larger and darker nodes indicate higher degree values and play more critical roles in the network. This protein-protein interaction network visually illustrates the interaction relationships among these potential targets. We performed module analysis on the lung cancer targets using the MCODE plugin in Cytoscape and screened for hub genes using the CytoHubba plugin. The MCODE analysis identified a total of 3 key subnetworks (Figure 3C); the CytoHubba analysis ranked nodes by degree and identified the top 10 core targets (Figure 3D); simultaneously, by intersecting this set with genes selected based on median values, we identified 7 core genes: RPS27A, RPS6, RPS2, RPS3A, ACTB, RPL5, and RPL4. Subsequently, GO and KEGG enrichment analyses were performed on these 71 potential targets using the DAVID database, with a focus on *Homo sapiens*. This analysis yielded 84 KEGG entries and 200 GO entries, including 122 BP, 34 CC, and 44 MF. In the GO enrichment results, we highlighted the top 10 entries in BP, CC, and MF, respectively, and plotted a GO enrichment bubble plot (Figure 3E). For the KEGG enrichment analysis, we selected the top 20 entries and plotted the corresponding chart (Figure 3F). As shown in Figure 3E, the BP categories are primarily associated with cytoplasmic translation, translation, and other

related processes. The CC categories are mainly related to cellular compartments such as the cytoplasm, ribosomes, and ribonucleoprotein complexes. The MF categories are primarily associated with the structural components of ribosomes and RNA binding. Figure 3F shows that in the KEGG pathway analysis, various disease pathways were identified, including the COVID-19 pathway, ribosome pathway, the mTOR signaling pathway, and the C-type lectin receptor signaling pathway. These findings provide critical experimental evidence at the molecular level for elucidating the pathogenesis of 6PPD-Q-induced lung cancer.

We also investigated the interaction characteristics between 6PPD-Q and seven core target genes through molecular docking analysis. The results showed that 6PPD-Q exhibits strong binding affinity with all core proteins. Figure 4 visually presents the structures of the lowest-energy complexes formed between these seven core targets and 6PPD-Q. Upon closer examination, the binding affinity between 6PPD-Q and ACTB was found to be -7.7 kcal/mol, indicating that the two form a stable complex. Molecular visualization analysis further revealed that 6PPD-Q forms a hydrogen bond with the key residue GLY-268 at a distance of 2.4 Å. 6PPD-Q binds to key residues such as LEU-235, ARG-121, ASP-147, THR-28, ARG-22, LEU-86, PRO-190, ARG-178, THR-181, and GLY-47. Binding energies range from -5.6 kcal/mol to -7.7 kcal/mol, with ACTB (-7.7 kcal/mol), RPS27A (-7.7 kcal/mol), RPL5 (-7.5 kcal/mol), RPS3A (-7.0 kcal/mol), RPS2 (-6.9 kcal/mol), RPL4 (-5.6 kcal/mol), and RPS6 (-5.6 kcal/mol). Among these, the binding energy of 6PPD-Q with ACTB and RPS27A was the lowest at -7.7 kcal/mol, indicating the most stable interaction between them; the binding energies with RPL4 and RPS6 are -5.6 kcal/mol, which is relatively higher than those with other core targets, but still capable of forming effective binding.

COPD is a preventable and treatable chronic respiratory disease that features persistent airflow limitation. Its pathogenesis is linked to chronic inflammatory responses in the airways and lung tissue. Prolonged exposure to harmful gases or particles, such as tobacco smoke and dust, causes airway epithelial injury. This activates inflammatory cells, which release a large amount of inflammatory mediators. These changes lead to airway wall thickening, excessive mucus secretion, and decreased lung elasticity, resulting in persistent airflow limitation that worsens over time. COPD is commonly associated with long-term smoking, air pollution, recurrent pulmonary infections, and genetic factors. Pulmonary fibrosis is a chronic, progressive lung disease characterized by pulmonary interstitial fibrosis and disrupted lung structure. It reflects an abnormal repair process following lung injury. Multiple triggers exist, including idiopathic factors, connective tissue diseases, drug-induced injury, radiation injury, and chronic infections. These

factors lead to the abnormal activation of pulmonary interstitial fibroblasts, resulting in excessive collagen deposition that replaces normal lung tissue. This reduces lung compliance and impairs gas exchange. The etiology of pulmonary fibrosis is multifactorial; genetic susceptibility and environmental exposure increase the risk of developing the disease.

We retrieved 13,799 targets associated with COPD from databases such as GeneCards and OMIM, integrated them with 576 6PPD-Q targets, and generated a Venn diagram (Figure 5A). This diagram reveals the overlap between 6PPD-Q targets and targets associated with COPD, highlighting 432 overlapping targets that are particularly relevant to 6PPD-Q-induced COPD. Using the STRING database, we constructed a PPI network for these 432 overlapping targets (Figure 5B) and imported the results into Cytoscape software for further analysis. We applied screening criteria to select key targets, identified 77 potential targets, and visualized the resulting protein-protein interaction network. We performed module analysis using the MCODE plugin and screened genes using the CytoHubba plugin. The MCODE analysis identified a total of 4 key subnetworks (Figure 5C); the CytoHubba plugin identified the top 10 core targets (Figure 5D); simultaneously, by intersecting this set with the genes selected based on the median screening criteria, we identified seven core genes: TP53, RPS27A, ACTB, HSP90AB1, HSP90AA1, H4C6, and HNRNPA1.

Subsequently, we performed GO and KEGG enrichment analyses on these 77 potential targets using the DAVID database, focusing specifically on humans. This analysis generated a comprehensive dataset containing 99 KEGG entries and 355 GO terms. For the GO enrichment results, we selected the top 10 entries from BP, CC, and MF, and plotted a GO enrichment bubble plot. For the KEGG enrichment analysis, we selected the top 20 entries and plotted the corresponding charts. As shown in Figure 5E, the BP categories are primarily related to cytoplasmic translation, translation, and chromatin organization. The CC categories mainly involve cytoplasmic ribosomes, ribosomes, ribonucleoprotein complexes, and cytoplasmic large ribosomal subunits. The MF categories primarily focus on ribosomal structural components and RNA binding. Figure 5F shows that in the KEGG pathway analysis, the vascular endothelial growth factor (VEGF) signaling pathway, the C-type lectin receptor signaling pathway, the T-cell receptor signaling pathway, and other related pathways were all significantly involved. These findings provide valuable insights into the molecular mechanisms underlying 6PPD-Q-induced COPD.

