A metabolic profiling study of pulmonary arterial hypertension

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Introduction. The main characteristic of arterial hypertension (PAH) is the gradual increase in pulmonary artery pressure and impaired vascular growth regulation. Increasingly, animal models of PAH and blood samples from patients with PAH and lung cells from patients with PAH have been found to have metabolic abnormalities. Because of this, changes in metabolism and biological functions are deemed to be common in PAHs. From the perspective of metabolomics, this review introduces metabolic research and possible treatments for pulmonary hypertension. Developing new therapeutics for treating patients can be strengthened by expanding our understanding of the metabolic mechanisms underlying PAH.

Keywords. pulmonary arterial hypertension; targeting drugs for pulmonary hypertension; Metabolomics; Pulmonary hypertension metabolism

INTRODUCTION

Pulmonary hypertension (PH) is a significantly hazardous disease, and gradually increasing pulmonary artery (PA) pressure is one of the principal characteristics. Symptoms were accompanied by high PA pressure included the remodeling of pulmonary vessels, enhanced vasoconstriction, and the compensatory hypertrophy of the right ventricle (RV). In the final phase of this disease, heart failure and even death

will occur[1]. Depending on the current clinical classification system of the World Health Organization(WHO), PH is classified into 5 clinical categories based on similar pathophysiological mechanisms, clinical manifestations, hemodynamic characteristics, and treatment management for PH-related clinical conditions. The first type of pulmonary hypertension, also known as arterial pulmonary hypertension (PAH), can be further subdivided into Idiopathic Pulmonary Arterial Hypertension (IPAH), Heritable Pulmonary Arterial Hypertension (HPAH), Drug and toxin-induced PAH, and pulmonary hypertension caused by other diseases. The other affects four categories are more reflective of the wide variety of global population, such as PH of left heart disease, PH of the disease, PH due to lung diseases and/or hypoxia, PH due to pulmonary artery obstructions, PH with unclear and/or multifactorial mechanisms[2]. Abnormalities in pulmonary vascular structure and/or function (pulmonary vascular remodeling) are the product of abnormal regulation of multiple signaling pathways that maintain the structural and functional integrity of the vasculature. It is currently believed that pulmonary vascular remodeling is the consequence of a combination of genetic and epigenetic factors as well as environmental factors. Multiple vasoactive molecules, multiple ion channels, and multiple signaling pathways play important regulatory roles in pulmonary vascular remodeling[3]. The fundamental pathophysiology of pulmonary hypertension is the proliferation and migration of smooth muscle cells, endothelial cells, and fibroblasts, which results in pulmonary vascular remodeling and luminal blockage of diminutive pulmonary arteries.

Current status of treatment with targeted drugs for pulmonary hypertension：

In recent years, the pharmacological treatment for PAH has emerged at a faster rate. Before 1995, the era of traditional PAH treatment was characterized by clinicians treating PH with traditional medications such digitalis, diuretics, and potassium supplements, as well as antihypertensive drugs, with a high mortality rate. In 1995, approval of injectable isoproterenol for marketing in Europe and the United States was open the curtain on the era of targeted therapy for PAH. With the progress of the

PAH research mechanism, targeted drugs with different action pathways have been released one after another, and the treatment of pulmonary arterial hypertension has entered the era of diversified treatment such as oral, inhalation, subcutaneous, and intravenous injections, etc. Therefore, the prognosis of PAH patients has been gradually improved[4, 5].

According to the Chinese Guidelines for the Diagnosis and Treatment of Pulmonary Hypertension in 2021, the goal of PAH treatment is to reach and maintain a low-risk state as soon as possible. At present, there are set at three classic approaches to the targeted drug treatment of PAH to regulate vasoconstriction or relaxation. (1) Endothelin receptor pathway: mainly includes selective endothelin receptor antagonists (Aniracetam) or non-selective endothelin receptor antagonists (Bosentan, Macitentan);

