ARTICLE

Network module-based drug repositioning for pulmonary arterial hypertension

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Abstract

Pulmonary arterial hypertension (PAH) is a progressive disorder characterized by pulmonary vascular remodeling leading to increased pulmonary vascular resistance and pulmonary arterial pressure. PAH is a highly morbid cardiopulmonary disease adversely affecting lifespan and quality of life. Despite increased awareness and advances of medical therapies in recent decades, long-term prognosis and survival remain poor for patients with PAH. Novel therapies that can target the underlying pathobiology of PAH and reverse pulmonary vascular remodeling are clearly needed. In this study, we develop a network module-based framework to examine potential drug repositioning for PAH. The rationale for this approach is that in order to have therapeutic effects, the targets of potential drugs must be significantly proximate to the disease module of interest in the human protein-protein interactome. Based on 15 existing drugs for treating PAH, our framework integrates drug-drug interactions, drug-drug chemical similarity, drug targets, and PAH disease proteins into the human interactome, and prioritizes candidate drugs for PAH. We identified 53 drugs that could potentially be repurposed for PAH. Many of these candidates have strong literature support. Compared to black-box-like machine learning models, network module-based drug repositioning can provide mechanistic insights into how repositioned drugs can target the underlying pathobiological mechanisms of PAH.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?
Pulmonary arterial hypertension (PAH) is a highly morbid cardiopulmonary disease. Despite increased awareness and advances of medical therapies in recent decades, long-term prognosis and survival remain poor.
WHAT QUESTION DID THIS STUDY ADDRESS?
We developed a network module-based framework, which integrates drug-drug interactions, drug-drug chemical similarity, drug targets, and PAH disease proteins as a means to examine drug repositioning for PAH, and identify potential novel therapies that can target the underlying pathobiology of PAH.
WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

We found 53 drugs that could potentially be repurposed for PAH. Many, but not all, of these candidates have strong literature support.

HOW MIGHT THIS CHANGE DRUG DISCOVERY, DEVELOPMENT, AND/OR THERAPEUTICS?

This drug repositioning framework might save time and reduce the cost of the drug discovery process (for PAH) by prioritizing potential drug candidates that have been approved for other purposes, and, thus, are very likely to be safe.

INTRODUCTION

Pulmonary arterial hypertension (PAH) is a progressive disease characterized by an obliterative vasculopathy involving distal pulmonary arteries and arterioles that ultimately leads to right heart failure and premature death.[1] The exact cause of PAH is unknown, with no known cure for the disease. Even in the contemporary era, PAH is a leading cause of mortality and morbidity and adversely affects lifespan and quality of life in children and adults. In the past 2 decades, substantial advances have been made in medical therapies for patients with PAH, with more than 10 drugs developed and approved for the treatment of the disease.[2,3] These drugs largely serve to improve vascular and endothelial dysfunction and can help lessen symptoms and improve quality of life; however, long-term prognosis and survival remain poor, with mortality rates of 25–60% over 5 years.[4] Novel therapies that can target the underlying pathobiology of PAH and reverse pulmonary vascular remodeling are clearly needed.

Despite remarkable advances in understanding basic disease mechanisms in the modern genomic era, clinical translation of these findings has been far slower than expected.[5] The cost and length of time required for new drug development are escalating, and the number of new drug approvals annually remains limited, which increases the need for innovative approaches for drug discovery. Drug repositioning (also called drug repurposing) is a strategy for identifying new uses for existing drugs to treat a disease or condition outside of the scope of the original medical indication and regulatory approval.[6] One of the great benefits of drug repositioning is that the pharmacokinetics and safety of known drugs do not need to be reassessed, provided that doses remain in the approved range. A variety of methods have been developed for drug repositioning. Straightforward, unbiased computational strategies, for example, can predict new therapeutic indications for existing US Food and Drug Administration (FDA)-approved drugs that can then rapidly enter clinical trials.

Network-based approaches offer important insights into the relationships between drugs and diseases, including network-based drug repositioning.[7] Previous studies have shown that the genes (gene products) associated with a disease tend to be clustered in the same network neighborhood of the human protein-protein interactome, denoted a disease module.[8] For a drug to be therapeutic for a disease, it must target proteins in the vicinity of the corresponding disease module.[9,10] With a deeper understanding of the pathobiology of PAH, many associated disease genes have been discovered,[11] which provides a good opportunity to examine the possibility of repositioning some existing drugs for the treatment of the disease. In this study, we developed a network module-based drug repositioning framework for PAH. Based on 15 existing drugs for PAH, we integrate drug-drug interactions, drug-drug chemical similarity, drug targets, and PAH disease proteins into the human interactome, and prioritize candidate drugs for PAH. We identified 53 drugs that could potentially be repurposed for PAH. Such a network module-based drug repositioning framework enables deeper molecular-level understanding of drug activity for PAH and offers the promise of improved therapies that can be rapidly tested.

METHODS

Approved drugs for the treatment of PAH

We searched drugs.com, drug databases in the FDA and European Medicines Agency (EMA), DrugBank, Therapeutic Target Database (TTD), PharmGKB, and the literature, and found 15 approved drugs as targeted therapies for PAH (Table 1).

