NETWORK PHARMACOLOGICAL ANALYSIS OF PRIMARY TARGETS UNDERLYING PULMONARY HYPERTENSION

Abstract

The emerging subject of network pharmacology uses networks of disease-gene-drug targets to better understand the bimolecular level pharmacological mechanism of active components, which in turn aids in drug discovery. When blood pressure rises in the pulmonary arteries, it's called hypertension. Appearing when pulmonary arterial pressure is extremely high. Globally, pulmonary hypertension is thought to affect about 1 percentage of the population. Using a network pharmacological strategy based on a string database, we identified particular genes associated in pulmonary hypertension, gene ontology, biological pathways, and performed a protein-protein interaction analysis on 100 proteins and 1910 PPI connections. The top 10 targets based on highest degree ratings in CytoScape_v3.9.1 could be considered central targets. This chapter focused on the top 5 gene targets associated with hypertension, including EDN1, VEGFA, AKT1, and TNF. With this approach, we found that most of AKT1's protein-protein interactions are similar to those of other genes. Our data implies that the AKT1 gene, via several pathways, may be a novel therapy option for pulmonary hypertension; however, more preclinical and clinical research is needed to verify our findings.

Keywords: AKT1, Network pharmacology, Cytoscape, Target, Hypertension

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I. INTRODUCTION

Pulmonary hypertension describes a group of diseases characterized by abnormal high pressures in the pulmonary arteries. Finding an effective treatment plan requires first identifying the source of the issue. Treatment for pulmonary hypertension typically focuses on the underlying condition, which is common in the left heart disease and advanced stages of chronic obstructive pulmonary disease (COPD).Right-sided cardiac catheterization confirms the diagnosis of pulmonary hypertension (PH) if the resting mean pulmonary arterial pressure (mPAP) is 20mm Hg or above(1).About one percent of people worldwide have pulmonary hypertension; up to ten percent of those over 65 have it; and at least half of those with heart failure (HF) have it. As a result, 2 PH is a common clinical problem encountered by cardiologists. The diagnosis and treatment of PH are discussed in detail in this article.(2).Primary vasculopathies and chronic organized thromboembolism are less common causes of pulmonary hypertension. Both can be treated with cutting-edge medical therapy, although the former requires examination for surgical surgery(3).Patients with pulmonary hypertension (PH) may fall into one of five clinical subgroups: those with pulmonary arterial hypertension (PAH), those with pulmonary hypertension caused by left-sided heart disease, those with PH caused by chronic lung illness, those with PH caused by chronic thromboembolic mechanisms, and those with PH caused by unclear and/or multifactorial mechanisms. These problems can develop from a wide variety of causes. About one percent of people worldwide have PH, and it's possible that more than half of those with heart failure have it. Therefore, PH is a common condition seen by cardiologists. Electrocardiography, chest radiography, and pulmonary function testing are the usual diagnostic procedures for individuals with symptoms and physical findings consistent with PH. To calculate a rough likelihood of PH, transthoracic echocardiography is employed. To rule out CTEPH, a ventilation-perfusion scan should be performed on all patients with suspected or proven PH who do not have left-sided heart or lung problems. For proper diagnosis and categorization, a right cardiac catheterization is required. It is mandatory to refer all patients with PAH or CTEPH to a specialised facility. CTEPH patients who are surgical candidates typically undergo pulmonary endarterectomy. Patients with PAH have access to a wide variety of approved therapies, including stimulators of soluble guanylate cyclase, specific inhibitors of phosphodiesterase type 5, antagonists of endothelin receptors, analogues of prostacyclin, and agonists of the prostacyclin receptor. The fundamental goal of treatment is to address the underlying cause of the issue due to left-sided cardiac disease(4) by identifying the specific target responsible for PH through network pharmacology.

II. NETWORK PHARMACOLOGY

Network pharmacology is a computational artificial intelligence (AI) technique developed in the 20th century, reduces the amount of time it takes to identify a potential drug discovery or protein/gene target for specific disease. It is similar to the mechanisms of multi constituents and multi targeting functionality of traditional medicine compounds, therefore it may be used to make predictions about how drugs will work in the treatment of diseases(5-7). In order to better understand the pharmacological mechanism of active components at the biomolecular level, a new field called network pharmacology is emerging, which is based on the network of disease: gene: drug targets. An increasingly comprehensive and useful method for understanding the intricate interplay between biological systems and drugs such illnesses, human organs, metabolic pathways, and target proteins is systems biology(8). New insights

into how specific chemicals work can be gained through the use of the Network pharmacology paradigm, which integrates computer prediction with experimental confirmation(6). Synergistic multi-compound network pharmacology and medication repurposing allow precise and effective therapeutic intervention, negating the need for drug research and accelerating clinical translation(9). Identifying the active natural biomolecule may be facilitated by combining a Network pharmacology approach with herbal medicine.