(2)Nitric oxide-cyclic guanosine monophosphate(NO-cGMP) pathway: phosphodiesterase type 5 inhibitor(PDE-5i) (Sildenafil, Tadalafil, Vardenafil) and soluble guanylate cyclase stimulators (sGCs) (Liothyronine); (3) Prostacyclin pathway: mainly include prostacyclin analogs (Beraprost, Ibuprostenol, Iloprost, Travoprost) or prostaglandin receptor agonists (Sellepag) [6, 7]. In addition, high-dose calcium channel blockers can be used alone for PAH patients with positive acute vasodilator test, with diltiazem preferred for a fast heart rate and amiodarone or nifedipine for a slow heart rate. Although pulmonary arterial hypertension has been improved in current targeted therapies, it still has the drawbacks of many adverse effects and high price, thus leading to poor compliance and high mortality in patients with pulmonary arterial hypertension, which is one of the incurable diseases. For example, adverse effects of endothelium receptor antagonists include abnormal liver function, peripheral edema, and increased chance of anemia, and bounden can produce hepatotoxicity, requiring regular monitoring of liver function during treatment [8, 9]. The most common adverse effects of Sildenafil are headache, diarrhea, and dyspepsia, and a 20% mortality rate is still observed in patients after 3 years of continuous use of sildenafil[2].

Chinese medicine has the advantages of multi-target, multi-pathway,and multi-pathway, etc. In recent years, more and more studies have shown that Chinese medicine has good effects on the prevention and treatment of pulmonary hypertension, and the in-depth study of the pharmacological effects of Chinese medicine and its mechanism of action is of great significance to the research and development of new drugs, which also brings new hope for the clinical treatment of pulmonary hypertension. However, compared with traditional Chinese medicine compound, Chinese herbal monomer has the advantages of clear active ingredients and easy to analyze and purify, and the research and development of new drugs in China is mainly focused on traditional Chinese medicine monomer, such as Resveratrol through miR-638 and NR4A3/cyclin D1 pathway for the treatment of pulmonary arterial hypertension[10].Shi et al[11] found that baicalin improved pulmonary artery endothelial function by partially inhibiting Endothelial-to-mesenchymal transition (EndoMT) through the NF-κB-BMPR2 pathway, which led to the treatment of wild lily soda-induced PAH in rats. Puerarin prevents hypoxia-induced pulmonary hypertension in rats by reducing autophagy and inhibiting proliferation of pulmonary artery smooth muscle cells and by inhibiting oxidative stress and activating BMPRII and PPARγ signaling pathways in endothelial cells [12, 13]. It was also found that Astragaloside could inhibit the development of wild lily soda-induced pulmonary hypertension in rats by attenuating the NLRP-3/calpain-1-mediated inflammatory response[14]. In contrast, ginsenoside Rb1 prevents wild lily soda-induced pulmonary hypertension by inhibiting calcium pool-manipulated calcium inward flow and pulmonary vasoconstriction[15]. Bao et al[16] found that Sodium Tanshinone II Sulfonate A attenuated hypoxia-induced pulmonary hypertension in rats by promoting apoptosis, inhibiting PI3K / AKT / mTOR signaling pathway, upregulating autophagy,and suppressing inflammatory responses. Echinacoside can be used to treat hypoxic pulmonary hypertension by modulating pulmonary artery endothelial and smooth muscle layer function and improving pulmonary artery remodeling[17].

OVERVIEW OF METABOLOMICS

With the development of genomics, transcriptomics,and proteomics, the emergence of metabolomics has become an inevitable trend in life science research. The concept of metabolomics was proposed by the British scholar Nicholson et al[18] based on extensive studies of the multicomponent metabolic components of biological fluids, cells and tissues by magnetic resonance bopping. In the last decade, metabolomics has exploded with improvements in mass spectrometry and magnetic resonance techniques, making it possible to detect hundreds or even thousands of metabolites in a single experimental study[19]. Metabolomics reflects a series of biological events of pathophysiological processes by revealing the trajectory of overall changes in the metabolism under the influence of intrinsic and extrinsic factors. It can perform dynamic qualitative and quantitative analysis of small molecule metabolites in organisms, identify the relative relationship between metabolite changes and physiological and biochemical changes in organisms, identify drug metabolites and metabolic changes in organisms induced by drug action, and thus obtain the corresponding biomarker groups to characterize or reveal the overall functional state of organisms at specific times and environments[20]. It has been used in numerous studies such as clinical diagnosis, clinical novel drug development, drug efficacy,and safety evaluation, and in the plant and microbial fields. For example, Liang et al[21] used non-targeted metabolomics techniques to examine 784 maternal blood samples to identify highly regulated metabolic changes during human pregnancy, and based on these results, they established a gestational metabolic molecular clock that can be used to predict the gestational age of the baby to organize the weekly changes in human gestational metabolism and can also provide some delivery-related information that cannot be given by ultrasound testing, providing a useful guide for the clinical understanding of the gestational process. Metabolism, as an in-depth phenotypic tool, has made great progress since the late 1990s[22]. However, the optimization and development of high-resolution platforms, conjoint