We further investigated the interaction characteristics between 6PPD-Q and the seven core target genes through molecular docking analysis. The results showed that 6PPD-Q exhibits strong

binding affinity with each of the core target proteins. Figure 6 visualizes the three-dimensional conformations of the lowest-energy complexes formed between 6PPD-Q and these 7 core targets. Upon magnification, it is evident that 6PPD-Q forms stable bonds with TP53, RPS27A, ACTB, HSP90AB1, HSP90AA1, H4C6, and HNRNPA1, with binding sites involving LYS-336, GLY-302, ASP-157, SER-14, GLY-15, GLY-225, ASN-298, HIS-299, ASP-94, LYS-166, VAL-177, TYR-139, ALA-420, ARG-456, GLU-465, GLN-49, ASP-52, ALA-22, ARG-34, PHE-33, and other key amino acid residues. The binding energies of 6PPD-Q to the aforementioned seven core target proteins range from -6.2 to -8.6 kcal/mol, with RPS27A at -6.2 kcal/mol, HSP90AB1 at -7.0 kcal/mol, HNRNPA1 at -7.1 kcal/mol, ACTB at -8.1 kcal/mol, HSP90AA1 at -8.3 kcal/mol, TP53 at -8.5 kcal/mol, and H4C6 at -8.6 kcal/mol. Among these, the binding energy between 6PPD-Q and H4C6 is the lowest, at -8.6 kcal/mol, suggesting that their interaction is the most stable; the binding energy with RPS27A is -6.2 kcal/mol, which, although relatively the highest among the seven target proteins, still allows for the formation of an effective and stable binding with the target protein.

The effect of 6PPD-Q on pulmonary fibrosis

A comprehensive dataset containing 10,745 targets was obtained from databases such as GeneCards and OMIM, integrated with 6PPD-Q targets, and visualized as a Venn diagram (Figure 7A). This diagram illustrates the targets associated with 6PPD-Q and pulmonary fibrosis, as well as their overlapping regions, which include 394 common targets potentially linked to 6PPD-Q-induced pulmonary fibrosis. To further investigate these targets, a PPI network was constructed using the STRING database and imported into Cytoscape for analysis. Using predefined criteria, a total of 79 potential targets were identified, and their PPI network was visualized (Figure 7B). Subsequently, another set of genes was obtained using different criteria. The MCODE plugin was used to cluster disease targets into modules, yielding three significant subnetwork modules (Figure 7C); the CytoHubba plugin was used to identify the top 10 hub genes by degree (Figure 7D); by intersecting the genes identified through the median screening criterion, nine core genes were identified: ACTB, HSP90AB1, TP53, RPS27A, HSP90AA1, RPS6, RPS3, RPL4, and RPL13A.

Subsequently, GO and KEGG enrichment analyses were performed on these 79 potential targets using the DAVID database (species restricted to humans), yielding a total of 1,102 GO terms (including 240 biological processes [BP], 51 cellular components [CC], and 73 molecular functions [MF]) and 113 KEGG terms. We selected the top 10 entries with the highest proportion of genes in BP, CC, and MF to construct a GO enrichment bubble plot (Figure 7E). The results showed that BP mainly involved cytoplasmic translation and translation; CC primarily included

cytoplasmic ribosomes, ribosomes, and ribonucleoprotein complexes; and MF mainly involved structural components of ribosomes and RNA binding. The top 20 KEGG entries by enrichment were selected for display (Figure 7F). The results showed significant enrichment of the vascular endothelial growth factor signaling pathway, apoptosis, and sphingolipid signaling pathways, providing important clues for elucidating the potential molecular mechanisms underlying 6PPD-Q-induced pulmonary fibrosis. We further investigated the interaction characteristics between 6PPD-Q and core target genes through molecular docking analysis. The results showed that 6PPD-Q exhibits strong binding affinity with each core target protein. Figure 8 visually presents the three-dimensional conformations of the lowest-energy complexes formed between 6PPD-Q and these nine core targets. Upon magnification, it is evident that 6PPD-Q forms stable complexes with the nine core target proteins ACTB, RPL4, HSP90AA1, HSP90AB1, RPL13A, RPS3, RPS6, RPS27A, and TP53, with binding sites involving HIS-279, ASN-278, GLU-103, LEU-103, LEU-107, SER-77, CYS-1237, ILE-108, ASP-106, GLU-157, THR-153, PHE-116, GLU-118, TYR-398, VAL-109, ASP-111, and ILE-102. The binding energies of 6PPD-Q with the above nine core target proteins range from -6.5 to -8.9 kcal/mol, with RPS27A at -6.5 kcal/mol, RPS3 at -6.7 kcal/mol, TP53 at -7.1 kcal/mol, RPL4 at -7.2 kcal/mol, RPL13A at -7.4 kcal/mol, RPS6 at -8.4 kcal/mol, HSP90AA1 at -8.6 kcal/mol, HSP90AB1 at -8.8 kcal/mol, and ACTB at -8.9 kcal/mol. Among these, the binding energy between 6PPD-Q and ACTB was the lowest, at -8.9 kcal/mol, suggesting that their interaction is the most stable; the binding energy with RPS27A was -6.5 kcal/mol, which, although the highest among the nine target proteins, still allows for an effective and stable binding with the target protein.

Discussion

6PPD-Q forms when ozone converts the tire antioxidant 6PPD in the environment. 6PPD is widely used in rubber and tire manufacturing to slow material aging and extend service life. However, 6PPD-Q is prevalent in various environmental media, including air, water, and sediments, raising significant ecological and health concerns. Studies have demonstrated that 6PPD-Q exhibits significant acute toxicity and hepatotoxicity in aquatic organisms and can cause neurological damage in mammals, which is linked to neurodegenerative diseases like Parkinson's disease[5]. Recently, the respiratory hazards of 6PPD-Q due to inhalation exposure have gained increasing attention. This contaminant can enter the human body through inhalation of tire wear particles and aerosols, leading to lung tissue damage, bioaccumulation, oxidative stress, inflammatory responses, and suppressed pulmonary immune function. Although studies have revealed the

multisystem toxicity of 6PPD-Q, the specific molecular mechanisms by which it induces respiratory diseases remain unclear. Given the widespread environmental distribution and potential health risks of 6PPD-Q, investigating its effects on the respiratory system and pathogenic mechanisms is crucial^[6].

We utilized several databases, including PubChem, SwissADME, GeneCards, OMIM, and STRING, to identify and analyze relevant targets. We conducted a comprehensive network analysis of these targets using Cytoscape software. Next, we performed GO and KEGG enrichment analyses on core targets using the DAVID database. Finally, we carried out molecular docking studies using the UniProt database, PDB, PyMOL, and AutoDock Vina to investigate the interactions between 6PPD-Q and core target proteins. This study elucidates the potential molecular mechanisms by which 6PPD-Q induces human lung diseases.

