Integrative bioinformatics approach for identifying key genes and potential therapeutic targets in the concurrent manifestation of hypertrophic cardiomyopathy and pulmonary hypertension

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Background: Hypertrophic cardiomyopathy (HCM), identified as a primary cause of sudden cardiac death (SCD), intertwines with pulmonary hypertension (PH) to amplify cardiovascular morbidity. This complex synergy poses significant therapeutic challenges due to the absence of drugs specifically targeting their concurrent manifestation. This study seeks to unravel the molecular intricacies linking HCM and PH, aiming to lay the groundwork for targeted therapeutic interventions.

Methods: Through the analysis of gene expression profiles from datasets GSE36961 (HCM) and GSE113439 (PH) within the public data repository of Gene Expression Omnibus (GEO), this research systematically identified differentially expressed genes (DEGs), conducted extensive functional annotations, and constructed detailed protein-protein interaction (PPI) networks to uncover crucial hub genes. Further, co-expression analyses, alongside drug prediction and molecular docking simulations, were employed to pinpoint potential therapeutic agents that could ameliorate the combined pathology of HCM and PH.

Results: Our comprehensive analysis unearthed 79 DEGs shared between HCM and PH, highlighting fourteen as pivotal hub genes. Validation across three additional datasets (GSE35229, GSE32453, and GSE53408) from GEO accentuated secreted phosphoprotein 1 (*SPP1*) as a key gene of interest. Remarkably,

the study identified tacrolimus, ponatinib, bosutinib, dasatinib, doxorubicin, and zanubrutinib as promising drugs for addressing the dual challenge of HCM and PH.

Conclusions: The findings of this investigation shed light on the genetic underpinnings of HCM and PH's simultaneous occurrence, emphasizing the central role of *SPP1* in their pathogenesis. The identification of six candidate drugs offers a hopeful vista for future therapeutic strategies targeting this complex cardiovascular interplay, marking a significant stride towards mitigating the compounded morbidity of HCM and PH. Future mechanistic and clinical studies are warranted for the investigation of this potential target and therapeutics.

Keywords: Hypertrophic cardiomyopathy (HCM); pulmonary hypertension (PH); differentially expressed genes (DEGs); secreted phosphoprotein 1 (*SPP1*)

Introduction

Hypertrophic cardiomyopathy (HCM) is a significant cause of sudden cardiac death (SCD), particularly prevalent among adolescents and young adults. This complex condition arises from interactions among various cell types, leading to a characteristic pathological state (1). Globally, unexplained left ventricular hypertrophy (LVH), a hallmark of HCM, is observed in approximately one in every 500 adults, representing 0.2% of the general population. This finding has been consistently reported across diverse regions, including the USA, Europe, Asia, and East Africa (2). The primary anatomical feature of HCM is asymmetric LVH, predominantly affecting the interventricular septum more than the left ventricular free wall (3-5). This hypertrophy stems from an increase in myocyte size rather than myocyte number and is accompanied by extensive myocardial fibrosis. The fibrotic changes are not only widespread throughout the myocardial interstitium but also manifest in discrete foci (6). Clinically, HCM presents with a spectrum of symptoms, including amaurosis, syncope, and chest tightness due to outflow tract obstruction. Other manifestations include chest pain, often attributed to relative myocardial ischemia and hypoxia, and palpitations, commonly resulting from atrial fibrillation or other malignant arrhythmias (7). If left unaddressed, HCM may progress to heart failure (HF) and is a potential precursor to SCD (8). This progression underscores the critical need for early detection and effective management of this condition.

While echocardiography and other imaging techniques can diagnose HCM by detecting a left ventricular end-diastolic wall thickness exceeding 13 mm, these methods present several limitations (9). Differentiating HCM from ventricular hypertrophy due to pressure overload can be challenging with imaging alone, as both conditions may appear similar (10). Notably, cardiac hypertrophy in HCM can have a late onset and may not reach the 13 mm diagnostic threshold (11). Moreover, a subset of patients exhibits a positive genotype for HCM but lacks corresponding phenotypic expressions (12), suggesting that the phenotype may emerge later or remain unexpressed

Highlight box

Key findings

• The current research successfully pinpointed *SPP1* as a crucial gene influencing the progression of hypertrophic cardiomyopathy (HCM) in tandem with pulmonary hypertension (PH), and identified potential drugs targeting this combined disease manifestation.

What is known and what is new?

• PH has been recognized as an independent risk factor contributing to the incidence rate of HCM. Recent studies have begun to unravel the underlying mechanisms potentially linking the co-occurrence of HCM with PH.

• The clinical mechanism underlying the concurrent manifestation of HCM and PH remains elusive, with no targeted therapeutic drugs currently available for treatment.

What is the implication, and what should change now?

• Our research offers initial insights into the genetic factors linked to the co-occurrence of HCM and PH, and proposes potential therapeutic drugs for treating HCM patients with PH in a clinical setting.

3154

Zheng et al. Key genes and therapeutic targets in HCM and PH

in some individuals. Gene detection plays a crucial role in HCM diagnosis. Research indicates that about 60% of HCM cases result from mutations in eight sarcomere-associated genes: *MYH7*, *MYBPC3*, *ACTC1*, *TPM1*, *MYL2*, *MYL3*, *TNNI3*, and *TNNT2* (13). Furthermore, mutations in non-sarcomeric protein genes have been identified in approximately 5% of patients with unexplained HCM by a molecular genetic study (14). Variability in phenotype expression among first-degree relatives of HCM patients highlights the potential influence of modifier genes, a domain that remains inadequately explored (15,16). This genetic complexity underscores the need for comprehensive genetic analysis in the assessment and management of HCM.

Pulmonary hypertension (PH) is clinically defined by a resting mean pulmonary artery pressure of 20 mmHg or higher (17-19). The World Health Organization categorizes PH into five distinct groups: pulmonary arterial hypertension (PAH), PH due to left heart disease, PH caused by chronic lung conditions, chronic thromboembolic PH (CTEPH), and PH resulting from unclear or multifactorial causes (17). Common symptoms associated with PH encompass exertional dyspnea, fatigue, weakness, anginal pain, precursors to syncope, and syncope itself (20). Diagnostic routines for suspected PH cases typically include electrocardiography, chest radiography, and pulmonary function tests (21). PH, a life-threatening condition, is linked to elevated mortality rates across all its classifications and etiologies (22). It is estimated to affect about 1% of the global population, with over half of HF patients potentially impacted (21). The coexistence of PH in patients with HCM is recognized; however, its clinical implications, pathophysiological mechanisms, effect on disease progression, response to therapies, and prognostic outcomes have only recently started to be understood (23-25). HCM concurrent with PH is known to elevate risks for thromboembolic events, atrial fibrillation, and HF (25). A study indicated that HCM patients with PH face higher incidence rates and mortality than those without PH, establishing PH as an independent risk factor for adverse outcomes in HCM (23). Current research offers preliminary insights into the mechanisms underlying the combination of obstructive and non-obstructive HCM with PH, noting asymmetric ventricular septal hypertrophy and diastolic dysfunction due to ventricular non-compliance, coupled with varying degrees of mitral regurgitation. In obstructive HCM, the heightened atrial pressure results from left ventricular outflow tract (LVOT) obstruction leading to

diastolic dysfunction and mitral regurgitation. Conversely, in non-obstructive HCM, the primary cause of increased atrial pressure is diastolic dysfunction (24,26). The interplay between post-capillary and precapillary pressures also plays a role in HCM patients with PH (27). Nevertheless, the full scope of the underlying mechanisms and prognostic significance of the concurrent occurrence of HCM and PH, particularly in terms of targeted gene identification, remains an area necessitating further exploration.

