Published in final edited form as:

Methods Mol Biol. 2018 ; 1783: 259–277. doi:10.1007/978-1-4939-7834-2_13.

A Review of Transcriptome Analysis in Pulmonary Vascular Diseases

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Abstract

Transcriptome analysis is a powerful tool in the study of pulmonary vascular disease and pulmonary hypertension. Pulmonary hypertension is a disease process that consists of several unique pathologies sharing a common clinical definition, that of elevated pressure within the pulmonary circulation. As such, it has become increasingly important to identify both similarities and differences among the different classes of pulmonary hypertension. Transcriptome analysis has been an invaluable tool both in the basic science research on animal models as well as clinical research among the various different groups of pulmonary hypertension. This work has identified new potential candidate genes, implicated numerous biochemical and molecular pathways in diseased onset and progression, developed gene signatures to appropriately classify types of pulmonary hypertension and severity of illness, and identified novel gene mutations leading to hereditary forms of the disease.

Keywords

Pulmonary hypertension; pulmonary vascular disease; animal models; BMPR2; Hereditary pulmonary arterial hypertension; lung disease

1 Pulmonary Hypertension

A disease characterized by elevated pressures in the pulmonary arterial circulation, pulmonary hypertension (PH) is a complex disease process with numerous subtypes. PH can arise spontaneously, yet also commonly complicates many diseases originating in the cardiopulmonary system as well as other areas of the body. In all of its forms, PH is associated with a high degree of morbidity and mortality. Even from early descriptions of PH, familial cases have been described such that a genetic link was inevitable. While working to understand pulmonary hypertension, the identification of a germline mutation in bone morphogenetic protein II (BMPR2) in these familial cases quickly advanced our understanding of the pathophysiology of pulmonary hypertension[1, 2]. Work on the genetics and genomics of pulmonary hypertension has grown quickly, with numerous mutations in BMPR2 and other genes being identified. This chapter will give an overview of

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the pathophysiology then will focus on transcriptome analysis in experimental models of pulmonary hypertension and in human disease. Much of the existing data in human disease has focused on hereditary pulmonary arterial hypertension, yet we will also discuss some of the gene expression analysis in other classes of pulmonary hypertension and the future directions of transcriptome analysis and gene therapies.

2 Pathophysiology of Pulmonary Hypertension

The diagnosis of pulmonary hypertension is based on hemodynamic derangements in the pulmonary vasculature that result from numerous types of pathophysiology. PH is hemodynamically defined on right heart catheterization with a mean pulmonary artery pressure (PAP) of equal to or greater than 25 mmHg. Elevations in PAP can arise from *i*) impaired venous return and left heart dysfunction, obstruction of the pulmonary artery blood flow by venous thromboembolism, *ii*) obliteration of the pulmonary vascular bed in diffuse parenchymal lung disease, *iii*) vasoconstriction as a result of severe lung disease or sleep-disordered breathing, *iv*) hyperkinetic blood flow due to congenital heart disease, or *v*) idiopathic small artery and arteriole vascular remodeling[3]. Pulmonary arterial hypertension (PAH) is one form of PH characterized by increased pulmonary vascular resistance (PVR) within the small arteries and arterioles leading to elevated PAP. PAH is hemodynamically defined with mean PAP $\geq$ 25 mmHg, with pulmonary artery wedge pressure (PAWP) $\leq$ 15 mmHg and PVR $>$ 3 Wood units. This pathology results from disease thought to be intrinsic to the pulmonary vasculature and has been used as the model both for basic and clinical research as well as drug development.

2.1 Pathogenic mechanisms of pulmonary arterial hypertension

Pulmonary vascular remodeling, sustained pulmonary vasoconstriction, *in situ* thrombosis, plexiform vasculopathy, and vascular wall stiffening all contribute both individually and collectively in the development and progression of PAH (Figure 1). Sustained vasoconstriction in small arteries and arterioles is the principal functional alteration encountered in PAH with profound impact on PVR. Pulmonary vascular remodeling affects all the different cell types of the pulmonary vasculature, including endothelial cell hyperplasia associated with intimal thickening, pulmonary artery smooth muscle cell hypertrophy, extracellular matrix stiffening as fibroblasts differentiate into myofibroblasts [4]. Pulmonary artery smooth muscle cell (PASMC) hypertrophy and hyperplasia is stimulated by sustained vasoconstriction and elevated PAP [5, 6]. This leads to muscularization of small non- or only partially muscularized arteries and arterioles. Classic structural alterations of the small arterioles in PAH are plexiform lesions. These complex lesions are characterized by aneurysmal dilation of arterioles associated with partial or fully occlusive intraluminal collections of various cells and matrix proteins [7, 8].