In our lung cancer study, we identified seven core targets and confirmed their biological relevance through multilevel analysis. Exposure to 6PPD-Q induces oxidative stress and chronic inflammation in lung cells^[7], acting as an upstream driver that disrupts the activity, stability, and expression levels of core ribosomal proteins like RPS27A and RPS68^[8]. Along with the synergistic effects of NF- κ B pathway activation, this disrupts ribosomal function and protein synthesis homeostasis, aligning with the enrichment analysis that identified the ribosomal pathway as a core signaling pathway. Abnormalities in ribosomal proteins allow DNA-damaged lung cells to evade apoptosis and proliferate abnormally by weakening the p53-mediated apoptotic pathway^[9]. Concurrently, 6PPD-Q impairs alveolar macrophage function and weakens pulmonary immune clearance, creating a favorable environment for tumorigenesis^[10]. Additionally, 6PPD-Q alters ACTB expression and function, enhancing the invasive and metastatic capabilities of lung cancer cells through cytoskeletal reorganization, collectively driving lung cancer development^[11]. Molecular docking results further support these mechanisms: 6PPD-Q stably binds to ACTB and also efficiently binds to ribosome-associated targets like RPS27A. Among these, the binding to ACTB and RPS27A is the most stable, although effective binding occurs with RPL4 and RPS6 as well. This confirms that 6PPD-Q can regulate key biological processes, such as cytoskeletal reorganization and ribosomal homeostasis by specifically binding to core targets like ACTB and RPS27A. This clarifies the molecular basis for 6PPD-Q's regulation of each target's function and confirms the synergistic role of these core targets in the 6PPD-Q-induced lung carcinogenesis process.

In COPD, we identified seven core targets by integrating disease-associated targets with drug-targeted molecules. Exposure to 6PPD-Q induces oxidative stress and DNA damage in lung cells,

activating stress pathways like TP53[12]. It disrupts the expression and function of ribosomal proteins such as RPS27A and affects molecular chaperones like HSP90AB1 and HSP90AA1[13]. This disruption impacts protein synthesis and folding, aligning with the central role of ribosomal pathways, DNA damage response, and histone modification functions identified in enrichment analysis. Additionally, it worsens airway inflammation and tissue remodeling by activating pathways such as Rap1 and VEGF. Simultaneously, 6PPD-Q alters ACTB-mediated cytoskeletal reorganization and regulates gene expression and RNA metabolism via H4C6 and HNRNPA1, driving the onset and progression of COPD[14]. Molecular docking results show that 6PPD-Q reliably interferes with ribosomal protein function, supporting the conclusion that it disrupts RPS27A expression. The stable binding of 6PPD-Q to TP53, ACTB, H4C6, and other core targets confirms its regulatory role in key processes like the DNA damage response, cytoskeletal reorganization, gene expression, and RNA metabolism. This provides important molecular evidence for investigating the synergistic regulatory mechanisms of each target and developing targeted interventions.

In the molecular network where 6PPD-Q regulates pulmonary fibrosis, our analysis of nine core targets revealed that exposure to 6PPD-Q induces oxidative stress in lung cells, consistent with oxidative stress as a central factor in pulmonary fibrosis[15]. It activates the TP53 stress pathway by inducing DNA damage and abnormal compensation of HSP90AB1 and HSP90AA1, disrupting ribosomal protein assembly and function. This disruption promotes senescence in alveolar epithelial cells and the secretion of senescence-associated secretory phenotype (SASP) components such as TGF- β , affecting autophagy and apoptosis balance[16]. This depletion of epithelial regenerative potential drives fibroblast activation and differentiation by activating pro-fibrotic pathways like TGF- β -SMAD. Furthermore, 6PPD-Q alters ACTB expression and function, promoting myofibroblast migration and excessive extracellular matrix deposition through cytoskeletal reorganization and pathways involving Rap1 and VEGF. This aligns with the pathological essence of pulmonary fibrosis as "abnormal wound healing," driving pulmonary scarring and remodeling[17]. Molecular docking results confirm the regulatory mechanisms, with the lowest binding energy between 6PPD-Q and ACTB, indicating a stable interaction. The binding energy with RPS27A was higher but still effective, confirming 6PPD-Q's interference with ribosomal protein function and its role in promoting pulmonary fibrosis progression.

Among the core targets identified across the three pulmonary diseases, ACTB and RPS27A emerged as common hub genes, highlighting their roles in mediating 6PPD-Q toxicity. ACTB encodes β -actin, a key component of the cytoskeleton affecting cell shape and motility. Our

molecular docking results showed the strongest binding affinity between 6PPD-Q and ACTB, suggesting it is a primary target. Disruption of ACTB function by 6PPD-Q induces cytoskeletal reorganization, enhancing lung cancer cell invasiveness, promoting myofibroblast migration, and contributing to airway remodeling in COPD. Such perturbations may amplify inflammatory and fibrotic cascades. RPS27A encodes a protein that is part of the 40S ribosomal subunit and functions in protein degradation. Our docking analysis confirmed stable binding of 6PPD-Q to RPS27A, impairing ribosomal assembly and leading to dysregulated protein synthesis and unfolded protein stress. The convergence of 6PPD-Q on both ACTB and RPS27A suggests it disrupts essential cellular functions, driving chronic inflammation and abnormal tissue repair. The presence of these targets across different lung diseases indicates that ACTB and RPS27A may serve as biomarkers for 6PPD-Q exposure and as targets for therapeutic interventions. Future studies should explore if genetic variations or post-translational modifications of ACTB and RPS27A affect individual susceptibility to 6PPD-Q-induced respiratory disorders and whether targeting these proteins could mitigate the multi-disease impact of this environmental toxicant.