In our comprehensive analysis, we meticulously examined two robust datasets, GSE36961 and GSE113439, to identify key genetic players in the overlapping pathology of HCM and PH. Through this examination, we pinpointed fourteen pivotal hub genes. To further validate these findings, we utilized three additional datasets, GSE35229, GSE32453 and GSE53408, reinforcing the significance of our initial observations. Notably, among these hub genes, *SPP1* emerged as the most significant differentially expressed gene (DEG), suggesting its pivotal role in the joint manifestation of HCM and PH. Delving deeper, we embarked on drug-gene interaction (DGI) analyses to unlock the therapeutic potential of *SPP1*. This included evaluating the affinity and interaction between small molecule drugs and the proteins encoded by *SPP1* through advanced molecular docking techniques. Our exploratory efforts have not only shed light on the genetic landscape underlying HCM and PH but have also paved the way for innovative therapeutic strategies. By highlighting *SPP1*'s role and identifying potential drug candidates, this study marks a significant stride towards developing tailored treatment options for patients suffering from the dual burden of HCM and PH. We present this article in accordance with the STREGA reporting checklist (available at https://jtd.amegroups.com/article/view/10.21037/jtd-23-1822/rc).

Methods

Data acquisition

Gene expression profiles (GSE36961, GSE35229, GSE113439, GSE53408) were downloaded from Gene Expression Omnibus (GEO) database (http://www.ncbi.nlm.nih.gov/geo/). The test samples of these gene expression profiles were of human origin. GSE36961 and GSE113439 were obtained to screen DEGs, while GSE35229 and GSE53408 were obtained to validate DEGs. GSE36961 contained 106 HCM and 39 non-HCM samples. GSE113439 contained fifteen PH and eleven

non-PH samples. In the validation dataset, nine HCM and two non-HCM samples were obtained in GSE35229. GSE53408 dataset was generated from PH (twelve samples) and matched unaffected tissue (eleven samples). We added another gene expression profile (GSE32453) to further validate DEGs. GSE32453 contained eight HCM and three non-HCM samples. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Identification of DEGs

GEO2R based on R language "limma" package, is a function recently updated by the GEO. It was used to screen DEGs by calculating and visualizing the differential expression multiple [settings: P<0.05, absolute fold change (FC) ≥1.5]. Volcano plot and hierarchical clustering were analysed and performed by the Hiplot platform (https://hiplot.cn/), which was an online diagram tool.

Functional enrichment analysis

Gene Ontology (GO, http://www.geneontology.org) and Kyoto Encyclopaedia of Genes and Genomes database (KEGG, http://www.genome.jp/kegg/) was used to classify the identified genes. Biological process (BP), molecular function (MF) and cellular component (CC) were identified as well as the pathways associated with the DEGs in the most significant modules.

Establishment of protein-protein interaction (PPI) network of DEGs

Search Tool for the Retrieval of Interacting Genes/Proteins database (STRING; version 12.0; http://string-db.org/) was used to construct PPI networks, which was a database for online analysis of protein interactions. Among these interactions, PPI plays a particularly important role due to their versatility, specificity, and adaptability.

Filtering and analysis of hub genes

The PPI networks were analysed by Cytoscape (version 3.6.1). According to the network topology, hub genes were screened by CytoHubba plug-in of Cytoscape. Maximal Clique Centrality (MCC), Maximum Neighborhood Component (MNC), Degree, Stress and Betweenness algorithms were used to identify hub genes.

Construction of a co-expression network and module analysis

GeneMANIA (http://www.genemania.org/) was used to construct co-expression networks of hub genes. GeneMANIA is a reliable tool for inferring gene function and conducting functional analysis of hub genes.

Validation of hub genes

The GSE35229 and GSE53408 datasets were applied to confirm the expression of the selected hub genes. The GSE35229 dataset consisted of nine HCM and two non-HCM (NHCM) samples. GSE53408 consisted of twelve PH tissue and eleven non-PH (NPH) tissue samples. Another gene expression profile (GSE32453) was downloaded to further validate DEGs. GSE32453 contained eight HCM and three non-HCM samples. Normality testing was initially conducted on the expression levels of the obtained hub genes. Heatmaps and bean plots were used for visual analysis.

DGI of potential genes

The DGI database (DGIdb: http://www.dgidb.org) was used to explore that how mutant genes can be targeted for therapy or drug development according to exploit the available resources. The significant genes, as the potential targets, were pasted into the drug-gene database to search for existing drugs or compounds. These potential genes with matching drugs were obtained.

The correlation between drugs and diseases was measured by network distance (28,29). Based on the drug target pairs in the Drugbank database and the PPI network (threshold score of 400) in the STRING database, the similarity between drugs and diseases was calculated. We used a similar method to evaluate distance between drugs and HCM with PH. Here, we gave S (the set of hub genes), D (degree of hub genes nodes in PPI), T (the set of drug target genes), and distance $d(s,t)$ the shortest path length between s (HCM with PH related genes in our case, $s \in S$) and t (drug target in our case, $t \in T$ as below:

$$d(s,T) = \frac{1}{|T|} \sum_{t \in T} \min_{s \in S} \left[d(s,t) + \omega \right] \quad [1]$$

where ω, the weight of a target gene, was defined as $\omega = -\ln(D+1)$ if the target was one of the HCM with PH

related genes; else, $\omega = 0$.

To evaluate the importance of the association between drugs and HCM with PH, we generated a simulated reference distance distribution corresponding to drugs. A set of proteins (represented as R) matching the size of drug targets were randomly selected in the network. Then the distance $d(S,R)$ was calculated between these simulated drug targets (represented simulated drugs) and HCM with PH related genes (defined by Eq. [1]). A reference distribution was generated by repeating the process 10,000 times. Average of reference distribution $\mu_d(S,R)$ and standard deviation $\sigma_d(S,R)$ was used to convert the observation distance corresponding to the real drug into a normalized distance, which is the approximate value:

$$z(S,T) = \frac{d(S,T) - \mu_d(S,R)}{\sigma_d(S,R)} \qquad [2]$$

We conducted visual analysis through online website (http://www.sxdyc.com/drugGeneset).

Molecular ligand docking analysis

The crystal structures of target proteins were downloaded from the RCSB Protein Data Bank (https://www.rcsb.org/) and Uniprot (https://www.uniprot.org/). Molecular Operating Environment (MOE, version 2022.02) software was used for molecular docking. The binding activities of small molecule drugs and target proteins were evaluated based on docking energy values. Score <−5 was considered a significant combination.

Statistical analysis

In this investigation, bioinformatics statistical evaluations were conducted utilizing the R programming environment (version 4.3.0) and the other aforementioned online analytical tools. A P value threshold of less than 0.05 was set to denote statistical significance.

Results

Identification of DEGs and functional enrichment analysis

A total of 629 DEGs were identified in GSE36961, in which 244 genes were upregulated while 385 genes were downregulated. A total of 3,113 DEGs were filtered from GSE113439. Volcano maps showed the total number of

upregulated and downregulated DEGs (*Figure 1A,1B*). GO analysis showed that the DEGs between HCM group and NHCM group were significantly enriched in "inflammatory response", "positive regulation of inflammatory response", "extracellular space", "extracellular exosome", "protein binding" and "RAGE receptor binding" (*Figure 1C*). KEGG pathway enrichment analysis showed that the DEGs mainly played a role in pathways about "Phagosome", "Apoptosis" and "Gap junction" in the HCM/NHCM comparison (*Figure 1C*). In the PH/NPH comparison, DEGs were closely related to "cellular response to DNA damage stimulus", "protein transport", "cytosol", "nucleoplasm", "protein binding" and "RNA binding" (*Figure 1D*). KEGG pathway enrichment analysis showed that DEGs in PH/NPH comparison were mainly clustered in "Nucleocytoplasmic transport", "NOD-like receptor signalling pathway" and "Cell cycle" (*Figure 1D*).

Functional enrichment analysis of common DEGs

Subsequently, a total of 79 DEGs were found to be common between HCM and PH using the Venn diagram (*Figure 2A*). Heatmap showed the expression of the 79 DEGs in HCM/NHCM group and PH/NPH group (*Figure 2B*). To further understand the function of the common DEGs, we performed GO and KEGG enrichment analysis. GO analysis showed that the common DEGs were significantly enriched in "positive regulation of tumor necrosis factor production", "platelet-derived growth factor receptor signalling pathway", "extracellular space", "extracellular matrix", "extracellular matrix structural constituent" and "receptor binding" (*Figure 2C*), while KEGG pathway enrichment analysis showed that the common DEGs were associated with "TGF-beta signalling pathway", "Focal adhesion" and "PI3K-Akt signalling pathway" (*Figure 2C*). These 79 common DEGs may be related to the simultaneous occurrence of HCM and PH.

