**Düzce University Journal of Science & Technology**

*Research Article*

# In Silico Analysis of Pulmonary Arterial Hypertension to Identify Key Biomarkers at Protein and RNA Levels

Sevinç AKÇAY [a,*]

[a] *Department of Molecular Biology and Genetics, Faculty of Art and Sciences, Kırşehir Ahi Evran University, Kırşehir, TURKEY*

** Corresponding author's e-mail address: sevinc.akcay@ahievran.edu.tr*

DOI:10.29130/dubited.1103902

## ABSTRACT

Pulmonary arterial hypertension (PAH) is a chronic cardiopulmonary disorder marked by a raised hypertension in the pulmonary arteries. There is no remedy for PAH, existing medications can help reduce the disease's progression. This research aimed to investigate potential protein and RNA biomarkers of PAH by bioinformatic analysis. Two PAH datasets accessed from the publicly available Gene Expression Omnibus (GEO) database were used to research differentially expressed genes (DEGs). Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses for common DEGs were conducted by the DAVID tool. Cytoscape was used to create the protein-protein interaction (PPI) and pick the top 10 hub genes. The transcription factors (TFs) and microRNAs (miRNAs) that target DEGs and hub genes were investigated using the JASPAR database. Potential therapeutics targeting the best hub genes were researched with DGIdb database. Ten hub genes were identified to be linked to the pathogenesis of PAH (*CCL5, TLR4, TLR1, SPP1, CYBB, HGF, IGF1, SELL, CD163,* and *POSTN*). "Positive regulation of tumor necrosis factor biosynthetic process" and a "toll-like receptor signaling pathway" are the most enriched GO term and KEGG pathway, respectively. "hsa-mir-26b-5p, hsa-mir-146a-5p, hsa-mir-335-5p" and FOXC1, YY1, GATA2 are the top TFs targeting hub genes. 21 drugs targeting ten hub genes have been identified. Our results would help to identify the pathogenesis of PAH and hub genes, miRNAs, and ten TFs that might serve as potential therapeutic targets at protein and RNA levels for PAH patients.

***Keywords:*** *Pulmonary Arterial Hypertension, Biomarkers, Transcription Factors, MicroRNAs, Differentially Expressed Genes*

# RNA ve Protein Seviyelerinde Temel Biyobelirteçleri Belirlemek İçin Pulmoner Arteriyel Hipertansiyonun In Silico Analizi

## ÖZET

Pulmoner arteriyel hipertansiyon (PAH), pulmoner arterlerde yüksek hipertansiyon ile işaretlenmiş kronik bir kardiyopulmoner bozukluktur. PAH hastalığı için kesin bir tedavi yöntemi yoktur, mevcut ilaçlar ise sadece hastalığın ilerlemesini azaltmaya yardımcı olabilir. Bu araştırma, biyoinformatik analiz yoluyla PAH'ın potansiyel protein ve RNA biyobelirteçlerini araştırmayı amaçlamıştır. Herkese açık Gene Expression Omnibus (GEO) veri tabanından erişilen iki PAH veri seti, diferansiyel olarak eksprese edilmiş genleri (DEG'ler) belirlemek için kullanıldı. Yaygın DEG'ler için Gen Ontoloji (GO) ve Kyoto Genler ve Genomlar Ansiklopedisi (KEGG) yolak analizleri DAVID aracıyla yapıldı. Cytoscape, protein-protein etkileşimini (PPI) oluşturmak ve ilk 10 aday genini seçmek için kullanıldı.

DEG'leri ve hub genlerini hedefleyen transkripsiyon faktörleri (TF'ler) ve mikroRNA'lar (miRNA'lar), JASPAR veri tabanı kullanılarak araştırıldı. En iyi ilk 10 aday geni hedefleyen potansiyel terapötikler DGIdb veritabanı ile araştırıldı. On aday geninin PAH patogeneziyle bağlantılı olduğu bulundu (*CCL5, TLR4, TLR1, SPP1, CYBB, HGF, IGF1, SELL, CD163 ve POSTN*). "Tümör nekroz faktörü biyosentetik sürecinin pozitif regülasyonu" ve "TOLL benzeri reseptör sinyal yolu" sırasıyla en zenginleştirilmiş GO terimi ve KEGG yolağıdır. "hsa-mir-26b-5p, hsa-mir-146a-5p, hsa-mir-335-5p" ve FOXC1, YY1, GATA2, aday genlerini hedefleyen en iyi TF'lerdir. On aday geni hedef alan 21 ilaç belirlendi. Sonuçlarımız, PAH hastaları için protein ve RNA seviyelerinde potansiyel terapötik hedefler olarak hizmet edebilecek PAH ve aday genleri, miRNA'lar ve on TF'nin patogenezini belirlemeye yardımcı olacaktır.

***Anahtar Kelimeler:*** *Pulmoner Arteryel Hipertansiyon, Biyobelirteçler, Transkripsiyon Faktörleri, MikroRNAlar, Diferansiyel Olarak Eksprese Edilen Genler*

---

Received: 15/04/2022, Revised: 16/11/2022, Accepted: 05/12/2022

# I. INTRODUCTION

Pulmonary arterial hypertension (PAH) is a chronic and rare disorder that reduces blood flow and enhances pulmonary arterial pressure (PAP) and pulmonary vascular resistance (PVR) [1]. The systolic pulmonary artery pressure in typical, healthy adults is 22–30 mmHg, the diastolic pulmonary artery pressure is 9–22 mmHg, and the mean PAP is 15–18 mmHg. The PAP in PAH patients is frequently > 25 mmHg at rest and > 30 mmHg with exercise [2].

Although recently, several investigations have been carried out to discover the etiology of PAH, its exact etiology of PAH is still unknown. The diverse etiology of PAH includes genetic, environmental, and biological components. Vasoconstriction, arterial wall remodeling, and in situ thrombosis are the important causes of PAH. Although there are different treatment options, PAH is still not completely curable. The rate of the clinical worsening of the disease can be reduced and the survival rate can be partially extended with PAH treatment. The goals of the PAH treatment are to restore the patient's functional stage, ease clinical deterioration, improve quality of life, and prolong survival. Although current treatment options improve the patient's symptoms, none of them fully treat the disease, and the mortality rate is still high. So, identifying new and reliable biomarkers that contribute to the pathogenesis of PAH is urgently needed.

Bioinformatics analysis has been extensively employed to uncover potential biomarkers of diseases by analyzing the differentially expressed genes (DEGs) in patients and normal samples [3]. Thanks to the advancement of microarray technology, identifying DEGs has become easier and faster. The quantity of Gene Expression Omnibus (GEO) data produced by microarray technology has risen recently. Several biomarkers and possible biological pathways linked to PAH have recently been uncovered [4; 5]. However, the mortality rate is still high. As a result, it's critical to find novel biomarkers for therapeutic and diagnostic purposes in PAH disease using more GSE datasets.

This study searches for biomarkers, biological pathways, and regulators using in silico tools in PAH patients. We found common DEGs across PAH patients and normal samples using two publicly available PAH GEO datasets. Additionally, we performed Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses, and PPI networks of the identified DEGs were established. miRNA and TFs were also identified. Finally, possible therapeutic targets for the 10 hub genes were examined to aid in the characterization of new PAH therapeutic techniques.

# II. MATERIALS AND METHODS

## A. IDENTIFICATION OF DEGs FROM THE TWO DATASETS

GSE113439 and GSE117261, publicly available datasets downloaded from the GEO database (https://www.ncbi.nlm.nih.gov/geo/) [6]. While the GSE113439 dataset includes 15 PAH patients and 11 controls, the GSE117261 dataset includes 58 PAH patients and 25 controls. Information about the datasets is given in Table 1.

DEGs were found in both the patient and control datasets by the GEO2R statistical tool. The following criteria were used to choose DEGs: *p*- value < 0.01, log2FC≥1 (up-regulated DEGs) or *p*-value < 0.01 and log2FC≤1 (down-regulated DEGs). The online Venn diagram tool was used to find common DEGs by detecting overlapping DEGs between two datasets. PANTHER database was utilized to discover the over-representation of protein categories [7]. Figure 1 illustrates the steps of our study.

