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Converging Paths of Pulmonary Arterial Hypertension and Cellular Senescence

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Abstract

Cellular senescence is recognized as a crucial contributor to the pathobiology of various degenerative and cardiovascular diseases, such as idiopathic pulmonary fibrosis and atherosclerosis. We describe the potential link between cellular senescence and the degenerative character of neointimal pulmonary vascular disease in pulmonary arterial hypertension (PAH). Senescence markers have been described in remodeled pulmonary arteries, and PAH and senescence share common triggers and pathogenic pathways, such as transforming growth factor-β/bone morphogenetic protein and TNF-α. In addition, interventions that target a senescence phenotype also target pulmonary vascular remodeling *in vivo*. These data provide a basis for further exploration of the role of senescence in the pathobiology of PAH and for preclinical trials with a senolytic class of drugs.

Keywords: pulmonary arterial hypertension; cellular senescence; senescence-associated secretory phenotype; senolysis; vascular remodeling

Pulmonary arterial hypertension (PAH) is a severe arteriopathy in which a characteristic form of neointimal vascular remodeling progressively occludes the pulmonary arteries. The vascular lesions observed in the various PAH etiologies typically consist of dysfunctional endothelial cells (EC) and smooth muscle cells (SMC) that have formed a neointima and a hypertrophied media. In severe lesions, the elastic laminae are disintegrated, and the vessel wall and perivascular area are inflamed and fibrotic (1). No drugs yet exist in the clinic that can reverse this sclerotic end-stage vascular phenotype, which is commonly referred to as irreversible (2). Primary pathologic features of vascular remodeling in PAH are hyperproliferation and apoptosis resistance of vascular cells, accompanied by the expression of an extensive profile of proinflammatory cytokines (3). Recently, a role for DNA damage has also been established (4). Combined, these mechanisms could also indicate a process of cellular senescence that has thus far remained relatively unexplored in the context of PAH (5). In the last decade, a fundamental role has been established for senescence in the pathogenesis of many degenerative and cardiovascular diseases, such as idiopathic pulmonary fibrosis (IPF) (6), chronic obstructive pulmonary disease (COPD) (5, 7–9), and atherosclerosis (10), which all share many parallels with the pathogenesis of PAH. In this review, we discuss the potential role of cellular senescence in PAH as a pathologic contributor to neointimal vascular remodeling and as a novel target for treatment.

Senescence and the Senescence-associated Secretory Phenotype

Cellular senescence describes the transition of a cell, induced by various forms of cellular stress, from a (potentially) proliferative or regenerative state into permanent, irreversible growth arrest (11). This transition is typically accompanied by context- and cell type–dependent phenotypic changes in morphology, chromatin structure, and secretome (12). Senescent cells also upregulate specific senescent cell antiapoptotic pathways such as survivin and Bcl2 (13), which makes them resistant to the proapoptotic environment in which they usually occur. The development of senescence is essentially regulated by the tumor

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suppressor protein p53 and the cell cycle inhibitors $p16^{ink4A}$ and $p21^{cip1}$ (14); however, various senescence-inducing programs are being identified (11). Senescence occurs in biological aging and is associated with many degenerative diseases of the elderly, such as Parkinson's disease, arthrosis, and IPF (6). Specific triggers for cellular senescence, some of which are not necessarily associated with old age, include replicative stress; telomere attrition; oncogene activation; and prolonged exposure to γ-irradiation, reactive oxygen species (ROS), or inflammation (15). The common denominator of these pathogenic triggers for senescence is that they are all genotoxic (i.e., converge to DNA damage) (15). Still, the occurrence of senescence in response to DNA damage is primarily physiologic. In cancer, senescence is recognized as an important tumor-suppressive mechanism that protects cells with accumulating DNA damage from further proliferating (12). Yet, the presence of senescent cells in tumors was also linked to a proinflammatory senescence-associated secretory phenotype (SASP) that paradoxically seemed to promote tumor progression and tissue degeneration and contributed to chemotherapy resistance (16–18). This implied that senescent cells, although unable to replicate, can still be metabolically active and, as such, influence their microenvironment in a cell-nonautonomous fashion (19). The SASP has since been identified as a critical driver of tissue degeneration and functional decline in various other senescence-associated pathologies (15). A SASP typically consists of numerous interleukins (e.g., IL-1α/IL-1β, IL-6, IL-8), cytokines and chemokines (e.g., TNF-α, monocyte chemoattractant protein 1), proteases (e.g., matrix metalloproteinases, cathepsins), and angiogenic growth factors (e.g., vascular endothelial growth factor, PAI-1). The exact secretome, however, is dynamic and depends on cell type, cause of senescence, microenvironment, and the biological pathway by which the SASP is activated (19). Activation of the SASP can be regulated by transcription factors, such as NF-κB, mTOR, and GATA4 (GATA-binding protein 4), or by epigenetic "super enhancers," such as BRD4 (bromodomain-containing protein 4) and HMGB2 (high-mobility group protein B2), which reorganize (open up) the chromatin structure to facilitate transcription of SASP genes (19). Combined, these data have established a role for senescent cells and the SASP as a driver for disease, which has also made these cells an opportune target for intervention in degenerative pathologies. Local clearance of senescent cells has, in multiple degenerative disease models, already been shown to attenuate disease progression by decreasing the SASP and creating a proregenerative environment (20–22).