These drugs are classified into six categories: endothelin receptor antagonists (ambrisentan, bosentan, and macitentan), phosphodiesterase 5 inhibitors (tadalafil and sildenafil), parenteral prostanoids (epoprostenol, iloprost, and treprostinil), soluble guanylyl cyclase stimulators (riociguat), prostacyclin receptor agonist (selexipag), and calcium channel blockers and vasodilators. Among many calcium channel blockers and vasodilators,[12] we only included five that are widely used. We did not include supportive therapies, such as oxygen, diuretics, digoxin, and warfarin, as we focused on the network relationships between specific drug targets and PAH disease proteins. For a robustness examination, we also repeated the drug repositioning

TABLE 1 Approved drugs for the treatment of PAH

Drug ID	Drug name	ATC code	Source
Endothelin receptor antagonists			
DB06403	Ambrisentan	C02KX02	Literature,[2] FDA, EMA
DB00559	Bosentan	C02KX01	Literature,[2] FDA, EMA
DB08932	Macitentan	C02KX04	Literature,[2] FDA, EMA
Phosphodiesterase 5 inhibitors			
DB00820	Tadalafil	G04BE08	DrugBank, literature,[2] FDA
DB00203	Sildenafil	G04BE03; G01AE10	Literature,[2] FDA, EMA
Parenteral prostanoids			
DB01240	Epoprostenol	B01AC09	DrugBank, literature,[2] FDA
DB01088	Iloprost	B01AC11	Literature,[2] FDA, EMA
DB00374	Treprostinil	B01AC21	Literature,[2] FDA
Soluble guanylyl cyclase stimulators			
DB08931	Riociguat	C02KX05	DrugBank; FDA, EMA
Prostacyclin (IP) receptor agonist			
DB11362	Selexipag	B01AC27	Literature,[2] FDA, EMA
Calcium channel blockers and other vasodilators			
DB00797	Tolazoline	C04AB02; M02AX02	TTD, DrugBank
DB00343	Diltiazem	C05AE03; C08DB01	DrugBank[12]
DB00622	Nicardipine	C08CA04	DrugBank
DB01115	Nifedipine	C08CA05, C08CA55, C08GA01	DrugBank[12]
DB00381	Amlodipine	C09DB05, C09DX01, C09DB01	DrugBank

Abbreviations: ATC, Anatomic Therapeutic Chemical classification system; EMA, European Medicines Agency; FDA, US Food and Drug Administration; PAH, pulmonary arterial hypertension; TTD, Therapeutic Target Database.

analysis after removing the five calcium channel blockers and vasodilators.

To select candidate drugs to reposition for PAH, we first focused on the drugs that interact with PAH drugs. Drug-drug interactions were retrieved from DrugBank. According to DrugBank, these drug-drug interactions are defined based on molecular mechanisms, including drug-target, drug-enzyme, and drug-transporter associations.[13] There are 8199 drug interactions involving 15 PAH drugs and 1329 non-PAH drugs. To reduce the number of drug-drug interactions and candidate drugs for repositioning, we first downloaded the compound structures of drugs as an SDF file from DrugBank and calculated extended connectivity fingerprints for each compound using the Morgan/circular method.[14] We next computed all pairwise chemical/structural similarities of drugs using the Dice similarity coefficient.[15] Using 0.3 as the cutoff for chemical similarity according to the distribution of similarity of all drug-interacting pairs (Figure S1), we identified 1820 drug interactions (646 drugs). This filtering is based on the assumption that drugs with similar structures tend to have similar therapeutic effects. We next focused on approved non-PAH drugs and removed those experimental and investigational drugs. After this filtering, we were left with 491 approved non-PAH drugs that interact with PAH drugs and were also found to be chemically similar to them (i.e., PAH-interacting drugs). These drugs are candidates for drug repositioning for PAH.

PAH disease genes

With a deeper understanding of the genetics and genomics of PAH, many disease genes associated with PAH have been discovered through genomewide association studies or other experiments. We first retrieved 131 disease genes directly implicated in the development of PAH from our previous work,[11] which were curated from the literature. We also collected 150 PAH disease genes from Phenopedia in the HuGE Navigator.[16] Other PAH genes were compiled from the Human Gene Mutation Database (HGMD),[17] Online Mendelian Inheritance in Man (OMIM),[18] and human phenotype ontology (HPO)[19] databases. Altogether, 328 PAH disease genes were obtained by integrating these resources. We next mapped them to the human interactome and denoted the subnetwork formed by the PAH disease proteins as a PAH disease module.

Consolidated human protein-protein interactome

To build a comprehensive human interactome, we compiled human physical, macromolecular interaction data from different sources, including protein-protein interactions, protein complexes, kinase-substrate interactions, and signaling pathways. High-quality protein-protein interactions from several high-throughput yeast-two-hybrid studies as well as the literature were compiled from CCSB Human Interactome.[20–24] Binary protein-protein interactions from other laboratories were incorporated, as well.[25,26] A protein complex is a group of two or more associated polypeptide chains linked by noncovalent protein-protein interactions. Protein-protein co-complex interactions were compiled from different high-profile publications.[27–33] In addition, we also incorporated experimental signaling interactions and kinase-substrate interactions, as well as high-quality literature-based signaling interactions.[34–37] The signaling interactions between proteins are derived from two sources.[34,35] In ref. 34, signaling interactions were generated by experimentally searching the protein interaction partners of signaling-related proteins by means of automated yeast two-hybrid interaction mating. Another resource is OmniPath, which is a comprehensive literature-curated human signaling pathway compendium.[35] We added the edges between proteins in the signaling pathways into our human interactome and collectively referred to them as physical protein-protein interactions. The latest large-scale binary protein-protein interactions were retrieved from HuRI.[38] This version of the consolidated human interactome has 16,470 proteins and 233,957 interactions, and displays a scale-free topology (Figure S2).

