Genetics and Genomics of Pulmonary Arterial Hypertension

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Major discoveries have been obtained within the last decade in the field of hereditary predisposition to pulmonary arterial hypertension (PAH). Among them, the identification of bone morphogenetic protein receptor type 2 (BMPR2) as the major predisposing gene and activin A receptor type II-like kinase-1 (ACVRL1, also known as ALK1) as the major gene when PAH is associated with hereditary hemorrhagic telangiectasia. The mutation detection rate for the known genes is approximately 75% in familial PAH, but the mutation shortfall remains unexplained even after careful molecular

investigation of these genes. To identify additional genetic variants predisposing to PAH, investigators harnessed the power of next-generation sequencing to successfully identify additional genes that will be described in this report. Furthermore, common genetic predisposing factors for PAH can be identified by genome-wide association studies and are detailed in this paper. The careful study of families and routine genetic diagnosis facilitated natural history studies based on large registries of PAH patients to be set up in different countries. These longitudinal or cross-sectional studies permitted the clinical characterization of PAH in mutation carriers to be accurately described. The availability of molecular genetic diagnosis has opened up a new field for patient care, including genetic counseling for a severe disease, taking into account that the major predisposing gene has a highly variable penetrance between families. Molecular information can be drawn from the genomic study of affected tissues in PAH, in particular, pulmonary vascular tissues and cells, to gain insight into the mechanisms leading to the development of the disease. High-throughput genomic techniques, on the basis of next-generation sequencing, now allow the accurate quantification and analysis of ribonucleic acid, species, including micro-ribonucleic acids, and allow for a genome-wide investigation of epigenetic or regulatory mechanisms, which include deoxyribonucleic acid methylation, histone methylation, and acetylation, or transcription factor binding.

Key Words

BMPR2; genetics; genomic; pulmonary hypertension

Abbreviations and Acronyms

BMP, bone morphogenetic protein; CHD, congenital heart disease; GINA, Genetic Information Non-Discrimination Act; GSD, glycogen storage disease; HDAC, histone deacetylase; HHT, hereditary hemorrhagic telangiectasia; HPAH, heritable pulmonary arterial hypertension; IL, interleukin; IPAH, idiopathic pulmonary arterial hypertension; mRNA, messenger ribonucleic acid; miRNA, micro ribonucleic acid; PAEC, pulmonary artery endothelial cell; PAH, pulmonary arterial hypertension; PASMC, pulmonary artery smooth muscle cell; SNP, single nucleotide polymorphism; TGF, transforming growth factor

Genetics of Pulmonary Hypertension

Hereditary predisposition to pulmonary arterial hypertension: from major genes to associated single nucleotide polymorphisms

Over 300 independent BMPR2 mutations (coding for a type II receptor member of the transforming growth factor [TGF]-β family) have been identified that account for approximately 75% of patients with a known family history of pulmonary arterial hypertension (PAH), and up to 25% of apparently sporadic cases have now unequivocally established defects in this gene as the major genetic determinant underlying PAH (1). Pathogenic mutations in the type I receptor ACVRL1 and, at a significantly lower frequency, the type III receptor endoglin in multiple kindreds cause PAH associated with hereditary hemorrhagic telangiectasia (HHT) (2). Together, these observations