Vascular Senescence

Aging is a risk factor for the vast majority of cardiovascular diseases, and many cardiovascular diseases are, *vice versa*, associated with premature vascular aging and vascular cell senescence (23). Typical age-related changes in the systemic vasculature include endothelial dysfunction, intimal thickening, medial hypertrophy, increased collagen deposition, and fractured elastin (23). In atherosclerosis especially, it is becoming evident that senescent cells play a deleterious role in disease development and progression (24). The principal risk factors for atherosclerosis are biological aging, smoking, and diabetes. Accumulating DNA damage and vascular cell senescence are most likely a consequence of these risk factors due to a combination of telomere attrition, oxidative stress, and glycation (10). During the development of atherosclerosis, senescent EC and SMC were shown to accumulate particularly in areas of plaque formation (25). Senescent ECs in these lesions produce less nitric oxide and prostacyclin, and both EC and SMC develop a proinflammatory, profibrotic SASP (26). Altogether, this leads to a dysfunctional vessel that is constricted and intrinsically inflamed, which further exacerbates plaque formation, creates a prothrombotic milieu, and leads to stiffening and systemic hypertension (23). Senescent EC also lose their ability to form new vessels and seem to impair normal angiogenesis in their vicinity (27). The hypothesis that DNA damage–induced vascular senescence may cause the development and progression of arteriopathies (rather than being a consequence of preexistent vascular disease) is underscored by rapid-aging syndromes such as Hutchinson-Gilford progeria syndrome (HGP) (28). In HGP, a mutation in laminin A compromises normal DNA repair and mitosis, which rapidly leads to a senescence phenotype with thorough effects on the cardiovascular system. These patients usually die of cardiovascular events during adolescence. The vascular morphology of patients with HGP is characterized by fibrotic acellular neointimal lesions, extensive adventitial fibrosis, and diffuse inflammation throughout the vessel wall (29). The senescence-associated cellular and vascular abnormalities found in atherosclerosis or biological and premature aging in the systemic vasculature are in many ways similar to those found in the pulmonary vasculature in PAH.

A Role for Senescence in PAH: Conceptual Model and First Observations

PAH is a progressive arteriopathy. The end-stage pulmonary vascular phenotype is characterized by neointimal formation; intimal, medial, and adventitial fibrosis; degradation of the internal elastic laminae; and perivascular inflammation. These culminate in the manifestation of plexiform lesions, the hallmark of irreversible PAH (3). The occurrence of this progressive, plexogenic type of vascular remodeling has been associated with various pathogenic triggers. These include mutations in the BMPR2 (bone morphogenetic protein receptor 2) pathway, autoimmune diseases, schistosomiasis or human immunodeficiency virus infection, alkylating chemotherapy or anorexigens, increased blood flow and pressure like in congenital heart disease (CHD), and irradiation (30). A common denominator for these triggers is that they induce a form of genotoxic stress that causes accumulating DNA damage to vascular cells (31).

The evidence for an early role of DNA damage in the pathology of PAH has been growing over recent years (4, 32, 33) (Figure 1C). The two main cellular responses to DNA damage beyond repair are *1*) apoptosis and *2*) survival with genomic abnormalities (13), which may happen simultaneously in the situation of genotoxic stress. This phenomenon is also observed in pulmonary EC treated with Sugen 5416, a compound used for the induction of PAH in rats (34, 35).

