Pulmonary Hypertension Associated With Scleroderma and Connective Tissue Disease: Potential Molecular and Cellular Targets

Maria Trojanowska, PhD
Arthritis Center
Boston University
Boston, MA

Systemic sclerosis (SSc) is characterized by autoimmunity, small-vessel vasculopathy, and fibrosis causing damage in multiple organ systems. Pulmonary arterial hypertension (PAH) is a serious and often fatal complication of SSc, occurring in patients with the limited (lcSSc) and diffuse (dcSSc) forms of the disease and affecting 8% to 15% of patients.[1,2] While pulmonary hypertension associated with connective tissue disease (CTD-PAH) has similar clinical features as idiopathic PAH, 1-year survival and freedom from hospitalization are lower in CTD-PAH.[3] SSc-PAH has the worst 1-year survival rate at 82% compared with other connective tissue diseases, including systemic lupus erythematosus, mixed connective tissue disease, and rheumatoid arthritis.[3,4] Despite the recent progress in the development of disease-targeted therapies, patients with SSc-PAH have a poorer response to treatment and a worse prognosis than other subgroups of PAH.[1] Autoimmunity and prolonged vasculopathy preceding the development of clinical manifestations of SSc-PAH may play a critical role in the poorer outcome of SSc-PAH patients.[1] This article will provide an overview of the recent findings related to cellular and molecular mechanisms associated with the development of PAH, with an emphasis on SSc-PAH.

AUTOIMMUNITY AND INFLAMMATION

Antinuclear antibodies (ANA) are commonly present in patients with systemic sclerosis (SSc) and are clinically useful in distinguishing specific disease subtypes.[5] Typically, anticentromere antibodies (ACA) are found in limited cutaneous systemic sclerosis (lcSSc) patients who may develop pulmonary arterial hypertension (PAH) later in life. Patients with diffuse cutaneous systemic sclerosis (dcSSc) but not lcSSc frequently present with anti-topoisomerase I antibody (ATA). Those patients have poorer prognosis, increased mortality, musculoskeletal and cardiac involvement, and are at risk of developing pulmonary fibrosis. Also highly specific to dcSSc are anti-RNA polymerase III antibodies (anti-RNAP III) that are associated with rapid progression of skin thickening, scleroderma renal crisis, and malignancy. In addition to ANAs, a group of functional pathogenic autoantibodies directed against cellular cell surface receptors and extracellular matrix proteins have been described in SSc.[6] Notably, such autoantibodies are also present in sera from healthy individuals, albeit at lower levels. Their function in a healthy immune system remains to be investigated.[7]

Agonistic Anti-AT1R and Anti-ETAR Antibodies

Increased activation of vasoactive mediators angiotensin II (Ang II) and endothelin-1 (ET-1) has been reported in SSc and implicated in both vasculopathy and fibrosis.[8,9] Angiotensin-converting enzyme (ACE) inhibitors, angiotensin 1 receptor (AT1R) antagonists, as well as ET-1 receptor blockers are routinely used to alleviate some of the vascular complications of SSc.[10] Importantly, functional pathogenic antibodies directed against AT1R and endothelin-1 type A receptors (ETAR) were identified in patients with SSc and linked to increased prevalence of SSc-related vascular and fibrotic complications and higher risk for SSc-induced mortality.[11,12] Studies have shown that those antibodies activate respective surface receptors on endothelial cells and fibroblasts and exert proinflammatory and profibrotic effects.[13] Recent studies by Becker et al have demonstrated higher levels of anti-ETAR and anti-AT1R antibodies in patients with SSc-PAH and connective tissue disease (CTD)-PAH compared to idiopathic pulmonary arterial hypertension (IPAH) and other forms of pulmonary hypertension (PH).[11] Concomitant presence of anti-ETAR and anti-AT1R antibodies in patients with SSc predicted development of PAH and its associated mortality. In functional studies, injections of antibody-positive human SSc-immunoglobulin G (IgG) into mice induced obliterative vasculopathy with lymphocyte infiltration, as well as increased alpha-smooth muscle actin (αSMA) expression around the wall of small pulmonary vessels; however, Fulton's index and pulmonary vascular resistance (PVR) were not different from controls.[11] Although findings from those studies suggest that the presence of antibodies alone is not sufficient to induce PAH, they nonetheless strongly support the pathogenic role of anti-ETAR and anti-AT1R antibodies in SSc-PAH and CTD-PAH patients.

Key Words—autoimmunity, connective tissue disease, fibrosis, macrophage, scleroderma
Correspondence: trojanme@bu.edu
Disclosures: Dr Trojanowska has nothing to disclose.

Stimulatory Anti-PDGF Receptor Antibodies

Platelet-derived growth factor (PDGF) is one of the key growth factors involved in the pathogenesis of SSc.[14,15] PDGF is also involved in the pathogenic vascular alterations in PAH.[16] Gabrielli and colleagues described agonistic pathogenic anti-PDGF receptor antibodies in SSc patients.[17] These autoantibodies functioned similarly to PDGF in inducing intracellular signaling and reactive oxygen species (ROS) production.[17] Furthermore, unlike natural ligands that elicit short-term effects, the effects of the anti-PDGF receptor autoantibodies were long lasting. With respect to PH, recent work from Svegliati el al demonstrated that autoantibodies targeting PDGF receptors induce proliferation and collagen synthesis, as well as NOX4 and ROS production in human pulmonary artery smooth muscle cells (HPASMC).[18] Furthermore, these effects were, in part, mediated through the activation of MTORC1. Although clinical association of anti-PDGF receptor antibodies with SSc-PAH have not yet been examined, the latter studies suggest that stimulatory antibodies targeting PDGF receptors could activate vascular smooth muscle cells and, thus, contribute to the development of vascular lesions.