support a prominent role for TGF-β family members in the development of PAH. Consequently, a series of studies have adopted a candidate gene approach to delineate novel genetic variants by examining TGF-β receptors and effectors in patient cohorts without mutations in the known PAH genes. With conventional analytical techniques, Shintani et al. (3) identified a truncating mutation in the bone morphogenetic protein (BMP)-responsive gene SMAD9 (p.C202X) in a panel of 23 Japanese cases. A second truncating mutation (p.R294X) has since been identified in another patient of Asian descent (4). A similar screen of the BMP-specific SMADs and SMAD4 described a series of 4 variants in 198 idiopathic pulmonary arterial hypertension (IPAH) patients. These variants in SMAD1 (p.V3A), SMAD4 (p.N13S; c.1448-6T>C), and SMAD9 (p.K43E) were described as being of unknown significance due to their moderate effects on canonical downstream BMP-mediated signaling outcomes (5). The SMAD9 variants are more compelling, because these data are supported by the development of clinical and histopathological features of pulmonary hypertension in a Smad9 knock-out mouse model (6). More recently, 2 missense mutations of the type I receptor BMPR1B (p.S160N and p.F392L) were reported in a cohort of 43 IPAH patients. Subsequent functional and reporter assays suggested that these variants generated an induction of SMAD9 and augmentation of transcriptional activity indicative of a gain-of-function mechanism. Because the preceding studies, in conjunction with the Smad9 mutant mouse model, suggest a molecular mechanism of haploinsufficiency for this gene, the observations described by Chida et al. (7) would seem to be contradictory and require further investigation on the functional level. Austin et al. (8) used whole exome sequencing to study a 3-generation family with multiple affected family members with PAH but no identifiable mutation in the known heritable pulmonary arterial hypertension (HPAH) genes and identified a novel gene for HPAH: Caveolin-1 (CAV1). They also identified a de novo frameshift mutation in a child with IPAH. CAV1 encodes a membrane protein of caveolae abundant in the endothelium and other cells of the lung. Caveolae are rich in cell surface receptors critical to initiation of a cellular signaling cascade such as the TGFβ superfamily, nitric oxide pathway, and G-protein coupled receptors. Aberrant signaling at the plasma membrane might be the mechanism for PAH pathogenesis. Their study demonstrates that mutations in CAV1 are associated in rare cases with familial PAH and IPAH, and it could provide new insight into the pathogenesis of PAH.

Exome sequencing in another family with multiple affected family members without identifiable HPAH mutations was found to have a heterozygous novel missense variant in the potassium channel KCNK3 (9). Analysis for additional familial PAH cases and IPAH cases identified 5 additional heterozygous novel missense variants. All 6 variants are located in highly conserved amino acids and are predicted to be damaging by in silico analysis. With transient transfection in COS-7 cells, whole patch clamp procedures demonstrated that each of the 6 mutations resulted in loss of function. Some, but not all, mutations were rescued by the phospholipase inhibitor, ONO RS-082. KCNK3 encodes a pH-sensitive potassium channel in the 2-pore domain superfamily (10). It has been reported that this potassium channel is sensitive to hypoxia and plays a role in the regulation of resting membrane potential and pulmonary vascular tone 11, 12 and 13. Identification of this gene as a cause of HPAH and IPAH and the possibility of rescuing specific mutations might provide a new target for PAH treatment.

Childhood-onset PAH shows some clinical and genetic differences from adult-onset PAH. The frequency of BMPR2 mutations found in sporadic cases is far lower than in adult-onset PAH 14, 15 and 16. Pulmonary hypertension is an uncommon complication in many genetic disorders, although in certain syndromes such as Down syndrome, PAH is more common (17). The increased risk for PAH with Down syndrome is due to left-to-right cardiac shunts; in addition, upper airway obstruction associated with obstructive sleep apnea might promote non-PAH pulmonary hypertension (18). Genetic syndromes more commonly but not necessarily associated with congenital heart disease (CHD) and pulmonary hypertension include DiGeorge syndrome, VACTERL syndrome, CHARGE syndrome, Scimitar syndrome (19), Noonan syndrome (20), and chromosomal anomalies associated with congenital diaphragmatic hernia. Genetic syndromes associated with pulmonary hypertension usually not associated with CHD include Adams-Oliver syndrome 21 and 22, neurofibromatosis type 1 23 and 24, long QT syndrome, hypertrophic cardiomyopathy, Cantu syndrome (25), autoimmune polyendocrine syndrome (26), mitochondrial disorders including mitochondrial encephalopathy lactic acidosis and stroke-like episodes (27), Gaucher disease (28), and glycogen storage diseases (GSDI and GSDIII) (29). The mechanism for development of pulmonary hypertension has not been definitely demonstrated for most genetic syndromes but could involve increased pulmonary blood flow with left-to-right shunts with CHD, upper airway obstruction, dysfunctional vascular smooth muscle cells with hyperproliferation leading to pulmonary vessel stenosis and remodeling (Adams Oliver syndrome 21 and 22 and neurofibromatosis type 1) 24 and 30, pulmonary venous obstruction (Cantu syndrome) (25), or production of diffusible hepatic factors increasing the pulmonary pressures (Gaucher disease and GSD) (29). Notably, pulmonary hypertension in patients with Gaucher disease has been reported to respond well to treatment of the primary metabolic disorders with enzyme replacement therapy (28).