### Construction of PPI network and identification of hub genes

The PPI network was constructed by STRING to explore the interactions between proteins encoded by those common DEGs (*Figure 3A*). We found that 79 common DEGs formed a complex interaction network, including 79 nodes and 101 edges. The average node degree was 2.56, and the local clustering coefficient was 0.372. The expected number of edges for this analysis was 37. Moreover, the

3158

Zheng et al. Key genes and therapeutic targets in HCM and PH
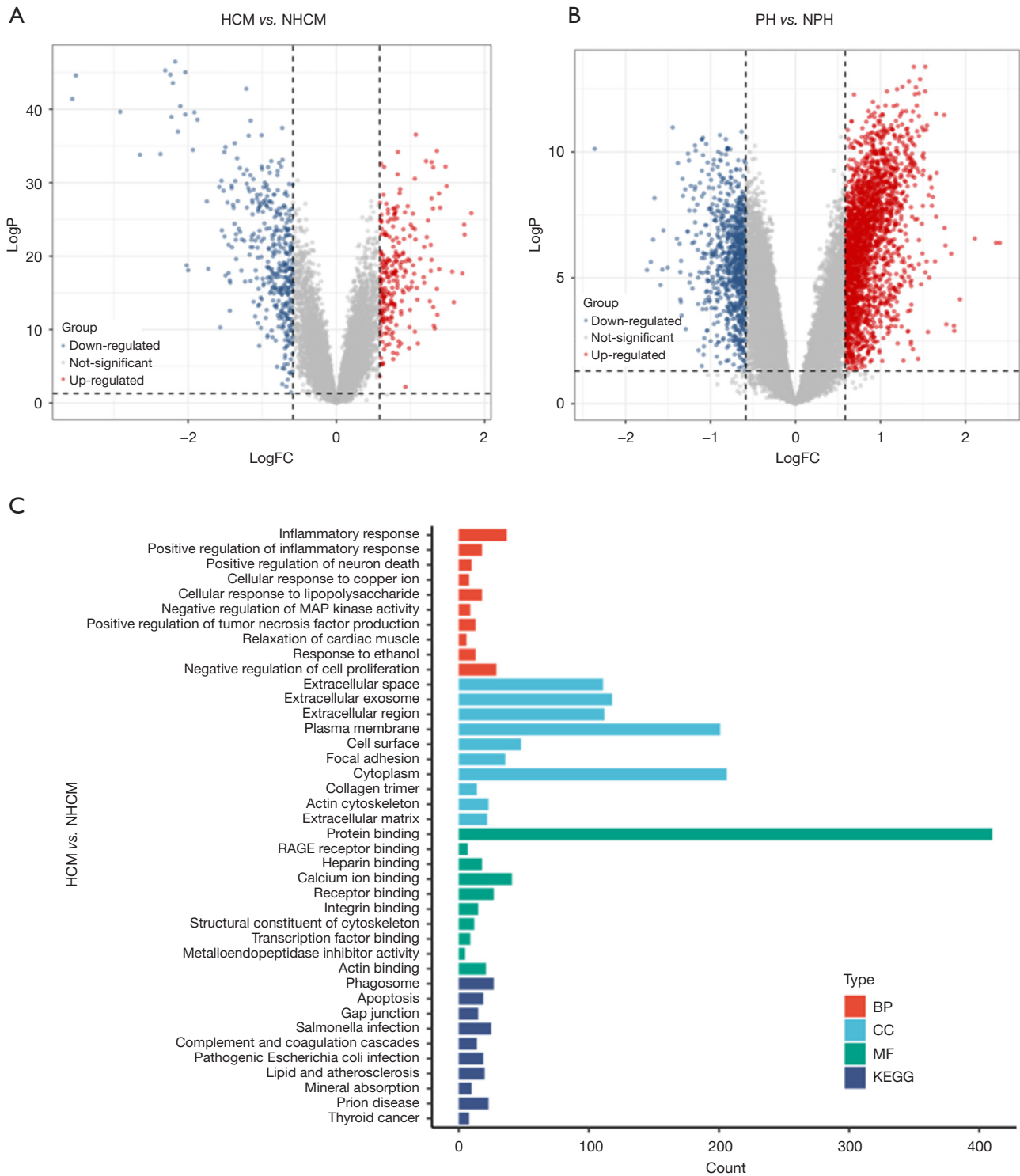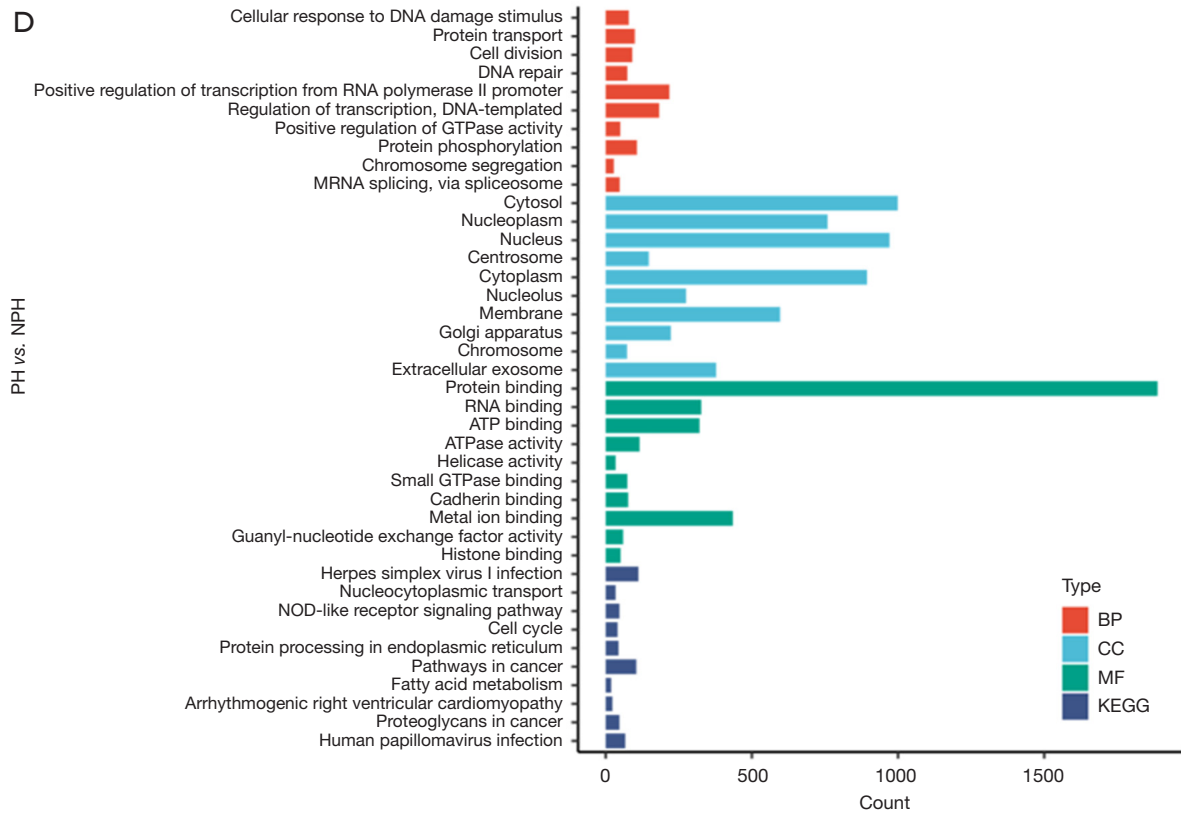
Figure 1 Enrichment analysis of DEGs in HCM/NHCM and PH/NPH. (A) Volcano plot of DEGs in HCM/NHCM; (B) Volcano plot of DEGs in PH/NPH; (C) GO and KEGG analysis of DEGs in HCM/NHCM; (D) GO and KEGG analysis of DEGs in PH/NPH. HCM, hypertrophic cardiomyopathy; NHCM, non-HCM; FC, fold change; PH, pulmonary hypertension; NPH, non-pulmonary hypertension; BP, biological process; CC, cellular component; MF, molecular function; KEGG, Kyoto Encyclopedia of Genes and Genomes; MRNA, messenger RNA; DEGs, differentially expressed genes; GO, Gene Ontology.