Notably, among the core targets identified across three pulmonary diseases, ACTB and RPS27A emerged as common hub genes. They underscore their roles in mediating 6PPD-Q toxicity. ACTB encodes cytoskeletal β -actin, a crucial component of the actin cytoskeleton that governs cell shape, motility, adhesion, and intracellular trafficking. Our molecular docking results showed strong binding affinity between 6PPD-Q and ACTB, with binding energies ranging from -7.7 to -8.9 kcal/mol across diseases. This suggests ACTB is a primary target of 6PPD-Q. Disruption of ACTB function by 6PPD-Q induces cytoskeletal reorganization. This not only enhances the invasive and metastatic capacity of lung cancer cells but also promotes myofibroblast migration, excessive extracellular matrix deposition in pulmonary fibrosis, and contributes to airway remodeling in COPD. Such cytoskeletal changes may further affect mechanotransduction and cell-matrix interactions, amplifying inflammation and fibrosis. On the other hand, RPS27A encodes a fusion protein that serves as a component of the 40S ribosomal subunit and as a ubiquitin carrier for protein degradation. Our docking analysis confirmed stable binding of 6PPD-Q to RPS27A, with energies from -6.2 to -7.7 kcal/mol. This indicates moderate affinity relative to ACTB but shows potential functional interference. By binding to RPS27A, 6PPD-Q can impair ribosomal assembly and translational fidelity. This leads to dysregulated protein synthesis and unfolded protein stress, common features in all three diseases. Additionally, the ubiquitin-related function of RPS27A may be compromised, affecting the proteasomal degradation of key regulatory proteins such as p53 and HIF-1 α . This, in turn, alters cell cycle control, apoptosis, and hypoxia responses. The convergence

of 6PPD-Q on both ACTB and RPS27A implies that this pollutant disrupts two essential cellular machineries: the cytoskeleton and the protein synthesis/degradation balance. Together, these drive shared pathological hallmarks of chronic inflammation, abnormal tissue repair, and malignant transformation.

Moreover, the presence of these two targets across different lung diseases suggests that ACTB and RPS27A may serve as potential biomarkers for 6PPD-Q exposure. They may also present promising nodes for developing broad-spectrum therapeutic interventions. Future studies should investigate whether genetic polymorphisms or post-translational modifications of ACTB and RPS27A modulate susceptibility to 6PPD-Q-induced respiratory disorders. Additionally, targeting these proteins could mitigate the multi-disease impact of this environmental toxicant. Through studies on the association between 6PPD-Q respiratory exposure and various respiratory diseases (including lung cancer, COPD, and pulmonary fibrosis)[18], we found that one core mechanism by which 6PPD-Q induces these conditions is the impairment of the innate immune function of alveolar macrophages. This mechanism is critical for maintaining pulmonary homeostasis and anti-infective capacity. Our findings suggest that 6PPD-Q downregulates the expression of chemokine genes such as *Ccl2*, *Cxcl1*, and *Cxcl2* in CD11c(+)CD11b(+) alveolar macrophages[19]. This downregulation impairs their chemotactic responsiveness and exacerbates pulmonary inflammatory damage.

Furthermore, we found that oxidative stress activation is a key response underlying 6PPD-Q-induced respiratory damage. This indicates that 6PPD-Q possesses a toxic mechanism that induces pulmonary oxidative damage and inflammatory dysregulation. By inducing oxidative stress, 6PPD-Q disrupts functions of core targets such as ribosomal proteins (RPS27A, RPS6, etc.), TP53, the HSP90 family, and ACTB. This disruption affects protein synthesis and folding homeostasis, activating pro-inflammatory, pro-fibrotic, and pro-oncogenic signaling pathways. This exacerbates the pathological progression of lung cancer, COPD, and pulmonary fibrosis. Thus, 6PPD-Q poses broad and significant health risks to the respiratory system, providing crucial evidence for the prevention and control of respiratory diseases it induces.

KEGG pathway analysis shows that 6PPD-Q is involved in the progression of various respiratory diseases, including lung cancer, COPD, and pulmonary fibrosis. This primarily involves key pathways such as oxidative stress, inflammatory responses, cell proliferation/apoptosis, fibrotic remodeling, and tumorigenesis. These findings align with network toxicology and molecular docking studies, indicating that 6PPD-Q can induce oxidative damage to lung tissue, chronic inflammation, destruction of alveolar structure, and abnormalities in tumor-related signaling.

These features correspond to those commonly observed in patients with lung cancer, COPD, and pulmonary fibrosis[20]. As a new environmental pollutant from tire wear, respiratory exposure to 6PPD-Q induces oxidative damage to lung tissue, chronic inflammation, destruction of alveolar structure, and abnormalities in tumor-related signaling. These effects align closely with the pathological features seen in lung cancer, COPD, and pulmonary fibrosis[21]. We identified two common genes, RPS27A and ACTB, among the core genes linked to these three respiratory diseases. This suggests that these genes may play a role in the onset and progression of each disease. Thus, there is a significant association among them.

This study may aid in developing prevention and treatment strategies for respiratory diseases. The core targets RPS27A and ACTB could lead to new insights for targeted therapies, including small-molecule inhibitors, gene-regulating drugs, and biologic agents. Additionally, it supports environmental monitoring and control of 6PPD-Q, enhancing public health measures and respiratory disease prevention strategies.

In summary, we identified multiple potential targets, primarily RPS27A and ACTB, associated with respiratory diseases using network toxicology and molecular docking techniques. These findings reveal potential mechanistic links between 6PPD-Q and three common respiratory diseases: lung cancer, COPD, and pulmonary fibrosis. This research offers valuable insights for toxicology and environmental health and paves the way for investigating the effects of environmental pollutants on human respiratory health.

We aim to build on this foundation to further explore how pollutants like 6PPD-Q influence respiratory disease onset and progression. Our core objective is to emphasize that protecting the environment and respiratory health is an ongoing challenge. We hope this study resonates globally and underscores the need for collaborative strategies to reduce toxic exposure to 6PPD-Q. Together, we must mitigate the impact of environmental toxins on ecosystems and public respiratory health.

Author Contributions

Liyun Gao: Concept and design. Shaoting Huang: Methodology. Jiaxin Gao: Resources. Jiayin He: Investigate. Weicheng He: Writing – review & editing.

Ethics approval and consent to participate

Ethics approval and consent to participate

Not applicable. This study did not involve human participants or animal subjects.

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Conflicts of Interest

The authors declare no competing financial interests or personal relationships that could influence the work reported in this manuscript.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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network illustrates biological interactions among targets in 6PPD-Q-induced disease. (C) GO enrichment (top 10 per category): BP – cytoplasmic translation, translation, ribosome biogenesis, chromatin organization; CC – cytoplasmic ribosomes, ribosomal subunits, ribosomes, nucleoli; MF – ribosomal structure, rRNA binding, mRNA 5'-UTR binding, methyltransferase activity. (D) Key gene KEGG Analysis. Top 20 enriched signaling pathways (by significance), including those linked to lung cancer, COPD, and pulmonary fibrosis.