2.2 Molecular mechanisms in the development and progression of PAH

There is complex interplay of numerous cellular and molecular mechanisms that influence these structural and functional changes during the development and progression of PAH. An imbalance of vasoactive mediators, altered expression and function of both mitogenic and angiogenic factors, and dysregulated cell survival proteins have all been implicated in the

various cell types of the pulmonary vasculature [3]. The imbalance of vasoactive mediators is characterized by a deficiency of vasodilators, primarily nitric oxide (NO) and prostacyclin, paired with excess vasoconstrictors, such as endothelin-1 and thromboxane A₂. NO, which is released by the vascular endothelial cells (EC), stimulates the production of cyclic guanosine monophosphate (cGMP) through soluble guanylate cyclase in PASMC resulting in relaxation and pulmonary vasodilation. Prostacyclin is synthesized in the endothelium as a product of the metabolism of arachidonic acid which is then secreted. Upon binding to the prostacyclin receptor (IPR) on PASMC, cyclic adenosine monophosphate (cAMP) is produced which promotes vasodilation as well as having anti-proliferative effects [9]. Endothelin (ET-1) plays a central role in the pathogenesis of PAH, exerting its effects as a potent vasoconstrictor as well as being shown to increase smooth muscle cell proliferation and inhibit apoptosis [10, 11]. Excess circulating levels of ET-1 have been clearly demonstrated in PAH patients [12]. Other vasoactive mediators such as thromboxane, serotonin, and vasoactive intestinal peptide appear have shown various roles in the development and progression of PAH.

Several ion channels and membrane receptors have been implicated in the pathogenesis of PAH. Rising cytosolic free Ca²⁺ concentration ([Ca²⁺]_{cyt}) in PASMC is a major trigger for PASMC contraction and vasoconstriction as well as an important mediator for PASMC proliferation and migration, essential to vascular remodeling. Ca²⁺-permeable cation channels and K⁺-permeable channels (e.g., KCNA5 and KCNK3) in the plasma membrane of PASMC, influence [Ca²⁺]_{cyt} both directly and indirectly, through membrane depolarization [7]. G protein- coupled receptors (GPCR) and tyrosine kinases receptors (TKR) have important effects on second messengers such as cGMP, cAMP, diacylglycerol, and inositol triphosphate leading to pulmonary vascular remodeling. Numerous other cellular and molecular mechanisms have also been identified, including: *i*) altered signaling pathways; *ii*) changes in expression and function of growth factors; *iii*) microparticle release; and *iv*) epigenetic modifications [7, 13, 14].

3 Gene Expression Analysis in Experimental Models of Pulmonary Hypertension

Numerous animal models of pulmonary hypertension have been developed in order to mimic the clinical characteristics and pathobiologic changes encountered in human disease. Although no single model recapitulates human disease, they can shed light on important aspects that may be applicable to human PH. Models that have commonly been used in rodents are hypoxia-induced PH (HPH, both in mice and rats), monocrotaline-induced PH (MCT, mostly effective as a model in rats), and a combined Sugen-hypoxia PH model (SuHx, mostly effective as a model in rats). Additionally there are unique models that have been used to a lesser extent that induce pressure overload on the pulmonary vascular system or more specifically on the right ventricular. There are also models in larger species that have been developed experimentally or fortuitously identified as a disease similar to human PH, such as Brisket disease in cattle. Gene expression analysis from these models have identified genetic alterations that are both similar to human disease and others that are more

unique and have not necessarily been identified in human populations but have been used to enlighten work on the function of the pulmonary vascular system (Table 1).