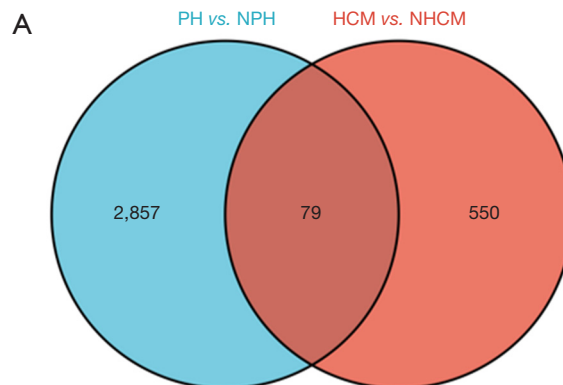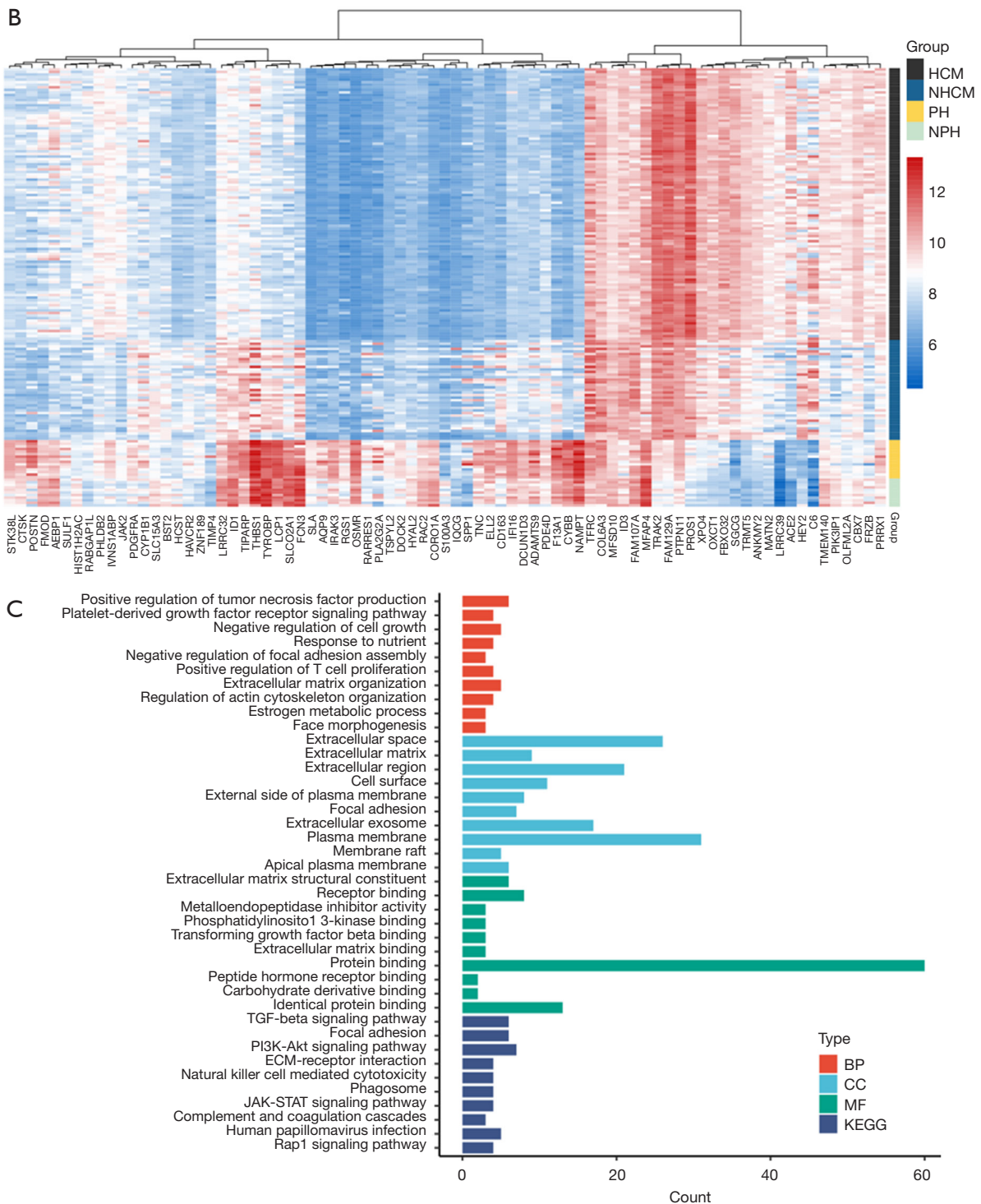
Figure 2 Identification of DEGs between HCM/NHCM and PH/NPH. (A) Venn diagram of overlap of common DEGs between HCM/NHCM and PH/NPH; (B) the heatmap and cluster analysis of common DEGs. Upregulated DEGs were in red, and downregulated DEGs were in blue. (C) GO and KEGG analysis of common DEGs. PH, pulmonary hypertension; NPH, non-pulmonary hypertension; HCM, hypertrophic cardiomyopathy; NHCM, non-HCM; BP, biological process; CC, cellular component; MF, molecular function; KEGG, Kyoto Encyclopedia of Genes and Genomes; TGF, transforming growth factor; ECM, extracellular matrix; DEGs, differentially expressed genes; GO, Gene Ontology.
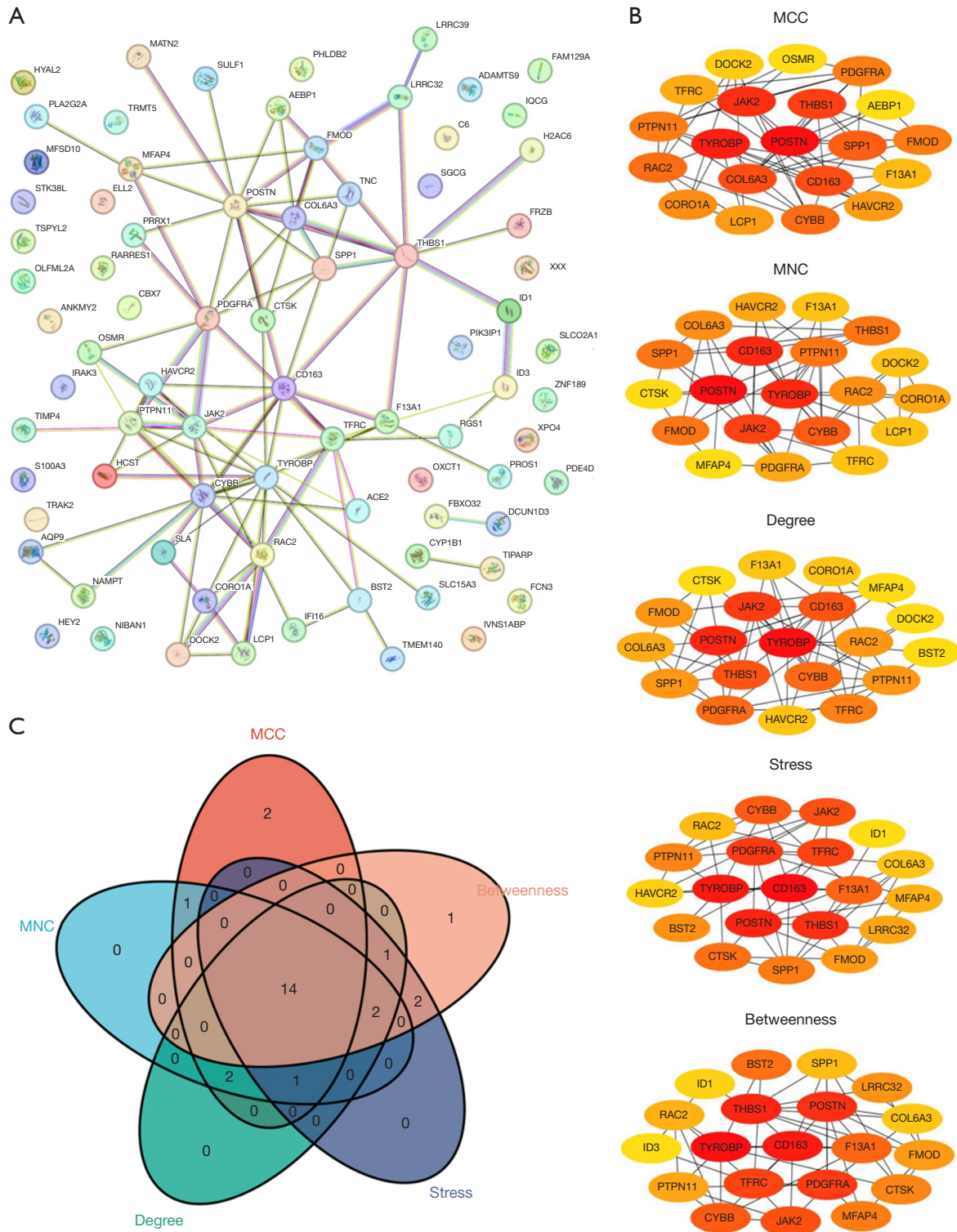
Figure 3 Construction of PPI network and identification of hub genes. (A) Mapping of DEGs onto a composite network based on predicted PPI; (B) the top 20 DEGs were screened by MCC, MNC, Degree, Stress and Betweenness algorithms of the cytoHubba plug-in; (C) Venn diagram of overlap of 20 DEGs among 5 algorithms. MCC, Maximal Clique Centrality; MNC, Maximum Neighborhood Component; PPI, protein-protein interaction; DEGs, differentially expressed genes.