Nimmakayalu et al. (31) reported a microdeletion encompassing TBX2 and TBX4 in a case of syndromic pulmonary hypertension associated with microcephaly thyroid and sensorineural abnormalities. Recently, Kerstjens-Frederikse et al. (32) studied 3 children with idiopathic or familial PAH associated with mental retardation and dysmorphic features by comparative genomic hybridization to identify deletions encompassing the same locus. They found 3 overlapping deletions at 17q23.2 involving also the TBX2 and TBX4 genes. These genes were subsequently sequenced in the 20 children, and 3 additional mutations were found in the TBX4 gene, which is responsible for the small patella syndrome. All patients with the TBX4 mutations present with signs of small patella syndrome. Inversely, careful investigation of patients known to have small patella syndrome did not reveal pulmonary hypertension.

Another approach for identifying genes predisposing for PAH is to perform association studies using polymorphic markers (single nucleotide polymorphisms [SNPs]) distributed throughout the whole genome. This approach requires a large number of patients and control subjects to compare the genotype frequencies in the 2 groups and look for a significant difference that can indicate association between the disease and the marker. With such an approach, Germain et al. (33) identified an SNP associated with IPAH and the familial form of PAH not caused by BMPR2

mutations. The risk allele of the SNP is associated with an odds ratio for PAH of 1.97 (95% confidence interval: 1.59 to 2.45; p = 7.47 × 10−10) and is close to the Cerebellin 2 (CBLN2) gene on Chr 18q22.3.

The molecular basis of the variation in penetrance observed for BMPR2 mutations has been addressed by several studies. The question is made difficult by the limited number of patients who can be included in this type of study, which requires large series of patients to reach statistical significance. Different approaches have been used. Philips et al. (34) studied a functional polymorphism of the TGF-β1 gene to investigate a possible disequilibrium between the BMPs and TGF signaling pathways that might influence the penetrance of the BMPR2 mutations. They proposed that the TGF-β1 polymorphism modulates the age at diagnosis and penetrance of the BMPR2 mutations. West et al. (35) used another approach by studying gene expression in immortalized B-lymphocyte cell lines of BMPR2 mutation carriers, either affected or unaffected. The most striking expression difference was observed for the CYP1B1 gene, with nearly 10-fold lower expression, but only in female patients (36). CYP1B1 is in the synthetic pathway of 2-OH estradiol metabolites that have anti-proliferative effects on pulmonary vascular smooth muscle cells and attenuate pulmonary hypertension in animal models 37 and 38. In contrast, when CYP1B1 is inhibited, 16β-OH-estradiol and -estrone are synthesized, which have proinflammatory, proangiogenic, and promitogenic effects (reviewed in Paulin and Michelakis [39]). However, mice with a disrupted Cyp1b1 gene do not exhibit differences in the development of experimental pulmonary hypertension, indicating an environmental context for the gene-effect (40). These results show the complexity of hormonal influences that might explain female predominance of PAH, which is observed in HPAH as well as in IPAH (41). With the same type of approach in cultured cells from patients carrying BMPR2 mutations leading to destruction of the mutated messenger ribonucleic acid (mRNA) by nonsense mediated ribonucleic acid (RNA) decay, Flynn et al. (42) have proposed a PAH penetrance signature on the basis of expression profiling of mRNAs in lymphocytes, and this profile suggests that reactive oxygen species formation would play an important role in the development of the disease. Concurrent inflammation can modify pathologic effects of the mutated BMPR2 gene 43 and 44.

