MMPs, inflammation and pulmonary arterial hypertension

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Abstract

Pulmonary arterial hypertension (PAH) is characterised by remodelling of small pulmonary arteries leading to a progressive increase in pulmonary vascular resistance and right ventricular failure [1]. PAH can be idiopathic, familial, or associated with a number of conditions or diseases, such as connective tissue disease. Its prognosis is poor, less than 3 yr from diagnosis. The aetiology of severe unexplained pulmonary hypertension remained largely unknown until a few years ago. The gene underlying familial PAH was identified in 2000, the *BMPR-2* gene. However its mutations are not always present, and it probably does not explained the full scope of the disease. PAH is associated with structural alterations in pulmonary arteries including intimal fibrosis, medial hypertrophy and adventitial changes, pointing towards extracellular matrix remodelling which have raised the question of involvement of the matrix degrading enzymes. Among them, serine proteases, such as plasmina and endogenous vascular elastase (EVE), and matrix metalloproteases have been studied. In experimental models, the three of them are increased. Accordingly, their inhibition has preventing and in some cases therapeutic effects. However it should be stressed that opposite consequence of protease inhibition on PAH can be observed depending on the experimental model, either chronic hypoxia-induced PAH (deleterious) or toxic moncrotalin-induced PAH (positive). In humans, only sparse reports exist, that found increase in the MMP inhibitor, TIMP-1, and MMP-2 expression and decreased collagenase (MMP-1). Inflammation is part of the PAH, and accordingly, protease production is a well known part of the inflammatory response. Answering the question whether protease inhibition might represent a therapeutic option in human PAH is however certainly too early.

Introduction

Pulmonary arterial hypertension (PAH) is characterised by remodelling of the small pulmonary arteries leading to a progressive increase in pulmonary vascular resistance and right ventricular failure [1]. PAH can be idiopathic, familial, or associated with a number of conditions or diseases, such as connective tissue disease, congenital heart disease, portal hypertension, HIV infection, and exposure to toxins

and drugs, including appetite suppressants [1, 2]. Severe unexplained idiopathic pulmonary arterial hypertension (IPAH), previously known as primary pulmonary hypertension, is a rare condition with an estimated incidence of 2–3 per million per year [3]. Its prognosis is poor, less than 3 yr from diagnosis before the advent of modern therapies. More recently, targeted therapy with endothelin receptor antagonists, phosphodiesterase inhibition, and prostanoids have been reported to improve symptoms of breathlessness and, in the case of epoprostenol, survival [4].

In most patients, the condition is believed to evolve over several years, with an initial asymptomatic increase in pulmonary arteriolar reactivity and remodelling. Signs and symptoms appear when the mean pulmonary artery pressure is in the range of 30–40 mmHg at rest (normal is < 20 mmHg). Gradual clinical deterioration occurs when the mean pulmonary artery pressure plateaus 60–70 mmHg and cardiac output progressively declines.

The aetiology of severe unexplained pulmonary hypertension remained largely unknown until a few years ago. Reports of a causal association between appetite-suppressant drugs [5] and the occurrence of severe pulmonary hypertension provided some insight into its pathogenesis. However, the identification of the gene underlying familial PAH (FPAH) in 2000 provided a firm basis for mechanistic studies [6–8]. After localisation of the disease gene to the long arm of chromosome 2 (2q33) [9], two independent groups identified heterozygous germline mutations in the bone morphogenetic protein (BMP) type II receptor (BMPR-II), a receptor for the transforming growth factor (TGF)-β superfamily [7, 8]. Mutations in the *BMPR2* gene have been found in approximately 70% of families [10]. In addition, up to 25% of patients with apparently sporadic IPAH have been found to harbour similar mutations [11].

PAH that occurs in association with collagen vascular disease or congenital left-to-right shunting and may be triggered by appetite suppressants (mainly fenfluramines and aminorex), human immunodeficiency virus (HIV) infection or portal hypertension, is indistinguishable from primary PAH, with regard to clinical course, histopathology and response to treatment. Therefore, a recent World Health Organization-sponsored consensus conference has suggested that the concept of primary PAH be extended to include these conditions and be renamed 'pulmonary arterial hypertension' (PAH).