PPI enrichment P value was less than $1.0×e^{-16}$, which indicated that the common DEGs were at least partially biologically connected as a group. Then the PPI network file was subsequently imported in Cytoscape. The top 20 DEGs were screened by MCC, MNC, Degree, Stress and Betweenness algorithms of the cytoHubba plug-in. We focused on the pivotal hub genes identified by five distinct, yet complementary, bioinformatics algorithms. This rigorous, multi-dimensional approach led us to the discovery of fourteen shared hub genes including *POSTN*, *TYROBP*, *JAK2*, *THBS1*, *COL6A3*, *CD163*, *SPP1*, *CYBB*, *RAC2*, *PTPN11*, *FMOD*, *PDGFRA*, *TFRC* and *F13A1* (*Figure 3B,3C*).

The co-expression network and related functions of hub genes were revealed through the GeneMANIA database. The complex PPI network showed 50.56% co-expression, 26.63% physical interaction, 20.67% prediction, 2.10% co-localization, and 0.04% shared protein domains (*Figure 4A*). GO analysis showed that in terms of biological processes, the most important GO terms were "positive regulation of tumor necrosis factor production" and "platelet-derived growth factor receptor signalling pathway". In terms of cell components, it mainly involved extracellular components. GO terms about molecular functions were "protein kinase binding" and "peptide hormone receptor binding" (*Figure 4B*). KEGG pathway enrichment analysis showed that 14 hub genes significantly enriched in fifteen signalling pathways, which were closely related to "Focal adhesion", "PI3K-Akt signalling pathway", "ECM-receptor interaction", TGF-beta signalling pathway", "Leukocyte transendothelial migration" and "Natural killer cell mediated cytotoxicity" (*Figure 4B*).

Validation of hub genes

Among the fourteen hub genes, the expression of the five genes including *POSTN*, *SPP1*, *COL6A3*, *PDGFRA* and *RAC2* were found to be statistically significant in two other validation databases (*Figure 4C*), in which *SPP1* was the sole most significant DEG (P<0.05 and absolute FC ≥1.5) in both GSE35229 and GSE53408 compared to healthy tissue (*Figure 4D-4G*). GSE32453 was added to illustrate the important role of *SPP1*, which was consistent with the results (Figure S1). Therefore, our findings highlight that *SPP1* can be a crucial gene closely associated with the co-morbidity of HCM and PH.

### DGI analysis of potential gene

SPP1 was loaded to DGIdb to search for potential drugs. We found that *SPP1* had interactions with some exist small molecule drugs (*Table 1*). To figure out the relationship between drug targets and the disease protein (osteopontin, OPN, encoded by *SPP1*), we applied relative proximity values to quantitatively measure the network-based relationship between drugs and disease related protein. Through this procedure, we excluded those irrelevant drugs with HCM combined with PH in the network and constructed a rank list of the proximal drugs (*Figure 5*, *Table 2*). We then selected the top ten drugs with the shortest distance and five drugs obtained from the DGIdb for subsequent analysis.

The molecular docking landscape on potential drugs against HCM combined with PH

We searched for the optimal conformation for the interaction between small molecule drugs and target protein through molecular docking. We downloaded the crystal structure of OPN (Uniprot ID: P10451) from the RCSB Protein Data Bank. MOE was used for molecular docking. Tacrolimus, ponatinib, bosutinib, dasatinib, doxorubicin and zanubrutinib were revealed as the top six candidate drugs to have a therapeutic potential on HCM combined with PH (*Figure 6*, *Table 3*).

Discussion

HCM is a key risk factor for HF, characterized by abnormal myocardial enlargement due to increased myocardial cell size and non-myocyte proliferation (30,31). It is widely acknowledged that various mutations in gene coding regions may potentially cause HCM (32). Therefore, employing bioinformatics to screen, analyze, and identify significant genes for the early diagnosis and treatment of HCM is of paramount importance. PH serves as an independent predictor of all-cause mortality in both obstructive and non-obstructive HCM cases. Pulmonary artery systolic pressure (PASP), as a non-invasive and readily accessible method, plays a crucial role in estimating pulmonary artery pressure. Elevated PASP levels are known to predict mortality in HCM patients and aid in stratifying embolism risk (25). In a pivotal study, Ahmed *et al.* explored the prevalence of PH in obstructive HCM patients undergoing septectomy,