1. **Goals of Pulmonary Hypertension Screening and Clinical Indications for Treatment:** Searches for "Pulmonary hypertension" in string database (https://string-db.org/) obtained list of genes were merged and deduplicated. After normalising the data on the UniProt database, we were able to gain the pertinent targets of pulmonary hypertension treatment. 100 identical targets were discovered from string database and further protein protein interaction analysis was performed.

2. **Constructing of Protein Protein Interaction (PPI) Network:** Analysis of Protein-Protein Interaction (PPI) is crucial for dissecting complex cellular mechanisms and gaining insight into biological processes. The PPI network was constructed using the STRING software. The aforementioned intersecting targets were loaded into STRING with the species set to "homo sapiens," and the interaction score set to 0.4, yielding the PPI network relationship. CytoScape_v3.9.1 was used to create the PPI network diagram. The network analyzer feature was used to determine the accuracy of the value. Central targets could be defined as the top 10 targets with the highest degree scores. In order to learn more about PPI networks, the STRING database was populated with data from 100 intersection targets. There are 100 proteins represented as nodes in the PPI network and 1910 PPI relationships represented as edges were illustrated in Fig.1. Cytoscape v_3.9.1 was used to visualise the information on protein interactions in the PPI network. After collecting the PPI network, we used the network analyzer to determine the degree value. EDN1, VEGFA, ALB, AKT1, TNF, NOS3, IL6, INS, IL1B, and ACTB were the top 10 targets according to degree value, with 77, 76, 75, 75, 74, 74, 74, 71, 67, and 67 degrees of separation, respectively and represented in Table 1. These targets serve as hubs at the core of the PPI network, linking together the various nodes.PPI of the top 5 targets were discussed in this chapter.

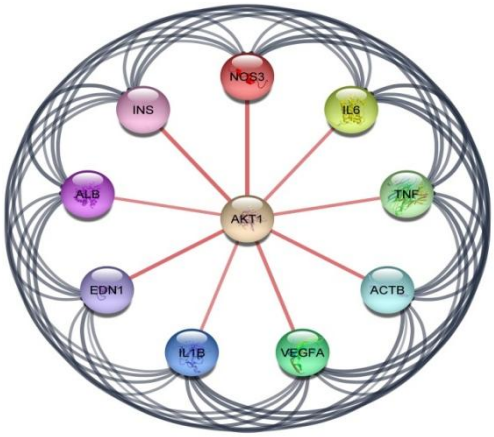

Figure 1: PPI of selected targets involved in PH

3. **Analysis of KEGG Pathways and Enrichment of GO Functions:** We used the Metascape platform to conduct a bioinformatics enrichment analysis on the prospective targets for pulmonary hypertension therapy, which included an analysis of biological processes, molecular activities, and cellular components using the GO and a pathway enrichment analysis using the KEGG. A significance level of P<0.01 was used, and "Homo sapiens" was selected as the species. The top 10 targets were selected for further analysis, the data was stored and analysed using a bioinformatics platform. Cytoscape v_3.9.1 software was used for representing the target networking in this chapter. Using the Metascape system, we analysed 65 CICS therapeutic candidates for migraines in terms of GO and KEGG pathway enrichment. The investigation yielded a total of 419 GO enrichment analysis comprising cellular component analysis (06), biological process analysis (407), and molecular function analysis (06). Results from the top ten GO characteristics were chosen, and the bioinformatics platform was used to preserve and visualize the results. The KEGG analysis yielded a total of 93 pathways was represented in Fig.2. The KEGG pathways are mainly related to Fluid shear stress,AGE-RAGE signaling pathway in diabetic complications, and atherosclerosis, HIF-1 signaling pathway, positive regulation of oxidoreductase activity, multicellular organismal-level homeostasis, Malignant pleural mesothelioma, vascular process in circulatory system, regulation of body fluid levels, positive regulation of MAP kinase activity,positive regulation of cell growth, maintenance of location, positive regulation of angiogenesis etc.