***Table 1.*** *GEO datasets analyzed in the study*

| GEO ID | Tissue type | Case-control group | Experiment Type | Platform |
|---|---|---|---|---|
| GSE113439 | Lung tissue | 15 PAHs - 11 Control | "Expression profiling by array" | "Affymetrix Human Gene 1.0 ST Array" |
| GSE117261 | Lung tissue | 58 PAHs - 25 Control | "Expression profiling by array" | "Affymetrix Human Gene 1.0 ST Array" |

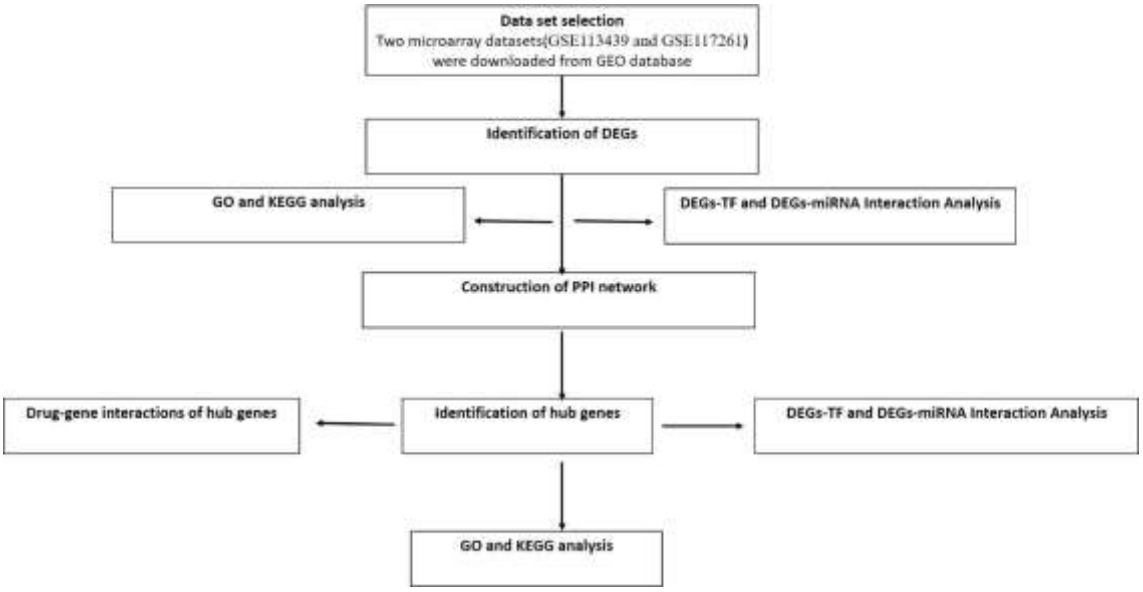


***Figure 1.*** *The workflow of the study*

### B. GENE ONTOLOGY AND KEGG ENRICHMENT ANALYSIS

The DAVID (The Database for Annotation, Visualization and Integrated Discovery) tool evaluated molecular pathways and their aggregation in biological processes in two datasets [8]. We accepted a *p-value* < 0.05 as the cut-off to choose GO enrichment terms (biological processes (BP), molecular functions (MF), and cellular components (CC)) and KEGG pathways.

### C. PROTEIN-PROTEIN INTERACTION NETWORK ANALYSIS AND 10 HUB GENE DETECTION

The STRING tool (https://string-db.org/) created the protein-protein interaction (PPI) network. The PPI network was created by the PPI confidence scores greater than 0.7. The Cytohubba plug-in in Cytoscape (Cytoscape v3.7.1) software was used to get the picture of the network, and hub genes were detected. 12 methods were used by the CytoHubba plugin to find hub genes. The "degree" technique was used to choose the 10 hub genes. Furthermore, the GeneMANIA database (https://genemania.org/) was utilized to predict gene function and identify functionally comparable genes [9].

### D. DEVELOPMENT OF TF-miRNA-GENE NETWORK

The JASPAR [10; 11] and miRTarBase [12] databases were used to look for TFs and miRNAs that influence DEGs at the transcriptional and posttranscriptional levels, respectively. The top 10 TFs and miRNAs that target common DEGs and hub genes were discovered. The NetworkAnalyst tool was also used to create DEG-TF and DEGs-miRNA networks [13].

### E. DRUG-HUB GENE INTERACTION

To find prospective therapeutic targets for the top hub genes identified in our investigation, we used the DGIdb (The Drug Gene Interaction Database) database (http://dgidb.genome.wustl.edu/) [14]. DGIdb is a database that uses DrugBank, TDD, Chembl, Drug Target Commons, and PharmGKB to provide probable drug-gene interactions.

## III. RESULTS

### A. DEG ANALYSIS AND IDENTIFICATION OF COMMON DEGs

Because of the analysis of the GSE113439 and GSE117261 datasets, 3870 DEGs in the GSE113439 datasets and 422 DEGs were identified in the GSE117261 dataset. By comparing DEGs in two datasets, 122 common DEGs were identified (Fig. 2A). While 41 DEGs were down-regulated and 81 DEGs were up-regulated in GSE113439 dataset, 57 DEGs were down-regulated and 65 DEGs were up-regulated in GSE117261 dataset. Based on their roles, 122 common DEGs were divided into 14 protein classes, including cell adhesion molecule (% 4.5), defense/immunity protein (% 4.5), extracellular matrix protein (% 4.5), gene-specific transcriptional regulator (% 10.2), intercellular signal molecule ( % 14.8), metabolite interconversion enzyme (% 27.3), nucleic acid metabolism protein (% 2.3), protein modifying enzyme (% 9.1), protein-binding activity modulator (% 5.7), scaffold/adaptor protein ( % 2.3), transfer/carrier protein (% 2.3), transmembrane signal receptor (% 4.5), transporter (% 6.8), and viral or transposable element protein (% 1.1) (Fig. 2B).

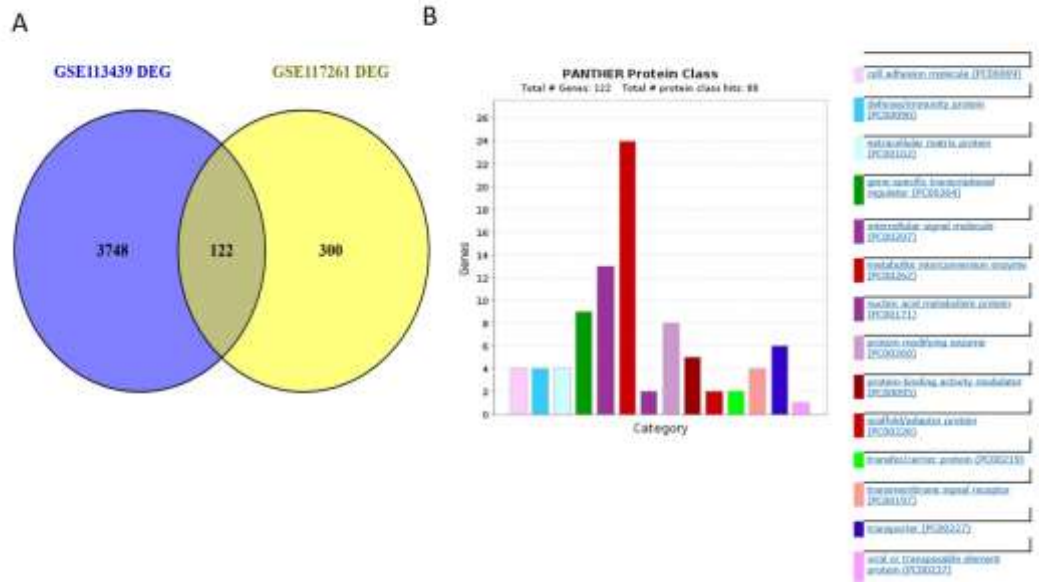


***Figure 2. (A)*** *Venn diagram of common DEGs detected in two datasets. In the Venn diagram, the two datasets overlapped by 122 genes* ***(B)*** *Categorization of common DEGS based on PANTHER [7] protein class.*

## B. FUNCTIONAL ENRICHMENT ANALYSIS OF COMMON DEGs

DAVID was used to define all DEGs based on their BPs, MFs, and CCs of the GO and KEGG pathways. Table 2 shows the top ten GO keywords in the biological process category. "Interleukin-1 beta secretion," "inflammatory response," and "cell adhesion" are the top GO terms in the BP category. "Lipopeptide binding," "heparin binding," and "growth factor activity" are the top GO keywords in the MF category. "Extracellular matrix," "extracellular exosome," and "extracellular space" are the top important GO terms in the CC category "African trypanosomiasis," "Malaria," and "Legionellosis" are the most important KEGG terms (Table 2).