3162

Zheng et al. Key genes and therapeutic targets in HCM and PH
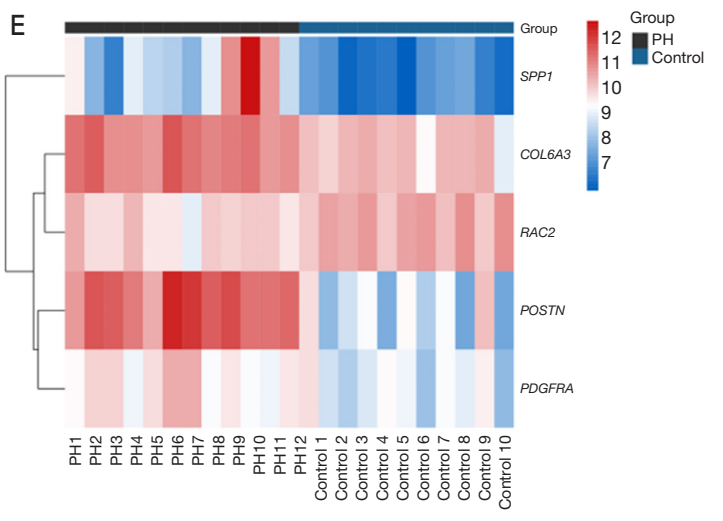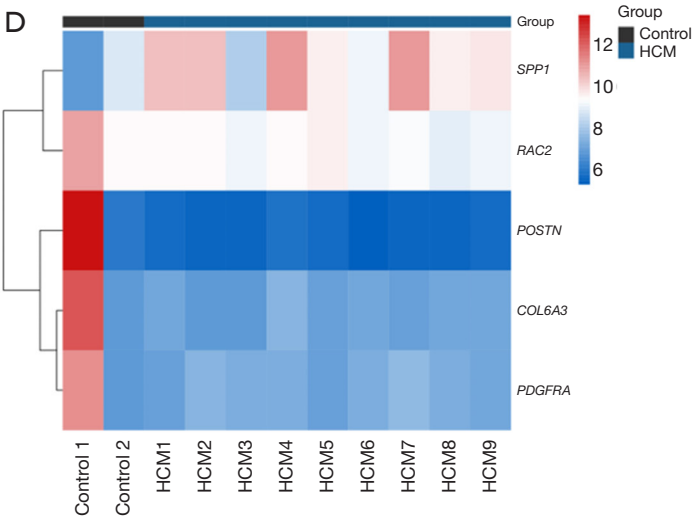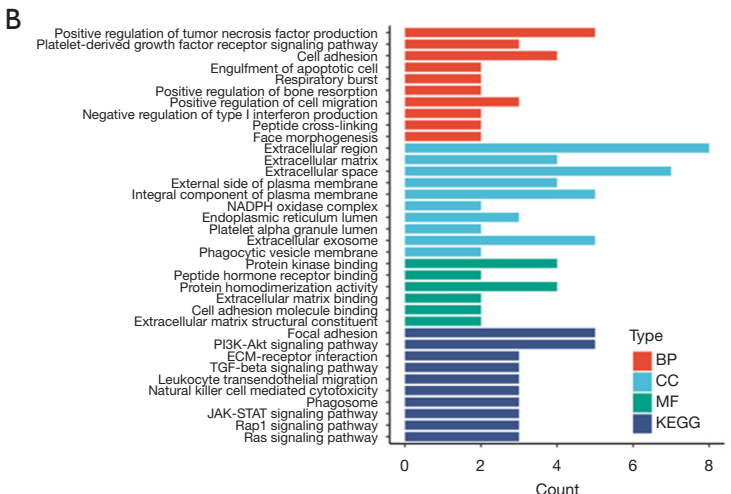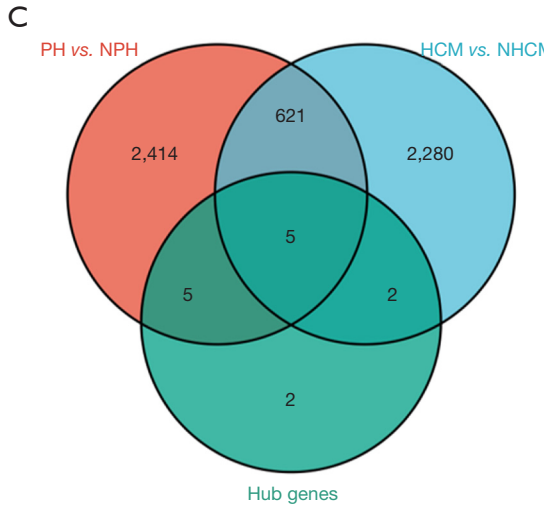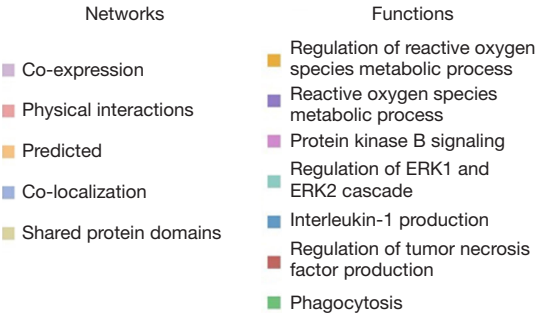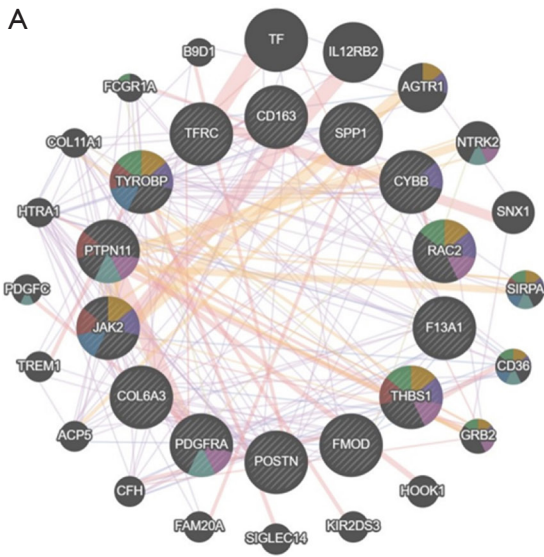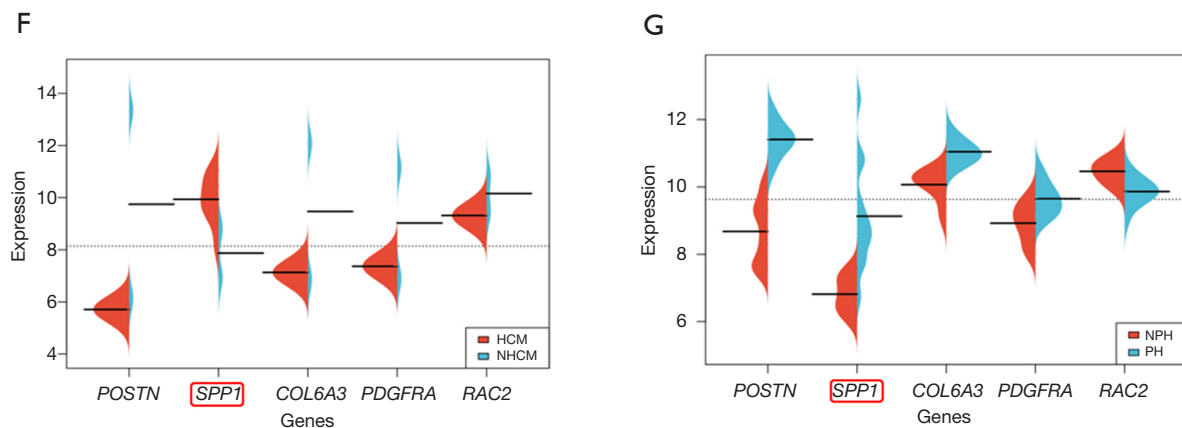
Figure 4 The co-expression network and validation of hub genes. (A) The co-expression network and related functions of hub genes; (B) GO and KEGG analysis of hub genes; (C) Venn diagram of overlap among hub genes and two other validated datasets; (D) the heatmap and cluster analysis of *POSTN*, *SPP1*, *COL6A3*, *PDGFRA* and *RAC2* in GSE35229. Upregulated DEGs were in red, and downregulated DEGs were in blue; (E) the heatmap and cluster analysis of *POSTN*, *SPP1*, *COL6A3*, *PDGFRA* and *RAC2* in GSE53408. Upregulated DEGs were in red, and downregulated DEGs were in blue; (F) the expression of *POSTN*, *SPP1*, *COL6A3*, *PDGFRA* and *RAC2* in GSE35229; (G) the expression of *POSTN*, *SPP1*, *COL6A3*, *PDGFRA* and *RAC2* in GSE53408. ECM, extracellular matrix; TGF, transforming growth factor; BP, biological process; CC, cellular component; MF, molecular function; KEGG, Kyoto Encyclopedia of Genes and Genomes; PH, pulmonary hypertension; NPH, non-pulmonary hypertension; HCM, hypertrophic cardiomyopathy; NHCM, non-HCM; GO, Gene Ontology; DEGs, differentially expressed genes.