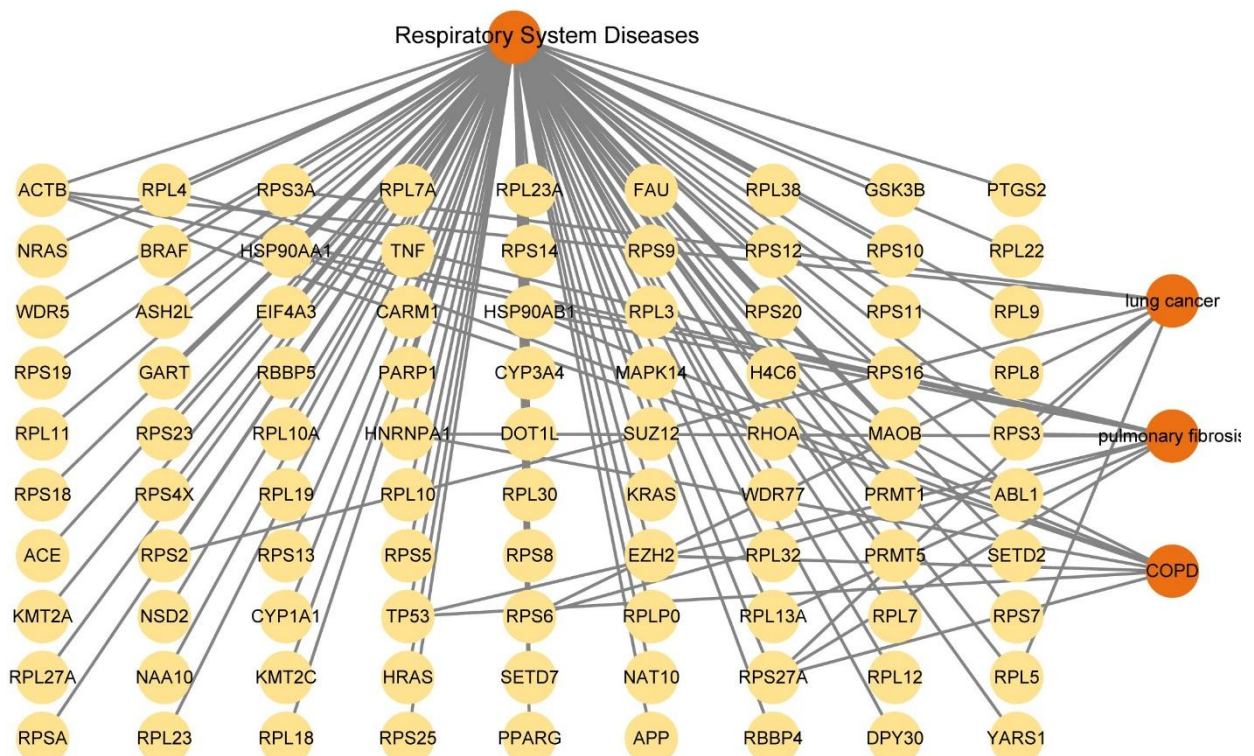


Figure 2. Network diagram of composite target pathways for 6PPD-Q-induced respiratory diseases. Red circles mark three key diseases (lung cancer, COPD, pulmonary fibrosis); yellow circles denote core target genes shared with 6PPD-Q. The diagram visualizes the interconnections among 6PPD-Q components, disease pathways, and associated genes

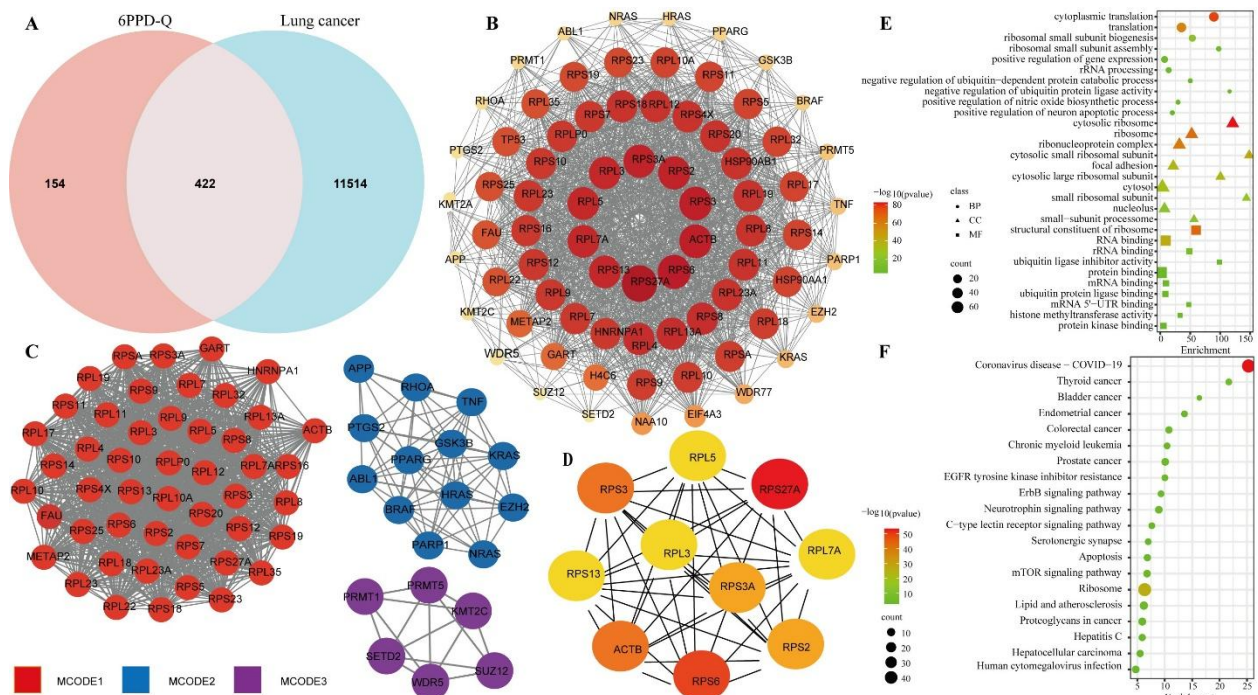


Figure 3. Relationship between 6PPD-Q and lung cancer. (A) Venn diagram of shared targets. 11,179 lung-cancer targets and 576 6PPD-Q targets share 408 overlapping targets. (B) Schematic representation of potential targets in a PPI network. Red nodes sized by degree illustrate interactions among the shared targets. (C) Three functional submodules identified via MCODE. (D) Top 10 core targets (CytoHubba) form a key regulatory subnetwork. (E) GO enrichment (top 10 per category): BP – cytoplasmic translation, translation, ribosome biogenesis, ubiquitin-dependent proteolysis; CC – cytoplasmic ribosomes, ribosomes, subunits, nucleocytoplasmic junction; MF – ribosomal structure, RNA binding, protein binding, ubiquitin ligase binding. (F) KEGG pathways: enriched in COVID-19, thyroid cancer, other tumors, ribosome, mTOR, and cancer pathways.