***Table 2.*** *The top 10 GO terms (BP, MF, CC) and KEGG pathways*

| Category | Term | Genes | *p*-value |
|---|---|---|---|
| **"BP"** | 'GO:005070' ~"Interleukin-1 beta secretion" | *NLRC4, GBP5, TLR4, TLR6* | 1.4E-5 |
| **"BP"** | 'GO:0006954'~"Inflammatory response" | *CCL5, NLRC4, NAIP, CYBB, GBP5, ORM1, RARRES2, SPP1, TLR1, TLR4, TLR6, VNN1* | 3.0E-4 |
| **"BP"** | 'GO:0007155'~"Cell adhesion" | *EPHA3, WISP2, CDON, CHL1, DPT, ITGA2, MFAP4, MFGE8, POSTN, SPP1, SELL, SEMA5A* | 1.7E-4 |
| **"BP"** | 'GO:0030335'~"Positive regulation of cell migration" | *CCL5, EDN1, HGF, IGF1, PDGFD, PDG, FA, SEMA3D, SEMA5A* | 1.8E-4 |
| **"BP"** | 'GO:0071560'~ "Cellular response to transforming growth factor beta stimulus" | *CLEC3B, ARG1, EDN1, POSTN, PDGFD* | 2.6E-4 |
| **"BP"** | 'GO:0014911'~ "Positive regulation of smooth muscle cell migration" | *CCL5, IGF1, ITGA2, POSTN* | 2.7E-4 |
| **"BP"** | 'GO:1900227'~ "Positive regulation of NLRP3 inflammasome complex assembly" | *GBP5, TLR4, TLR6* | 4.0E-4 |
| **"BP"** | 'GO:0002576'~"Platelet degranulation" | *CLEC3B, HGF, IGF1, ORM1, PDGFA, RARRES2* | 5.1E-4 |
| **"BP"** | 'GO:0048661'~ "Positive regulation of smooth muscle cell proliferation" | *CCL5, EDN1, IGF1, ITGA2, PDGFD* | 5.8E-4 |
| **"BP"** | 'GO:0014068'~ "Positive regulation of phosphatidylinositol 3*kinase | *CCL5, HGF, IGF1, PDGFD, PDGFA* | 7.8E-4 |

| | | | |
|---|---|---|---|
| | signaling” | | |
| **“MF”** | ‘GO:0071723‘~ “Lipopeptide binding” | *CD1C, TLR1, TLR6* | 1.0E-3 |
| **“MF”** | ‘GO:0008201‘~ “Heparin binding” | *CLEC3B, WISP2, CCDC80, LTBP2, POSTN, SELL* | 3.2E-3 |
| **“MF”** | ‘GO:0008083‘~ “Growth factor activity” | *MACC1, AREG, HGF, IGF1, PDGFD, PDGFA* | 3.4E-3 |
| **“MF”** | ‘GO:0004115‘~ “3’,5’-cyclic-AMP phosphodiesterase activity” | *PDE1A, PDE4D, PDE8B* | 3.8E-3 |
| **“MF”** | ‘GO:0004114‘~ “3’,5’-cyclic-nucleotide phosphodiesterase activity” | *PDE1A, PDE4D, PDE8B* | 8.9E-3 |
| **“MF”** | ‘GO:0004435‘~ “Phosphatidylinositol phospholipase C activity” | *CCL5, PLCB1, PLCB4* | 1.2E-2 |
| **“MF”** | ‘GO:0005509‘~ “Calcium ion binding” | *CLEC3B, LRP4, HMCN1, LTBP2, LTBP3, MATN2, MMP8, NKD1, PLCB1, PLCB4, SULF1* | 1.4E-2 |
| **“MF”** | ‘GO:0035663‘~ “Toll-like receptor 2 binding” | *TLR1, TLR6* | 1.9E-2 |
| **“MF”** | ‘GO:0030492‘~” Hemoglobin binding” | *HPR, HBB* | 2.5E-2 |
| **“MF”** | ‘GO:0005178‘~ “Integrin binding” | *WISP2, IGF1, ITGA2, MFGE8* | 2.8E-2 |
| **“CC”** | ‘GO:0031012‘~ “Extracellular matrix” | *CLEC3B, CDON, COL14A1, DPT, HMCN1, LTBP2, LTBP3, MATN2, MMP8, MFAP4, MFGE8, POSTN, RARRES2, TSHZ2* | 3.4E-8 |
| **“CC”** | ‘GO:0070062‘~ “Extracellular exosome” | *NT5E, ABCB1, CLEC3B, NAIP, SLC9A3R2, WISP2, ACAT2, ANO1, ARG1, CTSE, CTSF, CHL1, CLIC3, COL14A1, CR1, DPT, HPR, HMCN1, HBB, ITM2A, LTBP2, LTBP3, LRRN4, MFAP4, MFGE8, MNDA, ORM1, PI15, PGD, PLCB1, PDGFD, PTGDS, RARRES2* | 9.3E-7 |
| **“CC”** | ‘GO:0005615‘~ “Extracellular space” | *CCL5, CLEC3B, TIMP4, WISP2, AREG, ARG1, CTSF, CHIT1, COL14A1, DPT, EDN1, HGF, IGF1, LTBP2, MMP8, MFGE8, ORM1, POSTN, PDGFD, PDGFA, PTGDS, SOSTDC1, SPP1, SEMA3D, SULF1* | 1.8E-6 |
| **“CC”** | ‘GO:0005576‘~ “Extracellular region” | *CCL5, CLEC3B, CD163, EPHA3, CHIT1, COL14A1, EDN1, GZMK, HPR, HBB, HGF, IGF1, IL1R2, LTBP3, MMP8, MFAP4, MFGE8, ORM1, PDHFD, PDGFA, PTGDS, RARRES2, SPP1, UACA* | 1.0E-4 |
| **“CC”** | ‘GO:0005578‘~” Proteinaceous extracellular matrix” | *TIMP4, WISP2, CHL1, COL14A1, DPT, LTBP2, MATN2, MMP8, POSTN* | 2.5E-4 |
| **“CC”** | ‘GO:0009986‘~ “Cell surface” | *NT5E, ABCB1, FCER1G, LRP4, AREG, CR1, ITGA2, PDGFA, SLC7A11, SULF1, TLR4* | 1.9E-3 |
| **“CC”** | ‘GO:0031093‘~ ”Platelet alpha granule lumen” | *HFG, IGF1, ORM1, PDGFA* | 4.7E-3 |
| **“CC”** | ‘GO:0005887‘~ “Integral component of plasma membrane” | *CD163, CD1C, EPHA3, FCER1G, ADRA1A, AQP9, CDON, CR1, CYBB, IGSF6, LRRN4, MPP1, SELL, SLC7A11, TLR1, TLR4, TLR7* | 1.3E-2 |
| **“CC”** | ‘GO:0005791‘~ “Rough endoplasmic reticulum” | *CYBB, PTGDS, SLC7A11* | 3.9E-2 |
| **“CC”** | ‘GO:0009897‘~ “External side of plasma membrane” | *FCER1G, ITGA2, MFGE8, SELL, TLR4* | 4.2E-2 |
| **“KEGG Pathway”** | hsa05143~ “African trypanosomiasis” | *HPR, HBB, PLCB1, PLCB4* | 2.9E-3 |
| **“KEGG Pathway”** | hsa05144~ “Malaria” | *CR1, HBB, HGF, TLR4* | 8,7E-3 |
| **“KEGG Pathway”** | hsa05134~ “Legionellosis” | *NLRC4, NAIP, CR1, TLR4* | 1.1E-2 |
| **“KEGG Pathway”** | hsa05142~ “Chagas disease” | *CCL5, PLCB1, PLCB4, TLR4, TLR6* | 1.3E-2 |
| **“KEGG Pathway”** | hsa04620~ “Toll-like receptor signaling pathway” | *CCL5, SPP1, TLR1, TLR4, TLR6* | 1.3E-2 |
| **“KEGG Pathway”** | hsa05146~ “Amoebiasis” | *ARG1, IL1R2, PLCB1, PLCB4, TLR4* | 1.3E-2 |
| **“KEGG Pathway”** | hsa05218~ “Melanoma” | *ARG1, IL1R2, PLCB1, PLCB4, TLR4* | 2.4E-2 |
| **“KEGG Pathway”** | hsa04510~ “Focal adhesion” | *HGF, IGF1, ITGA2, PDGFD, PDGFA, SPP1* | 3.3E-2 |
| **“KEGG Pathway”** | hsa04015~ “Rap1 signaling pathway” | *HGF, IGF1, PLCB1, PLCB4, PDGFD, PDGFA* | 3.6E-4 |
| **“KEGG Pathway”** | hsa054640~ “Hematopoietic cell lineage” | *CD1C, CR1, ITGA2, IL1R2* | 4.0E-4 |