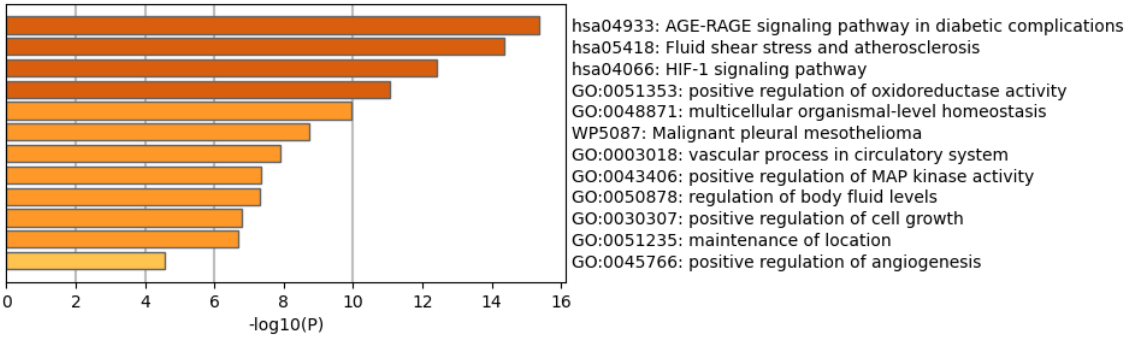

Figure 2: Kegg enrichment pathway

III. EDN1

In addition, the K198N (rs5370) in the endothelin 1 gene EDN1 has been linked to increased synthesis of endothelin 1 (ET-1), which may promote the development of PAH.(10).Therefore, we conduct a meta-analysis to examine the connection between polymorphisms in 5-HTT (rs25531), BMPR2 (rs1061157), END1 (rs5370), KCNA5 (rs10744676), and ENG (rs3739817), and the risk of PAH. Patients with PAH had different genotypes as compared to healthy controls when testing for the EDN1 gene-polymorphism. (ET-1) Endothelin-1 is a 21-amino acid peptide that is produced by the endothelial cells of vascular smooth muscle and acts as a potent vasoconstrictor. Multiple studies have linked ET-1's vasoconstrictive activity and effects on cell proliferation to PAH development. Since ET-1 is not stored but rather created when needed, it is able to physiologically modify vascular tone on demand. The plasma concentration of ET-1 is greatly increased in patients with PAH, and its action is mediated mostly by two types of receptors: ETB and ETA.

Because of their roles as regulators of receptor A, the former produce vasoconstriction and the latter, vasodilation (albeit the latter may also cause vasoconstriction in pathological contexts).(11). ET-1 is connected to the enzyme endothelial nitric oxide synthase, which generates the powerful vasodilator nitric oxide. In individuals with PAH and mutations in this gene, complicated genetic interactions have been observed. ET-1 is involved in this pathway.8 In addition, inhibiting ET-1 receptors can reduce inflammation, which is a major contributor to PAH.

Table 1: Degree analysis of top 5 genes

Target	Degree score	Closeness Centrality score	Between's Centrality score
EDN1	77	0.811475	0.036468
VEGFA	76	0.811475	0.033746
ALB	75	0.785714	0.023554
AKT1	75	0.798387	0.02952
TNF	74	0.779528	0.019528

The 212 amino acid pre-pro ET-1 serves as the starting point for the ET-1 synthesis process.(12). The 212 amino acid pre-pro ET-1 serves as a precursor for the mature protein ET-1, which is synthesised through a series of proteolytic processes. The 6386-base-pair ET-1 gene, EDN1, can be found in the 6p24-23 region of the human genome. An association between PAH and the G>T transversion at the +5665 position of the gene (K198N, rs3570), which changes Lys (K, lysine) to Asn (N, asparagine), has been found.(13). Blood pressure sensitivity, BMI, and plasma ET-1 levels have all been linked to this polymorphism. Researchers have found that those who carry the T allele have much higher levels of EDN1 activity, which may boost ET-1 production and promote PAH formation.

There are several endothelium mediators, but NO and EDN1 stand out. The NOS3 gene encodes the enzyme endothelium NO synthase, which catalyses the synthesis of NO from L-arginine. NO is a powerful endogenous vasodilator. Big EDN1 is a relatively inactive 39 amino acid peptide that is processed from a 212 amino acid pre-proendothelin. Endothelin converting enzyme-1(ECE-1) is a membranebound enzyme that converts the large EDN1 to a 21a.a. functional peptide. Since EDN1 receptor antagonists and NO agonists are now some of the finest alternatives available for therapy of IPAH, understanding the role of EDN-1 and NOS3 in IPAH has clinical value. Previously found polymorphisms in EDN1 include a G/T transversion at position +5665 (rs5370) affecting the 61st nucleotide of exon 5, which replaces Lys at the 198 codon with Asn (K198N), and a "A" insertion(I)/deletion(D) in exon1 at position +138 (rs10478694). This polymorphism is also known as Lys198Lys, Lys198Asn, and Asn198Asn. Table 1 provides the control and patient allelic and genotypic frequencies for the two EDN1 polymorphisms. In both the patient and control groups, no major disruption of Hardy-Weinberg equilibrium was seen. The rest of the EDN-1 gene's exons showed no evidence of genetic variation.