Other Autoantibodies Associated With SSc-PAH

Anti-endothelial cell antibodies (AECA) are detected in a large proportion of patients with SSc and are associated with vascular manifestations, including PAH.[19] Cellular targets of AECAs have recently been characterized using proteomic approaches and include ubiquitous cellular proteins such as lamin A/C, tubulin, and vinculin.[20] The presence of antifibroblast antibodies (AFA) has been described by several groups in patients with SSc-PAH.[21-23] Those antibodies recognize many cellular targets; however, their functional role in disease remains to be investigated. Bussone et al reported increased frequency of antibodies to vascular smooth muscle cells (VSMC) in patients with SSc-PAH compared to IPAH and healthy controls.[24] The authors also showed that serum IgG from those patients induced contraction of VSMCs in a collagen matrix, suggesting a pathogenic role. Given the widespread presence of self-reactive IgG autoantibodies in human sera, it may be expected that many additional functional autoantibodies will be characterized in SSc patients in the future.[7]

Inflammation

Animal models of PAH and clinical data support a role for perivascular inflammation in the pathogenesis of PAH.[25] Because human diseased lung tissue is not readily available, global gene expression has been analyzed by several investigators in SSc-PAH peripheral blood mononuclear cells (PBMC).[26-29] In general, those studies found elevated expression of inflammatory and vascular injury-related genes in SSc-PAH PBMCs compared to controls, although there was little overlap between the genes identified in the individual studies.

Increasing evidence supports the role of leukotrienes (LT), and, in particular, leukotriene B_4 (LTB_4), in the pathogenesis of PAH.[30] LTB_4, which is produced by perivascular macrophages, induced endothelial cell apoptosis by inhibiting expression and activation of sphingosine kinase and endothelial nitric oxide synthase (eNOS). LTB_4 also induced proliferation and hypertrophy of human pulmonary arterioles smooth muscle cells (PASMC). Furthermore, LTB_4 enhanced proliferation, migration, and myofibroblast differentiation of adventitial fibroblasts. The serum concentration of LTB_4 was found to be significantly increased in PAH, primarily in patients with SSc-PAH.[31]

Associations of other serum biomarkers with SSc-PAH have been reported. For example, elevated levels of pentraxin 3 were found in patients with digital ulcers and PAH.[32] A recent study revealed elevated levels of adipsin in lcSSc patients, which correlated with significantly increased risk for PAH and related cardiac dysfunction.[33] Adipsin, a serine protease produced by adipocytes, also known as a complement factor D, has not previously been associated with rheumatic diseases. Whether adipsin plays a pathogenic role in SSc-PAH remains to be determined.

Perivascular Inflammation

In PAH, immune and inflammatory cells reside primarily in the outer layer of the vessel wall, the adventitia. Vascular adventitia is composed of various cells, including fibroblasts, blood and lymphatic vessels, nerve cells, as well as immune cells embedded in the connective tissue.[34] Comprehensive comparison of the immune cells in control and IPAH lungs was recently reported by Savai et al.[35] The study showed a significant increase in monocytes and macrophages ($CD14^+$ and $CD68^+$), dendritic cells ($CD209^+$), T cells ($CD8^+$ and $CD4^+$) in IPAH lungs, as compared to controls. Notably, the immune cells were primarily localized to the adventitia. However, $Foxp3^+$ cells were significantly decreased, consistent with a reduced presence of regulatory T cells.

Of particular relevance to the pathogenesis of PAH are macrophages, a functionally heterogenous cell population, originally classified into M1 (classically activated, proinflammatory) and M2 (alternatively activated, anti-inflammatory) subsets.[36] It is now recognized that this classification does not adequately describe the spectrum of macrophage phenotypes, especially as it relates to the various disease conditions, including PAH. Studies have shown that in IPAH, as well as several experimental models of PH, adventitial macrophages were alternatively activated by interleukin (IL)-6 produced by the adventitial fibroblasts.[37] At the molecular level, macrophages activated in this fashion showed increased signal transducer and activator of transcription 3 (STAT3), hypoxia-inducible factor 1-alpha (HIF1α), and CCAAT/enhancer binding protein beta (C/EBPβ) signaling, as well as higher expression of arginase and other genes consistent with the phenotype of macrophages isolated from PH lesions.[37] This mode of activation differs from the established alternative macrophage activation pathways such as IL-4/IL-13/signal transducer and activator of transcription 6 (STAT6) or toll-like receptor (TLR)-myeloid differentiation primary response 88 (MyD88). Fibro-

blast-activated macrophages were also metabolically reprogrammed to aerobic glycolysis, an altered tricarboxylic acid (TCA) cycle, and reduced mitochondrial respiration, discussed below.

Macrophages play an essential role in the pathogenesis of PH and their depletion mitigates pulmonary vascular remodeling.[38] A recent study using hypoxia-induced PH in mice showed that M2 macrophages can be therapeutically targeted.[39] Global deletion of a chemokine receptor CX3CR1 or treatment with pharmacological inhibitor of CX3CR1 led to significant reduction of PH with concomitant decrease of M2 and increase of M1 markers, suggesting a role for CX3CR1 as a therapeutic target in PAH.

Genetic Factors

Mutations in the components of the transforming growth factor beta (TGFβ) superfamily, including bone morphogenetic protein receptor-2 (BMPR2), activin-receptor-like type I (ACVRL1), endoglin (ENG), SMAD8, as well as caveolin-1 (CAV1) have been identified in hereditary forms of PAH and in the subset of IPAH patients.[40] Although activation of TGFβ signaling is considered central to the pathogenesis of SSc,[41] to date only the association between polymorphism in the ENG gene and SSc-PAH has been reported.[42] Genetic variations in several other genes, including TLR2, tumor necrosis factor alpha-induced protein (TNFAIP) 3, and urokinase plasminogen activator (uPAR) have been associated with SSc vascular complications, as well as PAH.[43-45] In addition, HLA-B*35 (human leukocyte antigen class B) has been shown to be associated with increased risk for developing PAH in Italian SSc patients[46,47] and in Brazilian SSc patients[48]; however, it is unclear whether this correlation is present in other SSc cohorts. Functional studies in endothelial cells have demonstrated that the presence of HLA-B*35 contributes to endothelial cell dysfunction by increasing production of ET-1 and significantly decreasing nitric oxide synthase in conjunction with upregulation of endoplasmic reticulum (ER) stress and unfolded protein response (UPR).[49,50] The association of HLA-B*35 with increased expression of ER stress markers and inflammatory genes was further verified in PBMCs obtained from SSc patients.[51]