3.1 Gene expression in small animal models of pulmonary hypertension

Elevated PAP and increased PVR puts excess stress on the right ventricle (RV) which results in much of the morbidity and mortality attributed to pulmonary hypertension. Therefore, abundant gene expression profiling has focused on changes in the myocardium and adaptation of the right heart to stress through pressure overload and hypoxia induced pulmonary hypertension. Work with mice has revealed that gene expression profile changes after exposure to moderate chronic hypoxia predominated in the RV myocardium compared to the left ventricle (LV) [15]. These changes in the RV were associated with metabolic pathways (*Ldha*, *Slc2a1*, *Prkaa*), cell proliferation (*Igf2*), apoptosis (*Bnip3l*), vasculogenesis and angiogenesis (*Vegfa* and *Flt1*), and transcription (*Gata2*). A majority of the upregulated genes were also identified as targets of HIF1 α , the major transcription factor related to hypoxia known to have important implications in the development and progression of PH [16]. There were also interesting gene expression differences among male and female mice, such that the gene profiles identified after hypoxia exposure for one and four weeks were unique among different gender, likely contributing to the different phenotypic response of male and female mice to chronic hypoxia. In a rat model of MCT-induced PH, mRNA expression changes occur early in the development of PH and are related to the severity of PH as well as the adaptation of the RV to the stress [17]. This suggests that compensated RV hypertrophy has a unique gene signature, implicating specific pathways such as MAPK signaling and apoptosis being important to RV adaptability. Pulmonary artery banding, another model of RV pressure overload in mice in which blood flow from the RV is limited by placement of a surgical band in the pulmonary artery, has shown unique differences in gene expression profile both between the RV of control and diseased animals as well as between the RV and LV of diseased animals [18]. This work identified changes in matrix proteins associated with hypertrophy and vascular stiffness as well as in three signaling pathways: integrin, calcineurin-NFAT, and TGF β . These pathways have important implications on ECM stiffness, inflammation, and cardiac hypertrophy, all known to be disturbed in PAH. Work on the severity of RV stress and dysfunction has suggested distinct gene expression changes between normal RV, adaptive RV hypertrophy, and RV failure [19]. This expression profile also implicates a switch from aerobic metabolism to initial fatty acid utilization followed by glycolysis and lipid accumulation as a hallmark of RVH progressing to RV failure. Genes encoding fibrosis were also upregulated in RV failure. Interestingly, the pro-angiogenesis *IGF-1* was increased in models of RVH while it was decreased in RV failure, signaling inhibition of cell growth and angiogenesis at late stages of disease. Examining animal models that recapitulate other classes of PH, a genome-wide array study (GWAS) was performed on mice fed a chronic high-fat diet to induce PH identified 880 candidate genes [20]. This model was designed to simulate group 2 PH related to left heart disease. Of these 880 candidate genes, network analysis of genes known to be related to PH identified that epidermal growth factor receptor, *Egfr*, high the highest connectivity in the network, suggesting its importance in this experimental model of PH. In strains of mice that were most susceptible to PH from a high-fat diet, *Egfr* expression in lung tissue was significantly increased, further lending credence to its importance.

Early longitudinal analysis of a severe PH using MCT in pneumonectomized rats revealed that increased expression of genes encoding proteases correlate with the development of severe PH and vascular lesions [21]. Further work on the expression profile of vascular lesions in the severe PH models identify expression of transcription factors *ATF3* and *EGR-1* which were specifically associated with development of neointimal lesions and were found in abundance within these lesions [22]. Importantly, this work also identified changes in expression after treatment with prostacyclin, a mainstay treatment in humans. Prostacyclin treatment is shown to affect genes involved in the Wnt pathway as well other genes that have been described in severe human PAH. This implies that treatments have important consequences on transcriptome analysis, either as active therapeutic effects related to vasodilation and reversal of pulmonary vascular remodeling or as an inadvertent biomarker of treatment. As such, transcriptome analysis must take into account these key distinctions, particularly when used clinically on patients receiving various treatments.

3.2 Non-coding RNA in animal models of pulmonary hypertension

Rather than simply identifying encoding genes within DNA, there has been interest in identifying transcripts that may participate in epigenetic modifications (Table 2). Noncoding RNAs are implicated in genetic modification at both the transcriptional and post-transcriptional level. These modifications can affect any number of cellular mechanisms and have been linked to both cardiovascular and respiratory disease [23, 24]. Recent work has identified gene expression changes of long noncoding RNA in HPH rat model [25]. Similarly, work on post-transcriptional epigenetic modifications has identified differential expression of microRNA during the development of PH in rats [26-28]. A study examining the microRNA profile of lung tissue in a rat model of persistent pulmonary hypertension of the newborn, in which newborn rat pups were exposed to hypoxia, miR-126a-5p was identified then confirmed as a potential biomarker in human disease [29]. Interestingly, there are dynamic changes in the microRNA (miRNA) profile during development of PH and among different models of experimental PH there are both similarities and differences in microRNA expression [26]. These differences among miRNA expression profiles are also seen among mouse models of HPH and SuHx [30]. As with the dynamic changes that occur during development of experimental PH, animals resistant to MCT-PH show miRNA profiles similar to untreated rats [31]. The myocardial miRNA profile of RVH progressing to RV failure using murine pulmonary artery constriction is shown to differ from remodeling in LVH and LV failure, suggesting unique mechanisms at play [32]. A unique miRNA profile in the RV myocardium, distinct from LVH models, in the MCT rat also changes dynamically as RV fibrosis progresses [33]. Work on non-coding RNA and miRNA introduces a new aspect into expression profiling in PH, such that changes to functional coding genes are now clearly accompanied by factors contributing to transcription or post-transcriptional modification.