At present, it remains unknown whether this concept of 'PAH' corresponds to a common pathogenic mechanism. Although understanding of the pathobiological mechanisms underlying IPAH has progressed rapidly over the past few years, it is still unfeasible to classify patients on a pathogenic basis and to define therapeutic approaches accordingly. Current treatments, including continuous infusion of prostaglandin (PG)I_2 and oral endothelin receptor antagonists, probably address downstream manifestations of the disease rather than the central pathogenic mechanisms. The identification of the FPAH gene, bone morphogenetic protein (BMP) receptor (BMPR)-II, and the recognition of central pathobiological abnormalities

associated with IPAH, now provide a unique opportunity to develop a more robust understanding of the disease. In the near future, this should serve to assess new treatments aimed at correcting selective pulmonary vascular remodelling processes, and, simultaneously, to validate the pathophysiological concepts.

Identification of these molecular pathways might also provide insight into the understanding of secondary forms of PAH, including PAH secondary to chronic obstructive lung disease and left heart failure. In these conditions, as well as in persistent PAH in neonates, a genetic predisposition has been suggested. The severity of hypoxia-induced PAH also varies in intensity among individuals. Variations in expression and/or function of candidate genes involved in the process of pulmonary vascular remodelling might therefore improve understanding of secondary forms of PAH and also help to define susceptibility to PAH of various origins.

Pulmonary vascular remodelling

Pulmonary hypertension is associated with structural alterations in pulmonary arteries including intimal fibrosis, medial hypertrophy and adventitial changes [12].

Intimal lesions account for a great part of the reduction of luminal area of small arteries and potentially largely influence the overall pulmonary vascular resistance. Intimal lesions consist of eccentric intima thickening and fibrotic plexiform concentric and angiomatoid lesions. More advanced lesions acquire a fibrotic pattern, with interspersed myofibroblasts and marked accumulation of mucopolysaccharides. These lesions are widely present in explanted lungs of patients with severe PAH, both IPAH or PAH associated with CREST syndrome [13]. Variable degree of eccentric thickening can be seen also in cigarette smoker's lungs [14].

The increase in media thickness occurs by a combination of hypertrophy and hyperplasia of smooth muscle cells together with accumulation of extracellular matrix, including thicker but fragmented elastic laminæ. Fragmentation of elastic laminæ was initially described in PAH accompanying heart congenital defects [15] but is now recognised in all forms of PAH. In experimental PAH induced by the toxin monocrotaline, changes in elastin and collagen synthesis in the pulmonary artery walls, assessed both biochemically and ultrastructurally, were related to the development of progressive pulmonary hypertension with an increase in both insoluble elastin synthesis and total insoluble elastin content and an increase in collagen synthesis and total collagen content [16]. These changes have also been observed in other models of PAH, such as progressive pulmonary venous obstruction [17].

Noteworthy, the extracellular matrix is both a component of the thickened pulmonary vascular media and a regulator of smooth muscle cell growth. Tenascin, a glycoprotein involved in lung and vascular morphogenesis, which is strongly

expressed in remodelled intima and medial layers of human hypertensive pulmonary arteries [18], is a potent regulator of smooth muscle cell proliferation [18–20]. Fibronectin changes smooth muscle cell phenotype from contractile to migratory.

The adventitia is mostly composed of fibroblasts. There is growing evidence that, rather than just a structural support to pulmonary vessels, the adventitia may also play a role in the regulation of pulmonary vascular function from the 'outside-in' (as comprehensively reviewed in [21]). The normal adventitia represents approximately 15% of the external diameter of pulmonary arteries larger than 50 μm in diameter. In IPAH arteries, the adventitial thickness increases to 28% of artery diameter, predominantly due to collagen deposition [22]. It also contains a perivascular cuff of inflammatory cells, which might modulate the growth of or transdifferentiate themselves into vascular structural cells in the pulmonary vascular wall.