Network module identification and proximity measure

Drug targets interact with disease proteins, which form a large network consisting of only drug targets and disease proteins as well as all the direct protein-protein interactions between them. We assume that the action of a drug is either generated by directly targeting a disease protein or by targeting a direct neighbor that physically interacts with the disease protein. To dissect the network into relatively independent submodules based on its topology, we used the module detection method implemented in the Python package NetworkX.[39] The submodules contain only drug targets, PAH disease proteins, and the interactions between them, excluding other nodes in the interactome. We then attached the drugs to the submodules according to their binding relationships and denoted the resulting submodules as drug-target-disease submodules.

For drugs in the drug-target-disease submodules, we also characterized their proximity to the PAH disease module using a network proximity measure.[40] Each drug target can reach multiple disease proteins through shortest paths of different lengths in the human interactome. Among all the shortest path lengths, the minimum one is used in calculating network proximity (Figure 1c). If a drug has multiple targets, the minimum shortest path lengths were taken on average. In other words, network proximity from a drug to the PAH disease module is defined as the average minimum shortest path length (minimum signaling steps) in the interactome from its targets to the disease proteins:

$$P = \langle p_s \rangle \text{ and } p_s = min_d(L_{sd}),$$

where p_s is the minimum shortest path length in the human interactome from drug target s to the associated disease proteins of PAH. Network proximity is calculated based on all the targets of a particular drug, all the PAH disease proteins, and the entire interactome.

The significance of the overlap between the drug targets and the PAH disease proteins was calculated using the hypergeometric test. The significance of the proximity between the targets of a drug and the PAH disease module was evaluated by creating 1000 random modules of the same size and comparing the observed proximity value with the null model (random control) through fitting normal distributions; p values were adjusted by the Bonferroni procedure where applicable. All source codes and related data files have been deposited in a publicly available folder at github (https://github.com/bwh784/PAHdrugs).

RESULTS

Closeness between drug targets and PAH disease proteins

We mapped PAH disease genes (gene products), PAH drug targets, and the target proteins of PAH-interacting drugs to the human interactome and examined the closeness relationships between drug targets and PAH disease genes. According to DrugBank, 48 target proteins are associated with the 15 existing PAH drugs, and 44 can be found in the consolidated human interactome; 473 target proteins are associated with PAH-interacting drugs, and 449 can be found in our interactome. Among 328 PAH disease genes, 276 of their gene products can be found in the interactome. Forty of 44 PAH drug targets significantly overlap with the targets of PAH-interacting drugs ($p < 5.7E-59$; Figure 2a), and the targets of PAH and PAH-interacting drugs have significant overlap with PAH disease proteins, as well ($p < 9.6E-31$),
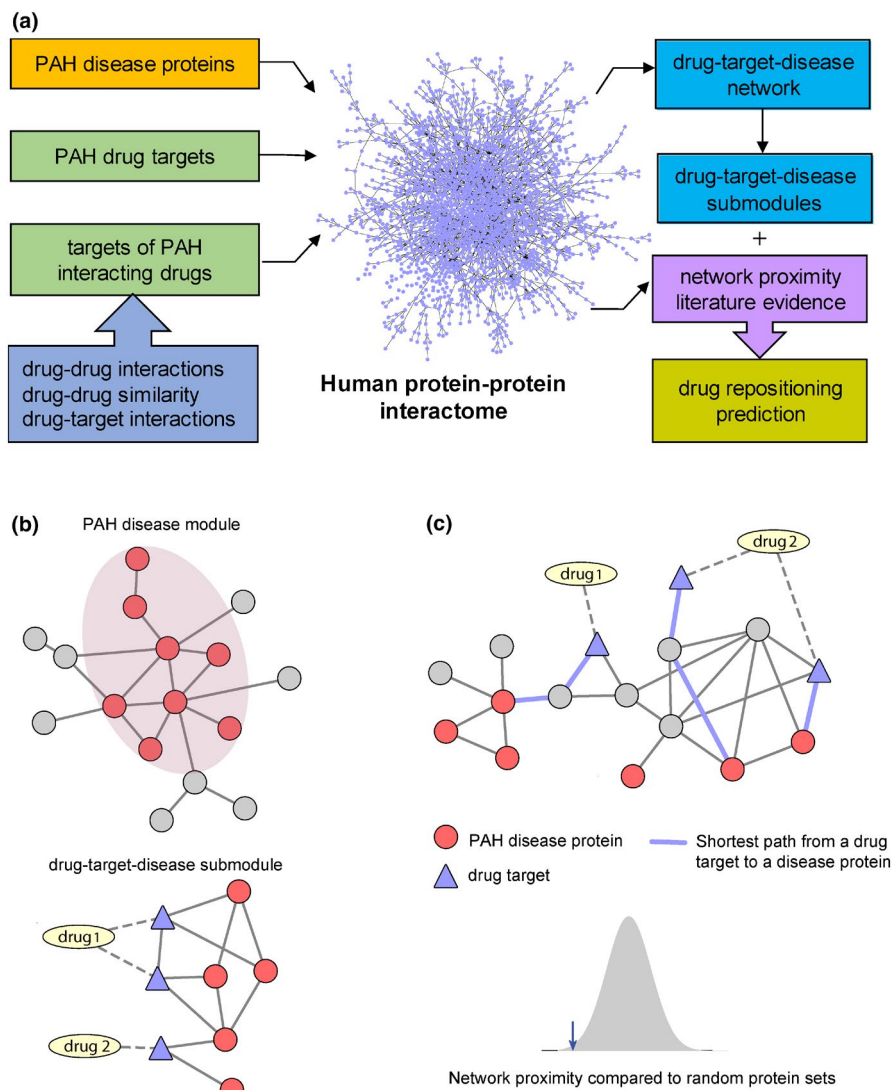
FIGURE 1 The framework for network module-based drug repositioning. (a) We first selected drugs that interact with pulmonary arterial hypertension (PAH) drugs and then filtered out those drug pairs that were not chemically similar. We mapped the drug targets and PAH disease genes into the human interactome and obtained a drug-target-disease network, which was then decomposed into individual submodules. Module drugs that were significantly proximate to the disease proteins comprised the list of drug repositioning predictions. (b) Illustration of the PAH disease module and drug-target-disease submodule. (c) Network proximity from drug targets to disease proteins