3.3 Gene expression in large animal models of pulmonary hypertension

A disease that has some similarities to human PH, brisket disease in cattle is pulmonary hypertension that develops at high-altitude in these animals causing significant mortality. It has long been known to be a heritable disease that is autosomal dominant with incomplete penetrance [34, 35]. Initial studies led to the identification of candidate genes and further

work identified a variant of *EPAS1* that is highly associated with the development of brisket disease [36, 37]. This gene encodes hypoxia-inducible factor 2a (HIF2a), a protein known to be important in the bodies response to low oxygen tension and the subsequent effects this has on the pulmonary vasculature in relation to hypoxic pulmonary vasoconstriction [16]. Variants of *EPAS1*, initially identified in a single breed of cattle, have since been shown to be important in the development of brisket disease in a diverse group of cattle representing multiple breeds [38]. Interesting, *EPAS1* mutations have also been identified in familial syndromes associated with erythrocytosis that also appear to carry an increased prevalence of pulmonary hypertension [39, 40]. Similar to the problems in the commercial cattle industry, ascites syndrome occurs as pulmonary hypertension in broiler chickens manifest as excess fluid accumulation in the abdomen, i.e. ascites, and increased mortality. The genetic predisposition to ascites syndrome observed in broilers led to genetic selection using exposure to hypobaric hypoxia to identify a commercially elite line of chickens [41]. Further work using gene expression analysis of susceptible and resistant strains led to the discovery of candidate genes that include *AGTRI*, angiotensin II type 1 receptor; *UTS2D*, urotensin receptor 2D; *5HT2B*, serotonin receptor/transporter type 2B; and *ACE*, angiotensinogen cleaving enzyme [42]. While variants of these particular genes have not been identified in human HPAH, they have been shown to play roles in vascular reactivity and the response to hypoxia. MiRNA profiles of broiler chickens with ascites syndrome has identified 29 differentially expressed miRNAs with predicted gene targets related to hypoxia sensing, endothelial dysfunction, and inflammation, all important aspects related to vascular remodeling [43]. This work in animal models of PH has advanced our understanding of the cellular and molecular mechanisms of PH as well as identified candidate genes that may be important to human disease.

4 Gene Expression Profiling in Pulmonary Arterial Hypertension

The genetic basis of PAH has long been described with familial clustering, yet it was the identification of genetic mutations in bone morphogenetic protein receptor II (*BMPR2*) that really advanced the knowledge on genetic susceptibility in pulmonary hypertension (Table 3) [44, 45]. Germline mutations in *BMPR2* have been identified in up to 70 percent of patients with familial PAH, and in 20 percent of patients with IPAH with nearly 300 different mutations identified [46-49]. Since this time, more rare mutations have been identified to cause HPAH. Mutations in the TGF- β pathway, for which *BMPR2* is a major component, include activin receptor kinase-like 1 (*ALK1*), endoglin (*Eng*), and Smad 8 (*Smad9*) [50-53]. Outside of the TGF- β superfamily there have been discovery of rare mutations in *KCNK3* and *CAVI* what are attributed to the development of HPAH [54, 55]. Patients with *BMPR2* mutations have also been identified with concomitant Thrombospondin-1 gene mutation (*THBS1*) which is proposed to promote pulmonary hypertension development and increase genetic penetrance [56].

4.1 Transcriptome analysis in pulmonary arterial hypertension

Gene expression profiling in PAH has been used both to identify specific mutations in HPAH as above as well as candidate genes for further study. Another powerful use of this technology is to identify gene signatures within specific cohorts of patients. Given that PH

exists as a spectrum of pathologies, gene expression profiling can help to distinguish between classifications of PH as well as distinguish individual subtypes within the same classification. Circulating peripheral blood mononuclear cells (PBMC) are often used as an abundant and easily accessible surrogate to establish gene expression differences. In PAH, a gene signature of 106 genes differentially expressed in PBMC was able to distinguish this disease from healthy controls [57]. Candidate genes can also be gleaned from these data sets based on the significance within the expression differential as well as biologic interest based on known function or previously published significance within the field. In this study, the genes *ADM* and *ECGF-1* were confirmed to be significantly dysregulated in PAH based on a follow-up prospective cohort and are implicated in pulmonary vasodilation and angiogenesis, both important in the development and progression of PAH. A gene signature from PBMC has also been used to distinguish between PAH subtypes such as IPAH and PAH related to systemic sclerosis (SSc- PAH) [58]. This nine gene signature includes genes involved in the immune response, *IL7R*, and in the TGF- β pathway, *NOG* and *TGFBR2*, which is a major pathway in all forms of PAH including IPAH, SSc-PAH, and HPAH. The severity of PAH is crucial in making treatment decisions on patients and gene expression profiling of PBMC has shown that there are important genetic differences among PAH patients with mild or severe disease [59]. Differential regulation of genes related to angiogenesis, chemotaxis, and inflammation corroborate the importance of these processes as PAH progresses. The genes *MMP9* and *VEGF* in this study have increased expression in mild disease but much lower or even undetectable levels in severe PAH suggesting that temporal changes in gene expression are important in the various stages of disease. Obtaining routine lung tissue samples from PAH patients is impractical and thus circulating PBMC signatures are employed. Work has also been done to identify cell lines from easily accessible body sites that carry a similar genetic signature to lung vascular cells and could potentially be used as a surrogate of lung tissue [60]. A common genetic signature identified in both lung and skin fibroblasts implicate the potential to employ skin biopsy in the clinical evaluation and management of PAH in the future. Expanding this work, gene expression from a specific mesenchymal progenitor cell lineage expressing *ABCG2* is highly correlated with expression of skin fibroblasts, identifying a similar diagnostic pattern for PAH [61]. Despite the overall similarities between gene expressions, there were important gene expression differences noted between the lung and skin mesenchymal progenitor cells with important distinctions among different subtypes of PAH, including IPAH and HPAH with varying genetic mutations.