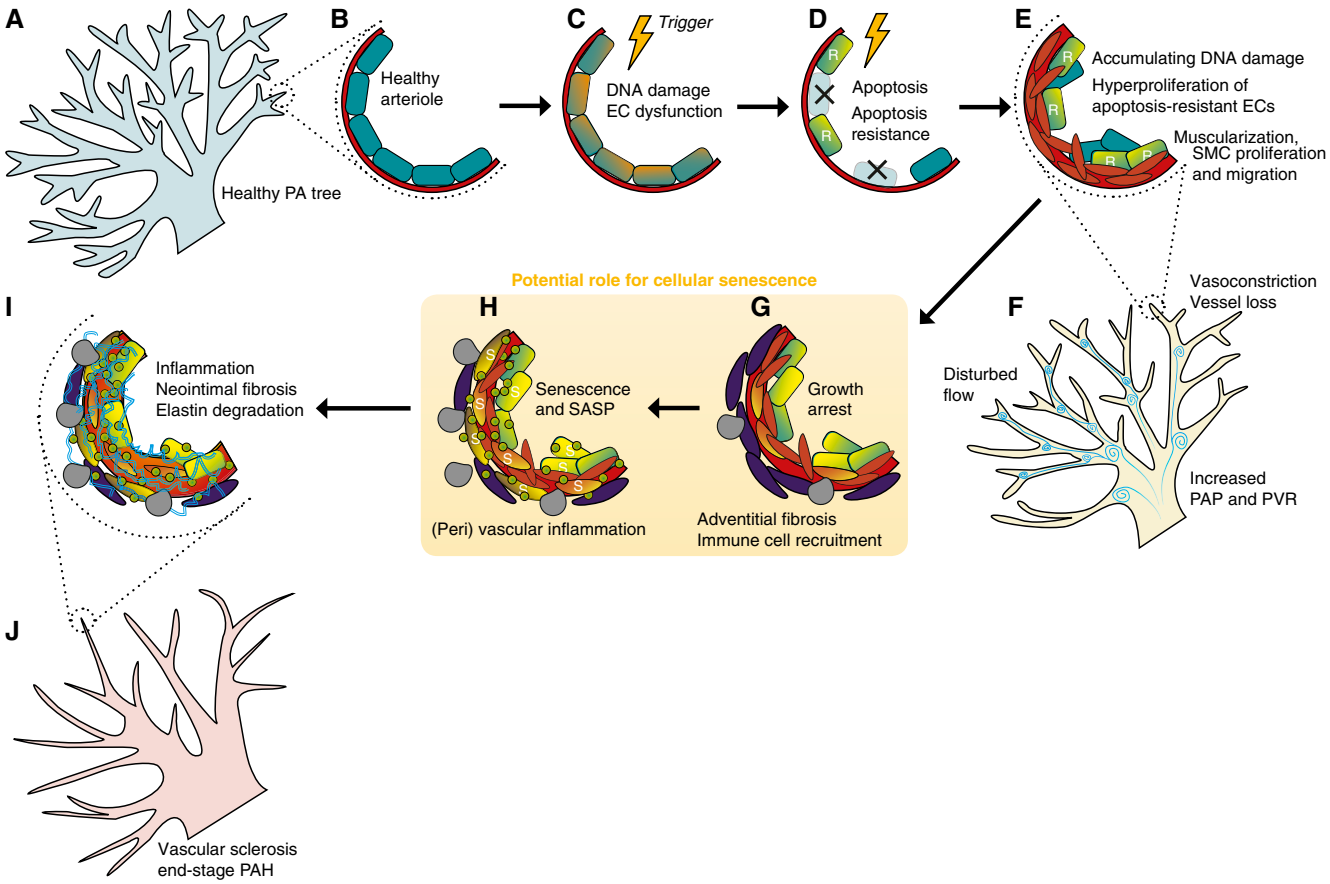

Figure 1. Conceptual representation of the potential role of cellular senescence in the progression of vascular remodeling in pulmonary arterial hypertension (PAH). (*A* and *B*) Healthy pulmonary vasculature. (*C*) PAH-associated trigger induces DNA damage and endothelial cell (EC) dysfunction. (*D*) When the trigger persists, DNA damage accumulates that can lead to apoptosis in the one cell or apoptosis resistance in the other. (*E*) Apoptosis-resistant cells with accumulating DNA damage hyperproliferate, leading to intimal hyperplasia, the media becomes muscularized, and smooth muscle cells (SMCs) proliferate and migrate into the intima. (*F*) EC dysfunction leads to vasoconstriction via reduced NO bioavailability; excessive vascular cell death causes loss of microvasculature and vascular remodeling stiffens the vessel wall. This disturbs local flow patterns and increases pulmonary arterial pressures (PAP) and pulmonary vascular resistance (PVR). (*G*) Hyperproliferation and accumulating DNA damage induces growth arrest. Inflammatory cells are recruited to the vessel. The adventitia becomes fibrotic. (*H*) Vascular cells become senescent and acquire a senescence-associated secretory phenotype, which promotes (*I*) inflammation, degradation, and fibrosis, leading to (*J*) a sclerotic end-stage. PA = pulmonary artery; peri = perivascular; R = apoptosis-resistant cell; SASP = senescence-associated secretory phenotype.