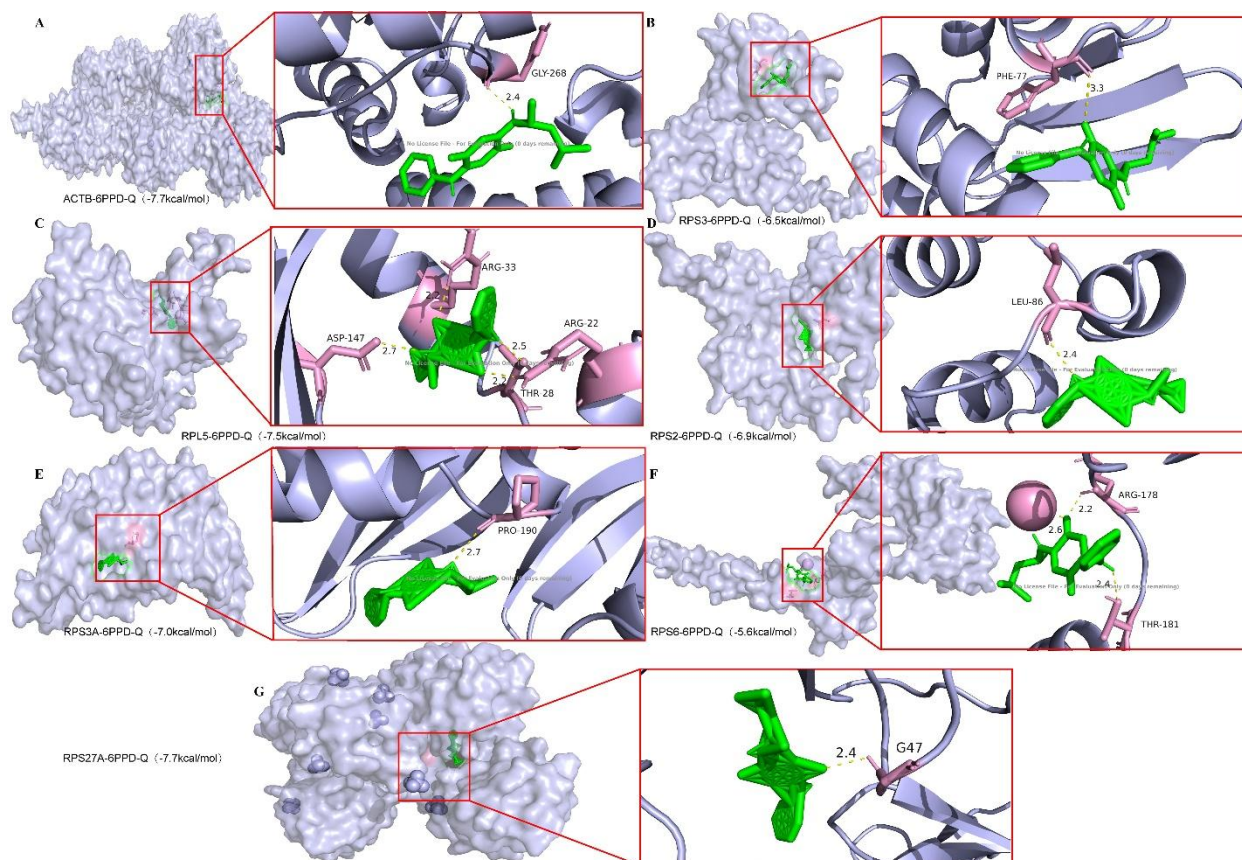


Figure 4. Visualization of the lowest binding energy during molecular docking between the core target protein and 6PPD-Q. Each subfigure shows docking details of the entire protein structure as well as a local zoom-in, highlighting key amino acid residues and their interaction distances (Å) with the 6PPD-Q molecule.