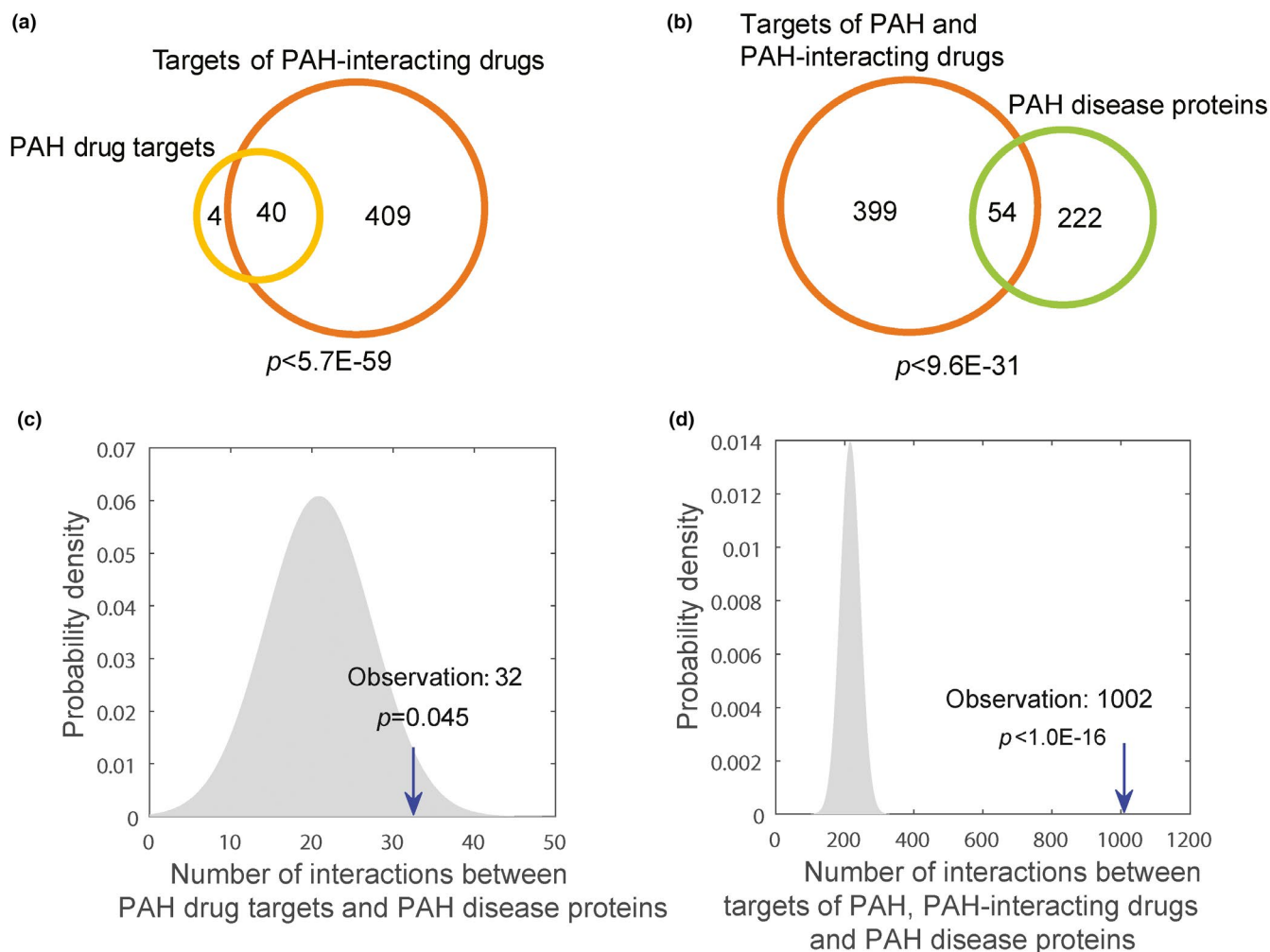suggesting the appropriateness of our candidate drug selection process for repositioning.