Widespread EC and SMC apoptosis, caused by inadequately compensated genotoxic stress, could thus lead to the vascular remodeling and rarefaction (Figure 1F) that are observed already early in the development of PAH (36, 37) (Figure 1D). Increased proliferation in this case could be viewed as a physiologic response of the surviving cells trying to restore the injured vasculature. Survival of cells (resistant to apoptosis) with genomic abnormalities, however, is also known to provoke a cancer-like hyperproliferative phenotype (35) (Figure 1E). Both types of this hyperproliferative response could create a form of replicative or oncogenic stress and are prone to lead to senescence (Figures 1G and 1H). Like in atherosclerosis, senescent cells could contribute to vascular remodeling, degeneration, and sclerosis in PAH (Figure 1J) by *1*) promoting inflammation and fibrosis in and around the vessel wall through the SASP, which inhibits regenerative mechanisms, promotes further senescence, and leads to vascular degeneration; and *2*) physically obstructing the vascular lumen owing to these cells' intrinsic resistance to apoptosis (Figure 1I). p16-Expressing cells have already been described in plexiform lesions in end-stage idiopathic pulmonary arterial hypertension (IPAH) (9, 38). We have summarized in the following sections further evidence for a role of senescence in PAH:

1. Triggers that induce senescence also induce PAH.
2. Pulmonary vascular remodeling occurs in chronic lung diseases associated with cellular senescence.
3. Pathways disturbed in PAH are also causally associated with senescence (*see also* Figure 2).
4. Interventions that target senescence also target pulmonary vascular remodeling *in vivo*.

Direct Triggers of Premature Senescence Also Induce PAH

In experimental settings, common methods to induce senescence are through

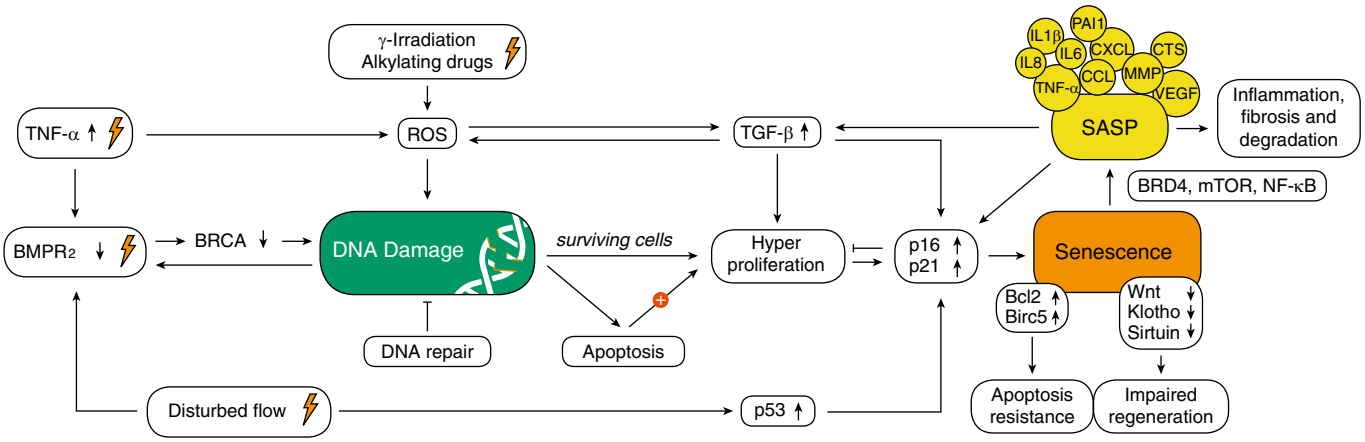

Figure 2. Various triggers associated with pulmonary arterial hypertension: increased TNF-α, reduced BMPR2 (bone morphogenetic protein receptor 2), γ-irradiation, and alkylating chemotherapy lead to DNA damage via increased reactive oxygen species (ROS) or reduced DNA damage repair signaling mediated by the BRCA family. Disturbed flow can lead to DNA damage via reduced BMPR2 and can upregulate the p53–p21 axis. Unrepaired DNA damage leads to either apoptosis or hyperproliferation of apoptosis-resistant cells. Increased apoptosis can also stimulate proliferation. Hyperproliferation leads to induction of the senescence genes p16 and p21. Senescence is characterized i.a. by persistent upregulation of apoptosis resistance genes such as Bcl2 or Birc5 (survivin) and downregulation of proregenerative pathways. After induction of senescence, epigenetic regulators such as BRD4 (bromodomain-containing protein 4), mTOR, and NF-κB induce the SASP, which stimulates further senescence, and inflammation, fibrosis, and degeneration. Birc5 = baculoviral inhibitor of apoptosis repeat containing 5; BRCA = breast cancer; CTS = cathepsins; MMP = matrix metalloproteinase; TGF-β = transforming growth factor-β; VEGF = vascular endothelial growth factor.

γ-irradiation and certain classes of drugs. Irradiation and these same drug classes have also been causally associated with PAH in the clinic.