Clinical presentation of HPAH

In approximately 75% of patients with a family history of PAH, a mutation in known PAH-causing genes has been identified 1, 15, 45 and 46 corresponding mostly with BMPR2 mutations. In patients without known family history (sporadic or idiopathic cases), approximately 20% harbor a germ-line mutation. In patients with a personal or familial history of HHT, ACVRL1 mutations were the major cause identified. Similar proportions of mutation carriers were observed in anorexigen-induced PAH. By contrast, BMPR2 mutations are not found in associated PAH (scleroderma and connective tissue diseases, portal hypertension, human immunodeficiency virus infection), with the exception of some reports in CHDs. Of note, familial cases of pulmonary veno-occlusive diseases are rarely associated with a BMPR2 mutation 47, 48 and 49.

Retrospective analysis from registries 1, 15, 45 and 46 and 1 prospective study (50) revealed that HPAH patients carrying a BMPR2 mutation, irrespective of the family history, develop PAH at a younger age than mutation-negative IPAH patients. Furthermore, HPAH patients have a more severe clinical and hemodynamic phenotype at diagnosis (less response to acute vasodilator challenge, lower cardiac index, and higher pulmonary vascular resistance), and they are more likely to progress to death or lung transplantation (at a younger age than noncarriers) 46, 50, 51, 52 and 53. However, the number of analyzed gene-carriers is so far relatively low. Further studies are needed to evaluate whether genetic testing might be helpful for risk stratification and clinical management. Similar findings are observed with ACVRL1 mutations with a significant number of pediatric cases and a dismal prognosis (50). Of note, ACVRL1 mutation carriers might develop both PAH and HHT. Because HHT has nearly complete penetrance at the age of 60 years, some ACVRL1 mutation carriers might not have clinical evidence of HHT at very young ages. Collecting information of personal and familial history of HHT, including “forme fruste,” seems important, especially in pediatric cases.

A more extensive evaluation of the Vanderbilt Pulmonary Hypertension Registry casts doubt on the likelihood of genetic anticipation in BMPR2-related familial PAH (54). Analysis of families with sibships that have lived at least 57 years from first family diagnosis allows >85% of mutation carriers to express disease. In these families, the apparent effect of lower age of onset in earlier generations disappears, because the time it takes for penetrance to occur in this illness can be up to 75 years of age in an apparently unaffected carrier. Thus, genetic anticipation is no longer supported by current data.

The penetrance of disease in the Vanderbilt Pulmonary Hypertension Registry has been re-evaluated (54): of a total number of 1,683 siblings, assuming a 50% carriage rate of the mutation, there were 232 affected individuals of 842 carriers (one-half of 1,683 siblings), or a 27% overall penetrance. There were 177 female subjects and 59 male subjects. The female/male ratio of PAH was 3:1, which was similar to previous estimates. The female penetrance was approximately 42%, and the male penetrance was approximately 14%. These sex differences should have an impact on disease and genetic counseling in families.

Genetic counseling and testing

Two consensus guidelines recommend that physicians offer professional genetic counseling and genetic testing to patients with a history that suggests HPAH 55 and 56. In addition, the authors of these guidelines have recommended that patients with IPAH be advised about the availability of genetic testing and counseling, because of the strong possibility that they carry a disease-causing mutation. The guidelines recommend that professionals offer counseling and testing to the affected IPAH patient before approaching other family members. The identification of a disease-causing

mutation in an affected family member allows less expensive testing of other family members, if they want such testing.