## C. PPI NETWORK CONSTRUCTION AND DETECTION OF THE TOP 10 HUB GENES

PPI network analysis was conducted to find the protein-protein associations between DEGs by the STRING database. Network visualization was done with Cytoscape, while the topological computations were done with Cytohubba. The PPI networks for common DEGs were constructed with 114 nodes and 166 edges (Supplementary Figure 1). The top 10 hub genes in our dataset were described using "degree topological words" in CytoHubba. The top 10 genes are "C-C motif chemokine ligand 5 (*CCL5*), Toll-like receptor 4 (*TLR4*), Toll-like receptor 1 (*TLR1*), Secreted phosphoprotein 1 (*SPP1*), Cytochrome B-245 beta chain (*CYBB*), Hepatocyte growth factor (*HGF*), Insulin-like growth factor 1 (*IGF1*), Selectin L (*SELL*), CD163 molecule (*CD163*) and Periostin (*POSTN*)" (Table 3). We observed that 2 hub genes (*IGF1, POSTN*) are regulated in the same direction and 8 hub genes (*CCL5, CYBB, TLR4, CD163, SELL, HGF, SPP1* and *TLR1*) are regulated in opposite directions in two datasets. *IGF1* and *POSTN* are up-regulated in two datasets. While CLL5 is down-regulated in GSE113439 dataset and up-regulated in GSE117261 dataset. *CYBB*, *TLR4*, *CD163*, *SELL*, *HGF*, *SPP1* and *TLR1* are up-regulated in GSE113439 dataset, they are down-regulated in GSE113439 dataset. Fig 3A shows the PPI network of 10 hub genes. 10 hub genes were also analyzed for GO and KEGG terms (Table 4). The top GO term in the BP category is "positive regulation of tumor necrosis factor biosynthetic process," the top GO term in the MF category is "protein binding," and the top GO term in the CC category is "extracellular space." The top KEGG term is 'toll-like receptors signaling pathway (Table 4). We also created a PPI network of common DEGs by the GeneMANIA to discover functionally similar genes and predict gene functions. "Detection of external biotic stimulus," "detection of biotic stimulus," "detection of other organism," "granulocyte chemotaxis," "myeloid leukocyte migration," "leukocyte migration," and "granulocyte migration" are the possible gene functions based on the PPI network (Fig. 3B).

***Table 3.*** *The top 10 hub genes in the PPI network*

| Gene ID | Betweenness | BottleNeck | Closeness | Clustering Coefficient | Degree | DNMC | EcCentricity | EPC | MCC | MNC | Radiality | Stress |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ***CCL5*** | 16.5 | 7 | 8.5 | 0.53 | 8 | 0.43736 | 0.5 | 4.841 | 72. | 8 | 3.33 | 50 |
| ***TLR4*** | 16.5 | 3 | 8.5 | 0.53 | 8 | 0.43736 | 0.5 | 4.859 | 72 | 8 | 3.33 | 50 |
| ***TLR1*** | 0.5 | 1 | 6.83 | 0.9 | 5 | 0.58344 | 0.3333 | 4.04 | 48 | 5 | 2.88 | 2 |
| ***SPP1*** | 4 | 2 | 7 | 0.8 | 5 | 0.51861 | 0.5 | 3.906 | 30 | 5 | 3 | 20 |
| ***CYBB*** | 0.5 | 1 | 6.83 | 0.9 | 5 | 0.58344 | 0.3333 | 4.082 | 48 | 5 | 2.88 | 2 |
| ***HGF*** | 4 | 1 | 7 | 0.8 | 5 | 0.51861 | 0.5 | 4.075 | 30 | 5 | 3 | 20 |
| ***IGF1*** | 4 | 1 | 7 | 0.8 | 5 | 0.51861 | 0.5 | 3.977 | 30 | 5 | 3 | 20 |
| ***SELL*** | 0 | 1 | 6.33 | 1 | 4 | 0.56839 | 0.3333 | 3.833 | 24 | 4 | 2.77 | 0 |
| ***CD163*** | 0 | 1 | 6.33 | 1 | 4 | 0.56839 | 0.3333 | 3.794 | 24 | 4 | 2.77 | 0 |
| ***POSTN*** | 0 | 1 | 5.33 | 1 | 3 | 0.546346 | 0.3333 | 3.069 | 6 | 3 | 2.33 | 0 |

***Table 4.*** *The top five enriched GO and KEGG terms of ten hub genes of PAH*

| Category | Term | p- value | Genes |
|---|---|---|---|
| **"BP"** | 'GO:0042535'~ "Positive regulation of tumor necrosis factor biosynthetic process" | 1.4E-5 | *TLR1, CYBB, TLR4* |
| **"BP"** | 'GO:0006954'~ "Inflammatory response" | 2.9E-5 | *TLR1, CCL5, SPP1, CYBB, TLR4* |
| **"BP"** | 'GO:0014911'~ "Positive regulation of smooth muscle cell migration" | 4.8E-5 | *POSTN, CCL5, IGF1* |

| | | | |
|---|---|---|---|
| **"BP"** | 'GO:0014068'~ "Positive regulation of phosphatidylinositol 3-kinase signaling" | 5.2E-4 | *CCL5, HGF, IGF1* |
| **"BP"** | 'GO:0000187'~ "Activation of MAPK activity" | 1.4E-3 | *HGF, IGF1, TLR4* |
| **"MF"** | 'GO:0005515'~ "Protein binding" | 2.8E-3 | *TLR1, CD163, POSTN, SELL, CCL5, HGF, SPP1, CYBB, IGF1, TRL4* |
| **"MF"** | 'GO:0042056'~ "Chemoattractant activity" | 1.4E-2 | *CCL5, HGF* |
| **"MF"** | 'GO:0046982'~ "Protein heterodimerization activity" | 2.4E-2 | *TLR1, HGF, CYBB* |
| **"MF"** | 'GO:0050839'~ "Cell adhesion molecule binding" | 3.3E-2 | *POSTN, SELL* |
| **"MF"** | 'GO:0008201'~ "Heparin binding" | 8.2E-2 | *POSTN, SELL* |
| **"CC"** | 'GO:0005615'~ "Extracellular space" | 2.8E-3 | *POSTN, CLL5, HGF, SPP1, IGF1* |
| **"CC"** | 'GO:0005887'~ "Integral component of plasma membrane" | 3.3E-3 | *TLR1, CD163, SELL, CYBB, TLR4* |
| **"CC"** | 'GO:0005576'~ "Extracellular region" | 5.3E-3 | *CD163, CCL5, HGF, SPP1, IGF1* |
| **"CC"** | 'GO:0031093'~ "Platelet alpha granule lumen 2" | 2.7E-2 | *HGF, IGF1* |
| **"CC"** | 'GO:0030670'~ "Phagocytic vesicle membrane" | 2.9E-2 | *TLR1, CYBB* |
| **"KEGG Pathway"** | hsa04620~ "Toll-like receptor signaling pathway" | 1.2E-4 | *TLR1, CCL5, SPP1, TLR4* |
| **"KEGG Pathway"** | hsa04151~ "PI3K-Akt signaling pathway" | 3.8E-3 | *HGF, SPP1, IGF1, TLR4* |
| **"KEGG Pathway"** | hsa05205~ "Proteoglycans in cancer" | 1.6E-2 | *HGF, IGF1, TLR4* |
| **"KEGG Pathway"** | hsa04510~ "Focal adhesion" | 1.7E-2 | *HGF, SPP1, IGF1* |
| **"KEGG Pathway"** | hsa05144~" Malaria" | 4.9E-2 | *HGF, TLR4* |