Altogether these data show that extracellular matrix remodelling is a key component of PAH pathophysiology.

Proteases in experimental pulmonary hypertension

Matrix deposition is the result of increased matrix degradation insufficient to counterbalance excessive matrix synthesis [16, 23]. The normally tightly regulated degradation of extracellular matrix results from the activity of several proteases that are active at neutral pH and act in concert. Based on their active sites, two main classes of neutral proteases are the focus of interest: the serine-proteases, including the endogenous vascular elastase (EVE) and the plasminogen activator/plasmin system on one hand and the matrix metalloproteases (MMPs) also called the matrixins on the other hand.

EVE, the endogenous vascular elastase

Early studies, analysing the ultrastructure of pulmonary arteries on lung biopsy from patients with PAH, showed that the internal elastic lamina, which normally separates endothelial from smooth muscle cells in muscular arteries, is fragmented [15]. This suggested that an elastinolytic enzyme might be involved in the pathophysiology of the disease. This was further explored in experimental PAH induced in rats by the toxin monocrotalin, in which an increased number of breaks in the internal elastic lamina was associated with the initiation of vascular structural changes as early as 4 days after the toxin injection [24]. Subsequently, early increase in elastinolytic activity that precedes vascular changes and a later increase associated with progressive disease were confirmed, and the inhibition profile of

enzymatic activity showed that it was attributable to a serine protease [23]. This increased elastinolysis was also shown in chronic hypoxia-induced experimental PAH [25]. Jacob et al. [26] and Hornebeck et al. [27] showed that a serine elastase was produced by aortic smooth muscle cells and associated with atherosclerosis, which was further characterised by Zhu et al. in pulmonary artery smooth muscle cells as the Endogenous Vascular Elastase (EVE) [28]. This elastase, like the polymorphonuclear neutrophil serine elastase, is inhibited by α_1-antitrypsin/α_1-proteinase inhibitor (α_1-PI), α_2-macroglobulin, elafin and by some synthetic inhibitors [29], the most relevant inhibitor in vascular biology being elafin. EVE is a powerful enzyme that, by virtue of its ability to degrade elastin, will also degrade proteoglycans that serve as storage sites for growth factors, such as basic fibroblast growth factor (FGF-2) and transforming growth factor. A study from Rabinovitch's group has shown that EVE releases FGF-2 in a biologically active form that stimulates smooth muscle cell proliferation [30]. Elastase activity either directly or *via* activation of matrix metalloproteinases (see below) can induce production of the matrix glycoprotein tenascin (TN), which optimises the mitogenic response to FGF-2 and is, in fact, a prerequisite for the response to epidermal growth factor (EGF) in cultured smooth muscle cells [19, 20]. The process of smooth muscle cell migration also appears to depend, at least in experimental animals, on the continued activity of elastase. Obviously, elastin peptides stimulate the production of the matrix glycoprotein fibronectin, which changes smooth muscle cells from a contractile to a migratory phenotype [31, 32].

The plasminogen system

Although these findings emphasise the important role of elastase in the pathogenesis of PAH, the question remains open regarding the role of other proteases specialised in extracellular matrix degradation, namely plasminogen activators/plasmin system and MMPs.

Several observations point to an additional role of the plasminogen system in pulmonary hypertension and to enhanced plasmin proteolysis that contributes to pulmonary vascular remodelling. The central reaction of the plasminogen activator (PA) system is the conversion of plasminogen to plasmin by plasminogen activators (PAs), the tissue plasminogen, tPA, and urokinase plasminogen activator, uPA [33]. The serine protease plasmin degrades fibrin to fibrin-degradation products. However, plasmin has several substrates other than fibrin, including blood coagulation factors, cell surface receptors, some matrix metalloproteinases, that plasmin activates, and structural components of the extracellular matrix such as fibronectin or laminin. While plasminogen resides primarily within the plasma, with the liver representing the primary site of plasminogen synthesis, plasminogen mRNA is present in several tissues, including adrenal, kidney, brain, testis, heart, lung, uterus, spleen,