γ-Irradiation

High-volume, high-dose γ-irradiation of the thorax for lung cancer, for example, can lead to radiation pneumonitis, resulting in serious complications or death (39). A rat study revealed that γ-irradiation also affects the small pulmonary arterioles, which were characterized by medial hypertrophy, adventitial fibrosis, and obliterative neointimal lesions with low cellularity, as well as by loss of capillaries. This effect was dose dependent, with the highest thoracic volume exposures even causing increased mean pulmonary artery pressure and right ventricular hypertrophy (40). The fact that γ-irradiation, one of the most straightforward inducers of senescence, also leads to neointimal remodeling reinforces the hypothesis that these mechanisms could also be causally involved in PAH development or progression.

Drugs Associated with PAH and Senescence

Drugs known to cause PAH in the clinical setting include anorexigens, amphetamines, alkylating chemotherapeutics such as cyclophosphamide and mitomycin C, and the tyrosine kinase inhibitor dasatinib (41). The pyrrolizidine alkaloid monocrotaline (MCT) and the vascular endothelial growth factor inhibitor Sugen 5416 are the most common substances used to induce vascular remodeling in preclinical models for PAH. All these drugs are also known to induce senescence via oxidative stress and ROS, leading to DNA damage and apoptosis (42). The alkylating agent mitomycin C is associated with senescence in lung cancer and is used to induce senescence *in vitro* (43). Treatment with MCT in dogs was associated with senescence of the endothelial progenitor cell population and an overall decrease in endothelial progenitor cell number (44), indicating a role for MCT-induced senescence in reduced capacity for vascular regeneration or repair. Low-dose dasatinib combined with the flavonoid quercetin can also serve as a senolytic, selectively killing senescent cells (6). Conversely, higher doses were shown to induce DNA damage leading to p21-dependent senescence intraarterially in non–small cell lung cancer cells (45). In rats, high-dose dasatinib treatment exaggerates the development of PAH induced by MCT or chronic hypoxia by aggravating EC dysfunction and by inducing apoptosis via increased ROS (46). In patients with chronic myeloid leukemia, high-dose dasatinib treatment can indeed lead to fatal PAH (47). However, reversible courses after early discontinuation of the drug have also been described (48).

Pulmonary Vascular Remodeling in Chronic Lung Diseases Associated with Senescence

A central and causal role for cellular senescence has been established in chronic lung diseases such as IPF and COPD (6, 49), both of which also predispose to vascular remodeling and pulmonary hypertension (PH) (5). This form of PH (classified as World Health Organization group 3) is usually less severe than group 1 PAH, and it is potentially reversible with treatment of the underlying disease. Still, the vascular phenotype in chronic lung disease PH resembles many of the features found in PAH during the earlier stages of disease development (2). Patients with COPD show increased pulmonary vascular p21 and p16 expression compared with control patients, and SMC isolated from patients with COPD show accelerated development of senescence and evidence of an SASP in culture (7). Interestingly,

this SASP seemed to stimulate hypertrophy, hyperplasia, and migration of nonsenescent SMC in culture (7), all of which could contribute to vascular remodeling in PH and PAH (5).

Common Pathways That Lead to Senescence and PAH

Biological processes fundamentally associated with the development of PAH, such as transforming growth factor-β (TGF-β)–BMPR2 imbalance, increased TNF-α and mTOR signaling, impaired vascular regenerative mechanisms, and pathways induced in disturbed flow conditions, are also associated with cellular senescence (*see also* Figure 2).

TGF-β–Bone Morphogenetic Protein Imbalance in PAH and Senescence

Mutations in the TGF-β/BMP (bone morphogenetic protein) pathway lead to the hereditary form of PAH, and TGF-β–BMP signaling imbalance is a central feature of all other PAH etiologies as well. TGF-β–BMP imbalance is therefore widely regarded as the primary instigator for PAH development. Still, pharmacologic restoration of the imbalance has not yet led to impressive reversal of vascular remodeling in preclinical trials (50–52), and variable results have been obtained thus far in the treatment of patients with end-stage PAH (53). We therefore hypothesize that prolonged TGF-β–BMP imbalance (perhaps accompanied by a second hit) induces DNA damage and senescence that, beyond a certain window, cannot be reversed by restoring TGF-β/BMP signaling alone.

The role of TGF-β in senescence may be both causal and consequential (54). In cancer, TGF-β is recognized as an important tumor suppressor capable of causing senescence through direct interaction with p16 and p21 (55) and indirectly by exacerbating ROS production in a positive feedback loop via the NOX (nicotinamide adenine dinucleotide phosphate, reduced form, oxidase) genes (56). Increased TGF-β can also be a consequence of senescence, however, because senescent cells are known to secrete TGF-β as part of the SASP (16). The TGF-β component of the SASP is recognized as an important mediator of fibrosis in age-related diseases such as IPF (6).