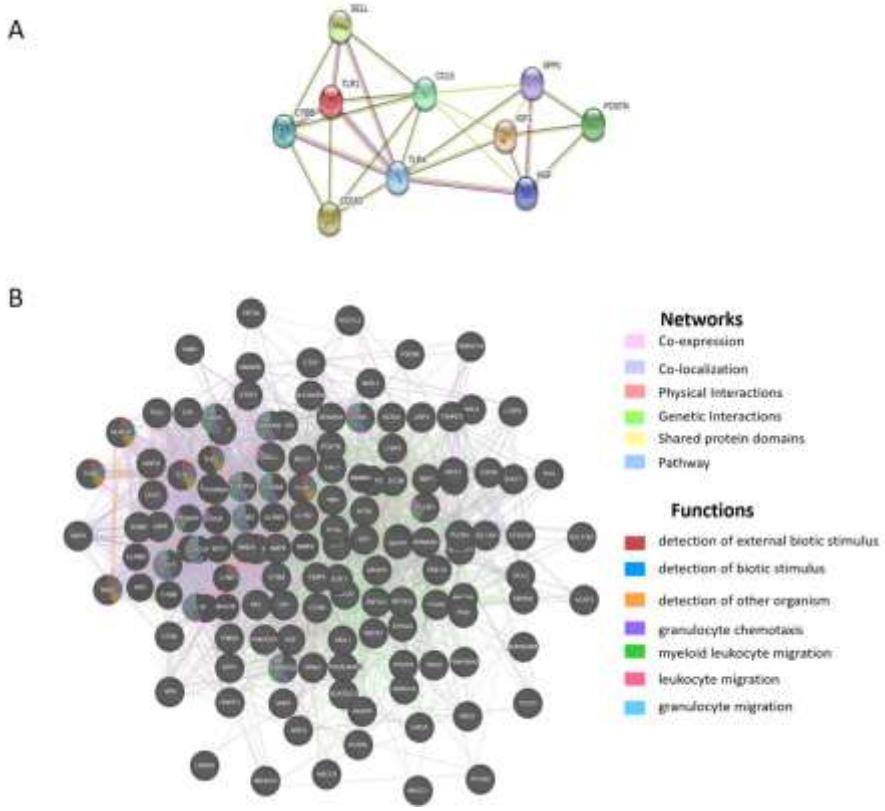


***Figure 3. (A)*** *The PPI network of hub genes created by STRING and presented by the Cytoscape, (**B**) The PPI network of common DEGs created by the GeneMANIA.*

## D. DEVELOPMENT OF TF-miRNA-GENE NETWORK

To discover the mechanisms and regulatory networks of the hub genes, DEGs, TFs, and miRNAs were analyzed. In this study, miRNA-DEGs and TF-DEGs were identified, and the top 10 TFs and miRNAs related to common DEGs are listed in Supplementary Table 1.

8 miRNAs ("hsa-mir-26b-5p, hsa-mir-146a-5p, hsa-mir-335-5p, hsa-mir-26a-5p, hsa-mir-98-5p, hsa-mir-199a-3p, hsa-mir-1-3p, hsa-mir-106b-5p") were detected that regulate two or more hub genes. FOXC1, YY1, GATA2, FOXL1, POU2F2, CREB1, STAT3, HINFP, NR3C1, and FOXI1 are top 10 TFs related to 10 hub genes in our study (Fig. 4A, 4B, 4C).

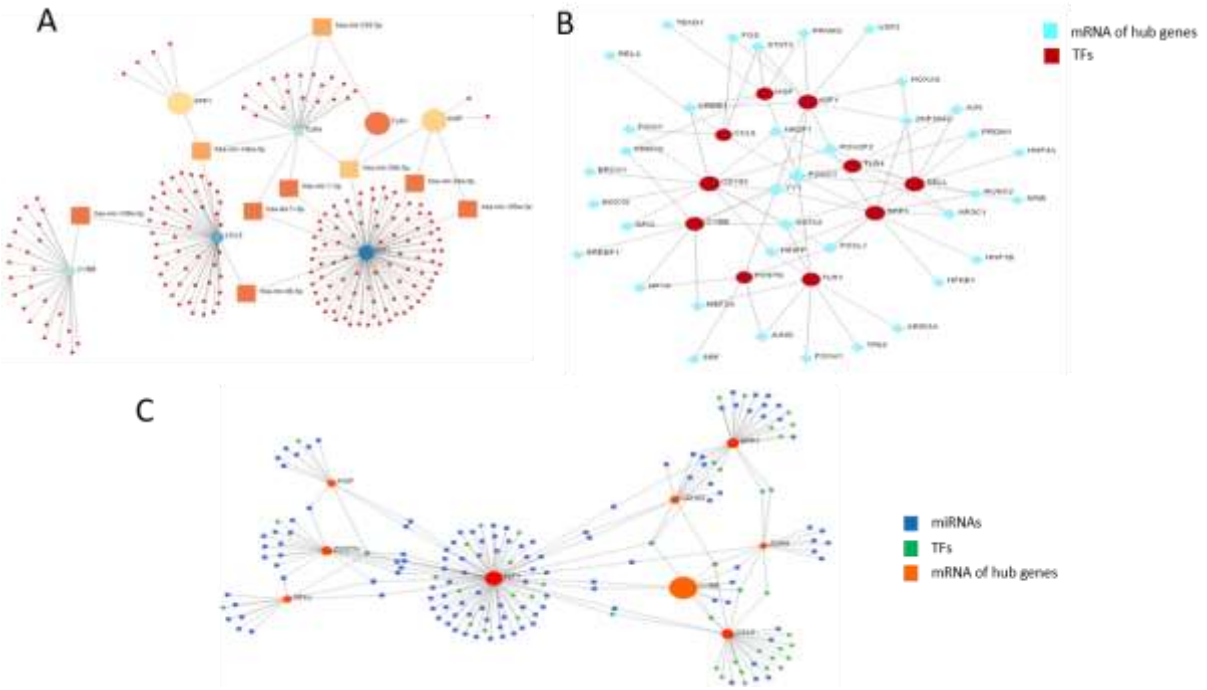


***Figure 4.*** ***(A)*** *miRNA-mRNA network of hub genes. The circle shows mRNA of hub genes and the square shows predicted miRNA,* ***(B)*** *TF-mRNA network. The red shows the mRNA hub genes, the blue shows the TF* ***(C)*** *TF-miRNA regulatory network. The blue shows predicted miRNAs, the green shows TFs, the orange shows mRNA of hub genes.*

## E. DRUG-GENE INTERACTION ANALYSIS

DGIdb results showed that 5 hub genes out of 10 hub genes showed interactions with drugs approved by the Food and Drug Administration (FDA). TLR4 has eight (Eritoran, Nelfinavir, Saquinavir, Tacrolimus, Ritonavir, Infliximab, Pravastatin and Methotrexate) drug targets, SPP1 has five (Calcitonin, Tacrolimus, Wortmannin, Alteplase and Gentamicin) drug targets, CYBB has three (Chrysin, Apigenin and Luteolin) drug targets, HGF has four (Imatinib Mesylate, Wortmannin, Reserpine and Epigalocatechin Gallate) drug targets, and SELL has one (Rivipansel) drug target (Table 5).