VASCULAR DISEASE

Vascular disease, a major pathologic manifestation of SSc, affects mainly microcirculation and small arterioles. Injury to the endothelial cells leads to widespread progressive capillary malformation and rarefaction, as well as fibroproliferative changes in arterioles and small arteries. Vascular abnormalities are not limited to the skin and also occur in the involved organs, including the kidneys, heart, lung, and muscles.[52] Microvascular manifestations, including increased number of telangiectasias and reduced capillary nailfold density have been linked to the increased risk of PAH in patients with SSc.[53,54] Despite the significant research efforts, the nature of the initial insult and the mechanisms underlying defective angiogenesis and vasculogenesis in SSc is not fully understood. Several comprehensive reviews on SSc vasculopathy have recently been published; readers are referred to those articles for additional information.[52,55-59]

Endothelial to Mesenchymal Transition

Endothelial to mesenchymal transition (EndoMT) is a process whereby endothelial cells undergo molecular changes that result in a gradual loss of endothelial cell characteristics and emergence of mesenchymal cell markers. A number of studies using various experimental models of fibrosis have implicated EndoMT as a potential source of fibroblasts/myofibroblasts.[60,61] The presence of EndoMT has been demonstrated in human PAH and in 2 experimental models of PAH.[62] Similar observations were also made in SSc-PAH. Good et al demonstrated the presence of cells coexpressing von Willebrand factor (vWF) and αSMA in pulmonary vessels of patients with SSc-PAH.[63] EndoMT may also contribute to the fibrotic process in SSc-associated pulmonary fibrosis.[64] Likewise, coexpression of endothelial cell markers cluster of differentiation (CD31) or vascular endothelin cadherin (VE-cad) and αSMA was frequently observed in SSc dermal capillaries and arterioles.[65] Further in vitro comparison of SSc and healthy control microvascular endothelial cells showed higher expression of mesenchymal markers, such as collagen, αSMA, and S100 calcium-binding protein A4 (S100A4)/fibroblast-specific protein-1 (FSP1) and lower expression of CD31 and VE-cad in SSc endothelial cells. Interestingly, sera from SSc patients but not healthy controls induced EndoMT in control endothelial cells with a potency similar to that of TGFβ, suggesting that a factor(s) in SSc sera is contributing to the EndoMT in vivo. Inflammatory cytokines have been implicated in inducing EndoMT, suggesting that perivascular inflammation may contribute to this process in PAH.[61] EndoMT appears to represent a common manifestation of vascular dysfunction; however, it is not yet clear whether endothelial cells serve as a primary source of αSMA expressing cells in the pulmonary lesions.

Transcriptional Regulation of Vascular Cells

Dysregulated transcription factor network has been associated with altered behavior of vascular smooth muscle and endothelial cells in PAH.[66] Recent studies have demonstrated that the level and activity of one of the key regulators of VSMC proliferation and apoptosis, transcription factor forkhead box protein O-1 (FoxO1), is reduced in VSMCs from IPAH patients, as well as 2 experimental models of PH.[67] Genetic or pharmacological inhibition of FoxO1 in VSMCs recapitulated PH characteristics in vivo, while re-expression of FoxO1 reversed the development of PAH and restored normal lung and heart function. Thus, FoxO1 deficiency in VSMCs appears to play a key role in the development of PH and its restoration may have a beneficial therapeutic effect. Additional transcription factors implicated in pro-proliferative and anti-apoptotic behavior of smooth muscle cells include Krüppel-like factor 5 (KLF5) and Oct-4.[68,69] Both factors are expressed at elevated levels in PASMCs isolated from IPAH patients, while elevated KLF5 expression was also confirmed in IPAH lung biopsies. Functional studies have shown reduced proliferation

and increased apoptosis in response to KLF5 inhibition in PAH-PASMCs.[68] Furthermore, in vivo inhibition of KLF5 improved PAH in a monocrotaline-induced PAH in rats.

Pulmonary veno-occlusive disease (PVOD) is characterized by the preferential involvement of the pulmonary venous system resulting in progressive elevation in pulmonary arterial pressure, leading to right heart failure and death.[70] PVOD appears to be relatively common in patients with CTD and my affect up to 75% of patients.[71] Heritable PVOD is caused by biallelic mutation in EIF2AK4 (eukaryotic initiation translation factor 2 alpha kinase 4) gene.[70] EIF2AK4, also known as GCN2, is 1 of 4 kinases responsible for phosphorylating eukaryotic initiation phosphorylation factor 2 (eIF2), a key regulator of cellular response to various forms of stress. Studies have shown that GCN2 protects against hepatoxicity during aspariginase treatment, while the loss of GCN2 promotes oxidative stress and inflammation-mediated DNA damage.[72] The role of GCN2 deficiency in the development of PVOD awaits further investigation.

Recent studies have linked suppression of the erythroblast transformation-specific (ETS)-related gene (ERG) to PVOD.[73] ERG, a member of the ETS family of transcription factors, is involved in several developmental processes, including endothelial cell specification and blood vessel formation.[74] ERG is highly expressed in differentiated endothelial cells, including large arterial, venous, and microvascular endothelium. Lathen et al have demonstrated that protein levels of ERG were significantly decreased in patients with PVOD.[73] They further showed that ERG regulates vein-specific expression of Apelin receptor (APLNR). Mice with the homozygous deletion of ERG or APLNR developed lethal PVOD. These studies support the key role for the ERG-APLNR axis in maintaining pulmonary venous endothelial homeostasis. The potential interactions between ERG-APLNR and GCN2 in the pathogenesis of PVOD remain to be established. Interestingly, recent studies have shown that ERG could also be involved in regulating inflammation in pulmonary vasculature.[75] Looney et al showed that endothelial ERG protein expression was reduced in patients with IPAH and SSc-PAH, as well as in the lungs of hypoxic mice.[75] Loss of ERG in the pulmonary endothelial cells led to de-repression of interferon beta 1 (IFNβ-1) gene and marked upregulation of interferon-responsive proinflammatory genes. Friend leukemia integration factor 1 (FLI1) is a close homolog of ERG and, like ERG, is involved in regulation of vascular stability.[76] Simultaneous depletion of ERG and FLI1 had a synergistic or additive effect on expression of the inflammatory genes. Furthermore, ERG and FLI1 double-heterozygous mice showed increased inflammation in the lung.[75] It has been proposed that ERG and FLI1 may represent the "master" transcription factors regulating the crucial manifestation of PH such as inflammation and small artery remodeling.[77] Further support for the central role of ERG in regulating vascular inflammation came from the work of Hogan et al.[78] Using a combination of computational and experimental approaches the authors identified activator protein 1 (AP1), ETS, and GATA family members as central transcriptional regulators of endothelial cell homeostasis. Among the multiple members of those families, JUN, JUNB, JUND, and ERG were highly expressed in human aortic endothelial cells. Consistent with other studies, knockdown of ERG in human aortic endothelial cells elicited proinflammatory gene expression.[78]