Affected individuals and “at risk” family members might want to know their mutation status for family planning purposes. Pre-natal screening or pre-implantation diagnosis and management are possible. Reproductive medicine allows several options for preventing transmission of HPAH to the next generation. Indeed, current reproductive options for couples with a BMPR2 mutation carrier are to remain childless, to have no genetic pre-natal testing (reproductive chance), to undergo pre-natal or pre-implantation genetic diagnosis, to use gamete donation, or to adopt. Pre-natal diagnosis allows the detection of an in utero fetus carrying a mutation predisposing to PAH. Pre-natal diagnosis requires that the familial mutation has been identified molecularly. If the familial mutation is identified, a medical abortion is an option.

Another option is pre-implantation genetic diagnosis, medically-assisted reproduction with selection and implantation of embryos that do not carry the familial mutation, thus avoiding the distress of a medical abortion. Pre-implantation genetic diagnosis requires in vitro fertilization and might require multiple cycles before leading to successful delivery of a baby. Pre-implantation genetic diagnosis is not available in all countries and is not a covered insurance benefit in all countries or by all insurers. These methods are used in many other diseases but are controversial in conditions in which penetrance is incomplete, such as HPAH. Due to the psychological impact of abortion on prospective parents, especially in the setting of an incompletely penetrant genetic disease, many patients prefer pre-implantation genetic diagnosis in selected HPAH families after multidisciplinary discussion when it is financially feasible and medically available. In France, pre-implantation genetic diagnosis is currently offered to selected families with highly-penetrant BMPR2 mutations causing HPAH 57 and 58. Because pregnancy is a risk factor of PAH, pre-implantation genetic diagnosis is currently proposed in couples where the future father carries the causal mutation.

Genetic testing allows identification of pre-symptomatic carriers of PAH-causing mutations who are at high risk of developing PAH. However, because of incomplete penetrance of mutations in PAH-predisposing genes, it is currently not possible to identify which carriers of a mutation will develop PAH. There are currently no proven effective interventions or medications available to prevent disease in mutation carriers. Associated genetic or environmental factors modifying penetrance of PAH in these mutation carriers to improve risk stratification are still unknown. Thus, genetic testing in relatives will effectively identify mutation noncarriers who have no increased risk of the heritable disease and potentially provide significant relief; however, mutation carriers currently face many uncertainties, because physicians cannot determine which patients will develop the disease or when. Such patients are currently offered yearly screening echocardiography with Doppler as well as immediate evaluation for symptoms such as exercise dyspnea. Because of the psychological impact of the positive or negative genetic results in asymptomatic relatives, pre-symptomatic genetic testing should be provided in the setting of a multidisciplinary team with availability of pulmonary hypertension specialists, genetic counselors, geneticists, psychologists, and nurses.

In France, up to 200 relatives of mutation carriers have volunteered for pre-symptomatic genetic testing. This led to the identification of dozens of asymptomatic BMPR2 mutation carriers. An ongoing study is currently evaluating the efficacy of pre-symptomatic screening and follow-up in this cohort. In this study, all carriers have yearly complete evaluation, including exercise testing, Doppler echocardiography, and measurement of circulating biomarkers (and rest and exercise right heart catheterization) (NCT01600898). Long-term follow-up might allow investigators to identify predictors of progression to PAH in pre-symptomatic BMPR2 mutation carriers. This active screening approach remains investigational and should help to refine future guidelines.