Table 1 The DGI of potential genes

Gene	Drugs	Mechanism	Interaction score
SPP1	Gentamicin	Gentamicin is an aminoglycoside used to treat a wide variety of aerobic infections in the body	0.98
	Wortmannin	Wortmannin is a steroid metabolite of *Penicillium funiculosum* and *Talaromyces wortmannii* fungi. This drug acts as a nonspecific, covalent inhibitor of PI3Ks	0.74
	Tacrolimus	Tacrolimus is a calcineurin inhibitor used to prevent organ transplant rejection and to treat moderate to severe atopic dermatitis	0.61

DGI, drug-gene interaction; PI3Ks, phosphoinositide 3-kinase enzymes.

investigating their survival rates and the postoperative progression of PH. The study unveiled that preoperative PH independently correlates with increased late mortality post-septectomy, and the magnitude of preoperative right ventricular systolic pressure aligns with reduced postoperative pulmonary pressure (33). Furthermore, Chakraborty *et al.* examined the influence of PH on hospitalization outcomes among HCM patients admitted for acute decompensated HF or cardiogenic shock. Their findings suggest that PH in HCM patients is linked with heightened incidence rates, including an increased risk of transient ischemic attack and respiratory failure (34). Collectively, these insights affirm PH as a recognized complication of HCM and a strong mortality predictor.

The utilization of bioinformatics for identifying key genes in HCM combined with PH is not only pivotal for diagnosis and treatment but also enhances our comprehension of the disease's pathogenesis. A notable case report involving a male Caucasian patient with recurrent c.1168G>A (p.Ala390Thr) and a novel biallelic missense variant c.2758T>C (p.Tyr920His) in the *VARS2* gene, as revealed by whole exome sequencing, exemplifies this. The patient presented with clinical symptoms of HCM and PH. The *VARS2* gene, known for encoding mitochondrial Valyl-tRNA synthase, saw an expansion in its mutation spectrum and phenotypic expression with this new discovery (35). Another gene, *TMEM70*, typically associated with nuclear adenosine triphosphate (ATP) synthase deficiency, leads

3164

Zheng et al. Key genes and therapeutic targets in HCM and PH
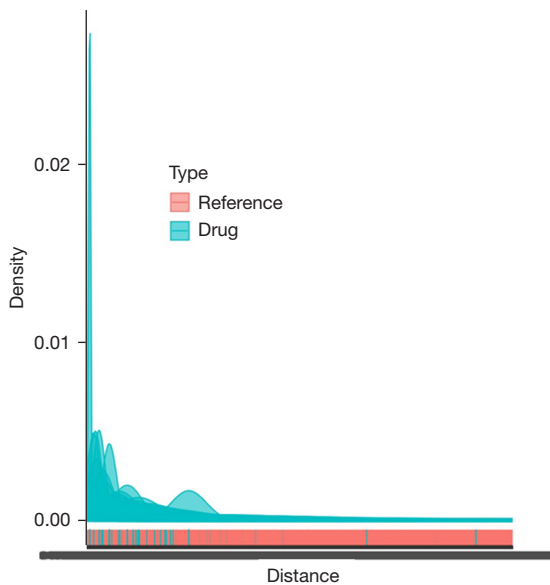
Figure 5 Proximity between drug targets and disease proteins (OPN, encoded by *SPP1*). OPN, osteopontin.

Table 2 The rank list of the proximal drugs

Drugs	Distances	P value	FDR
Bosutinib	1.13	7.06E−09	3.87E−05
Minocycline	1.37	2.48E−12	1.36E−08
Dextromethorphan	1.37	1.78E−13	9.78E−10
Ponatinib	1.40	1.09E−09	5.95E−06
Foreskin keratinocyte (neonatal)	1.40	1.62E−07	8.89E−04
Dasatinib	1.50	1.49E−10	8.17E−07
Zanubrutinib	1.53	1.58E−07	8.62E−04
Tamoxifen	1.56	7.32E−08	4.01E−04
Marimastat	1.61	3.11E−09	1.70E−05
Resveratrol	1.62	2.93E−10	1.60E−06
Acetylsalicylic acid	1.70	1.77E−08	9.68E−05
Zinc chloride	1.74	1.70E−21	9.34E−18
Zinc sulfate, unspecified form	1.74	1.70E−21	9.34E−18
Zinc	1.76	3.56E−26	1.96E−22
Zinc acetate	1.76	3.56E−26	1.96E−22
Guanosine-5'-diphosphate	1.77	2.09E−08	1.14E−04
Fostamatinib	1.84	5.97E−39	3.28E−35
Copper	1.84	3.10E−20	1.70E−16
Artenimol	1.89	2.48E−10	1.36E−06

FDR, false discovery rate.

to a distinct phenotype characterized by severe neonatal hypotonia, HCM, facial dysmorphisms, and acute lactate acidosis. Catteruccia *et al.* highlighted that severe persistent pulmonary hypertension of the newborn (PPHN) was observed in children with *TMEM70* deficiency. Intriguingly, PPHN can manifest in these children even in the absence of apparent cardiomyopathy, thus serving as an early indicator and diagnostic clue. This finding broadens both the clinical and genetic spectrum of the syndrome (36), emphasizing the complexity and the interconnectedness of genetic factors in HCM and PH.

In our integrative bioinformatics analysis, we identified *SPP1* as a key hub gene co-expressed in both HCM and PH. *SPP1* encodes OPN, a primary non-collagenous bone protein that tightly binds to hydroxyapatite, playing a crucial role in the mineralized matrix and cell-matrix interactions. As a cytokine, OPN is pivotal in type I immunity and is expressed in diverse tissues, including epithelia, kidneys, bones, and teeth, and is detectable in all bodily fluids such as blood (37). OPN is implicated in numerous diseases, including myocardial infarction, atherosclerosis, kidney injury, diabetes, and chronic inflammatory diseases, as observed in various animal models (38-41). Notably, OPN serves as a potent predictor of adverse outcomes in cardiovascular diseases (42-44). In myocardial infarction patients, macrophages infiltrating the myocardium are a major source of OPN (39). One previous study has shown