technologies, miniaturization, and data acquisition toolkits in metabolism have brought new challenges to data pre-processing, analysis, interpretation, and integration methodologies[23].

Application of Metabolomics in Drug Research

Metabolomics has a unique role in evaluating drug toxicity, revealing its mechanism of action and drug-related functions, and is now widely used in various drug studies[24, 25]. Wang et al[26] explored the mechanism of action of *Bidens bipinnata* L. (BBL) in the treatment of hyperlipidemia (HLP) by a metabolomic method and found a clear separation between the model, control, and BBL groups, and that BBL modulates six metabolites in the intervention of HLP, which are considered as potential biomarkers for BBL treatment while showing that linoleic acid metabolism, AA metabolism, and terpenoid backbone biosynthesis were considered as the most dominant metabolic pathways in the interventional effect of BBL on hyperlipidemia. Ryu et al[27] applied cisplatin-treated mice and then performed the metabolomic analysis of their blood and urine and found that the bird concentrations of alanine, glucose, glycine, guanidinoacetate, acetate, and lactate were significantly increased, while the concentrations of citrate and marinate in urinary metabolites were significantly decreased after cisplatin treatment. By correlating serum and urinary ^{1}H NMR OPLS-DA with serum biochemistry and renal histological Correlation analysis of serum and urinary ^{1}H NMR OPLS-DA with serum biochemical and renal histological alterations revealed that urinary ^{1}H NMR analysis can be used as a reliable indicator for predicting and screening cisplatin nephrotoxicity.

In addition, researchers have used metabolomics-based high-resolution mass spectrometry (MS) on liver samples and various software and Bioinformatics analysis methods to identify and analyze the endogenous nature of mice between the control group and different bisphenol A (BPA) dose groups, The difference in liver metabolites, it is found that BPA may affect the metabolism and synthesis of fatty acids and glucose in the liver, block the TCA cycle, and BPA also regulates the nuclear receptor LXR caused by hypoglycemia, thereby affecting the normal

metabolic function of the liver[28]. Li et al[29] established a rat model of type 2 (T2DM) diabetes mellitus by feeding it on a high-fat diet (HFD) and by a single intraperitoneal injection of a small dose of streptozotocin (STZ), Subsequently, metabolites in urine samples were analyzed using a UPLC- ESI-Q-TOF-MS system combined with quadrupole time of Thus, metabolomic approaches are proving to be a promising tool to explore the antidiabetic effects of ST and provide a better understanding of its mechanism of action. Kang et al[30] comprehensively evaluated the changes in chemical composition and efficacy of smallpox powder induced by sulfur fumigation based on non-targeted metabolomics and extensive metabolomics approaches. Seven fumigation markers were initially identified by non-targeted metabolomics, while Trichosanthis Radix samples were tested for different levels of sulfur fumigation by chemical transference analysis and sulfur dioxide residue testing, and sulfur fumigated Trichosanthis Radix samples showed significantly lower levels of differential metabolites, Besides, 20 kinds of non-sulfur marker metabolites were tested to evaluate the quality of TR samples before and after sulfur fumigation, predominantly including phenolic acids, amino acids, lipids, and nucleotides. Looking at the hot issues related to drug toxicity experiments and new drug research and development at home and abroad, metabolomics technology provides a good technical platform with its high sensitivity, accuracy, and characteristics intuitively reflecting cell life activities.