In the United States, physicians, PAH patients, and their family members have rarely embraced pre-symptomatic genetic testing for several reasons. First, genetic testing is relatively expensive. Second, the psychological impact of either a positive test (anxiety and depression) or a negative test (survivor guilt) is important for some individuals. These effects might have unintended consequences for other family members who do not wish to know their mutational state. Third, in the United States, concerns about discrimination remain, in spite of the passage of the Genetic Information Non-Discrimination Act (GINA) (HR 493). Although GINA protects against discrimination by insurers and employers, there are gaps in GINA protections (e.g., when applying for life, disability, or long-term insurance). In contrast, the French Network of Pulmonary Hypertension has launched a genetic counseling clinic with more than 1,000 subjects volunteering for “free” genetic counseling in the last 10 years (M. Humbert, personal communication, June 2013).

In a German proof of concept approach (59) and a subsequent larger study in the European Union, screening of family members with echocardiography at rest and during exercise and hypoxia revealed a significantly higher frequency of an elevated tricuspid regurgitation velocity response to exercise and to prolonged hypoxia than in control subjects, especially in those relatives who shared a BMPR2 mutation with the index patients (60). This suggests that elevated estimated pulmonary artery pressure response to exercise and hypoxia might be genetically determined with a familial clustering. Further studies are needed to analyze the clinical value of noninvasive screening assessments in relatives of IPAH and HPAH patients and to develop an algorithm for early diagnosis in this cohort.

Genomics of PAH

Besides the investigation of constitutional genetic variations or mutations underlying PAH, molecular investigation of lung tissue or specific cell types when possible or surrogate blood cells can provide important information concerning the mechanisms of the disease.

Somatic genetic changes in PAH lungs

Considerable evidence has accumulated over the past decade to advance the hypothesis that the pathogenesis of PAH is a neoplastic-like process 61, 62 and 63. Microdissection of plexiform lesions from the lungs of idiopathic and anorexigen-induced PAH cases showed that endothelial cells have a monoclonal pattern of X-inactivation 62 and 64. Some lesions also showed microsatellite instability, a hallmark of hereditary non-polyposis colon cancer, and mutations of the apoptosis regulator BAX (65). Many of the abnormal properties observed in pulmonary artery endothelial cells (PAECs) and pulmonary artery smooth muscle cells (PASMCs) are analogous to cancer, including increased proliferation, decreased apoptosis, activation of hypoxia-inducible factor-1-alpha, mitochondrial abnormalities, and a shift from oxidative to glycolytic metabolism 66, 67, 68, 69, 70, 71 and 72.

Use of SNP arrays or comparative genomic hybridization array data to assess copy number variations can provide important information in PAH. Analysis of hyperproliferative PAECs and PASMCs from patients with PAH identified large-scale genomic alterations in the endothelial cells, which were confirmed in patient lung tissue by fluorescent in-situ hybridization (73). Abnormalities were detected across heritable, idiopathic, and associated cases of PAH, providing the first evidence for a second genetic hit in patients with germline BMPR2 mutations and also suggesting that somatic changes might represent a shared feature across different types of the disease. However, there is no evidence for direct loss of heterozygosity at the BMPR2 locus (74). In some cases, PAECs seem to be clonal even before the acquisition of the cytogenetically abnormal sub-clone (73). This suggests that another underlying genetic mutation or other population bottleneck precedes the chromosome rearrangement, a finding that fits well with the hypothesis that endothelial apoptosis in the early stages of PAH leads to subsequent selection of proliferative, apoptosis-resistant endothelial cells (75).

The PASMC proliferation is also a critical component of vascular remodeling in PAH, yet the incidence of chromosome abnormalities seems to be much lower than in PAECs. PASMCs are also usually polyclonal (62). The reasons for these differences are presently unclear.