***Table 5.*** Candidate drugs targeting hub genes with PAH

| Gene | Drug | Sources | PMID |
|---|---|---|---|
| TLR4 | Eritoran | TTD | 16820585 |

| | | | |
|---|---|---|---|
| TLR4 | Nelfinavir | NCI | 15388451 |
| TLR4 | Saquinavir | NCI | 15388451 |
| TLR4 | Tacrolimus | PharmGKB | 24820765 |
| TLR4 | Ritonavir | NCI | 15388451 |
| TLR4 | Infliximab | NCI | 12847679 |
| TLR4 | Pravastatin | PharmGKB | 12742999 |
| TLR4 | Methotrexate | PharmGKB | 20136356 |
| SPP1 | Calcitonin | NCI | 8013390 |
| SPP1 | Tacrolimus | NCI | 16103732 |
| SPP1 | Wortmannin | NCI | 14703434 |
| SPP1 | Alteplase | NCI | 12009309 |
| SPP1 | Gentamicin | NCI | 11274264 |
| CYBB | Chrysin | DTC | 23786520 |
| CYBB | Apigenin | DTC | 23786520 |
| CYBB | Luteolin | DTC | 23786520 |
| HGF | Imatinib Mesylate | NCI | 113439348 |
| HGF | Wortmannin | NCI | 9603913 |
| HGF | Reserpine | NCI | 16081063 |
| HGF | Epigalocatechin Gallate | NCI | 16449979 |
| SELL | Rivipansel | TTD | 20508165 |

# III.DISCUSSION

PAH is one of the rare forms of pulmonary hypertension (PH) that is marked by a rise in average pulmonary artery pressure and vascular resistance. Although significant improvement has been achieved in the pathogenesis of PAH made recently, diagnosing PAH remains challenging. The pathogenesis of PAH includes mutations, immune dysfunction, and inflammation.

Thanks to the development of microarray technology, several biomarkers for PAH have been identified recently. Although some novel biomarkers for PAH have been identified recently, greater knowledge of the disease's molecular pathogenesis will help the discovery of new treatment options. Reliable and specific biomarkers for PAH are urgently needed to diagnose this disease. Our study aims to find possible biomarkers and molecular pathways for PAH by analyzing the gene expression data. We searched for PAH GSE datasets in the NCBI GSE website. Although there are several PAH datasets [4; 15; 16], we chose two datasets (GSE113439 and GSE117261) based on the following criteria: a. datasets which used the same platform (GPL6244), b. datasets include a minimum of 15 PAH samples. One recent study which only used GSE117261 dataset identified potential hub genes, biomarkers, miRNAs and TFs [4], but our study used two datasets which confirm and strengthen the results of that study.

Hub genes, TFs, mRNAs, and miRNAs were identified to be involved in the pathobiology of PAH. There were 122 common DEGs found between the two datasets. We uncovered 10 genes that may have roles in the molecular etiology of PAH *(CCL5, TLR4, TLR1, SPP1, CYBB, HGF, IGF1, SELL, CD163,* and *POSTN*). *CLL5* and *SPP1* are also found to be the hub genes related to PAH previously [4]. We also looked into GO and KEGG pathway analysis of common DEGs and identified 10 hub genes to interpret the genes biologically. The most enriched GO terms of common DEGs in the BP, MF, and CC categories are “interleukin-1 beta secretion”,“lipopeptide binding” and “extracellular matrix” respectively. The most enriched GO terms of 10 hub genes in the BP, MF, and CC categories are “positive regulation of tumor necrosis factor biosynthetic process”, “protein binding”, and “extracellular space.” One of the top GO terms in the BP category is “inflammatory response” in our study. Previous research has shown inflammation as a significant factor in the progression of PAH.

[17]. Inflammatory response and extracellular matrix were also revealed to be the most enriched GO terms in BP and CC terms, according to a recent study [18]. Our finding support that inflammation may be an important disease-related factor in PAH [17]. In previous research, extracellular matrix remodeling was found to contribute to the molecular etiology of PAH. [19].This data, combined with our data, confirms that the extracellular matrix also plays a crucial role in the pathobiology of PAH. Because of the inflammatory changes, PAH is also accepted as an auto-inflammatory disease [20]. Immune imbalances play a key part in the development of IPAH [21]. Overall, our findings on the inflammatory response, TLR signaling pathway, and PI3K-Akt signaling pathway changes support the idea that inflammation is an important pathobiological mechanism of PAH.

We found that the TLR pathway is one of the top 10 enriched KEGG terms related to common DEGs and the most enriched KEGG term related to 10 hub genes. Previous research has shown the relevance of the TLR pathway in PAH, which is supported by our findings. [22]. PI3K-Akt signaling pathway was the second most enriched KEGG term related to 10 hub genes. Previous research has shown the important role of the PI3K-Akt signaling pathway in PAH [23; 24].
The interactions between DEGs, TFs, and miRNAs were also analyzed. We identified ten TFs (FOXC1, GATA2, YY1, FOXL1, NFKB1, PDEUD, POU2F2, NFK, HINFP, and JUN) and ten miRNAs ("hsa-mir-335-5p, hsa-mir-92a-3p, hsa-mir-1-3p, hsa-mir-124-3p, hsa-mir-155-5p, hsa-mir-190a-3p, hsa-mir-5011-5p, hsa-mir-16-5p, hsa-mir-19b-3p, and hsa-mir-15b-5p") that regulate the common DEGs of PAH. The miRNAs are important in the etiology of PAH, particularly affecting cell proliferation of pulmonary artery smooth muscle cells and fibroblasts and apoptosis [25]. Previous studies showed that PAH patients had downregulation in miR-124 [26]. Additionally, the interactions between 10 hub genes and TFs and miRNAs were analyzed. We detected 8 miRNAs ("hsa-mir-26b-5p, hsa-mir-146a-5p, hsa-mir-335-5p, hsa-mir-26a-5p, hsa-mir-98-5p, hsa-mir-199a-3p, hsa-mir-1-3p, hsa-mir-106b-5p") that regulate the two or more hub genes. We also identified 10 TFs (FOXC1, YY1, GATA2, FOXL1, POU2F2, CREB1, STAT3, HINFP, NR3C1, and FOXI1) as the top 10 TFs related to the identified 10 hub genes in our study. One previous study identified hsa-mir-199a-3p and has-mir-26b-5p as miRNAs related to IPAH [27]. FOXC1, FOXL1, GATA2, YY1, and JUN were compatible with previous studies [28; 29]. Identified miRNAs may serve as great biomarkers for PAH.

The identified hub genes in our study might contribute to the pathobiology and treatment of PAH. SPP1 gene is a matrix phosphoglycoprotein that has roles in cell adhesion, inflammatory response, and osteoblast differentiation. SPP1 gene was also identified as a hub gene for PAH by a previous comprehensive gene expression analysis, which verifies our findings [28; 29]. The role of the SPP1 gene in the proliferation of pulmonary vascular smooth muscle cells (PVSMCs) has previously been demonstrated [29]. The increase in the expression of the SPP1 gene in PAH has also been shown [30]. SPP1 has also been identified as a hub gene for idiopathic pulmonary arterial hypertension (IPAH) [27]. Previous studies showed that CCL5 was also identified as a hub gene in PAH [31]. CCL5 has been proven to have a significant function in pulmonary vascular remodeling in the past. [31].CLL5 has also been identified as a hub gene for IPAH [32].

Although the association between PAH and inflammation is strong, the exact mechanism contributing to PAH is still unclear. One of the top 10 KEGG pathways we found in our research is the toll-like receptor signaling (TLR) pathway.TLR3 and TLR4 appear to have crucial roles in the pathobiology of PAH, according to recent research. TLR4 may play a role in endothelial-to-mesenchymal transition and pulmonary vascular remodeling (EndMT) [33; 34]. PAH is linked to pulmonary vascular inflammation and immune dysregulation, both of which are influenced by TLR4 signaling pathways. [35]. One study also showed that PAH patients have TLR3 deficiency [36].TLR1 and TLR4, which we identified as hub genes in our study that play an important role in TLR signaling, confirmed by previous studies. Based on these results, TLR4 might be a crucial therapeutic target for treating PAH patients. HGF is a hepatocyte growth factor. One study showed that HGF has an anti-inflammatory activity that makes it a great treatment target for PAH [37]. IGF1 has been linked to the proliferation of smooth muscle cells in the pulmonary artery in PAH patients. [38]. CD163 was modulated in PAMCS [39] POSTN is involved in vascular injury and smooth muscle cell proliferation [40]. High

expression of POSTN was discovered in PA and IPAH patients [41]. POSTN was shown to be a great treatment target for pulmonary hypertension [42].