In the field of traditional Chinese medicine, metabonomics technology can be used to evaluate the efficacy of Chinese medicinal materials, scientific and theoretical research on the compatibility of traditional Chinese medicines and their laws, and biomarker screening can also be used to discover disease-related organisms through this technology. Markers and determining the metabolic pathways involved have important reference significance for TCM diagnosis and accurate evaluation of curative effects. For example, Quan et al[31] integrated the data of the gut microbiome, serum metabolome, and brown adipose tissue (BAT), and proved that GE-E.faecalis-LCFA (especially ginseng extract Myristic acid) can activate BAT and

beige Fat is formed to reduce obesity. In addition, they also proved for the first time that as a long-chain fatty acid, myristoleic acid (MA) produced by Enterococcus has great potential in the treatment of metabolic syndrome disorders. Traditional Chinese medicine saposhnikovia root extract Prim-O-glucosylcimifugin (POG) could bind well to the target proteins and inhibit the proliferation, metabolism, and immunosuppressive ability of PMN-MDSCs by inhibiting arginine metabolism and the tricarboxylic acid cycle (TCA cycle), POG could also increase CD8 T-lymphocyte infiltration in the tumors and enhance the antitumor effect of PD-1 inhibitor[32]. Sheng et al[33] analyzed the exposed fractions of *Dengzhan Shengmai* (DZSM) in brain tissue using high-performance liquid chromatography-mass spectrometry (HPLC-MS/MS) in chronic cerebral hypoperfusion (CCH) model rat and analyzed the exposed fractions in glutamate-induced neuronal excitability The exposed fractions were examined on a cellular model of glutamate-induced neuronal excitatory damage to verify the active ingredients and mechanism of action of DZSM. These studies provided definitive clues of the mechanism for the neuroprotective effect of DZSM for CCH treatment and the active compounds regulating glutamatergic and GABAergic synapses.

Research on metabolomics in the field of pulmonary hypertension research

In recent years, domestic and foreign scholars have achieved good results in the evaluation of pulmonary hypertension and the mechanism of action using metabonomics technology. Chen et al[34] serum samples from 40 patients with idiopathic PAH (IPAH), 20 patients with congenital heart disease-associated PAH (CHD-PAH) , and healthy controls were collected and analyzed by ultra-high-performance liquid chromatography coupled with high-resolution mass spectrometry (UPLC-HRMS). Orthogonal partial least square discriminate analysis (OPLS-DA) was applied to screen potential biomarkers. These results were validated in the monocrotaline (MCT)-induced PAH rat model , the analysis showed dysregulation of the different metabolic pathways, including lipid metabolism, glucose metabolism, amino acid metabolism , and phospholipid metabolism pathways

in PAH patients compared to their healthy counterparts. Among the metabolites of these disordered metabolic pathways, a group of metabolites of lipid metabolism and fatty acid oxidation is closely associated with PAH. Recently, Professor Jing Zhicheng's team[35] published a metabolomic study of PAH in Eur Respir J. The study found that spermine levels in the plasma of patients with idiopathic PAH were significantly higher than those of healthy controls, that the addition of spermine promoted the proliferation and migration of pulmonary artery smooth muscle cells and worsened vascular remodeling in a rat model of pulmonary hypertension, and that spermine-mediated poor vascular remodeling was caused by the upregulation of spermine synthase The up-regulation of spermine synthetase was observed. In vitro experiments showed that inhibition of the spermine synthase inhibited platelet-derived growth factor BB-mediated proliferation of pulmonary artery smooth muscle cells, and animal experiments showed that inhibition of the spermine synthase reduced wild lily base mediated pulmonary hypertension. This study suggests that spermine can promote pulmonary vascular remodeling, and inhibition of the spermine synthase may become a new target for PAH treatment. Professor Christopher J Rhodes' team[36] studied a population of patients with chronic thromboembolic pulmonary hypertension (CTEPH) and performed metabolite analysis by non-targeted plasma metabolomics LC-MS techniques on plasma from CTEPH patients before and after PEA surgery and from different parts of the patient's body to distinguish CTEPH patients from healthy individuals and other patients with similar diseases The specific metabolic profile of CTEPH patients was identified, and the analysis of plasma metabolite gradients showed that cardiopulmonary tissue metabolites were associated with pulmonary hypertension and that the two metabolites that overlapped with the differential metabolites of CTEPH patients and healthy individuals before and after surgical treatment for PEA could be used as metabolites for the evaluation of appropriate non-invasive markers of targeted therapeutic interventions for pulmonary hypertension. Another researcher, through metabolomics techniques in serum and lung tissues, found that resveratrol plays a therapeutic role in pulmonary hypertension