There is also evidence that dysregulated expression of GATA-6 may contribute to PAH. It has been reported that GATA-6 protein levels are markedly reduced in pulmonary endothelial cells and smooth muscle cells in patients with IPAH and SSc-PAH.[79] GATA-6 is a direct transcriptional regulator of genes controlling vascular tone, including ET-1, ETAR, and eNOS, as well as CX-3CL1. Mice with endothelial deletion of GATA-6 (GATA-6 knockout [KO]) spontaneously develop elevated pulmonary pressure and right ventricular hypertrophy.[79] After exposure to hypoxia, GATA-6 KO mice display marked worsening in pulmonary pressure that is paralleled by extensive vascular remodeling.

Metabolism and Mitochondrial Dynamics

In recent years, it has been recognized that there are striking similarities between phenotypic changes occurring in PAH vasculature and cancer.[80] These findings gave rise to the metabolic theory of PAH.[81] Like cancer cells, PAH-PASMCs and endothelial cells are hyperproliferative and resistant to apoptosis.[81-83] Furthermore, akin to cancer cells, vascular cells in PAH undergo a metabolic switch from mitochondrial oxidative phosphorylation to aerobic glycolysis. Mitochondrial alterations, which are both structural and functional, have been primarily investigated in PASMCs. PH-PASMCs have hyperpolarized mitochondria that produce less ROS. The changes in the mitochondrial metabolic redox signaling are associated with activation of HIF1 and nuclear factor of activated T cells (NFAT). Once activated, HIF1 upregulates transcription of most of the glycolytic enzymes. Furthermore, mitochondrial pyruvate dehydrogenase complex (PDH), a key enzyme in glucose metabolism that links glycolysis to the TCA cycle, is inactivated by pyruvate dehydrogenase kinase (PDK), also a target of HIF1. Both HIF1 and NFAT contribute to the downregulation of Kv channel expression, a well-known feature of PAH. Structural alterations of mitochondria include fragmented mitochondrial network due to the imbalance of mitochondrial biogenesis characterized by dysregulated expression of mitochondrial fission and fusion genes.[83]

PH fibroblasts also exhibit a hyperproliferative and inflammatory phenotype that is caused by metabolic reprogramming.[82,84] The cellular basis underlying these changes was recently elucidated by Stenmark and colleagues. They found a switch of pyruvate kinase isoenzymes PKM1 to PKM2 in PH fibroblasts. PKM1/2 is an enzyme that catalyzes the last step of glycolysis in which pyruvate and adenosine triphosphate (ATP) are formed. A switch to a less active PKM2 isoform leads to elevated glycolytic reprogramming and facilitates generation of macromolecule

precursors that are necessary for rapid cell proliferation.[82] Of note, PKM2, which also has many nonmetabolic functions, is widely overexpressed in tumors and supports tumor cell growth.[85] Metabolic reprogramming toward aerobic glycolysis in PH fibroblasts led to enhanced levels of free reduced nicotinamide adenine dinucleotide (NADH), which in turn upregulated a transcriptional co-repressor C-terminal binding protein 1 (CtBP1).[84] As a negative regulator of several tumor suppressor genes, CtBP contributes to proliferation and resistance to apoptosis in both tumor cells and PH fibroblasts.

Numerous studies support the role of excessive oxidative stress in the pathogenesis of SSc.[86,87] Fibroblasts isolated from SSc lesions spontaneously produce increased amounts of ROS.[88] Likewise, circulating monocytes from SSc patients release more superoxide than controls.[89] In addition, sera obtained from SSc patients but not from healthy controls induce ROS production in cultured fibroblasts and endothelial cells.[90] Oxidative stress has also been implicated in the development of SSc-PAH; for example, SSc-PAH patients have lower levels of exhaled nitric oxide (NO),[91,92] as well as elevated plasma levels of asymmetrical dimethylarginine (ADMA), which may further contribute to lower NO production.[93] SSc PBMCs express significantly higher levels of genes representing all 3 branches of the UPR, including immunoglobulin heavy-chain binding protein (BiP), activating transcription factor (ATF) 6, ATF4, spliced X-box binding protein 1 (XBP1), and chaperone DNAJB1.[29] Interestingly, there was a significant correlation between pulmonary arterial pressure and DNAJB1, as well as between IL-6 expression and BiP, suggesting that chronic UPR may contribute to increased inflammation in SSc. Analysis of plasma obtained from SSc patients with and without PAH revealed unfavorable metabolic profile characterized by deficiency of metabolites with protective effects on endothelial cells.[94]