We next examined the closeness relationships between drug targets and PAH disease proteins at the network level. We found 32 direct interactions between PAH drug targets and PAH disease proteins, which is significant compared to two random protein sets of the same sizes ($p = 0.045$). There are 1002 direct interactions between the targets of PAH and PAH-interacting drugs and PAH disease proteins, further indicating that the targets of PAH-interacting drugs are close to PAH disease proteins and can be considered for repositioning. We also analyzed the overlap and interactions between drug targets and PAH disease proteins when we focused on the 10 approved drugs, and summarized the results in Figure S3. These key findings remain: the targets of PAH and

PAH-interacting drugs have significant overlap with PAH disease proteins ($p < 3.9E-20$), and the number of interactions between the targets of PAH and PAH-interacting drugs and PAH disease proteins is significant compared to two random protein sets of the same sizes ($p < 1.0E-16$).

Construction of a drug-target-disease network for PAH

With evidence that the target proteins of PAH drugs and PAH-interacting drugs are significantly close to PAH disease proteins at the network level, we constructed a network consisting of direct interactions between drug targets and disease proteins in order to obtain mechanistic insights on

(a)

Targets of PAH-interacting drugs

PAH drug targets

4 40 409

$p<5.7E-59$

(b)

Targets of PAH and PAH-interacting drugs

PAH disease proteins

399 54 222

$p<9.6E-31$

(c)

Probability density

Observation: 32
$p=0.045$

Number of interactions between
PAH drug targets and PAH disease proteins

(d)

Probability density

Observation: 1002
$p<1.0E-16$

Number of interactions between
targets of PAH, PAH-interacting drugs
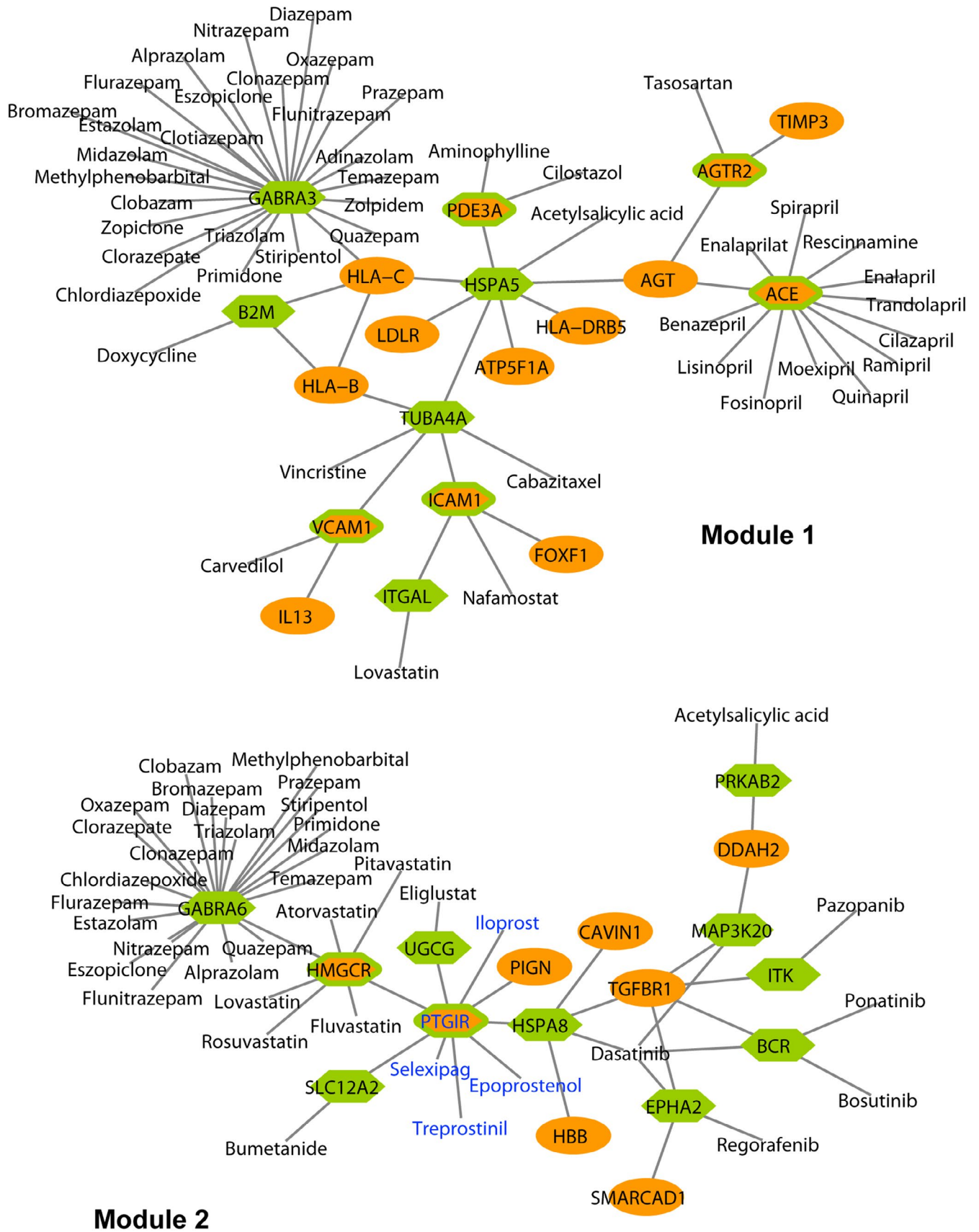and PAH disease proteins