in rats through amino acids, TCA cycle, choline, and linoleic acid[37]. Yao Li[38] focused on rat serum intestinal flora metabolites and analyzed the differential metabolites and their metabolic pathways in the development process of pulmonary arterial hypertension using untargeted metabolomics and targeted metabolomics, and found that 55 differential metabolites were modified in LC/MS negative ion mode to support the development process of PAH, and the intestinal flora metabolites showed the most significant increase in the level of bilinogenic substances, while six lipids metabolic pathways were found to show significant modifications, and the metabolic modification mechanism of PAH was effectively understood from the perspective of studying small molecule metabolism, and intestinal flora metabolites were found to be involved in the metabolic reprogramming of pulmonary arterial hypertension.

Potential treatments for pulmonary hypertension metabolism:

The metabolic changes in patients with arterial hypertension provide a new therapeutic approach for the treatment of pulmonary hypertension. The metabolic pathway of regulating redox balance can treat pulmonary hypertension. In monocrotaline-induced pulmonary hypertension rats, crocin can reduce the lung injury caused by oxidative stress by modulating the antioxidant 1 (OXR1) signaling pathway[39]. Aldosterone can oxidize cysteine thiol in endothelin-B receptor (ETB) to promote pulmonary vascular tone, and it can also act as a redox switch to reduce endothelin-1 (ET-1) stimulated pulmonary endothelial nitric oxide levels to regulate pulmonary arteries high pressure[40]. In the experiments of Alamri et al[41] cholecalciferol (D3, or vitamin D) and tocopherol (vitamin E) have been shown to restore the redox state of many metabolic pathways. The ability of superoxide dismutase mimics to eliminate extracellular oxidation/anti-oxidation imbalance and its role in inflammation in hypoxia-induced pulmonary hypertension[42]. Redox signaling triggered by NADPH oxidase (Nox1) promotes transcription factor CREB activation via oxidation reductase 1 (Ref-1), transactivates Gremlin1 transcription, and initiates endothelial cell proliferation and migration responses that are critical for vascular remodeling in rat pulmonary hypertension[43]. In patients with pulmonary

arterial hypertension, Coenzyme Q (CoQ) is one of the most widely used cofactors in the treatment of mitochondrial-related diseases, and also acts as an antioxidant through the redox cycle and improves hemoglobin and erythrocyte maturation in patients with pulmonary arterial hypertension[44].

Using human blood, RV tissue, and non-invasive imaging, Brittain et al[45] demonstrate that human PAH is associated with abnormalities in systemic and myocardial FA metabolism. Sildenafil can adjust fatty acid (FAs) ingredients to reduce oleic acid, linoleic (LA), DPA, docosahexanoic (DHA) acids in oils, thereby reducing oxidative stress[46]. Hou et al[47] protected mice from hypoxia-induced PAH by inhibiting FAs through activation of the PI3K/AKT signaling pathway. Omega-3 fatty acids prevent the development of heart failure by inflammation by improving the composition of FAs in the heart[48]. Electrophilic nitroalkenes can improve pulmonary vascular remodeling and glucose tolerance induced by a high-fat diet (HFD) and significantly attenuated HFD-induced RVESP, PVR, RV hypertrophy, lung XO activity, oxidative stress, and pro-inflammatory pulmonary cytokine levels[49]. Pioglitazone is a PPARγ receptor agonist, which can reverse severe pulmonary hypertension and vascular remodeling through fatty acid oxidation, and prevent right heart failure[50].

Mitochondria provide the sites for the metabolism of many substances and the enzymes they require, such as glucose metabolism and glutamine metabolism. Mitochondrial dysfunction has been identified as a metabolic cause of pulmonary hypertension, according to research[51]. There have been many results in preclinical and early clinical data to improve the severity of the disease through the mitochondrial approach. He et al [52] confirmed that the natural dietary flavonoid Apogenin hypoxia-induced factor 1α (HIF-1α) -Kv1.5 pathway is involved in the process of PASMCs mitochondrial-dependent apoptosis, thereby effectively inhibiting the occurrence and development of hypoxia-induced PAH. By boosting A2a receptor (A2aR)-related mitochondrial-dependent pathways, salidroside can promote PASMCs apoptosis, thereby inhibiting chronic hypoxia-induced PAH and pulmonary vascular