thymus, and gut, supporting a broadly distributed functional role of the PA system [34]. Tissue-type PA and u-PA activate plasminogen by cleaving a specific Arg-Val peptide bond located within the protease domain. The activation of plasminogen by t-PA is highly dependent on the presence of cofactors, such as fibrin, that bind and alter the conformation of plasminogen [35]. The activation of plasminogen by u-PA is tightly regulated at the cell surface, due to anchorage of u-PA to cell surface *via* a specific receptor, the uPA-R. Plasmin formation is intensely regulated by PA inhibitors, which inhibit t-PA and u-PA, most notably PA inhibitor-1 (PAI-1) [33]. Plasmin is directly inhibited by α_2-antiplasmin, which circulates in plasma.

In addition to functioning within the vascular lumen to control fibrinolysis, the PA system is active within the blood vessel wall, where it plays an important role in controlling vascular remodelling. The development of intimal hyperplasia after vascular injury is diminished in plasminogen-deficient mice, supporting the concept that plasmin associated with vascular smooth muscle cells enhances cell migration by fostering extracellular matrix degradation, either directly or indirectly by activating matrix metalloproteinases [36]. Vascular smooth muscle cells express u-PA and its receptor. Urokinase (u-PA) deficiency and pharmacological inhibition of u-PA receptor, but not t-PA deficiency, inhibit neointima formation in mice, suggesting that u-PA-triggered plasmin formation drives vascular smooth muscle cell migration [37–39]. Regarding pulmonary vascular system and pulmonary hypertension, hypoxia increases expression of u-PAR, enhances plasma fibrinolytic activity, and upregulates expression of plasminogen activators during ventricular hypertrophy in response to hypoxia or overloading [40–42]. Interestingly, and also similar to elastase, plasmin could also participate in smooth muscle cell proliferation indirectly as it has been shown to induce the release of FGF-2 from the extracellular matrix [43].

The matrix metalloproteases

The matrixins form a family of >20 members known in humans, initially identified based on their ability to degrade extracellular matrix proteins (e.g., collagenases degrade fibrillar collagens, metallo-elastase, elastin, etc.), and are known to have many other roles [44]. One of the striking features of the matrixins is that many of those genes are 'inducible'. The effectors include growth factors, cytokines, chemical agents (e.g., phorbol esters, actin stress fibre-disrupting drugs), physical stress and oncogenic cellular transformation. MMP gene expression may be downregulated by suppressive factors (e.g., transforming growth factor, retinoic acids, glucocorticoids). Their proteolytic activities are precisely controlled during activation from their precursors and inhibition by endogenous inhibitors, α-macroglobulins and tissue inhibitors of metalloproteinases (TIMPs). All matrixins are synthesised as pre-pro-enzymes, the loss of the 'pre' peptide is a signal of secretion. Apart from a few members activated

intracellularly by furin, most are secreted from the cell as inactive zymogens. Secreted promatrixins are activated *in vitro* by proteinases, such as plamin, and by nonproteolytic agents, such as *in vivo* reactive oxygen species and *in vitro*, thiol-reactive agents, mercurial compounds, and denaturants. In all cases, activation requires the disruption of the Cys-Zn^{2+} (cystein switch) interaction between the zinc of the active site and the cystein present within the 'pro'-domain. Subsequently, the removal of the propeptide proceeds often in a step-wise manner [45]. *In vivo*, most promatrixins are likely to be activated by tissue or plasma proteinases, such as neutrophil elastase [46] or plasmin, or opportunistic bacterial proteinases. Using transgenic mice deficient in uPA, Carmeliet et al. have shown that the uPA/plasmin system is, *in vivo*, a pathophysiologically significant activator of promatrixins [45]. By contrast, no data exist regarding activation of promatrixin by EVE. Other activation cascades are known; for example, the activation of pro-MMP-2 takes place primarily on the cell surface and results from the action of membrane-anchored MMPs, the 'membrane-type MMPs' (MT-MMPs). Studies have shown that this activation process requires both active MT1-MMP and the TIMP-2-bound MT1-MMP [47].