The first genome-wide association study (GWAS) conducted on IPAH and HPAH individuals compared to normal controls was able to identify overexpression of *CBLN2* in diseased individuals and implicate it as a candidate gene in the development of PAH [62]. GWAS has also been used to delineate vasodilator-responsive IPAH in order to better understand molecular mechanisms related to therapeutic response [63]. In this work, IPAH was highly associated with genetic variability in genes involved in the Wnt signaling pathway which is important for cell survival, proliferation, and migration. Vasodilator-responsive PAH was enriched with genes involved in PASMC contraction, an important target of current pulmonary vasodilator therapy.

4.2 Non-coding RNA expression in pulmonary arterial hypertension

Examining non-coding RNA, a microRNA profile of IPAH, both circulating miRNA and isolated from PASMC, identifies numerous differentially expressed microRNA compared to healthy controls (Table 4.) [28, 64, 65]. The differentially expressed miRNA can be correlated with physiologic parameters such as walk distance and pulmonary hemodynamic measurements. Comparing differentially expressed microRNA to encoding gene expression patterns the authors were able to identify that miR-23a could potentially control expression of 17% of the identified encoding genes in IPAH, suggesting that this miRNA is highly relevant to the disease process. Interestingly, a network biology based approach, in which known differentially expressed genes in PAH can be connected to miRNA which potentially target these important genes, was able to come up with 29 candidate miRNA [66]. MiR-21 was predicted by this model and shown to be increased in remodeled pulmonary vessels from both animal models of PH and human PAH, as well as have implications in vasoconstrictive mechanisms in human pulmonary endothelial cells.

Gene expression profiling in PAH has led initially to a much clearer understanding of HPAH, including the identification of both common and uncommon mutations leading to this disease. The role has now expanded to shed new light on novel mechanisms, help to better understand differences that exist among PAH subtypes, and identify important clinical distinctions such as severity of illness. There is an often unclear clinical distinction between PH classifications; transcriptome analysis is being used to understand the genetic differences among these entities.

5 Gene Expression Profiling in non-PAH Pulmonary Hypertension

While pulmonary arterial hypertension and in particular HPAH has led the way to investigating a genetic linkage to the development and progression of PAH, non-group 1 PH carries a much larger burden clinically, effecting much larger numbers of patients. PH associated with cardiac disease, lung disease, or any number of other systemic illnesses is a major source of morbidity and mortality. Furthermore, it is often unclear why PH develops in these conditions. While compounding comorbidities or severe forms of a disease can lead to PH, it has also been shown to develop early or with less severe disease in certain patients [67]. This paradox would suggest that genetic susceptibility may play a role in the development of PH, yet limited data exists with regard to gene expression profiling in these individuals. While logic would dictate that genetic susceptibility may likely play a role in the development of PH associated with left heart disease, particularly when identifying patients with out-of-proportion PH (diastolic pressure gradient >7 mmHg), there has been little work on gene expression profiling in human subjects. While it is clear from experimental animal models discussed earlier that left ventricular and right ventricular response to pressure overload appear to be genetically divergent and that adaptive and maladaptive RV response have distinct genetic profiles, much of this work is lacking in human disease. This has not been the case for PH associated with respiratory disorders and CTEPH.

5.1 Pulmonary hypertension owing to respiratory diseases

Chronic obstructive pulmonary disease (COPD) has now become the third leading cause of death in the United States. PH is often encountered as a complication in advanced stages of disease, though only 1% of patients present with PAP ≥ 40 mmHg [68]. The development of pulmonary hypertension in these individuals is thought to arise from pulmonary vascular remodeling as a response to vascular injury, hypoxia, and shear stress [69]. Gene expression analysis of these remodeled vessels in COPD patients has shown the gene *ANGPT-2* to be the most significantly upregulated in areas of vascular remodeling and higher circulating levels of ANGPT-2 was discovered in patients with PH complicating the diagnosis of COPD [70]. In idiopathic pulmonary fibrosis (IPF), gene expression profiles from small pulmonary arterioles could distinguish IPF patients from controls but showed no significant genetic variability among patients with mild PH versus those without PH [71]. Differing gene expression patterns have also been identified between PH complicating either COPD or IPF [72]. Genes involved in retinol metabolism and extracellular membrane-receptor metabolism appear to differentiate these two diseases.