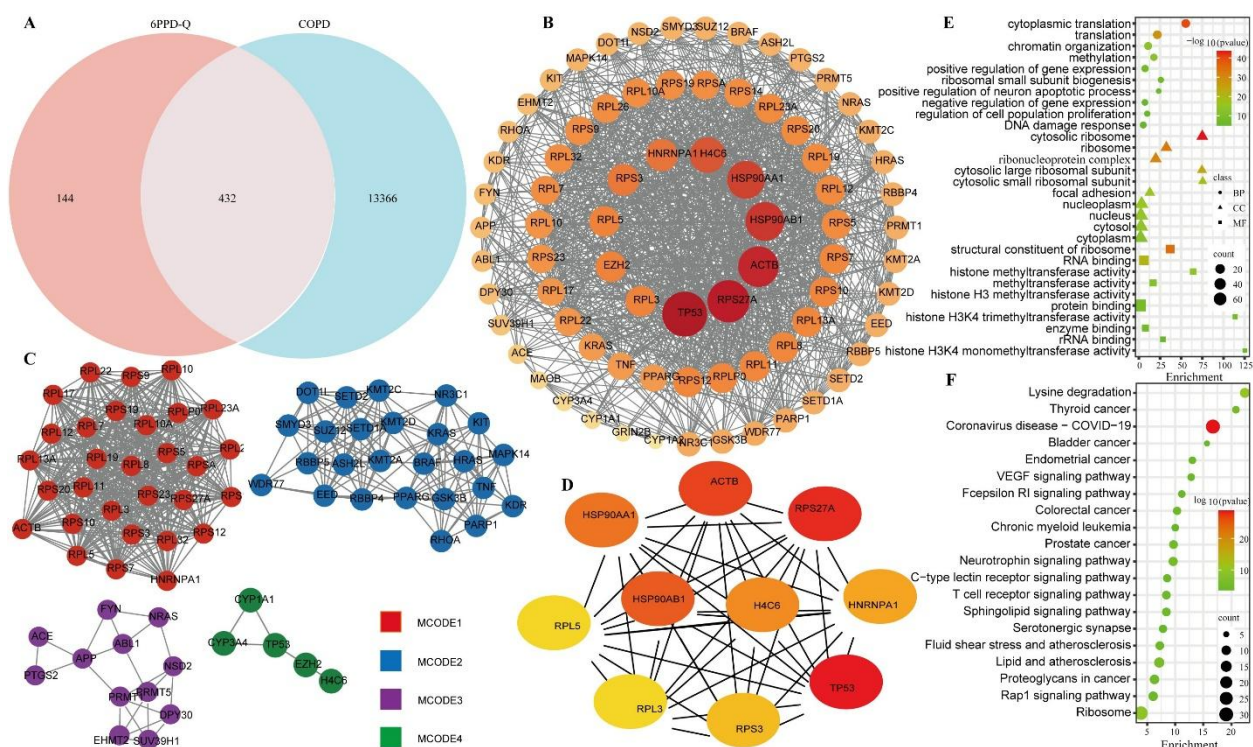


Figure 5. Relationship between 6PPD-Q and COPD. (A) Venn diagram of shared targets. 13,798 COPD targets and 576 6PPD-Q targets share 432 overlapping targets. (B) Schematic representation of potential targets in a PPI network. Red nodes sized by degree illustrate interactions among shared targets. (C) Four functional submodules identified via MCODE. (D) Top 10 core targets (CytoHubba) form a key regulatory subnetwork. (E) GO enrichment (top 10 per category): BP – cytoplasmic translation, translation, ribosome biogenesis, chromatin organization and methylation; CC – cytoplasmic ribosomes, ribosomes, subunits, nucleoplasm, nucleus; MF – ribosomal structure, RNA binding, protein binding, histone methyltransferase activity. (F) KEGG pathways: enriched in COVID-19, thyroid cancer, other tumors, ribosome, VEGF, and Rap1 signaling.

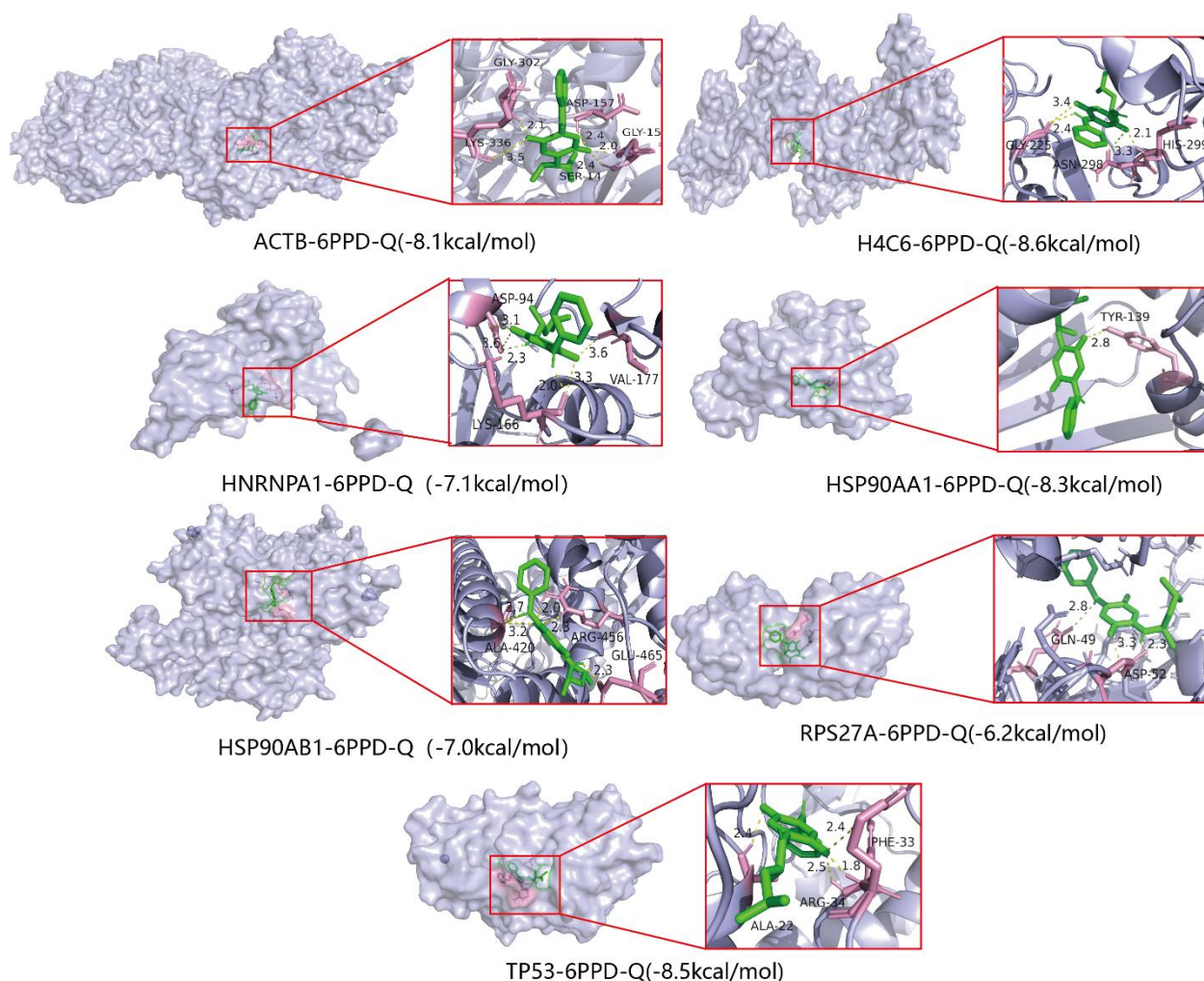


Figure 6. Visualization of the lowest binding energy during molecular docking between the core target protein and 6PPD-Q. Each subfigure shows docking details of the entire protein structure as well as a local zoom-in, highlighting key amino acid residues and their interaction distances (Å) with the 6PPD-Q molecule.