IV. VEGFA

Pulmonary hypertension (PH) is a cluster of diseases marked by right heart failure and vascular dysfunction in the lungs. Many other pathogeneses, including BMPR2 gene variations, pulmonary fibrosis, and left heart disorders, could be to blame(2) and Increased expression of the growth factors vascular endothelial growth factor A and its receptor VEGFR2 (14).In both healthy and diseased states, VEGF-A plays a crucial role as an angiogenic growth factor. It is necessary for endothelial cell survival, proliferation, migration, and permeability, and it binds to its primary receptor VEGFR2 on vascular endothelial cells, causing tyrosine phosphorylation of VEGFR2 at specific sites and triggering a complex network of downstream signalling processes(15, 16).Tyrosines Y1054 and Y1059 are essential for kinase activity and tyrosines Y949 (Y951 in humans), Y1173 and Y1212 directly bound to differential intracellular adaptor proteins to transduce VEGF-A signals upon phosphorylation. Endothelial cell proliferation and differentiation depend on Y1175 phosphorylation and subsequent activation of the PLC-ERK axis(17, 18)endothelial cell proliferation (through activation of GRB2/ERK and PI3K/AKT pathways) and CDC42-mediated front-back polarisation (through recruitment of NCK) during migration have all been linked to Y1214 phosphorylation(19, 20).Here, we show that VEGF-A-induced vascular leak is linked to PH in both human patients and hypoxia-induced mice models.

For normal angiogenesis to occur, the main angiogenic factor, vascular endothelial growth factor, is required. Proliferation of ECs has been linked to increased VEGF expression in severe PAH. Higher levels of plasma VEGF were found in PAH patients and higher expressions of VEGF and its receptor were seen in animal models (hypoxia or MCT-induced PH). Protein levels of vascular endothelial growth factor (VEGF) in the lungs of TGF- mice. The protein level of vascular endothelial growth factor A in the lungs of 2-day-old TGF- mice was reduced by 45% compared to that of WT mice of the same age. The protein level of VEGF164 was decreased in the lungs of both 2-day-old and adult TGF- mice, as measured by Western blotting. Placental growth factor (PlGF), a member of the vascular endothelial growth factor (VEGF) family, binds specifically to FGF receptor type 1 (Flt-1) and activates Flt-1 and its downstream target genes directly inside the cell, and transphosphorylates VEGFR-2 to generate pro-angiogenic signalling. Flt-1 can be bound and activated by the PlGF/VEGF-A heterodimer, which also induces Flt-1/VEGFR-2 dimerization.

Target	Mechanism of action	Reference
EDN1	The endothelial cell-derived vasoconstrictor endothelin-1 promotes the growth of smooth muscle cells in the pulmonary artery. Both endothelin receptor A (ETA) and endothelin receptor B (ETB) are important targets for endothelin-1. In PAH, ETA is highly expressed in VSMCs, where it promotes VSMC contraction and proliferation. Enhanced vasodilation is achieved by ETB because it increases prostacyclin and nitric oxide (NO) production and decreases ET-1 levels in the blood.	(21, 22)
VEGFA	Vasodilatory mediators are the primary means through which VEGF exerts its effect. Nitric oxide (NO) is the primary	(23, 24)

	downstream mediator of the vascular smooth muscle (VSP) and is increased by VEGF signalling through VEGFR. The binding of VEGF causes autophosphorylation of VEGFR2, which in turn elevates intracellular calcium via PI3K/Akt.	
TNF	In pulmonary artery smooth muscle cells (PASMCs), it specifically suppresses BMPR-II transcription and mediates post-translational BMPR-II cleavage via the sheddases ADAM10 and ADAM17. By inhibiting BMPR-II through TNF, BMP signalling is subverted, and BMP6 causes PASMC proliferation through the preferential activation of an ALK2/ACTR-IIA signalling axis. TNF also decreases BMPR-II expression and upregulates pro-proliferative NOTCH2 signalling in HPAH PASMCs via SRC family kinases.	(25, 26)
AKT1	Diastolic blood pressure is greatly elevated when Akt1 is depleted specifically in endothelial cells. Shear stress-mediated endothelial NO generation plays a crucial role in arterial pressure's maintenance of vascular function and responsiveness.	(27)