FIGURE 2 The closeness between targets of pulmonary arterial hypertension (PAH) drugs/interacting drugs and PAH disease proteins at the network level. (a) Overlap between PAH drug targets and the targets of PAH interacting drugs. (b) Overlap of the targets of PAH, PAH-interacting drugs, and PAH disease proteins. (c) Number of interactions between PAH drug targets and PAH disease proteins. (d) Number of interactions between the targets of PAH, PAH-interacting drugs, and PAH disease genes

drug action. We assumed that the action of a drug is either generated by directly targeting a disease protein or by targeting a direct neighbor that physically interacts with the disease protein. Therefore, only drug targets, PAH disease proteins, and the direct interactions between them are retained in this network, with all other nodes in the human interactome excluded. This network has 399 proteins and 1002 interactions (Figure S3).

To obtain a higher resolution of the network, we decomposed it into individual, densely connected submodules using a module detection method.[39] The submodules contain only drug targets, PAH disease proteins, and the direct interactions among them. We then attached the drugs to the submodules according to their binding relationships and obtained 11 drug-target-disease submodules (Figure S4). These submodules now consist of drugs, drug targets, and PAH disease proteins, and provide a detailed view of how the drugs act on target proteins, the pathways they may

affect, and their neighborhoods. Figure 3 illustrates two drug-target-disease modules. Although some of these drugs have well-known targets that can affect vascular function (e.g., ACE inhibitors), which are not necessarily effective in PAH, others represent novel target pathways with novel therapeutic potential (e.g., tasosartan and doxycycline). Imatinib is a tyrosine kinase inhibitor used to treat certain cancers. Six of the targets of imatinib lie in module 3 (KIT, CSF1R, PDGFRA, DDR1, ABL1, and PDGFRB; Figure 4a). PDGFRA and PDGFRB are disease proteins associated with PAH, and their mRNA expression was increased in pulmonary arteries from patients with idiopathic PAH.[41] Imatinib can inhibit platelet-derived growth factor receptor (PDGFR) downstream signaling pathways, suggesting that it may have therapeutic potential for PAH. Indeed, some clinical trials and experimental studies have tested the use of imatinib for PAH,[42] although the outcomes have been mixed.