PAH Therapies: Present and Future

Rapid progress in the understanding of the pathogenesis of PAH led to development of specific therapies targeting deficiencies in either NO signaling (phosphodiesterase type-5 inhibition or soluble guanylyl cyclase agonism), PGI2 (prostacyclin analogs), or excessive ET-1 (ET receptor blocker).[95] Furthermore, a double or triple combination therapy offered benefits to patients with severe PAH.[95] Despite these promising treatment options, the disease remains poorly managed, especially in SSc patients. In the final stages of PAH, lung transplant is the only option and, therefore, there is a significant need for alternative treatment options. The complexity of PAH, including impaired redox homeostasis, abnormal vascular remodeling, and immune cell activation suggests that a therapeutic agent capable of modulating several key pathways would be an attractive addition to the treatment regimen of PAH. The transcriptional regulator nuclear factor (erythroid-derived 2)-related factor 2 (NRF2) is a key regulator of the antioxidant genes.[96] Importantly, NRF2 function is linked to nuclear factor kappa beta (NFκB) signaling with activation leading to an anti-inflammatory response. In support of NRF2-based therapy in PAH, bardoxolone methyl (CDDO-Me), an NRF2 inducer, has shown encouraging results in a phase 2 study in PAH.[97] Dimethyl fumarate (DMF, BG-12,Tecfidera®), another inducer of NRF2, has recently been approved in the United States for the treatment of multiple sclerosis (MS).[98] DMF has an excellent safety record in MS and 2 decades of use in psoriasis, also an inflammatory condition. DMF has been evaluated in experimental models of PH[99] and mitigated PH by targeting multiple signaling pathways including oxidative damage, inflammation, and fibrosis. Efforts are underway to conduct a pilot randomized controlled trial with Tecfidera in SSc-PAH.[100]

References

1. Gashouta MA, Humbert M, Hassoun PM. Update in systemic sclerosis-associated pulmonary arterial hypertension. *Presse Med*. 2014;43(10 Pt 2):e293-e304.
2. Hachulla E, Launay D, Mouthon L, et al; French PAH-SSc Network. Is pulmonary arterial hypertension really a late complication of systemic sclerosis? *Chest*. 2009;136(5):1211-1219.
3. Chung L, Liu J, Parsons L, et al. Characterization of connective tissue disease-associated pulmonary arterial hypertension from REVEAL: identifying systemic sclerosis as a unique phenotype. *Chest*. 2010;138(6):1383-1394.
4. Ahmed S, Palevsky HI. Pulmonary arterial hypertension related to connective tissue disease: a review. *Rheum Dis Clin North Am*. 2014;40(1):103-124.
5. Mehra S, Walker J, Patterson K, Fritzler MJ. Autoantibodies in systemic sclerosis. *Autoimmun Rev*. 2013;12(3):340-354.
6. Cabral-Marques O, Riemekasten G. Functional autoantibodies directed against cell surface receptors in systemic sclerosis. *J Scleroderma Relat Disord*. 2017 Jun 5. [Epub ahead of print]
7. Nagele EP, Han M, Acharya NK, DeMarshall C, Kosciuk MC, Nagele RG. Natural IgG autoantibodies are abundant and ubiquitous in human sera, and their number is influenced by age, gender, and disease. *PLoS One*. 2013;8(4):e60726.
8. Kawaguchi Y, Takagi K, Hara M, et al. Angiotensin II in the lesional skin of systemic sclerosis patients contributes to tissue fibrosis via angiotensin II type 1 receptors. *Arthritis Rheum*. 2004;50(1):216-226.
9. Yamane K, Miyauchi T, Suzuki N, et al. Significance of plasma endothelin-1 levels in patients with systemic sclerosis. *J Rheumatol*. 1992;19(10):1566-1571.
10. Moinzadeh P, Riemekasten G, Siegert E, et al; German Network for Systemic Scleroderma. Vasoactive Therapy in Systemic Sclerosis: Real-life Therapeutic Practice in More Than 3000 Patients. *J Rheumatol*. 2016;43(1):66-74.
11. Becker MO, Kill A, Kutsche M, et al. Vascular receptor autoantibodies in pulmonary arterial hypertension associated with systemic sclerosis. *Am J Respir Crit Care Med*. 2014;190(7):808-817.
12. Riemekasten G, Philippe A, Nather M, et al. Involvement of functional autoantibodies against vascular receptors in systemic sclerosis. *Ann Rheum Dis*. 2011;70(3):530-536.
13. Kill A, Tabeling C, Undeutsch R, et al. Autoantibodies to angiotensin and endothelin receptors in systemic sclerosis induce cellular and systemic events associated with disease pathogenesis. *Arthritis Res Ther*. 2014;16(1):R29.
14. Makino K, Makino T, Stawski L, et al. Blockade of PDGF Receptors by Crenolanib Has Therapeutic Effect in Patient Fibroblasts and in Preclinical Models of Systemic Sclerosis. *J Invest Dermatol*. 2017;137(8):1671-1681.
15. Trojanowska M. Role of PDGF in fibrotic diseases and systemic sclerosis. *Rheumatology (Oxford)*. 2008;47 Suppl 5:v2-v4.
16. Grimminger F, Schermuly RT. PDGF receptor and its antagonists: role in treatment of PAH. *Adv Exp Med Biol*. 2010;661:435-446.
17. Baroni SS, Santillo M, Bevilacqua F, et al. Stimulatory autoantibodies to the PDGF receptor in systemic sclerosis. *N Engl J Med*. 2006;354(25):2667-2676.
18. Svegliati S, Amico D, Spadoni T, et al. Agonistic Anti-PDGF Receptor Autoantibodies from Patients with Systemic Sclerosis Impact Human