that macrophages in the hearts of injured neonates secrete significant amounts of OPN, stimulating cardiac cells to enhance scar formation, left ventricular remodeling, and cardiac function post-myocardial infarction (45). In the diseased heart, myocardial cells are also a primary source of OPN, with its expression elevated in hypertrophic myocardium (46). OPN regulates the activation of the p38 kinases and c-Jun N-terminal kinases (JNK), influencing myocardial hypertrophy development in response to chronic pressure overload in mice (47). Furthermore, plasma OPN levels are elevated in patients with coronary artery disease, inversely correlating with left ventricular ejection fraction (48-50). Plasma OPN and lymphocytes expressing OPN are associated with the severity of HF (51). Serum OPN levels also predict the incidence of ventricular fibrillation and tachycardia in chronic HF patients (52). Circulating OPN levels are linked to PH development in coronary heart disease patients (53) and increased OPN levels are reported in other heart diseases with concurrent PH and right
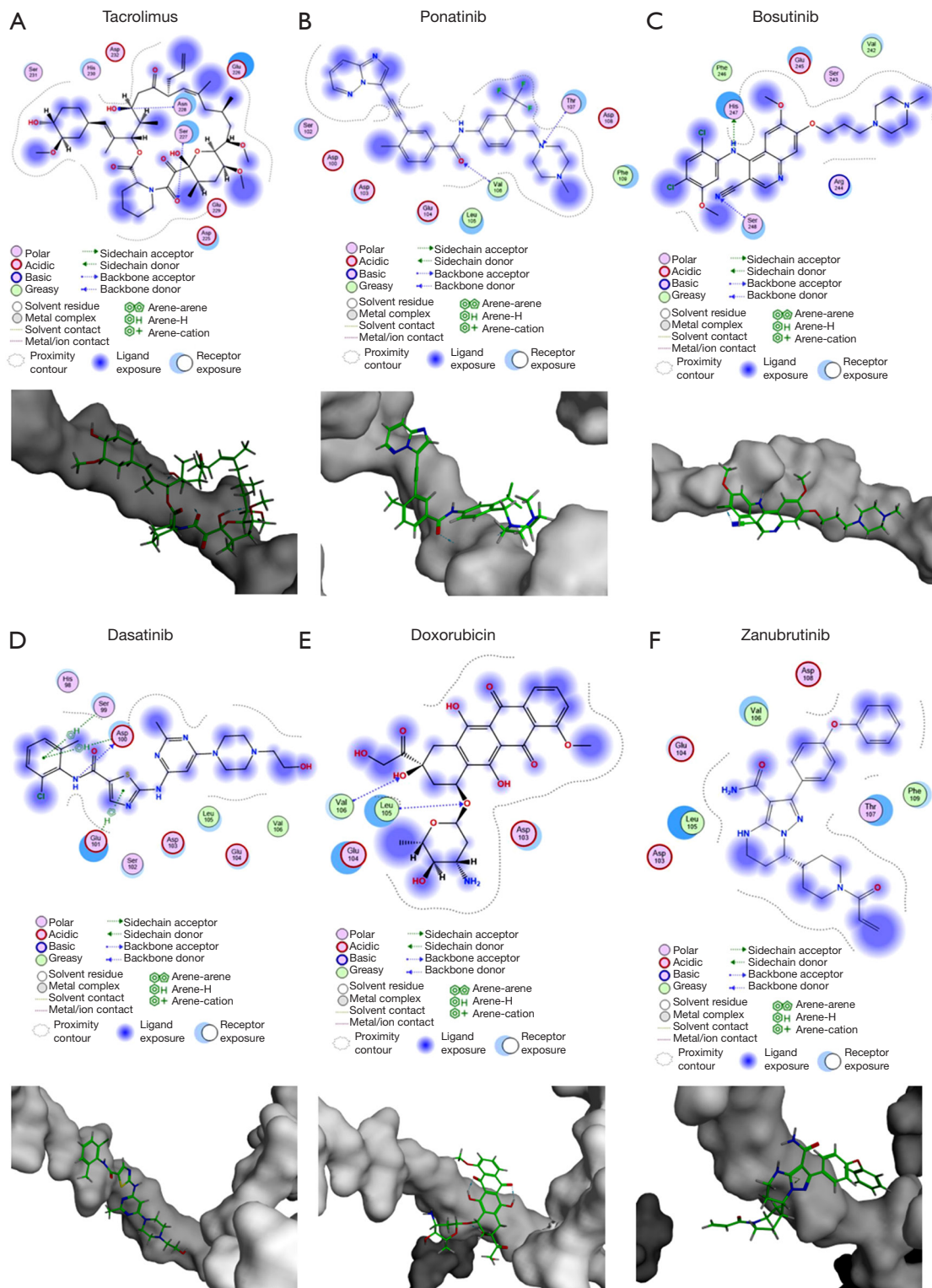
Figure 6 Binding sites and 3D binding patterns of OPN and small molecule drugs. (A) Binding sites and 3D binding patterns of OPN and tacrolimus; (B) binding sites and 3D binding patterns of OPN and ponatinib; (C) binding sites and 3D binding patterns of OPN and bosutinib; (D) binding sites and 3D binding patterns of OPN and dasatinib; (E) binding sites and 3D binding patterns of OPN and doxorubicin; (F) binding sites and 3D binding patterns of OPN and zanubrutinib. OPN, osteopontin.

3166

Zheng et al. Key genes and therapeutic targets in HCM and PH

Table 3 The results of MOE

Drugs	Score (OPN)
Tacrolimus	−6.7
Ponatinib	−5.95
Bosutinib	−5.87
Dasatinib	−5.85
Doxorubicin	−5.63
Zanubrutinib	−5.53
Idarubicin	−5.32
Gentamicin	−5.06
Minocycline	−4.89
Tamoxifen	−4.88
Wortmannin	−4.41
Marimastat	−4.36
Dextromethorphan	−4.24
Resveratrol	−4.24

MOE, molecular operating environment; OPN, osteopontin.

HF (54). Elevated circulating OPN can prognosticate right ventricular dysfunction and remodeling in PAH patients (55). The accumulating evidence underscores OPN's role as an effective biomarker and mediator for cardiovascular disease, PH progression, severity, and prognosis (37). Given that OPN is a secreted circulating protein, it offers the advantage of non-invasive assessment through peripheral blood sampling (56).

In our investigation, we utilized datasets GSE36961 and GSE113439 to identify DEGs associated with HCM and PH. After comprehensive functional annotation, hub gene identification, and co-expression analysis, we pinpointed fourteen hub genes. Among these, *SPP1* consistently demonstrated significant upregulation in GSE35229, GSE32453 and GSE53408 datasets, indicating its potential as a therapeutic target for HCM combined with PH. To explore treatment possibilities, we executed drug prediction and small molecule docking simulations. This led to the identification of several potential therapeutic agents, including tacrolimus, ponatinib, bosutinib, dasatinib, doxorubicin, and zanubrutinib. Tacrolimus, known for its immunosuppressive properties, has shown promise in preclinical models where activation of bone morphogenetic protein (BMP) signalling and pharmacological application of tacrolimus ameliorate right ventricular function by diminishing right ventricular afterload (57). Additionally, tacrolimus has stabilized conditions in patients with end-stage PAH (57). Ponatinib, bosutinib, and dasatinib, primarily used in the treatment of chronic myeloid leukemia, have also shown potential. For instance, low-dose dasatinib has been effective in improving HCM in Noonan syndrome with multiple lentigines (58). However, it is important to note that dasatinib may cause pulmonary vascular injury, induce endoplasmic reticulum (ER) stress, and elevate mitochondrial reactive oxygen species (ROS) production, thereby increasing the risk of developing PH (59). Zanubrutinib, utilized in treating B-cell malignancies such as chronic lymphocytic leukemia and small lymphocytic lymphoma (60), along with doxorubicin, an anti-tumor antibiotic that inhibits RNA and DNA synthesis, have also been identified as potential treatments (61). These drugs, selected based on molecular docking results, offer promising therapeutic avenues for HCM combined with PH, although their efficacy and safety need further validation in clinical settings.

While our study offers novel insights and potential therapeutic targets for HCM combined with PH, it also acknowledges certain limitations. The primary gene of interest, *SPP1*, along with the OPN protein it encodes, were identified through bioinformatics analysis. However, their efficacy as therapeutic targets requires further clinical validation. Additionally, the specific effectiveness of the identified drugs—selected through molecular docking simulations—on HCM combined with PH demands thorough clinical evaluation. While these drugs present promising avenues for treatment, their actual impact on patient outcomes in real-world clinical scenarios remains to be ascertained.

Conclusions

In conclusion, this integrative bioinformatics study establishes a foundational framework by pinpointing potential biomarkers and guiding the development of clinical interventions for HCM coexisting with PH. Our findings underscore the importance of *SPP1* as a crucial gene in the progression of this comorbidity. Furthermore, the drugs we identified, namely tacrolimus, ponatinib, bosutinib, dasatinib, doxorubicin, and zanubrutinib, emerge as promising candidates for targeting this specific pathological nexus. This research not only contributes to the understanding of the molecular interplay in HCM and PH but also opens avenues for future clinical trials to validate these potential therapeutic agents.

Footnote

Reporting Checklist: The authors have completed the STREGA reporting checklist. Available at https://jtd.amegroups.com/article/view/10.21037/jtd-23-1822/rc

Peer Review File: Available at https://jtd.amegroups.com/article/view/10.21037/jtd-23-1822/prf

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://jtd.amegroups.com/article/view/10.21037/jtd-23-1822/coif). X.B. reports that this work was partially supported by Shanghai Pujiang Program (No. 22PJ1412400 to X.B.), Science and Technology Development Fund of Shanghai Pudong New Area (No. PKJ2022-Y50 to X.B.), Young Clinical Investigator Training Program of Fudan University Shanghai Medical College (No. DGF828019-3 to X.B.), and Cultivation Program for Single-cell Sequencing Scientific Research of Shanghai Pudong Hospital (No. YJDXB2023-01 to X.B.). The other authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

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3168

Zheng et al. Key genes and therapeutic targets in HCM and PH

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