5.2 Chronic thromboembolic pulmonary hypertension

Chronic thromboembolic pulmonary hypertension results from persistence of organized thrombi within the pulmonary vasculature leading to elevations in pulmonary vascular resistance at the area of obstruction as well as vascular remodeling in other areas of the lung [73]. This is a relatively uncommon event, with estimates suggesting an incidence in about 3% of clinically apparent acute pulmonary embolism events. Gene expression analysis of endothelial cells isolated from CTEPH patients during thromboendarterectomy compared to control lung donor tissue was able to show a wide variety of genetic variability among these groups [74]. Several of the most upregulated genes in CTEPH were associated with cardiovascular disorders and inflammation, while downregulated genes were involved in tumor regulation and immune mediators. The genetic profile identified has suggested that in addition to thrombosis; cell signal transduction, cell proliferation, and inflammation are also likely to be important in the development of CTEPH. Additionally, some of the most highly upregulated genes, *ORL1* and *IL8*, have already been implicated in the pathogenesis of CTEPH [75, 76]. PASMC isolated from patients undergoing pulmonary thromboendarterectomy has also shown a unique miRNA profile compared to lobectomy tissue from non-PH subjects [77]. The miRNA that were differentially expressed had target genes known to be important to cell survival and proliferation in PASMC, key factors in vascular remodeling.

5.3 Pulmonary hypertension with multifactorial mechanisms

Within group 5 PH exists numerous pathologies which are thought to have multifactorial mechanisms as the source of PH. PH related to sickle cell disease (SCD) falls under this category and appears to affect at least 10% of sickle cell disease patients and is associated with increased mortality [78]. Examining the gene expression profile of PBMC from SCD at highest risk of pulmonary hypertension, those with elevated tricuspid regurgitation, identifies a gene signature of 10 genes [79]. Many of these genes have published citations related to PH pathophysiology and this work has suggested a high probability of *GALNT13*

and *ADORA2B* being candidate genes for further study in SCD-PH. Similarly, PBMC expression profiling of sarcoidosis patients reveals an 18-gene signature that differentiates sarcoidosis with PH from sarcoidosis without PH and from healthy controls [80]. Interestingly there was no overlap between previously published gene signatures from other PH types and only 3 of the 18 genes were similarly dysregulated from an existing gene expression data set of PAH patients, suggesting specificity of this gene signature to sarcoidosis-PH.

6 Conclusion

The ability to sequence large amounts of genetic material, both in basic science and clinical research, has paved the way for a greater understanding of the development and progression of pulmonary hypertension. While familial cases of PAH have been described for decades, it has not been until the beginning of the 21st century that *BMPR2* was identified as the causative gene for a majority of these individuals. Since that time, and with widespread use of transcriptome analysis, there has been multiple additional gene mutations linked to HPAH. This information is invaluable to these families, in which appropriate genetic screening and counseling is an integral part of management. Transcriptome analysis in experimental models of PH has led to discovery of novel genes involved in the development and progression of PH. This work identifies potential therapeutic targets that will lead the way for translational of new therapies to the bedside. A new understanding of the genetic differences between the various classifications of PH has allowed for a more precise diagnosis. These findings will also shape our management of the disparate pathologies encompassing PH that often present clinically in such similar ways. The goal of precision medicine is to better understand each of us by involving all of us; transcriptome analysis is expanding our understanding of PH by examining specific genetic differences that make up this deadly disorder.

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Figure 1. Major pathogenic components in the development of pulmonary arterial hypertension Pathogenic factors contributing to the development and progression of PAH include sustained vasoconstriction, pulmonary vascular remodeling, in situ thrombosis, vascular wall stiffening, and inflammation. These pathways are commonly implicated in gene expression profile analysis.

Table 1

Unique genes in animal models of pulmonary hypertension.

Publication	Genes Identified	Animal Model
Bohuslavova, R. et al. 2010 [15]	<i>Ldha, Slc2a1, Prkaa, Igf2, Bnip3l, Vegfa, Flt1, Gata2</i>	Hx - Mouse
Drake, J.I. et al. 2011 [19]	<i>IGF-1, Apelin, VEGF, HK1, ADH7, PFKM, Ucp2</i>	Hx, SuHx, PAB - Mouse
van Albada, M.E. et al. 2010 [22]	<i>ATF3, EGR-1</i>	MCT, MCT+shunt - Rat
Kreymborg, K. et al 2010 [18]	<i>Cilp, Col8a1, Postn, Col8a1, Meox1, Bgn, Mybpc2, Bgn, Adcy7, Rcan1, Pcdh17, Ankrd23, Mest, Masp1, Ccnb2, Col4a1, Pmepa1, Abra, Dct, Mllt11, Lxn, Tubb2a, Fscn1, Synpo2l, Cox19, Fhl1, Haus8, Nlrc3, Sez6l2, Edn3, Gcat, Sorcs2, Aldob</i>	PAC - Mouse
Newman, J.H. et al. 2015 [36]	<i>EPAS1</i>	Brisket disease - cattle
Muir, M.W. et al. 2008 [42]	<i>AGTR1, UTS2D, 5HT2B, ACE</i>	Ascites syndrome - chicken