TIMPs (21–30 kDa) are the major endogenous inhibitors of MMP activities in tissue, and four homologous TIMPs (TIMPs 1–4) have been identified to date [48]. TIMPs exhibit additional biological functions. As detailed above, TIMP-2 plays a role in MMP-2 activation. TIMP-1 and TIMP-2 have mitogenic activities on a number of cell types, whereas overexpression of these inhibitors reduces tumour cell growth, and TIMP-2, but not TIMP-1, inhibits FGF-2 induced human endothelial cell growth. These biological activities of TIMPs are independent of MMP-inhibitory activities [49].

MMPs, particularly gelatinase A/MMP-2, which degrade the type IV collagen of basement membranes, are increased in the pulmonary vascular bed, during both toxin- and hypoxia-induced experimental PAH [50]. Increases in interstitial collagenase (MMP-13), stromelysin-1 (MMP-3) and gelatinases A (MMP-2) and B (MMP-9) have also been described following return to normoxia [51, 52]. Interestingly, inhibition of MMPs by either a synthetic inhibitor, doxycycline, or adenovirus-mediated human TIMP-1 gene transfer during chronic hypoxia is associated with exacerbation of PAH and vascular remodelling. Either of two MMP-inhibiting treatments increased muscularisation and collagen accumulation in small pulmonary arteries [53], providing strong support for the argument that MMPs play a crucial protective role in hypoxic PAH. In keeping with these results is the demonstration that deficiency of uPA-mediated plasmin generation impairs vascular remodelling in hypoxic PAH [54]. MMPs and plasmin probably protect against PAH by limiting matrix deposition. Another important hypothesis concerns angiogenesis, which represents an important protective mechanism, as demonstrated by increase in lung vascular endothelial growth factor (VEGF) during hypoxic PAH, improvement of PAH after VEGF gene transfer and worsening of PAH following angiostatin gene transfer [55, 56].

In contrast to hypoxic PAH, inhibition of MMPs in an *ex vivo* model of organ culture of pulmonary artery rings obtained from rats treated by monocrotalin, showed regression of medial thickness to control levels [57]. Similar results in this study, obtained by either serine-protease inhibitors or metalloprotease inhibitors, come from tight interactions between the two proteolytic systems, as elastase can activate some pro-MMPs [46] and degrade the MMP-inhibitor, TIMP-1 [57]. In contrast, most MMPs degrade the major elastase inbihitor, α_1-PI [46]. In keeping with the *ex vivo* results, *in vivo* experiments in toxic monocrotaline-induced PAH have shown that inhibition of MMPs by adenovirus-mediated human TIMP-1 gene transfer in the lung leads to less severe PAH with decreased muscularisation and increased lung-cell apoptosis, as compared to controls [58]. The effect of TIMP-1 on PAH is consistent with an ability of MMP inhibition to prevent monocrotaline-induced pulmonary vascular remodelling and PAH, in part by reducing smooth muscle cell migration and proliferation. All together, these data support a synergistic and deleterious role for serine- and metalloproteases in toxic PAH and indicate that MMPs may have opposite effects in different PAH models.

Proteases and BMPs

BMPs are the largest group of cytokines within the TGF-β superfamily and were originally identified as molecules regulating growth and differentiation of bone and cartilage [59]. BMPs regulate growth, differentiation, and apoptosis in a diverse number of cell lines, including mesenchymal and epithelial cells, acting as instructive signals during embryogenesis and contributing to the maintenance and repair of adult tissues [59, 60]. TGF-β superfamily type II receptors are constitutively active serine-threonine kinases and form homodimers that exist constitutively or are recruited to receptor complexes on ligand stimulation. BMPR-II is distinguished from other TGF-β superfamily type II receptors by a long carboxyl-terminal sequence following the intracellular kinase domain [61]. BMPR-II initiates intracellular signalling in response to specific ligands: BMP 2, BMP 4, BMP 6, BMP 7, growth and differentiation factor-5 (GDF 5), and GDF 6 [61].