One limitation of these studies is their reliance on explant or autopsy lung tissue, which by definition represents end-stage disease. However, it is not feasible to obtain tissue by lung biopsy in the earlier stages of PAH. Another important consideration is to demonstrate that these abnormalities are not simply artifacts of in vitro cell culture. Several lines of evidence argue against this, including confirmation of 2 chromosome deletions in uncultured lung tissue by fluorescent in-situ hybridization and the absence of any detectable abnormalities in multiple control subjects or cells from explant lungs of patients with cystic fibrosis or chronic obstructive pulmonary disease (73).

mRNA expression studies

Early expression studies on lung tissue were limited by small sample sizes. Alternative strategies with surrogate tissue (peripheral blood) have suggested the utility of transcriptional profiling (76). The effectiveness of expanding cohort sizes and using well-defined phenotypes for array-based classification was demonstrated with blood and examining markers that differentiate “scleroderma only” from “systemic sclerosis-associated PAH” patients (77). There is a clear benefit to using large, well-characterized cohorts when examining lung tissue gene expression profiles. Several newer efforts have focused on this approach. A larger sample of lung tissue array analysis demonstrates similar pathway disruption between pulmonary hypertension and pulmonary fibrosis (78). Perhaps the largest study to date using lung tissue microarray profiling demonstrated that, in patients with pulmonary fibrosis, the presence of pulmonary hypertension is characterized by a specific gene expression profile in both a training and testing algorithm (79).

Cell-based expression studies have been useful in characterizing selected pathways as well as determining differences in selected cell populations. For systemic sclerosis-associated PAH, pulmonary fibroblasts and lung tissue from patients with PAH and those from systemic sclerosis patients without PAH demonstrate characteristic gene expression signatures (80). Several studies have used global gene expression signatures to determine a more robust pathway analysis, including the effects of BMPR2 deficiency (81). The novel role of interleukin (IL)-13 in PAH pathobiology has been investigated, on the basis of array-generated data (82) and mouse model studies (83). Potential new therapeutic targets, such as apelin and peroxisome proliferator-activated receptor-gamma, have been extensively studied with array-based platforms 84 and 85.

One significant challenge to all genomic approaches is leveraging data into novel systems-based analysis approaches. Putting all of the relevant information into a systems model of pulmonary vascular disease might provide unique insights (86).

Role of miRNAs in PAH

Microribonucleic acids (miRNAs) are small non-coding sequences of RNA that have the capacity to regulate many genes, pathways, and complex biological networks within cells, acting either alone or in concert with one another (87). In diseases such as cancer and cardiac disease, the role of miRNAs in disease pathogenesis has been well-documented (88). The application of miRNA technologies and their therapeutic potential in cardiovascular diseases is most elegantly summarized by Small and Olson (89). The most extensive global investigation, leading to mechanistic studies and potential therapeutic implications for miRNAs in PAH centers, was performed on miR-204 (90). In this study, the investigators provided a comprehensive model linking abnormal miRNA expression to already known pathophysiologic processes in PAH, including nuclear factor of activated T cells activation, BMPR-II down-regulation, IL-6 production, the Rho pathway, PASMC proliferation, and resistance to

apoptosis. This study not only demonstrates the importance of miRNAs in PAH but also suggests that re-establishing normal miR-204 levels might represent a novel therapeutic approach for human PAH (90). Brock et al. (91) showed that BMPR2 is directly targeted by miR-17-5p and miR-20a and that IL-6 induces miR-17/92 through STAT3 induction. A highly conserved and functional STAT3-binding site in the promoter region of miR17/92 was found, and persistent activation of STAT3 leads to repressed protein expression of BMPR2 (91).

The BMP/TGF-β signaling itself regulates multiple different miRNAs through an interaction between Smads and the primary miRNA transcript, which leads to up-regulation of mature miRNAs in response to BMP ligand (92). This response was lost in lung vascular cells from patients with BMPR2 or SMAD9 mutations, suggesting that abnormal miRNA regulation plays an important role in HPAH (4). A systems biology approach supports a central role for miR-21, 1 of the miRNAs regulated by this BMP-mediated pathway (93). Abnormalities of miRNA processing in HPAH cells can be corrected by increasing the amount of BMPR-II protein at the cell surface or by promoting readthrough of nonsense mutations in BMPR2 or SMAD9 94 and 95. These approaches have the advantage of correcting the levels of multiple different miRNAs as well as other aspects of BMP signaling and, therefore, could represent promising therapeutic approaches in HPAH. Other studies in human tissues and animal models of pulmonary hypertension have implicated additional miRNAs, including the miR-17-92 cluster and miR-145 91, 96 and 97.