Hx indicates chronic hypoxia; SuHx, Sugen 5416/Hypoxia; PAB, pulmonary artery banding; MCT, monocrotalline; PAC, pulmonary artery constriction.

Table 2

Non-coding RNA identified in animal models of pulmonary hypertension.

Publication	Non-coding RNA Identified	Animal Model
Caruso, P. et al. 2010 [26]	<i>miR-22, miR-30c, Let-7f, miR-322, miR-451, miR-21, Let-7a</i>	MCT, Hx - Rat
Reddy, S. et al. 2012 [32]	<i>miR-34a, miR-28, miR148a, miR-93</i>	PAC - Mouse (RV tissue)
Schlosser, K. et al. 2013 [28]	Upregulated <i>miR-27b, miR-15b, miR-16, miR-223, miR-195, miR-29c, miR-22, miR-29a, miR-21, miR-365, miR-423, miR-30b-5p, miR-192, miR-505, miR-30a</i> Downregulated <i>miR-10a-5p, miR-26a, let-7d, miR-125b-5p, let-7a, let-7c, miR-140, miR-434, miR-145, miR-124</i>	MCT - Rat
Paulin, R. et al. 2015 [33]	<i>miR-200b, miR-338-3p, miR155, miR-92a, miR-208a</i>	MCT - Rat (RV tissue)
Xu, Y. et al. 2017 [29]	<i>miR-19a, miR-218a, miR-3588, miR-532, miR-551b, miR-126a, let-7b, miR-210</i>	PPHN - Rat
Liu, P et al. 2017 [43]	Upregulated <i>miR-146b-5p, miR-146b-3p, miR-30e-5p, miR-10b-5p, miR-32-5p, miR142-5p, miR-142-3p, miR-460b-5p, miR-460b-3p, miR-34a-5p, miR1662, miR-147, miR-155, miR-148a-5p, miR-489-3p, miR-1684a-3p, miR-33-5p, miR-144-3p</i> Downregulated <i>miR-365b-5p, miR-2954, miR-23b-3p, miR-30e-3p, Mir-187-3p, miR-200b-3p, miR-100-5p, miR-125b-5p, miR-499-5p, miR-460a-5p, miR-99a-5p</i>	Ascites syndrome - chicken
Xiao, T. et al. 2017 [27]	<i>miR-125-3p, miR-148-3p, miR-193</i>	MCT - Rat
Wang, X. et al. 2016 [25]	<i>lncRNA</i>	Hx - Rat

Hx indicates chronic hypoxia; SuHx, Sugen 5416/Hypoxia; MCT, monocrotalline; PAC, pulmonary artery constriction; PPHN, primary pulmonary hypertension of the newborn.

Table 3

Unique genes identified in human pulmonary hypertension.