Pulmonary Artery Smooth Muscle Cells Function In Vitro. *Front Immunol*. 2017;8:75.
19. Negi VS, Tripathy NK, Misra R, Nityanand S. Antiendothelial cell antibodies in scleroderma correlate with severe digital ischemia and pulmonary arterial hypertension. *J Rheumatol*. 1998;25(3):462-426.
20. Dib H, Tamby MC, Bussone G, et al. Targets of anti-endothelial cell antibodies in pulmonary hypertension and scleroderma. *Eur Respir J*. 2012;39(6):1405-1414.
21. Chizzolini C, Raschi E, Rezzonico R, et al. Autoantibodies to fibroblasts induce a proadhesive and proinflammatory fibroblast phenotype in patients with systemic sclerosis. *Arthritis Rheum*. 2002;46(6):1602-1613.
22. Tamby MC, Humbert M, Guilpain P, et al. Antibodies to fibroblasts in idiopathic and scleroderma-associated pulmonary hypertension. *Eur Respir J*. 2006;28(4):799-807.
23. Terrier B, Tamby MC, Camoin L, et al. Identification of target antigens of antifibroblast antibodies in pulmonary arterial hypertension. *Am J Respir Crit Care Med*. 2008;177(10):1128-1134.
24. Bussone G, Tamby MC, Calzas C, et al. IgG from patients with pulmonary arterial hypertension and/or systemic sclerosis binds to vascular smooth muscle cells and induces cell contraction. *Ann Rheum Dis*. 2012;71(4):596-605.
25. Price LC, Wort SJ, Perros F, et al. Inflammation in pulmonary arterial hypertension. *Chest*. 2012;141(1):210-221.
26. Bull TM, Coldren CD, Moore M, et al. Gene microarray analysis of peripheral blood cells in pulmonary arterial hypertension. *Am J Respir Crit Care Med*. 2004;170(8):911-919.
27. Christmann RB, Hayes E, Pendergrass S, et al. Interferon and alternative activation of monocyte/macrophages in systemic sclerosis-associated pulmonary arterial hypertension. *Arthritis Rheum*. 2011;63(6):1718-1728.
28. Grigoryev DN, Mathai SC, Fisher MR, et al. Identification of candidate genes in scleroderma-related pulmonary arterial hypertension. *Transl Res*. 2008;151(4):197-207.
29. Lenna S, Farina AG, Martyanov V, et al. Increased expression of endoplasmic reticulum stress and unfolded protein response genes in peripheral blood mononuclear cells from patients with limited cutaneous systemic sclerosis and pulmonary arterial hypertension. *Arthritis Rheum*. 2013;65(5):1357-1366.
30. Tian W, Jiang X, Sung YK, Qian J, Yuan K, Nicolls MR. Leukotrienes in pulmonary arterial hypertension. *Immunol Res*. 2014;58(2-3):387-393.
31. Qian J, Tian W, Jiang X, et al. Leukotriene B4 Activates Pulmonary Artery Adventitial Fibroblasts in Pulmonary Hypertension. *Hypertension*. 2015;66(6):1227-1239.
32. Shirai Y, Okazaki Y, Inoue Y, et al. Elevated levels of pentraxin 3 in systemic sclerosis: associations with vascular manifestations and defective vasculogenesis. *Arthritis Rheumatol*. 2015;67(2):498-507.
33. Korman BD, Marangoni RG, Hinchcliff M, et al. Brief Report: Association of Elevated Adipsin Levels With Pulmonary Arterial Hypertension in Systemic Sclerosis. *Arthritis Rheumatol*. 2017;69(10):2062-2068.
34. Stenmark KR, Yeager ME, El Kasmi KC, et al. The adventitia: essential regulator of vascular wall structure and function. *Annu Rev Physiol*. 2013;75:23-47.
35. Savai R, Pullamsetti SS, Kolbe J, et al. Immune and inflammatory cell involvement in the pathology of idiopathic pulmonary arterial hypertension. *Am J Respir Crit Care Med*. 2012;186(9):897-908.
36. Lawrence T, Natoli G. Transcriptional regulation of macrophage polarization: enabling diversity with identity. *Nat Rev Immunol*. 2011;11(11):750-761.
37. El Kasmi KC, Pugliese SC, Riddle SR, et al. Adventitial fibroblasts induce a distinct proinflammatory/profibrotic macrophage phenotype in pulmonary hypertension. *J Immunol*. 2014;193(2):597-609.
38. Frid MG, Brunetti JA, Burke DL, et al. Hypoxia-induced pulmonary vascular remodeling requires recruitment of circulating mesenchymal precursors of a monocyte/macrophage lineage. *Am J Pathol*. 2006;168(2):659-669.
39. Amsellem V, Abid S, Poupel L, et al. Roles for the CX3CL1/CX3CR1 and CCL2/CCR2 Chemokine Systems in Hypoxic Pulmonary Hypertension. *Am J Respir Cell Mol Biol*. 2017;56(5):597-608.
40. Ma L, Chung WK. The role of genetics in pulmonary arterial hypertension. *J Pathol*. 2017;241(2):273-280.
41. Allanore Y, Simms R, Distler O, et al. Systemic sclerosis. *Nat Rev Dis Primers*. 2015;1:15002.