Mutations of *BMPR-II* have been found in FPAH [10]. Approximately 30% of mutations are missense mutations occurring in highly conserved amino acids with predictable effects on receptor function. For example, many of these involve the serine-threonine kinase domain of BMPR-II or the extracellular ligand binding domain. However, the majority (~70%) of *BMPR2* coding mutations are frameshift and nonsense mutations, many of which would be expected to produce a transcript susceptible to nonsense-mediated mRNA decay. Thus, haploinsufficiency for BMPR-II represents the predominant molecular mechanism underlying inherited predisposition to FPAH. Further genetic analysis is revealing an increasing number of families in which BMPR-II mutation is implicated, including the identification

of gene deletions and rearrangements. Interestingly, BMPR-II alterations are also involved in experimental PAH, either induced by chronic hypoxia [62] or by the toxic model, monocrotalin-induced [63].

Clearly, one major mechanism by which BMPR-II plays a role in FPAH is smooth muscle proliferation. However, interference with extracellular matrix remodelling can be hypothesised. Very recently, induction of uPA upon BMP 4 stimulation of BMPR-II has been reported in tumoral cell line [64]. This report is the only one to date that has explored induction of protease(s) after engagement of the BMPr-II receptor but is certainly a promising direction.

MMPs in human pulmonary hypertension

Very few studies are available in the human disease, regarding MMPs. Based on studies using cultured human smooth muscle cells isolated from elastic pulmonary arteries from IPAH patients obtained surgically, we reported MMP/TIMP production by smooth muscle cells *in vitro*. We also performed immunolocalisation in whole medium-sized pulmonary arteries. *In vitro*, TIMP-1 was overexpressed and MMP-3 underexpressed by IPAH cells, whereas MMP-1 expression was similar in the two groups. Total MMP-2 overexpression was also found, with a greater amount of the active form in IPAH cells as compared with controls. *In situ* studies showed gelatinolytic activity in tissue sections and strong MMP-2 immunostaining along the inner elastic lamina up to the lamina break. TIMP-1 immunostaining was found in both control and IPAH arteries, whereas MMP-3 staining was detected only in the media of a few control specimens [65]. It is unclear whether a similar pattern is present in distally remodelled pulmonary arteries; however, endothelial cells express moderate/intense immunohistochemical expression of MMP-2, while myofibroblasts display low levels of this extracellular protease [66]. Membrane type-1-MMP was also expressed in endothelial and myofibroblastic cells of concentric and plexiform lesions.

Demonstration of a TIMP-1–MMP imbalance conducive to extracellular matrix accumulation does not rule out a role for active MMP-2 in IPAH. Proteolysis may be effective in limited areas even when TIMP levels are high in the extracellular milieu, because MMP-2 tethering and activation at the cell surface focuses the catalytic activity on limited targets on the cell membrane. This hypothesis is supported by immunohistology and *in situ* zymography data, which clearly show that gelatinolytic activity colocalised with MMP-2 immunostaining in arteries from IPAH patients [65]. This pattern of MMP and TIMP expression, characterised by increased TIMP-1 levels coexisting with evidence of extracellular matrix degradation, has been found in other fibrotic diseases, such as adult respiratory distress syndrome (ARDS). In bronchoalveolar lavage (BAL) fluid from ARDS patients, TIMP-1 levels were significantly higher than in healthy controls. Despite the high TIMP-1

levels, extracellular matrix degradation by MMPs is suggested by the presence of active MMP-2 in epithelial lining fluid [67] and of basement membrane disruption markers in BAL fluid of ARDS patients [68]. Altogether, these data suggest that a TIMP-1–MMP imbalance promoting extracellular matrix accumulation within the interstitial tissue may coexist with the presence of active MMP-2 confined to the cell surface.