BMPR2 downregulation has not yet been linked directly to senescence. BMPR2 loss in PAH has, however, been specifically associated with abnormal DNA damage and repair. Pulmonary EC with reduced BMPR2 are more susceptible to DNA damage (32), possibly because BRCA1, a DNA repair gene and protector against genotoxic stress, is concomitantly reduced in these ECs (33). *Vice versa*, DNA damage was also shown to induce BMPR2 downregulation itself, suggesting a vicious cycle of progressively impaired DNA repair (33). One could hypothesize that the interplaying vicious cycles of BMPR2 loss and DNA damage on the one hand, and of TGF-β and ROS on the other, are highly implicated to lead to senescence, especially when a second genotoxic hit is present.

Antiregenerative Pathways in PAH and Senescence

The potential to regenerate declines with age, and deactivation of pathways involved in regeneration, such as Klotho, sirtuin, or Wnt, is associated with the development of premature senescence (57). Klotho deficiency in mice leads to changes in the systemic vasculature similar to those observed in biological aging, such as medial hypertrophy and calcification, EC dysfunction, and neointimal hyperplasia (58). Klotho expression is also decreased in MCT-PAH rats, whereas intravenous delivery of mesenchymal stem cells that overexpress Klotho was shown to prevent vascular remodeling, potentially by increasing sirtuin 1 expression (59). Indeed, sirtuins are recognized as a protective factor in vascular aging (60) and cardiovascular disease (61), and they are also downregulated in PAH (62). Deactivation of Wnt signaling is also associated with many degenerative diseases, including Alzheimer's disease and atherosclerosis (63), as well as with the onset of cellular senescence (64). In the lung, proangiogenic Wnt signaling is essential for vascular homeostasis and regeneration in response to injury (63). Stimulation of vascular regeneration by activating Wnt signaling was therefore proposed as a therapeutic strategy in PAH (63). However, experimental studies aimed at restoration of Wnt have yet to be conducted. This delay may be due to the observation that Wnt signaling is in fact increased in plexiform lesions of patients with PAH (65), which could reflect the process of disorganized neoangiogenesis that is believed to lead to plexus channel formation, characteristic of the plexiform lesion (66).

TNF-α in PAH and Senescence

Inflammatory diseases known to cause PAH, such as schistosomiasis, human immunodeficiency virus, systemic sclerosis, and systemic lupus erythematosus, are all associated with increased concentrations of TNF-α, a key inflammatory mediator (67). Recent evidence indicates that increased TNF-α directly promotes pulmonary vascular remodeling by reducing BMPR2 signaling (68). Transgenic mice that overexpress TNF-α in the lung spontaneously develop PAH (69). Therefore, TNF-α is recognized as a critical trigger for PAH (70). Prolonged exposure of TNF-α is also known to induce senescence and an SASP in fibroblasts (71) and EC via increased ROS and activation of the NF-κB pathway (72). In systemic sclerosis, TNF-α–associated EC and fibroblast senescence is recognized to exacerbate the progression of liver and kidney fibrosis (73). The occurrence of PAH in systemic sclerosis could arise in a similar way.

mTOR Signaling in COPD-PH, PAH, and Senescence

The mTOR pathway, an intracellular pathway involved in cell cycle regulation, is also involved in the induction of cellular senescence and the SASP (74). Recent evidence shows that the mTOR pathway is upregulated in EC, SMC, and epithelial cells of patients with COPD, together with markers for senescence (8). mTOR signaling was amplified in transgenic mice to confirm a causal role of mTOR-induced senescence in COPD-PH. This upregulated the expression of p16 and p21 and a cytokine profile suggestive of an SASP, as well as a COPD-like pulmonary phenotype with mild elevation in mean pulmonary pressure and medial hypertrophy of the distal pulmonary arterioles. Increased mTOR signaling is also found in the pulmonary arteriolar SMC of patients with IPAH, in whom it was shown to stimulate apoptosis resistance and vascular remodeling (75). A relationship with senescence, however, was not assessed. mTOR inhibition by various drugs (including rapamycin, known to decelerate the development of senescence

Table 1. Overview of Different Types of Senotherapy, Some of Which Have Been Evaluated in the Context of Pulmonary Arterial Hypertension