For normal angiogenesis to occur, one of major angiogenic factors is vascular endothelial growth factor (VEGF), is required. Proliferation of ECs has been linked to increased VEGF expression in severe PAH. Higher levels of plasma VEGF were found in PAH patients, and higher expressions of VEGF and its receptor were seen in animal models (hypoxia or MCT-induced PH). Protein levels of vascular endothelial growth factor (VEGF) in the lungs of TGF- mice. The protein level of vascular endothelial growth factor A in the lungs of 2-day-old TGF- mice was reduced by 45% compared to that of WT mice of the same age. The protein level of VEGF164 was decreased in the lungs of both 2-day-old and adult TGF- mice, as measured by Western blotting. Placental growth factor, a member of the vascular endothelial growth factor family, binds specifically to FGF receptor type 1 and induces pro-angiogenic signalling through multiple mechanisms, including direct intracellular stimulation of Flt-1 and downstream target genes and transphosphorylation of VEGFR-2 by activated Flt-1. Flt-1 can be bound and activated by the PlGF/VEGF-A heterodimer, which also induces Flt-1/VEGFR-2 dimerization.

V. AKT1

Activation of the downstream signalling molecule mTOR has been associated to the regulation of cell proliferation, migration, and death initiated by the Akt signalling pathway.Activation of the downstream signalling molecule mTOR has been linked to the regulation of cell proliferation, migration, and death initiated by the Akt signalling pathway(28, 29).Silencing Akt1 in pulmonary vascular endothelial cells and using Akt1 knockout mice reduce microvascular permeability and edoema formation, however Akt2 appears to play no major function in vascular tissue(30).Akt1, Akt2, and Akt3 are all members of the Akt kinase family, and they're extremely similar to one another. In contrast to Akt3, which is primarily expressed in brain tissue, Akt1 and Akt2 are expressed in a wide variety of tissues and cell types(31, 32). Akt isoforms regulate protein synthesis, cell cycle

progression, apoptosis, and proliferation in separate but overlapping ways(33). The proliferation of PASMCs can be dramatically reduced by blocking the Akt or mTOR signalling with Rapamycin/Akt inhibitor(34). Vascular remodelling occurs when PTEN is deleted in mouse smooth muscle cells, and severe pulmonary hypertension develops in PTEN conditional knockout (KO) mice when they are exposed to hypoxia(35), (36).