Publication	Gene(s) identified	PH Classification
Thomson, J.R. et al. 2000 [45] Newman, J.H. et al. 2001 [44]	<i>BMPR2</i>	PAH
Trembath, R.C. et al. 2001 [53]	<i>ALK1</i>	PAH
Chaouat A. et al. 2004 [52]	<i>Eng</i>	PAH
Shintani, M. et al. 2009 [50]	<i>Smad9</i>	PAH
Austin, E.D. et al. 2012 [54]	<i>CAV1</i>	PAH
Ma, L. et al. 2013 [55]	<i>KCNK3</i>	PAH
Risbano et al. 2010 [58]	<i>IL7R, LRRN3, NOG, NMT2, TUBE1, MAP9, CCR7, TGFBR2</i>	SSc-PAH
Gaskill et al. 2016 [61]	<i>SPON2, PEAR1, TNC, NEO1, DKK1, PDK4, WLS, RGS5, PTGS2</i>	PAH (fibroblasts)
Garcia-Lucio, J. et al. 2016 [70]	<i>ANGPT-2</i>	COPD-PH
Hoffman, J. et al. 2014 [72]	Upregulated <i>FOSB, BTG2, NPTXR, MYOM1, ZNF776, RERGL, EGR1, LOC572558, NTRK3, SIK1</i> Downregulated <i>UBE2C, CMTM3, SNX24, CLDN4, NEDD4L, ZNF521, SLC34A3, SLC34A2, COL6A3, NCEH1, SERPINA1, HOPX, DHCR24, 1RX3, NKX2-1, SCNN1A, TJP3, POSTN, GGT5</i>	COPD-PH
Hoffman, J. et al. 2014 [72]	Upregulated <i>S100A2, MMP1, SCGB1A1, MMP7, C20orf85, BCAS1, CXCL14, KRT17, WDR38, TRIM29, SIX1, BDKRB2, GPX2, CL1C6, TUBB3, HMGB3, SCGB3A1, CLCA2, C6orf165, SOX2, CBX8, STK33, SAA2, MUC4, FANK1</i> Downregulated <i>TMEM88, PDE1B, MMRN2, PLA1A, C1orf115, PTPRR, IDO1, CLDN5, CLEC14A, PNMT, PCYT2, SDPR, HBA2, ADRB2</i>	IPF-PH
Hoffman, J. et al. 2014 [72]	Upregulated <i>UGT1A6, ALDH1A1, CYP2A7, RDH10, LAMC2, SDC1, LAMB3, COL3A1, COL6A3, TNC, ITGB6, THBS2</i> Downregulated <i>CYP4A11, CYP2C19, ADH5, PNPLA4, ALDH1A2, CYP26B1, VWF, ITGA8</i>	IPF-PH vs COPD-PH
Gu, S. et al. 2014 [74]	<i>ORL1, IL8</i>	CTEPH
Desai, A.A. et al. 2012 [79]	<i>ADORA2B, CIQBP, ADC, MYL10, PRELP, SPDEF, GALNT13, SLC27A5, NAT8L, C9orf16</i>	SCD-PH
Singla, S. et al. 2016 [80]	<i>HIST1H4C, ETHE1, WASF1, RGS19, CORO1B, CACHD1, SUMO4, LOC100128751, RASSF3, STOX1, CD177, SERPINB8, FXVD6, NRCAM, RCVRN, BLOC1S1, SH3BGRL3, SLC22A16</i>	Sarcoidosis-PH

PAH indicates pulmonary arterial hypertension; SSc-PAH, pulmonary arterial hypertension related to systemic sclerosis; COPD-PH, pulmonary hypertension related to chronic obstructive pulmonary disease; IPF-PH, pulmonary hypertension related to idiopathic pulmonary fibrosis; CTEPH, chronic thromboembolic pulmonary hypertension; SCD-PH, pulmonary hypertension related to sickle cell disease.

Table 4

Non-coding RNA identified in human pulmonary hypertension.

Publication	Non-coding RNA Identified	PH Classification
Courboulin, A. et al. 2011 [65]	<i>miR-204, miR-450a, miR-145, miR-302b, miR-27b, miR-367, miR-138</i>	IPAH
Schlosser, K. et al. 2013 [28]	Upregulated <i>miR-2191-3p, miR-3180-3p, miR-2117, miR-3622b-3p, miR365, miR-3622a-5p, miR-324-3p, miR-1231, miR-4302, miR-188-5p, miR-720, miR505, miR-887, miR-1306</i> Downregulated <i>miR-423-3p, miR-30b, miR-589, miR-340, miR-150, let-7g, miR-485-3p, miR-26a, miR-181b, miR-30a, miR-342-3p</i>	IPAH
Sarrion, I. et al. 2015 [64]	<i>miR-7-1, miR-20, miR-138-1, miR-520h, miR-559, miR-593, miR-601, miR-616, miR-543, miR-1184, miR-1285, miR-1286, miR-3153, miR-3156, miR-4301, miR-4304, miR-4313, miR-1-2, miR-1259, miR-1263, miR-193a, miR-195, miR-30c-2, miR-3120, miR-3145, miR-3184, miR-340, miR-4261, miR-524, miR-606, miR-634, miR-921, miR-99a, miR-181d, miR-1893, miR-1934, miR-1944, miR-1957, miR-1981, miR-20a, miR-145, miR-27a, miR-328, miR-23a, miR-2145-2, miR-23b, miR-291a, miR-191, miR-327, miR-423, miR-465b-2, miR-719, miR-130, miR-124-1, miR-184, miR-199a, miR-30e, miR-330, miR-362, miR-513, miR-666</i>	IPAH
Wang, L. et al. 2013 [77]	Upregulated <i>miR-320c, miR-149, miR-4288, miR-191, miR-425, miR-151-5p, miR-29a, miR361-5p, miR-3676, miR-1246, miR-155, miR-1290</i> Downregulated <i>miR-30a, let-7c, miR-140-5p, miR-27b, let-7d, miR-152</i>	CTEPH

miR indicates microRNA, IPAH, idiopathic pulmonary arterial hypertension, CTEPH, chronic thromboembolic pulmonary hypertension.