FIGURE 3 Two drug-target-disease submodules. The nodes without geometric shapes represent drugs. The hexagonal nodes are drug targets and the oval nodes are pulmonary arterial hypertension (PAH) disease proteins. Disease proteins that are also drug targets are indicated by hexagonal nodes with orange centers. The drugs with blue labels are PAH drugs, and the targets with blue labels represent PAH drug targets
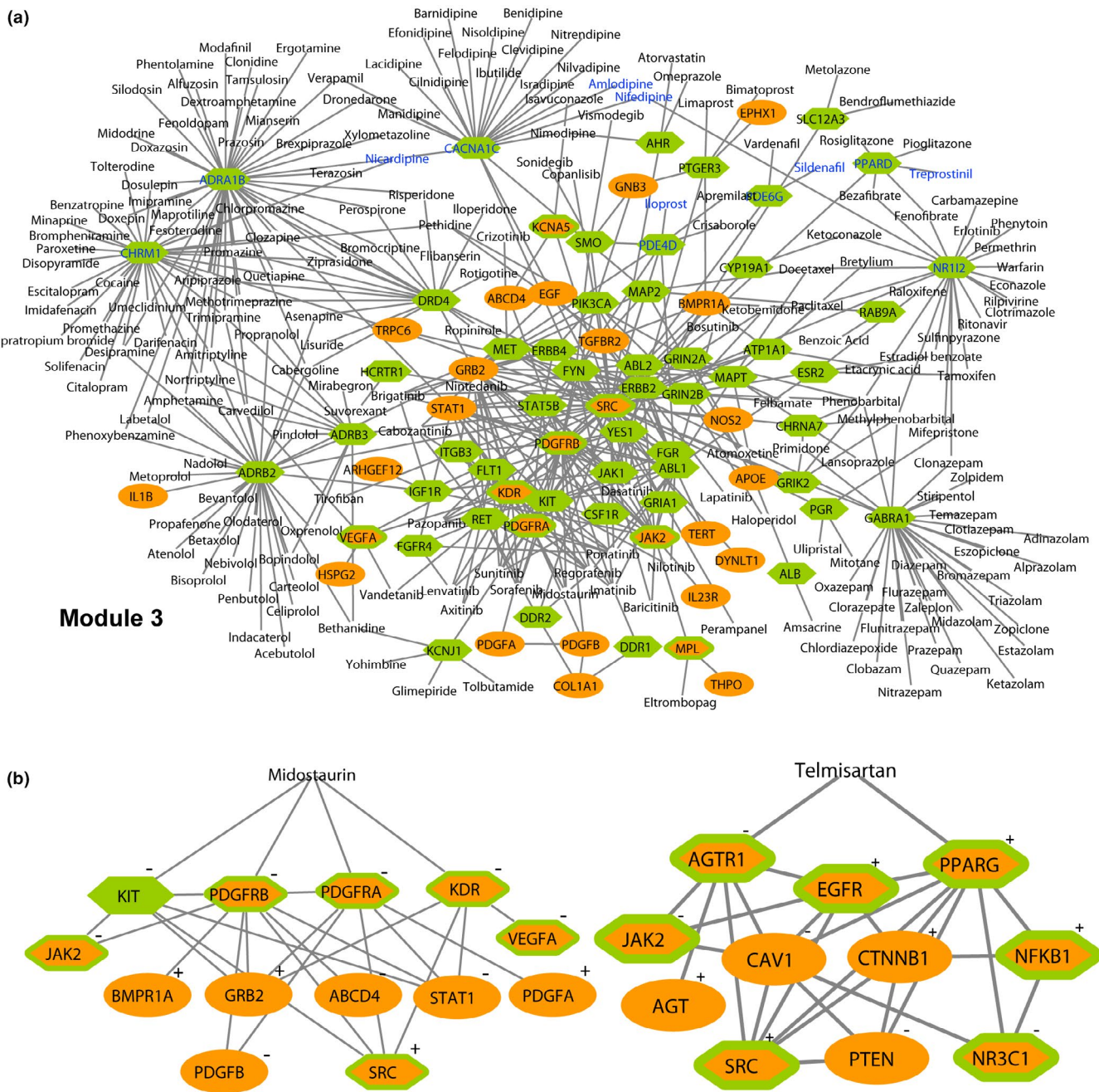
FIGURE 4 Illustration of one drug-target-disease submodule and downstream pathways of drugs. (a) Drug-target-disease submodule 3; (b) The downstream proteins of midostaurin and telmisartan. The nodes without geometric shapes represent drugs. The hexagonal nodes are drug targets and the oval nodes are pulmonary arterial hypertension (PAH) disease proteins. Disease proteins that are also drug targets are indicated by hexagonal nodes with orange centers. "+" ("−") on the upper right corner of a node means the Z-score of the gene is positive (negative) in the drug treatment based on the signatures from Library of Integrated Network-based Cellular Signatures (LINCS)

Network proximity and literature search

Among 491 candidate drugs for repositioning, there are 386 drugs included in these drug-target-disease modules, and we refer to these drugs as module drugs. We focus on module drugs, as the targets of other candidate drugs are at least two interaction steps away from a PAH disease protein. Some module drugs have multiple targets, but only one target has a disease protein neighbor; whereas other module drugs have multiple targets and all of their targets are disease proteins or have disease proteins as neighbors. To narrow down the list of drugs to be considered for repositioning for PAH further, we use a network proximity measure that we developed previously[40] to quantify the closeness of the targets of each module drug to PAH disease proteins. This step uses all the targets of a particular drug, all the PAH disease proteins, and

the entire interactome to ensure that drugs closely associated with the PAH disease module are selected as drug repositioning candidates. The significance of the closeness between the targets of a drug and the disease module is evaluated by constructing a random disease module of the same size as the background. Among 386 module drugs, 105 are significantly close to the PAH disease module after the Benjamini-Hochberg correction for multiple testing ($p < 0.01$). We found that among 12 PAH drugs that lie in the drug-target-disease modules, three are significantly close to PAH disease proteins in the network. The enrichment of PAH drugs in the high ranked drug pool is statistically significant compared to that of non-PAH drugs (Table 2; $p = 0.0043$), demonstrating that PAH drug targets tend to be in the vicinity of the PAH disease module. Calcium channel blockers and vasodilators are not specific for PAH, and their targets may not necessarily be close to the PAH disease module; after we excluded them, the enrichment remained significant (Table 2; $p = 0.002$).

Among all module drugs, 305 have more than one target and 70 of them are significantly proximate to the PAH disease proteins, including seven PAH drugs. The enrichment of PAH drugs with more than one target in the high ranked drug pool is significant (Table 2; $p = 0.0035$). Therefore, we focused on those module drugs whose targets are significantly close to PAH disease proteins and performed a literature search on their targets and downstream effectors. Among 63 significant drugs that are not PAH drugs, 14 drugs can reverse or attenuate PAH, and 10 may induce PAH according to the literature, leading to a list of 53 drugs that have repositioning potential for PAH (Table S1). Many drug candidates have strong literature support for their beneficial effects in reversing pulmonary vascular remodeling or attenuating the symptoms of patients with PAH (Table 3), and others represent drugs with novel therapeutic potential for PAH (Table S1).