Drug (Class)	Mode of Action	Data of Trials in PAH*
Geroprotectors (96)		
Any drug that prevents disruption of normal homeostasis, such as: Antidiabetics Antihypertensives Lipid-lowering drugs Antioxidants Preventers of telomere shortening	Decelerate the development of senescence or aging phenotypes and prevent genotoxic stress or DNA damage	Various examples, such as: (C) Vasodilatory therapy decelerates the progression of PAH (97). (P) Antioxidant treatment attenuates the development of PAH in MCT rats (98). (C + P) Metformin prevents MCT PAH (92), and improved PVR in patients with PAH-CHD (93).
SASP inhibitors (88, 99, 100)		
mTOR inhibitors, such as: Rapamycin Everolimus	Suppression of the SASP	(C) Everolimus decreased PVR in 8 of 10 patients with IPAH (82). (P) Rapamycin attenuated the development of PAH in SuHx and MCT rats (78–80).
NF-κB inhibitors: Metformin Elafin	Suppression of the SASP	(C + P) Metformin prevents MCT PAH (92) and improved PVR in patients with PAH-CHD (93). (P) Elafin reverses SuHx PAH (51).
p38 MAPK inhibitors: GS-444217 SB203580 BIRB 796	Suppression of the SASP	(P) GS-444217 attenuates the development of PAH in SuHx and MCT rats by decreasing inflammation via p38 MAPK (101).
BRD4 inhibitors: JQ1 Apabetalone/Rvx208	Prevents/reduces the induction of the SASP	(P) JQ1 reverses PAH in SuHx rats by decreasing apoptosis resistance and inflammation (89).
Glucocorticoids, such as: Dexamethasone	Suppression of the SASP	(P) Dexamethasone reversed PH in MCT rats, associated with decreased IL-6 (102).
Statins, such as: Simvastatin	Reduces expression of cytokines, such as IL-6, IL-8, and MCP-1	(C) No significant effect of statin therapy in patients with PAH (meta-analysis) (103).
IL1-α/β (receptor) inhibitors: Anakinra Canakinumab Rilonacept	Neutralize the effects of secreted IL-1α or IL-1β	(P) Anakinra reversed PH in MCT rats and decreased inflammation (104).
IL-6 inhibitors: Siltuximab Tocilizumab	Neutralize the effects of secreted IL-6	(P) Monoclonal antibody against IL-6 receptor attenuated the development of PH in hypoxia (105).
TNF-α inhibitors: Adalimumab Infliximab Etanercept	Neutralize the effects of secreted TNF-α. Reduce secretion of SASP components (e.g., IL-6, IL-1α)	(C) Infliximab reduced pulmonary artery pressures in one patient with systemic sclerosis–associated PAH (106).
Senolytics (88)		
Dasatinib + quercetin	Targets multiple antiapoptotic pathways. Induces senescent cell apoptosis	(C) High-dose dasatinib led to fatal PAH in patients with myeloid leukemia (47). (P) High-dose dasatinib treatment aggravates MCT and hypoxic PAH (46).
Bcl inhibitors such as: Navitoclax (Abt263) Abt737	Inhibits Bcl2/BclXl antiapoptotic pathways. Induces senescent cell apoptosis	No data in PAH.
UBX0101	Stimulates p53-mediated apoptosis of senescent cells	No data in PAH.
HSP90 inhibitors	Induces apoptosis in senescent cells	(P) Improved vascular remodeling and reduced inflammation in MCT-PAH by inducing apoptosis (92).
DRI-FOXO4	Stimulates p53-mediated apoptosis of senescent cells	No data in PAH.
Immunotherapy (88)		
CAR T cells	Patient-derived cytotoxic T cells targeted at senescent cells	No data in PAH.
PDL1 inhibitors	*Hypothetical*: PDL1 may suppress $CD4^+$ T cell–mediated clearance of senescent cells, like that observed in cancer cells (107)	No data in PAH.

Definition of abbreviations: BRD4 = bromodomain-containing protein 4; C = clinical study; CAR = chimeric antigen receptor; CHD = congenital heart disease; IPAH = idiopathic pulmonary arterial hypertension; MAPK = mitogen-activated protein kinase; MCP-1 = monocyte chemoattractant protein 1; MCT + Shunt rats = progressive PAH induced by monocrotaline and an aortocaval shunt; MCT rats = a milder form of PAH induced by monocrotaline; P = preclinical study; PAH = pulmonary arterial hypertension; PDL1 = programmed death ligand 1; PVR = pulmonary vascular resistance; SASP = senescence-associated secretory phenotype; SuHx rats = progressive PAH induced by Sugen 5416 and hypoxia.
Note: None of the studies in pulmonary hypertension/PAH investigated the effect of the treatments in relation to senescence or the SASP.
*None of the trials has assessed the drugs in direct relation to senescence or SASP.

[76] and reduce the SASP [77]) has been shown to attenuate the development of vascular remodeling and improve hemodynamics in multiple PAH animal models, but it has thus far not proved effective in reversing the disease (75, 78–80). Everolimus was the first mTOR inhibitor evaluated in a small trial of end-stage human PAH (81). Although a mean decrease of 31% in pulmonary vascular resistance was observed in 8 of 10 patients after 6 months (81), no follow-up trial has been planned, perhaps owing to a report of severe everolimus-induced pulmonary toxicity published that same year (82).