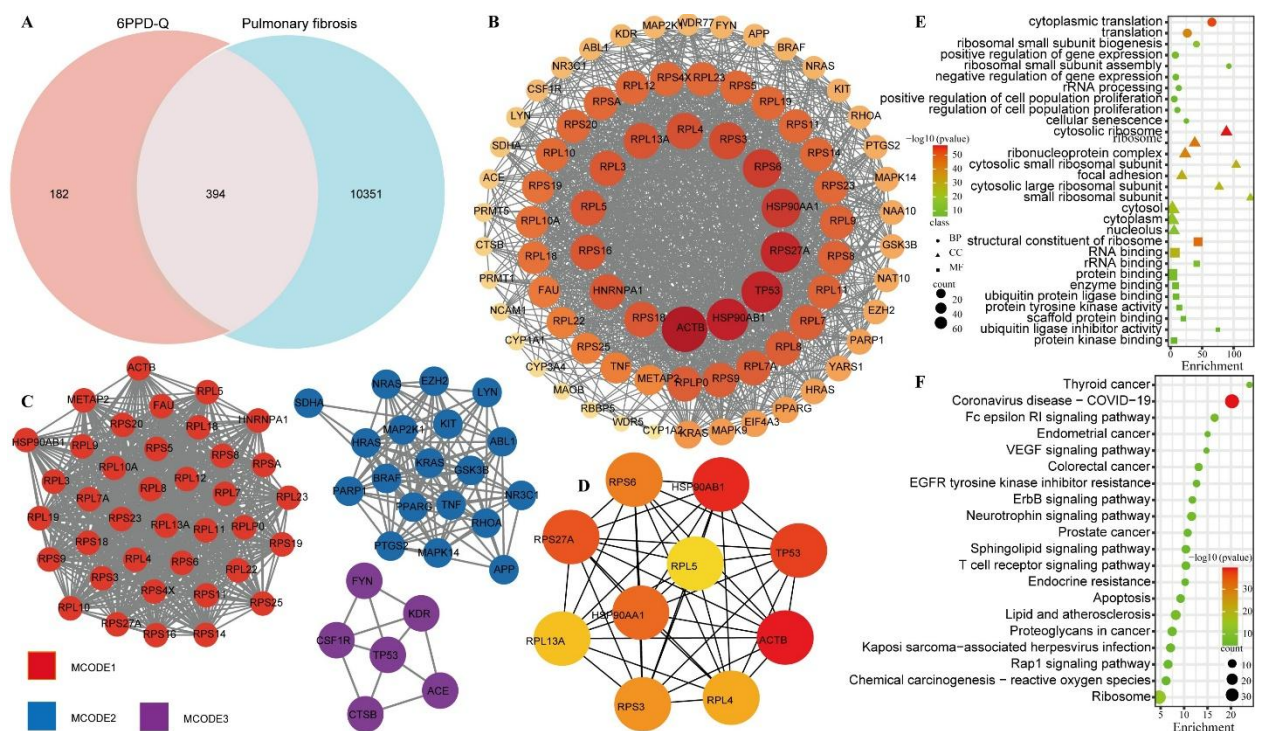


Figure 7. Relationship between 6PPD-Q and COPD. (A) Venn diagram of shared targets. 13,798 COPD targets and 576 6PPD-Q targets share 432 overlapping targets. (B) Schematic representation of potential targets in a PPI network. Red nodes sized by degree illustrate interactions among shared targets. (C) Four functional submodules identified via MCODE. (D) Top 10 core targets (CytoHubba) form a key regulatory subnetwork. (E) GO enrichment (top 10 per category): BP – cytoplasmic translation, translation, ribosome biogenesis, chromatin organization and methylation; CC – cytoplasmic ribosomes, ribosomes, subunits, nucleoplasm, nucleus; MF – ribosomal structure, RNA binding, protein binding, histone methyltransferase activity. (F) KEGG pathways: enriched in COVID-19, thyroid cancer, other tumors, ribosome, VEGF, and Rap1 signaling.

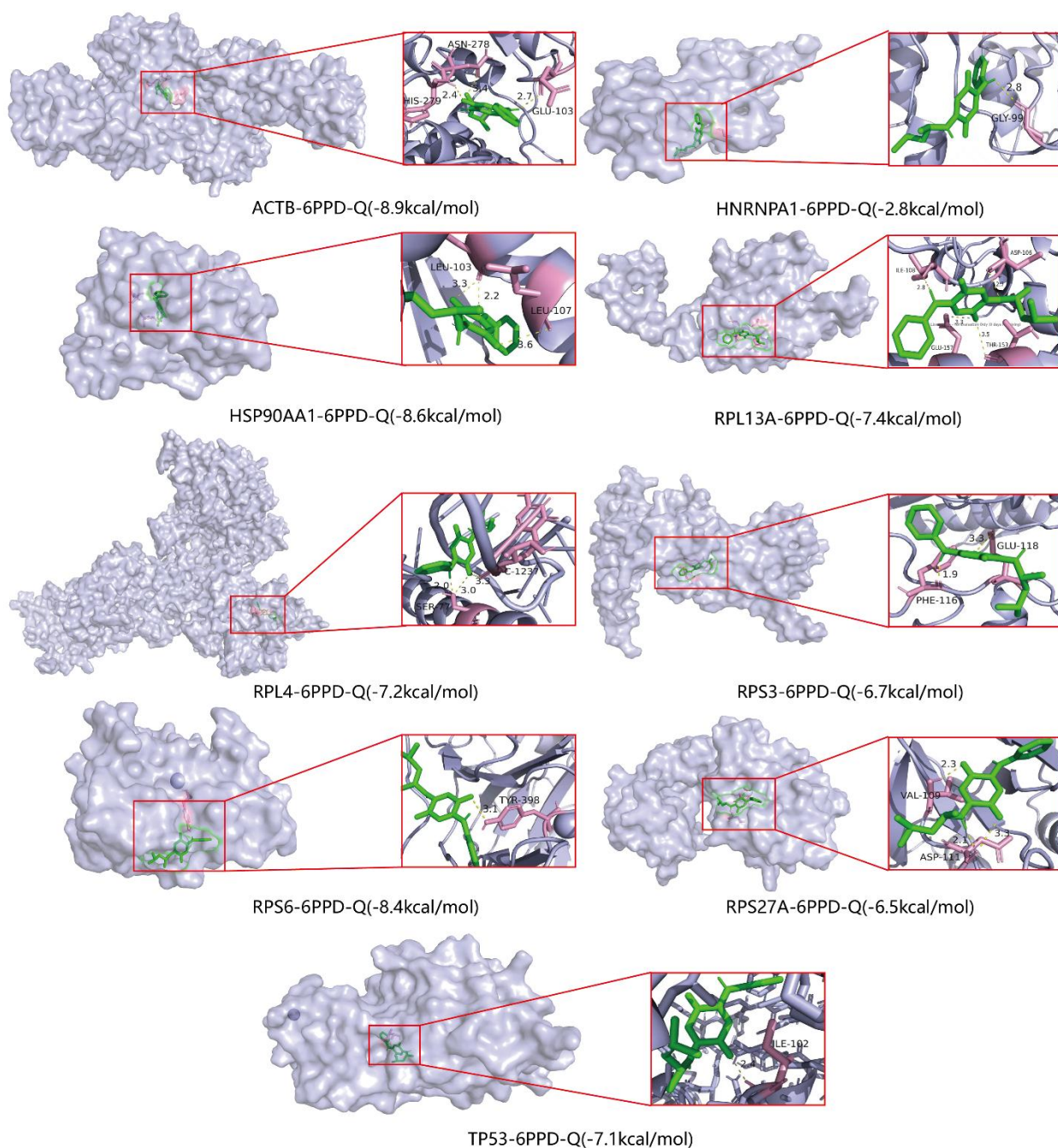


Figure 8. Visualization of the lowest binding energy during molecular docking between the core target protein and 6PPD-Q. Each subfigure shows docking details of the entire protein structure as well as a local zoom-in, highlighting key amino acid residues and their interaction distances (Å) with the 6PPD-Q molecule.