The five calcium channel blockers in Table 1 are used to treat PAH,[12] but their efficacy and safety are unproven, and only a small percentage of PAH patients benefit from calcium

TABLE 2 The contingency table of Fisher's exact test for the enrichment of PAH drugs in the high ranked drug pool that are significantly proximate to PAH disease proteins

	PAH drugs	Non-PAH drugs	PAH drugs with more than one target	Non-PAH drugs with more than one target
Significant proximity	8 (7)	97	7	63
Insignificant proximity	4 (2)	277	4	231
Column total	12 (9)	374	11	294

Abbreviation: PAH, pulmonary arterial hypertension.

The figures in the brackets are those excluding calcium channel blockers and vasodilators.

TABLE 3 A list of drugs with strong literature support that have biologically plausible repositioning potential for PAH

Module drugs	No. of targets	Proximity to PAH disease proteins	p value	Literature evidence and clinical trial ID
Nintedanib	10	0.60	2.4E−7	Ref.45
Sorafenib	10	0.70	3.5E−5	NCT00452218, Ref. 46
Sunitinib	8	0.63	3.5E−5	Ref. 47
Imatinib	7	0.71	2.9E−4	NCT00902174, Ref. 42
Tamoxifen	16	1.13	0.002	NCT03528902
Telmisartan	2	0.00	5.0E−4	NCT02242344
Carvedilol	16	1.13	0.001	NCT01586156
Fluoxetine	4	0.75	0.006	NCT00942708
Fenofibrate	4	0.75	0.005	Ref.48
Acetylsalicylic acid	18	1.17	0.001	NCT00384865
Rosiglitazone	7	1.00	0.006	Ref. 49
Pioglitazone	4	0.75	0.006	Refs.49,50
Paclitaxel	6	1.00	0.009	Ref. 49
Docetaxel	6	1.00	0.007	Ref. 51
Atorvastatin	3	0.67	0.006	NCT00615823

Abbreviation: PAH, pulmonary arterial hypertension.

channel blockers long term. As a robustness test, we conducted an additional drug repositioning analysis based on the 10 approved drugs. Specifically, we removed the five calcium channel blockers, retrieved the drug-drug interactions of the 10 approved drugs, and determined PAH interacting drugs using drug-drug chemical similarity. Thereafter, we obtained 360 drug candidates and examined the network proximity between these drugs and the PAH disease module. We found that among 53 drugs in Table S1, 37 drugs remain on the list of significant drugs even after we removed the five calcium channel blockers. We highlighted the remaining drugs in Table S1.

Network-based drug repositioning can provide pharmacological mechanisms for the mode of action of drugs. We illustrate the targets and downstream effectors of some of these drugs as drug-centered subnetworks shown in Figures 4b and S6. Considering upregulation or downregulation of drug targets and gene products allows us to decipher better the mechanistically signal transduction from drug targets to downstream proteins. This requires induced gene expression profiles in pulmonary arterial cells treated by the candidate drugs, which are currently not available for inclusion in this study. The Library of Integrated Network-based Cellular Signatures (LINCS) project profiled gene expression changes following pharmacologic or genetic perturbation of cell lines in high-throughput assays.[43] However, its core set of cell lines does not include pulmonary arterial cells. Nevertheless, we examined the gene signatures of some drugs using the lung cancer cell lines (A549) and added upregulation and downregulation of genes in the downstream pathways of these drugs (Figure S6).

DISCUSSION

In this study, we integrated the targets of PAH drugs, interacting PAH drugs, and PAH disease genes using the human interactome. We evaluated potential drug repositioning for PAH based on network module and network proximity analyses and prioritized 53 drugs that potentially can be repurposed for PAH. This drug repositioning framework offers an unbiased way to screen and narrow down a drug candidate list computationally for PAH, which could save time and reduce the cost of the drug discovery process for PAH. The efficacy of these high-ranked candidates is subject to subsequent experimental validation in an animal model or clinical trial. Here, we used the published literature as evidence to demonstrate the power of our network-based computational approach.

A potential limitation of this study is that we cannot distinguish the direction of the effect of the non-PAH drugs, as the interactome used is not directed. Although many candidate drugs have strong literature support for their beneficial and therapeutic effects for PAH, some drugs that are significantly close to PAH modules may not be therapeutic; rather, they may have side effects or may, in fact, induce pulmonary arterial hypertension. These adverse effects are not predicted a priori because of the undirected nature of the interactome. For example, dasatinib is an oral dual Bcr/Abl and Src family tyrosine kinase inhibitor approved for use in patients with chronic myelogenous leukemia. It is significantly close to PAH disease proteins, but it may induce rather than treat pulmonary arterial hypertension based on previous studies.[44] As a future direction, we will explore how to incorporate more biological or chemical information into the analysis to distinguish positive and negative effects of these drugs. We will use tissue-specific gene expression data from GTEx to create the action pathways of some candidate drugs that facilitate experimental validation. These additional filtering processes should provide more refined information with which to select drugs for further testing in a clinical repositioning program.

CONFLICT OF INTEREST
J.L. is scientific co-founder of Scipher Medicine, Inc., which uses network medicine analyses to identify disease biomarkers and potential therapies. R.S.W. has no conflicts of interest to declare.

AUTHOR CONTRIBUTIONS
R.S.W. and J.L. wrote the manuscript. R.S.W. and J.L. designed the research. R.S.W. performed the research and analyzed the data.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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