Osteopontin in COPD-PH, PAH, and Senescence

Senescent pulmonary SMC were recently shown to secrete high concentrations of osteopontin as a key component of their SASP (9). Osteopontin expression was also increased in the pulmonary arterioles of patients with COPD-PH and patients with IPAH (9, 83), as well as in patients with advanced but not early PAH associated with CHD (PAH-CHD) (84). Osteopontin, secreted by senescent SMC, was shown to stimulate vascular remodeling by promoting SMC growth and migration in their vicinity (9). Aged mice with an osteopontin knockout did not develop medial hypertrophy in response to chronic hypoxia, and they had significantly lower vascular expression of p16 and p21 than the aged wild-type mice (9). These data indicate that osteopontin stimulates age-driven vascular remodeling and may further promote a prosenescence vascular phenotype. It remains to be determined whether osteopontin plays a similar role in PAH, which typically affects a younger patient group.

Disturbed Flow in PAH and Senescence

In PAH-CHD, increased flow due to left-to-right shunting is regarded as the essential trigger for neointimal vascular remodeling (85). In the other etiologies of PAH, in which pulmonary flow is initially normal, neointimal lesions still typically occur at branching points where flow is most likely to be disturbed (85). Studies in atherosclerosis have shown a direct relationship between disturbed flow and EC senescence in plaques (86), which may explain the focal nature of these plaques in the systemic vasculature (87). *In vitro*, disturbed flow alone is able to induce full p21-mediated EC senescence. *In vivo*, disturbed flow alone induced a moderate senescence response, but the process was strongly exacerbated when the vasculature was sensitized by an inflammatory trigger (TNF-α) (86).

Senotherapy in PAH

The pharmacologic arsenal used to target cellular senescence or aging phenotypes, senotherapy collectively, has expanded greatly in recent years. The three contemporary classes of senotherapy are *1*) geroprotectors, comprising drugs that prevent DNA damage and senescence by reducing oxidative and other genotoxic stressors or by protecting normal homeostasis; *2*) SASP inhibitors, comprising drugs that reduce the proinflammatory phenotype of senescent cells, such as mTOR inhibitors and NF-κB or BRD4 inhibitors; and *3*) senolytics, comprising drugs that selectively induce apoptosis in senescent cells by targeting (anti)apoptotic pathways. Gene therapies and immunotherapies are being explored as well (88). Table 1 provides an overview of the various classes of drugs that have been developed as senotherapies or were proven to have geroprotective, SASP-modulating or senolytic effects. Some of these drugs have already been evaluated in the context of PAH. However, none of the trials in PAH investigated the effect of the treatments in direct relation to senescence or the SASP. A number of drugs that had positive effects in preclinical trials for PAH, have now also been identified as potent SASP inhibitors. The BRD4 inhibitor JQ1, for instance, significantly improved pulmonary hemodynamics and vascular remodeling in Sugen 5416/hypoxia PAH rats (89). Later, BRD4 was also indicated as an essential regulator for the SASP in senescent cancer cells (90). Metformin inhibits the SASP by reducing NF-κB signaling (91), which also both prevented experimental PAH (92) and improved pulmonary vascular resistance and 6-minute walking distance in patients with PAH-CHD (93). mTOR inhibitors likewise both are effective in reducing the SASP and attenuate vascular remodeling in different PAH rat models (75, 78–80). Exploration of the SASP phenotypes that are specific to the context of PAH could help to establish a role for these drugs in attenuating vascular remodeling or preventing further vascular degeneration.

One trial has been conducted in PAH using a drug that has also been identified as a senolytic. Heat shock protein 90 inhibitors both delay the development of senescence and eliminate senescent cells selectively (94), and they improve vascular remodeling and reduce inflammation in experimental MCT-PAH (95). An interesting feature of other senolytics, such as ABT-263 or FOXO4-DRI, is that these drugs, besides clearance of senescent cells, seem to stimulate tissue regeneration (20, 22). This could also benefit patients with PAH, in whom vascular occlusion and rarefaction contribute critically to the severity of the disease. Together, these data indicate that senotherapy may have a place in the treatment of PAH and merits further exploration in animal models.

Conclusions

Cellular senescence is recognized as a crucial pathogenic factor in various degenerative and cardiovascular diseases, such as IPF and atherosclerosis. Cumulative evidence indicates that senescence could also contribute to the degenerative character of neointimal pulmonary vascular disease in PAH. These data provide a basis for further exploration of the role of senescence in the pathobiology of PAH and for preclinical trials with senolytic drugs. ■

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