There are several methods to assess global miRNA expression, and both array-based and polymerase chain reaction-based methods represent biased approaches, relying on “known” miRNA sequences. Because miRNA processing can result in changes of miRNA sequences, the most unbiased approach and one that is increasingly adopted is the use of massively parallel sequencing strategies targeting small RNA species.

Epigenetic modifications and pulmonary hypertension

Epigenetic traits are stably heritable phenotypes resulting from changes in a chromosome without alterations in deoxyribonucleic acid sequence (98). Epigenetic changes are thought to lead to cellular reprogramming, the process by which a differentiated cell type can be induced to adopt an alternate cell fate. This idea seems to be consistent with observations in pulmonary hypertension, in which PAECs, PASMCs, and adventitial fibroblasts have all been demonstrated to acquire significantly altered characteristics, including stable increases in proliferation, resistance to apoptosis, metabolic switching, and pro-inflammatory gene expression. Recent studies have documented that downregulation of superoxide dismutase-2 in the fawn-hooded rat model of pulmonary hypertension results from tissue-specific hypermethylation of just 2 CpG positions in the SOD2 promoter and an intronic enhancer (99). Another candidate for epigenetic study is BMPR2, with significantly down-regulated expression in many PAH lungs, even in the absence of a germline mutation 78 and 100.

Histone deacetylases (HDACs) catalyze removal of acetyl groups from lysine residues in a variety of proteins. The HDACs have mainly been studied in the context of chromatin, where they regulate gene transcription by deacetylating nucleosomal histones. The 18 mammalian HDACs are grouped into 4 classes (101). Dysregulation of HDACs is associated with a variety of pathophysiological processes, including cancer and inflammatory signaling in rheumatoid arthritis.

Expression of class I HDACs, particularly HDAC1, is dramatically elevated in pulmonary arteries of humans with pulmonary hypertension and in lungs and vessels from pulmonary hypertensive models. On the basis of these findings, recent studies have begun to address the role of class I HDACs in the pathogenesis of pulmonary hypertension. In a 3-week rat model of hypobaric hypoxia, the class I HDAC-selective inhibitor, MGCD0103, reduced pulmonary artery pressure through a mechanism involving suppression of PASMC proliferation (102). The anti-proliferative effect of MGCD0103 was due, in part, to up-regulation of the FoxO3a transcription factor and induction of a downstream target gene encoding the p27 cyclin-dependent kinase inhibitor. In addition it has become increasingly clear that HDAC inhibitors can be used to reduce cardiac hypertrophy and fibrosis (103).

Conclusions

Pathophysiological changes occurring during the development of PAH are extremely complex and probably involve many genetic and epigenetic mechanisms that lead to changes in gene expression and proliferative and metabolic changes in cells. Until now, approaches have been fragmentary and did not allow a holistic view of disease development. Recent high-throughput techniques, including genomics, metabolomics, and proteomics, can be performed simultaneously for a given patient and in different cells and biological fluids and can be repeated longitudinally as disease progresses. Such an approach was described for 1 subject and generated useful information (104). Such an approach would be invaluable for understanding the disease evolution, particularly in BMPR2 mutation carriers.

We can also expect that next-generation sequencing in selected families will identify new important genes for explaining heritable forms of PAH. Although the identification of novel PAH genes might not account for a large percentage of patients, recent findings would suggest that these data have potential to elucidate pathogenesis and provide novel targets for therapy. Equally, the analysis of common variation in large, well-characterized PAH groups has been demonstrated to yield important insights, and the replication and extension of these genome-wide association studies should serve to further define the PAH genetic landscape.

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