In IPAH, disruption of the internal elastic lamina, extracellular matrix disorganisation and smooth muscle cell migration are strong arguments supporting a direct role for active MMP-2. This enzyme not only degrades nonfibrillar collagen, but also cleaves elastin. Moreover, latent-MMP-2 may both bind to elastin and undergo auto-activation, subsequently degrading elastin [69]. These results on MMP-2 expression in PAH are consistent with previous data, as *in situ* zymography and MMP-2 immunolocalisation showed colocalisation of gelatinolytic activities and MMP-2 along elastic fibres. Also, active MMP-2 may contribute not only to extracellular matrix remodelling but also to important processes in IPAH, such as smooth muscle cell migration and proliferation [70].

Inflammation and pulmonary hypertension

There is compelling evidence of global immunological alterations in IPAH patients [71] and PAH occurs in the setting of profound immune deregulation underlying HIV infection and collagen vascular diseases. The recognition of an inflammatory component in PAH [12] supports the investigation of expression of cytokines that might potentially drive perivascular inflammation and thus contribute to the disease. Remodelled pulmonary arteries express IL-1, IL-6, and PDGF in infiltrating inflammatory cells [78, 79], the chemokine RANTES (acronym for regulated upon activation, normal T cell expressed and secreted), an important chemoattractant for monocytes and T cells, and the macrophage inflammatory protein-1α (MIP-1α). Lungs of IPAH patients have increased expression of fraktaline, a chemokine involved in T cell trafficking and monocyte recruitment, and their circulating CD4 and CD8 T cells have higher levels of the fraktaline receptor CX3CR1 when compared with controls or samples of patients with thromboembolic PAH.

Inflammatory cells infiltrating remodelled pulmonary arteries may include subpopulations of vascular precursor or early-progenitor cells, also potential contributors to pulmonary vascular remodelling in PAH. Pulmonary arteries in PAH caused by chronic hypoxia contain an infiltrating subpopulation of fibrocytes, identified by the expression mononuclear cell markers CD45, CD11b, CD14, and the fibroblast marker α1-procollagen. About 15% and 20% of these cells also undergo proliferation and express smooth muscle α-actin, respectively [72, 73]. These studies also document that depletion of circulating monocytic cells alleviates pulmonary vascular remodelling caused by chronic hypoxia. Endothelial cell precursors may play a

beneficial role in PAH since their administration to monocrotaline-treated rats has dramatic healing effects in remodelled pulmonary arteries, notably when transfected with the endothelial nitric oxide synthase gene [74]. Pulmonary vascular inflammation has also been documented in chronic obstructive pulmonary disease with or without coexisting PAH [14, 75–78], and vascular progenitor cells identified in lung vessels [77].

Within the scope of the present review, it should be stressed that protease production is part of the inflammatory response. Sources of protease are circulating cells, leukocytes [79, 80] and platelets [81, 82], together with resident endothelial [83] and smooth muscle cells [84].

Therapeutic consequences and future directions

In experimental models, protease inhibition, especially elastase inhibition has clearly proven its efficacy, both at preventing and curing PAH induced by monocrotalin [18, 57]. Accordingly, overexpression of the EVE inhibitor elafin protects partially transgenic mice from hypoxic PAH [85]. However, conflicting results have been obtained regarding metalloprotase inhibition, depending on the considered model: worsening hypoxic PAH [53] and preventing toxic monocrotalin-induced PAH [58].

It is certainly worth asking the question "should we expect an improvement in PAH when using protease inhibitors?". It seems quite clear-cut when thinking of elastic lamina fragmentation, but not so obvious when taking into account the large increase in extracellular matrix deposition observed in pulmonary vascular beds and regarding all other roles for these proteases in different cellular processes, such as angiogenesis, cell migration and cell differentiation. All these considerations leave the question of their beneficial or deleterious role open. The tight interplay between the three proteolytic systems further complicates the answer.

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