We found that eritoran was the top drug target for the TLR4 gene. Eritoran a TLR4 receptor antagonist was found to heal inflammation by inhibiting the TLR4 signaling pathway in a mouse model with chronic liver injury [43]. Our finding, that the TLR4 signaling pathway is one of the top-enriched KEGG pathways, also supports Eritoran may be a good treatment candidate for PAH, especially for treating inflammation-based symptoms of the disease. We found that chrysin is the top drug target for the CYBB gene. Previous studies have shown that chrysin heals chronic hypoxia-induced pulmonary hypertension in rat models [18; 44]. Imatinib, another top drug target for HGF, was shown to alleviate PAH patients' symptoms [45], Overall, these medicines that target the hub genes identified in our investigation may be good candidates for future PAH treatment studies.

Our study has two main limitations. Our study's first limitation is the small sample size; we used two GEO datasets of PAH. Our results need to be confirmed by more datasets. The second limitation of our study is that our study is only an in silico analysis. Our in silico findings have to be validated in vivo and in vitro experiments.

# IV.CONCLUSION

In conclusion, we detected DEGs and associated functional terminology pathways using in silico analysis. *CCL5*, *TLR4*, *TLR1*, *SPP1*, *CYBB*, *HGF*, *IGF1*, *SELL*, *CD163*, and *POSTN* genes were identified to be strong prospective therapeutic biomarkers at the protein level in our study. The enrichment analysis of hub genes identified RNA-based therapeutic biomarkers "hsa-mir-26b-5p, hsa-mir-146a-5p, hsa-mir-335-5p, hsa-mir-26a-5p, hsa-mir-98-5p, hsa-mir-199a-3p, hsa-mir-1-3p, hsa-mir-106b-5p." We also identified 10 TFs (FOXC1, YY1, GATA2, FOXL1, POU2F2, CREB1, STAT3, HINFP, NR3C1, and FOXI1) that regulate the top 10 hub genes in our study. Our findings will aid in the understanding of PAH pathophysiology and molecular processes.

# V. REFERENCES


[1] R.M. Tuder, S.L. Archer, P. Dorfmuller, S.C. Erzurum, C. Guignabert , E. Michelakis, M. Rabinovitch, R. Schermuly, K.R. Stenmark, N.W. Morrell, "Relevant issues in the pathology and pathobiology of pulmonary hypertension," *J Am Coll Cardiol*, 62(25 Suppl):D4–12, 2013.

[2] G. Simonneau, D. Montani, D.S. Celermajer, et al., "Haemodynamic definitions and updated clinical classification of pulmonary hypertension," *Eur Respir*, *53*(1), 2019.

[3] J. Gauthier, A.T. Vincent, S.J. Charette, & N. Derome, "A brief history of bioinformatics," *Briefings in Bioinformatics*, *20*(6), 1981–1996, 2019.

[4] Y. Zeng, N. Li, Z. Zheng, R. Chen, M. Peng, W. Liu, J. Zhu, M. Zeng, J. Cheng, C. Hong, "Screening of Hub Genes Associated with Pulmonary Arterial Hypertension by Integrated Bioinformatic Analysis," *BioMed Research International*, vol. 2021, 16 pages, 2021.

[5] R.Q. Wei, W.M. Zhang, Z. Liang, C. Piao, G. Zhu, "Identification of Signal Pathways and Hub Genes of Pulmonary Arterial Hypertension by Bioinformatic Analysis," *Canadian Respiratory Journal*, vol. 2022, 12 pages, 2022.

[6] R. Edgar, M. Domrachev, A.E. Lash, "Gene Expression Omnibus: NCBI gene expression and hybridization array data repository," *Nucleic Acids Res*, *30*(1):207-10, 2002.

[7] P. Thomas, D. Ebert, A. Muruganujan, T. Mushayahama, L.P. Albou and H. Mi, "PANTHER: Making genome-scale phylogenetics accessible to all," *Protein Society*, 31(1):8-22, 2022.

[8] D.W. Huang, B.T. Sherman, R.A. Lempicki, "Systematic and integrative analysis of large gene lists using DAVID Bioinformatics Resource," *Nature Protoc, 4*(1):44-57, 2009.

[9] D. Warde-Farley, S.L. Donaldson, O. Comes, K. Zuberi, R. Badrawi, P. Chao, M. Franz, C. Grouios, F. Kazi, C.T. Lopes, A. Maitland, S. Mostafavi, J. Montojo, Q. Shao, G. Wright, G.D. Bader, & Q. Morris, "The GeneMANIA prediction server: biological network integration for gene prioritization and predicting gene function," *Nucleic acids research 38*(Web Server issue), W214–W220, 2010.

[10] J. Xia, E.E. Gill, and R.E. Hancock, "NetworkAnalyst for statistical, visual and network-based meta-analysis of gene expression data," *Nat. Protoc, 10*,823–844, 2015.

[11] A. Khan, O. Fornes, A. Stigliani, M. Gheorghe, J.A. Castro-Mondragon, R. Van Der Lee et al., "JASPAR 2018: update of the open-access database of transcription factor binding profiles and its web framework," *Nucleic Acids Res, 46*, D260–D266, 2018.

[12] H. Sheng-Da, L. Feng-Mao, W. Wei-Yun, L. Chao, H. Wei-Chih, C. Wen-Ling, T. Wen-Ting, C. Goun-Zhou, L. Chia-Jung, C. Chih-Min, C. Chia-Hung, W. Ming-Chia, H. Chi-Ying, T. Ann-Ping, H. Hsien-Da, " miRTarBase: a database curates experimentally validated microRNA–target interactions*," Nucleic Acids Research Volume, 39*, Issue suppl_1, Pages D163–D169, 2011.

[13] J. Xia, E.E. Gill, R.E. Hancock., "NetworkAnalyst for statistical, visual and network-based meta-analysis of gene expression data," *Nat Protoc, 10*(6):823-44, 2015.

[14] K.C. Cotto, A.H. Wagner, Y.Y. Feng, S. Kiwala, A.C. Coffman, G. Spies, A. Wollam, N.C. Spies, O.L. Griffith, & M. Griffith, "DGIdb 3.0: a redesign and expansion of the drug-gene interaction database," *Nucleic Acids Research 46*(D1), D1068–D1073, 2018.

[15] Y.D. Zhao, H.Z.H. Yun, J. Peng, L. Yin et al., "De novo synthesize of bile acids in pulmonary arterial hypertension lung," *Metabolomics*, 10(6):1169-1175, 2014.

[16] R. Rajkumar, K. Konishi, T.J. Richards, D.C. Ishizawar et al., "Genomewide RNA expression profiling in lung identifies distinct signatures in idiopathic pulmonary arterial hypertension and secondary pulmonary hypertension," *Am J Physiol Heart Circ Physiol*, 298(4):H1235-48, 2010.

[17] E. Soon, A. Crosby, M. Southwood, P. Yang, T. Tajsic, M. Toshner, S. Appleby, C.M. Shanahan, K.D. Bloch, J. Pepke-Zaba, P. Upton, N.W. Morrell N, "Bone morphogenetic protein receptor type II deficiency and increased inflammatory cytokine production. A gateway to pulmonary arterial hypertension,"*Am J Respir Crit Care Med, 192*:859–72, 2015.

[18] H. Dong, X. Li, M. Cai, C. Zhang, W. Mao, Y. Wang, Q. Xu, M. Chen, L. Wang, X. Huang, "Integrated bioinformatic analysis reveals the underlying molecular mechanism of and potential drugs for pulmonary arterial hypertension," *Aging* (*Albany NY), 13*:14234-14257, 2021.

[19] T. Thenappan, S.Y. Chan, E.K. Weir, "Role of extracellular matrix in the pathogenesis of pulmonary arterial hypertension," *Am J Physiol Heart Circ Physiol, 315*:H1322–31, 2018.

[20] M. Rabinovitch, C. Guignabert, M. Humbert, et al, "Inflammation and immunity in the pathogenesis of pulmonary arterial hypertension," *Circ Res, 115* (1):165–175, 2014.

[21] L.M. Marsh, K. Jandl, G. Grünig, et al., "The inflammatory cell landscape in the lungs of patients with idiopathic pulmonary arterial hypertension," *Eur Respir J*, 51 (1):1701214, 2018.

[22] R.S. Stearman, Q.M. Bui, G. Speyer, et al., "Systems analysis of the human pulmonary arterial hypertension lung transcriptome," *Am J Respir Cell Mol Biol, 60*: 637–649, 2019.