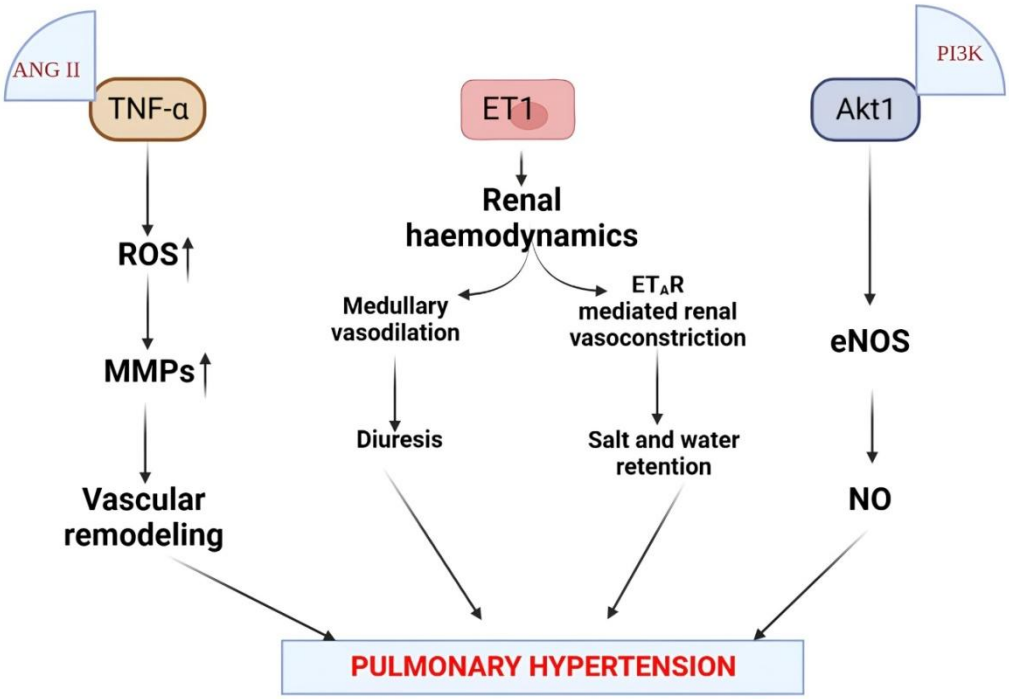

Figure 3: Screened genes and the pathophysiology of pulmonary hypertension

We hypothesised that PTEN/Akt/mTOR signalling is involved in the pathogenesis of PH and that the deletion of specific Akt kinase (Akt1/Akt2) isoforms might have different effects on the pathogenesis and progression of pulmonary vascular remodelling in animals with experimental PH. Endothelial nitric oxide synthase is translocated into mitochondria in response to asymmetric dimethylarginine (ADMA) via nitration-mediated activation of Akt1. Threonine (T) 308 and serine (S) 473 phosphorylation processes are necessary for Akt1 activation, however. Therefore, the current study was conducted to better understand how ADMA may influence Akt1 phosphorylation. Finally, in the model of pulmonary hypertension, we discovered that mitochondrial translocation of eNOS is linked to elevated levels of eNOS and Akt1 phosphorylation and decreased levels of Akt1-CTMP protein interactions. As a result of our findings, we hypothesise that elevating CTMP levels could be a therapeutic strategy for PH treatment and that CTMP playing a direct role in ADMA induced Akt1 phosphorylation both inin vitro and in vivo.

VI. TNF

Both caspase-11 and its human counterpart caspase-4 were activated in pulmonary arterial hypertension (PAH) animal models and in human pulmonary artery endothelial cells caused by TNF (tumour necrosis factor). In addition, blocking the activation of pyroptosis

effectors GSDMD (gasdermin D) and GSDME (gasdermin E) by inhibiting caspase-4 in human pulmonary artery endothelial cells reduced the onset of TNF-induced pyroptosis. As a traditional pro-PAH factor, tumour necrosis factor alpha (TNF-) is found at increased levels in the plasma of patients with PAH and animal models, therefore we employ TNF- as a stimulator of HPAECs to study caspase-4/11-mediated pyroptosis(37, 38). Some recent research has also linked TNF- to pyroptosis by showing that it can activate caspase-3 to cleave GSDME (gasdermin E)(39); Because of its importance in the aetiology of PAH, this study aims to investigate the effect of TNF-induced caspase-11-mediated pyroptosis on endothelial function. Here, we showed that caspase-4/11 was activated in the PAH animal models and that TNF-induced HPAECs, deletion of caspase-11, or pharmacological regulation of caspase-11 may provide protection against PAH.

Not only that, but it's possible that inflammatory molecules like IL-1, NF-B, and TNF- may stabilise HIF-1. Many researchers consider NF-B to be the master regulator of inflammation under hypoxic conditions due to its ability to stabilise HIF-1 following its release from inhibitory kinase b (IKb) via nuclear factor kinase subunit b (Ikk) activation. Increased expression of 12(s)-hydroxyeicosatetraenoic acid (12(s)-HETE), produced by activation of leukocyte-type 12 lipoxygenase (12-LO), was seen in the lungs of rats subjected to chronic hypobaric hypoxia. By activating ERK1/2 and p38 MAPK, this stimulation contributes to inflammatory pathways via smooth muscle cells (SMCs). Then, HAPH develops after the proliferation process is fanned. Short-term and long-term exposure to high altitudes, as will be seen in the next sections, activates inflammatory pathways, contributing to the development of pulmonary high-altitude illnesses including HAPE and HAPH. Serum levels of TNF- and IL-6 are also increased in HAPE patients. After that, Sharma et al. discovered that people with HAPE had higher than usual blood levels of TNF-, and that this difference could impair lung permeability(40).

VII. CONCLUSION

The network pharmacological strategy for hypertension presented in this chapter identified a group of genes with a potential role in the condition. The bulk of AKT1's protein-protein interactions were found to be comparative with those of other genes using this method. We argue that our study provides a fresh perspective on the investigation of such problems and improves our knowledge of the genes involved in hypertension by illuminating their functional and pathway links. While more in-depth clinical studies and preclinical are required to confirm our results, our work does provide preliminary evidence that the AKT1 gene, acting in a number of different ways, may be an effective in the PH treatment.

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