remodeling [53].Tian et al [54] demonstrated that epigenetic pathways that induce miRNAs and DNA methyltransferase 1 (DNMT1) by altering the mitochondrial redox signaling are located upstream of metabolic changes. The mitochondrial inhibitor 3-Bromopyruvate (3-BrPA) improves mitochondrial metabolism, reduces pulmonary artery pressure, and reverses pulmonary vascular remodeling in an animal model of PAH [55]. Dichloroacetate (DCA) and Atorvastatin (ATO) combined with the p38 signaling pathway inhibit excessive proliferation, regulate mitochondria-associated apoptosis and reduce oxidative stress in pulmonary arterial hypertension [56]. There are also the results of the treatment of PAH for glucose metabolism that have been proposed and have achieved certain success. The Warburg effect is a reduction in oxidative glucose phosphorylation and an increase in aerobic glycolysis. The Warburg effect is observed in both patients and animal models of pulmonary hypertension and plays a key role in pulmonary vascular remodeling and right ventricular hypertrophy [57, 58].

Poly (lactic acid-co-glycol) acid (PLGA) microparticles that deliver verteporfin simultaneously inhibit Yes-associated Protein 1 (YAP1), and in combination with the glutamine inhibitor CB-839, glutaminase activity, and intravascular PCNA levels And pulmonary artery collagen deposition (non-polarization) can be significantly reduced. Light) and collagen cross-linking (polarization) improve monochromatalin-induced pulmonary hypertension in rats[59]. Oxymatrine reduces NG-dimethyl-L-arginine (ADMA) metabolism pathway to prevent monocrotaline-induced pulmonary hypertension by improving pulmonary vascular remodeling and inhibiting the expression of protein arginine methyltransferase 1 (PRMT1)[60]. Ahmed et al found that Naringenin can significantly increase L-arginine's effect on MCT-induced pulmonary arteries in rats by reducing oxidative stress, preserving NO beneficial effects, stimulating eNOS expression as well as inhibiting the deleterious iNOS overexpression and its related inflammation and proliferation regulation. The protective effect of high pressure[61]. Arginase inhibitors may increase the bioavailability of NO in animal models of PAH, reverse pulmonary vascular

remodeling, and reduce right ventricular contractile pressure (RVSP) [62, 63].

These study results on the effectiveness of metabolic interventions in the treatment of pulmonary hypertension are exciting. However, more research is needed to see whether metabolic interventions can improve survival and/or reverse pulmonary vascular remodeling. To ensure no or limited adverse effects, metabolic interventions almost certainly necessarily require a precise approach to care and close monitoring over time. Our current knowledge of how to incorporate the best metabolic therapies into PAH therapeutic approaches is limited. Despite the availability of treatments, PAH survival and mortality rates are still low. These new metabolic concepts have the potential to expand the scope of patient-care research.

SUMMARY AND PROSPECT

In recent years, significant advances have been made in the study of metabolomic techniques for PAH metabolism including reduction and oxidative cellular capacity, fatty acid oxidation, glycolysis and glucose oxidation, glutamine metabolism, and the metabolic impact of PAH-related mitochondrial mutations. The metabolic phenotype of PAH is attributed to proliferative cells, such as cancer. Thus, treatment plans being used in goal metabolism in most cancers may have relevance in addressing the hyperproliferation considered in vascular cells in PAH. Currently, with the continuous upgrading and improvement of metabolomics technology for metabolic alterations in pulmonary hypertension, metabolomics technology is expected to become a new tool to guide the diagnosis, assessment, detection, and treatment of pulmonary hypertension in humans.

CONFLICTS OF INTEREST STATEMENT

The authors declare that there are no conflicts of interest.

FUNDING

This project was supported by Ningxia Hui Autonomous Region Key Research and Development Project (Grant No. 2019BFG02027); Ningxia Hui Autonomous Region Key Research and Development Project (grant no. 2020BEG03040); 2017 Ningxia Hui Autonomous Region Science and Technology innovation leader training project(Grant No. KJT2017005) ; National Natural Science Foundation of China (Grant No. 82160018) ; National Natural Science Foundation of China (Grant No.81402990) .

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