42. Wipff J, Kahan A, Hachulla E, et al. Association between an endoglin gene polymorphism and systemic sclerosis-related pulmonary arterial hypertension. *Rheumatology (Oxford)*. 2007;46(4):622-625.
43. Broen JC, Bossini-Castillo L, van Bon L, et al; Spanish Systemic Sclerosis Group. A rare polymorphism in the gene for Toll-like receptor 2 is associated with systemic sclerosis phenotype and increases the production of inflammatory mediators. *Arthritis Rheum*. 2012;64(1):264-271.
44. Dieude P, Guedj M, Wipff J, et al. Association of the TNFAIP3 rs5029939 variant with systemic sclerosis in the European Caucasian population. *Ann Rheum Dis*. 2010;69(11):1958-1964.
45. Manetti M, Allanore Y, Revillod L, et al. A genetic variation located in the promoter region of the UPAR (CD87) gene is associated with the vascular complications of systemic sclerosis. *Arthritis Rheum*. 2011;63(1):247-256.
46. Santaniello A, Salazar G, Lenna S, et al. HLA-B35 upregulates the production of endothelin-1 in HLA-transfected cells: a possible pathogenetic role in pulmonary hypertension. *Tissue Antigens*. 2006;68(3):239-244.
47. Scorza R, Caronni M, Bazzi S, et al. Post-menopause is the main risk factor for developing isolated pulmonary hypertension in systemic sclerosis. *Ann N Y Acad Sci*. 2002;966:238-246.
48. Bondarenko AL, Ustiuzhaninov VN, Vozhegova NP, Strazhnikova GA. [Correlation between HLA antigens with clinical features of mixed infection of hepatitis A and HBV-carriers]. *Zh Mikrobiol Epidemiol Immunobiol*. 2007;(3):30-34.
49. Lenna S, Chrobak I, Farina GA, et al. HLA-B35 and dsRNA induce endothelin-1 via activation of ATF4 in human microvascular endothelial cells. *PLoS One*. 2013;8(2):e56123.
50. Lenna S, Townsend DM, Tan FK, et al. HLA-B35 upregulates endothelin-1 and downregulates endothelial nitric oxide synthase via endoplasmic reticulum stress response in endothelial cells. *J Immunol*. 2010;184(9):4654-4661.
51. Lenna S, Assassi S, Farina GA, et al. The HLA-B*35 allele modulates ER stress, inflammation and proliferation in PBMCs from Limited Cutaneous Systemic Sclerosis patients. *Arthritis Res Ther*. 2015;17:363.
52. Matucci-Cerinic M, Kahaleh B, Wigley FM. Review: evidence that systemic sclerosis is a vascular disease. *Arthritis Rheum*. 2013;65(8):1953-1962.
53. Ong YY, Nikoloutsopoulos T, Bond CP, Smith MD, Ahern MJ, Roberts-Thomson PJ. Decreased nailfold capillary density in limited scleroderma with pulmonary hypertension. *Asian Pac J Allergy Immunol*. 1998;16(2-3):81-86.
54. Shah AA, Wigley FM, Hummers LK. Telangiectases in scleroderma: a potential clinical marker of pulmonary arterial hypertension. *J Rheumatol*. 2010;37(1):98-104.
55. Asano Y, Sato S. Vasculopathy in scleroderma. *Semin Immunopathol*. 2015;37(5):489-500.
56. Kavian N, Batteux F. Macro- and microvascular disease in systemic sclerosis. *Vascul Pharmacol*. 2015;71:16-23.
57. Pattanaik D, Brown M, Postlethwaite AE. Vascular involvement in systemic sclerosis (scleroderma). *J Inflamm Res*. 2011;4:105-125.
58. Trojanowska M. Cellular and molecular aspects of vascular dysfunction in systemic sclerosis. *Nat Rev Rheumatol*. 2010;6(8):453-460.
59. Wigley FM. Vascular disease in scleroderma. *Clin Rev Allergy Immunol*. 2009;36(2-3):150-175.
60. Piera-Velazquez S, Mendoza FA, Jimenez SA. Endothelial to Mesenchymal Transition (EndoMT) in the Pathogenesis of Human Fibrotic Diseases. *J Clin Med*. 2016;5(4).
61. Varga J, Trojanowska M, Kuwana M. Pathogenesis of systemic sclerosis: recent insights of molecular and cellular mechanisms and therapeutic opportunities. *J Scleroderma Relat Disord*. 2017;2(3):137-152.
62. Ranchoux B, Antigny F, Rucker-Martin C, et al. Endothelial-to-mesenchymal transition in pulmonary hypertension. *Circulation*. 2015;131(11):1006-1018.
63. Good RB, Gilbane AJ, Trinder SL, et al. Endothelial to Mesenchymal Transition Contributes to Endothelial Dysfunction in Pulmonary Arterial Hypertension. *Am J Pathol*. 2015;185(7):1850-1858.
64. Mendoza FA, Piera-Velazquez S, Farber JL, Feghali-Bostwick C, Jimenez SA. Endothelial Cells Expressing Endothelial and Mesenchymal Cell Gene Products in Lung Tissue From Patients With Systemic Sclerosis-Associated Interstitial Lung Disease. *Arthritis Rheumatol*. 2016;68(1):210-217.