[23] Q. Zhang, Y. Cao, Q. Luo, P. Wang, P. Shi, C.E.M. Song, J. Ren, B. Fu, H. Sun, "The transient receptor potential vanilloid-3 regulates hypoxia-mediated pulmonary artery smooth muscle cells proliferation via PI3K/AKT signaling pathway," *Cell Prolif, 51*:e12436, 2018.

[24] J. Wu, Z. Yu, D. Su, "BMP4 protects rat pulmonary arterial smooth muscle cells from apoptosis by PI3K/AKT/Smad1/5/8 signaling," *Int J Mol Sci, 15*:13738–54, 2014.

[25] A. Courboulin, B. Ranchoux, S. Cohen-Kaminsky, F. Perros, S. Bonnet, "MicroRNA networks in pulmonary arterial hypertension: share mechanisms with cancer*?," Curr Opin Oncol, 28*(1):72-82, 2016.

[26] P. Caruso, B.J. Dunmore, K. Schlosser, S. Schoors, C. Dos Santos, C. Perez-Iratxeta, J.R. Lavoie, H. Zhang, L. Long, A.R. Flockton, G. Frid, P.D. Upton, A. D'Alessandro, C. Hadinnapola, F.N. Kiskin, M. Taha, L.A. Hurst, M.L. Ormiston, A. Hata, K.R. Stenmark, N.W. Morrell, "Identification of MicroRNA-124 as a Major Regulator of Enhanced Endothelial Cell Glycolysis in Pulmonary Arterial Hypertension via PTBP1 (Polypyrimidine Tract Binding Protein) and Pyruvate Kinase M2," *Circulation, 136*(25), 2451–2467, 2017.

[27] C. Li, Z. Zhang, Q. Xu, R. Shi, "Comprehensive Analyses of miRNA-mRNA Network and Potential Drugs in Idiopathic Pulmonary Arterial Hypertensioni," *BioMed Research Internationa*l, vol. 2020, Article ID 5156304, 10 pages, 2020.

[28] X. Yao, T. Jing, T. Wang, C. Gu, X. Chen, F. Chen, H. Feng, H. Zhao, D. Chen, & W. Ma, "Molecular Characterization and Elucidation of Pathways to Identify Novel Therapeutic Targets in Pulmonary Arterial Hypertension," *Frontiers in Physiology, 12*, 694702, 2021.

[29] Y. Zeng, N. Li, Z. Zheng, R. Chen, M. Peng, W. Liu, J. Zhu, M. Zeng, J. Cheng, & C. Hong, "Screening of Hub Genes Associated with Pulmonary Arterial Hypertension by Integrated Bioinformatic Analysis," *BioMed Research International*, 6626094, 2021.

[30] M. Saker, L. Lipskaia, E. Marcos, S. Abid, A. Parpaleix, A. Houssaini, P. Validire, P. Girard, H. Noureddine, L. Boyer, N. Vienney, V. Amsellem, L. Marguerit, B. Maitre, G. Derumeaux, J.L. Dubois-Rande, C. Jourdan-Lesaux, M. Delcroix, R. Quarck, & S. Adnot, "Osteopontin, a Key Mediator Expressed by Senescent Pulmonary Vascular Cells in Pulmonary Hypertension Arteriosclerosis*," Thrombosis, and Vascular Biology, 36*(9) 1879–1890, 2016.

[31] S. Abid, E. Marcos, A. Parpaleix, V. Amsellem, M. Breau, A. Houssaini, N. Vienney, M. Lefevre, G. Derumeaux, S. Evans, C. Hubeau, M. Delcroix, R. Quarck, S. Adnot, & L. Lipskaia, "CCR2/CCR5-mediated macrophage-smooth muscle cell crosstalk in pulmonary hypertension," *The European Respiratory Journal, 54*(4), 1802308, 2019.

[32] J. Xu, Y. Yang, Y. Yang, & C. Xiong, "Identification of Potential Risk Genes and the Immune Landscape of Idiopathic Pulmonary Arterial Hypertension via Microarray Gene Expression Dataset Reanalysis," *Genes,* 12(1), 125, 2021.

[33] Y. Sadamura-Takenaka, T. Ito, S. Noma, Y. Oyama, S. Yamada, Kawahara KI, et al., "HMGB1 promotes the development of pulmonary arterial hypertension in rats," *PLoS ONE, 9*:e102482, 2014.

[34] T. Suzuki, E.J. Carrier, M.A. Talati, A. Rathinasabapathy, X. Chen, R. Nishimura, et al., "Isolation and characterization of endothelial-to-mesenchymal transition-cells in pulmonary arterial hypertension," *Am J Physiol Lung Cell Mol Physiol, 314*:L118–126, 2017.

[35] C. Gerges and I. Lang, "Targeting inflammation and immunity in pulmonary arterial hypertension: any easier after the CANTOS proof-of-concept that anti-inflammation cuts cardiovascular events?," *Pulm Circ, 8*: 2045893218754855, 2018.

[36] D. Farkas, A.A. Roger Thompson, A.R. Bhagwani, S. Hultman, H. Ji, N. Kotha, et al., "Toll-like receptor 3 is a therapeutic target for pulmonary hypertension," *Am J Respir Crit Care Med, 199*:199–210, 2019.

[37] Y. Pang, M.T. Liang, Y. Gong et al., "HGF Reduces Disease Severity and Inflammation by Attenuating the NF-κB Signaling in a Rat Model of Pulmonary Artery Hypertension*," Inflammation, 41*, 924–931, 2018.

[38] L. Dewachter, C. Dewachter, A. Belhaj, S. Lalande, B. Rondelet, M. Remmelink, J. Vachiery, R. Naeije, "Insulin-like growth-factor-1 contributes to the pulmonary artery smooth muscle cell proliferation in pulmonary arterial hypertension," *European Respiratory Journal, 44*:P316, 2014.

[39] L. Ramakrishnan, S. Mumby, J. Wort, G. Quinlan, "CD163 is expressed and modulated in human pulmonary artery smooth muscle cells: Implications for pulmonary artery hypertension," *European Respiratory Journal, 46* (suppl 59), 2015.

[40] J.A. Schwanekamp, A. Lorts, R.J. Vagnozzi, D. Vanhoutte, J.D. Molkentin, "Deletion of periostin protects against atherosclerosis in mice by altering inflammation and extracellular matrix remodeling," *Arterioscler Thromb Vasc Biol*, 36:60–68, 2016.

[41] V.B. Abdul-Salam, J. Wharton, J. Cupitt, M. Berryman, R.J. Edwards, M.R. Wilkins, "Proteomic analysis of lung tissues from patients with pulmonary arterial hypertension," *Circulation, 122*:2058–2067, 2010.

[42] X. Nie, C. Shen, J. Tan, Z. Wu, W. Wang, Y. Chen, Y. Dai, X. Yang, S. Ye, J. Chen, & J.S. Bian, "Periostin: A Potential Therapeutic Target For Pulmonary Hypertension?," *Circulation Research, 127*(9), 1138–1152, 2020.

[43] Y.C. Hsieh, K.C. Lee, P.S. Wu, T.I. Huo, Y.H. Huang, M.C. Hou, H.C. Lin, "Eritoran Attenuates Hepatic Inflammation and Fibrosis in Mice with Chronic Liver Injury," *Cells, 10*(6):1562, 2021.

[44] F. Dong, J. Zhang, X. Chen, S. Zhang, L. Zhu, Y. Peng, & Z. Guo, "Chrysin Alleviates Monocrotaline-Induced Pulmonary Hypertension in Rats Through Regulation of Intracellular Calcium Homeostasis in Pulmonary Arterial Smooth Muscle Cells," *Journal of Cardiovascular Pharmacology, 75*(6), 596–602, 2020.

[45] M.M. Hoeper, R.J. Barst, R.C. Bourge, J. Feldman, A.E. Frost, N. Galié, M.A. Gómez-Sánchez, F. Grimminger, E. Grünig, P.M. Hassoun, N.W. Morrell, A.J. Peacock, T. Satoh, G. Simonneau, V.F. Tapson, F. Torres, D. Lawrence, D.A. Quinn, & H.A. Ghofrani, "Imatinib mesylate as add-on therapy for pulmonary arterial hypertension: results of the randomized IMPRES study," *Circulation, 127*(10) 1128–1138, 2013.