65. Manetti M, Rosa I, Milia AF, et al. Inactivation of urokinase-type plasminogen activator receptor (uPAR) gene induces dermal and pulmonary fibrosis and peripheral microvasculopathy in mice: a new model of experimental scleroderma? *Ann Rheum Dis*. 2014;73(9):1700-1709.
66. Pullamsetti SS, Perros F, Chelladurai P, Yuan J, Stenmark K. Transcription factors, transcriptional coregulators, and epigenetic modulation in the control of pulmonary vascular cell phenotype: therapeutic implications for pulmonary hypertension (2015 Grover Conference series). *Pulm Circ*. 2016;6(4):448-464.
67. Savai R, Al-Tamari HM, Sedding D, et al. Pro-proliferative and inflammatory signaling converge on FoxO1 transcription factor in pulmonary hypertension. *Nat Med*. 2014;20(11):1289-1300.
68. Courboulin A, Tremblay VL, Barrier M, et al. Kruppel-like factor 5 contributes to pulmonary artery smooth muscle proliferation and resistance to apoptosis in human pulmonary arterial hypertension. *Respir Res*. 2011;12:128.
69. Firth AL, Yao W, Remillard CV, Ogawa A, Yuan JX. Upregulation of Oct-4 isoforms in pulmonary artery smooth muscle cells from patients with pulmonary arterial hypertension. *Am J Physiol Lung Cell Mol Physiol*. 2010;298(4):L548-L557.
70. Montani D, Lau EM, Dorfmuller P, et al. Pulmonary veno-occlusive disease. *Eur Respir J*. 2016;47(5):1518-1534.
71. Dorfmuller P, Humbert M, Perros F, et al. Fibrous remodeling of the pulmonary venous system in pulmonary arterial hypertension associated with connective tissue diseases. *Hum Pathol*. 2007;38(6):893-902.
72. Wilson GJ, Bunpo P, Cundiff JK, Wek RC, Anthony TG. The eukaryotic initiation factor 2 kinase GCN2 protects against hepatotoxicity during asparaginase treatment. *Am J Physiol Endocrinol Metab*. 2013;305(9):E1124-E1133.
73. Lathen C, Zhang Y, Chow J, et al. ERG-APLNR axis controls pulmonary venule endothelial proliferation in pulmonary veno-occlusive disease. *Circulation*. 2014;130(14):1179-1191.
74. Shah AV, Birdsey GM, Randi AM. Regulation of endothelial homeostasis, vascular development and angiogenesis by the transcription factor ERG. *Vascul Pharmacol*. 2016;86:3-13.
75. Looney AP, Han R, Stawski L, Marden G, Iwamoto M, Trojanowska M. Synergistic Role of Endothelial ERG and FLI1 in Mediating Pulmonary Vascular Homeostasis. *Am J Respir Cell Mol Biol*. 2017;57(1):121-131.
76. Asano Y, Stawski L, Hant F, et al. Endothelial Fli1 deficiency impairs vascular homeostasis: a role in scleroderma vasculopathy. *Am J Pathol*. 2010;176(4):1983-1998.
77. Thistlethwaite PA. Linking Vascular Remodeling and Inflammation in Pulmonary Arterial Hypertension: Is There a Common Root Cause? *Am J Respir Cell Mol Biol*. 2017;57(1):15-17.
78. Hogan NT, Whalen MB, Stolze LK, et al. Transcriptional networks specifying homeostatic and inflammatory programs of gene expression in human aortic endothelial cells. *Elife*. 2017;6.
79. Ghatnekar A, Chrobak I, Reese C, et al. Endothelial GATA-6 deficiency promotes pulmonary arterial hypertension. *Am J Pathol*. 2013;182(6):2391-2406.
80. Voelkel NF, Cool C, Lee SD, Wright L, Geraci MW, Tuder RM. Primary pulmonary hypertension between inflammation and cancer. *Chest*. 1998;114(3 Suppl):225S-230S.
81. Paulin R, Michelakis ED. The metabolic theory of pulmonary arterial hypertension. *Circ Res*. 2014;115(1):148-164.
82. D'Alessandro A, El Kasmi KC, Plecita-Hlavata L, et al. Hallmarks of Pulmonary Hypertension: Mesenchymal and Inflammatory Cell Metabolic Reprogramming. *Antioxid Redox Signal*. 2017 Aug 14. [Epub ahead of print]
83. Ryan J, Dasgupta A, Huston J, Chen KH, Archer SL. Mitochondrial dynamics in pulmonary arterial hypertension. *J Mol Med (Berl)*. 2015;93(3):229-242.
84. Li M, Riddle S, Zhang H, et al. Metabolic Reprogramming Regulates the Proliferative and Inflammatory Phenotype of Adventitial Fibroblasts in Pulmonary Hypertension Through the Transcriptional Corepressor C-Terminal Binding Protein-1. *Circulation*. 2016;134(15):1105-1121.
85. Dong G, Mao Q, Xia W, et al. PKM2 and cancer: The function of PKM2 beyond glycolysis. *Oncol Lett*. 2016;11(3):1980-1986.
86. Dooley A, Bruckdorfer KR, Abraham DJ. Modulation of fibrosis in systemic sclerosis by nitric oxide and antioxidants. *Cardiol Res Pract*. 2012;2012:521958.
87. Gabrielli A, Svegliati S, Moroncini G, Amico D. New insights into the role of oxidative stress in scleroderma fibrosis. *Open Rheumatol J*. 2012;6:87-95.
88. Sambo P, Baroni SS, Luchetti M, et al. Oxidative stress in scleroderma: maintenance of scleroderma fibroblast phenotype by the constitutive up-regulation of reactive oxygen species generation through the NADPH oxidase complex pathway. *Arthritis Rheum*. 2001;44(11):2653-2664.
89. Sadamura-Takenaka Y, Ito T, Noma S, et al. HMGB1 promotes the development of pulmonary arterial hypertension in rats. *PloS One*. 2014;9(7):e102482.
90. Servettaz A, Guilpain P, Goulvestre C, et al. Radical oxygen species production induced by advanced oxidation protein products predicts clinical evolution and response to treatment in systemic sclerosis. *Ann Rheum Dis*. 2007;66(9):1202-1209.
91. Kharitonov SA, Cailes JB, Black CM, du Bois RM, Barnes PJ. Decreased nitric oxide in the exhaled air of patients with systemic sclerosis with pulmonary hypertension. *Thorax*. 1997;52(12):1051-1055.
92. Malerba M, Radaeli A, Ragnoli B, et al. Exhaled nitric oxide levels in systemic sclerosis with and without pulmonary involvement. *Chest*. 2007;132(2):575-580.
93. Dimitroulas T, Giannakoulas G, Sfetsios T, et al. Asymmetrical dimethylarginine in systemic sclerosis-related pulmonary arterial hypertension. *Rheumatology (Oxford)*. 2008;47(11):1682-1685.
94. Deidda M, Piras C, Cadeddu Dessalvi C, et al. Distinctive metabolomic fingerprint in scleroderma patients with pulmonary arterial hypertension. *Int J Cardiol*. 2017;241:401-406.
95. Lajoie AC, Bonnet S, Provencher S. Combination therapy in pulmonary arterial hypertension: recent accomplishments and future challenges. *Pulm Circ*. 2017;7(2):312-325.
96. Tebay LE, Robertson H, Durant ST, et al. Mechanisms of activation of the transcription factor Nrf2 by redox stressors, nutrient cues, and energy status and the pathways through which it attenuates degenerative disease. *Free Radic Biol Med*. 2015;88(Pt B):108-146.
97. Reata's Bardoxolone Methyl as PAH Therapy Shows Promise in Initial Phase 2 Data. *Pulmonary Hypertension News*. https://pulmonaryhypertensionnews.com/2015/10/27/reatas-bardoxolone-methyl-pah-therapy-shows-promise-initial-phase-2-data/. Accessed September 2017.
98. Lee DH, Gold R, Linker RA. Mechanisms of oxidative damage in multiple sclerosis and neurodegenerative diseases: therapeutic modulation via fumaric acid esters. *Int J Mol Sci*. 2012;13(9):11783-11803.
99. Grzegorzewska AP, Seta F, Han R, et al. Dimethyl Fumarate ameliorates pulmonary arterial hypertension and lung fibrosis by targeting multiple pathways. *Sci Rep*. 2017;7:41605.
100. Dimethyl Fumarate (DMF) in Systemic Sclerosis-Associated Pulmonary Arterial Hypertension. TrialBulletin.com. https://trialbulletin.com/lib/entry/ct-02981082. Accessed September 2017.